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Ethnic comparisons, microbiomes and more: relevant research for better health

This issue of the journal contains seventeen interesting articles comprising three reviews and fourteen original research papers. They cover various topics on communicable and non-communicable disorders. The latter are of particular interest because of changing disease patterns attributable to adoption of western lifestyles with dietary changes. We have increased the number of papers published per issue as a result of high patronage.

The dearth of radiotherapy facilities for managing cancers was highlighted in one of the review articles which serves to remind everyone about the increasing challenges of cancer management in resource-poor settings. Cross-cultural studies are important for teasing out environmental factors in disease phenotypes. Odusan and colleagues compared the patterns of diabetes mellitus in Nigerians and Basotho. They showed that anthropometric measurements correlated with age, blood pressure measurements and glycated haemoglobin levels irrespective of ethnicity. It would be necessary to follow up with genetic studies. Genomic comparison of the major Nigerian tribes by Adetona and Shokunbi is an interesting and challenging paper particularly with the finding that only 2% molecular variance was present between the ethnic groups, although the use of microsatellites could differentiate the ethnic groups. A retrospective-prospective analysis of data on muscular dystrophy by Oyinlade and colleagues confirmed the preponderance of Duchene muscular dystrophy. The challenges with diagnosis and management were highlighted. Jaiyeoba_Ojigho reported on the bilateral symmetry of the mandibular ramus in Nigerians which was not the experience in some populations. It may thus have forensic value. The pathogenesis and mechanics as well as the surgical management of acetabular protrusion was elegantly reviewed by Ayekoloye and colleagues.

Human immunodeficiency virus (HIV) is a disease associated with considerable stigma and this was dealt with in some details by Lawal *et al;* while Onifade and Salako presented a preliminary report on A-Zam - a herbal remedy used for treating 9 HIV cases with significant reduction in viral load. Recent literature on neurodegenerative diseases has firmly placed gut microbiomes in the pathogenetic mechanisms of many, and a first of its kind in our environment is the paper by Nwaokorie and others comparing microbiomes in rural vs. urban populations. This should lead to more exploration of microbiota in various diseases.

With regards to ethnopharmacy, one manuscript highlighted the use of mango derivatives in the management of diabetic wounds in experimental animals while the second reported on the therapeutic benefits of *Drymaria cordata* in reversing glutamate-induced lesions. Okunlola and colleagues in their open-labelled, randomized prospective study reported that the use of oral bicarbonate could be cost-effective in retarding the progression of end-stage kidney disease. The rightful place of improved education in averting various disorders or complications was the theme of some of the articles including Adeyera and others warning against the use of skin lightening creams by pregnant women because of the deleterious effects on both the mothers and their babies while Oke and Balogun emphasized its importance for educating young female graduates on preconception care; Olajide and colleagues focused on its importance on warning about safe sex; and for educating patent medicine vendors on reporting adverse drug reactions. Lastly, Michael and colleagues noted a high frequency of rodenticide use without conformity with safe practices that could result in environmental and clinical toxicity.

This issue of the journal will wet readers' appetite on genomic and ethnic comparisons, microbiomes, traditional/alternative medicine, appropriate and cost-effective treatment modalities and the importance of education in improving health. These little steps in health care should translate to giant strides in making life better for all.

A. Ogunniyi
Editor-in-Chief

Contents

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Factors associated with inconsistent condom use and multiple sexual partners among adolescents in South Western Nigeria

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Abstract
Objective: The study examined factors associated with inconsistent condom use and multiple sexual partners among adolescents in Osun State.
Methods: Descriptive cross-sectional study of 450 adolescents interviewed using a pre-tested, semi-structured questionnaire. Focus group discussions (FGDs) were conducted among this in-school and out-of- school adolescents. Main outcomes were inconsistent condom use and multiple sexual partners within previous year. Qualitative data was used to triangulate findings from the quantitative data which was analyzed using SPSS version 16.
Results: The mean age of respondents was 15 (SD 2.6) years. One hundred and thirty adolescents (28.9%) have had sexual intercourse and only 36 (27.7%) of these used condom consistently. Fifty-five respondents (42.3% of the sexually active) had multiple sexual partners in the last year. Most participants in FGD agreed that adolescents get involved in high-risk sexual practices secondary to peer pressure and lack of parental care. Pornography, male gender and castigation of chastity among peers were some of the factors significantly associated with multiple sexual partners ($p < 0.05$).
Conclusion: Sexually active adolescents have low risk perception of condom use and have multiple sexual partners. Preventive education could increase their risk perception and reduce risk taking among this vulnerable group.

Keywords: *adolescents, inconsistent condom use, multiple sexual partner.*

Abstrait
Objectif : L'étude a examiné les facteurs associés à l'utilisation irrégulière du préservatif et aux partenaires sexuels multiples parmi les adolescents de l'État d'Osun.
Méthodes : Une étude transversale descriptive de 450 adolescents interrogés à l'aide d'un questionnaire semi-structuré pré-testé. Des discussions de groupe focus (DGF) ont été organisés parmi ces adolescents scolarisés et non scolarisés. Les principaux résultats étaient l'utilisation irrégulière du préservatif et la multiplicité des partenaires sexuels au cours de l'année précédente. Des données qualitatives ont été utilisées pour trianguler les résultats des données quantitatives qui ont été analysées à l'aide de SPSS version 16.
Résultats : L'âge moyen des répondants était de 15 ans (ET 2,6). Cent trente adolescents (28,9%) ont eu des rapports sexuels et seulement 36 (27,7%) d'entre eux ont utilisé le préservatif régulièrement. Cinquante-cinq répondants (42,3% des sexuellement actifs) ont eu plusieurs partenaires sexuels au cours de la dernière année. La plupart des participants aux discussions de groupe focus ont convenu que les adolescents s'impliquent dans des pratiques sexuelles à haut risque en raison de la pression des pairs et le manque de soins parentaux. La pornographie, le genre masculin et la fustigation de la chasteté entre les pairs étaient quelques-uns des facteurs significativement associés aux partenaires sexuels multiples ($p < 0,05$).
Conclusion : Les adolescents sexuellement actifs ont une faible perception du risque de l'utilisation du préservatif et ont de multiples partenaires sexuels. L'éducation préventive pourrait accroître leur perception du risque et réduire la prise de risque au sein de ce groupe vulnérable.

Mots clés : *adolescents, utilisation irrégulière du préservatif, partenaire sexuel multiple.*

Introduction

Adolescents are defined by the World Health Organization as those between the ages of 10 and 19 years [1]. This group of people possesses desire to be

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free from strict parental control; defiant behaviour and high level of desire for the excitement and sophistication of modern life styles [1-3]. Adolescents are disproportionately affected by the reproductive health morbidity of high-risk sexual practices such as unwanted pregnancies, abortion, sexually transmitted infections (STI) including HIV/AIDS [1,4,5]. A study in South East Nigeria reported that pre-marital sex is common among adolescents as three-quarter (75%) had sex before 19 years, with majority (69%) having multiple sexual partners. Most adolescents reported having had sex in exchange for money or gifts with only 13.5% ever using condoms. Many adolescents' experienced major stressors due to unwanted pregnancy, including school and job termination, partner rejecting the pregnancy, religious sanction, discrimination, and stigmatization [5].

Adolescents account for a significant proportion of unsafe abortions globally. According to the World Health Organization (WHO) at least one-third of all women seeking hospital care for abortion complications are less than 20 years [4]. Several reports had showed that majority of abortion seekers are adolescents [6]. Also, young person participates in risky sexual activities including early sexual debut [7], multiple sexual partners and inconsistent use of condoms [8,9].

Sub-Saharan Africa has the highest prevalence of HIV infection worldwide with 22.5 million HIV positive people resident out of 33.3 million people living with HIV worldwide in 2009 [10]. Nigeria rank second to South Africa with 3.1 million people living with HIV/AIDS [10]. The HIV prevalence in Nigeria has evolved with the first case reported in 1986, subsequent antenatal sentinel surveys showed rates of 1.8% in 1991, 5.8% in 2001, 4.4% in 2005, 4.6% in 2008 and 4.1% in 2010 [10]. Majority (80%) of people living with HIV were infected via the unprotected heterosexual intercourse while rest become infected through transfusion of blood and blood products, and mother-to-child transmission in pregnancy, delivery and breast feeding [10]. HIV infection through other means are few but becoming important. The key drivers of HIV epidemic in Nigeria are low risk perception, multiple concurrent partners, informal transactional and intergeneration sex, lack of effective services for sexually transmitted infections (STIs), poor quality of health services, gender inequalities, poverty, and HIV/AIDS stigma and discrimination.

The lack of family life education in most schools and treating sex as a taboo had encouraged adolescents to consult their peer for sex education with virginity becoming unpopular especially among these vulnerable group [11-14]. A central problem in the planning of communication campaigns to change behaviours is identification and application of appropriate communication, persuasion, and behaviour change theories to overcome obstacles to behaviour change [14,15]. Persistence of high-risk sexual behaviour among adolescents despite various forms of interventions by diverse organizations makes it imperative for studies focusing on factors associated with these behaviours so that interventions can be strategically directed.

he study aimed at elucidating the factors associated with inconsistent condom use and multiple sexual partners among adolescents in Osun State with a view to providing a basis for effective intervention.

Materials and methods

The study employed a descriptive cross-sectional study design among adolescents in Osun State, South Western Nigeria. Osun State was created in 1991 from old Oyo State. It has three senatorial district and 30 Local Government Areas (LGAs).

The sample size was determined using an appropriate statistical formula for estimating the minimum sample size in descriptive health studies [$n=Z^2pq/d^2$] [16], Where, n= Sample Size, Z= standard normal deviate corresponding to confidence level at 95% =1.96, d= degree of accuracy desired =0.05, p= 36% of adolescents had multiple sexual partners in a study conducted in Osun state [17] while q=1-p=1-0.36=0.64. $n=1.96^2*0.36*0.64/(0.05^2) = 354$. A sample size of 400 was used after non-responders were taken into consideration.

Multi-stage sampling technique was used to select respondents. In the first stage, simple random sampling technique utilizing the ballot method was used to select one Local Government Area (LGA) out of each of the three senatorial districts in Osun State, giving a total of three LGAs altogether for the study. Simple random sampling technique utilizing the ballot method was used to select twenty enumeration areas from each of the selected LGAs in the second stage. This gave a total of sixty enumeration areas. Systematic random sampling method was then used to select eight houses from each of the enumeration areas. The total number of houses in each enumeration area was divided by 8 to get the sampling interval. The first house was randomly selected and the sampling interval calculated

was used to determine the houses to be selected. In the last stage, simple random sampling technique utilizing ballot method was used to select one out of all the adolescents in each house. All adolescents who were not married were included in the study while the married ones were excluded.

Quantitative and qualitative methods of data collection were employed. The quantitative aspect made use of a pre-tested semi-structured interviewer administered questionnaire divided into various sections including socio-demographic characteristics, sexual behaviour, factors influencing sexual behaviour, perceptions about unsafe sexual practices and its consequences. The qualitative aspect made use of a focus group guide exploring factors associated with unsafe sexual practices and condom use. The questionnaire and the focus group guide were derived from literature review and validated by the authors using face validity and making necessary adjustments where required to the questions before use in the study. Research assistants were trained on the use of the questionnaire before data collection. The research assistants were graduate students awaiting youth services and some officers of the national population commission that have previous experience in quantitative method of data collection. Focus group discussions were conducted among the adolescents in the selected three LGAs in the State. Four focus group discussions (FGD) were conducted per LGA among in-school male, in-school female, out-of-school male and out-of-school female adolescents. The number of participants in each FGD was between 8 and 10. In-school adolescents were purposely selected from some secondary schools located within the LGA not included in the quantitative aspect of the study while out-of-school respondents were selected from among the apprentices in each LGA. The apprentices selected included those learning tailoring, hairdressing; glass cutting. The instruments used in this study were translated into Yoruba, the prevalent local language spoken in the study area and back translated into English. The FGD was conducted by the researchers and notes were taken by a research assistant. The discussions were recorded on a tape and were transcribed immediately. For the purpose of the study, adolescents that did not use condom at every sexual exposure were categorized under inconsistent condom users.

The questionnaire was checked and sorted out manually, and the data obtained were entered and analyzed using SPSS statistical software package version 16. The data was edited and frequency distribution tables were generated for the variables to determine their socio-demographic distribution and sexual behaviour. Bivariate analysis was done to determine factors associated with sexual behaviour. The significant level was set at $p < 0.05$. Responses from the focus group discussion were manually summarized to triangulate the results of the quantitative method of data collection.

Permission to conduct this study was obtained from the Ethical and Research Review Committee of the Ife Central Local Government, Ile-Ife, Osun State, Nigeria. The purpose of the study was explained to the head of the households that the selected adolescents belonged to and also to the respondents. Also, written consent was taken from the parents/guardians and respondents 18 years and above while assent was taken from participants less than 18 years. The researchers ensure privacy with the data secured in a passworded computer.

Results

The mean age (SD) of respondents was 15 (3) years. Their ages were almost equally distributed across the age groups with 155 (34.4%) in the early adolescence and 148 (32.9%) in the late adolescence. There were 230 (51.1%) males and 220 (48.9%) females. Two hundred and fifty eight respondents (57.3%) were Muslims while 167 (37.2%) were Christians. More than half of them (54.7%) were in secondary school while 101 (22.4%), 95 (21.1%) and 8 (1.8%) had primary, tertiary and no education respectively. Only 139 respondents (30.9%) came from polygamous family setting while 151 (33.6%) were not living with both parents (Table 1).

About one third of the respondents (30.9%) have ever worked to earn money, 18 (4.0%) have ever drank alcohol, 173(38.4%) of them have had previous premarital sex, 157 (34.9%) of them have friends that have previous premarital sex, while 88 (19.6%) of them have friends that have been pregnant or made someone pregnant. Only 22 (4.9%) think that their parents support premarital sex.

One participant stated: 'My parents do not support premarital sex; my mother always warns me about having sex while in school as I might get pregnant hence ending my education' 12 year old female.

Over one quarter 130 (28.9%) have had sexual intercourse, 47.8% used condom inconsistently while 71.3% had multiple sexual partners in the previous 12 months (Figure 1). Only 27.7% of the respondents used condom at last sexual intercourse in this study.

A participants stated: 'My parents do not know I have a boyfriend, my mother always check my uniform pockets and school bag for condoms; she also warn me to abstain from premarital sex' 16 year old female.

Table 1: Socio-demographic characteristics of respondents (n=450)

Socio demographic characterisitics	Frequency	%
Age group (years)		
10-13 (early adolescent)	155	34.4
14-16 (middle adolescents)	147	32.7
17-19 (late adolescents)	148	32.9
Sex		
Male	230	51.1
Female	220	48.9
Religion		
Christinity	167	37.2
Islam	258	57.3
Traditional	23	5.1
Others	2	3
Level of education of respondents		
None	8	1.8
Primary	101	22.8
Secondary	246	54.7
Tertiary	95	21.1
Level of educationf fathers		
None	15	3.3
Primary	77	17.1
Secondary	129	28.7
Tertiary	229	50.9
Level of education of mothers		
None	24	5.3
Primary	98	21.8
Secondary	130	28.9
Tertiary	198	44.0
Who respondents live with		
Both parents	299	66.4
One of the parent	113	25.1
None of the parents	38	8.5
Numher of wives of respondents' father		
None	20	4.4
One	291	64.7
More than one	139	30.9
Birth Order of respondents		
1st Born	121	26.9
2nd born	105	23.3
3rd born	86	19.1
4th born and above	138	30.7

A smaller proportion used condom during their first sexual intercourse (26.9%) compared to their most recent sexual intercourse (41.5%). Over two fifth of the sexually active (42.3%) had multiple sexual partners with 19% becoming pregnant or getting somebody pregnant in the last twelve months. Although 28.4% of them have been forced into sexual intercourse, only 9.2% have ever forced somebody to have sex with him or her.

One participant stated: I was forced to have sex by my master that I am learning from. He took special interest in me when I joined his outfit to learn the trade' 15-year-old female.

Another stated: 'I learnt from friends that it is more common for men to force the females to have sex with them than otherwise; but I stated having sex at a very young age with an older female' 17-year-old male.

About a quarter of the sexually active respondents (26.2%) have had abnormal discharge or itching in their private part while 19.2% of them have had sore or ulcer in their private parts before. Almost a quarter (22.2%) were not aware that high risk sexual practices predispose to sexually transmitted infections.

One participant stated: 'I never knew the dangers of premarital sex till I had gonorrhoea, I was lucky to get treatment in the hospital, since then, I have stopped having premarital sex' 14 year old male.

Majority 260 (57.8%) could not resist sexual advances while 376 (83.6%) believed that there is no divine approval of premarital sex. Over one third 172 (38.2%) believed that society disapproved of chastity while 70 (15.6%) could not reveal avoiding premarital sex to their peers. Only 15% of these college students perceived themselves to be at moderate-to-high risk of acquiring HIV infection compared with 85% who perceived themselves to be at little or no risk.

Another participant stated: 'It is difficult to resist sexual advances from your master, my formal master had sex with me several times before I left him, he never took NO for an answer' 15 year old female. 'It is easy to be lured into sex if your master is very nice to you especially if you are not experienced' 13 year old female.

Table 2 reported that multiple sexual intercourse was significantly associated with respondents' perception of parental approval of premarital sex, (p=0.004), males, alcohol use, (p=0.001), watching pornography (p=0.004). Also, 68.0% of those that were unable to tell their friends about abstinence from premarital sex had multiple sexual partners (p=0.022).

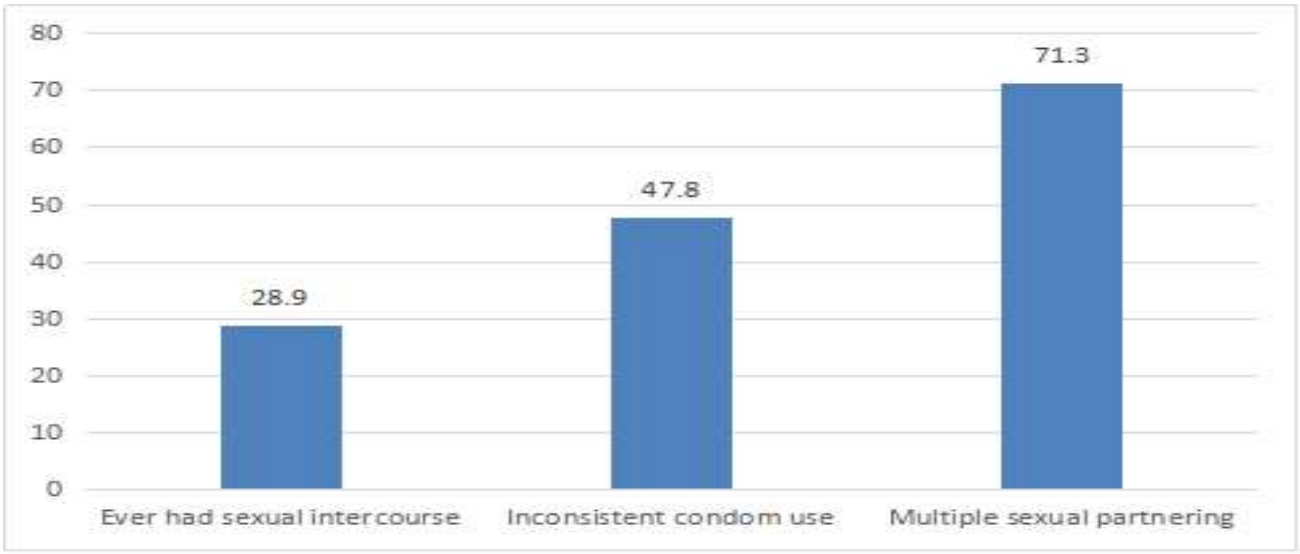

Fig. 1: Sexual history of respondents

Table 2: Factors significantly associated with having multiple sexual partners among respondents that were sexually active in the last 12 months (n=115)

Variable	Had mutiple sexual partners		Total	Error! Reference source not found 2	p value
Sex					
Male	35(57.4)	26(42.6)	61	4.75	0.030
Female	20(37.0)	34(63.0)	54		
Ever drank alcohol					
Yes	16(88.9)	2(11.1)	18		0.001*
No	39(40.2)	58(59.8)	97		
Watch pornographic films					
Yes	20(71.4)	8(28.6)	28	8.26	0.004
No	35(40.2)	52(59.8)	87		
Perceived parental disposition towards premarital sex among adolescents					
Approve	13(81.3)	3(18.7)	16	8.32	0.004
Diapprove	42(42.4)	57(57.6)	87		
Boldness to tell peers about chastity					
Bold	38(42.2)	52(57.8)	90		
Not bold	17(68.0)	8(32.0)	25	5.21	0.022
Perception of societal belief					
Believe that society favours premarital sex among adolescents.	33(57.9)	24(42.1)	57	4.59	0.032
Believe that society does not favour premarital sex among adolescents	22(37,9)	36(62.1)	58		
Perception on divine approval					
Believe that there is divine approval of premarital sex	20(66.7)	10(33.3)	30	5.77	0.016
Believe that there is no divine approval of premarital sex	35(41.2)	50(58.8)	85		

* = *Fishers Exact value*

Table 3: Factors significantly associated with inconsistent condom use among sexually active respondents (n=130)

Variable	Consistent use of condom (%)	Inconsistent use of condom (%)	Total	Error! Reference source not found 2	p value
Watch pornographic films					
Yes	13(46.4)	15(53.6)	28	4.75	
No	23(2.5)	79(77.5)	102	6.26	0.012
Had abnormal discharge and/or itching of the private part in the last twelve months					
Yes	14(41.2)	20(58.8)	34		
No	22(22.9)	74(77.1)	96	4.18	0.041
Ever being forced to have sexual intercourse					
Yes	15(40.5)	22(59.5)	37	4.12	0.042
No	21(22.8)	72(77.2)	92		
Ever forced someone to have sexual intercourse with him/her	10(71.4)	4(28.6)	14	14.9	0.001
Never forced someone to have sexual intercourse with him/her	26(22.4)	90(77.6)	116		
Believe that condom is effective against STI and unwanted pregnancy	35(33.4)	64(64.6)	99		<0.001*
Believe that condom is ineffective against STI and unwanted pregnancy	1(3.2)	30(96.8)	31		
Believe that he/she is able to resist sexual advances	34(37.8)	56(62.2)	90		<0.001*
Believe that he/she is unable to resist sexual advances	2(5.0)	38(95.0)	40		
Believe that there is divine approval of premarital sex	17(58.6)	12(41.4)	29	17.8	0.0002
Believe that there is no divine approval of premaritral sex.	19(18.8)	82(81.2)	101		

* = *Fishers exact value*

Table 3 reported that higher proportion of sexually active respondents from a polygamous family setting compared with those from monogamous family setting did not use condom at last sexual intercourse (86% vs. 62.5%; p= 0.008). Also, higher proportion of respondents with lower education compared with those with higher education used condom at last sexual intercourse (48.1% vs. 25.7%; p=0.024). Other factors associated with condom use at last sexual intercourse include knowledge of dual role of condom in prevention of STIs and unwanted pregnancy ($p < 0.001$). Inconsistent condom use and sexual intercourse with more than one partner were generally identified as acts that may predispose to sexually transmitted infections including HIV/AIDS and unwanted teenage pregnancy (high-risk sexual practices) but males out-of-school included men having sex with men in the list. Majority of them agreed that some adolescents have multiple sexual partners.

A participant stated: 'I first had sex with my mother's driver several years ago in primary school, since then I have had several boyfriends including my lesion teacher, we hardly use condom' 16-year-old female. Also, some participants asserted that people laugh at somebody of their age that have never had sexual intercourse. For instance, a participants said: 'It is seen as strange that you do not have sex, it means you do not belong' 15-year-old male.

Table 4: Logistic regression analysis of selected variables associated with having multiple sexual partners

Variable	Odd ratio	95% CI	p-cvalue
Sex			
Male	2.29	1.08-4.86	0.031
Female (ref.)			
Ever drank alcohol			
Yes	11.90	2.59-54.67	0.001
Watch pornographic films			
Yes	3,71	1.47-9.37	0.005
No (*ref.)			
Perceivedparental disposition towards premarital sex among adolescents			
Approve	5.88	1.58-21.95	0.008
Do not approve (ref)			
Boldness to tell peers about chastity			
Bold	2.91	1.14-7.43	0.026
Not bold (ref.)			
Believe that society favours premarital sex among adolescents			
Yes	2.25	1.07-4.75	0.033
No (ref.)			
Believe that there is divine approval of premarital sex			
Yes	2.86	1.19-6.84	0.018
No (ref.)			

Lack of proper parental care, joblessness, peer pressure, deception by men and poverty were the factors believed to be responsible for these high risk sexual practices. A participant stated: 'Many of my mates engaged in premarital sex but my mother talked to me about waiting till I grow up and get married, I think home training will reduce premarital sex' 12-year-old female.

Another participant stated: 'High level of poverty, joblessness and inducement by older males make many young females succumb to premarital sex, some parents do not complain when they see their wards living above their means' 17-year-old female while good parental care, love for parents and fear of God are the major reasons popularly accepted as protective factors against high-risk sexual practices among the adolescents. A participant stated: 'Not engaging in premarital sex is possible with good watchful loving parents with fear of God as pregnancy resulting from such behaviour leads to abortion' 17-year-old male. More in-school participants are particular about prevention of diseases like HIV/AIDS as a reason for abstaining from such practices than the out-of-school respondents.

Table 4 shows the logistic regression analysis of selected variables associated with having multiple sexual partners. The statistically significant factors include male gender, alcohol use, watched phonographic films, parental, societal and divine approval of premarital sex.

Table 5 reported the logistic regression analysis of selected variables associated with inconsistent condom use. The statistically significant factors include watched phonographic films, history of STI, forced sex, do not believe that condom is effective against STI and unwanted pregnancy, do not believe that he/she is able to resist sexual advances, and believe there is divine approval of premarital sex.

Discussion

Risky sexual practices are common among these adolescents. Some adolescents were sexually active while over two-fifth had more than one sexual partner. These findings were similar to the report of a study conducted among in-school adolescents in Nigeria where about 36% of the sexually active respondents had more than one partner and about 14.8% were aware that their partners had other partners [17]. Risky sexual

Table 5: Logistic regression analysis of selected variables associated with inconsistent condom use

Variable	Odd ratio	95% CI	p-cvalue
Watch pornographic films			
Yes	3.35	1.41-7.99	0.006
No (ref.)			
Had abnormal discharge and/or itching of the private part in the last twelve months			
Yes	2.36	1.02-5.41	0.044
No (ref.)			
Ever being forced to have sexual intercourse			
Yes	2.34	1.03-5.29	0.042
No. (ref.)			
Ever forced someone to have sexual intercourse with him/her.			
Yes	8.65	2.51-29.87	0.001
No (ref.)			
Believe that condom is effective against STI and unwanted pregnancy			
No.	16.41	2.15-125.49	0.007
Yes (ref.)			
Believe that he/she is able to resist sexual advances			
No	11.54	2.61-50.90	0.001
Yes (ref.)			
Believe that there is divine approval of premarital sex			
Yes	6.11	2.51-14.91	0.0001
No (ref.)			

behaviour, transactional sex and nonconsensual sex were also reported in another study conducted among out-of-school female adolescents in Lagos, Nigeria [18].

Only 27.7% of the respondents used condom at last sexual intercourse in this study. This is higher than a similar study conducted also in Nigeria where 8.6% used condom at last sexual intercourse while 41.9% had never used a condom [17]. This study reported low risk perception and high risky behaviour among these adolescents. This finding is similar to previous studies in Nigeria. For instance, another study reported that despite appropriate knowledge of HIV/AIDS, risky behaviour was common while protective behaviour was poor because only 14% of males and 5% of females used any form of protection [19]. This risky behaviour usually results in unwanted pregnancy and sexually transmitted diseases' including HIV/AIDS since condom is the only available method of barrier contraception that offers 'dual protection' against STI and unwanted pregnancy. This is also corroborated by the fact that almost one quarter of the sexually active respondents had symptoms suggestive of sexually transmitted infections in the year preceding this study. A similar study in South Western Nigeria also documented a high rate of sexually transmitted infections reported by 27% of male and 10% of female youth [19]. These symptoms are also risks for transmission of HIV among the adolescents.

The improvement observed in this study in the proportion of respondents that used condom during the first (26.9%) and last sexual exposure (41.5%) is encouraging. A survey in South Western Nigeria among out-of-school youths also reported that only 29% and 38.9% of the sexually active respondents used condom at first and last sexual intercourse respectively [20]. This could be as a result of programs organized by various governmental and non-governmental organizations to promote the use of condoms. However, adolescents could have fallen victims of consequences of unsafe sexual practices during the first sexual exposure and could have had adverse effect on their

life. It is therefore very important that interventions start early to protect these people as more people tend to engage in risky practices during the first sexual experience.

Many of the respondents had a wrong perception that inconsistent use of condoms during sexual intercourse and having more than one faithful partner do not put somebody at risk of contracting sexually transmitted infections. Similar findings were documented among college students in another study where it was discovered that many of them underestimate their risk of contracting HIV/AIDS. Only 15% of these students perceived themselves to be at moderate-to-high risk of acquiring HIV infection compared with 85% who perceived themselves to be at little or no risk. Whereas, an assessment of risk status of the participants revealed that 77% of the participants were actually at high risk of infection and only 23% at low risk [21]. A study conducted among youths in Nigeria also reported that only two thirds of the sexually active respondents used condom during sexual intercourse and 27% of them believed that some sexually transmitted diseases like HIV/AIDS are caused by witches or the anger of 'God' [22].

The report of another study revealed that perception of peers is associated with a higher frequency of sexual initiation and commitment, including oral sex. It also discovered that sexual permissiveness of peers is associated with a higher frequency of sexual practices considered risky [23]. This suggests that the social environment especially the attitude of peers influences the sexual behaviour of these adolescents and unfortunately many of the respondents in this study do not have good perception about social acceptability of abstinence since about thirty eight percent of them felt that people will laugh at adolescents of their age group who do not have sex.

Good perception of self-efficacy is one of the factors that have been found to be associated with possibility of behaviour change, but more than half of the respondents did not think that they will be able to resist pressure to have sexual intercourse if they found themselves in such conditions. Similar findings were documented in a study conducted among out-of-school female adolescents in Lagos, Nigeria where respondents engaging in risky sexual behaviour were exposed to sexual abuse and lacked skills to resist sexual pressure [18]. More than half of the respondents in this study did not think that they can get condom easily in case they needed it. This is important for programme planning so that access of sexually active adolescents to contraceptives (especially condom) may be improved since this is essential to the prevention of high-risk sexual practices among them.

Being a male was significantly associated with having multiple sexual partners in this study and this may be connected with the fact that female gender in this environment is expected to be more reserved and might not have been bold enough to go into multiple sexual practices freely compared with males. A recent study conducted in Ibadan also revealed that male respondents were 3 times more likely than females to have had sexual experience [24]. Results of the hierarchical regression model in another study provided support for the influence of gender on sexual behaviour of young Nigerians since male participants reported a greater extent of risky sexual behaviour orientation than their female counterparts [25].

Cigarette smoking, drinking of alcohol and watching of pornographic films were factors found to be significantly associated with inconsistent condom use and multiple sexual partners among those that have been sexually exposed. A study conducted among newly admitted undergraduates in a University in South-Western Nigeria also reported that alcohol use was significantly associated with sexual risk behaviour [26]. Physiological and behavioural research indicates that alcohol independently affects decision-making concerning sex and skills for negotiating consistent and correct use of condoms. The reasons given for engaging in high risk sexual practices in another study were peer influence, financial reward, drug influence, fun, or experimentation [17]. Perception of the adolescents that their parents disapprove of premarital sexual exposure was found to be associated with safer sexual practices and responses from the focus group discussions showed that lack of proper parental care predisposes to the risky sexual practices. This buttresses the fact that parents need to be carried along in interventions to curb the menace of such risky sexual practices among the adolescents.

Factors associated with condom use at last sexual intercourse were perceived susceptibility to sexually transmitted infections, perception that consequences of high-risk sexual practices can lead to termination of education and death and perception that consistent and regular use of condom can prevent unwanted pregnancy. A similar study conducted in South Western Nigeria showed that among girls, those who perceived social support from peers and non-parental

figures were more likely to use condoms while among boys, earning an income, high risk perception and self-efficacy were associated with higher odds of condom use and it was recommended that programs aiming to increase condom use among young people should address these factors through community based strategies [27]. Having street based peer who was a condom user was associated with a 70% reduction in the odds of having unprotected sex at last intercourse in a recent study conducted among young people in America [28]. Factors impeding decisions to use protection by young people in another study included lack of knowledge about prevalence of STI, ambiguity around contraception and safer sex practices, and lack of self-efficacy in negotiating safer sex among the females [29]. This buttresses the fact that social support and influences can affect condom use among the sexually active young people.

Factors that significantly prevented having multiple sexual partner among those that had been sexually exposed from this study included perceived susceptibility to pregnancy from high-risk sexual practices, perception that consequences of high-risk sexual practices can lead to termination of education and death, perception of self-efficacy to resist pressures to have sex, perception of ability to disclose chastity to peers and perception that it is not God's will for them to have sex before marriage. On the other hand, studies conducted among young people identified risk factors for multiple sex partners to include working in a place of entertainment, having current close friends that were living with boyfriends, poor academic performance, and positive attitudes toward having multiple partners [30,31]. Male adolescents also affirmed that having multiple sexual partners bolsters their sense of maleness and boosts their acceptance and ranking among peers [31,32]. These shows that social acceptability and perceptions contributes to whether young people will have multiple sexual partners or not.

This study finding provides more information on the subject matter but is limited in being a cross-sectional survey as no cause-effect relationship could be established. Also, a smaller proportion of the respondents were sexually active thereby resulting in the relatively small sample size on which the main analysis (association between the outcomes and the independent characteristics) was based.

Conclusion

It can be concluded from this study that many adolescents in Osun State are sexually active and are still involved in high-risk sexual practices since more than one third of those that were sexually exposed had multiple sexual partners in the last twelve months and only about a quarter of them used condom consistently. Factors significantly associated with these included being a male, watching of pornographic films and perception of inability to declare chastity among peers. These sexual practices were also significantly affected by various families and peers' social factors. Many of the adolescents harbour wrong perceptions about high-risk sexual practices and these misconceptions significantly affected their involvement in some of the practices.

It was recommended that health education programs among the adolescents should emphasize the importance of some social activities like watching of pornographic films and peer influence on their sexual behaviour. So also, behaviour change communication programs should aim at correcting misconceptions about high-risk sexual practices among them and interventions should also be targeted towards parents and care-givers of adolescents. Further research can be conducted to evaluate some of the proposed interventions.

References

1. Blum R and Nelson-Mmari K. The Health of the Young People in a Global Context. Journal of Adolescent Health. 2004; 35: 402-418.
2. Adeboyejo T and Onyeonoru I. Aspects of Home Environment and Adolescent Sexual behaviour. African Population Studies 2002;12:47-53.
3. John D and Catherine T. Guidelines for Comprehensive Sexuality Education in Nigeria. Action Health International 2003:4-9.
4. Barnet B. Youth often risk unsafe abortion. Network 1998;14:212-215.
5. UNFPA. Nigeria HIV/AIDS Report 2009.1-2
6. Ilika A and Anthony I. Unintended Pregnancy among Unmarried Adolescents and Young Women in Anambra State, Southeast Nigeria. African Journal of Reproductive Health. 2004;3:8.
7. Otoide V, Oronsaye F and Okonofua F. Why Nigerian Adolescents Seek Abortion Rather than Contraception: Evidence from Focus-Group Discussions. International Family Planning Perspectives. 2001;27:77-81.
8. Gail B Slap LL, Bin Huang, Comfort A Daniyam, Therese M Zink, Paul A Succop. Sexual behavior of adolescents in Nigeria: cross sectional survey

of secondary school students. British Medical Journal. 2003; 326: 15-18.

9. Iwuagwu SC, Olaseha IO and Ajuwon AJ. Sexual behavior and negotiation of male condoms by female students of the University of Ibadan. Journal of Obstetrics and Gynaecology 2000;20:507-513.
10. Olaseha IO, Ajuwon AJ and Onyejekwe C . Reproductive health knowledge and use among mothers in a sub-urban community. African Journal of Medicine Medical Sciences. 2004;33:139-143.
11. Moronkola OA and Idris OM. Sexual health knowledge, determinants of sexual behavior and use of contraceptives among female secondary school students in Ibadan. School Health Journal. 2000;12:27-35.
12. Izugbara CO. Risks and Benefits of Multiple Sexual Partnerships: Beliefs of Rural Nigerian Adolescent Males. American Journal of Men's Health. 2007;1:197-207.
13. Imrie J and Johnson A. Strategies for Prevention In: Michael W. (Ed), ABC of AIDS, 5th edition. British Medical Journal 2001:99-100.
14. Slater MD. Integrating Application of Media Effects, Persuasion, and behavior Change Theories to Communication Campaigns: A Stages-of-Change Framework Health Communication 1999 (11):335-354.
15. Fatusi A and Jimoh A. The role of behavior change communication and mass media. In: Adeyi O, Kanki J, Oluwole O, Idoko J.(Eds), AIDS in Nigeria; A Nation on the Threshold Havard Centre for population and Development Studies, Cambridge, MA 02138, USA 2006:323-347.
16. Araoye M. Research Methodology with statistics for Health and social sciences. Nathadex Publishers 2003:115-128.
17. Bamidele JO, Abodunrin OL and Adebimpe WO. Sexual behavior and risk of HIV/AIDS among adolescents in public secondary schools in Osogbo, Osun State, Nigeria. International Journal of Adolescent Medical Health 2009;21:387-394.
18. Odeyemi K, Onajole A and Ogunowo B. Sexual behavior and the influencing factors among out-of-school female adolescents in Mushin market, Lagos, Nigeria. International Journal Adolescent Medical Health. 2009 21:101-109.
19. Adedimeji AA, Omololu FO and Odutolu O. HIV risk perception and constraints to protective behavior among young slum dwellers in Ibadan, Nigeria. Journal of Health Population Nutrition 2007;25:146-157.
20. Adebiyi AO and Asuzu MC. Condom use amongst out-of-school youths in a local government area in Nigeria. African Health Sciences. 2009;9:92-97.
21. Ijadunola K, Abiona T, Odu O and Ijadunola Y. College students underestimate their risk of contracting HIV/AIDS infection. The European Journal of Contraception and Reproductive Health Care. 2007;12:131-137.
22. Ike SO, Aniebue PN. HIV/AIDS perception and sexual behavior among Nigerian University students. Nigerian Journal of Clinical Practice. 2007 ;10(2):105-110.
23. Potard C, Curtoise R and Rusch E. The influence of peers on risky sexual behavior during adolescence. The European Journal of Contraception and Reproductive Health Care 2008 Sep;13(3):264-70 2008;13:264.
24. Oladokun A, Morhason-Bello IO, Enakpenea CA, *et al.* Sexual behavior and Contraceptive Usage of Secondary School Adolescents in Ibadan, Nigeria. Journal of Reproduction and Contraception 2007;18:279-288.
25. Adebayo DO, Udegbe IB and Sunmola AM. Gender, Internet Use, and Sexual behaviour Orientation among Young Nigerians. CyberPsychology and Behavior 2006; 9: 742-752.
26. Olley BO. Child sexual abuse, harmful alcohol use and age as determinants of sexual risk behaviors among freshmen in a Nigerian university. African Journal of Reproductive Health. 2008; 12: 75-88.
27. Adedimeji AA, Heard NJ, Odutolu O and Omololu FO. Social factors, social support and condom use behavior among young urban slum inhabitants in southwest Nigeria. East African Journal of Public Health 2008;5:215-222.
28. Rice E. The positive role of social networks and social networking technology in the condom-using behaviors of homeless young people. Public Health Reports 2010;125:588-595.
29. East L, Jackson D, O'Brien L and Peters K. Use of the male condom by heterosexual adolescents and young people: literature review. J Adv Nurs 2007;59:103-110.
30. Olowookere S.A., Adeleke N.A., Fatiregun A.A. and Abioye-Kuteyi E.A. Pattern of condom use among clients at a Nigerian HIV Counseling and Testing Centre. BMC Research Notes. 2013; 289 (6): 1-5.

31. Yan H CW, Wu H, Bi Y, Zhang M, Li S, Braun KL. Multiple sex partner behavior in female undergraduate students in China: a multi-campus survey. BMC Public Health 2009;9:305.
32. Izugbara CO. Risks and Benefits of Multiple Sexual Partnerships: Beliefs of Rural Nigerian Adolescent Males. *A*merican Journal of Men's Health 2007;1:197-207.
33. Apata C., Olowookere S.A., Esimai O.A., Ilori T. Determinants of early sexual initiation and contraceptive use among in-school adolescents in an urban Local Government Area in Southwest Nigeria. The Nigerian Journal of Public Health. 2016; 1: 15-19.

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Muscular dystrophies in a developing economy: clinical presentations and challenges to management

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Abstract

Background: Muscular dystrophy (MD) is a recognized cause of neurodisability in children. However, the prevalence, presentation and outcome in Nigerian children had been poorly documented.

Methods: A retrospective review of the records of cases of muscular dystrophies seen over a period of 11 years was undertaken to describe the frequency, types, clinical presentation and outcomes of MD in our centre.

Results: Thirty cases of MD were seen out of 2607 new cases and this accounted for 1.2% of new cases seen over the period. Six cases were excluded from further analysis due to missing or incomplete information. Among the 24 cases analysed, males (83.3%) were more frequently affected than females (16.6%) giving a male to female ratio of 5:1. The first symptom was noticed at 7.3 ± 4.1 years while mean age at diagnosis was 10±3.9years. Diagnosis was mainly clinical with 71% having features of Duchenne muscular dystrophy (DMD). Only 2 (8.3%) were able to have serum creatinine kinase assay and muscle biopsy for confirmation and only 2 (8.3%) had access to assisted locomotive device. Twenty-two (91.7%) were lost to follow-up after 1 to 2 clinic visits while one died at home.

Conclusion: DMD is the commonest type of MD seen in Nigerian children and it is associated with delayed presentation and a high default rate. Although the prognosis of MD is poor even in the best centres, public awareness can enhance early presentation and compliance with follow-up schedules while multidisciplinary support can improve the quality of life of patients with MD during the course of management.

Keywords: *Muscular dystrophies, children, multidisciplinary*

Abstrait

Contexte **:** La dystrophie musculaire (DM) est une cause reconnue de neurodisabilité chez les enfants. Cependant, la prévalence, la présentation et les résultats chez les enfants nigérians ont été mal documentés.

Méthodes: Une revue rétrospective des dossiers des cas de dystrophies musculaires observés sur une période de 11 ans a été entreprise pour décrire la fréquence, les types, la présentation clinique et les résultats de la DM dans notre centre .

Résultats: Trente cas de DM ont été vus sur 2607 nouveaux cas et cela représentait 1,2% des nouveaux cas vus sur la période. Six cas ont été exclus de l'analyse approfondie en raison d'informations manquantes ou incomplètes. Parmi les 24 cas analysés, les garçons (83,3%) étaient plus fréquemment touchés que les filles (16,6%), donnant un ratio garçon : femme de 5 : 1. Le premier symptôme a été remarqué à 7,3 ± 4,1 ans alors que l'âge moyen au diagnostic était de 10 ± 3,9 ans. Le diagnostic était principalement clinique avec 71% présentant des caractéristiques de dystrophie musculaire de Duchenne (DMD). Seulement 2 (8,3%) ont pu subir un test de créatinine kinase sérique et une biopsie musculaire pour confirmation et seulement 2 (8,3%) ont eu accès à un dispositif de locomotive assistée. Vingt-deux (91,7%) ont été perdus de vue après 1 à 2 visites à la clinique tandis qu'un était mort à domicile.

Conclusion : La DMD est le type de DM la plus courante observée chez les enfants nigérians et elle est associée à un retard de présentation et à un taux de défaut élevé. Bien que le pronostic de la DM soit médiocre même dans les meilleurs centres, la sensibilisation du public peut améliorer la tôt présentation et le respect des calendriers de suivi, tandis que le soutien multidisciplinaire peut améliorer la qualité de vie des patients atteints de DM au cours de la prise en charge.

Mots clés: *Dystrophies musculaires, enfants, multidisciplinaire*

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Introduction

Muscular dystrophies are inherited, progressive skeletal muscle disorders resulting in muscle degeneration and loss of strength [1,2]. The onset of clinical symptoms ranges from the neonatal period to late adulthood. Six major phenotypes have been identified. These include: Duchenne muscular dystrophy involving shoulder and pelvic girdles, neck flexor muscles and calf hypertrophy; Emery-Dreifuss type, with a triad of scapulohumeral distribution, peroneal involvement and early contractures; Limb-girdle muscular dystrophy (pelvic and shoulder distribution); Facioscapulohumeral muscular dystrophy with peroneal involvement; Distal myopathy(distal muscle involvement) and Oculopharyngeal muscular dystrophy with or without shoulder and limb girdle involvement[1-3] Although no cure is available presently for the various forms of muscular dystrophy, extensive laboratory supports for definitive diagnoses are available in developed economies [4-7] while several measures have been instituted for proper management which includes robust multidisplinary setting, pharmacological therapy such as steroid therapy and physical rehabilitation all in a bid to improve the quality of life in these patients. [4-5, 8-10] Similarly, several studies are ongoing in developed countries to explore the molecular basis of muscular dystrophies in order to further enhance genotypic / phenotypic correlations, diagnoses and discovery of new therapies [11-13].

Individuals with muscular dystrophy can have delayed motor milestones and often experience more falls than their peers [1-2]. Gait abnormalities, difficulties rising from the floor and problems negotiating the stairs usually become evident at 3-4 years of age [1-3]. At the onset of muscle weakness, affected children would need to turn on their side when getting up from a supine position on the floor, which is the initial component of the Gower's manoeuver [1]. Majority of patients with muscular dystrophies, if not properly managed, are wheelchair dependent by early adolescent and die in their late teens or early twenties from multi-organ failures, especially respiratory and cardiac decompensations [.1-2].

Earlier reports on muscular dystrophies in Nigeria dated back to the sixties when Dada *et al* reported 11 cases over a period of 5 years in UCH, Ibadan during which failure of early recognition and late presentation were identified as major contributors to poor prognosis [14]. Osuntokun *et al* in the seventies also reported an incidence of 0.13 per thousand out of 9600 patients suffering from neurological diseases seen at the University College Hospital (UCH) Ibadan over a 12-year period (1957-1969) [15]. Krahe *et al* in the nineties also reported myotonic dystrophy mutation in a Nigerian kindred [16]. However, these earlier reports were mainly on the adult population. In an analysis of disorders seen at the Paediatric Neurology Clinic, University College Hospital Ibadan over a 20 months period (May 2004 to December 2005), Lagunju *et al* reported three cases of Duchenne muscular dystrophy among the cases seen during the period under review [17] while Eseigbe *et al* in 2006 reported two cases of Muscular Dystrophy in Nigerian children [18]. Odinaka *et al* in 2013 also reported another case of Duchenne muscular dystrophy in another Nigerian child. In this report, some of the challenges militating against the management of muscular dystrophies in Nigeria were highlighted [19]. Among the few reports that have been published on muscular dystrophy in Nigeria over the years, there has been paucity of information on the incidence, mode of presentation, clinical profile, challenges to management and outcomes in the paediatric age group. This study therefore set out to address this research gap by overviewing the pattern of presentation of muscular dystrophy in Nigerian children as seen in a single centre, identifying the challenges to management and providing recommendations for improvement of the quality of life of children with muscular dystrophy in our environment.

Methodology

A retro-prospective descriptive study of cases of muscular dystrophies seen at the Paediatric Neurology Clinic, University College Hospital, Ibadan was carried out. The University College Hospital Ibadan is a first generation Teaching Hospital in Nigeria with referral catchment spanning through the entire southwest geopolitical zone of Nigeria and beyond. New referrals are seen by the paediatric neurologist who engages in history taking and meticulous clinical evaluation of the new patients. Findings are then documented in the patients' case notes. A structured questionnaire was utilized to obtain information from both the old and new cases seen at the clinic over a period of 11 years. (2005-2016). Information obtained included age, sex, onset of illness, presenting complaints, clinical findings, investigations, results, intervention(s) and outcomes. The diagnosis of muscular dystrophy was clinical and based on history of progressive muscle weakness, a

likely genetic basis for the disease with or without presence of muscle wasting and evidence of muscle degeneration [1,2].

Data was stored and analysed with the Statistical Package for the Social Sciences version 20 software. The mean, range, standard deviations and proportions were determined.

Results

Thirty new cases were seen over 11-year period (2005-2016) out of 2,607 new cases which presented at the Paediatric Neurology Clinic, University College Hospital, Ibadan giving a frequency of 1.2%. Six cases were excluded from analysis due to missing clinical information. The age range among the 24 cases analysed was 7 months to 18 years with a mean age of 10 ± 3.9 years. Twenty (83.3%) of the study population were males while 4 (16.7%) were females giving a male to female ratio of 5.1:1. Age at onset of symptoms ranged from 1 month to 14 years with mean age of 7.3 ± 4.1 years. Two (8.3%) of the patients had features of congenital myopathy, having been found to be floppy since the first month of life. Average duration of onset of symptoms before presentation in the clinic was 4± 2.6 years while 79.2% had only 1-2 clinic visits.

All the patients presented with of progressive weakness of the limbs at presentation while 8 (33.3%) of the study population presented with inability to walk with only 2 (8.3%) of them having assistive device for mobility. Although 79.2% of them were enrolled in school, 25% of them were noticed to have intellectual impairment evidenced by parental report of poor school performance. A positive family history was found in 4 (16.7%) of the population among which was a twin pair. Ten (41.7%) of the patients had delayed developmental milestones in early childhood. Table 1 shows the physical signs elicited during clinical evaluation.

Examination of the respiratory system and the abdomen revealed normal findings in the study group while 1 (4.2%) had a cardiac murmur. Table 2 shows the phenotypic diagnosis among the study groups. Majority of the patients had clinical features in keeping with Duchenne muscular dystrophy out of which 2 cases were confirmed with serum creatinine kinase and muscle biopsy results.

Table 3 shows the type of investigations requested and the proportion of those who carried out the investigations.

Interventions and Outcome

After clinical evaluation, 16 (66.6%) commenced physiotherapy, 2(8.3%) were commenced on anti-failure regimen while 17 (70.8%) were commenced on steroid therapy with oral prednisolone. However, 22(91.7%) of the patients made only 1 to 2 clinic visits before being lost to follow up and therefore not giving the clinician the sufficient time to evaluate the effect of the therapeutic measures on the progression and prognosis of the conditions. One patient (4.2%) died while one (4.2%) was still on follow-up as at the time of this study.

Table 1: Physical signs in 24 children with muscular dystrophy

Types of Muscular Dystrophy	Male n (%)	Female n (%)	Total n (%)	P-Value
Muscle wasting	14 (73.7)	3 (100.0)	17 (77.3)	0.312
Calf pseudohypertrophy	14 (73.7)	1 (25.0)	15 (65.2)	0.063
Positive Gower sign	13 (68.4)	2 (50.0)	15 (65.2)	0.609
Waddling gait	12 (70.6)	3 (75.0)	15 (71.4)	0.861
Tongue hypertrophy	2 (10.5)	0 (0.0)	2 (8.7)	0.695
Scoliosis	7 (36.8)	3 (75.0)	10 (43.5)	0.162
Pes cavus	6 (31.6)	0 (0.0)	6 (27.3)	0.254
Kyphosis	4 (20.0)	1 (25.0)	5 (20.8)	0.822
Facial involvement	4 (21.1)	0 (0.0)	4 (17.4)	0.313

Majority of the patients had calf pseudohypertrophy, muscle wasting, positive Gower's sign and waddling gait

Table 2: phenotypic diagnoses in 24 children with muscular dystrophy

Diagnosis	Male n (%)	Female n (%)	Total n (%)	P-Value
Duchenne muscular dystrophy	17 (85.0)	0 (0.0)	17 (70.8)	
Congenital muscular dystrophy	1 (5.0)	1 (25.0)	2 (8.3)	<0.001
Facioscapulohumeral dystrophy	1 (5.0)	0 (0.0)	1 (4.2)	
Limb girdle muscular dystrophy	0 (0.0)	3 (75.0)	3 (12.5)	

Two of the DMD cases had elevated serum creatinine kinase and evidence of muscle degeneration on muscle biopsy.

Table 3: Investigations and results in 24 children with muscular dystrophy

Investigation	n (%)	results		remark
		Normal n (%)	Abnormal n (%)	
ECG	5 (21.8)	4 (17)	1 (4.2)	RVH
ECHO	2 (8.3)	1(4.2)	1(4.2)	ASD + VSD
Chest X ray	2 (8.3)	2 (8.3)	0(0)	Normal result
Creatinine Kinase	4 (17)	2 (8.3)	2 (8.3)	Markedly elevated abnormal values
Nerve conduction velocity	1 (4.2)	1(4.2)	0(0)	Normal result
EMG	0(0)	0(0)	0(0)	Investigation not done
Muscle Biopsy	2(8.3)	0(0)	2(8.3)	DMD confirmed
AMA	1 (4.2)	0(0)	1(4.2)	Seropositive

The 2 patients with elevated creatinine kinase had DMD confirmed on muscle biopsy. ECG: Electrocardiography; ECHO: Echocardiography; EMG: Electromyography; AMA: Antimuscarinic antibody; RVH: right ventricular hypertrophy; ASD: Atrial septal defect; VSD: Ventricular septal defect; DMD: Duchenne Muscular Dystrophy

Discussion

This study illustrates the basic challenges encountered by clinicians managing patients with muscular dystrophies in developing economies like Nigeria. The frequency of 1.2% in this study differs from 0.13 per thousand earlier reported by Osuntokun *et al* [15] over 4 decades ago. While the present study was conducted on paediatric populations alone, the former was conducted on the general population. Population dynamics over the years could also have added to the difference. The fact that most (83.3%) of the study population were males with phenotypic features in keeping with Duchenne muscular dystrophy further corroborate earlier documentations in the literature that DMD is the commonest form of muscular dystrophies [1,2]

It is notable that all the patients in this study presented with progressive weakness of the limbs, evidenced by worsening difficulty with walking, a fact which attests to the progressive nature of muscular dystrophies. The presence of positive family history in 16.7 % of the population among which was a twin pair reflects the genetic basis of muscular dystrophies. This proportion could have been higher if facilities had been available to screen the family members of the study group for subclinical forms of muscular dystrophies which may be present among the pedigrees [16]. Result from this study shows a significant delay of 4± 2.6 years between the onset of symptoms and presentation at the paediatric neurology clinic, with 8 (33.3%) of the patients being unable to walk at presentation. This delay in presentation may not be far from the complex interplay between ignorance, poverty and disease which is prevalent in developing economies like Nigeria and is usually responsible for poor health seeking behavior among our people. Due to poverty, majority of those who are unable to walk among these patients had no access to assistive motility devices, a situation which has made them to be nuisance to their caregivers as

they would have to be carried manually everywhere. This no doubt is one of the major factors which has contributed to their poor clinic attendance and eventual lost to follow up.

The presence of intellectual impairment in a quarter (25%) of these patients agrees with earlier reports on the presence of intellectual impairments in patients with muscular dystrophies, especially the Duchenne type [1-2] while positive history of delayed developmental milestone in some of them suggest that the degenerative process might have gradually evolved from early childhood until striking clinical manifestations compelled the caregivers to eventually present in the hospital. Most of the diagnoses in this study were based on phenotypic features of the patients with only two of the cases showing elevated serum creatinine kinase and evidence of muscle degeneration via muscle biopsy. Most of the patients did not perform the investigations requested before being eventually lost to follow up. This underscores the need for government supports through social welfare schemes, provision of free medical and laboratory services for children, rich multidisciplinary cohorts of health workers, establishment of cohesive support groups and generous networking system among the caregivers of patients with muscular dystrophies. When all these are in place, it will go a long was to improve the prognosis and quality of life of children with muscular dystrophies despite the lack of cure for these conditions at present [4-7, 9-10, 20].

This study should be viewed in the light of its limitations which include the relatively small number of the patients involved and the fact that diagnoses were largely based on phenotypic features in most of the patients with only few being able to perform confirmatory laboratory investigations. Nevertheless, the challenges militating against effective management of these patients are illustrated, a fact which the study set out to achieve.

Conclusion

Muscular dystrophies, though incurable at present, require a multidisciplinary approach to management with provision of adequate financial support for screening and provision of physical therapy, pharmacological interventions and other supportive care. Although these means are generously available in developed countries, situations in developing countries like Nigeria are grossly inadequate and need to be improved upon through provision of funds for laboratory supports, physical therapy and assistive ambulatory devices, psychotherapy, creation of social support groups and networking among patients with muscular dystrophies. All these, if done, will definitely improve their Quality of Life as the quest to unravel curative approach to management continues in research landscape. Pending the availability of definitive treatment, genetic counseling will be of immense benefit to families at risk of having children with Muscular dystrophies.

References

1. Escolar DM and Leshner RT. Muscular Dystrophies. In: Swaiman KF, Ashwal S, Ferriero DM, Schor NF eds. Swaiman's Paediatric Neurology, Fifth edition. Elsevier Saunders 2012. e1520-e1606.
2. Sarnat HB. Muscular Dystrophies In: Kliegman RM, Stanton BF, Schor NF, Geme JWS, Behrman RE eds. Nelson textbook of Paediatrics, 19th edition. Elsevier Saunders 2011; 2119-2129.
3. Emery AEH. Population frequencies of inherited neuromuscular diseases- a world survey. Neuromuscular Disorders 1991; 1(1): 19-29.
4. Bushby K, Finkel R, Birnkrant DJ *et al*. Diagnosis and management of Duchenne Muscular Dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. The Lancet Neurology. 2010; 9(1):77-93.
5. Bushby K, Finkel R, Birnkrant DJ *et al*. Diagnosis and management of Duchenne Muscular Dystrophy, part 2: implementation of multidisciplinary care. The Lancet Neurology. 2010;9(2):177-189.
6. Upadhyaya M, Maynard J, Rogers MT *et al* Improved molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD): validation of the differential double digestion for FSHD. Journal of medical genetics. 1997;34(6):476-479.
7. Peat RA, Smith JM, Compton AG *et al*. Diagnosis and etiology of congenital muscular dystrophy. Neurology. 2008 Jul 29;71(5):312-321.
8. Manzur AY, Kinali M and Muntoni F. Update on the management of Duchenne Muscular Dystrophy. Archives of disease in childhood. 2008; 93(11):986-990.
9. Bushby K, Bourke J, Bullock R *et al*. The multidisciplinary management of Duchenne Muscular Dystrophy. Current Paediatrics. 2005;15(4):292-300.

10. Bushby K, Lochmüller H, Lynn S *et al*. Interventions for Muscular Dystrophy: molecular medicines entering the clinic. The Lancet. 2009; 374(9704):1849-1856.
11. Clemens PR, Fenwick RG, Chamberlain JS *et al*. Carrier Detection and Prenatal Diagnosis in Duchenne and Becker Muscular Dystrophy Families, Using Dinucleotide Repeat Polymorphisms. Am.J. Genet.1991; 49:951-60.
12. Jacobs PA, Hunt PA, Mayer M *et al*. Duchenne Muscular Dystrophy(DMD) in a female with an X / Autosome Translocation: Further Evidence That the DMD Locus is at Xp21. Am J Hum Genet 1981; 33: 513-518.
13. Cossu G, Sampaolesi M. New therapies for Duchenne muscular dystrophy: challenges, prospects and clinical trials. Trends in Molecular Medicine 2007; 13 (12): 520-526.
14. Dada TO, Elliott BA. Muscular Dystrophy of Duchenne type in Nigerians. Journal of the neurological sciences. 1967;4(3):435-444.
15. Osuntokun BO. The pattern of neurological illness in tropical Africa: experience at Ibadan, Nigeria. Journal of the neurological sciences. 1971. 30;12(4):417-442.
16. Krahe R, Eckhart M, Ogunniyi AO *et al*. De Novo Myotonic Dystrophy Mutation in a Nigerian Kindred. Am. J. Genet. 56: 1067-1074.
17. Lagunju IA and Okafor OO. An Analysis of Disorders seen at the Paediatric Neurology Clinic, University College Hospital, Ibadan Nigeria. West African Journal of Medicine, 2009; 28(1): 38-41.
18. Eseigbe EE, Anyiam JO and Wammanda RD. Care of the Child with special health care needs: A report on 2 Nigerian Chidren with Muscular Dystrophy. Annals of Nigerian Medicine. 2006; 2(2): 29-31.
19. Odinaka KK and Nwolisa EC. Challenges in the management of the child with Duchenne Muscular Dystrophy in a resource poor setting: a case report. Pamj 2014. Available at: http://www.panafrican-med-journal.com/content/article/19/227/full/
20. Baiardini I, Minetti C, Bonifacino S *et al*. Quality of life in Duchenne Muscular Dystrophy: Subjective impact on children and parents. Journal of child neurology. 2011 Apr 11: 0883073810389043.

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GCMS analysis and Phytoprotective effect of chloroform fraction of methanol leaf extract of *Drymaria cordata* against MSG-induced lesions in specific tissues

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Abstract
Background: Monosodium glutamate (MSG) is a food additive whose toxicity has been demonstrated in experimental animals. *Drymaria cordata,* is traditionally used as antidote. The protective effect of chloroform fraction of methanol extract of *drymaria cordata* LINN. (CFDC) against MSG-induced lesions in rat liver, brain and prostate was investigated in this study.
Methods: Twenty four male Wistar rats were equally divided into four groups and orally treated for twenty-eight days as follows; A(control), B(CFDC (100 mg/kg)), C(MSG (200 mg/kg)) and D(MSG+CFDC). The animals were sacrificed 24 hours after the final exposure and blood was collected by cardiac puncture into EDTA-sterilized sample bottles. The liver, brain and prostate were harvested and subjected to histological examination. The effect of CFDC was also investigated on lipid peroxidation, DNA fragmentation, caspase 9 and caspase 3. The GC-MS analysis of the chloroform fraction was also carried out.
Results: The results show that MSG caused injury to the hepatocytes, brain and the prostate which was significantly ameliorated in the group co-administered with CFDC. In addition, CFDC protected against the increased malondialdehyde level, elevated caspases 9 & 3 activities and increased percentage hepatic DNA fragmentation caused by MSG administration. The GC-MS analysis revealed the presence of some phytochemical compounds that might be the cause of its pharmacological effect in protecting against lesions in liver, brain and prostate of MSG-treated rats.
Conclusion: These results suggest that CFDC contains phytochemicals that might be relevant in the chemopreventive and therapeutic approach to MSG-induced cellular damage. However, further studies need to be carried out in order to investigate its mechanism of action

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Key words: *Drymaria cordata, monosodium glutamate, cellular injury, chemoprevention*

Abstrait
Contexte: Le glutamate monosodique (MSG) est un additif alimentaire dont la toxicité a été démontrée chez les animaux de laboratoire. *Drymaria cordata* , est traditionnellement utilisée comme antidote. L'effet protecteur de la fraction chloroforme de l'extrait de méthanol de *drymaria cordata* LINN (CFDC) contre les lésions induites par MSG dans le foie, le cerveau et la prostate du rat a été étudiée dans cette étude.
Méthodes: Vingt-quatre rats mâles Wistar ont été répartis également en quatre groupes et traités par voie orale pendant vingt-huit jours comme suit; A (contrôle), B (CFDC (100 mg / kg)), C (MSG (200 mg / kg)) et D (MSG + CFDC). Les animaux ont été sacrifiés 24 heures après l'exposition finale et le sang a été collecté par ponction cardiaque dans des bouteilles d'échantillons stérilisées à l'EDTA. Le foie, le cerveau et la prostate ont été prélevés et soumis à un examen histologique. L'effet de la CFDC a également été étudié sur la peroxydation lipidique, la fragmentation de l'ADN, la caspase 9 et la caspase 3. L'analyse GC-MS de la fraction chloroforme a également été réalisée.
Résultats: Les résultats montrent que le MSG a causé des lésions aux hépatocytes, au cerveau et à la prostate qui ont été considérablement améliorées dans le groupe coadministré avec la CFDC. En outre, CFDC a protégé contre l'augmentation du niveau de malondialdéhyde, les activités élevées de caspases 9 et 3 et l'augmentation du pourcentage de fragmentation de l'ADN hépatique causée par l'administration MSG. L'analyse GC-MS a révélé la présence de certains composés phytochimiques qui pourraient être la cause de son effet pharmacologique

dans la protection contre les lésions du foie, du cerveau et de la prostate de rats traités au MSG.
Conclusion: Ces résultats suggèrent que la CFDC contient des composés phytochimiques qui pourraient être pertinents dans l'approche chimio-préventive et thérapeutique des dommages cellulaires induits par le MSG. Cependant, d'autres études doivent être menées afin d'étudier son mécanisme d'action

Mots clés: *Drymaria cordata, glutamate monosodique, lésion cellulaire, chimio-prévention*

Introduction

Monosodium glutamate (MSG) is used commercially as a food additive and is commonly marketed as a flavour enhancer [1]. Various trade names like Ajinomoto, vedan, Vetsin and Tasting Powder are associated with monosodium glutamate [2]. The part of MSG that negatively affects the human body is the "glutamate", not the sodium. [3].

In general, the natural glutamic acid found in food does not cause problems, but the synthetic free glutamic acid formed during industrial processing is a toxin. In addition, when MSG is formed using hydrochloric acid the final product includes carcinogens [4]. MSG has also been reported to have neurotoxic effects resulting in brain cell damage [5], retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis [6]. According to Farombi and Onyema [7], MSG has been shown to be genotoxic. It has been shown to cause uterine hyperplasia in experimental female rats [8].

Epidemiological studies have revealed protective action of natural compounds, with important application in disease prevention or treatment. [9,10]. *Drymaria cordata* (Linn.) Willd. (Caryophyllaceae) is a creeping herb growing in dense patches in moist shady places and also in dry sun-exposed areas. The anti-inflammatory [11,12], antitussive [13], antibacterial [14], cytotoxic [15], anxiolytic activity [16], analgesic, anti-nociceptive and antipyretic properties [17-19] of *Drymaria cordata* extract have been reported. *Drymaria cordata* has been traditionally used in various parts of the world like Africa, and Asia as folk medicine. In tropical Africa, its preparations are used for the treatment of diverse ailments including cold, headache, coryza, bronchitis, as poultice on sore (to treat aching, inflamed or painful parts), leprosy, tumors, as fumigant for eye troubles, as cerebral stimulant and antifebrile agent [20]. In North East India, the plant has been traditionally used as an antidote, appetizer, depurative, emollient, febrifuge, laxative and stimulant in both human and animals [21]. In our laboratory, it has been shown that methanol extract and chloroform fraction of *drymaria cordata* ameliorated MSG-induced uterine hyperplasia in female rats. It is on this premise that we decided to investigate the effect of oral administration of chloroform fraction of methanol extract of *drymaria cordata* on the liver, brain and prostate of MSG-treated male rats.

Materials and methods

Monosodium Glutamate

MSG was obtained from Sigma Aldrich Chemical Co. St Louis USA and a stock solution was prepared by dissolving 40g of MSG in 400mls of distilled water. Based on the weight of the animals, 200mg/kg dosages of MSG were administered to the group that took MSG alone and the group that received co-administration with CFDC.

Collection of Fresh Drymaria cordata

The whole plant of *Drymaria cordata* was freshly harvested and obtained from Department of Botany, University of Ibadan, Nigeria. Samples were authenticated and identified at the Herbarium, Department of Botany, University of Ibadan, Ibadan, Oyo State and a specimen Voucher No.UIH-22555 was deposited in the Herbarium. The plants were washed, air-dried for four weeks in the laboratory after which they were powdered and weighed.

Preparation of Crude Methanol Extract and Chloroform Fraction of Drymaria cordata

6-kilogramme air-dried, whole plant of *Drymaria cordata* were extracted with sufficient methanol (Sigma Aldrich Chemical Co. St Louis USA) in all- glass jars at room temperature for seventy-two hours. The filtrate was decanted, filtered and concentrated under reduced pressure using a rotary evaporator (Stuart United Kingdom). The crude methanol extract was heated over a water bath at 40^0C to obtain a solvent free extract. The crude methanol extract was further partitioned successively between n-hexane, chloroform, ethyl acetate and methanol using vacuum liquid chromatography technique to obtain the fractions. All these fractions were concentrated to dryness under pressure using rotary evaporator at 40^oC to obtain the

n-hexane (HF), chloroform (CF), ethylacetate (EF) and the methanol (MF) fractions.

Experimental Animals

Male Wistar strain albino rats weighing between 100–120g were purchased from the Preclinical Animal House of the College of medicine, University of Ibadan, Ibadan, Nigeria. All the animals were allowed two weeks period of acclimatization in the Animal House of the Department of Biochemistry, University of Ibadan. The animals were placed under a 12hr light/dark cycle and fed commercial pelletized rat chow and water *ad libitum* throughout the experimental period. All experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Animals Groupings, Treatment and Sample Collections

Rats were divided into 4 groups: A (control group), B (CFDC: 100mg/kg), C (MSG : 200mg/kg), D (MSG :200mg/kg + CFDC :100mg/kg). The administrations were carried out as a single dose daily by oral gavage for 28 days. One day after the final exposure, the animals were sacrificed. Blood was collected by cardiac puncture into EDTA sample bottles.

Tissue Preparation for Histopathology

Liver, brain and prostate were used for histopathology. They were quickly removed and trimmed and were placed in 10% formalin for about five days for proper fixation, dehydrated by ascending grades of isopropyl alcohol for an hour. The dehydrated organs were cleared in xylene and transferred into two changes of liquid paraffin wax. The tissue sections were stained in Ehrlich's hematoxylin for eight minutes, washed in tap water and dipped in acid alcohol to remove excess stain. These were counter stained in 10% aqueous eosin, incubated and mounted for photomicrography. The Histological pictures were taken with a Digital Microscope, VJ-2005 DN model bio-microscope.

Sample preparation and analysis of caspases 9 & 3 using Elisa Technique

The rat liver was excised, weighed and rinsed with phosphate buffered saline thoroughly until a clear wash was obtained. The washed livers were homogenized on ice and the homogenates were centrifuged at 8,000 rpm for 5 minutes. The supernatant thus obtained were then put in sample bottles and freezed. After freezing for two days, the samples were brought out to thaw. This was done twice after which the samples were used for caspases 9 and 3 analysis, respectively.

Analysis of caspases 9 and 3

Analysis of caspases 9 and 3 were carried out using an ELISA kit, a product of Elabscience biotechnology Ltd., Technology Industry Park, WuHan, Peoples Republic of China. This kit uses Sandwich-ELISA as the method. A microplate reader (DNM-9602A from China) was used to read the optical density at 450nm wavelength.

DNA Fragmentation

The percentage hepatic DNA fragmentation was determined according to the method of Wu *et al*., [22]. Liver tissues were sliced with scissors and homogenized in 10 volumes of Tri-EDTA Triton buffer (TET) pH 8.0. Homogenates were centrifuged at 27000g for 20mins to separate intact chromatin (pellet A) from fragmented (pellet B). Pellet A was suspended in Tris-EDTA buffer of pH 8.0. 1ml aliquot of each sample (pellet and supernatant) was placed in separate test tubes and then 1ml of freshly prepared diphenylamine solution was added to each. Reaction mixture was incubated at 37p C for 20 hours. Absorbance of the mixture was then measured at 620nm.

Calculation

Quantity of fragmented DNA was estimated by using the formula

% fragmented DNA = B/(A+B) X 100

Determination of protein concentration. Protein concentration was determined according to the method of Lowry *et al*. [23] using bovine serum albumin as standard.

Assessment of lipid peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample (isolated rat liver mitochondria) according to the method of Varshney and Kale [25]. Under acidic conditions, malondialdehyde (MDA) produced from the peroxidation of fatty acids reacts with the chromogenic reagent 2-thiobarbituric acid to yield a pink coloured complex with maximum absorbance at 532 nm.

An aliquot of 0.4 ml of the test sample was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 0.75% TBA was

added and placed in a water bath for 45 minutes at 80°C. This was then cooled in ice to room temperature and centrifuged at 3000 rpm for 10 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm.

Calculation

The MDA level was calculated using an extinction coefficient of 0.156 $\mu M^{-1}cm^{-1}$

Lipid peroxidation (nmole MDA/mg protein) = Absorbance × volume of mixture

$$E_{532nm} \times \text{volume of sample} \times \text{mg protein/ml}$$

GC-MS analysis of the chloroform fraction of methanol

Extract of drymaria cordata

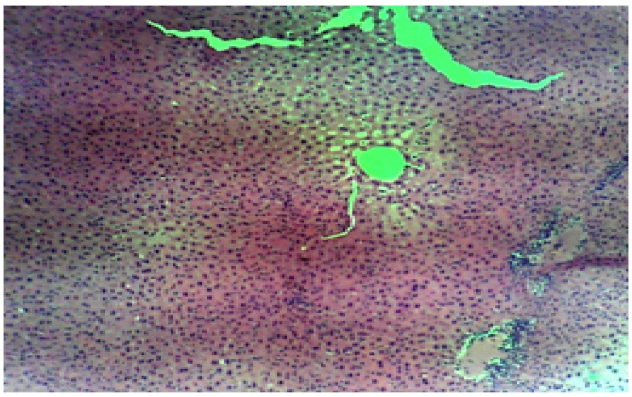
The chloroform fraction of methanol extract of *drymaria cordata* was subjected to GC-MS analysis using Agilent technologies 7890 GC system and the model of the detector is Agilent technologies 5975 MSD (Mass Spect. Detector). The principle behind the GC analysis is separation techniques. In separation techniques, there are two phases – the mobile and the stationary phase. The mobile phase is the carrier gas (Helium, 99.99% purity), while the stationary phase is the column. The model of the column is HP5 MS with length 30 m, internal diameter 0.320 mm, while the thickness is 0.25 µm. The oven temperature program is initial temperature of 80°c to hold for 1 minute. It increases by 10^0 per minute to the final temperature of 240^0c to hold for 6 minutes. The injection volume is 1 microlitre and the heater or detector temperature is 250^0C.

Statistical analysis of data

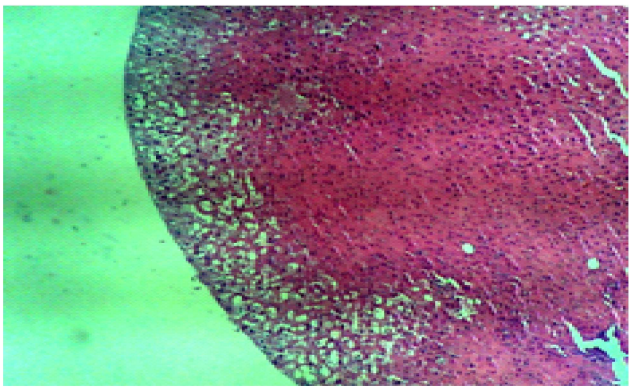
Statistical analysis was performed using one way analysis of variance (ANOVA). Level of significance was set at $p < 0.05$ and all the results were expressed as mean ± standard deviation (SD).

Results

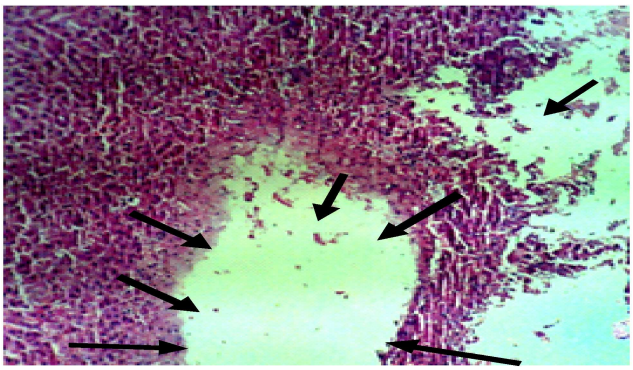
As depicted in figure 1 which shows the histological assessment of the liver after oral administration of CFDC for twenty-eight days, the control group had a normal architecture of the hepatocytes as well as the CFDC-treated group. However, the MSG-treated group showed a marked degeneration and necrotic damage of the hepatocytes which was ameliorated in the co-



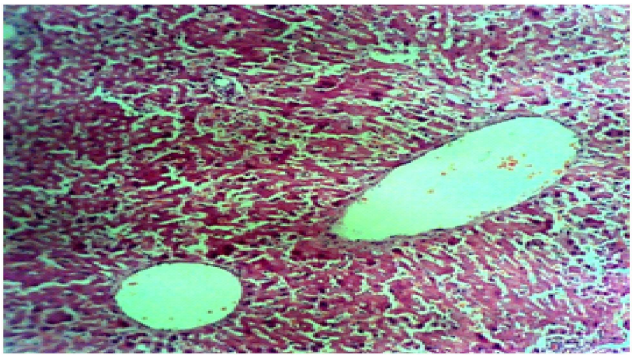
CONTROL: Hepatocytes are normal



CFDC: Liver showing normal hepatocytes

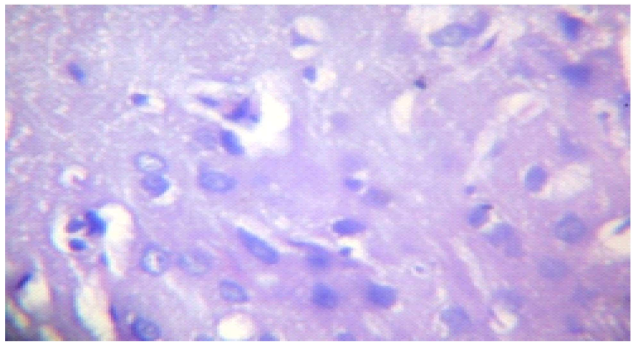


MSG: There is loss of hepatic lobules coupled with onset of necrotic damage with obvious cytoplasmic degeneration

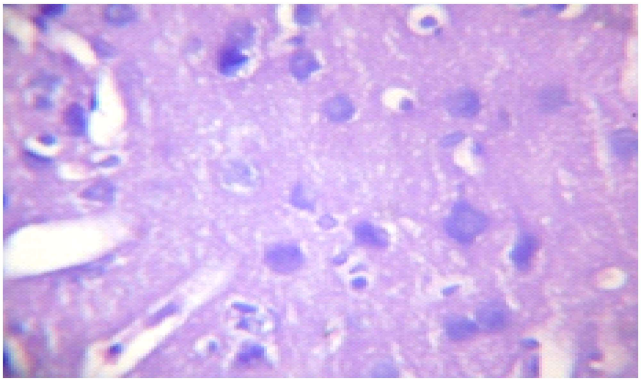


CFDC &MSG : There is increased central vein with loss of hepatocyte features

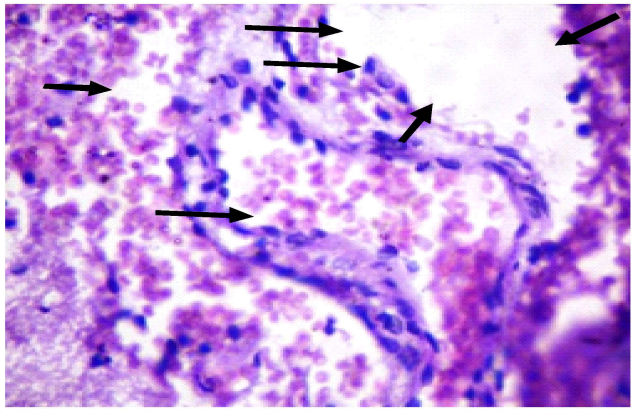
Fig. 1: Histological assessment on the liver Mag x 100 (H & E staining)



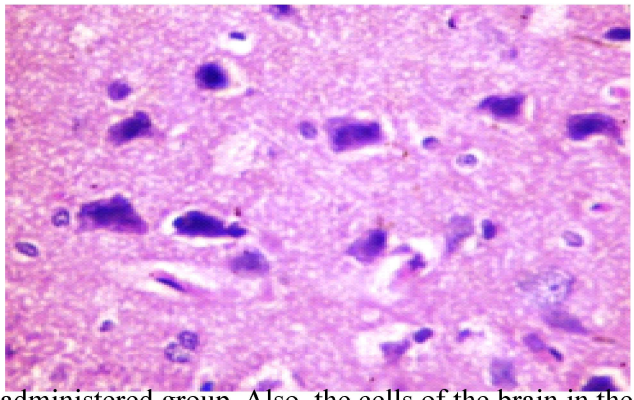
CONTROL: Plates show no significant lesion



CFDC: There is no lesion



MSG: Plates show marked focal area of congestion haemorrhage lesion and necrosis of the pyramidal layer

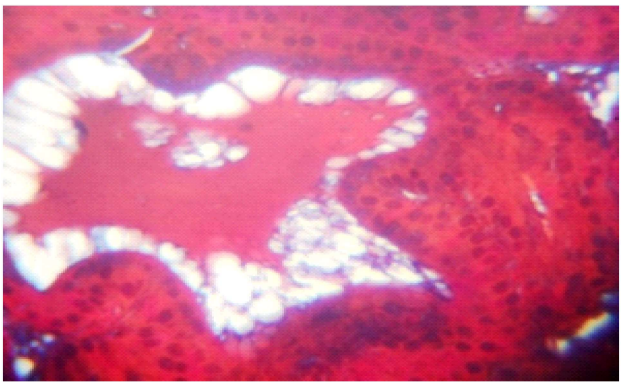


MSG&CFDC: Plates show mid focal congestion

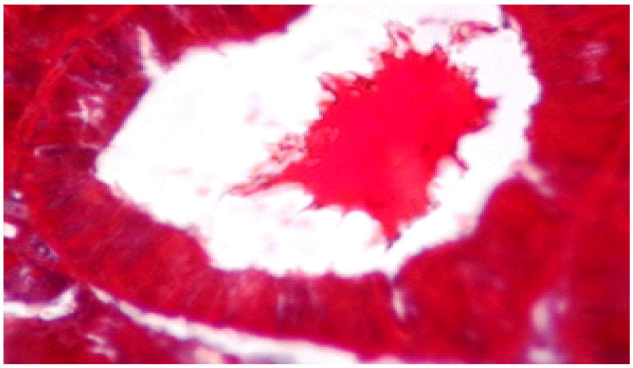
Fig. 2: Histological assessment of the brain X400 (H & E staining

administered group. Also, the cells of the brain in the control and CFDC-treated group show no lesion.

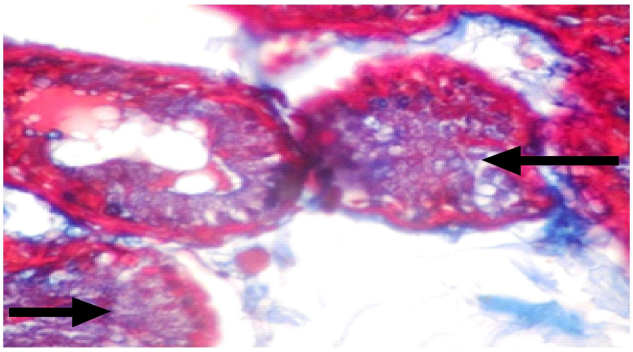
However, in the MSG-treated group, there was hemorrhagic lesion, congestion, swollen pyramidal cells with vesicular nuclei and necrosis of the pyramidal cells of the cerebellum as well as neurons of the cortex. This toxic effect of MSG on the cells of the brain was also mitigated by the co-administration of CFDC. On the histological assessment of the prostate as shown in figure 3, both the control and CFDC-treated group show normal morphology of the prostate gland. Reverse was the case in the MSG-treated group as the plates show area of hyperplasia of the gland. This effect was ameliorated in the group that received co-administration with CFDC.



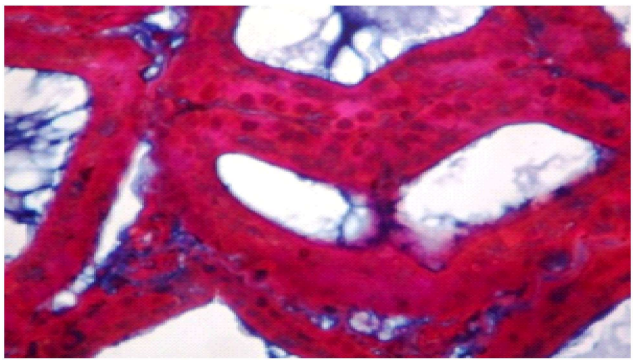
CONTROL: Plates show ormal morphology



CFDC: Plates show normal morphology



MSG: Plates show area of hyperplasia of the glands



CFDC & MSG: Plates show area of fibrosis as a result of reparative process

Fig. 3: Histological assessment of the prostate glands X400 (Masson trichome staining

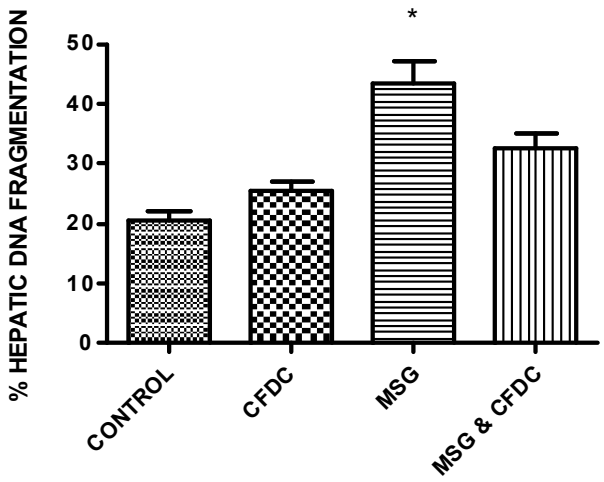


Fig. 4: Effect of oral administration of CFDC on hepatic DNA fragmentation in normal and MSG-treated rats

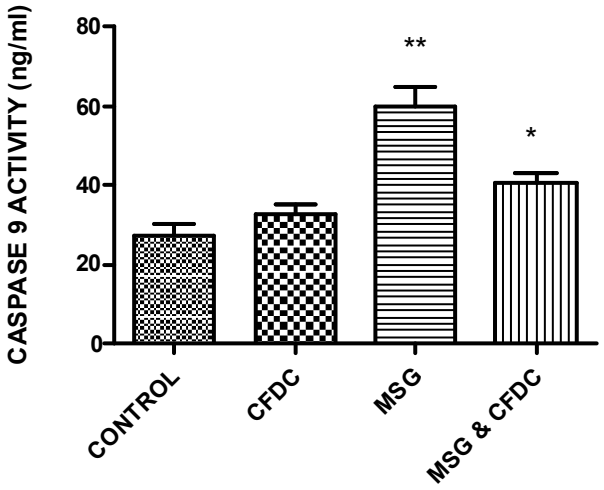


Fig. 5: Effect of oral administration of CFDC on caspase 9 activation in normal and MSG-treated rats

The Effect of oral administration of CFDC on hepatic DNA fragmentation was depicted in figure 4. The results show an elevated percentage of hepatic DNA fragmentation in the MSG-treated rats while the co-administration with CFDC ameliorated this effect. Both caspases 9 & 3 are significantly activated by the administration of MSG when compared with the control as shown in figure 5 and 6. This effect was lowered by the co-administration with CFDC. The MSG-induced increase in the level of malondialdehyde generated was significantly lowered in the group that received co-administration with CFDC as shown in figure 7.

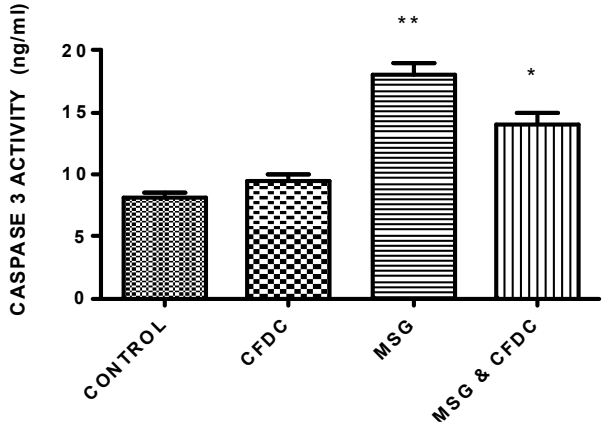


Fig. 6: Effect of oral administration of CFDC on caspase 3 activation in normal and MSG-treated rats

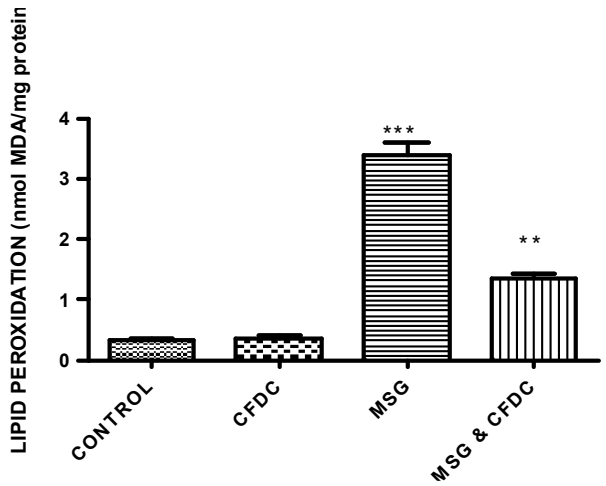


Fig. 7: Effect of oral administration of CFDC on lipid peroxidation in normal MSG-treated rats

The GC-MS analysis of chloroform fraction of *drymaria cordata* revealed the occurrence of some phytochemicals like hexadecanoic acid methyl ester (4.03%), hexadecanoic acid (33.75%), 9-Octadecenoic acid (12.16%) and 3, 7, 11, 15 tetramethyl-2- hexadecen-1-ol (18.88%).

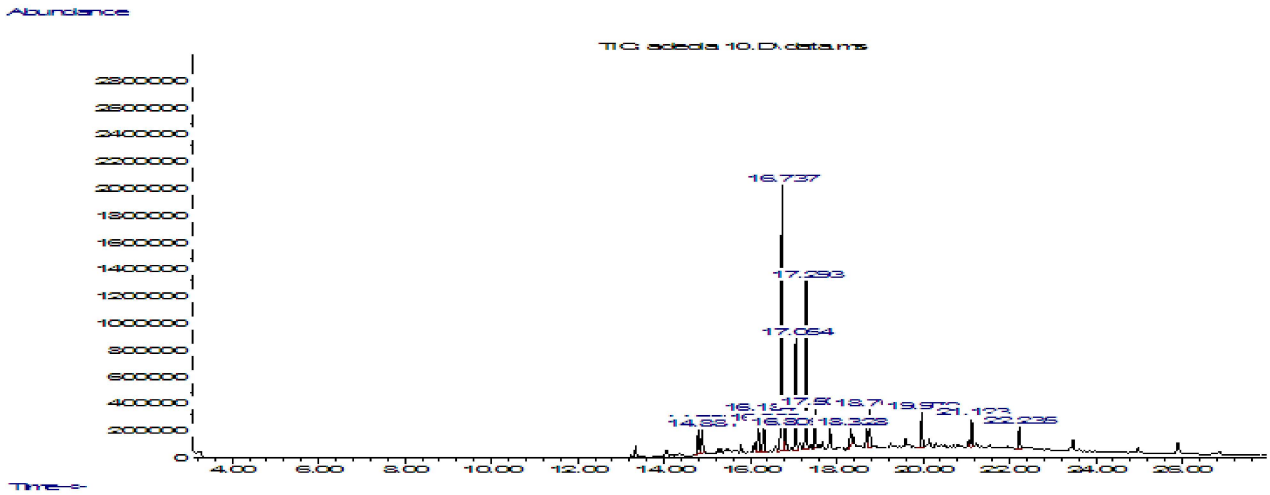


Fig. 8a: GC-MS chromatogram of chloroform fraction of methanol extract of *drymaria cordata*

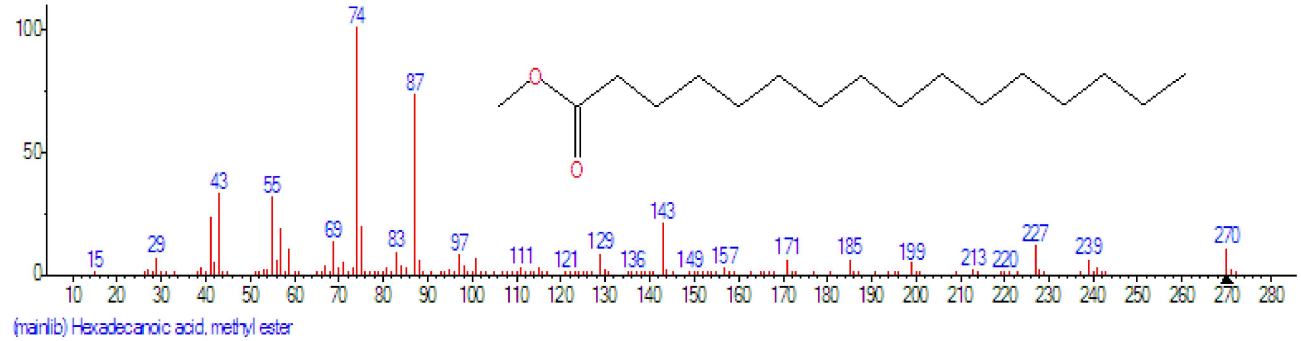


Fig. 8b: Mass spectra of the peak having retention time 16.18 (4.03%)

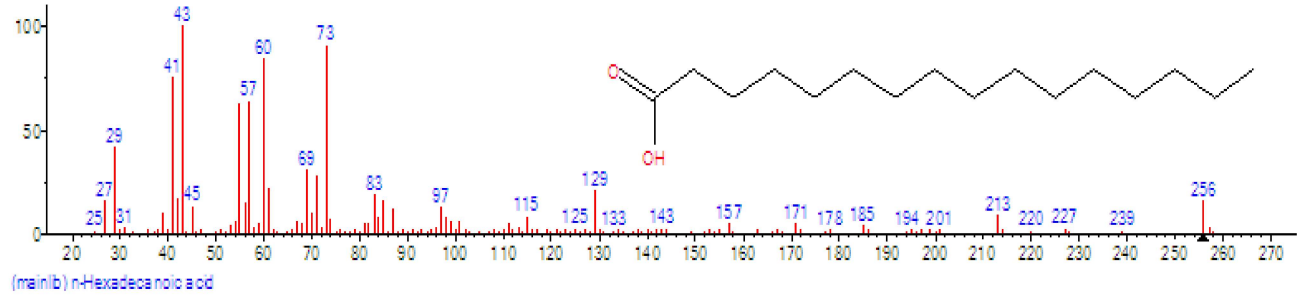


Fig. 8c: Mass spectra of the peak having retention time 16.74 (33.75%)

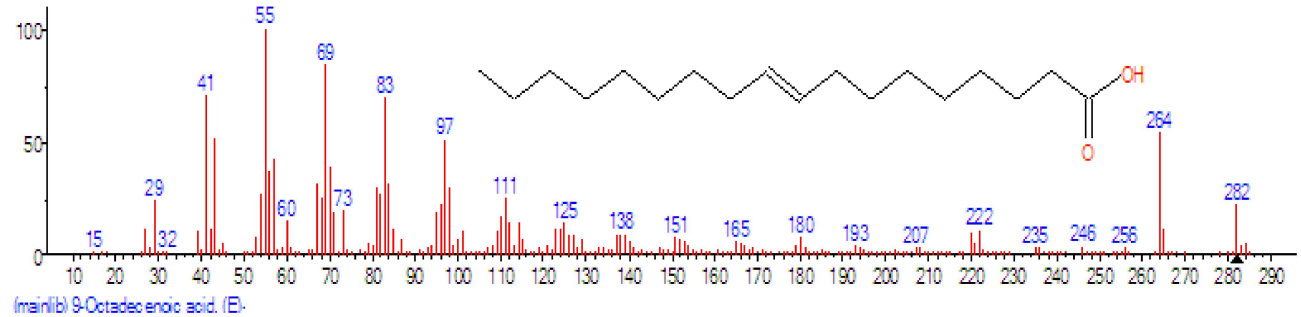


Fig. 8d: Mass spectra of the peak having retention time 17.05 (12.16%)

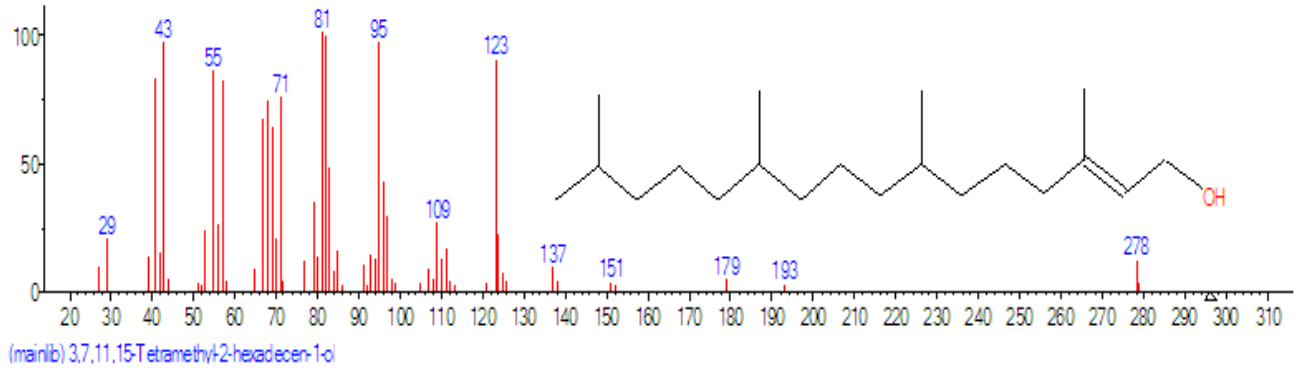


Fig. 8e: Mass spectra of the peak having retention time 17.29 (18.88%)

Discussion

Monosodium Glutamate (MSG) is a salt of glutamate, synthesized from L-glutamic acid and used as a flavour enhancer in foods. Though MSG improves taste stimulation and enhances appetite, studies have shown that it is toxic to human and experimental animals [25]. MSG has also been reported to have neurotoxic effects resulting in brain cell damage [26], retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophiclateral sclerosis [27]. Several studies have also shown MSG as an inducer of fibroid [28,29] and uterine hyperplasia [30,8]. Based on these experimental proofs of toxicity of MSG administration, we investigated the effect of chloroform fraction of methanol extract of *drymaria cordata* on monosodium glutamate-induced cellular damage. The choice of chloroform fraction of methanol extract of *drymaria cordata* for this study was based on previous results in our laboratory researching on the plant extract [31] and also on some findings involving the protective role of the plant in some chemical-induced toxicological studies [17-19] .

The histological assessment of the chloroform fraction on the hepatocytes revealed that there was no lesion on the control and CFDC-treated group at the dosage administered. However, the MSG-treated group showed some areas of degeneration and necrotic damage. This could be due to high dosage and long exposure to MSG treatment. Interestingly, co-administration with CFDC ameliorated the cellular damage noticed in the MSG-treated group. Similar pattern of results were recorded on the effect on brain and prostate gland. There were no lesions in the brain and prostate of the control and CFDC-treated rats. However, in the brain of the MSG-treated group, necrosis of the pyramidal cells of the cerebellum as well as in neurons of the cortex was noticed while co-administration mitigated this effect. Also, hyperplasia was recorded in the prostate of MSG-treated rats which was also reversed by the co-administration with CFDC. The cellular damage found in the MSG-treated rats could be as a result of high dosage and long exposure to MSG treatment. This is in consonance with the findings of Olowofolahan *et al.*[8] where MSG-induced uterine hyperplasia was significantly ameliorated by the chloroform fraction of methanol extract of *dyrmaria cordata.*

The histological findings show that the chloroform fraction of methanol extract of *drymaria cordata* contains some bioactive agents which can protect against MSG-induced cellular damage.

A variety of stress stimuli including growth factor withdrawal, heat shock, permeabilisation of mitochondrial membrane and DNA damage activate apoptosis. The formation of distinct DNA fragments of oligonucleosomal size (180-200 bp lengths) is a biochemical hallmark of apoptosis in many cells. In this study, the percentage hepatic DNA fragmentation was determined using diphenylamine (DPA) method [22] in normal and MSG-treated rats. The data show that there was significant ($P<0.05$) induction of hepatic DNA fragmentation in the MSG-treated group when compared with the control. However, this effect was ameliorated by the co-administration with CFDC. Many phytochemicals like epigallocatechin gallate, catechins and curcumin have been shown to induce DNA damage at higher concentrations while they protect against

DNA damage at lower concentrations. [32]. This result shows that CFDC contains some phytochemicals that can protect against MSG-induced DNA damage.

Caspases are crucial for the initiation, propagation and execution of apoptosis. Perturbation of organelles such as nuclei, endoplasmic reticulum and mitochondria results in the activation of caspases. [33,34]. Excessive apoptosis could result to neurodegenerative disease. In this study, the effect of CFDC was investigated on caspases 3 and 9 in normal and MSG-treated rats. The results show that there was significant upregulation of caspases 9 & 3 in the MSG-treated rats when compared with the control and this was ameliorated in the group that received co-administration with CFDC. This result is similar to Eman.[35], who reported that MSG caused upregulation of caspase 3 expression and downregulation of anti-apoptotic Bcl-2 in rat liver and testes but was normalized by Annona Muricata Linn. (Annonaceae) Leaf Extract. Also, Nia *et al*., [36] reported that MSG-induced apoptosis in the brain cells of zebrafish (*Ddanio rerio*).

In this study, we also investigated the effect of oral administration of CFDC on lipid peroxidation in normal and MSG-treated rats. The results of the lipid peroxidation as depicted in figure 4 showed a significant ($P \leq 0.05$) increase in the level of malondialdehyde produced in the MSG-treated rats when compared with the control group. The CFDC-treated group was not statistically different from the control group. Interestingly, the co-administered group showed a significant reduction in the level of malondialdehyde produced when compared with the MSG-treated group. The results from the lipid peroxidation suggest that CFDC can prevent lipid peroxidation-induced damage. This result is in agreement with the findings of Olowofolahan *et al*.,[31] where CFDC was found to inhibit lipid peroxidation. Compounds that inhibit lipid peroxidation may be exerting a pharmacological effect in the prevention of radical-induced oxidative pathological events [37]. The results show that chloroform fraction of *Drymaria cordata* has an antioxidant property which enables it to reduce lipid peroxidation induced in MSG-treated rats. This shows the potential of CFDC to reverse the elevated levels of malondialdehyde caused by MSG administration. This study also suggests that CFDC possesses free radical scavenging activity that could protect against lipid peroxidation-induced tissue damage.

The GC-MS analysis of chloroform fraction of *drymaria cordata* revealed the occurence of some phytochemicals like hexadecanoic acid methyl ester (4.031%), hexadecanoic acid (33.754%), 9-Octadecenoic acid (12.156%) and 3, 7, 11, 15 tetramethyl-2- hexadecen-1-ol (18.880%). The hexadecanoic acid has been shown to have nematicide, 5-Alpha-Reductase-Inhibitory, antialopecic antiandrogenic, antifibrinolytic, hypocholesterolemic and antioxidant properties [38,39]. Also, the hexadecanoic methyl ester has been shown to possess flavour enhancing with antioxidant and hypocholesterolemic activities as well as 5-α reductase inhibitory activity [40,41]. Oleic acid (9-Octadecenoic acid) has been shown to have cancer preventive, anemiagenic, insectifuge, antiandrogenic and dermatitigenic [42,43] . In addition, 3, 7, 11, 15 tetramethyl-2- hexadecen-1-ol (Phytol) has been reported to possess cancer-preventive, antimicrobial, anti-inflammatory, anti-diuretic, antioxidant, antifungal, antiallergic and antinociceptive activities [44,45]. Studies have also revealed phytol to be involved in the activation of both innate and acquired immunity [46]. The presence of these various phytochemicals in CFDC might be responsible for its protective role and also as an antidote against MSG-induced cellular injury/damage.

In conclusion, this study suggests that CFDC contains phytochemicals that can protect against MSG-induced cellular damage. The pharmacological activities shown by the chloroform fraction in this study might be due to the presence of antioxidants phytochemical identified in the fraction.

References

1. Leung A Y and Foster S. "Monosodium Glutamate". Encyclopedia of Common Natural Ingredients: Used in Food, Drugs, and Cosmetics. 2003:.2nd ed., New York: Wiley. pp. 373-375.
2. Beyreuther K, Biesalski HK and Fernstrom JD. "Consensus meeting: monosodium glutamate -an update". Eur. J. Clin. Nutr. 2007. 61 (3): 304–313.
3. Stevenson D D .Monosodium glutamate and asthma". J. Nutr. 2000. 130: 1067S-1073S.
4. Geha R, Beiser A, Ren C *et al.* Multicenter multiphase double blind placebo controlled study to evaluate alleged reactions to monosodium glutamate (MSG). J. Allergy Clin. Immunol. 1998.101:S243
5. Eweka AO and Adjene JO. Histological studies of the effects of monosodium Glutamate on themedial

geniculate body of adult Wister rat. Electron J Biomed. 2007. 22:9–13.
6. Samuels A. The Toxicity/Safety of MSG: A study in suppression of information. Acctabil Resch. 1999. 6(4):259–310
7. Farombi E.O and Onyema OO. Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Toxicol. 2006. May;25 (5):251-259.
8. Olowofolahan A. O., Aina O.O, Hassan E.T and Olorunsogo O.O. AmeliorativePotentials of Methanol Extract and Chloroform Fraction of Drymaria cordata on MSG-induced Uterine hyperplasia in Female Wistar Rats. European Journal of Medicinal Plants. 2017. 20(4): 1-9.
9. Braicu C., Pilecki V., Balacescu O. and Irimie, A. Neagoe, I.B. The relationships between biological activities and structure of flavan-3-ols. Int. J. Mol. Sci. 2011. 12, 9342–9353.
10. Petric R, Braicu C. Raduly L, *et al.* Phytochemicals modulate carcinogenic signaling pathways in breast and hormone-related cancers. Onco Targets Ther. 2015. 8, 2053–2066.
11. Adeyemi O.O., Akindele A.J. and Ndubuisi N. Anti-inflammatory activity of *Drymaria cordata* extract. J Nat Remedies. 2008. 8 (1): 93– 100,
12. Barua C.C., Barua A.G., Roy J.D., Buragohain B. and Borah P. Studies on the Anti-Inflammatory Properties of *Drymaria cordata* Leaf Extract. The Indian Journal of Animal Sciences. 2010.80, 1268-1270.
13. Mukherjee PK, Mukherjee K, Das J , Pal M and Saha BP . Studies on the anti- inflammatoryeffects of Drymaria cordata Willd. Natural Product Sciences 1997; 63: 367- 369.
14. Mukherjee P.K., Bhattacharya S., Saha K., *et al*. Antibacterial Evaluation of *Drymaria cordata* Willd (Fam. Caryophyllaceae) Extract. Phytotherapy Research. 1998. 11, 249-250.
15. Sowemimo A., Van de Venter M., Baatjies L and Koekemoer T. Cytotoxic Activity of Selected Nigerian Plants. African Journal of Traditional Complementary and Alternative Medicine. 2009. 6, 526-528.
16. Barua C.C., Roy J.D., Buragohain B., *et al*. Anxiolytic Activity of Hydroethanolic Extract of *Drymaria cordata* Willd. Indian Journal of Experimental Biology. 2009. 47, 969-973.
17. Akindele A.J., Ibe I.F. and Adeyemi O.O. Analgesic and Antipyretic Activities of *Drymaria cordata* (Linn.) Willd (Caryophyllaceae) Extract. African Journal of Traditional Complementary and Alternative Medicine. 2012. 9, 25-35.
18. Barua C.C., Roy J.D., Buragohain B., *et al*. Analgesic and Anti-Nociceptive Activity of Hydroethanolic Extract of *Drymaria cordata* Willd. Indian Journal of Pharmacology. 2011. 43, 121-125.
19. Barua C.C., Roy J.D., Buragohain B., *et al.* Anxiolytic Activity of Hydroethanolic Extract of *Drymaria cordata* Willd. Indian Journal of Experimental Biology. 2009. 47, 969-973.
20. Burkill H.M. The Useful Plants of West Tropical Africa. 2nd Edition, Vol. 1. Royal Botanic Gardens, Kew. 1985. 343.
21. Saklani A. and Jain S.K. In Cross Cultural Ethnobotany of North East India. Deep Publisher, India. 1994.
22. Wu Chin-Chung Mei-Ling Chan, Wen-Ying C, Ching-Yi T, Fang-Rong C and Yang-Chang W. DOI: 10.1158/1535-7163.MCT-05-0027 Published August 2005
23. Lowry O.H., Rosebrough N.J., Farr A.I. and Randall R.J. Protein measurements with the folin-phenol reagent. J. Biol.Chem. 1951. 193:260-265.
24. Varshney R. and Kale R.K. Effect of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int. Radiat Biol. 1990. 58;773-743.
25. Belluardo M, Mudo G and Bindoni M. Effect of early destruction of the mouse arcuate nucleus by MSG on age dependent natural killer activity. Brain Res. 1990. 534:225– 333
26. Eweka AO and Adjene JO. Histological studies of the effects of *Monosodium glutamate* on the medial geniculate body of adult wister rat. Electron J Biomed. 2007;22:9–13.
27. Samuels A. The toxicity/safety of MSG: A study in suppression of information. AcctabilResch. 1999. 6(4):259–310.
28. Obochi GO, Malu SP, Obi-Abang M, Alozie Y and Iyam M. Effect of garlic extracts on MSG induced fibroid in wistar rats. Pak J Nutr. 2009. 8:970–976.
29. Eweka AO, Eweka A and Om'Iniabohs FAE.. Histological studies of the effects of *Monosodium glutamate* on the fallopian tube of adult female wistar rats. N Am J Med Sci. 2010;2(3):146–149.

30. George Asumeng Koffuor*, Kofi Annan, James Oppong Kyekyeku, Hope Korshie Fiadjoe1 and Ernest Enyan . Effect of Ethanolic Stem Bark Extract of *Blighia unijugata* (Sapindaceae) on Monosodium Glutamate-Induced Uterine Leiomyoma in Sprague-Dawley Rats. British Journal of Pharmaceutical Research 3(4)*:* 2013. 880-896, 2013
31. Olowofolahan A.O, Adeoye O.A, Offor G.N, Adebisi L.A and Olorunsogo O.O. Induction of Mitochondrial Membrane Permeability Transition Pore Opening and Cytochrome C Release by Fractions of *Drymaria cordata.* Arch. Bas. App. Med. 3 (2015) 135 – 144
32. Merve BA. Ahmet B and Nurşen B. Are all phytochemicals useful in the preventing of DNA damage? Food and Chemical Toxicology Volume 109, Part 1, November 2017, Pages 210-217.
33. Susin SA, Lorenzo HK, Zamzami N, *et al*. Mitochondrial release of caspase-2 and -9 during the apoptotic process J. Exp. Med. 1999. 189: 381–394.
34. Qin ZH, Wang Y, Kikly KK, *et al*. Pro-caspase-8 is predominantly localized in mitochondria and released into cytoplasm upon apoptotic stimulation J. Biol. Chem. 2001. 276: 8079–8086.
35. Eman Mohammed Mohammed Abd-Ella , Abd-Elkarim Mohammed Abd-Lateif Mohammed . Attenuation of Monosodium Glutamate-Induced Hepatic and Testicular Toxicity in Albino Rats by Annona Muricata Linn. (Annonaceae) Leaf Extract. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. IV (Nov. - Dec.2016), PP 61-69 .
36. Nia K, Juliyatin PU, Nurdiana N and Diana L Monosodium glutamate exposure at early developmental stage increases apoptosis and stereotypic behavior risks on zebrafish (danio rerio) larvae. Indonesia Journal of Pharmacy .Vol 27 No 3, 2016 : 128-138.
37. Ruberto G, Baratta MT, Deans SG and Dorman HJ. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta Med. 2000. 66: 687–693
38. Sermakkani M and Thangapandian V. GC-MS analysis of *Cassia italic* leaf methanol extract. Asian J Pharm Clin Res 5: 2012. 90-94.
39. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K and Uma C . GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides*(L.) L. J Food Sci Techno. 2015. 52: 1212-1217.
40. Dandekar R, Fegade B and Bhaskar VH . GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxipetalum* leaves. J Pharmacogn Phytochem. 2015. 4: 149-154.
41. Vijisaral ED and Subramanian A. GC-MS analysis of ethanol extract of *Cyperus rotundus* leaves. Int J Curr Biotechnol; 2014; 2: 19-23.
42. Rajeswari G, Murugan M and Mohan VR. GC-MS analysis of bioactive components of *Hugonia mystax* L (Linaceae). Res J Pharmaceut Bio Chem Sci 2012; 3: 301-308.
43. Rajab MS, Cantrell C, Franzblau SG and Fischer NH. Antimycobacterial activity of phytol and derivatives: a preliminary structure-activity study. Planta Med, 1998; 64: 2-4.
44. Ryu KR, Choi YJ, Chung S and Kim DH. Antiscratching behavioral effect of the essential oil and phytol isolated from Artemisia princeps Pamp. In mice. Planta Med, 2011; 77: 22-26.
45. Saikia D, Parihar S, Chanda D, Ojha S and Kumar JK. Antitubercular potential of some semisynthetic analogues of phytol. Bioorg Med Chem Lett, 2010; 20: 508-512.
46. Lim SY, Meyer M, Kjonaas RA and Ghosh SK. Phytol-based novel adjuvants in vaccine formulation: 1. assessment of safety and efficacy during stimulation of humoral and cell-mediated immune responses. J Immune Based Ther Vaccines, 2006; 4: 6.

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Knowledge and reporting of adverse drug reactions among patent medicine vendors in Ibadan South west local government area, Oyo State, Nigeria

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Abstract

Background: Adverse drug reactions (ADRs) are one of the leading causes of morbidity and mortality. Detection and spontaneous reporting of ADRs by private providers, in which patent medicine vendors can be categorized, could reduce their consequences. Little is known about the knowledge and reporting of ADRs among private health care professionals in Oyo State, Nigeria. This study was designed to assess the level of knowledge and reporting of adverse drug reactions (ADRs) among patent medicine vendors (PMVs) in Ibadan South West LGA, Oyo State, Nigeria.

Methods: The study design was descriptive and cross-sectional. All available PMVs (128) who consented to participate were enrolled in the study and completed a 29- item validated questionnaire on socio-demographic characteristics, ADRs knowledge, experiences and ADRs practices. Knowledge of the respondents to ADRs was measured on a 13-point scale, while practice was measured on a 5 point scale. Knowledge scores of $\leq$6 and >7 were classified as poor and good respectively. Practice scores of $\leq$ 2 and > 3 were classified as poor and good respectively.

Results: A majority of PMVs were knowledgeable of causes (84.4%) and the risk factors (85.2%) of ADRs; some of which included patient sensitivity to a drug (77.6%), drug-drug reaction (86.1%) and patient using alcohol to swallow medication (91.1%). Familiarity with the ADR reporting process was low as most (75.1%) PMVs claimed not to have encountered ADRs. Of the 21.0 % PMVs who reportedly encountered ADR four weeks preceding the survey, only 2.7% ever reported. Major reasons for underreporting of ADRs were not knowing where to report (56.8%), insufficient knowledge (32.2%) and ADR reporting being a time-wasting activity (25.4%). While few PMVs (29.0%) reported to have been trained on reporting ADRs, the respondents who had received training reported to have been trained through NAFDAC (30.6%), UCH (14.3%) and NDLEA (20.4%)

Conclusion: Patent medicine vendors on the average are knowledgeable of adverse drug reactions but not on the reporting process. Improving knowledge - practice gap will require educational interventions in form of formal training and seminars.

Keywords: A*dverse drug reaction, knowledge, reporting, patent medicine vendors*

Abstrait

Contexte: : Les effets indésirables des médicaments (EIM) sont l'une des principales causes de morbidité et de mortalité. La détection et la notification spontanée des effets indésirables par des prestataires privés, dans lesquels les fournisseurs de médicaments brevetés peuvent être classés, pourraient réduire leurs conséquences. On sait peu de choses sur la connaissance et le signalement des effets indésirables parmi les professionnels de la santé privés de l'État d'Oyo, au Nigéria. Cette étude a été conçue pour évaluer le niveau de connaissance et de déclaration des effets indésirables des médicaments (EIM) chez les vendeurs de médicaments brevetés (VMB) à Ibadan South West LGA, Etat d'Oyo, Nigeria.

Méthodes : La conception de l'étude était descriptive et transversale. Tous les VMB disponibles (128) qui ont consenti à participer ont été inscrits à l'étude et ont rempli un questionnaire validé de 29 éléments sur les caractéristiques sociodémographiques, les connaissances, les expériences et les pratiques en matière de EIM. La connaissance des répondants aux EIM a été mesurée sur une échelle de 13 points, tandis que la pratique a été mesurée sur une échelle de 5 points. Les scores de connaissance $\leq$6 et> 7 ont été classés respectivement comme mauvais et bon. Les scores de pratique $\leq$2 et> 3 ont été classés respectivement mauvais et bon.

Résultats : La majorité des VMB connaissaient les causes (84,4%) et les facteurs de risque (85,2%) des EIM; certains d'entre eux comprenaient la sensibilité des patients à un médicament (77,6%), la réaction médicamenteuse (86,1%) et les patients consommant de l'alcool pour avaler des médicaments (91,1%). La

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familiarité avec le processus de déclaration des EIM était faible, car la plupart (75,1%) des VMB affirmaient ne pas avoir rencontré d'EIM. Sur les 21,0% de VMB qui auraient rencontré un EIM quatre semaines avant l'enquête, seulement 2,7% l'ont signalé. Les principales raisons de la sous-déclaration des EIM étaient de ne pas savoir où déclarer (56,8%), les connaissances insuffisantes (32,2%) et la déclaration d'EIM à être une activité de perte de temps (25,4%). Tandis que peu de VMB (29,0%) ont déclaré avoir été formés à la déclaration des EIM, les répondants qui avaient reçu une formation ont déclaré avoir été formés par le NAFDAC (30,6%), UCH (14,3%) et NDLEA (20,4%) *Conclusion : En* moyenne, les vendeurs de médicaments brevetés connaissent les effets indésirables des médicaments, mais pas le processus de déclaration. L'amélioration du fossé entre les connaissances et les pratiques nécessitera des interventions éducatives sous forme de formation formelle et de séminaires.

Mots-clés : *Effets indésirables des médicaments, connaissances, rapports, vendeurs de médicaments brevetés*

Introduction

Patent Medicine Vendors (PMVs) are "persons without formal pharmacy training selling orthodox pharmaceutical products on a retail basis for profit" [1]. They form a significant proportion of the private sector involved in the sale of modern medicines. PMVs include individuals, owners, or attendants working in private shops that may be registered or unregistered. These shops may legally sell over-the-counter drugs, and generally they also illegally sell prescription drugs, such as antibiotics, sedatives among others [2]. With the current population of Nigeria and the limited number of trained health care providers, PMVs are inevitable and highly needed especially in the rural areas for the supply of drugs in treating minor illnesses [3].

The Medicines and Healthcare Products Regulatory Agency (MHRA) defined an adverse drug reaction (ADR) as "an unwanted or harmful reaction experienced following the administration of a drug or combination of drugs, and is suspected to be related to the drug. The reaction may be a known side effect of the drug or it may be new and previously unrecognized" [4]. Adverse drug reactions (ADRs) occur frequently and globally accounting for a significant number of fatalities each year. It has been estimated that fatalities directly attributable to ADRs are the fourth to sixth leading cause of death in hospitals in the United States, exceeding deaths caused by pneumonia and diabetes [5]. In addition to the human costs, ADRs have a major impact on public health by imposing a significant financial drain on the society and the already stretched healthcare systems.

At the point new medicines are registered for use in humans, not much is known about those medicines beyond data obtained from clinical trials in controlled settings. Clinical trials for the evaluation of safety, efficacy and quality of new medicines are conducted in patients that may not necessarily represent all type of patients that will use the medicines when they are approved. Limited numbers of patients are exposed to the medicine during clinical trials and research settings differ from the conditions of use when the drug is marketed. Lack of complete understanding of the effects of long-term exposure, co morbid conditions, and use in elderly, racial groups, children and pregnant women are other limitations of preapproval clinical trials [6]. Post-marketing surveillance and Pharmacovigilance activities i.e. reports of adverse drug reactions can help in obtaining real-life information of safety and effectiveness of medicines when they are being used in the population. These post-marketing surveillance activities have resulted in the reappraisal of indications, identification of risk factors and characterization of users, identification of long-term toxicities, quality problems, etc. Gatti in 2012 reported that many drugs studied in clinical trials have limited experience in the general population and in special populations such as children and older adults; therefore, reporting an adverse events from real-life use in clinical practice is invaluable [7].

Under-reporting of ADRs is a worldwide phenomenon and this has been established from previous studies [8]. It is estimated that only 6% of ADRs are reported worldwide; which implies that ongoing evaluation of the risk-benefit ratio of medications in the market is largely unavailable [9, 10]. The importance of reporting adverse drug reactions can be seen in a case study of reported cases of renal failure observed in children in the Ahmadu Bello University Teaching Hospital Zaria Nigeria who were said to have ingested a teething mixture called "My Pikin" in 2012. As at the time the report was made, about 84 children were said to have died but further deaths were averted through this reports as the harmful batch of the mixture were immediately withdrawn from circulation [11].

Gross underreporting of ADRs is a cause of concern, the reason for which may be inadequate funds, lack of trained staff and lack of awareness about detection, communication and spontaneous monitoring of ADRs [12]. Other factors include lack of knowledge of the forms for reporting, ignorance of the rules and procedure for reporting, and not being sure of the type of reactions to be reported. The results are similar to the studies carried out in China, Nigeria, and Malaysia [13-15].

PMVs can play an important role in ADR reporting and pharmacovigilance by increasing the number as well as the quality of submitted reports [16]. When a reaction is observed, it is important to classify it as serious and report it to the appropriate body. By identifying and reporting adverse drug events, PMVs may influence drug labeling or alerts that impact prescribing practices and help protect the public's health. Besides, good pharmacovigilance programs are expected to identify the risks and the risk factors in the shortest possible time so that harm can be avoided or minimized. When communicated effectively, this information allows for the intelligent, evidence-based use of medicines and has the potential for preventing many adverse reactions [8]. The most important function of spontaneous reporting systems is the early identification of signals and formulation of hypotheses, leading to further confirmatory investigations or sometimes regulatory warnings and changes of product information leaflet [17].

Of several measures suggested to improve ADR reporting, worthy of note amongst them include creating awareness about ADR monitoring among health care professionals and consumers, through appropriate educational interventions [e.g. seminars, sensitizations], making ADR reporting forms easily available and simplifying the process of reporting. Feedback from ADR monitoring centers about the causality and severity of ADRs reported would also encourage them to continue reporting [8]. The use of new technologies such as the mobile phones as well as the internet for reporting ADRs will be favorably accepted while the involvement of religious and community leaders in the communication of the importance of pharmacovigilance can also increase awareness of their followers and the entire community at large.

Educational interventions were mentioned in majority of studies that have been carried out previously with inclusion of courses on ADR and its reporting process in undergraduate programs as well as in continuous professional development courses [4, 12]. As part of the effort in minimizing ADRs in Nigeria, the National Agency for Food & Drug Administration & Control (NAFDAC) in Nigeria has developed a National Pharmacovigilance (Drug Safety Monitoring) Centre (NPC) that monitors and controls reports of adverse drug reactions through the yellow reporting forms. In addition, the body developed a step by step approach to help in assessing possible drug- related ADRs [18].

Studies carried out on underreporting of ADRs have been on health care professionals in the hospitals; though, many patients have more direct contacts with patent medicine vendors in the purchase of their mediations in the country at the present. The goal of this study was to generate evidence on ADRs awareness, knowledge and reporting practices among PMVs in Ibadan South West LGA, Nigeria so as to stimulate needed actions on pharmacovigilance in the private sector market.

Study location

The study was carried out among patent medicine vendors in Ibadan South West Local Government Area of Ibadan, Oyo State. Ibadan South West Local Government Area was carved out of the defunct Ibadan Municipal Government (IMG) in 1991 with its administrative headquarters located at Oluyole Estate. It covers a landmass of 133,500 square kilometers with a population density of 2,401 persons per square kilometer. The 2010 estimated population for the area was projected as 320,536 people, using a growth rate of 3.2% from 2006 census [19].

Methods

The study was a descriptive cross-sectional survey using a non-probability sampling technique. Snowballing was used to locate all the Patent Medicine Vendors from Ibadan South West Local Government Area of Oyo State, Nigeria. A 29- item validated questionnaire. on socio-demographic characteristics, ADRs awareness, experiences and ADRs practices was first translated from English to the local language (Yoruba) and back translated into English with a view to ensure accuracy of translation followed by pilot testing and production of final copies which were subsequently self-administered to all the 128 PMVs in the LGA over a period of seven days. Verbal informed and voluntary consent was obtained from each study participant.

Table 1: Respondent's demographic charasteristics (n=125)

		Frequency
Respondent's sex	Male	37(28.9)
	Female	91(71.1)
Age distribution*	15-24	22(17.7)
	25-34	50(40.3)
	35-44	41(33.1)
	45-54	8(6.5)
	55-84	3(2.4)
Mean age of participants		33.13(±9.79)
Ethnic Group	Yoruba	124(96.9)
	Igbo	4(3.1)
Religion:	Christianity	67(52.3)
	Islam	61(47.7)
Highest level of education completed	Tertiary	
	M.Sc./Postgraduate	25(19.5)
	Senior Secondary	94(73.4)
	Others	9(7.2)
Others		
Adult education		
Junior secondary		
Post-secondary (grade 2)		
Standard 6		
School of hygiene		
School of nursing		
School of health		
Type of professional qualification		
	School certificate	98(76.6)
*Others	OND	14(10.9)
Health assistant	Others	16(12.6)
HND		
NCE		
Marketer		
Nursing		
Community Health Extension Worker		

*Missing values were excluded

Analysis

PMV awareness on ADRs was measured on a 13-point scale, while practice was measured on a 5-point scale. Knowledge scores of ≤6 and >7 were classified as "poor" and "good" respectively. Practice scores of ≤ 2 and > 3 were classified as "poor" and "good" respectively. The information in the filled copies of the questionnaire were coded with the aid of the developed coding guide and entered into SPSS version 16 for analysis and subjected to descriptive (frequencies, mean, median, mode) and inferential statistics using Chi-square test to detect statistically significant differences (p value<0.05)

Results

Respondents' Demographic characteristics

The mean age of respondents was 33.13(±9.79) years and was mainly from the Yoruba ethnic group (96.9%). A majority (73.4%) had Senior Secondary School education. A total of 87 (72.5%) PMVs had one Patent Medicine Store (PMS), 4 (3.3 %) had two; while 29 (24.2%) didn't own a PMS (Table 1).

A majority of PMVs (71%) had never received training on reporting Adverse Drug Reaction (ADR). Of those that did, sources of training included the National Agency for Food and Drug Administration and Control (NAFDAC) (30.6%) followed by the National

Drug Law Enforcement Agency (NDLEA) (20.4%), and College of Medicine (UCH) Ibadan (14.3%) (Table 2).

A high majority of respondents were aware that reduced kidney or liver functions (78.9%), use of herbal supplements with orthodox medicines (87.2%),

Table 2: Respondents* previous training experience on ADR (N=129)

		n(%)
Training on how to report ADRs*	Yes	36(29.0)
	No	88(71.0)
Specification of where respondent was treated	College of Medicine (UCH)	7(14.3)
	Association for Reproductive and Family Health (ARFH)	6(12.2)
	NAFDAC	15(30.6)
	Company seminars	3(6.1)
	NDLEA	10(20.4)

**Missing responses were excluded*
+Multiple responses were allowed

use of medicines borrowed from other people (76.6%) are risk factors that can facilitate ADR while 98.4% considered consumption of fake drugs as risk factors that can facilitate ADR. A mean knowledge score of 8.95± 2.33 was obtained.

Respondents' Awareness about Adverse Drug Reaction (ADR)

As depicted in table 3, a high proportion of respondents (77.6%) were aware of a patient being sensitive to a drug as being a cause of ADR, as well as a drug-drug reaction (86.1%). Most (91.1%) PMVs were aware that a patient using alcohol to swallow his/her medication was a cause of ADR while 93.5% said a patient using water to swallow his/her medication will not have an ADR.

Familiarity with ADR reporting process in Ibadan

Out of the population involved in the study, 98 (83.8%) of the patent medicine vendors reported unfamiliarity with the reporting process and 50 (40.0%) didn't know about the online ADRs submission.

Table 3: Respondents' awareness about adverse drug reaction (N=128)

	Yes (%)	No (%)
A patient being sensitive to a drug*	97 (77.6)	28(22.)
Drug-drug reaction*	105(86.1)	17(13.9)
A worsening of an existing medical problem*	80(65.0)	43(35)
Increasing the dosage of medication being taken*	93 (75.6)	30(24.4)
Adding a new drug to the ones being taken (Polypharmacy)*	88 (71.0)	36(29)
A patient using alcohol to swallow his/her medication*	113(91.1)	11(8.9)
A patient using water to swallow his/her medication*	5(4.0)	119(96)
Age of the patient*	47 (37.9)	77(62.1)
Reduced kidney or liver function*	97(78.9)	26(21.1)
Use of herbal supplements with orthodox medicines*	109(87.2)	16(12.8)
Use of medicines borrowed from other people*	95(76.6)	29(23.4)
Consumption of fake drugs*	122(98.4)	2(1.6)
Timing of use of the medicine*	33(28.2)	84(71.8)

** Missing values were excluded*

Measures adopted to comfort a patient complaining of ADR or side effect

When asked about measures that could be adopted to comfort a patient complaining of ADR or side effect, 80.8% of the PMVs correctly answered to referring the patient to see a physician/doctor, 48.8% also rightly answered to asking him/her to stop taking the medicine causing the ADR or side effect and 32.0% reported that they would give him/her a medication to treat his/her condition while asking him/her to stop the medication causing the ADR.

preceding the study while 11.7% had one case of ADR encountered, and 6.2% had two cases encountered. Slightly over two fifths (42.9%) PMVs reported that itching was one of the types of ADRs encountered in the month preceding the study; while 32.1% PMVs reported swelling of different parts of the body and weakness as the types of ADRs encountered.

Only 2 (2.7%) of the PMVs had ever reported ADRs they came across and indicated a Primary Health Centre and a close by hospital as the places they reported to. Over a third (44.2%) explained they

Table 4: Common drug classes associated with the ADRs encountered by respondents[+*] (N=128)

	Frequency (n)	Percentage (%)
Sulphonamides (Septrin, Fansidar)	50	57.5
Diruetics	9	10.3
Penicillins	17	19.5
Cephalosporins	2	2.3
Chloroquine	66	75.9
Antihistamines	6	6.9
Arthemeter Combination Therapy (ACT) (Lonart, Combisunate, Artequineetc)	29	33.3
Non-Steroidal Anti-inflammatory drugs (Ibuprofen, Diclofenac, Aspirin)	63	72.4
Family Planning pills	37	42.5

+ Multiple responses were allowed
** Missing values were excluded*

Table 5: Reported reasons for under-reporting ADRs by respondents[+] (N=128)

	Frequency (n)	Percentage (%)
Only safe drugs are available in the market.	12	10.2
Reporting does not influence the treatment scheme.	18	15.3
Busy schedule.	17	14.4
Lack of incentives.	16	13.6
Doctor should rather collect data and publish himself/ herself.	7	5.9
Difficult to pin point suspected drug.	22	18.6
ADR reporting is a time-wasting activity with no outcome	30	25.4
ADR is known to the doctor alone.	14	11.9
Don't know whom to report	67	56.8
Reporting could show ignorance.	7	5.9
Difficult to admit injury (harm) to the patient.	9	7.6
Insufficient medical knowledge.	38	32.2
Submitting one report doesn't make any difference.	16	13.6
Other causes of under-reporting of ADRs	10	7.8

+ Multiple responses were allowed

Adverse drug reaction reporting practice

Experiences with Adverse Drug Reactions (ADR)

A majority of the respondents (75.1%) had not encountered any ADR reported to them in the month had not come across any ADRs in their practice while 36.5% reported not knowing where to report to.

As shown in table 4, majority of the PMVs reported chloroquine (75.9%) and Non-Steroidal Anti-

inflammatory drugs (72.4%) as common drug classes associated with the ADRs ever encountered, while 57.5% said sulphonamides and 42.5% mentioned family planning pills.

Factors influencing under-reporting of suspected ADRs
Table 5 summarizes the reported reasons for under-reporting ADRs by PMVs. These include not knowing whom to report to (56.8 %), insufficient medical knowledge (32.2%) and ADR reporting believed to being a time-wasting activity with no outcome (25.4%).

Respondents' suggestions for improving ADR reporting
In addition, various submissions were made as possible ways of improving ADR reporting. A vast majority of the respondents (83.3%) suggested seminars and education on ADR for both patients and store owners. Another majority (62.3%) feel that an increased sensitization and awareness on ADR reporting will be beneficial.

Discussion

In this study majority of PMVs disclosed that chloroquine and Non-Steroidal Anti-inflammatory drugs were the commonest drug classes associated with the ADRs ever encountered, while nearly half also identified Sulphonamides and family planning pills. This is similar to what was reported in a study in 2011[9, 20]. When clients react to these drugs, especially chloroquine and sulphonamides, they tend to stop using the drug half way into the treatment, thereby building up drug resistance if used in such ways many times. Though it may not be possible to know who will react adversely to a drug, it is important that a reaction is reported and documented so that such patient will not be given the drug again so as not to build up drug resistance and another class of medication can be given.

Majority of the population involved in this study reported unfamiliarity with the ADR reporting process and two fifths did not know it was possible to submit ADRs. This may be due to it not being emphasized during training. This can be highlighted in the significant increase in the knowledge about adverse drug reactions and it's reporting among patent medicine vendors in a study carried out by Awodele et al in Ekiti [3]. To address this key finding, it is important that PMVs be trained effectively on ADRs and its reporting process as well as make available the reporting forms for them to use when need be.

In this study, the respondents' awareness on causes and risk factors of Adverse Drug Reactions was high but had very low awareness on the process of reporting. One of the most reported causes of under-reporting ADRs identified in this study was not knowing whom to report to. These factor mentioned was similar to those mentioned in studies carried out among physicians in India [7] and among pharmacists in India too by [21].

Other barriers to reporting ADRs identified among the PMVs included insufficient medical knowledge and a feeling that ADR reporting is a time-wasting activity with no outcome. The fear of shops being locked or sealed and apprehension by law enforcement agencies for selling fake drugs also came into play as barriers to reporting adverse drug reactions.

These factors and or causes of under-reporting revealed in this study were similar to those mentioned in studies carried out among Patent Medicine Vendors in Ekiti State [3], physicians in India [7] and among pharmacists in India [21]. Hager et al concluded that there is poor knowledge and reporting practice of adverse drug reactions among health care professionals but there is good attitude and states that there is the need for appropriate training of healthcare professionals for improved knowledge and practice [22]. Of note is that the authors are unaware of any seminar of training for PPMVs on ADR in the LGA by government health care establishments or agencies.

Findings from this study have health promotion and education consequences and propose the need for multiple interventions directed at confronting the occurrence. Awareness on the importance of reporting Adverse Drug Reactions must be raised among patent medicine vendors as well as patients. This can be achieved through public enlightenment and community health education in the provision of seminars and health talks. Information and communication materials such as posters promoting messages on the importance of reporting any suspected adverse drug reaction should also be used.

Regulatory bodies through the Government such as the Pharmacist Council of Nigeria (PCN) and the Ministry of Health, Non-Governmental Organizations and PMV associations should carry out capacity building of the PMVs through improvements in the training process. This is a very important step in improving ADR reporting. Refresher courses which can include new and recent developments in the various types of ADRs, the provision of the yellow forms for reporting ADRs and training PMVs on how to use them would likely stimulate use. Follow up should be done to

monitor the improvements in ADR reporting and good reporting practice should be rewarded.

The evidence obtained from this study will be further disseminated to policy makers so as to stimulate policy debates that will in turn generate policies guiding the reporting of Adverse Drug Reactions by PPMVs and its benefits. Also, PMV associations will be encouraged to formulate association policies that will mandate all PMVs to report ADRs experienced by their clients with incentives for reporting incorporated.

References

1. Brieger WR, Osamor PE, Salami KS, Oladepo O and Otusanya SA., Observations of patent medicine vendor and customer interactions in urban and rural areas of Oyo state, Nigeria. Health Policy Plan 2004; 19:177-182.
2. Oladepo O., Salami .K.K., Adeoye .B.W., Oshiname F., Ofi .B. and Oladepo M. Malaria treatment and policy in three regions in Nigeria: The role of patent medicine vendors. London: Future Health Systems 2007; 29. (FHS working paper no. 1; Nigeria series).
3. Awodele O, Adeniran A and Awodele DF. Pharmacovigilance amongst patent medicine vendors (PMVs) in Ekiti State, Nigeria. Int J Risk Saf Med., 2012, 24: 65–72.
4. Board of Science, Reporting adverse drug reactions: A guide for healthcare professionals Reporting adverse drug reactions A guide for healthcare professionals, 2006.
5. Oshikoya KA., Chukwura H., Njokanma OF., Senbanjo IO and Ojo I. Sao Paulo Medical Journal - Incidence and cost estimate of treating pediatric adverse drug reactions in Lagos, Nigeria. Sao Paulo Medical Journal, 2011, 129(3).
6. Nwokike J. Monitoring Adverse Drug Reactions in the Public Health Programs/ : the case of the Nigeria TB program. 2008.
7. Gatti JC. The Importance of Reporting Adverse Drug Events. American Family Physician, 2004.
8. Kamtane R. and Jayawardhani V. Knowledge, attitude and perception of physicians towards adverse drug reaction reporting: a pharmacoepidemiological study. Asian Journal of Pharmaceutical and Clinical Research, 2012, *5*, 210–214.
9. Inman W.H. Assessment of drug safety problems. In: Gent M, Shigmatsu I, editors. Epidemiological issues in reported drug-induced illnesses. Honolulu (ON): McMaster University Library Press; 1976. p. 17-24.
10. Bello SO and Umar MT. Knowledge and attitudes of physicians relating to reporting of adverse drug reactions in Sokoto, north-western Nigeria. Annals of African Medicine, 2011, 10(1), 13–18.
11. Akuse R M. and Garnett FF. Spontaneous reporting of paediatric adverse drug reactions in a Nigerian tertiary health centre – any relationship to severity/ ? International Journal of Pharmaceutical Science Invention, 2013, 2(1), 5–11.
12. Isfahani M.E., Mousavi S., Rakhshan A., Assarian M and Kuti L., Adverse Drug Reactions/ : Knowledge, Attitude and Practice of Pharmacy Students. Journal of Pharmaceutical Care, 2013, (6), 145–148.
13. Li Q, Zhang S.M., Chen H.T., *et al.* Awareness and attitudes of healthcare professionals in Wuhan, China to the reporting of adverse drug reactions. Chin. Med. J. 2004, 117(6): 856-861.
14. Aziz Z, Siang TC and Badarudin N.S. Reporting of adverse drug reactions: predictors of under reporting in Malaysia. Pharmacoepidemiol. Drug Saf., 2007, 16(2):223-228.
15. Okezie E.O., Adverse drug reactions reporting by physicians in Ibadan, Nigeria. Pharmacoepidemiol. Drug Saf. 2008, 17(5):517-522.
16. Kees V.G., Olsson S., Couper M. and Berg L. Pharmacists' role in reporting adverse drug reactions in an international perspective. Pharmacoepidemiol.Drug Saf. 2004, 13, 457– 464.
17. Pal S. N., Duncombe C., Falzon D. and Olsson S. WHO Strategy for Collecting Safety Data in Public Health Programmes/ : Complementing Spontaneous Reporting Systems. Drug Saf, 2013, 75–81.
18. www.nafdac.gov.ng (Accessed November 2014).
19. www.ibadanland.net (Accessed February 2015)
20. Mahmoud M. A., Alswaida Y., Alshammari T., *et al.* Community pharmacists' knowledge, behaviors and experiences about adverse drug reaction reporting in Saudi Arabia. Saudi Pharmaceutical Journal, 2013, doi:10.1016/j.jsps.2013.07.005
21. Akram A., Patel I. and Manna P. K. An evaluation of knowledge & attitudes of Indian phamacists to ADR. Perspective in Clinical Research, 2013, 4, 204–210.
22. Hager AS, Albert F and Fourrier-Réglat A. Knowledge, Attitude and Practice of Health Professionals Towards Adverse Drug Reactions Reporting European Journal of Pharmaceutical and Medical Research, 2016, 3(8), 12-21.

Rodent control methods and knowledge of potential human toxicity in Ibadan: a pilot study

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Abstract

Background: Studies on the rodent situation and methods of rodent control in households in Nigeria are scarce. This pilot study examined types of rodents, methods of their control, and participants' knowledge of potential clinical toxicities of rodenticides used.

Methods: A cross-sectional study that involved a random interview of participants aged 10 years and above at urban markets along roads leading to the University of Ibadan, Nigeria. A structured, interviewer-administered, questionnaire was used to obtain data on the presence and types of rodents in households, methods of control, and knowledge of potential human toxicity.

Results: One hundred and eight participants were interviewed. Thirty four participants (34/108, 31.6%) were in the age category of 31 to 40 years. Ninety six (88.9 %) stated that they had rodents in their households and used rodent control methods. The house rat and mouse were the rodents said to be present in households. Sixty nine participants (69/96, 71.9 %) responded that they used rodenticides, 24 (24/96, 25.0%) claimed the use of adhesive rat glue, and a few (3/96, 3.1 %) used both rodenticides and adhesive rat glue. Sixty one (61/69, 88.4%) stated that they threw dead rats into surface waste dumps or gutters, 2 (2/69, 2.9%) stated that they buried the dead rats. Rodenticides were left in corners of households without removal. Sixty (60/69, 87%) stated that they were aware of potential toxicity to humans. However, none stated that they were aware of any user instructions for appropriate use, prevention of harm, or appropriate disposal of rodenticides.

Conclusion: Majority of those interviewed had house rats and mice in their households. There was a high use of rodenticides with near zero compliance to safe practices. This has implications for public health, environmental and clinical toxicity.

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Key words: *Rodent, house rat, house mouse, rodenticides, toxicity*

Abstrait

Contexte: : Les études sur la situation des rongeurs et les méthodes de lutte contre les rongeurs dans les ménages au Nigéria sont rares. Cette étude pilote a examiné les types de rongeurs, les méthodes de contrôle et les connaissances des participants sur les toxicités cliniques potentielles des rodenticides utilisés.

Méthodes : Une étude transversale qui impliquait une interview aléatoire de participants âgés de 10 ans et plus dans les marchés urbains le long des routes menant à l'Université d'Ibadan, au Nigéria. Un questionnaire structuré administré par un intervieweur a été utilisé pour obtenir des données sur la présence et les types de rongeurs dans les ménages, les méthodes de contrôle et la connaissance de la toxicité humaine potentielle.

Résultats : Cent huit participants ont été interrogés. Trente-quatre participants (34/108 ; 31,6%) étaient dans la catégorie d'âge de 31 à 40 ans. Quatre-vingt-seize (88,9%) ont déclaré avoir des rongeurs dans leur foyer et utiliser des méthodes de contrôle des rongeurs. Le rat et la souris domestique étaient les rongeurs dit à être présents dans les ménages. Soixante-neuf participants (69/96 ; 71,9%) ont répondu qu'ils utilisaient des rodenticides, 24 (24/96 ; 25,0%) ont déclaré utiliser de la colle adhésive pour rats, et quelques-uns (3/96 ; 3,1%) ont utilisé à la fois des rodenticides et de la colle adhésive de rat. Soixante et un (61/69, 88,4%) ont déclaré avoir jeté des rats morts dans des poubelles de surface ou des caniveaux, 2 (2/69 ; 2,9%) ont déclaré avoir enterré les rats morts. Les rodenticides ont été laissés dans les coins des ménages sans être enlevés. Soixante (60/69 ; 87%) ont déclaré être conscients de la toxicité potentielle pour les humains. Cependant, aucun n'a déclaré connaître les instructions d'utilisation appropriées, la prévention des dommages ou l'élimination appropriée des rodenticides.

Conclusion : La majorité des personnes interrogées avaient des rats et souris domestiques dans leur

ménage. Il y avait une utilisation élevée de rodenticides avec une conformité presque nulle aux pratiques sécuritaires. Cela a des implications pour la santé publique, la toxicité environnementale et clinique.

Mots clés: *rongeur, rat domestique, souris domestique, rodenticides, toxicité*

Introduction

The control of rodents in households and farms in many countries of the world is a challenging venture. Rodents consume farm products, contaminate drinking water, and are known vectors of certain infectious diseases. Rodents are mammalian species characterized by a pair of broad, sharp-edged incisor teeth firmly inserted in both jaws. There are about 400 genera and over 2,000 species of rodents in the world [1, 2]; these include the squirrel, guinea pig, chipmunk, jerbea, and rat. The *Rattus* rodent (among which is the house rat) is known to live in human communities and households worldwide. Left unchecked the house rat can multiply to large numbers and cause significant economic losses and health hazards.

Rodents are reservoirs and hosts for several zoonotic diseases such as plague, leptospirosis, and leishmaniasis. In a study conducted at Iran in 2018, rodents found to be involved in disease transmission were *Rattus norvegicus, Mus musculus, Rattus rattus, Meriones persicus, Apodemus spp., Tatera indica, Meriones libycus, Rhombomys opimus, Cricetulus migratorius, and Nesokia indica* [3]. Studies have also shown that increase in outdoor rodent populations have resulted in outbreaks of disease epidemics [4]. Climate and vectors borne??? diseases in relation with the rise in prevalence of old and emerging infectious diseases have been reported from different countries [5]. In addition to zoonotic diseases transmitted by rodents, methods of rodent control may pose additional hazards to humans. In a retrospective study conducted in Hong Kong, occult exposure to anticoagulant-type rodenticides accounted for a worrisome proportion of hospital cases presenting with unexplained coagulopathy [6]. These and other negative impacts of uncontrolled rodent populations have resulted in the development of different methods of rodent control.

The process of control of rodent populations has continued to evolve. Methods of control include the use of ingenious techniques such as traps, glues, and rodenticides (commonly known as rat poison in some countries). Effective preservation of public health, with respect to zoonotic diseases of rodents and potential hazards of methods of control, requires the availability of reliable data on methods, potential hazards, knowledge, and practices of those exposed to rodents and to methods of rodent control. In Nigeria, where rodenticides and other methods of rodent control are widely used, publications on the pattern of use, knowledge, attitudes, and methods used are scarce. The absence of adequate research on the interface of rodents and human communities poses a limitation to effective control of rodents and rodent-borne zoonotic diseases. With recurrent outbreaks of emerging viral hemorrhagic diseases such as Lassa fever [7], there is a need for studies on how rodents are controlled and to what extent human populations are exposed to hazards of rodent-borne zoonotic diseases and of methods of control. The research reported here examined the types of rodents in homes of an urban area in Ibadan city, methods of their control, and the knowledge of potential clinical toxicities of chemical rodenticides which are widely used by the populace.

Methods

Study period and site

The study was carried out between the months of January to July 2018. It was conducted at two urban areas in Ibadan metropolis (Bodija market and Agbowo, opposite the University of Ibadan, Oyo state, Nigeria. Participants were traders and those making purchases at the selected urban areas. The study was a cross-sectional study that involved random selection and interviews of participants.

Inclusion and exclusion criteria

Participants were interviewed if they were aged 10 years and above, accepted to voluntarily participate in the study, gender either male or female, and could understand the study requirements and communicate with the study team. Those who refused to participate or could not communicate with the study team were not included in the study.

Study questionnaire

A structured, interviewer-administered, questionnaire was used to minimize variation in information requested. Aspects of practices during the use of rodenticides assessed included age of participant (categorized into 10-20, 21-30, 31-40, 41-50, 51-60, 61-70, and >70 years), gender, occupation, presence and type of rodents in households, methods of rodent

control (rodenticides, also known as chemical rat poisons, adhesive traps for rodents, or other rat traps), types of rodenticides (locally made or imported), sources of control methods, appropriate knowledge of use of rodent control method, disposal of dead rodents, disposal of used rodent control method, and knowledge of potential human toxicity of stated rodent control methods. Types of rodents inquired of the participants included the common black house rat (*Rattus norvegicus*), white bush rat (*Rattus fuscipes*), cane rat (*Thyonomys gregorianus*), mouse (*Congosorex phillisorum*), African giant rat (*Cricetomys gambianus*), squirrel (*Xterus inauria*), and rabbit (*Oryctotagas cuniculus*). Control measures mentioned as options included use of rodenticides (locally made and imported brands), use of rat traps, and use of rat glue.

Administration of the questionnaire

Traders and buyers conducting businesses at the markets visited were approached and interviewed on the above-mentioned aspects. The description of rodents and types of rodents were explained to participants. Participants were asked to name and describe the kinds of rodents at their homes and offices. Rodent choices described are as mentioned above, under the study questionnaire. These are rodents found in southwest Nigeria. Interview questions were followed by described options and, where required, left open ended. Responses were written on apportioned spaces on the hard copies of the questionnaire. The interviews were conducted in English and/or local language (Yoruba) as appropriate.

Sample size calculation

Literature search conducted during the planning stage of the study did not yield similar studies with reported proportions that could be used for sample size calculation. In the absence of a reported proportion, we decided to conduct this pilot study to determine the proportion of the selected population with rodents in their homes and requiring rodent controlled measures. The project targeted a sample size of 100 participants aimed at providing preliminary proportions that will aid workers in the estimation of sample size for similar studies in the future.

Data management and analysis

Responses were entered unto a spreadsheet and analysed with SPPS version 17. Categorical data was summarized as simple proportions; continuous data was summarized as means and standard deviations.

Ethical considerations

Permission was sought from potential participants before the interview was conducted. Participants were assured of confidentiality of the findings and identifiers traceable to them were not requested during the interview. Authors had no conflict of interest to declare.

Results

Table 1: Age distribution of all participants interviewed during the study

Age category (years)	Frequency (n/N)	%
10 – 20	6/108	5.6
21 – 30	26/108	24.1
31 – 40	34/108	31.6
41 – 50	21/108	19.4
51 – 60	13/108	12
61 – 70	6/108	5.6
> 70	2/108	1.9

Table 2: Occupational status of the study participants at the period of the study

Occupation	Proportion of participants (%) N = 108
Petty trading	54.9
Artisans and manual workers	25
Undergraduate students	5.6
Not employed	9
Did not disclose occupation	5.5

Demographic details and occupation of study participants

One hundred and eight participants were interviewed during the study. Forty six of them (46/108, 42.6%) were males while 62 were females 62/108, 57.4%). Thirty four participants (34/108, 31.6%) were in the age category of 31 to 40 years. The age distribution is shown in table 1. Fifty-six (56/102, 54.9%) of the respondents said that they were petty traders while the remaining (46/102, 45.1%) said they were engaged in other businesses. Six of the participants did not disclose their occupation. Table 2 is a representation of the various occupations of the study participants.

Presence of rodents and control measures

One hundred and eight (108) participants were interviewed during the study, 96 (96/108, 88.9 %), (Figure 1) of the respondents stated that they had rodents in their households. Of all the rodents mentioned and described to the participants, the house rodenticides (chemical rat poisons) while 24 participants (24/96, 25.0%) claimed the use of adhesive rat glue, while a few of them (3/96, 3.1 %) use both rodenticides and adhesive rat glue. The other proportion of the participants (12/108, 11.1 %) stated that they did not have rodents in their homes and did not use any rodent

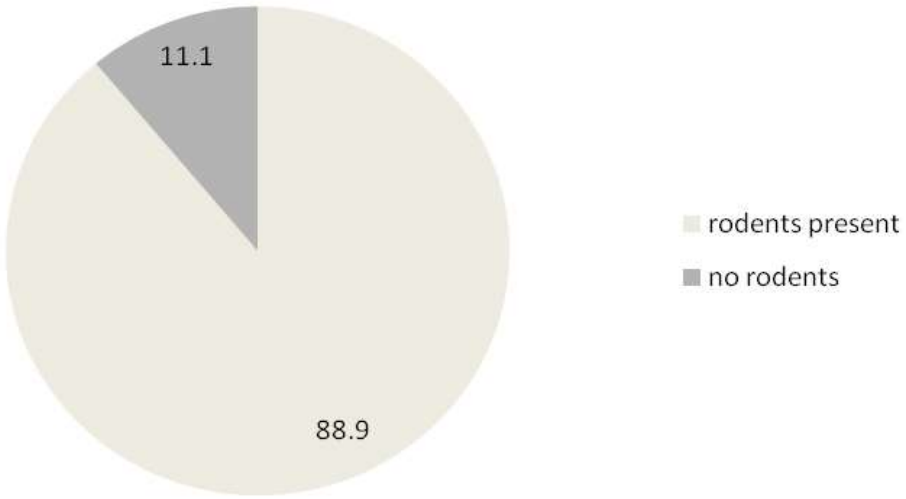

Fig.1: Proportion (%, N = 108) of study participants with rodents (house rat) present in their homes

rat and mouse were the rodents chosen as present in their households requiring repeated control measures. All ninety-six participants (96/108, 88.9%) responded that they used a method of rodent control. Sixty nine participants, (69/96, 71.9 %) responded that they used control measure. Of the 69 participants who responded that they used chemical rodenticides, the majority (50/69, 72.5%) stated that they used locally made brands. The result of types of rodenticides (local/imported types) used is shown in figure 2. When asked of their opinion

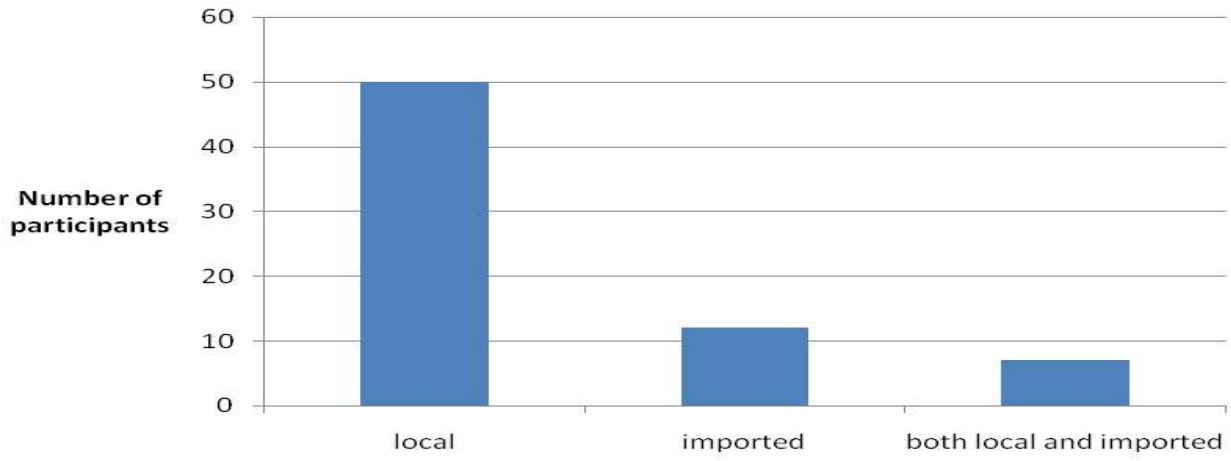

Fig. 2: Types of chemical rodenticides (local, imported, and use of both types) by number of study participants.

on which type had superior efficacy, 34 (34/69, 49.37%) participants provided no answer, 23 (23/69, 33.32%) stated that locally made rodenticides were more effective, four (4/69, 5.8%) stated that imported types were more effective, and eight (8/69, 11.6%) stated that both types were equal in effectiveness. All participants stated that they purchased rodenticides from vendors at market places or roadsides close to their households or at markets places.

Use of Rodenticides and disposal of dead rodents

All study participants who used rodenticides stated that the rodenticides were placed on the ground by the corners of the house or outside homes. No participant stated that they read or knew of any guidelines of use before application of rodenticides. Participants had no plans for removal of rodenticides placed on surfaces or baits. All participants stated that the chemicals were left indefinitely after placement until swept away accidentally or washed away by rain water. Majority of those that use rodenticides (39/69, 56.5%) stated that the offensive odour of dead rats was a feature they considered a major unpleasant challenge with the use of rodenticides. All participants who used adhesive rat glue stated that the method eliminated the offensive odour of dead rats, as the dead rats are trapped in the glue and can be removed and disposed before decaying. Two methods were selected as the means of disposal of dead rats; throwing them into rubbish bins and surface waste dumps or gutters, and/or burying the dead rats. Sixty one participants (61/69, 88.4%) stated that they threw dead rats into rubbish bins and surface waste dumps, or gutters, two participants (2/69, 2.9%) stated that they buried the dead rats. Six participants (6/69, 8.7%) could not provide answers to how they disposed of the dead rodents. One participant stated that his dog died from consuming a poisoned rat. There was no other claim of death of domestic animals from incidental or accidental contact with chemical rodenticides or from ingestion of dead poisoned rats.

Knowledge of potential human toxicity

Sixty-nine (69) participants stated that they used chemical rodenticides for rat control, sixty (60/69, 87%) stated that they were aware of potential toxicity to humans. Nine (9/69, 13%) participants stated that they were not aware that chemical rodenticides posed any potential harm to humans. When asked to mention possible ways that chemical rodenticides could come into contact with humans causing harm, 57 (57/69, 82.6%) stated that the chemicals could cause harm if they contaminate food and water. Twelve participants (12/69, 17.4%) stated that they were not aware of any route through which chemical rodenticides could cause any harm to humans. On whether they were aware of the possibility of infection transmission through rat urine contaminating food and water, 52 (52/69, 75.4%) said they were aware, while 17 (17/69, 24.6%) were not aware that rat urine could be a source of infections. When asked to mention practices that could prevent potential harm from use of chemical rodenticides, sixty-seven (67/69, 97.1%) participants stated that they were not aware of any action that could prevent harm; one (1/69, 1.4%) participant stated that good hygiene and covering food and water during use of chemical rodenticides are preventive. One (1/69, 1.4%) other participant stated that there was no need to apply any preventive measure. None of the participants stated that they were aware of any user instructions for appropriate use, prevention of harm to user, or appropriate disposal of chemical rodenticides.

Discussion

The study revealed that the majority of those interviewed (88.9%) stated that they had a challenge of house rats in their households. There was also a correspondingly high use of chemical rodenticides in the population with a worrisome poor compliance to safe practices. While majority of the population studied was aware of the potential for human toxicity that may occur from exposure to and wrong use of chemical rodenticides, only one participant could mention a method of preventing harmful exposure or contact with the chemicals. These findings have significant public health impact as chemical rodenticides have been reported as poisonous to humans, have been used by depressed people in suicidal attempts, and on account of recurrent sporadic and epidemic outbreaks of Lassa virus infection in Nigeria.

Rodents have a worldwide distribution and are known to have the capacity to affect the existence of other species negatively [8]. Their connections to zoonotic diseases and destruction of agricultural products make them a group of mammals with significant impact on the health and economic outcomes of many nations. Despite the widespread presence of rodents in many countries, the prevalence of rodents in households in communities within sub-Saharan Africa remains largely unknown. Our study revealed that about 88.9% of households of those

interviewed had a rodent problem. The participants stated that rodents found in the households where the house rat and house mice. There was no differentiation between the rat and mouse species during the study. Other rodents were not mentioned as being present in the households. Rats and mice are known vectors of zoonotic diseases and can contaminate food and water with their urine, faeces, and saliva [9]. The high prevalence of rats and mice in household poses risks of disease transmission, thus the widespread availability of rodenticides in the community.

Chemical rodenticides were the commonest method of rodent control in the study. Seventy five percent (75%, including the 3.1% that use both adhesive glue and chemical rodenticides) of the study participants stated that they used chemical rodenticides to control household rats and mice. This was followed by the use of rat adhesive glue by 25% of the participants interviewed. It is noteworthy that the use of metal traps or cats as methods of rodent control was not mentioned by any of the study participants. According to those interviewed for the study, chemical rodenticides are available on sale at markets and road sides. While study participants held varied views on efficacies of the chemicals in rodent control, these claims will require further objective evaluation. The study participants were ignorant of safe practices when using chemical rodenticides. The dead rodents were thrown onto surface rubbish bins which may be sources of environmental contamination. The placement of rodenticides on surfaces amenable to rain water, which can wash them into underground sources of water, may pose hazardous risks to human populations in the community. In Nigeria, as in many regions in sub-Saharan Africa, water for drinking and cooking is obtained mainly from wells and boreholes [10-12]. Studies have shown that drinking water contaminated with heavy metals is sold as sachet water available on sale in cities [13, 14]. It remains to be evaluated if residual chemical components of rodenticides are also present in available sources of water for domestic use in cities with high use of chemical rodenticides.

Environmental contamination by indiscriminate use of chemical rodenticides is worrisome, as their continuous use may have cumulative effects that are yet to be determined in communities in sub-Saharan Africa. Studies from advanced countries have attributed poisoning of some wildlife and domestic animals to incidental and accidental contact with chemical components of rodenticides. Romano and co-authors in 2018 published a report of fatal bromethalin intoxication in 3 cats and 2 dogs from accidental ingestion [15]. Primary and secondary poisoning on non-target animals in Spain was reported by Sanchez-Babudo and co-authors in 2012 [16]. The authors recommended that the use of accumulative second generation anticoagulant rodenticides and their application on baits should be discontinued. Lin J and co-authors in 2015 published results that showed the primary toxic effects of bromodiolone (a second generation anticoagulant rodenticides used for field control of rodents) on earthworms [17]. They proposed that the poisoned worms may be a source of secondary poisoning to insectivores and scavengers. The widespread use of rodenticides in Ibadan (and very likely all over Nigeria) ought to raise concerns about potential environmental contamination, poisoning of animals, and human toxicity.

Fifty-seven (82.6%) of those interviewed during our study (reported in this paper) stated that they were aware of potential clinical toxicity of the rodenticides they used in their households. However, sixty-seven (97.1%) of the participants who use chemical rodenticides for control of rodents stated that they were unaware of measures that may prevent harm to users and members of the household. This is indicative of significant ignorance of practices that can ensure safe use of rodenticides. This gap in knowledge requires intervention by all stakeholders to safeguard environmental, animal, and human health. Reports of accidental and intentional exposure to rodenticides with accompanying harm to humans are not uncommon [18, 19]

Wu li *et al* in 2012 reported a misdiagnosis of ectopic pregnancy in a patient who turned out to be bleeding on account of rodenticides poisoning [20]. Paradoxical thrombosis and haemorrhage in a 48 year old woman on account of exposure to anticoagulant rodenticides was reported by Franco, Everett, and Manoucher in case report published in 2013 [21]. Fatalities from exposure to anticoagulant and thallium-based rodenticides have been and continue to be reported by many workers globally [22,23]. Okeniyi and Lawal in 2007 reported a fatal accidental ingestion of a locally made rodenticide by a three year old child on account of improper storage of the rodenticide [24]. Reports have suggested that children are the most at risk of rodenticides poisoning [25] and recommendations for risk reduction include focusing

on packaging and positioning of rodenticides baits to prevent accidental ingestion by children, product reformulation, and the distribution of management guidelines for health workers and users [25]. These and other relevant measures aimed at risk reduction are urgently needed in our communities where there is a high use of rodenticides. Further studies may reveal the need to place restrictions on availability and access to highly poisonous rodenticides.

Related to the high prevalence of rodents in households in Ibadan, and likely other parts of the country, is the occurrence of sporadic and epidemic outbreaks of Lassa fever virus infection in Nigeria [26, 27]. Lassa fever is an acute viral zoonotic illness caused by Lassa virus, an arenavirus known to be responsible for a severe haemorrhagic fever characterised by fever, muscle aches, sore throat, nausea, vomiting, chest, and abdominal pain. Some studies indicate that 300,000 to 500,000 cases of Lassa fever and 5000 deaths occur yearly across West Africa and that the scarcity of resources available for health care delivery and the political instability that characterise the West African countries would continue to impede efforts for the control of Lassa fever in the sub-region [28]. Reports from Nigeria have implicated rodents in sporadic and epidemic cases of Lassa fever disease outbreaks with evidence that other rodents other than the natal multimammate rat (*Mastomys natalensis*) may be vectors of the disease [29-31]. There is thus a need for epidemiologic studies of the prevalence, types, infectivity of rodents in households and methods of rodent control to enhance efforts towards the effective control of Lassa virus disease in Nigeria.

In conclusion, the study showed that the prevalence of rodents (house rats and mice) in households of the population interviewed was high. There was also a high use of chemical rodenticides in the control of house rats in the households of study participants. The use of rodenticides was unregulated with disposal of poisoned rats into bushes and surface rubbish dumps. While a good proportion of study participants were aware of potential human toxicity of rodenticides, there was poor knowledge of measures aimed at reducing the risks of harm to humans from the use of rodenticides. There was also a complete absence of safe practices. The pilot study was limited by small sample size and coverage of a small area of the city. There is a need to extend the study to other urban and rural areas for a more comprehensive evaluation.

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References

1. Alderton D. Rodents of the world: Blandford; 1996.
2. Delany M. The biology of small rodents in Mayanja Forest, Uganda. Journal of Zoology. 1971;165(1):85-129.
3. Rabiee MH, Mahmoudi A, Siahsarvie R, Krystufek B and Mostafavi E. Rodent-borne diseases and their public health importance in Iran. PLoS Negl Trop Dis. 2018;12(4):e0006256.
4. Diaz JH. Rodent-borne infectious disease outbreaks after flooding disasters: Epidemiology, management, and prevention. J Emerg Manag. 2015;13(5):459-467.
5. Githeko AK, Lindsay SW, Confalonieri UE and Patz JA. Climate change and vector-borne diseases: a regional analysis. Bulletin of the World Health Organization. 2000;78:1136-1147.
6. Ng WY, Ching CK, Chong YK, *et al*. Retrospective Study of the Characteristics of Anticoagulant-Type Rodenticide Poisoning in Hong Kong. J Med Toxicol. 2018;14(3):218-228.
7. Akhuemokhan OC, Ewah-Odiase RO, Akpede N, *et al*. Prevalence of Lassa Virus Disease (LVD) in Nigerian children with fever or fever and convulsions in an endemic area. PLoS Negl Trop Dis. 2017;11(7):e0005711.
8. Amori G and Clout M. Rodents on islands: a conservation challenge. ACIAR MONOGRAPH SERIES. 2003;96:63-68.
9. Meerburg BG, Singleton GR and Kijlstra A. Rodent-borne diseases and their risks for public health. Critical reviews in microbiology. 2009;35(3):221-270.
10. Bello O, Osho A, Bankole S and Bello T. Bacteriological and physicochemical analyses of borehole and well water sources in Ijebu-Ode, Southwestern Nigeria. Int J Pharm Biol Sci. 2013;8:18-25.
11. Itama E, Olaseha I and Sridhar M. Springs as supplementary potable water supplies for inner city populations: a study from Ibadan, Nigeria. Urban Water Journal. 2006;3(4):215-23.

12. Fayiga AO, Ipinmoroti MO and Chirenje T. Environmental pollution in Africa. Environment, Development and Sustainability. 2018;20(1):41-73.
13. Orisakwe OE, Igwilo IO, Afonne OJ, *et al.* Heavy metal hazards of sachet water in Nigeria. Arch Environ Occup Health. 2006;61(5):209-213.
14. Mayomi I and Elisha I. Water quality analysis of the commercial boreholes in Mubi Metropolis, Adamawa State, Nigeria: geographic information system approach. East Afr J Public Health. 2011;8(4):263-270.
15. Romano MC, Loynachan AT, Bolin DC, *et al*. Fatal bromethalin intoxication in 3 cats and 2 dogs with minimal or no histologic central nervous system spongiform change. J Vet Diagn Invest. 2018;30(4):642-645.
16. Sanchez-Barbudo IS, Camarero PR and Mateo R. Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. Sci Total Environ. 2012;420:280-8.
17. Liu J, Xiong K, Ye X, *et al*. Toxicity and bioaccumulation of bromadiolone to earthworm Eisenia fetida. Chemosphere. 2015;135:250-256.
18. Yogendranathan N, Herath H, Sivasundaram T, Constantine R and Kulatunga A. A case report of zinc phosphide poisoning: complicated by acute renal failure and tubulo interstitial nephritis. BMC Pharmacol Toxicol. 2017;18(1):37.
19. Sanchez-Villegas MC and Barcena-Ruiz A. [Zinc phosphide poisoning in pediatric patients from a Toxicology Center at Mexico City]. Rev Med Inst Mex Seguro Soc. 2017;55 Suppl 1:S44-S52.
20. Wu L, Lu X, Yin R and Lu D. Misdiagnosis of rodenticide poisoning as ectopic pregnancy: a case report. Eur J Obstet Gynecol Reprod Biol. 2012;163(1):120-121.
21. Franco D, Everett G and Manoucheri M. I smell a rat: a case report and literature review of paradoxical thrombosis and hemorrhage in a patient with brodifacoum toxicity. Blood Coagul Fibrinolysis. 2013;24(2):202-204.
22. Riyaz R, Pandalai SL, Schwartz M and Kazzi ZN. A fatal case of thallium toxicity: challenges in management. J Med Toxicol. 2013;9(1):75-78.
23. Card DJ, Francis S, Deuchande K and Harrington DJ. Superwarfarin poisoning and its management. BMJ Case Rep. 2014;2014.
24. Okeniyi J and Lawal O. Accidental Poisoning with Otapiapia: a Local Organophasphate-Containing Rodenticide: A Case Report. Nigerian Medical Practitioner. 2007;52(4):100-101.
25. Parsons BJ, Day LM, Ozanne-Smith J and Dobbin M. Rodenticide poisoning among children. Aust N Z J Public Health. 1996;20(5):488-492.
26. Roberts L. Nigeria hit by unprecedented Lassa fever outbreak. Science. 2018;359(6381):1201-1202.
27. Akhiwu HO, Yiltok ES, Ebonyi AO, *et al.* Lassa fever outbreak in adolescents in North Central Nigeria: report of cases. J Virus Erad. 2018;4(4):225-227.
28. Ogbu O, Ajuluchukwu E and Uneke CJ. Lassa fever in West African sub-region: an overview. J Vector Borne Dis. 2007;44(1):1-11.
29. Siddle KJ, Eromon P, Barnes KG, *et al.* Genomic Analysis of Lassa Virus during an Increase in Cases in Nigeria in 2018. N Engl J Med. 2018;379(18):1745-1753.
30. Agbonlahor DE, Erah A, Agba IM, *et al*. Prevalence of Lassa virus among rodents trapped in three South-South States of Nigeria. J Vector Borne Dis. 2017;54(2):146-150.
31. Olayemi A, Cadar D, Magassouba N, *et al.* New Hosts of The Lassa Virus. Sci Rep. 2016;6:25280.

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Unripe *mangifera indica* pulp and rind: novel therapy for diabetic wounds in rat.

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Abstract
*Background:Mangifera indica*is a naturally occurring plant having many medicinal and health values attributable to its fruit (mango). This study investigated the wound healing benefits of the pulp and rind of the unripe mango fruit in diabetic wistar rats.
Methods:The excisional wounds of twenty four diabetic rats in four equal groups were dressed respectively with pulp extract, rind extract,sofratulle and normal saline till healed.Specific wound contraction rates were derived from the dimensions of the wounds. Granulation tissue biopsies and the matured scars were processed for histological evaluation.
Results: The specific wound contraction rates of the extract groups compared favourably with those of the sofratulle and normal saline. Both granulation tissues and scars of extracts and control had similar histology.
Conclusion: This study showed that the extract of demonstrated that the extracts of unripe *Mangifera indica* pulp and rind when used as wound dressing materials had the same end point comparable to those of sofratulle and normal saline in diabetic wistar rats. Hence they may serve as substitutes to sofratulle in the management of wounds with diabetic co-morbidity.

Keywords: *Diabetes, Wound healing, Unripe Mangifera indica pulp and rind*

Abstrait
Contexte: Mangifera indica est une plante naturelle ayant de nombreuses valeurs médicinales et sanitaires attribuables à son fruit (mangue). Cette étude a examiné les avantages de la pulpe et la peau de la mangue non mûre dans la cicatrisation des blessures chez des rats diabétiques Wistar.
Méthodes : Les plaies excisionnelles de vingt-quatre rats diabétiques en quatre groupes égaux ont été habillées respectivement avec un extrait de pulpe, un extrait de la peau, du sofratulle et une solution saline normale jusqu'à guérison. Les taux de contraction des plaies spécifiques ont été dérivés des dimensions des plaies. Les biopsies de tissu de granulation et les cicatrices mûres ont été traitées pour une évaluation histologique.
Résultats : Les taux spécifiques de contraction des plaies dans les groupes d'extraits se comparaient favorablement à ceux du sofratulle et de la solution saline normale. Le tissu de granulation et les cicatrices des extraits et du contrôle ont une histologie similaire.
Conclusion : Cette étude a ainsi démontré que les extraits de pulpe et de peau de *Mangifera indica* non mûres en tant que matériel de pansement avaient le même point final comparable à ceux du sofratulle et de la solution saline normale chez les rats iabétiques wistar. Par conséquent, ils peuvent servir de substituts au sofratulle dans la prise en charge des plaies avec comorbidité diabétique.

Mots clés: *Diabète, cicatrisation des plaies, pulpe et peau de Mangifera indica non mûres*

Introduction

The World Health Organization Global Report on diabetes put the world prevalence rate of diabetes amongst adults at 8.7 % which translated to 422 million. Also about 3.7 million adults world-wide were reported to have died from diabetes and its complications in 2012 [1].

Diabetes mellitus is a systemic chronic disease with multifaceted complications such as foot ulcerations which may ultimately be an indication foramputation. Diabetic foot ulcer is a chronic wound that requires dressing for a considerable period and may invariably need surgical intervention such as skin grafting or amputation. An appropriate dressing material should promote healing with resultant reduction in exudate and odour, alleviate pain and prevent infection. Though dressing materials that meet these criteria are commercially available, cost and affordability remain major determinants in the extent of their utilisation.

Plants are of immense benefits in the management of wounds and they induce healing via multiple mechanisms. The medicinal value of plants is inherent in the bioactive phytochemical constituents that produce definite physiological actions[2].Such

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constituents include alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds [3]. *Mangifera indica L.* (mango) is a juicy stone fruit of the Anacardiaceae family in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. Mango fruits are an important source of micronutrients, vitamins and other phytochemicals[4].Moreover, they provide energy, dietary fibre, carbohydrates, proteins, fats and phenolic compounds [5], which are vital to normal human growth and development [6].*Mangifera indica* rind has pigments of high biological potency; such asprovitamin A, beta-carotene, lutein and alphacarotene [7].Diabetic wound is an enigmatic and debilitating complication and poses a serious challenge in the clinical practice. Although, there are several studies on the medicinal values of*Mangifera indica* pulp and rind, those on wound healing in diabetics are scrimpy.Thus, this study investigated the wound healing activities of unripe *Mangifera indica* pulp and rind in excisional wounds of Wistar rats with induced diabetes mellitus.

Materials and methods

Plant materials

Unripe *Mangifera indica* fruits were harvested from mango trees within the premises ofUniversity of Ibadan. Identification and authentication of the fruits were done at the Botany Department, University of Ibadan with voucher number UIH - 226608. The fruits were washed, peeled and sliced while the seeds were discarded. The sliced fruit (pulp) and rind were dried separately at room temperature with good natural sunlight illumination till the moisture content was nil. These were subsequently and separately milled into powdery texture.

Preparation of extracts

Six hundred grammes (600 g) and 550 g of the powdered pulp and rind respectively were used for the ethanolic extractions with 100 % ethanol. The resultant extracts were in gel form and the pulp yield was 11.70 % and that of the rind being 14.98 %. They were stored under optimal conditions till use.

Phytochemical analyses

The qualitative and quantitative analyses of the pulp and rind extracts for saponins, tannins, cardiac glycosides, flavonoids, terpenoids, steroids, antraquinones, and alkaloids were carried out with the methods previously described by others [8-11].

Animals

Forty eight adult female wistar rats weighing 150-230 g procured from the Central Animal House, University of Ibadan were used for the study. They had an initial two weeks of preconditioning under controlled environmental temperature, humidity and adequate aeration and illumination were ensured. They had pelletized feed and water.

Induction of diabetes

Diabetes mellitus was induced by the technique previously described by Ajani and Ogunbiyi. [12].The pre induction fasting blood sugar levels were estimated with single touch glucometer (ACCU-CHECK®, Roche Diagnostics, Germany) using the blood obtained from the tails of the rats and a blood sugar level of 55-75 mg/dl was considered normal. A single dose of 100 mg/kg body weight of alloxan monohydrate dissolved in normal saline and administered intraperitoneally was used to induce diabetes mellitus. Forty eight hour post-induction blood sugar levels above 200 mg/dl were considered diabetic.

Design of the Experiment

Using induction of diabetes mellitus and dressing materials as criteria for group allotment, the animals were allotted into eight groups of six each. These were mango pulp control (MPC), mango rind control(MRC), sofratulle control (SFC), normal saline control (NSC), mango pulp diabetic (MPD), mango rind diabetic (MRD), sofratulle diabetic (SFD) and normal saline diabetic (NSD) were created.

Wound creation and management

In sequence, each rat was placed on a flat board after sedation with intramuscular ketamine hydrochloride (120 mg/kg). The skin over the dorsal thoracic region was cleansed with salvon antiseptic liquid. A 2cm by 2cm full thickness skin about 1.5 cm from the vertebral column was excised. Unripe mango pulp extract, unripe mango rind extract, sofratulle and normal saline were used as wound dressing materials for the respective group (MPC, MPD; MRC, MRD; STC, STD; NSC, NSD). The wounds were dressed daily till the scab dropped off which indicated healing. Prior to change of dressing, wound size estimation was done by taking measurement along two perpendicular axes every three days.

On day 3, granulation tissue was harvested from one animal from each group for the purpose of histological examination using Heamatoxylin and Eosin

(H&E). This procedure was repeated in all the groups on day 6 and 9. The processed granulation tissues were used for evaluation of healing in terms of cellularity, angiogenesis, fibroplasia and collagensynthesis.

The removal of granulation tissue was the end point for such animal. The wounds of remaining animals in all the groups were allowed to heal and the scars were similarly processed for light microscopy. Falling off of the scab without any residual wound indicated the endpoint of complete epithelization and the days required for this connoted the duration of healing.

Data analysis and processing

The numerical aspects of the results were analysed with Graph Pad Prism version 5 and expressed as percentages, means plus standard deviation of means (SD). The student t- test was used for inter group comparison with significance level set at < 0.05

Results

Anthraquinones, terpenoids, alkaloids, flavonoids, tanins and saponinis were present in both the pulp and rind of unripe *M. indica* at about equal magnitude. However, steroids were detected in the rind but absent in the pulp. Both the pulp and the rind were lacking in cardiac glycosides (Table 1).

Table 1. Phytochemical analyses of unripe pulp and rind of *Mangiferaindica*

TEST	Anthra quinones		Terpenoids		Alkaloids		Flavinoids		Tanins		Cardiac glycosides		Saponins		Steroids	
	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind
Quali tative	+	+	+	+	++	+	++	+	++	++	-	-	++	++	+	-
Quantita tive (%)					3.8	5.98	44.6	25					4.5	4.25		

+ (scanty), ++ (moderate), +++ (abundant) and – (absent)

All the diabetic groups had weight loss of less than 6 % at 14-day post induction (3.3 % for NSD, 5.5 % for STD, 1.7% for MPD and 4.3 % for MRD) and

Table 2 Mean fasting blood sugar levels and mean body weight

Para meter	NSC	NSD	STC	STD	MPC	MPD	MRC	MRD
Pre induction sugar level (mg/dl)	NA	68.50	NA	82.00	NA	68.75	NA	60.60
Post induction sugar level (mg/dl)	NA	361.00	NA	317.75	NA	303.50	NA	311.75
Pre induction weight (g)	165.5±3.82	203.3±4.27	180.0±3.74	201.8±14.10	180.0±11.87	188.8±7.26	161.6±6.39	187.6±7.15
Post induction-14 days (g)	166.3±3.73	196.5±3.07	182.5±4.59	190.8±15.04	181.0±11.90	185.5±9.14	166.6±6.08	179.6±4.92
Post induction-28 days (g)	169.2±3.31	198.8±15.5	185.3±5.41	206.8±16.97	182.6±12.50	186.0±9.71	170.0±6.08	178.8±5.21

NA= Not applicable

Table3. Interval mean values of wound contraction rates in percentages (%)

Group	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30
NSC	8.30±3.05	32.72±6.19	61.53±7.03	78.98±3.02	90.88±3.78	93.08±2.93	97.40±0.45	99.01±0.37		
NSD	6.72±2.13	16.71±3.04	25.02±6.40	40.36±1.36	53.33±9.01	69.41±4.88	82.74±1.45	92.99±0.60	95.85±0.91	97.03±0.40
STC	17.60±2.87	29.63±4.66	43.94±7.84	60.71±4.26	83.07±2.75	93.94±1.32	97.22±0.76	*	*	
STD	1.54±2.77	9.65±5.39	23.98±1.85	38.09±0.72	38.01±10.79	62.57±5.53	76.19±6.78	90.67±1.98	97.30±0.47	99.01±0.37
MPC	8.30±2.58	21.17±4.14	30.95±8.65	33.04±11.73	49.24±9.01	58.60±7.92	81.6±4.35	92.83±2.10	98.26±0.26	*
MPD	3.47±0.98	11.00±1.51	19.24±3.82	33.57±1.99	51.64±4.61	62.48±3.83	73.00±2.83	84.19±2.23	92.41±1.58	96.99±0.03
MRC	13.73±2.69	36.47±6.12	62.15±5.36	83.45±5.51	90.47±3.27	95.98±0.43	98.51±0.29	*	*	*
MRD	6.51±2.96	27.63±4.89	47.23±8.27	58.78±9.89	68.91±9.82	81.51±8.83	92.80±2.26	97.76±0.61	99.45±0.62	*

the least weight loss was recorded amongst the MPD group. However, by 28-day post induction they all had recorded relative weight gain. While all the control groups gained weight during the periods under consideration (Table 2).

Wound sepsis and mortality were recorded in a small proportion of the diabetic groups

Table 4: Highest and lowest mean contraction values for specific period

Period	Contraction rates (%)
Day 3	17.60±2.87 (STC)[a**]1.54±2.77 (STD)
Day 6	36.47±6.12 (MRC)[b**]9.65±5.39 (STD)
Day 9	62.15±5.36 (MRC)[c**]19.68±12.07 (MPD)

Wound contraction rates

On day 3, the STC group had the overall highest mean wound contraction rate of 17.60±2.87 % while the STD had the least value of 1.54±2.77 %. Amongst the diabetic groups, the MRD rate was similar to that of NSD (6.51±2.96**:** 6.72±2.13%). Also on day 3, it was observed that the values for the STC, MPC and MRC groups were more than double their respective diabetic group (Table 3). On day 6, the highest rate of 36.47±6.12 % was by the MRC group and the lowest from the STD group (9.65±5.39 %). Only the NSC, STC and MPC had contraction rates that were double those of their respective diabetic group. The difference between the MRC and MRD groups was about 9 % (36.47±6.12 vs 27.63±4.8%). On day 9, both the NSC and MRC had the highest comparable rates of 62.15±5.36 and 61.53±7.03 % respectively while the least value (19.24±3.82%) was from the MPD group. For the diabetic subgroups, the highest day 9 value of 47.23±8.27% was from the MRD. For day 12, the MRC group still maintained the lead with 83.45±5.51% and the highest amongst the diabetic subgroups was the MRD (58.78±9.89). By day 15, the MRC, NSC and STC had mean wound contraction rates that were above 80 % while the MRD still maintained the lead (68.91±9.82 %) in the diabetic category.

The MRD group had a mean wound contraction rate of 81.51±8.83 % by day 18 while groups NSD and MPC achieved above 80 % mean contraction rate by day 21. It was noted that the wounds had healed in all the groups by day 24 (Table 3).

When the highest and lowest mean contraction rates were compared for specific periods; it was observed that STC had significantly higher value than STD on day 3, MRC higher than STD on day 6 and MRC than MPD on day 9 (Table 4).

Table 5: Intra and Inter group comparisons of wound mean contraction rates

Compared groups	Day 3 mean rates	Day 6 mean rates	Day 9 mean rates
MPD vs MRD	3.47±0.98	11.00±1.51[c**]	19.24±3.82[h**]
	6.51±2.96	27.63±4.89	47.23±8.27
MPD vs NSD	3.47±0.98	11.00±1.51	19.24±3.82
	6.72±2.13	16.71±3.04	25.02±6.40
MPD vs STD	3.47±0.98	11.00±1.51	19.24±3.82
	1.54±2.77	9.65±5.39	23.98±1.85
MRD vs NSD	6.51±2.96	27.63±4.89	47.23±8.27[j**]
	6.72±2.13	16.71±3.04	25.02±6.40
MRD vs STD	6.51±2.96	27.63±4.89**	47.23±8.27[j**]
	1.54±2.77	9.65±5.39	23.98±1.85
MPC vs MPD	8.30±2.58	21.17±4.14[e**]	30.95±8.65
	3.47±0.98	11.00±1.51	19.24±3.82
MRC vs MRD	13.73±2.69[a**]	36.47±6.12	62.15±5.36[l**]
	6.51±2.96	27.63±4.89	47.23±8.27
NSC vs NSD	8.30±3.05	32.72±6.19[f**]	61.53±7.03[m**]
	6.72±2.13	16.71±3.04	25.02±6.40
STC vs STD	17.60±2.87[b**]	29.63±4.66[g**]	43.94±7.84[n**]
	1.54±2.77	9.65±5.39	23.98±1.85

Intra and Inter group comparisons of wound mean contraction rates

On day 3, it was only the comparisons between (i) MRC and MRD and (ii) STC vs STD that showed significant differences. However, on day 6 MPD vs MRD; MRD vs STD; MPC vs MPD; NSC vs NSD and STC vs STD all showed significant differences. On day 9, MRD was significantly higher than MPD, MRD higher than NSD, MRD higher than STD, MRC higher than MRD, NSC higher than NSD and STC higher than STD (Table 5).

Granulation tissues and wound scars.

Sections of the granulation tissues in both MPC and MPD on day 3 showed intense inflammatory cellular infiltration of about equal magnitude. The rind groups also exhibited cellular infiltration but slightly more in the MRC group than the MRD. The cellular infiltration that characterised day 3 granulation tissues was of least intensity in the normal saline subgroups but moderate in the Sofratulle subgroups i.e. STC and STD (Plate 1). On day 6, the granulation tissues of MPC and MPD

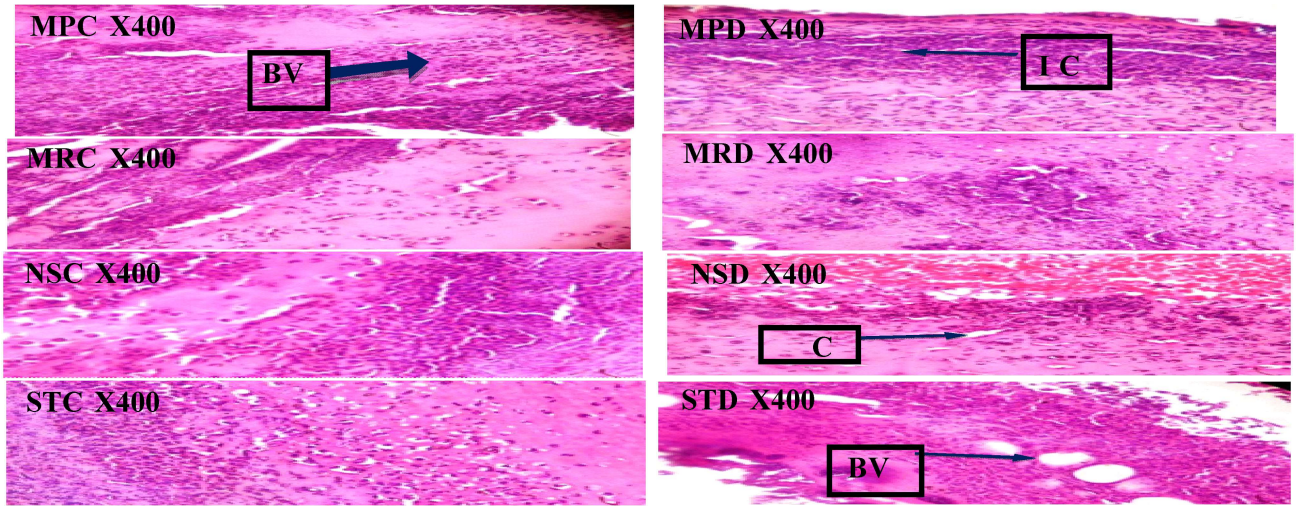

Plate 1: Granulation tissue at Day 3 (H & E).
MPC (Unripe mango pulp control), MPD (Unripe mango pulp diabetic), MRC (Unripe mango rind control), MRD (Unripe mango rind diabetic), NSC (Normal saline control) NSD (Normal saline diabetic), STC (Sofratulle control), STD (Sofratulle diabetic), BV (Blood vessel), C (Collagen) and IC (Inflammatory cells).

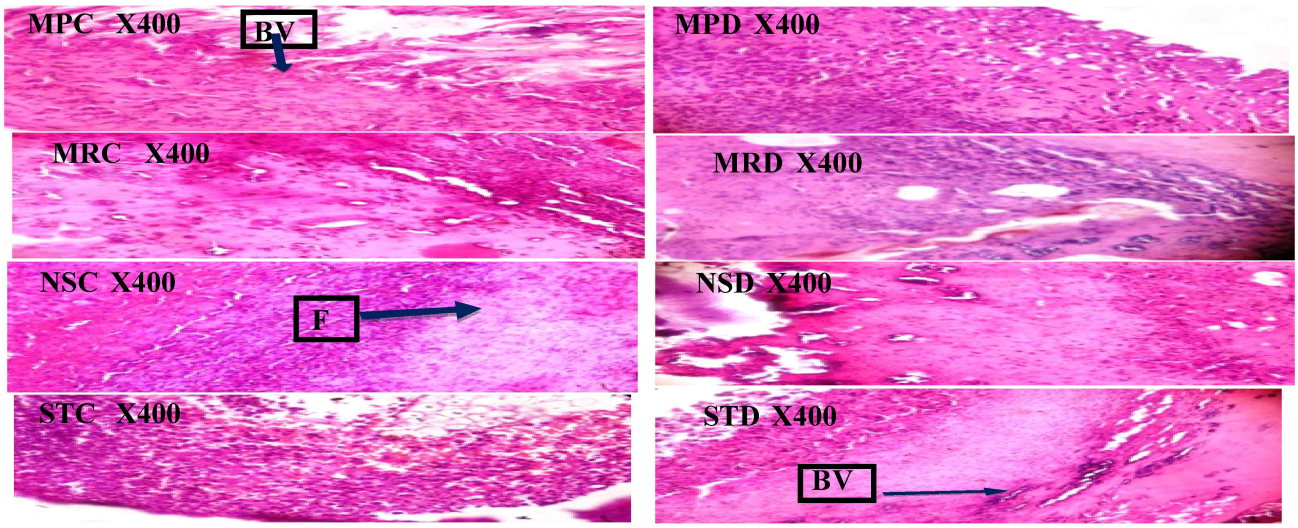

Plate 2.Granulation tissue at Day 6 (H & E).
MPC (Unripe mango pulp control), MPD (Unripe mango pulp diabetic), MRC (Unripe mango rind control), MRD (Unripe mango rind diabetic), NSC (Normal saline control) NSD (Normal saline diabetic), STC (Sofratulle control), STD (Sofratulle diabetic), BV (Blood vessel), C (Collagen) and F (Fibroblast).

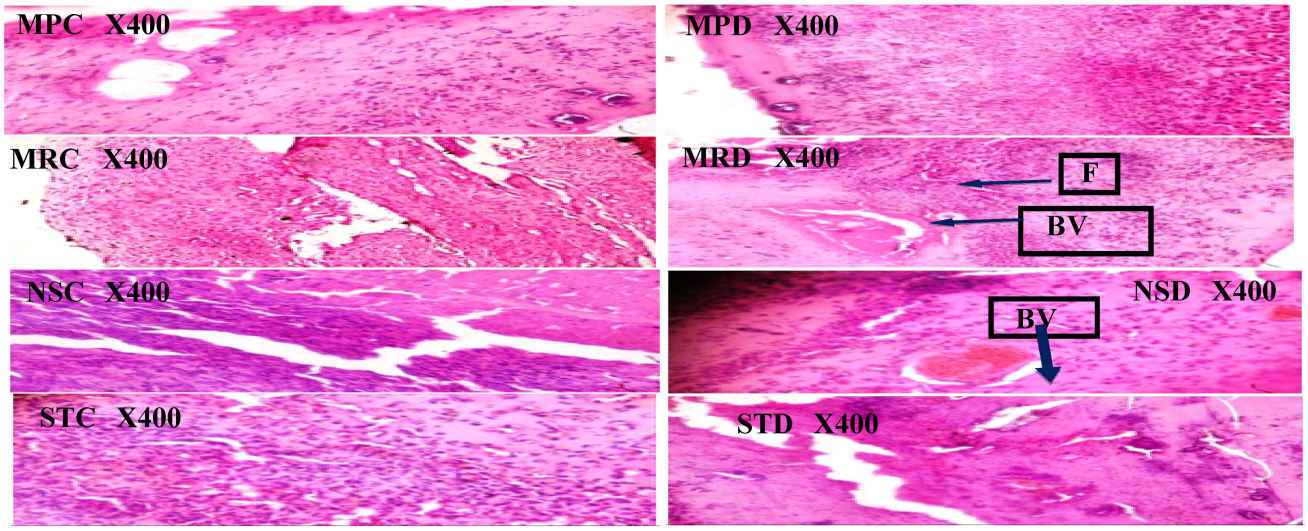

Plate 3: Granulation tissue at Day 9 (H & E).
MPC (Unripe mango pulp control), MPD (Unripe mango pulp diabetic), MRC (Unripe mango rind control), MRD (Unripe mango rind diabetic), NSC (Normal saline control) NSD (Normal saline diabetic), STC (Sofratulle control), STD (Sofratulle diabetic), BV (Blood vessel), C (Collagen) and F (Fibroblast).

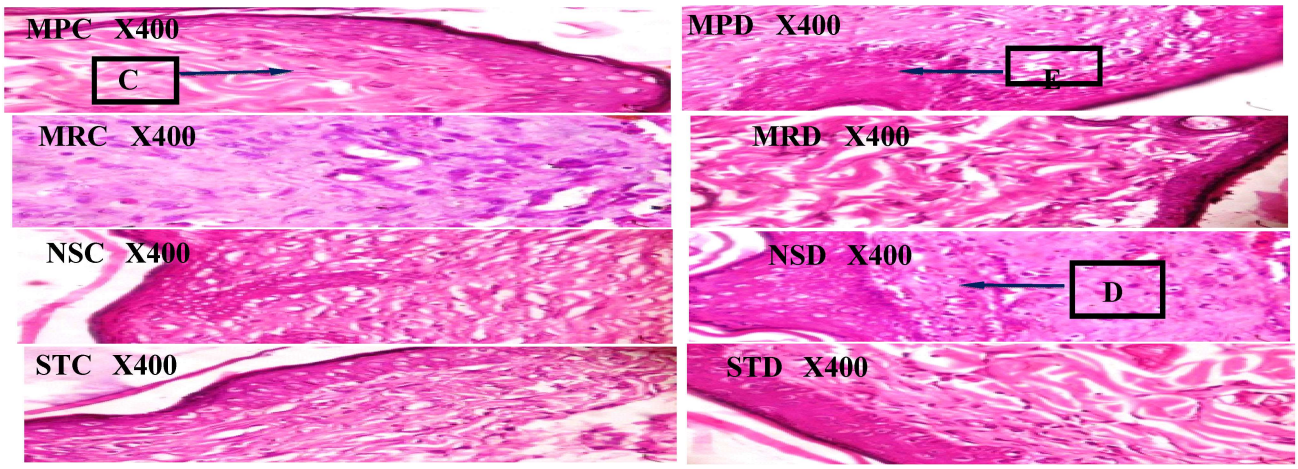

Plate 4.Sections from the wound scars (H & E).
MPC (Unripe mango pulp control), MPD (Unripe mango pulp diabetic), MRC (Unripe mango rind control), MRD (Unripe mango rind diabetic), NSC (Normal saline control) NSD (Normal saline diabetic), STC (Sofratulle control), STD (Sofratulle diabetic),C (Collagen), D (Dermis)and E (Epidermis).

still showed considerable cellular infiltration while this was of lesser magnitude in control groups (MPC and MRC). Fibrillary structures suggestive of collagen were seen in MPC. Single layered blood vessels suggestive of angiogenesis were visible all the subgroups (control and diabetic).Also noted on day 6 was moderate cellular infiltration in both NSC and STC groups (Plate 2)

On day 9, section of granulation tissue from the MPD still showed predominantly cellular infiltration while its control i.e. MRC showed vessels with wider diameter. In all the groups, fibroblasts and collagen were visible with the STC group having the highest cellular density (Plate 3). Sections obtained from the scars of all the groups showed well defined skin layers with abundance of collagen fibres and decreased cellularity when compared with those of the granulation tissues (Plate 4)

Following the induction of diabetes, the blood sugar levels were markedly elevated in all the four diabetic groups. The post induction blood sugar level

was significantly higher than the respective pre induction value ($P < 0.05$).

The asterisks connote that the wounds had healed. Amongst the mango groups, the rind control (MRC) was the first to achieve healing while the diabetic groups had delayed healing. On day 3and 6, the sofratulle diabetic (STD) group had negative values as wound contraction rates this implied that the wounds expanded rather than contracted.

Comparisons between the highest mean contraction rates on days 3,6 and 9 showed that the rate for the STC group was significantly higher than that of STD on day 3 (a); while that of MRC group were significantly higher than that of STD group (b) and MPD group (c) on day 6 and 9 respectively $^{**}P < 0.05$.

The significant values for the intergroup and intra group comparisons were: (a) the mango rind control (MRC) was higher than its diabetic group (MRD) and the Sofratulle control (STC) than STD (b) both on day 3. On day 6, the mango rind diabetic (MRD) was higher than MPD (c) and STD (d); the MPC was higher than MPD (e), NSC higher than NSD (f) and STC higher than STD (g). However, on day 9 the MRD was higher than the MPD (h), NSD (j) and STD (k); MRC higher than MRD (l), NSC higher than NSD (m) and STC higher than STD (n) $^{**}P < 0.05$.

Discussion

One of the symptoms of diabetes mellitus is emaciation, thus the weight loss observed in all the diabetic groups fourteen days following induction was not unexpected. It was however observed that the least weight loss occurred in the group that had unripe *M.indica* pulp treatment (MPD). This could infer that *M.indica* has anti diabetic property as previously suggested by Amrita [13], Perpétuo [14] and Aderibigbe [15]. However, by twenty eight day post induction, all the diabetic groups recorded relative weight gain. This observation could suggest that the pancreatic islet destruction caused by alloxan monohydrate was self-limiting and reversible. This assertion may be a mere conjecture as nothing in this study could validate it. The recorded sepsis and mortality that occurred only in the diabetic groups might be attributable to the induced hyperglycaemia.

Following injury to a tissue or an organ, immediate attempt is made at restoration of the morphology. This restoration could either be by regeneration or repair; the latter is known as wound healing. In the excisional model used in this study, healing involves four sequential phases namely haemostasis, inflammation, proliferative and remodelling. Wound contraction is the chief component of the remodelling phase and it entails progressive reduction in the size of the wound thus it is a very reliable measurable parameter in the wound healing study. Both the control and diabetic groups had low mean wound contraction rates on day 3, this observation was not unexpected as the predominant cells at this period were inflammatory cells.

Also on day 3, it was noted that each of the control group had a rate that was more than double the respective diabetic group; this could be attributed to the inhibitory effect of the induced hyperglycaemia on wound healing. By day 6, all the groups had more than doubled their respective day 3 rate, though the diabetic groups still lagged behind. This upsurge in the rate might have been due to fibroblasts which at this period of the study had increased in quantity with reference to what obtained on day 3. The mean rate for the MRD group was higher than those of the NSD, STD and MPD. This might connote that the rind of *M. indica* could exert ameliorative action on the inhibitory effect of the induced hyperglycaemia on wound healing.

The trend in the wound contraction rates on day 9 was similar to that of day 6. Also on day 9, the rate for the MRC group compared favourably with that of the NSC this might tend to suggest that the rind of M. *indica* could be equipotent to normal saline as dressing material in non-diabetic wound. On day 12, the MRC group had the highest mean wound contraction rate of over eighty percent. Thus it can be reasonably concluded that the rind of *M. indica* possesses an excellent wound healing potential. By day 15, both the MRC and NSC groups had mean wound contraction rates above ninety percent this observation strengthened further the earlier deduction that the rind of *M. indica* accelerate*s* wound healing especially in non- diabetic rats and may be considered as alternative dressing material to normal saline and sofratulle. Wound contraction is dependent on the content of myofibroblasts; these cells have features of fibroblasts and smooth muscle [16].

The probable mechanism by which mango rind accelerates wound healing could be through increased myofibroblast production which resulted in enhanced wound contraction and thus into healing. Diabetes has been documented to inhibit collagen synthesis[17,18].

It is also pertinent to note that the diabetic rind group (MRD) had the highest rate amongst the diabetic groups throughout the duration of the study. This observation might suggest that mango rind was able to reverse the inhibition of collagen synthesis caused by diabetes mellitus. A comparison of the highest and lowest mean contraction rates showed that the value for the

diabetic sofratulle group (STD) was significantly lower than that of STC and MRC on day 3 and 6 respectively. Could this imply that the antibiotic (framycetin) impregnated in sofratulle heightened the inhibitory effect of diabetes on wound healing or was source dependent? Sofratulle is a synthetic material unlike mango which is naturally occurring plant. Thus the issue of adulteration and substandard is very possible with sofratulle but definitely not with mango.

Further study is needed to ascertain this probability of framycetin-in-sofratulle on wound healing in a diabetic setting. The mean wound contraction rates of the MRD group were significantly higher than those of the MPD group on days 6 and 9 thus *M. indica* rind was a better dressing material than its pulp in diabetic rat. Also the rates for the MRD group were significantly higher than those of the NSD and STD on days 6 and 9 thus mango rind was a more efficacious dressing material than normal saline and sofratulle in diabetic rats. Comparisons between a diabetic group with its respective control showed that control had a significantly higher rate than its diabetic counterpart in all the four paired groups on days 3,6 and 9; thus this study had been able to show that diabetes mellitus inhibited wound healing in rats.

Granulation tissue

Granulation tissue is the macroscopic appearance of the wound connective tissue and is usually pinkish and granular forming the new matrix of the skin being repaired. During this phase, formation of new blood vessels (angiogenesis) occurs, fibroblasts lay down collagen and extracellular matrix under the influence of some cytokines. The composition and rate of formation of granulation tissue can be affected by several factors either positively or negatively and this will either accelerate or retard wound healing. Thus the histology of granulation is a very important and reliable tool in the study of wound healing propensity of a material be it natural or synthetic. Light microscopy of the granulation tissues obtained at day 3 revealed inflammatory cellular infiltration in the diabetic groups especially in MPD while blood vessels (capillaries) and fibrillary structures that appeared like collagen were largely observed in the control groups. In clean, non-contaminated and non-diabetic wound, fibroblasts become functional seventy two hours post injury; thus the non observation of fibroblast activities in the diabetic groups was very likely due to the induced hyperglycaemia. By day 6, fibroblastic activities had become visible in the granulation tissue sections from the diabetic groups as evidenced by the presence of blood vessels and collagen. This pattern was also observed in sections obtained on day 9 at an increased magnitude. Also on day 9, sections of the granulation tissues of the diabetic groups had similar appearance to the respective control, since this observation cut across all the paired subgroups this probably might had been due to the self-limiting physiologic effects of the induced hyperglycaemia.

Sections of the scars obtained from all the groups revealed well defined layers of the skin thus the extracts of mango pulp and rind as wound dressing materials had the same end point comparable to those of sofratulle and normal saline in diabetic wistar rats.

The results of the phytochemical analyses of both the pulp and rind of *M. indica* were similar to those of relevant studies [19-23]. These phytochemicals have been reported to have antioxidant properties [24-27]. Thus the effects of the extracts of the pulp and rind of *M. indica* on excisional wounds in diabetic rats might be due to these phytochemicals. Further studies using specific isolates of the mango pulp and rind are needed to validate this assertion.

Conclusion

Results of this study showed that extracts of the pulp and rind of unripe *M. indica* accelerated wound healing in diabetic Wistar rats. When compared with sofratulle and normal saline; they had higher wound contraction rates at any point in time and their granulation tissues and scars had microscopic features akin to those of the former. However, the rind appeared to be more efficacious as a wound dressing material if wound contraction was the end point determinant. This could be of environmental and clinical importance considering the fact that mango rind is normally discarded. Thus it can be put to a beneficial use rather than being a source of environmental nuisance and thus hazardous to human health. If clinical trials on diabetic patients with cutaneous ulcers prove successful, commercial production of the extracts of unripe mango pulp and rind will be cheaper and thus affordable by the patients. This will ultimately result in better prognosis for diabetic foot ulcers and thus reduce the lower limb amputation rate for diabetic foot.

References

1. World Health Organization. Global Reports on Diabetes .2016;) http://www.who.int
2. Akinmoladun AC, Ibukun EO, Afor E, *et al*. Chemical constituents and antioxidant activity of *Alstoniaboonei*. African Journal of Biotechnology 2007; 6 (10):1197–1201

3. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants.African Journal of Biotechnology 2005; 4(7):685–688.
4. Ajila CM, Bhat SG and PrasadaRao UJS. Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem 2007; 102:1006–1011
5. Tharanathan RN, Yashoda HM and Prabha, TN. Mango (*Mangifera indica*L.), the king of fruits – A review. Food Reviews International 2006; 22:95-123.
6. Jahurul MHA, Zaidul ISM, Ghafoor K, *et al*. Mango (*Mangiferaindica*L.) by-products and their valuable components: A review. Food Chemistry 2015; 183:173-180.
7. Gouado I, Schweigert FJ, Ejoh RA, Tchouanguep MF and CampJV. Systemic levels of carotenoids from mangoes and papaya consumed in three forms (juice, fresh and dry slice). European Journal of Clinical Nutrition 2007; 61(10):1180-1188.
8. Harborne J B. Phytochemical Methods. In: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK 1973.
9. Boham BA and Kocipai AR. "Flavonoids and condensed Tannins from Leaves of Hawaiian *Vacciniumvaticulatum* and *V. calycinium*," Pacific Science 1994; 48: 458–463.
10. Obadoni BO andOchuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 2002;8(2):203–208.
11. Ejikeme CM, Ezeonu CS and Eboatu AN. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. European Scientific Journal 2014;10(18): 247–270.
12. Ajani, RS and Ogunbiyi, KI. *Carica papaya* latex Accelerates Wound Healing in diabetic Wistar rats. European Journal of Medicinal Plant 2015;9(3):1-12.
13. Amrita B, Liakot A, Masfida A and Begum R. Studies on the antidiabetic effects of *Mangifera indica* stem-barks and leaves on nondiabetic, type 1 and type 2 diabetic model rats. Bangladesh J Pharmacol 2009; 4:110–114.
14. Perpétuo GF and Salgado JM.Effect of mango (*Mangifera indica*, L.) ingestion on blood glucose levels of normal and diabetic rats. J Plant Foods Hum Nutr 2003; 58 (3):1–12.
15. Aderibigbe AO Emudianughe TS and Lawal BAS. Evaluation of the antidiabetic action of *Mangiferaindica* in mice.Phytotherapy Res 2001; 15: 456-458.
16. Junqueira LC andCarneiro J. Basic Histology. New York: McGraw-Hill, 2005; 93
17. Schaper NC, Almqvist J and Bakker K. The international consensus and practical guidelines on the management and prevention of the diabetic foot.CurrDiab Rep. 2003;3:475–479.
18. Becks PJ, Mackaay AJ, de Neeling JN, *et al*. Peripheral arterial disease in relation to glycemic level in an elderly Caucasian population: The Hoorn study. Diabetologia.1995;38(1):163-136.
19. Ajila CM, Naidu K.A, Bhat SG and PrasadaRao UJS. Bioactive compounds and antioxidant potential of mango peel extract. Food Chem.2007;105: 982–988.
20. Shivashankara K, Isobe S, Al-Haq M, Takenaka, M and Shiina, T. Fruit antioxidant activity, ascorbic acid, total phenol, quercetin, and carotene of Irwin mango fruits stored at low temperature after high electric field pretreatment. Journal of Agricultural and Food Chemistry.2004; 52: 1282–1286.
21. Vilela, C, Santos SAO, Oliveira L *et al*. The ripe pulp of *Mangifera indica*L.: A rich source of phytosterols and other lipophilic phytochemicals. Food Research International 2013; 54:1535–1540.
22. Saby K, BhatAndu SG and Prasada JS. Involvement of Peroxidase and polyphenol oxidase in mango sap injury. J Food Biochem 2002; 26: 403–414
23. Ribeiro, S and Schieber, A . Bioactive compounds in mango (*Mangifera indica* L.). In Bioact.*Foods Promot. Heal*.; Elsevier: USA, pp 507–523; 2010.
24. Sánchez S, Lizárraga D, Miranda A, *et al*. Grape antioxidant dietary fiber inhibits intestinal polyposis in ApcMin/+ mice: Relation to cell cycle and immune response. Carcinogenesis 2013;8:1881–1888.
25. De Vos P, Faas MM, Spasojevic M and Sikkema J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int. Dairy J 2010; 20:292–302.
26. Rocha-Ribeiro SM, Queiroz JH, Lopes-Ribeiro de Queiroz ME, Campos FM and Pinheiro-Sant'ana HM. Antioxidant in mango (*Mangiferaindica*L.) pulp. Plant Foods and Human Nutrition 2007;62(1):13-17.
27. Saura F. Antioxidant dietary fiber product: A new concept and a potential food ingredient. J.Sci 1998; 46: 4303–4306.

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Genome screening for specific microsatellite markers in Nigerian ethnic populations

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Abstract

*Background***:** DNA markers are very effective for distinguishing among individuals. The abundance and high variability of microsatellites in diverse genomes are useful tools for identification, linkage analysis, forensic investigations and phylogeny reconstruction. This study analyzed microsatellites in three Nigerian ethnic groups in order to detect unique microsatellites for estimation of ethnic affiliation.

*Materials and methods***:** Five hundred and sixty adult Nigerians of Hausa (175), Igbo (163) and Yoruba (222) extraction participated in the study. Deoxyribonucleic acid (DNA) was extracted from dry blood spots on filter paper and microsatellites loci were amplified by Inter Simple Sequence Repeat (ISSR) primers. Amplicon bands were scored for distinct microsatellite alleles. The result was analyzed for phylogeny, cluster analysis, genetic distance, and genetic identity. Sequencing was performed on microsatellite loci within ethnic clusters.

Results: The phylogenetic tree and the principal coordinate analysis of amplified loci revealed clustering of the three ethnic populations with a 2% molecular variance. Pairwise population matrix of Nei genetic distance was 0.032 between Hausa and Igbo, 0.027 between Igbo and Yoruba, and 0.024 between Hausa and Yoruba. Fixation index was 0.022 for Hausa and Igbo, 0.020 for Igbo and Yoruba, and 0.019 for Hausa and Yoruba. Nei genetic identity was 0.969 between Hausa and Igbo, 0.973 between Igbo and Yoruba, and 0.977 between Hausa and Yoruba. Allelic sequencing revealed 1484 microsatellites, 2.8% (41) of which were specific for the ethnic groups either trimeric, tetrameric, pentameric or hexameric.

Conclusion: Findings of this study are suggestive of microsatellites being able to confirm the identity of ethnic populations despite close genetic distance.

Keywords: *Genome screening, microsatellite markers, Nigerian populations.*

Abstrait

Contexte **:** les marqueurs ADN sont très efficaces pour distinguer les individus. L'abondance et la grande variabilité des microsatellites dans divers génomes sont des outils utiles pour l'identification, l'analyse de liaison, les enquêtes forensiques et la reconstruction de la phylogénie. Cette étude a analysé des microsatellites dans trois groupes ethniques nigérians afin de détecter des microsatellites uniques pour l'estimation de l'appartenance ethnique.

Matériel et méthodes **:** Cinq cent soixante Nigérians adultes d'extraction haoussa (175), Igbo (163) et yoruba (222) ont participé à l'étude. L'acide désoxyribonucléique (ADN) a été extrait de taches de sang sec sur papier filtre et les locus de microsatellites ont été amplifiés par des amorces Inter Simple Séquence Répéter (ISSR). Les bandes d' amplicon ont été évaluées pour les allèles microsatellites distincts. Le résultat a été analysé pour la phylogénie, l'analyse des groupes, la distance génétique et l'identité génétique. Le séquençage a été effectué sur des loci microsatellites au sein de groupes ethniques.

Résultats : L'arbre phylogénétique et l'analyse des coordonnées principales des locus amplifiés ont révélé un regroupement des trois populations ethniques avec une variance moléculaire de 2%. La matrice de population par paires de la distance génétique Nei était de 0,032 entre Haoussa et Igbo, 0,027 entre Igbo et Yoruba et 0,024 entre Haoussa et Yoruba. L'indice de fixation était de 0,022 pour haoussa et Igbo, de 0,020 pour Igbo et yoruba et de 0,019 pour haoussa et yoruba. L' identité génétique Nei était de 0,969 entre Haoussa et Igbo, 0,973 entre Igbo et Yoruba et 0,977 entre Haoussa et Yoruba. Le séquençage allélique a révélé 1484 microsatellites, dont 2,8% (41) étaient spécifiques des groupes ethniques trimériques, tétramériques, pentamériques ou hexamèriques.

Conclusion : Les résultats de cette étude suggèrent que les microsatellites sont capables de confirmer l'identité des populations ethniques malgré une distance génétique proche.

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Mots clés : *Dépistage du génome, marqueurs microsatellites, populations nigérianes.*

Introduction

There is a lot of genetic variation among African populations, this is as a result of demographic history, changes in population size because of short and long range migration, admixture and genetic mutation [1]. Africa population as a region of genetic, linguistic, cultural and phenotypic diversity can be genetically studied and characterized based on genetics among individuals and ethnic populations. This will reveal differential history of human origin, complex interaction of genetic and environmental factors that produce the phenotypes. Genomic studies of ethnic variations will also ascertain levels of variable traits for ethnic population identity, admixture and adaptation to diverse environment and those that confer protective roles in ethnic populations. Genetic finger printing has been utilized for the identification of individuals and ethnic populations [2]. Among the DNA finger printing methods, species specific microsatellite analyses are of major importance [3]. The variation in number of repeats affects the overall length of the microsatellite, a characteristic readily measured by laboratory techniques. In humans, the mutation rate of these markers is estimated to be around 10^{-3} to 10^{-4} per locus per generation [4]. The higher mutation rate allows smaller fraction of the genome to be sampled to make inferences with microsatellite data. Microsatellites vary in length between individuals and generally have many distinct alleles within a related population [5,6]. Microsatellites high level of variability has led to their use as markers in linkage analysis, forensic investigations, human population genetics and phylogeny reconstruction. Microsatellites regions of "unordered" DNA (Inter simple sequence repeats - ISSR) on each side of the repeat units can be used for the development of locus-specific primers to amplify microsatellites. The unordered DNA between two repeated sequences - Inter simple sequence repeats (ISSR) – can be used to design primers which will extend outside of ISSR to amplify a unique region for individuals and ethnic populations.

Kashyap *et al.* [7] used autosomal microsatellite markers on ethnically, linguistically and geographically diverse human populations in India to decipher intra-population diversity. The population genetic study of Jewish population by autosomal microsatellite markers, revealed that Jewish populations shared Middle East ancestry compared to Europeans with varying degree of admixture [8]. The genotypes of the microsatellite markers from Sudanese populations have shown that the microsatellite analysis was a standard robust forensic tool; it showed ethnic differentiation, personal identification and parentage analysis [9]. Pemberton *et al*., [10] used microsatellite systematic genotypic analysis on human population to reveal genetic relatedness, detect intra-population and inter-population pairs of previously unidentified close relatives. Matrix metalloproteinase-9 microsatellite candidate markers were used to demonstrate interethnic variation among populations. Camacho-Mejorado *et al* [11] and Algee-Hewitt *et al* [12] had posited that microsatellite markers can provide information that might be used to characterize individual ancestry-related traits. Genetic variations between the three Nigeria ethnic populations was demonstrated with microsatellite loci [13]. He *et al* [14] also used autosomal microsatellites analysis to suggest that genetic affinity and genetic distinction exist among ethnic Chinese population. Nigeria ethnic groups lack data that reveal genetic affinity and genetic distinction.

This study elucidated genomic markers that are similar or differ among Hausa, Igbo and Yoruba ethnic populations of Nigeria and its application in forensic and public health.

Materials and methods

Sample Collection:

Sample size was based on optimum sample size for population genetic studies [15]. Sampling was taken based on volunteers' availability and satisfying the inclusion criteria: descendants of parents, grandparents and great grandparents should come from the same ethnic groups, and must come from the appropriate zones of the country: northwest zone for the Hausa, southeast zone for Igbo and southwest zone for Yoruba.

Blood sample collection

After cleansing of the fingertip with ethanol swab, it was subsequently pricked with a sterile single use lancet and three drops of blood were deposited on a Whatman filter paper. This was allowed to dry at room temperature for few minutes before storage in a sterile labelled paper bag containing silica gel desiccant.

DNA extractions and quantification

Four 3mm spots were excised from the dried blood spot with a single-hole paper puncher into 1.5ml

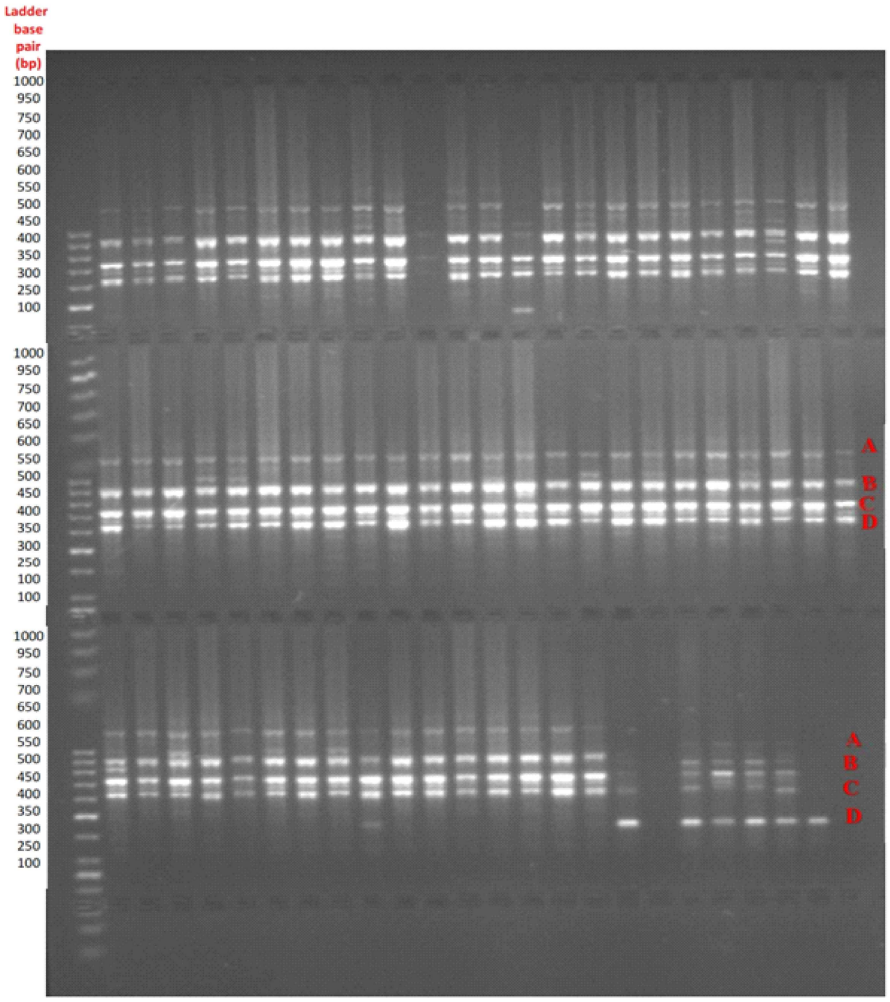

Fig.1: Agarose gel picture showing bands of amplicons

Eppendorf tube using Haris Uni-core and Haris cutting mat. The puncher was cleansed with 100% alcohol after each use for a Whatman filter paper. Human genomic DNA was extracted on the basis of Qiagen manufacturer's Dried Blood Spot Protocol.

Qualitative and Quantitative estimation of extracted DNA

Extracted DNA samples (2µl) were loaded into the wells of the prepared Agarose gel to ascertain quality of DNA. The gel was placed in tank, covered with 0.5% TBE buffer and run at 100V. Electrophoretic gel picture showed the presence of DNA. Nanodrop spectrophotometer was used to check the purity and quantity of the DNA. The samples purity range between 1.50-4.76 and the concentration level was between 110.39ng to 382.76ng/µl. The DNA was stored at "20 °C for subsequent amplification and analysis.

DNA amplification, genome screening for microsatellites loci and genotyping

Twelve single and 3 combined Inter Simple Sequence Repeat (ISSR) primers were used to screen for the microsatellites loci of the extracted nuclear DNA using the technique of Korpelainen *et al* [3]. Amplification reactions were conducted in a volume of 25µl. The reaction mixture contained 3µl nuclear DNA, 0.3µl Taq DNA polymerase (Promega, USA), 5µl PCR buffer, 2µl 10 mM dNTP, 2µl of a single primer or 1µl each of two combined primers, 2µl of $MgCl_2$ and 10.7µl of sterile water. Multi Gene Gradient Thermal Cycler was programmed for 2minute denaturation at 95°C, followed by 40 cycles of denaturation at 95°C for 30seconds, annealing at 52°C for 45s, and extension at 72°C for 45. Final extension for 7min. All amplification products were separated by electrophoresis on 2% agarose gels (Figure 1).

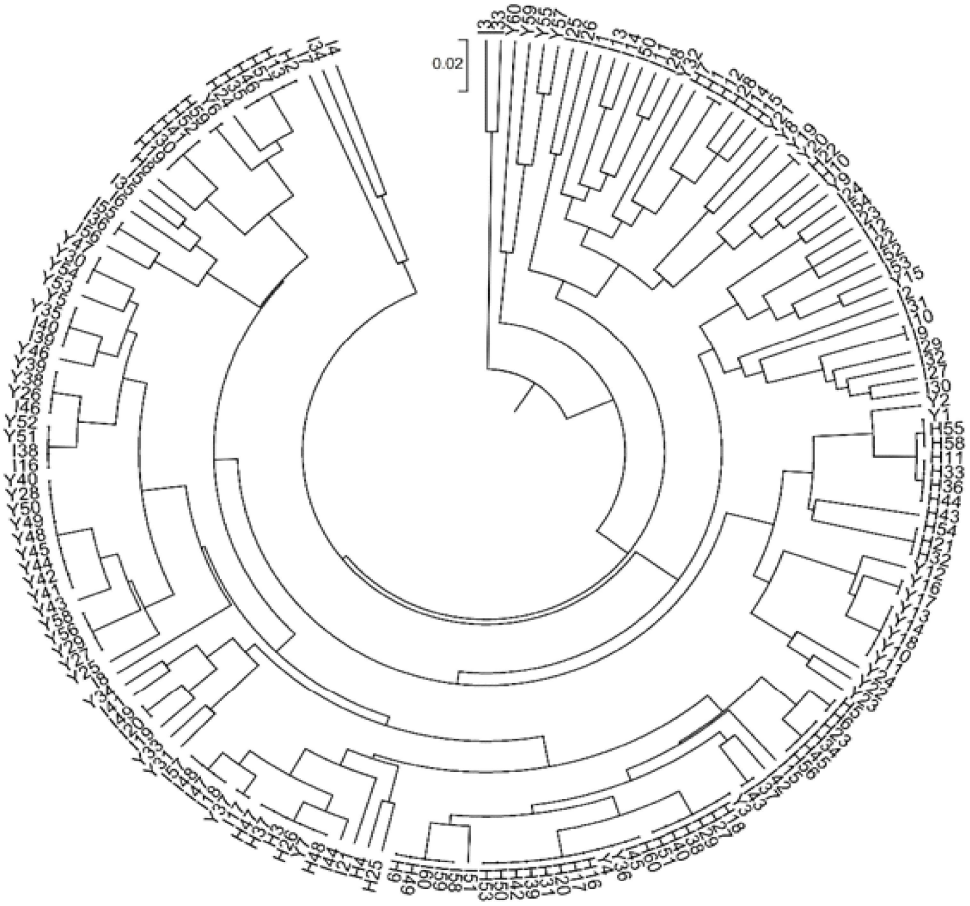

Fig. 2: Neighbor-joining tree of microsatellite allelic pattern of the ethnic populations showing genetic relationship (H – Hausa, I – Igbo, Y – Yoruba).

Data management and Statistical analysis

Amplicons were scored for all the samples under the primer names: (AG-T), (CA-A), (GA-T), (AG-C), (CA-G), (GA-C), (AG-G), (GA-A), (AC-T), (AC-C), (AC-G), (CA-T), GAT/CAT, GAC/AGC, and AGT/GAT.

Distinct microsatellite alleles were designated A, B, C, D, E on gel pictures with marker lane indicating the number of sample allele base pairs (Fig. 1). The presence of allele was designated "1" and absence as "0", and missing data as "9" during scoring. Scored amplicons were analyzed by GeneAlex, PowerMarker and Clustal softwares for MANOVA, phylogeny, cluster analysis, genetic distance and genetic identity. Scored amplicons that form ethnic clusters were purified and sequenced to detect microsatellites unique for each ethnic population.

This work was reviewed and approved by the Ethics Committee of the Ministry of Health.

Results

The mean allelic pattern across the populations was as shown in Table 3. The mean allelic patterns across the population showed the number of different alleles with frequency greater than or equal to 5%, the number of effective alleles, the expected and observed gene diversity for the three ethnic populations. The frequency pattern of allelic scored bands across the ethnic populations' yielded graphically illustrated Neighbour-joining phylogenetic tree. The Neighbour-joining genetic tree demonstrated the clustering of ethnic populations: Hausa (H) ethnic population, Igbo (I) ethnic population and Yoruba (Y) ethnic populations (Fig.2). Analysis of molecular variance (AMOVA) of the loci revealed 2% variation between ethnic populations, zero percent variation among the individuals within populations assigned to the correct ethnic populations and 98% variation between individuals within ethnic populations.

Table 1: Microsatellite Primers

Inter Simple Sequence Repeat (ISSR) Primers for genome screening of microsatellite loci		
1	5′-AGAGAGAGAGAGAGAGT-3′	(AG-T)
2	5′-CACACACACACACACAA-3′	(CA-A)
3	5′-GAGAGAGAGAGAGAGAT-3′	(GA-T)
4	5′-AGAGAGAGAGAGAGAGC-3′	(AG-C)
5	5′-CACACACACACACACAG-3′	(CA-G)
6	5′-GAGAGAGAGAGAGAGAC-3′	(GA-C)
7	5′-AGAGAGAGAGAGAGAGGT-3′	(AG-G)
8	5′-GAGAGAGAGAGAGAGAA-3′	(GA-A)
9	5′-ACACACACACACACACT-3′	(AC-T)
10	5′-ACACACACACACACACC-3′	(AC-C)
11	5′-ACACACACACACACACG-3′	(AC-G)
12	5′-CACACACACACACACAT-3′	(CA-T)
	Combined Primers	
1	GAT/CAT	
2	GAC/AGC	
3	AGT/GAT	

Table 2: Populations genetic distance and identity

Matrix of net genetic distance			
H	I	Y	
0/000			H
0.022	0.000		I
0.019	0.020	0.000	Y
Matrix of net gentic identity			
H	I	Y	
1.000			H
0.900	1.000		I
0.977	0.973	1.000	Y

Fst = 0.001 H, Hausa: I, Igbo: Y, Yoruba

Matrix of Nei genetic distance showed that genetic distance differentiation between the populations was not negligible (Fst < 0.05). Matrix of Nei genetic identity revealed genetic closeness of Hausa to Igbo, Yoruba to Igbo and Hausa to Yoruba (Table 2).

The sequencing of the clustered alleles from each of the three ethnic groups yielded 1484 microsatellites. 82.28 % (1221) were dinucleotides, 13.78 % (205) were trinucleotides, 3.3 % (50) were tetranucleotides, 0.5% (7) were pentanucleotides, and 0.07% (1) was hexanucleotide (Table 4). (AA) dinucleotides microsatellites (2.48%) was highest among all dinucleotide repeat motifs for the Hausa ethnic population, while (AT) and (GC) dinucleotide motifs had zero percent. (TT) dinucleotide motifs (4.91%) was highest and (CG) dinucleotide motif (0.07%) was lowest for Igbo ethnic population. (TT) dinucleotide motifs (4.84%) was highest and (CG) dinucleotide motif (0.07%) was lowest for Yoruba ethnic population. (TCT) trinucleotides microsatellites (0.47%) was highest for Hausa among all trinucleotide repeat motifs, (TGT) trinucleotide motifs (1.15%) was the highest within Igbo ethnic population, (TGT) trinucleotide repeats (0.6%) was also the highest within Yoruba population.

(TTTG) tetranucleotides microsatellites (0.27%) was highest among tetranucleotides within the Hausa ethnic population, (TTTG) tetranucleotides motifs (0.34%) was also the highest of tetranucleotides microsatellites within the Igbo ethnic population while (CCCT) (0.13%), (CTTT) (0.13%) and (TTTG) (0.13%) were the highest within the Yoruba ethnic population.

Pentanucleotide microsatellite was absent within Hausa ethnic population, (TCTTT) pentanucleotide repeat

Table 3: Mean Allelic Patterns across the Populations

Population	H	I	Y
No. of Different Alleles (Na)	2.33±0.23	2.27±0.21	2.47±0.24
No. of Different Alleles with Freq. ≥ 5%	2.20±0.22	2.20 ± 0.20	2.20±0.24
No. of Effective Alleles (Ne)	1.81±0.14	1.80±0.11	1.85±0.15
Shannon's Information Index (I)	0.64±0.09	0.62±0.07	0.62±0.09
No. Private Alleles (No. of Alleles Unique to a Single Population)	0.12±0.09	0.13±0.09	0.13±0.09
Expected Heterozygosity (He) (Gene diversity)	0.41±0.05	0.41±0.05	0.40±0.06
Observed Heterozygosity (Ho)	0.42±0.06	0.41±0.05	0.40±0.06

H, Hausa; I, Igbo; Y, Yoruba

Table 4: Distribution of Nucleotide Repeat Motifs

DI NUCLEOTIDE	TRI NUCLEOTIDE	TETRA NUCLEOTIDE	PENTA NUCLEOTIDE	HEXA NUCLEOTIDE	TOTAL
1221(82.28%)	205 (13.78%)	50(3.37%)	07(0.50%)	01(0.07%)	1484

motifs (0.13%), was highest within Igbo ethnic population while (TCTTT) pentanucleotide microsatellite (0.07%) was present within Yoruba ethnic population. Hexanucleotides microsatellite, (GAAATAGAAATA) (0.07%) was present only within Hausa ethnic population.

Table 5: Specific Alleles loci Unique to a Single Population

Population	Locus	Allele	Frequency
H	GAT	2	0.07
I	CAT	3	0.06
I	AGT/GAT	2	0.20
Y	CAA	4	0.06
Y	GAC/AGC	2	0.02

H, Hausa; I, Igbo; Y, Yoruba

Of the 1484 microsatellites, 2.8 % were unique for the three ethnic populations, these were located at loci GAT for Hausa (0.5%), CAT, AGT/GAT for Igbo (1.9%) and CAA, GAC/AGC for Yoruba (0.4%) (Table 5). The unique microsatellites were trimeric, tetrameric, pentameric and hexameric short tandem repeats (STRs) (Tables 6).

The primary nucleotides of DNA sequences information from three ethnic populations was NCBI blast to find regions of local similarity between ethnic microsatellites sequences to human sequence databases. The blasts identify sequences that resembled the query sequence on Homo sapiens chromosomes 1, 9 and 10 of Genome Reference Consortium Human Build 38 patch release 2 (GRCh38.p2) primary assembly with one or two ranges of nucleotide sequences on both 5' end and 3' end [29]. The blasts for the Hausa ethnic population showed the gene coding for Tumor Necrosis Factor receptor (TNFR) and Cholesterol 25 hydroxylase (CH25H) on chromosome 10q23. Igbo ethnic population showed Tumor Necrosis Factor receptor (TNFR) and Cholesterol 25 hydroxylase (CH25H) on chromosome 10q23, brain-derived neurotrophic factor/ neurotrophin-

Table 6: Ethnic population unique microsatellites

Hausa Unique Microsatellites	
Microsatellites	Abbreviation
ACAACA	(ACA)2
CCCACCCA	(CCCA)2
CTTCCTTG	(CTTT)2
CTTTCTTT	(CTTT)2
GAAATAGAAATA	(GAAATA)2
TATTAT	(TAT)2
TTATTA	(TTA)2

Igbo Unique Microsatellites	
Microsatellites	Abbreviation
AACAAC	(AAC)2
ACATACAT	(ACAT)2
ACTCACTC	(ACTC)2
AGGAGG	(AGG)2
ATAAATAA	(ATAAA)2
ATAATA	(ATA)2
ATGATG	(ATG)2
ATTATT	(ATT)2
CACAGCACAG	(CACAG)2
CCGCCG	(CCG)2
CCTGGCCTGG	(CCTGG)2
CCTTCCTT	(CCTT)2
CTCCTC	(CTC)2
GAAAGAAA	(GAAA)2
GATGAT	(GAT)2
GCCGCC	(GCC)2
TCCCTCCC	(TCC)2
TCTTTTCTTT	(TCTTT)2
TGATGA	(TGA)2
TGCTGC	(TGC)2
TGCTTGCT	(TGCT)2
TGGTTGGT	(TGGT)2
TGTATGTA	(TGTA)2
TGTTGT	(TGT)2
TTCTTC	(TTC)4
TTGTTG	(TTG)2
TTTCTTTC	(TTTC)2
TTTGTTTG	(TTTG)2

Yoruba Unique Microsatellites	
Microsatellites	Abbreviation
AACTAACT	(AAACT)2
AATAAT	(AAT)2
ACCAACCA	(ACCA)2
CGCCGC	(CGC)2
TCCTCC	(TCC)2
TGGTGG	(TGG)2

3 (BDNF/NT-3) isoform X3 and X5 on chromosome 9q22, and Neuron navigator 1 isoform X1 and X7 on chromosome 1q22.Yoruba ethnic population had Tumor Necrosis Factor receptor (TNFR) and Cholesterol 25 hydroxylase (CH25H) also on chromosome 10q23, and Neuron navigator 1 isoform X1 and X1 on chromosome 1q22.

Discussion

The biases on populations, based on the phenotypic parameters that may show subjectivity due to errors in measurements readings and dermatoglyphic analysis, cryptic population structures difficult to detect using visible characters may be significant in genetic terms. This study analyzed DNA allelic frequencies and microsatellite uniqueness for each population. Paetkau *et al.* [16], Rannala and Mountain [17] had reported that DNA samples estimation of allelic frequencies for each population at a series of unlinked loci can be used to assign each population.

Frequency pattern of the allele across the three ethnic populations showed the clustering of the ethnic populations which depicted the level of genetic variation and the level of gene flow between the populations thus revealing the level of admixture among the ethnic populations.

The matix of Nei genetic distance between the ethnic groups (FST < 0.05) confirmed low level of genetic variation among the three ethnic populations while the matrix of Nei genetic identity revealed their closeness. Within this low level of differentiation, the number of alleles unique to a single population was elucidated. Neel [18] was the first to propose that there were genetic markers that exist in one population and not in others. Reed [19] also described hypothetical genetic marker loci at which different alleles were fixed in different populations. Chakraborty *et al*. [20] called those variants that were found in only one population unique alleles. Chakraborty *et al*. [21], Stephens *et al*. [22] described genetic markers with large allele-frequency differentials. Shriver *et al.* [23] also identified a panel of population-specific genetic markers that enable ethnic-affiliation estimation for major U.S. resident populations with significant statistical power for ethnic-affiliation estimation, forensic ethnic-affiliation estimation, populations and individual level admixture estimation and in mapping of genes. Sun *et al* [24] using 10 autosomal microsatellites to demonstrate that most ethnic populations who speak the same language demonstrate a similar genetic composition.

In the language classification, Hausa belong to Afro-Asiatic classification while Igbo and Yoruba belong to Niger-Congo classification [25]. The low genetic distance and identity among the three ethnic populations could only be explained by these ethnic three groups' inhabiting a region with a history of long migrations and settlements with such a mixture of social and cultural relationships. It is difficult to separate these ethnic populations within these settlements into neat socio-cultural groups. Consistency of genetic and linguistic evolution had been known to be broken by factors such as less isolation, language replacement or intermarriage. [24]. This relationship was exhibited in the phylogenetic tree of the three ethnic populations. Phylogenies trace patterns showed shared ancestry between the three ethnic populations (common ancestor), but each ethnic population has a part of its history that is unique to it alone. Hausa and Yoruba had parts in their history that were shared more recently than with the Igbo ethnic population that had earlier speciation. Mutations of alleles at microsatellite loci tend to result in alleles with repeat scores similar to those of the alleles from which they were derived.

The difference in repeat score between alleles carries information about the amount of time that has passed since they shared a common ancestral allele. Edwards *et al* [26], found trimeric and tetrameric microsatellites loci as useful markers for the study of new mutations and genetic linkage analysis and for application to personal identification in the medical and forensic sciences. Shriver *et a*l [23] noted that most phenotypic characteristics and self-claim of an individual within a population offer little power to distinguish ethnicity. Ethnic-affiliation estimation (EAE) by DNA markers are very powerful for distinguishing among individuals to support physical characteristics and statement about the ethnicity. In 1997, the FBI announced the selection of 13 STR loci to constitute the core of the United States national database, Combined DNA Index System (CODIS), the national DNA information repository maintained by the FBI, allows state and local crime laboratories to store and compare DNA profiles from crime-scene evidence and convicted offenders; they are also used routinely to identify human remains, establish or exclude paternity. All CODIS STRs were tetrameric repeat sequences [27]. The ethnic unique microsatellites for the Hausa, Igbo and Yoruba were trinucleotides, tetranucleotides, pentanucleotides and hexanucleotides. With the determination of the frequencies

of these markers within the ethnic populations, they could become source of index system for Nigeria.

The study revealed identification of the common gene location on chromosomes by blasts which also revealed the statistical significance of matches, infer functional, evolutionary relationships between sequences and identify members of gene families [28]. The common gene have different nucleotide ranges for the three ethnic populations which confirm the common shared ancestry that was revealed by the phylogenetic tree. Igbo and Yoruba shared the gene coding for Neuron navigator 1 on chromosome 1q22. This showed that though Igbo speciation was earlier on the phylogenetic tree and that the Hausa and Yoruba shared more recent ancestry than with the Igbo ethnic population. The language classification that showed Igbo and Yoruba as belonging to Niger-Congo classification was of genetic significance. Tishkoff, *et al* [30] had stated that genetic diversity within Africa significantly correlated with estimates from microsatellite variance and this varies by linguistic, geographic, and subsistence classifications. The NCBI BLAST also confirmed closeness in identity and reduced genetic distance among the three ethnic populations. The microsatellite identification reports through the Combined DNA Index system (CODIS) and the characterization of genetic variation and relationships among populations across west Africa and the continent of Africa have no particular reference to multi-linguistic, multicultural, pluralistic nation like Nigeria with ethnic identities.

The study also showed the level of gene flow and admixture among the Nigerian three ethnic populations by identifying ethnic unique microsatellites. This study located shared and differential genes on chromosomes 1, 9 and 10 for the three ethnic populations as an implication of closeness and relatedness but with useful distinguishable different nucleotide ranges. The ethnic unique microsatellites may be markers for the three ethnic populations and may be a useful tool for identification, studying mutations and genetic linkages among the populations. These findings will further both anthropological and genetic studies among Nigeria ethnic populations with its applications.

References

1. Campbell, MC and Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. Annual review of Genomics and Human Genetics 2008; 9: 403-433.
2. Jeffreys AJ, Royle NJ, Wilson V and Wong Z. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature1988; 332(6161), 278.
3. Korpelainen H, Kostamo K and Virtanen V. Microsattelites marker identification using genome screening and restriction-ligation. BioTechniques 2007; 42(4):479-486.
4. Ellegren H. Microsatellites: Simple Sequences with complex evolution. Nature Reviews Genetics 2004; 5: 435- 445.
5. Weissenbach J, Gyapay G, Dib C, *et al*. Second-generation linkage map of the human genome. Nature 1992; 359: 794–801.
6. Broman KW, Murray JC, Sheffield VC, White RL and Weber JL. Comprehensive human genetic maps: individual and sex-specific variation in recombination. American Journal Human Genetics 1998; 63, 861–869.
7. Kashyap VK, Ashma R, Gaikwad S, Sarkar BN and Trivedi R. Deciphering diversity in populations of various linguistic and ethnic affiliations of different geographical regions of India: Analysis based on 15 microsatellite markers. Journal of Genetics 2004; 83(1): 49-63.
8. Kopelman NM, Stone L, Wang C, *et al*. Genomic microsatellites identify shared Jewish ancestry intermediate between Middle Eastern and European populations. BMC Genetics 2009; 10:80.
9. Babiker HMA, Schlebusch CM, Hassan HY and Jakobsson M. Genetic variation and population structure of Sudanese populations as indicated by 15 Identifiler sequence-tagged repeat (STR) loci. Investigative Genetics 2011; 2:12.
10. Pemberton TJ, DeGiorgio M, and Rosenberg NA. Population structure in a comprehensive genomic data set on human microsatellite variation. G3: Genes, Genomes, Genetics 2013; 3(5), 891-907.
11. Camacho-Mejorado R, Noris G, Santana C, *et al* Interethnic variation of the MMP-9 microsatellite in Amerindian and Mexican Mestizo populations: considerations for genetic association studies. Genetics and Molecular Research 2015; 14(1), 2929-2939.
12. Algee-Hewitt BF, Edge MD, Kim J, Li JZ and Rosenberg NA. Individual identifiability predicts population identifiability in forensic microsatellite markers. Current Biology 2016; 26(7), 935-942.

13. Agbo BU, Ebuehi OAT and de Osuntoki AA. Genetic Variations at 15 Forensically Relevant Microsatellite Loci (STRs) in the Three Major Ethno-Linguistic Population Groups in Nigeria. The FASEB Journal 2017; 31(1_supplement), 591-610.
14. He G, Wang Z, Wang M, *et al*. Genetic variations and forensic characteristics of Han Chinese population residing in the Pearl River Delta revealed by 23 autosomal STRs. Molecular biology reports 2018; 45(5), 1125-1133.
15. Bashalkhanov S, Pandey M and Rajora OP. A simple method for estimating genetic diversity in large populations from finite sample sizes. BMC genetics 2009; 10(1): 84.
16. Paetkau D. Calvert W, Stirling I, Strobeck C. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 1995; 4(3): 347–354.
16. Rannala B and Mountain JL. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA 1997; 94: 9197– 9201.
17. Neel JV. "Private" genetic variants and the frequency of mutation among South American Indians. Proceedings of National Academy Science 1973; 70(12):3311–3315.
18. Reed TE. Number of gene loci required for accurate estimation of ancestral population proportions in individual human hybrids. Nature 1973; 244:575-576.
19. Chakraborty R, Fornage M, Gueguen R and Boerwinkle E. Population genetics of hypervariable loci: analysis of PCR based VNTR polymorphism within a population. In DNA fingerprinting: approaches and applications 1991; (pp. 127-143). Birkhäuser Basel.
20. Chakraborty R, Kamboh MI, Nwankwo M and Ferrell RE. Caucasian genes in American blacks: new data. American Journal of Human Genetics 1992; 50(1):145–155.
21. Stephens JC, Briscoe D, O'Brien SJ. Mapping by admixture linkage disequilibrium in human populations: limits and guidelines. American Journal Human Genetics 1994; 55(4):809–824.
22. Shriver MD, Smith MW, Jin L, *et al*. Ethnic-affiliation estimation by use of population-specific DNA markers. American journal of human genetics 1997; 60(4), 957.
23. Sun H, Zhou C, Huang X, *et al*. Correlation between the linguistic affinity and genetic diversity of Chinese ethnic groups. Journal of human genetics 2013; 58(10): 686-693.
24. Otitie O. Nigeria's Identifiable Ethnic Groups. https://www.onlinenigeria. com/tribes/tribes.asp. Extracted on 20/9/2016.
25. Edwards AL, Hammond H.A, Jin L, Caskey CT and Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992; 12(2): 241-253.
26. Norrgard K. Forensics, DNA fingerprinting, and CODIS. Nature Education 2008; 1(1):35.
27. Madden T. The BLAST sequence analysis tool 2013.
28. Coordinators NR. Database resources of the national center for biotechnology information. Nucleic acids research 2015; 43(Database issue), D6.
29. Tishkoff SA, Reed FA, Friedlaender FR, et *al*. The Genetic Structure and History of Africans and Afrcan Americans. Science 2009; 324(5930): 1035-1044.

Appendix

Few sequenced products of clustered microsatellites loci and blast results

H_35_GAT

TAACCCATGCTTGCCTAGGATCGTTGCTAACTTAGGCTGACTCTCTTATTTGTCGTTGTATGACATCAGAAAGTCTTTTCT
TCCCTTTGCACCTTACTTCTCCTAAGTATCAACTCCTATTCTGAACTAAATGATTCTGCCCTAATGTCTAGGTG
GAGGTTAAAATCTGACTCGTGGAGGTCCACTGATTATCTCTCTGGATACCCTCTTGCGTCAACAGGTCCCTGT
TGTCTTTTCCAGGTGTGTATGATTGCATGGTACTGATGACCAGAACTTAACAACATTCTTCTCCCTGTGCTTG
ATGTGCTTGGGGCCAATTGCTTTGGGCCTCTTTTCTTTACCTCCAGGCCTTTGTTTGGGCTGTTTTCAATAAA
TGGTTGCCACACACCAATTGGGACCCTGGCCTGGTCTAGGCCTAGGGCAAAAATGTCTTCTCTTTCCTGCCACT
GCCTATTGTTGAGATGGATCCCCTGGAAATCAGAATGCACCCATCTTGAAAAAAGTGGACCGTGAATGATTAT
CAAGAGATCTACCATTGTATTATTTTAACCTCCACCGATACATTAGGCTTTGATCATTTAATTCCACTCCCTCT
CTTTATACATAATAGAACTAAGGTCCAGGACTAGGAAAAGACTTGCTGATGTTGATACAACTTGCTAACTGCAGAGTTAGT
ACTTGAATAGTACTTTCCTGGGCCAAGATAAGTGCCTTGTCTGCCAGACTGATCTCTCTCTCTCTCTCTCATCAAA

Homo sapiens chromosome 10q23, GRCh38.p2 Primary Assembly
Sequence ID: ref |NC_000010.11| Length: 133797422 Number of Matches: 2
Related Information

Range 1: 89098905 to 89099388

Score	Expect	Identities	Gaps	Strand Frame
363 bits(196)	2e-97 3	92/487(80%)	12/487(2%)	Plus/Minus

Features:
84455 bp at 5' side: tumor necrosis factor receptor superfamily member 6 isoform
107086 bp at 3' side: cholesterol 25-hydroxylase

```
Query  40        ACTCTCTTATTTGTC-GTTGTAT-GACATCAGAAAGTCTTTTCTTCCCTTTGCACCTTAC  97
Sbjct  89099388  ACTCTCTTATTTGCAAGTTGTATCAACATCAGCAAGTCTTTTCCTCTCTTTGGACCTTAG  89099329

Query  98        TTCTCCTAAGTATCAACTCCTATTCTGAACTAAATGATTCTGCCCTAATGTCTAGGTGGA  157
Sbjct  89099328  TTCTCTTATGTATAAAGTGAGGGAATGAACTAAATGATTCTGCCCTAATGTCTAGGTGGA  89099269

Query  158       GGTTAAAATCTGACTCGTGGAGGTCCACTGATTATCTCTCTGGATACCCTCTTGCGTCAA  217
Sbjct  89099268  GGTTAAAATCTGACAAGTGTAGGTCCACTGATTATCATTCTGGATACACTCTTGTGTCAG  89099209

Query  218       CAGGTCCCTGTTGTCTTTTC-CAGGTGTGTATGATTGCATGGTACTGATGACCAGAACTT  276
Sbjct  89099208  CTGGTCCCTGTTGTCTTTGAGCTGGAGC-TATGATTGCATGGTACTGATGACAGGAAAGA  89099150

Query  277       AACAACATTCTTCTCCCTGTGCTTG-ATGTGC-TTGGGGCCAATTGCT-TTGGGCCTCTT  333
Sbjct  89099149  GACAACATTCTTCTCCCTGTGCTACCATGTGCATTGGGGCCAATTGGTGTTGGGCCTCCA  89099090

Query  334       TTCTTTACCTCCAGGCCTTTGTTTGGGCTGTTTTCAATAAATGGTTGCCACACACCAATT  393
Sbjct  89099089  CTCTTTACCTCCAGGCCTTTGTTTGTGCTCTTTTCGGTAAAAAGTTGTGACAAAGCAATC  89099030

Query  394       GGGACCCTGGCCTGGTCTAGGCCTAGGGCAAA-AATGTCTTCTCTTTCCTGCC-ACTGCC  451
Sbjct  89099029  AGGAGCCTGGGCTGGTCAAGGCCTAAGGCTGAGAGAG-CTT-TAAGTTCTGTCTACTGCC  89098972

Query  452       TATTGTTGAGATGGATCC-CCTGGAAATCAGAATGCACCCATCTTGAAAAAAGTGGACCG  510
Sbjct  89098971  TATAGTTGAGATGGACACACCTGGAAATAGGAGTACATACATCTTGGAGAAAGTGGACTG  89098912

Query  511       TGAATGA  517
Sbjct  89098911  TGACTGA  89098905
```

Range 2: 89099229 to 89099463

Score	Expect	Identities	Gaps	StrandFrame
246 bits(133)	2e-62	203/237(86%)	4/237(1%)	Plus/Plus

Features:
84779 bp at 5' side: tumor necrosis factor receptor superfamily member 6 isoform
107011 bp at 3' side: cholesterol 25-hydroxylase

```
Query  512       GAATGATTATCAAGAGATCTACCATTGTATTATTTTAACCTCCACCGATACATTAGGCTT        571
                  |||||||  ||||      ||  ||||    ||||      ||||||||||||||||  |  ||||||||
Sbjct  89099229  GAATGATAATCAGTGGACCTACACTTGTCAGATTTTAACCTCCACCTAGACATTAGGGCA  89099288

Query  572       TGATCATTTAATTCCACTCCCTCTCTTTATACATAATAGAACTAAGGTCCA-GGACTAGG        630
                  ||||||||  ||  ||  ||||||  ||||||||||||  ||||||||||||||    ||    |||
Sbjct  89099289  GAATCATTTAGTT-CATTCCCTCACTTTATACATAAGAGAACTAAGGTCCAAAGA-GAGG  89099346

Query  631       AAAAGACTTGCTGATGTTGATACAACTTGCTAACTGCAGAGTTAGTACTTGAATAG-TAC        689
                  |||||||||||||||||||||||||||||||  ||        ||||||||||||||||||    ||
Sbjct  89099347  AAAAGACTTGCTGATGTTGATACAACTTGCAAATAAGAGAGTTAGTACTTGAATAGCAAC  89099406

Query  690       TTTCCTGGGCCA AGATAAGTGCCTTGTCTGCCAGACTGAtctctctctctctctctc          746
                      ||||||||||||||||  ||||||||||||||||||||||||||||||||||||||||||
Sbjct  89099407  TTTCCTGGGCCAAGATAGGTGCCTTGTCTGCCAGACTGATCTCTCTCTCTCTCTCTC  89099463
```

I_2_GAT

GAGCCTAGTCTTGCCTGGGAAGTTGCTATTCTCGGCTGACTCTCTTAGTTGCCAGTTGCCTGACATTGAGAGACTTTTC
CTCCTCATGCACCTCCCTTCTCCCTATCTCCCTCTCCTATTCTGAACTAATCCCTTCTGCCCTCGCTTT
AGGTGGAGGTTAAAATCTGACTCGTGGAGGTCCACTGATTATTTTTCTGGATACCCTCTTGCGCC
AACAGGTCCCTGTTGTCTTTTCCAGGTGTGTCCATCTGCATGGTACTGATGAACAG
AAATAAACAACATTCTTCTCCCTGTGCTTGATGTGCTTGCTCCTGATGGTGTTGGGCCTCTTTT
CTTTACCTCCAGGACT TTGTTTGGGCTGTTTTCAAAAAATG GAGGCCACACACCAATTGGGACCCTGGCCTGGTCAA
GGCCTAGGGCAAAAAGAGCTTCTCTTTCCTGTCACTGCCTATTGCAATCATGGATCCCCTGGAAAACAA
AATGCACCAGCTGAGAAAAAAGTG GATTGTGAATGATTTTCAAGAGATCTACC CTTGAATTATTTTAACCCCC
CCCTATACATTAGGCTTTGCTCATTTAATTCATTCCCTCACTTTGAACATAATAGAAAT GAGGTACAGGA
CTAAGAAAAGACTTGCTGATGTTGATACAACTTGCATGACTGCCTGTTAGAACTTG AATAAGTCTGTTCCTG
GCCAAG ATAGGTGCCT TGTCTGATCAGA CTGATCTCTCCCCTTCTCTCTACACCTCA

Homo sapiens chromosome 10q23, GRCh38.p2 Primary Assembly
Sequence ID: ref|NC_000010.11| Length: 133797422 Number of Matches: 1
Related Information
Range 1: 89099148 to 89099464

Score	Expect	Identities	Gaps	Strand
167 bits(90)	2e-38	247/322(77%)	14/322(4%)	Plus/Plus

Features:
84698 bp at 5' side: tumor necrosis factor receptor superfamily member 6 isoform
107010 bp at 3' side: cholesterol 25-hydroxylase

```
Query  431         TCTCTTTCCTGTCA-C—TGCCTATTGCAATCATGGATCC-CCTGGAAAACAAAATGCAC  486
           |||||||||||||||  |    |  ||  |  |||||||||  |  |||    ||    ||  ||||  |  |  ||
Sbjct  89099148  TCTCTTTCCTGTCATCAGTACC-A-TGCAATCATAGCTCCAGCTCAAAGACAACAGGGAC  89099205

Query  487         CAGCTGAGAAAAAAGTGGATTGTGAATGATTTTCAAGAGATCTACCCTTGAATTATTTTA  546
           |||||||  |  ||  ||||  ||     |||||||   |||     ||  ||||  ||||      ||||||
Sbjct  89099206  CAGCTGACACAAGAGTGTATCCAGAATGATAATCAGTGGACCTACACTTGTCAGATTTTA  89099265

Query  547AccccccccTATACATTAGGCTTTGCTCATTTAATTCATTCCCTCACTTTGAACATAATA  606
           |||  ||  ||||  ||||||||          |||||||  ||||||||||||||||   ||||||  |
Sbjct  89099266  ACCTCCACCTAGACATTAGGGCAGAATCATTTAGTTCATTCCCTCACTTTATACATAAGA  89099325

Query  607         GAAATGAGGTACA-GGACTAAGAAAAGACTTGCTGATGTTGATACAACTTGCATGACTGC  665
           |||  |  ||||  ||    ||    |  |||||||||||||||||||||||||||||||||         |
Sbjct  89099326  GAACTAAGGTCCAAAGA-GAGGAAAAGACTTGCTGATGTTGATACAACTTGCAAATAAGA  89099384

Query  666         CTGTTAGAACTTGAATA-AGTCTGTTCCT-GGCCAAGATAGGTGCCTTGTCTGATCAGAC  723
              |||||  |||||||||       ||  |||||  |||||||||||||||||||||||    |||||
Sbjct  89099385  GAGTTAGTACTTGAATAGCAACT-TTCCTGGGCCAAGATAGGTGCCTTGTCTGC-CAGAC  89099442

Query  724       TGATCTCTCCC-CT-TCTCTCT  743
           |||||||||  |  ||  |||||||
Sbjct  89099443  TGATCTCTCTCTCTCTCTCTCT  89099464
```

Y_44_GAT

ATCAATACCCCCTTTATGACTCTATTCTAGTGGAATCCATTCTGCCCTAATGTCTATCCGGAGGTTAT AACCTAAT TCGCTGAGGTACCTCGATTATCTTTCTG GATACCCTCTTTTGTCAACAGGTCCCTACTCCTATTTCC CTG GAG TCCATATTGCATGGTACTGATG AACAGAAAGAGACAACATTCTTCTCCCTGTGCTTGATGTGC TTGCTC CTG TTGCTGTTGGGCCTCCTTTCTTTACCTCCAGGACATTCTTTGG GCTGGTTTCAAAAAAT GGAGGCCACACA CCAATTGGGACCCT GGCACATGGTAGGCCTAAGAGAAGAAGAGCTTCTCTTTCCG TCTACTGCCCCATGCAA ACATGGCTCCAGCTGGAAGTCAAAATACACCCACCGTGACAAAAGTGGACCGTGACTGATTTT CAATAGATCTACAATCGTCTGA TGTTAACACTTCCTATACATTAGGGCAG AATCATTGGATT CATTCCCTC TAT TTATAGATA ATAGAACTGAGGGAGAGGACTGA GAAAAGACAGTGCTCTATGTTGTCTCTAC TTGCAT GAC TGCCTGA TAGTATCTTGCATAGTACGTT CCTGGGCCAAGATAGCGCCATGT CTGCCAGACTGA TCTCTC TCTCTCT CTCATCTCAC

Homo sapiens chromosome 10q23, GRCh38.p2 Primary Assembly
Sequence ID: ref|NC_000010.11| Length: 133797422 Number of Matches: 1
Related Information
Range 1: 89099055 to 89099292

Score	**Expect**	**Identities**	**Gaps**	**Strand**
159 bits(86)	3e-36	193/243(79%)	13/243(5%)	Plus/Minus

Features:
84605 bp at 5' side: tumor necrosis factor receptor superfamily member 6 isoform
107182 bp at 3' side: cholesterol 25-hydroxylase

```
Query  81           ATTCTGCCCTAATGTCTATCCGGAGGTTATAACCTAATTCGCTG-AGGTACCTC-GATTA  138
                    |||||||||||||||||||      ||||||||  ||  ||  |      |  ||  ||||  ||  |  |||||
Sbjct  89099292  ATTCTGCCCTAATGTCTAGGTGGAGGTTAAAATCTGACAAG-TGTAGGT-CCACTGATTA  89099235

Query  139          TCTTTCTGGATACCCTCTTTTGTCAACAGGTCCCTACTCCTATTT-CCCTGGAGTCCAT-  196
                    ||  ||||||||||  |||||  |||||  |  |||||||    |    |  |||      ||||||  |  ||
Sbjct  89099234  TCATTCTGGATACACTCTTGTGTCAGCTGGTCCCT-GTTGTCTTTGAGCTGGAG-CTATG  89099177

Query  197          ATTGCATGGTACTGATGAACA-GAAAGAGACAACATTCTTCTCCCTGTGCTTG-ATGTGC  254
                      ||||||||||||||||||  ||  ||||||||||||||||||||||||||||||      ||||||
Sbjct  89099176  ATTGCATGGTACTGATGA-CAGGAAAGAGACAACATTCTTCTCCCTGTGCTACCATGTGC  89099118

Query  255        - TTGCT-CCTGTTGCTGTTGGGCCTCCTTTCTTTACCTCCAGGACATTCTTTGGGCTGGT  312
                     |||      ||    |||  ||||||||||||    ||||||||||||||  |  ||  ||||  |||    |
Sbjct  89099117   ATTGGGGCCAATTGGTGTTGGGCCTCCACTCTTTACCTCCAGGCCTTTGTTTGTGCTCTT  89099058

Query  313        TTC  315
                   |||
Sbjct  89099057  TTC  89099055
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Differences in demographic and clinical variables among some African ethnic groups with T2DM

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Abstract

*Background***:** Diabetes mellitus, the commonest endocrine disease is approaching epidemic proportion worldwide driven by age, nutrition, genetic admixture, socio-economy and ethnicity.

Objective: To determine differences in demographic and clinical outcome of two ethnic African populations with type 2 diabetes.

Methodology: A cross sectional study of 240 type 2 diabetic persons (in two groups of 120 each) diagnosed using WHO criteria recruited from diabetes out-patient clinics of tertiary health facilities in Nigeria, West Africa and Lesotho, Southern Africa. They had no apparent diabetic complication(s). Information on age, sex, blood pressure, body mass index (BMI) and glycosylated haemoglobin determined by standard procedure were obtained from hospital records and analyzed using Statistical Package for Social Sciences (SPSS) version 20.

Results: Participants age ranged 32-82 years with mean of 52.4(±13.9) years. Nigerians were significantly older than Basotho (57.40±10.72 vs 47.40±14.94; p=0.0001). There were no significant differences in gender and mean BMI ((p=0.267; p=0.264 respectively). Basotho had higher proportion of obese diabetics than Nigerians (40 % vs 20%) who were more in the overweight category [45% vs 25%], (p=0.003). Mean HbA1c was higher in Basotho than Nigerians [10.64±3.95 vs 8.27±1.53] (p=0.0001) while mean blood pressure of study participants were within normal though Nigerians had non-significantly higher mean systolic pressure [135.58±20.13 vs 133.33±26.71; p=0.603]. BMI of study participants correlated positively with age, BP and HbA1c irrespective of ethnicity.

Conclusion: There are differences in age, BP, BMI and HbA1c of ethnic African diabetics. Improved education, access to quality health care and social insurance policies should drive preventing DM and improve management outcomes.

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Keywords: *Ethnicity, clinical outcomes, socio-demography, Type 2 diabetes.*

Abstrait

Contexte **:** Le diabète sucré, la maladie endocrinienne la plus courante, s'approche de la proportion épidémique dans le monde en raison de l'âge, de la nutrition, du mélange génétique, de la socio-économie et de l'ethnicité.

Objectif : Pour déterminer les différences dans les résultats démographiques et cliniques de deux populations ethniques africaines atteintes de diabète de type 2.

Méthodologie : Une étude transversale de 240 personnes diabétiques de type 2 (en deux groupes de 120 chacun) diagnostiquées selon les critères de l'OMS recrutées dans les cliniques externes de diabète des établissements de santé tertiaires au Nigéria, en Afrique de l'Ouest et au Lesotho, en Afrique Australe. Ils n'avaient aucune complication (s) diabétique apparente (s). Les informations sur l'âge, le sexe, la pression artérielle, l'indice de masse corporelle (IMC) et l' hémoglobine glycosylée déterminées par la procédure standard ont été obtenues à partir des dossiers hospitaliers et analysées à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 20.

Résultats: L'âge des participants variait de 32 à 82 ans avec une moyenne de 52,4 (± 13,9) ans. Les Nigérians étaient significativement plus âgés que les Basotho (57,40 ± 10,72 vs 47,40 ± 14,94 ; p = 0,0001). Il n'y avait pas de différences significatives dans le sexe et l'IMC moyen ((p = 0,267; p = 0,264 respectivement). Basotho avait une proportion plus élevée de diabétiques obèses que les Nigérians (40% vs 20%) qui étaient plus dans la catégorie du surpoids [45% vs 25 %], (p = 0,003). L'HbA1c moyenne était plus élevée parmi les Basotho que chez les Nigérians [10,64 ± 3,95 vs 8,27 ± 1,53] (p = 0,0001) alors que la pression artérielle moyenne des participants à l'étude était dans la normale, bien que les Nigérians aient une systolique moyenne non significativement plus élevée pression [135,58 ± 20,13 vs 133,33 ± 26,71 ; p = 0,603]. L'IMC des

participants à l'étude était corrélé positivement avec l'âge, la PA et l'HbA1c indépendamment de l'origine ethnique. *Conclusion:* Il existe des différences d'âge, de PA, d'IMC et d'HbA1c des diabétiques ethniques africains. Une éducation améliorée, l'accès à des soins de santé de qualité et des politiques d'assurance sociale devraient favoriser la prévention du diabète et améliorer les résultats de gestion.

Mots clés : *Ethnicité, résultats cliniques, socio-démographie, diabète de type 2.*

Introduction

Noticeable shift in disease pattern from communicable to non-communicable diseases is dependent on factors like age, ethnicity, nutrition, sedentary lifestyle, genetic admixture, migration history and socio-economic factors. [1, 2]

Diabetes is defined as chronic hyperglycaemia from Insulin secretory defect, resistance or both with consequent long term complications [3]. In 2015, between 9.5 and 29.3 million Africans lived with diabetes of which an estimated two thirds are undiagnosed, the highest for any region [4]. The country specific prevalence of diabetes for Nigeria ranged from 2.1 to 6.7% with prevalence of 3.2% in Jos, and 6.8% in Port Harcourt [4,5,6]. Ghana had a prevalence of 1.9%, Lesotho 2.9% and South Africa 7.0% [4]. In 2017, worldwide prevalence of diabetes was 425 million projected to increase to 629 million by 2045[7]. Drivers of this diabetes epidemic include family history of diabetes and hypertension, increased intake of high calorie food, sedentary lifestyle, obesity, foetal programming and socioeconomic factors related to rapid development and urbanization [2].

While ethnicity classification is based on shared cultural values, customs and their meaning, there is evidence that its combination with other social determinants of health disproportionately affect the process and quality of care and clinical outcomes in diabetes particularly of body mass index (BMI), glucose, lipids and blood pressure control [8]. In a systematic review, Campbell et al reported significant impact of race and ethnicity on differences in monitoring of glycaemic and blood pressure control in diabetics [9].

DM is recognized as a leading cause of blindness, kidney failure, cardiovascular disease and non-traumatic limb amputation from increased risk of microvascular and macrovascular damage due to poor glycaemic, blood pressure and lipid control with higher risk of infections [3,10]. Maintaining good blood pressure, glycaemic and lipids control, compliance to clinic visits, medication adherence and self-care activities can reduce T2DM morbidity and mortality [11,12]. Ongoing strategies to reduce or eliminate racial and ethnic disparities in health has focused on improving access to quality care [13]. However, an important but often ignored component is the role of social determinants of health on outcomes and health disparities [14]. Factors that interact as social determinants of health (SDOH) are psychosocial, political, economic, cultural, socioeconomic circumstances and neighborhood environment [15,16]. SDOH have also been found to influence health inequalities within and between countries suggesting they may help explain racial and ethnic differences in health outcomes [15]. Health care professionals are therefore expected to be skilled in assessing social determinants of health and also take them into consideration in clinical care [14,17].

There is dearth of study on ethnic disparity in anthropometric indices, clinical outcomes and access to quality health care. Such study may help better understanding of mechanisms and relationship that will help to develop culture related and cost effective program for diabetes care, prevention and management [18].

Objectives

This study sought to determine and compare demographic and clinical outcome variables among cohort of adult Nigerian (West African) and Basotho (Southern African) ethnic population with type 2 Diabetes.

Subjects and methods

A total of 240 apparently well T2DM patients (120 from each study center) attending the Endocrine clinic of Medical Outpatient Department of Queen Elizabeth II Hospital, Maseru Kingdom of Lesotho and the Dame Adebutu Diabetes Care Center, OOUTH, Sagamu, Nigeria were recruited consecutively over a 4-month period of August to November 2010 for the study. Excluded from the study were patients with concurrent diabetes complications including hyperglycaemic emergency, infections or limb amputation. Diabetes was defined using WHO criteria [19]. Information on age, sex, anthropometry (BMI), blood pressure (BP) and laboratory parameter HbA1c determined by standard procedure were culled from hospital medical records of participants.

Data obtained were collated and analyzed using SPSS (IBM compatible) version 20. The results are presented as mean (±SD) and proportions (%). Tests of association and correlation were done with Chi-square and Pearson correlation coefficient respectively while level of statistical significance was set at $p \leq 0.05$.

Ethical approval

Ethical approval was sought and obtained from the Olabisi Onabanjo University Teaching Hospital, Sagamu, Health Research and Ethics Committee (OOUTH-HREC).

Table 1: Demographic, anthropometric, clinical and laboratory characteristics of study participants.

Variables	Nigeria (N=120) Mean±SD (n= %)	Basotho (N=120) Mean±SD (n=%)	t-value/χ^2-value	*P*-value
Age (years)	57.40±10.72	47.40±14.94		
Age group				
32-39 (yrs) %	2 (1.7)	26 (21.7)	4.212	0.0001*
40-60 (yrs) %	76 (63.3)	68 (56.6)		
≥61 (yrs) %	42 (35.0)	26 (21.7)	12.390 (χ^2)	0.002*
Sex				
Male (%)	56 (46.7)	44 (36.7)	1.234 (χ^2)	0.267
Female (%)	64 (53.3)	76 (63.3)		
BMI(Kg/m2)	26.66±4.79	27.96±7.57	-1.122	0.264
BMI Group				
underweight < 19.0	4 (3.3)	18 (15)		
normal 20.0- 24.9	38 (31.7)	24 (20.0)		
overweight 25-29.9	54 (45.0)	30 (25)	14.285 (χ^2)	0.003**
obese > 30.0	24(20.0)	48 (40)		
SBP(mmHg)	135.58±20.13	133.33±26.71	0.521	0.603
DBP(mmHg)	80.83±12.99	84.17±13.35	-1.386	0.168
HbA1c (%)	8.27±1.53	10.64±3.95	-4.333	0.0001*

BMI=Body Mass Index; SBP= systolic blood pressure; DBP= diastolic blood pressure; HbA1c= Glycosylated haemoglobin

Results

The study involved 240 T2DM participants divided into two groups of 120 each of Nigerians and Basotho recruited for the study.

Among the study population, Nigerian diabetics were significantly older, age range 32-84 years, mean age 57.4% (±10.72) than their Basotho counterparts, age range 31-74 years, mean age 47.4 (±14.94) respectively (p=0.0001). Twenty six (21.7%) Basotho below age 40 years had type 2 diabetes compared with only 2 (1.7%) Nigerians (p=0.002). Though both ethnic groups had higher proportion of females than males with type 2 diabetes, this difference was not statistically significant (p=0.267). Nigerians with type 2 diabetes above age 60 years were more compared with the Basotho [42 (35.0%) vs 26 (21.7%), (p=0.002)]. [Table 1].

The mean body mass index (BMI) of both ethnic population ranged 15.80-47.65 with mean of 26.66 (± 4.79) in Nigerians and 27.96 (±7.57) in Basotho with no significant difference (p=0.264). Classifying obesity using WHO criteria revealed a higher proportion of obese type 2 diabetes (BMI>30) among Basotho compared with Nigerians (40% vs 20%). There were also more Basotho at extremes of weight categories, (underweight and obese) than Nigerians (55% vs 23.3%). The Nigerians were more in the overweight category (45% vs 25%), (p=0.003). [Table 1]

The study participants long term glycaemic control assessed by HbA1c level ranged between 5.7-19.5 and was better in Nigerians than the Basotho, HbA1c 8.2% (±1.53) vs 10.64 (±3.95) respectively (p=0.001).

The mean systolic and diastolic blood pressure among studied ethnic group were within normal limits [135±20.13 vs 133.33± 26.71 and 80.83± 12.99 vs 84.17 ±13.35 mmHg] with no significant difference (p=0.603 and 0.168 respectively). Nigerians had higher

mean SBP than the Basotho who had higher mean DBP (Table 1). Irrespective of ethnic grouping, our study participants' BMI correlated positively with age, blood pressure and HbA1c, [0.0001, 0.001, 0.030 and 0.000] respectively (Table 2).

Table 2: Correlation matrix (r) ofAge, BMI, blood pressure and HbA1c among Participants.

		BMI(Kg/m2)	SBP(mmHg)	DBP(mmHg)	HbA1c(%)
BMI(kg/m2)	Pearson Correlation				
	Sig. (2-tailed)				
SBP(mmHg)	Pearson Correlation	0.345**			
	Sig. (2-tailed)	0.000			
DBP(mmHg)	Pearson Correlation	0.370**	0.750**		
	Sig. (2-tailed)	0.000	0.000		
HbA1c(%)	Pearson Correlation	-0.277**	-0.390**	-0.281**	
	Sig. (2-tailed)	0.002	0.000	0.002	
Age (yrs)	Pearson Correlation	0.349**	0.306**	0.198*	-0.448**
	Sig. (2-tailed)	0.000	0.001	0.030	0.000

*** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).*

Discussion

This was a cross sectional, hospital-based study seeking to determine differences in demographic and clinical outcomes in 240 patients of 2 ethnic groups with type 2 Diabetes that were apparently free of diabetes related complications.

Mean age of study participants with type 2 diabetes was above 40 years with the Nigerian being significantly older than the Basotho similar to earlier reported age prevalence of Type 2 diabetes patients worldwide [1,2,4]. Our study had excluded patients below age 30years, the diagnostic cut off age for T1 diabetes which has a worldwide low prevalence, Nigeria inclusive [3-5]. This study finding of age differential presupposes that ethnic Basotho might have early age onset of type 2 diabetes compared with their Nigerian counterparts. This may be predicated on factors such as diet, environmental and socioeconomic differences alongside other social determinants of health (SDOH) coupled with possible genetic predisposition [2,7,8]. These presumptions will need further study to elucidate factors and underlying mechanism for the presumed early age of onset of type 2 diabetics. The increased prevalence of type 2 diabetes after age 40 may have been preceded by about 10 years of symptoms of glucose intolerance, the pre-diabetes state which may account for the high proportion of undiagnosed diabetics in the African population [2,7,20].

Our study showed no significant gender difference in prevalence of T2 diabetes between the 2 ethnic groups similar to earlier reports of no gender difference in diabetes prevalence worldwide though more women than men are reportedly afflicted with diabetes [3,5,7]

There are reports of higher prevalence of type 2 diabetes in urban compared with rural area consequent on rapid urbanization, sedentary lifestyle, occupation, unhealthy diet and obesity [5,19-21]. Sagamu is a Nigerian suburban town where farming is the main occupation while the Basotho population study location of Queen Elizabeth II Hospital, Maseru, Lesotho's national capital (an urban area) has predominant occupation in the civil service with its readily available support services of fast foods outlets and public transportation system [22,23]. The factors promoting increasing diabetes prevalence is being addressed by increasing awareness, health education and regular population screening [24].

Our study revealed poor long term glycaemic control among the studied groups that is worse among the Basotho ethnic population using HbA1c. This finding agrees with earlier reports of differences in glycaemic control among ethnic groups ascribed to diet, access to medications and health care services with social insurance schemes and food insecurity among others [13,25]. It is noteworthy that in Nigeria, health insurance scheme is in its infancy with health expenditure being mainly out of pocket [22]. On the contrary, the Lesotho government has a social security health insurance scheme in place whereby patients' health expenses are mostly borne by government [23]. Prevention of chronic diabetes complications and glycaemic control depend on such factors as knowledge and attitude of diagnosed diabetics and lifestyle modification. The poorer

glycaemic control among the Basotho may therefore be predicated on patient centered factors of poor clinic and medication compliance, unhealthy nutrition and physical inactivity [23].

Though mean body mass index of the 2 ethnic populations was not significantly different, classifying BMI using WHO criteria [26] revealed more obese Basotho than Nigerians in our study population. This can be ascribed to the high fat diet, physical inactivity and socio-economic status of the Basotho [23]. However, Nigerians with type 2 diabetes are more in the overweight category which may probably be due to their being more physical active and with healthier dietary habits [22]. This is in consonance with the Healthy Peoples 2020 advisory committee report on promotion and disease prevention objectives [17]. Normalizing BMI therefore becomes an important goal for type 2 diabetes management and control [24, 26,27]. The occurrence of T2DM in lean individuals has recently attained prominence particularly in underweight and normal weight individuals depending on the ethnic population [20,26]. A possible pathway of Type 2 diabetes in lean weight adults may be autoimmune as in Latent Autoimmune Diabetes in adults (LADA) [28]. Differences in T2DM prevalence in the absence of overweight and obesity calls for more studies on pathophysiology of T2DM in lean individuals.

The mean blood pressure of the 2 ethnic groups falls within normal with no significant differences (p=0.168; 0.603). However, the mean systolic pressure of Nigerian group is higher than Basotho with no significant difference in the mean diastolic blood pressure. Earlier studies on blood pressure occurrence with diabetes reported 45-55% of coexisting hypertension with diabetes [29,30]. A larger proportion of Nigerians in this study have elevated systolic blood pressure.

Among our study population, BMI correlated positively with blood pressure, HbA1c and age irrespective of ethnic grouping. This may be ascribed to factors like lifestyle, high salt and fatty diet, socio-economic and other social determinants of health factors prevalent in the African society [8,9,13,29].

Conclusion

Ethnic differences in age, BMI and long term glycaemic control among Nigerian and Basotho T2DM patients may be consequent on biomedical and social factors. Intervention in social determinants of health to improve access to facility, quality care, redistributive social policies and circumstances will reduce prevalence and improve outcome of type 2 diabetes.

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References

1. Centers for Disease Control and Prevention: National Diabetes Statistics Report, 2014. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services; Atlanta: 2014.
2. Zimmet P Z. Diabetes and its drivers; the largest epidemic in history? Clin Diabetes and Endocrinol 2017; 3:1.
3. Alberti KG and Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications Part 1: Provisional report of a WHO consultation. Diabet Med 1998; 15 (7): 539-553.
4. International Diabetes Federation; International Diabetic Federation Atlas; 7th edition, 2015 Executive summary: Diabetes: A global emergency. http;//www.diabetesatlas.org
5. Pueppet F. H. The prevalence of diabetes mellitus and associated risk factors in adults in Jos; National Postgraduate Medical College of Nigeria. 1996, Part 2 FMCP dissertations
6. Nyenwe EA, Odia JO, Anele EI and Aarono SB. Type 2 diabetes in adult Nigerians; A study of its prevalence and risk factors in Port Harcourt, Nigeria; Diab Res Clin Prac 2003; 62; 177-185.
7. International Diabetes Federation; International Diabetic Federation Atlas; 8th edition, 2017. http;// www.diabetesatlas.org.
8. Walker RJ, Williams JS and Egede LE. Impact of Race/Ethnicity and Social Determinants of Health on Diabetes Outcomes. Am J Med Sci. 2016 April; 351(4): 366–373. doi:10.1016/j.amjms.2016.01.008.
9 Campbell JA, Walker RJ, Smalls BL and Egede LE. Glucose control in diabetes: the impact of racial differences on monitoring and outcomes. Endocrine. 2012; 42:471–482. [PubMed: 22815042]
10. Ferreira LT, Saviolli IH, Valenti VE and Abreu LC. Diabetes mellitus: hyperglycemia and its chronic complications. *ABCS* Health Sciences 2011; 36(3):182-188.

11. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. BMJ. 1998; 317:703–713. [PubMed: 9732337]
12. American Diabetes Association (ADA). Standards of medical care in diabetes-2015. Diabetes Care 2016; 39(Supl.):1-112.
13. Kurian AK and Cardarelli KM. Racial and ethnic differences in cardiovascular disease risk factors: a systematic review. Ethn Dis. 2007; 17:143–152. [PubMed: 17274224]
14. Solar O and Irwin A. A conceptual framework for action on the social determinants of health. Social Determinants of Health Discussion Paper 2 (Policy and Practice). World Health Organization; Geneva: 2010.
15. Marmot M. Social determinants of health inequities. The Lancet. 2005; 365:1099–1104
16. Williams DR, Costa MV, Odunlami AO and Mohammed SA. Moving upstream: how interventions that address the social determinants of health can improve health and reduce disparities. J Public Health Manag Pract. 2008; 14(Suppl):S8–S17. [PubMed: 18843244]
17. Healthy People. Secretary's Advisory Committee on Health Promotion and Disease Prevention Objectives for 2020. Healthy People 2020: An Opportunity to Address the Societal Determinants of Health in the United States. Jul 26. 2010 Available from: http://www.healthypeople.gov/2020/topicsobjectives2020/overview .aspx?topicid=39
18. Marmot M. Fair society, healthy lives (the Marmot review). Department of Health; London: 2010.
19. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications; Part 1: Diagnosis and classification of diabetes mellitus. Department of Non-communicable Disease Surveillance, Geneva, 1999.
20. Gning SB, Thiam M, Fall F, *et al*. diabetes mellitus in Sub-Saharan Africa epidemiological aspects and management issues. Med Trop (Mars). 2007 Dec; 67 (6): 607-611
21. Raimi TH, Odusan O and Fasanmade OA. Correlation of Anthropometric Indices and Age with Fasting Plasma Glucose among Inhabitants of Ogun State, South-West Nigeria. 2016. British Journal of Medicine & Medical Research. 2017; 19 (5): 1-11
22. Odusan O, Amoran OE, Olubodun AB and Salami BA. Type 2 Diabetes Mellitus: Awareness, Knowledge and Associated Risk factors among Commercial Bank workers in Sagamu, Nigeria. Nigerian Medical Practitioner. 2017; vol 71, No 5-6, 81-87.
23. Shonubi AMO, Odusan O, Oloruntoba DO, Agbahowe SA and Siddique MA; Health for All in a Least-Developed Country. Journal of the National Medical Association. 2005; vol. 97 (7):1020-1026.
24. American Diabetes Association. Strategies for improving care. Sec.1: In Standards of Medical Care in Diabetes—2015. Diabetes Care. 2015; 38(Suppl. 1):S1–S94.
25. Lynch CP, Strom Williams JL, Reid J, *et al*. Racial/Ethnic differences in multiple diabetes outcomes in patients with type 2 diabetes in the southeastern United States. Ethn Dis. 2014; 24(2):189–194. [PubMed: 24804365]
26. World Health Organization: Obesity;www.who.int/features/factfiles/obesity/facts.
27. Ali MK, Bullard KM, Saaddine JB, *et al*. Achievement of goals in U.S. diabetes care, 1999-2010. N Engl J Med 2013; 368(17):1613-1624.
28. Strenstrom G**,** Gottsater A. Bakhtadze E, Berger B and Sundkvist G,; Latent autoimmune diabetes of adults; Definition, Prevalence, β cell function and treatment; Diabetes 2006 Dec; 54(supp 2), s68-72. Available from http;//lup.lub.lu.se/record/147737 DOI; 10.2337/diabetes.54 suppl-2.s68
29. Unadike BC, Eregie A and Ohwovoriole AE. Prevalence of hypertension among persons with diabetes mellitus in Benin City, Nigeria. Niger J Clin Pract 2011; 14 (3): 300-302 doi:10.4103/1119-3077.86772
30. Liu X and Song P. Is the association of diabetes with uncontrolled blood pressure stronger in Mexican Americans and blacks than in whites among diagnosed hypertensive patients? Am J Hypertension. 2013; 26(11):1328–1334.

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Radiation Oncology in Nigeria; rescuing a fast receding, yet crucially important medical specialty

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Abstract
Radiotherapy is an important treatment modality in the management of malignant tumours. It is estimated that more than half of cancer patients diagnosed in Nigeria will require radiotherapy as a component of their treatment. This translates to about a hundred thousand cancer cases requiring radiotherapy in Nigeria on an annual basis. However, radiation facilities in the country are unable to meet this need. There are only eight treatment centres serving the whole country. Each of these centres have limited facilities and are often bedevilled with various problems including equipment breakdown. The travails of radiotherapy practice in Nigeria is discussed in view of the increasing cancer burden in the country. Recommendations are made on how to improve access to radiotherapy care in the country as we go forward.

Keywords: *Radiotherapy Oncology Nigeria*

Abstrait
La radiothérapie est une modalité de traitement importante dans la prise en charge des tumeurs malignes. On estime que plus de la moitié des patients cancéreux diagnostiqués au Nigéria nécessiteront une radiothérapie dans le cadre de leur traitement. Cela se traduit par une centaine de milliers de cas de cancer nécessitant une radiothérapie au Nigeria sur une base annuelle. Cependant, les installations de radiation du pays ne sont pas en mesure de répondre à ce besoin. Il n'y a que huit centres de traitement desservant tout le pays. Chacun de ces centres a des installations limitées et est souvent confronté à divers problèmes, y compris la panne d'équipement. Les difficultés de la pratique de la radiothérapie au Nigéria sont discutées compte tenu de la charge croissante du cancer dans le pays. Desrecommandations sont formulées sur la manière d'améliorer l'accès aux soins de radiothérapie dans le pays à mesure que nous progressons.

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Mots clés: *Radiothérapie Oncologie Nigeria Amélioration*

Introduction

Radiation Oncology, previously called radiotherapy is a specialty in crisis, in Nigeria, at least, possibly in most part of Africa. [1] Yet, it makes a crucial and irreplaceable contribution to the treatment of cancer cases in Nigeria. Since its inception in the seventies at the Lagos University Teaching Hospital (LUTH), it spread out to other centres in the country culminating today in eight centres in the country with a centre in each of the country's geopolitical zones.

Radiation oncology is an important component of the multimodal treatment of cancer. It has been estimated that about 50% of cancer patients will require radiation treatment as a component of their cancer care modalities. [2] This is from data collected in developed countries where many patients are seen with early disease stages. Early cancer cases are more likely to be amenable to surgery and are less likely to require radiotherapy and systemic treatment like chemotherapy or hormone therapy. In many developing and underdeveloped countries like Nigeria, most cancer cases are seen at advanced stages where palliative radiotherapy plays much more significant roles. [3] It is therefore not inconceivable that a larger proportion of patients in low to medium income countries like Nigeria will require radiotherapy in the course of managing their malignancies. This will put more pressure on the equipment and the care-givers. One will therefore understand the fact that there may indeed be greater need for radiotherapy facilities per unit population in the less developed countries.

Cancer is a growing worldwide problem. In 2018, it was estimated that over 18 million new cancer cases were diagnosed. About 40% of these were in low to medium development area including a large part of Africa. However, of the over 9.5 million cancer deaths recorded in the year, more than half were in the less developed countries. [8] Cancer incidence is estimated to rise progressively to about 22 million worldwide over the next two decades.

Reliable cancer statistics are hard to come by in Nigeria. Most of the figures available are hospital-based data and do not reflect the true state of things in the population. There is a gross under-reporting of cancer cases in Nigeria and most parts of Africa. In a review of literature on cancer registry from across the world, only 1% of the literature was from Africa compared to Europe 34% and Asia, 42%. [8]

In a 2012 paper reporting cancer rates in two population-based cancer registries in Nigeria, the Ibadan and Abuja Population Based Cancer Registries, 4521 cases of invasive malignancies were reported from the two centers over a two-year period giving age standardized incidence rate (ASR) for all cancers in males and females of 62.4 and 131.1 per 100 000 respectively. [10] With a population estimated at about 200 million [9], over 200,000 cases of cancer will be seen per year in the country. Some have even suggested that the figure may be as high as half a million cancer cases in the country per annum. [11]

From the forgoing, it is safe to suggest that about 100,000 Nigerians will require radiotherapy services in Nigeria annually. The International Atomic Energy Agency (IAEA) recommends a radiotherapy machine for 500 new cancers cases [12] or 4-8 radiotherapy centers per million people. [13] It is therefore estimated that Nigeria requires a minimum of 200 radiation treatment equipment installed and working. This is a far cry from the current situation where the whole country has only eight machines with only a small fraction working at any point in time. Indeed, there were occasions when none of the equipment was working, though, this is rare. It is more common to have either two or three working at one time.

Role of radiotherapy in patients care

Radiotherapy involves the use of ionizing radiation in the management of medical conditions most of which are due to malignant tumours and a few non-malignant diseases like keloid, Radiotherapy can be used as the sole treatment or in conjunction with other treatment modalities. Cancer treatments are usually multimodal involving specialties in surgery, radiation oncology, medical oncology, internal medicine, radiology, pathology, gynaecology, haematology, etc.

Radiotherapy is particularly suited for patients with advanced diseases for which the rigors and possible complications of surgery might not be desirable because of its non-invasiveness and also being an essentially painless procedure. It is therefore used quite well in advanced cases where palliation, rather than cure is the main expected (desired) outcome.

Radiotherapy can also help in reducing the scale and extent of surgery. Pre-operative radiation treatment can reduce the size of tumour and improve or ensure its resectability. It may also help to reduce tumour spread as a result of surgical handling.

Post-operatively, radiation treatment can help in improving local control by sterilizing the tumour bed following radical surgery. In settings where frozen sections are not routinely taken from resection margins at surgery, post-operative radiation treatment is an invaluable tool in preventing tumour recurrence from residual lesions left at surgery.

Another area of strength for radiotherapy is in treating tumour in cosmetically sensitive parts of the body like the face. Radiotherapy usually have better cosmetic and functional outcome than surgery.

Radiotherapy is used in the treatment of bone pain from malignant infiltration, it can be used to relieve symptoms in patients with spinal metastases, and it is also used to achieve haemostasis in cervical cancer and other bleeding tumours. Radiotherapy can also be applied intraoperatively to reduce the chance of local recurrence and scar implantation.

Radiotherapy also plays important roles in the management of non-cancers/non-malignant cases. The most non-malignant use of radiation treatment in our setting is for keloid. Post-operative radiation treatment to the keloid bed is a common practice in Nigeria. [4] However, some European countries including Germany have developed extensive protocol for the use of radiation therapy in non-malignant cases. It has been estimated that up to 30% of radiation treatment in some academic (i.e. teaching) hospitals in Germany are for benign non-malignant diseases. [5]

The benign disorders for which radiotherapy is being used are broadly classified into inflammatory, degenerative, proliferative and functional disorders. These include painful arthrosis in the knees, hip, elbows and fingers, painful bursitis or fasciitis, keloids, Mobus Dupuytren, Desmoid tumours, haemangiomas etc. [6]

Kilovoltage radiation beams and electrons from Linear Accelerators (LINAC) are particularly suited for treating superficial benign ailments like keloid. Unfortunately, most Nigeria radiotherapy centres have no kilovoltage equipment and those with LINACs are not using electrons routinely due to technical difficulties.

Radiotherapy treatment of benign tumours other than keloid and a few others, is poorly developed in

Nigeria. No center in Nigeria is offering radiation treatment for osteoarthritis and other benign painful degenerative diseases which are common in our elderly. There is a need to take a look at this area and pay some attention to it.

Types of Radiotherapy

There are basically two types of radiotherapy; these are external beam radiotherapy (EBRT) and Brachytherapy. External beam radiotherapy involves the use of radiation sources at a measurable distance from the area to be treated. EBRT directs the radiation beam to the area of the body to be treated. This is the most common type of radiation treatment and is the most widely available.

EBRT is suitable for treating large tumours or field sizes and can encompass whole or multiple body regions within a single large field. EBRT is delivered using Kilovoltage X-ray equipment, Cobalt-60 based EBRT equipment or linear and other types of particle accelerators.

The radiation for EBRT can be derived from radioactive sources like Cobalt 60, or can be generated using electrical equipment. This uses the basic principle of x-ray production in which accelerated electrons are brought to a halt which then transform their kinetic energy into x-ray and thermal energy. In Linear Accelerators, the accelerated electrons (and other particles) can be used to treat patients directly or the electrons can be used to produce x-rays.

Photons (e.g. x-rays) and electrons have differing properties that make them useful in different settings. Electrons have poor penetrating power and are therefore more useful in treating superficial lesions especially in the skin. Photons (x-rays) on the other hand are better suited for more deeply situated tumours.

EBRT is also used in many of the recently developed improved treatment delivery modalities like Intensity Modulated Radiotherapy (IMRT), Image-Guided Radiotherapy (IGRT) etc.

Brachytherapy on the other hand involves the use of radiation sources that are located within the tumour or the area to be treated. These sources can be in form of needles, wires, seeds and plaques. The sources used in brachytherapy are contained in an inert material and therefore do not react chemically with the body tissue. These are called sealed sources, unlike unsealed sources that are used in nuclear medicine practice for diagnosis and treatment.

Brachytherapy is most suitable for treating small lesions with high doses of radiotherapy without significantly affecting nearby organs and structures. Brachytherapy can be used as the sole radiotherapy modality or it can be used in conjunction with EBRT. In such circumstances, EBRT is used to treat the bigger tumour with bigger fields usually encompassing regional lymph nodes while brachytherapy will be used to give a boost to the area primarily affected.

Brachytherapy is widely used in the management of cancers in different parts of the body. It is used to give boost to the tumour bed in breast cancer after minimal surgery, in cervical cancer, prostate cancer, oesophageal cancer etc.

Intraoperative radiotherapy is another useful way by which radiation is delivered to the required areas while patient is undergoing surgery. This could be in form of delivery of EBRT to the tumour bed following resection or could involve insertion of "permanent" brachytherapy sources in the operated areas. These sources are usually in form of seeds with short half-lives. This have the benefit of improving the rate of local control and overall treatment outcome.

Effects of inadequate facilities

Cancer is a progressive disease. It requires immediate treatment to avoid morbidity, mortality and disease progression to more advanced stages. Delay in treatment will invariable lead to disease progression and poorer outcomes. Every case of cancer should therefore receive prompt, relevant and adequate treatment.

However, due to inadequate equipment, most cancer cases requiring radiotherapy cannot be treated promptly. The waiting time for radiotherapy can be as long as three months in some cases. In crisis periods when there is breakdown of machines in multiple centers waiting time can be as long 6 months or more.

The consequences of these inadequacies are many and grave for the cancer patients in the country. For some, it is a matter of life and death and many patients will be lost as a result of their inability to receive radiation treatment. Others will end up with severe morbidity and some will have poor quality of life as a result of inability to treat their symptoms including bleeding, severe pain, limb paralysis as a result of cord compression etc.

Training is another area that suffers greatly from poor and inadequate radiation equipment. Nigeria has active training programs in radiotherapy

and radiation oncology. There are postgraduate residency training programs in radiotherapy and clinical oncology leading to the awards of fellowships of the West African College of Surgeons (FWACS) and National Postgraduate College of Radiology (FMCR). This training is conducted in all the centers with at least one radiation treatment equipment. There are also training programs for Therapy Radiographers and Medical Physicists.

However, most of the centers are unable to meet the required standards for training world class radiation oncologists due to poor facilities. It follows therefore that most radiation oncologists and other radiotherapy staff trained in Nigeria are not adequately exposed to modern radiotherapy practices including IMRT, IGRT, brachytherapy etc. They will all require further intensive hands-on training in more developed radiotherapy centers abroad to work outside Nigeria. This has grave implications for the quality of treatment received by cancer patients in Nigeria.

Radiotherapy facilities in Nigeria

Nigeria is a sub-Saharan country located in the west coast of Africa. Nigeria is Africa's most populous country with an estimated population of about 200 million. [9] It is also an oil-producing country with a strong presence in the Organization of the Petroleum Exporting Countries (OPEC). However, a pervasive culture of corruption in the country over the years have led to poor infrastructural developments in all the sectors of Nigeria's economy.

In the health sector, radiotherapy and radiation oncology is particularly well hit by lack of funds for infrastructural development. Africa as a whole has the least number of radiation treatment centers per population. According to IAEA figures, Africa has about 2% of megavoltage equipment in the world with Europe and Latin American (and the Caribbean) having 34% each, Asia 27% and North America 26%. Figure 1 [14]. In addition, more than half of the 2% in Africa are located in South Africa and Egypt [15] leaving the rest of Africa including Nigeria with less than 1% of the entire world megavoltage radiation treatment equipment.

Nigeria has nine radiotherapy centers with megavoltage external beam radiation treatment facilities. These are distributed around the major geopolitical zones with one each in Lagos University Teaching Hospital (LUTH), Lagos and University College Hospital (UCH), Ibadan, both in the South West, one at the University of Benin Teaching Hospital (UBTH), Benin in the South-South, one at the University of Nigeria Teaching Hospital (UNTH), Enugu, South East, one at the National Hospital, Abuja, Middle Belt,

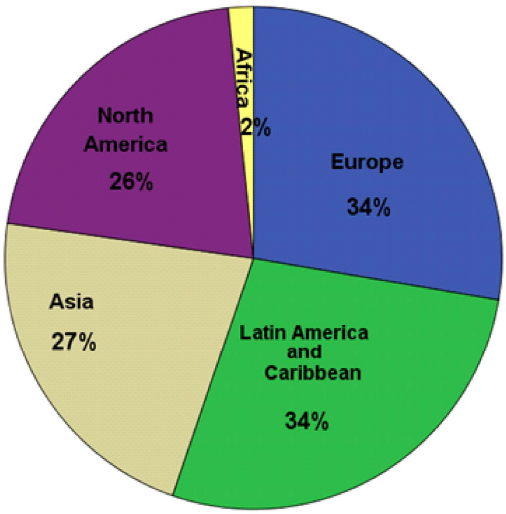

Fig. 1: Distribution of EBRT Facilities across the Continents

one at Ahmadu Bello University Teaching Hospital, ABUTH, Zaria in the North Central, one at the Uthman Dan-Fodio University Teaching Hospital (UDUTH), Sokoto in North West and the only privately owned radiotherapy center in Nigeria at the EKO Hospital, also in Lagos. The only brachytherapy-only centre is located at the Federal Medical Center, Gombe in North Eastern geopolitical zone of Nigeria. (Figure 2)

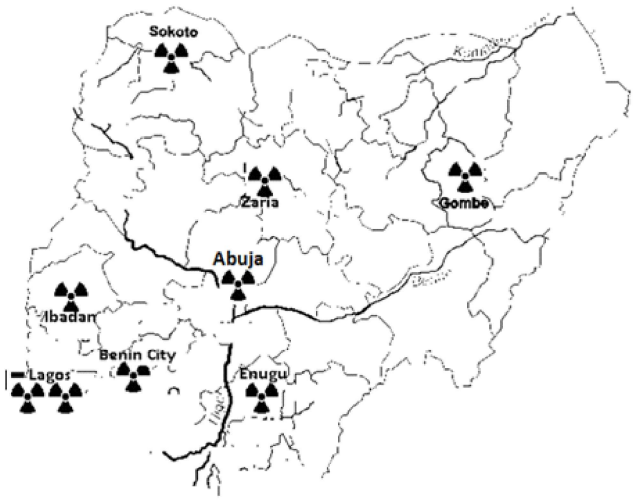

Fig. 2: Radiotherapy Centres in Nigeria

These eight centers serve the Nigeria's teeming patients with some affluent patients going to other countries including Egypt, India, United Arab Emirates, South Africa, United Kingdom, United States of America and other countries for their radiotherapy needs. All the centers have a single external beam equipment without any fall back when equipment develop faults. Ibadan, Zaria and EKO Hospital have Cobalt-60 machines while the rest have linear accelerators. Ibadan

and Gombe are the only two centers with High Dose Rate (HDR) brachytherapy equipment. The rest have either a Low/Medium Dose rate (L/MDR) or no brachytherapy equipment. Most of the brachytherapy treatment are limited to intracavitary insertions for cervical cancer and occasional endometrial and vaginal cancers.

Interstitial brachytherapy and radioactive seed implants are currently unavailable in any center in Nigeria. Patients who require or desire such treatments have to obtain it elsewhere. Other important ancillary facilities including simulators, mold room facilities, treatment planning systems are mostly not available or not working optimally.

Important support infrastructure like power and maintenance engineering are also lacking. Electricity failure is one of the major contributors to down time. Unstable power supply can also lead to equipment failures.

Put together, the lack of relevant equipment, poor maintenance ability, inadequate training and the long waiting time ensure that cancer patients receive sub-optimal radiation treatment in Nigeria. Nigeria has a long way to go in meeting the potential needs of its citizens for adequate, effective, reliable and efficient radiation treatment for its current crops of cancer patients and those yet to be diagnosed.

Addressing the Nigeria radiation treatments problems

To meet its population's needs for radiotherapy, Nigeria needs to pay greater attention to this oft-neglected areas. It is easy to suggest more radiotherapy centers, but this is unlikely to be helpful if they will receive similar neglects like the existing ones. Nigeria can significantly improve access to radiation treatment by ensuring that the existing centers are working optimally. This can be achieved by auditing the current facilities and replacing the obsolete ones with new and modern equipment. Redundancy is a cardinal principle in ensuring uninterrupted or minimally-interruptible system. This can be achieved by ensuring that each of the existing centers have a minimum of two working megavoltage machines.

Electricity is a major disruptive agent in the workings of most radiotherapy centers in the country. Big hospitals can set up their own independent power plant to permanently address the problem associated with lack of and unstable power. Government can explore the place of renewable energy sources like solar energy to serve critical areas in the hospitals.

Each radiotherapy center should have the full complement of a modern radiation treatment centers including mold room facilities, 3-D conformal treatment planning system, CT-simulator in addition to modern teletherapy equipment.

Each of the treatment centers should have HDR brachytherapy for cervical cancer. A few centers can be selected to provide more comprehensive brachytherapy services.

Government can liaise with donor agencies like IAEA, UICC, Bill and Melinda Gates Foundation for procurement of new radiotherapy equipment for the country. Decommissioned equipment in developed countries can also be solicited for refurbishment and deployment in Nigeria and other developing countries. The country can also cut down waste in the procurement of new radiotherapy equipment by collaborating with IAEA in her equipment purchases rather than going through high-priced contractors and suppliers.

At present Nigeria is far behind in the quality of radiotherapy services it offers to its population. Our equipment are mostly outdated and inadequate. Sadly, this situation is likely to get worse as developments in radiation medicine practice is happening at a very rapid pace. New treatment techniques are being developed in brachytherapy and external beam radiotherapy. New radiation sources like proton therapy have already started proving their worth in improving treatment outcomes. In addition to fixing the current radiotherapy practice in the country, there is also a need to keep abreast of developments in the field. Otherwise, by the time we finished fixing the existing problems, we will find out that the world has left us, again, very far behind.

Conclusion

Radiotherapy is an important treatment modality in the management of cancer patients in Nigeria. More than half of all cancer cases will require radiotherapy at some point in their treatment. Unfortunately, many of them will not be able to receive treatment due to inadequate and inefficient treatment setup in Nigeria. There is an urgent need for government intervention in improving the quality of radiotherapy services in Nigeria.

References

1. Adewuyi S, Campbell O, Ketiku K, *et al*. Current status of radiation oncology facilities in Nigeria. West African Journal of Radiology 2013; 20(1):30.

2. Delaney G, Jacob S, Featherstone C and Barton M. The Role of Radiotherapy in Cancer Treatment: Estimating Optimal Utilization from a Review of Evidence-Based Clinical Guidelines. Cancer 2005; 104(6):1129–1137.
3. Abdus-Salam A, Ogunnorin O and Abdus-Salam R. HIV Seroprevalence in Patients with Carcinoma of the Cervix in Ibadan, Nigeria. Ghana Medical Journal 2008;42(4):141–3.
4. Durosinmi-Etti FA, Olasinde TA and Solarin EO. A short course postoperative radiotherapy regime for keloid scars in Nigeria. West African Journal of Medicine 1994;13(1):17-19
5. Seegenschmiedt MH, Micke O, and Muecke R. Radiotherapy for non-malignant disorders: state of the art and update of the evidence-based practice guidelines. The British Journal of Radiology 2015;88(1051):20150080.
6. Harbour R and Miller JA new system for grading recommendations in evidence based guidelines. BMJ (Clinical Research Ed.) 2001; 323(7308), 334-336.
7. Ferlay J., Soerjomataram I., Ervik M., *et al*. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase. No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. 2013.
8. Bray F, Ferlay J, Soerjomataram I, *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin [Internet]. 2018 Nov 1 [cited 2019 May 12];68(6):394–424. Available from: https://onlinelibrary.wiley.com/doi/full/10.3322/caac.21492
9. UNFPA - United Nations Population Fund [Internet]. [cited 2019 May 16]. Available from: https://www.unfpa.org/data/world-population/NG
10. Jedy-Agba E., Curado M. P., Ogunbiyi O.*et al*. . Cancer incidence in Nigeria: a report from population-based cancer registries. Cancer Epidemiology, 2012; 36(5), e271–8.
11. Ogundipe S and Obinna C. Why cancer is on the rise in the country. Vanguard Newspaper *report*, http://allafrica.com/stories/200806170258.html 2008 (Retrieved January 27, 2016)
12. Design and implementation of a radiotherapy programme: Clinical, medical physics, radiation protection and safety aspects. IAEA-TECDOC-1040 pg 3.
13. Abdel-Wahab M., Bourque JM., Pynda Y. *et al*. Status of radiotherapy resources in Africa: An International Atomic Energy Agency analysis. The Lancet Oncology. 2013; 14(4):168-175.
14. International Atomic Energy Agency. Inequity in cancer care: A global Perspective. IAEA Human Health Reports, 2011; 1–37.
15. Grover S., Xu M. J., Yeager A. *et al*. A Systematic Review of Radiotherapy Capacity in Low- and Middle-Income Countries**.** Frontiers in Oncology, 2014; 4, 380.

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A survey of preconception care among young female graduates in Ibadan, Southwest Nigeria

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Abstract

Background: Young female graduates are mostly young women with pregnancy intentions. Utilization of preconception care (PC) by women will reduce maternal mortality which is very high in Nigeria. The aim of the study was to assess knowledge, perception and practice of PC among female graduates. The study was carried out in Ibadan, Southwest Nigeria.

Methods: The study was cross-sectional in design and carried out in the National Youth Service Corps Secretariat, Ibadan North Local Government Area (LGA), Agodi Area, Ibadan, Oyo State. A total sampling of 426 females was done. A structured questionnaire adapted from 3 previously validated instruments was used.

Results: Their mean age was 24.4± 2.6 years. Majority, 344(80.8%) were single and 295 (69.2%) were University graduates. Majority, 354 (83.1%) had never been pregnant. Mean knowledge score was 12.0 ± 4.5 out of 25. Only 41.4% had good knowledge. Mean perception score was 62.2 ± 7.6 out of 77 and 426 (100%) had positive perception. Mean practice score was 2.9 ± 3.8 out of 14. Only 21.2% of those intending to be pregnant had good practice of PC. A higher proportion of respondents aged 25-30 years (47.8%) had good knowledge of PC. Older age, 25-30years, was a predictor of good knowledge of PC (OR: 1.5, 95% CI:1.04-2.27). A higher proportion of respondents who had good knowledge of PC had good practice. This was statistically significant. Knowledge of PC was a predictor of good practice (OR: 1.83, 95% CI: 1.10 – 3.06).

Conclusion: Knowledge and practice of PC among young female graduates is poor. Health education intervention on all aspects of PC is needed among these young women.

Keywords: *Preconception care, female graduates, youth corps members*

Abstrait

Contexte : Les jeunes femmes diplômées sont pour la plupart des jeunes dames ayant des intentions de grossesse. L'utilisation des soins pré-conceptionnels (SP) par les femmes réduira la mortalité maternelle qui est très élevée au Nigéria. Le but de l'étude était d'évaluer les connaissances, la perception et la pratique de SP parmi les femmes diplômées. L'étude a été réalisée à Ibadan, dans le sud-ouest du Nigéria.

Méthodes : L'étude était transversale dans sa conception et réalisée au Secrétariat du Corps de la Jeunesse pour le Service National, Ibadan North Local Government Area (LGA), Agodi Area, Ibadan, Oyo State. Un échantillonnage total de 426 femmes a été effectué. Un questionnaire structuré adapté de 3 instruments préalablement validés a été utilisé.

Résultats : Leur âge moyen était de 24,4 ± 2,6 ans. La majorité, 344 (80,8%) étaient célibataires et 295 (69,2%) étaient des diplômés universitaires. La majorité, 354 (83,1%) n'avaient jamais été enceintes. Le score moyen des connaissances était de 12,0 ± 4,5 sur 25. Seulement 41,4% avaient de bonnes connaissances. Le score de perception moyen était de 62,2 ± 7,6 sur 77 et 426 (100%) avaient une perception positive. Le score de pratique moyen était de 2,9 ± 3,8 sur 14. Seulement 21,2% des femmes ayant l'intention d'être enceintes avaient de bonnes pratiques de SP. Une proportion plus élevée de répondantes âgés de 25 à 30 ans (47,8%) avait une bonne connaissance de SP. L'âge plus avancé, 25-30 ans, était un prédicteur de bonne connaissance de SP (OR: 1,5, IC à 95% : 1,04 -2,27). Une proportion plus élevée des répondantes ayant une bonne connaissance de SP avait de bonnes pratiques. Ceci était statistiquement significatif. La connaissance de SP était un prédicteur de bonnes pratiques (OR : 1,83, IC à 95% : 1,10 - 3,06).

Conclusion : La connaissance et la pratique de SP chez les jeunes femmes diplômées sont faibles. Une intervention d'éducation sanitaire sur tous les aspects de SP est nécessaire chez ces jeunes femmes.

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Mots clés : *soins pré-conceptionnels, femmes diplômées, membres du corps des jeunes*

Introduction

Nigeria and India accounted for over one third of all estimated global maternal deaths in 2015 [1]. Nigeria has a high maternal mortality ratio of 814 maternal deaths per 100,000 live births [1]. Preconception care is a key strategy in achievement of the Sustainable Development Goals (SDG) of reducing global maternal mortality ratio [2]. It has been described as the next frontier for improving maternal and child health [3]. The key components of preconception care include: nutritional health care, genetic counseling, assessment for potential future pregnancy risks, HIV screening, screening for Sexually Transmitted Infections (STIs), screening for chronic diseases, premarital counseling, mental health care, vaccination against infectious diseases, behavioural interventions (such as advice on intimate partner violence, psychoactive substance use and tobacco use) and environmental health care [2]. It provides continuous health surveillance and early intervention to ensure women are fit for pregnancy and has the advantages of promoting maternal and child health, reducing morbidity and mortality among neonates, presenting opportunity for genetic counseling, identifying people with potential for high risk pregnancy and enables them to get early interventions [4,5].

Antenatal care could be too late an intervention because organogenesis takes place even before antenatal care begins [6]. The most appropriate time for providing health interventions targeted at ensuring healthy mothers and children is before conception, through preconception care [7]. The WHO recommends that preconception care should target individuals contemplating a pregnancy. They are likely to be receptive to inputs on what they could do to increase the likelihood of positive maternal and child health outcomes [2]. In a systematic review of the impact of preconception care interventions, it was reported that it is not too early to provide information on preconception care to adolescents [8].

Studies on preconception care have been carried out among undergraduates [9]; and high school adolescents in Lebanon [10]. In Nigeria, few studies on preconception care have focused on women attending ANC clinics [11-15].These women are already pregnant and will not benefit maximally from the advantages of preconception care. Knowledge of preconception care was found to be poor among Nigerian women attending antenatal and immunization clinics [11,12,14]. The health belief model shows that good knowledge of health behaviour and its benefits could lead to positive perception which could increase the likelihood of practicing it [16]. Women of reproductive ages in Nigeria were reported to have positive perceptions toward preconception care but do not practice it [11,14]. Utilisation of preconception care was as low as 2.7% among women of reproductive age group in Northwestern Nigeria who delivered in the 24 months preceding the survey.

Little is documented on knowledge of preconception care among young women who have just graduated from tertiary institutions. Preconception care will be most beneficial to this group of young women during this stage of their lives. Young female graduates comprise women in the reproductive age group who are mostly singles and will soon be getting married and pregnant. Preconception care provides continuous health surveillance and early intervention to ensure women are fit for pregnancy.. The most appropriate time for providing health interventions targeted at ensuring healthy mothers and children is before conception, through preconception care.

The study was aimed at assessing the knowledge, perception and practice of preconception care, among young female graduates who are National Youth Service Corps (NYSC) members in Ibadan North Local Government Area, Oyo State, South-Western Nigeria. Respondents' preferred components of preconception care were also identified.

Materials and methods

The study was cross-sectional in design. A total sampling of all female NYSC members in the National Youth Service Corps Secretariat, Ibadan North Local Government Area (LGA), Agodi Area, Ibadan, Oyo State was done . National Youth Service Corps scheme is a one-year mandatory program for all Nigerian graduates aged 30 years and below. The program was created to encourage the development of common ties among Nigerian youths (NYSC, 2015). Each year, eligible Nigerian graduates from universities and polytechnics are enrolled into this compulsory scheme for a period of one year. The NYSC secretariat is situated within the Ibadan North LGA secretariat building. Youth corps members serving in Ibadan North LGA converge in the NYSC secretariat for administrative purposes and various activities that are required of them throughout the service year. Permission to carry out the study was obtained from the Ibadan North Local Government NYSC Inspector.

Ethical approval was granted by the Oyo State Ministry of Health Ethical Review Committee.

A structured, written questionnaire was used to collect information on socio-demographic characteristics of respondents' knowledge, perception, and practice of preconception care. The questionnaire was also used to identify preferred component of preconception care. Questions were adapted and modified from Three (3) previously validated instruments namely: Reproductive Health Attitudes and Behaviours (RHAB) Questionnaire [17], Preconception /prenatal Family Health History Questionnaire [18] and Reproductive Health Knowledge Scale for Women (RHKS-W) [19] (Charron-Prochownik *et al.*, 2006).

The questionnaire was pretested among female corps members serving in Ibadan Southwest LGA. Questions that were unclear or ambiguous were rephrased. The questionnaire was in English because all respondents were graduates and able to communicate in English.

The purpose of the study was explained to corps members at their meetings. A total sampling of all female corps members in the secretariat was done. All female corps members posted to Ibadan North LGA were included in the study. Celibates and those who have had hysterectomy were excluded from the study. Respondents were told about the inclusion and exclusion criteria at the time of informing them about the study. They were also asked privately at the time of obtaining informed consent Questionnaires were administered by 6 trained research assistants who were graduates of tertiary institutions who provided information and clarifications of medical terms in the questionnaire to the respondents. They were also trained to ensure privacy. Written informed consent was obtained from each corps member. All consenting corps members filled the questionnaires with the help of trained research assistants and were interviewed away from their colleagues.

Study variables

The dependent variables for the study were knowledge, perception and practice of preconception Care.

Twenty-five questions were used to assess knowledge of preconception care such as 'preconception care is the health intervention before conception, preconception care involves genetic counselling, a mother's mental health and emotional well-being can affect a baby's health'. Each correct response was scored 1(one) and incorrect and "don't know" responses were scored 0 (zero). The maximum obtainable score for knowledge of preconception care was 25. A 50th percentile score of 13 was used to categorize knowledge into poor or good knowledge. Female youth corps members with scores <13 were considered as having poor knowledge of preconception care while those with scores e"13 were considered as having good knowledge.

Fifteen statements were used to assess perception of preconception care using three point Likert scales. Each correct statement in the 3-point Likert scales was scored 5 giving a total of 75. Two other statements were added to assess if respondents were willing to receive preconception care and if they were willing to advice others to receive preconception care. These other two statements were scored 2. The total maximum obtainable score for perception of preconception care was 77. A 50th percentile score of 39 was used to categorize perception into negative or positive perception. Female youth corps members with scores < 39 were considered as having negative perception of preconception care while those with scores $\geq$39 were considered as having positive perception.

Practice of preconception care was defined as the practice of all the components of preconception care. Fourteen questions were used to assess the practice of preconception care such as: nutritional health care, advice on environmental health issues, screening for chronic conditions and assessment for potential pregnancy risks in the future. Each question was scored one. The maximum obtainable score was 14. 50th percentile score of 7 was used to categorize practice into poor or good practice. Female youth corps members with scores < 7 were considered as having poor practice of preconception care, while those with scores e" 7 were considered as having good practice.

Respondents were also asked which of the components of preconception care they preferred. The component of preconception care with the highest frequency was taken to be the most preferred, while that with the least frequency was taken to be the least preferred. The independent variables were demographic characteristics assessed using the following indicators: Age of respondents, marital status, religion, tribe, highest level of education, course of study, number of children and number of pregnancies.

The data were analysed with Statistical Package for Social Sciences (SPSS) software programme version

20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Bivariate analysis was done to test for associations between categorical variables using the chi-square test. Multivariable logistic regression analysis was done to identify factors associated with knowledge, perception and practice of preconception care. Odds ratios (ORs) and their 95% confidence intervals (CIs) were reported. Variables significantly associated with outcome variables in bivariate analysis at p= 0.05 were included in the logistic regression model. The 'enter' option of the logistic regression in SPSS was used for variable selection where all variables in a block were entered in a single step. Level of significance for all tests was at 5%.

Results

A total sampling of 470 young female graduates (NYSC members) in Ibadan North LGA was done but 426 responded giving a response rate of 90.6%. Their ages ranged between 19 and 30 years with a mean age of 24.4 ± 2.6 years. Over half of them, 244 (57.3%) were aged between 19 and 24 years. Majority of the respondents were Christians, 300 (70.4%), 297 (69.7%) were of the Yoruba tribe and 344 (80.8%) were singles.

Over two-thirds of the respondents 295 (69.2%) were University graduates and 131(30.8%) were polytechnic graduates. Seventy-two (16.9%) of them had ever been pregnant of which 10(13.9%) were singles and 62(86.1%) were married. Among those pregnant, 27(37.5%) had 1 child while 45(62.5%) had 2 or more children. (Table 1)

Knowledge of preconception care

Table 2 presents a point-by-point response to questions on knowledge of preconception care. Most 380 (89.2%) knew that preconception care is the health intervention before conception and 308 (72.3%) knew that preconception care involves genetic counseling. Very few women, 93 (21.8%) knew that women planning to conceive are encouraged to consume any kind of fish. Mean knowledge score of preconception care score was 12.0 ± 4.5 out of 25, 41.8% of them had good knowledge while 58.2% had poor knowledge.

Perception of preconception care

Table 3 reports respondents' perception of preconception care.

Almost all 393(92.3%) believed that seeking preconception care when planning pregnancy canimprove their chances of having a healthy baby and 379 (89.0%) believed getting preconception care can improve their chances of having a healthy pregnancy. However, 137(32.2%) believed it is difficult to access preconception care. Majority, 372 (87.3%) were willing to receive preconception care and 380 (89.2%) were willing to advice others to receive preconception care. Mean perception score was 62.2 ± 7.6 out of 77 and 426 (100%) had positive perception.

Table 1: Socio-demographic and reproductive characteristics of respondents N=426

Variables	n %
Age in group (years)	
19-21	59 (13.9)
22-24	185 (43.4)
25-27	119 (27.9)
28-30	63 (14.8)
Marital status	
Single	344 (80.8)
Married	67 (15.7)
Cohabiting	10 (2.3)
Separated	5 (1.2)
Religion	
Christianity	300 (70.4)
Islam	126 (29.6)
Tribe	
Yoruba	297 (69.7)
Igbo	67 (15.7)
*Others	62 (14.6)
Highest Level of Education	
University	295 (69.2)
Polytechnic	131 (30.8)
Ever been pregnant	
Yes	72(16.9)
No	354(83.1)
Number of children	
1	27(37.5)
≥2	45(62.5)

Preferred components of Preconception care

Table 4 shows components of preconception care preferred by respondents. Preferred components were Nutritional healthcare (e.g. advice on use of folic acid) 371(87.1%); Genetic counseling, 354 (83.1%); Advice on environmental health issues (e.g. sanitation, exposure to pollutants) 342(80.3%); Screening for HIV, 340 (79.8%); screening for chronic conditions e.g. diabetes), 338 (79.3%); Assessment for potential pregnancy risks in the future, 337 (79.1).

Table 2: Knowledge of preconception care among female graduates N=426

Statement	n (%)
Preconception care is the health intervention before conception	380 (89.2)
Preconception care involves genetic counseling	308 (72.3)
A mother's mental health and emotional well-being can affect her baby's health	277 (65.0)
Preconception care is useful for screening for Sexually transmitted disease	272 (63.8)
Diagnosis and management of diabetes before getting pregnant can reduce the likelihood of some adverse pregnancy outcomes	270 (63.4)
Alcohol use before pregnancy can cause damage to the child when pregnant	270 (63.4)
Smoking is associated with increased risk of infertility	248(58.2)
Women who are breastfeeding cannot become pregnant	234(54.9)
Immunization is included in preconception care	229(53.8)
Women planning to conceive can consume folic acid to prevent birth defects	213(50.0)
Preconception care is the same as antenatal care	208(48.8)
A mother with HIV/AIDS cannot pass it on to her baby by breastfeeding	203(47.7)
Sexually transmitted infections such as Hepatitis B and HIV are not transmitted between a mother and her developing baby	190 (44.6)
Women who are overweight or obese are predisposed to negative pregnancy outcomes	187 (43.9)
Intimate partner violence may not be a risk for poor pregnancy outcomes	182 (42.7)
Premarital counseling is not a component of preconception care	174 (40.8)
Preconception care involves only clinical examination and investigation	167(39.2)
Withdrawal is an effective method of birth control	159 (37.3)
Vaccines are not recommended for women who are planning to become pregnant	158 (37.1)
Infants of diabetic mothers are 2 to 3 times more likely to have heart defects than those of non-diabetic mothers	156 (36.6)
Asthma is a chronic condition that has no impact on the overall health of pregnancy	156 (36.6)
IUDs are effective birth control options for up to 10 years of insertion	153(35.9)
Women trying to conceive are encouraged to discontinue a regular exercise routine	133(31.2)
Women planning to conceive are encouraged to consume any kind of fish	93(21.8)
Women should begin taking preconception vitamins after they become pregnant	83(19.5)

Practice of preconception care

Majority 382 (89.7%) of the respondents had pregnancy intention. One third of these 123 (32.2%) had received preconception guidance. More than two thirds 84(68.3%) actively sought for preconception guidance while 39 (31.7%) received it coincidentally. Mean practice score was 2.9 ± 3.8 out of 14. Eighty-one (21.2%) of those with pregnancy intention had good practice of preconception care.

Sources of received preconception care includeD Health care workers; Doctors and nurses (32.2%), Family members/friend (22.3%), Media/internet (15.4%), Religious leaders (12.0%), community health officers (7.6%).

Figure 1 shows component of preconception care received by respondents. These included nutritional health care (25.7%), premarital counseling (23.0%), screening for HIV (22.0%) and genetic counseling (20.9%). Least received components of preconception care were mental health care, 54 (14.1%); screening for chronic conditions like diabetes, 52 (13.6%) and vaccination (against rubella virus, hepatitis B, tetanus or diphtheria) 49 (12.8%).

Bivariate analysis

*Factors associated with knowledge and practice of preconception care***.**

Table 5 presents bivariate analysis of sociodemographic characteristics of respondents and good knowledge of preconception care. A higher proportion of respondents aged 25-30 years (47.8%) significantly had good knowledge of preconception care.

Table 6 presents bivariate analysis of sociodemographic characteristics of respondents and practice of preconception care. A higher proportion of respondents who were married (35.3%) had good practice of preconception care compared to respondents who were unmarried (19.0%). A higher proportion of respondents

Table 3: Perception of preconception care N= 426

Statements	Agree n(%)
The following items are important in helping a young woman to be prepared to have a healthy pregnancy and healthy baby:	
Eating healthy foods	422(99.1)
Maintaining a healthy weight	371(87.1)
Taking Vitamins	367(86.2)
Getting regular exercise	360(84.5)
Seeing a doctor annually for a health checkup	356(83.6)
Being smoke and tobacco free	304(71.4)
Avoiding unplanned pregnancies	272(63.8)
Engaging in unprotected sexual intercourse	159(37.3)
Drinking Alcohol	79(18.5)
Using hard drug such as cocaine	58(13.6)
Seeking preconception care when planning pregnancy can improve your chances of having a healthy baby	393(92.3)
Getting preconception care can improve your chances of having a healthy pregnancy	379(89.0)
Most of the components of preconception care counseling and advice are practicable	316(74.2)
There is no additional risk attached to the health of the mother and baby in an unplanned pregnancy	182(42.7)
It is difficult to access preconception care	137(32.2)

Table 4: Preferred components of Preconception care N =426

Component of preconception care	n (%)
Nutritional healthcare (e.g.) advice on use of folic acid	371 (87.1)
Genetic counseling	354 (83.1)
Advice on environmental health issues (e.g. sanitation, exposure to pollutants)	342 (80.3)
Screening for HIV	340 (79.8)
Screening for chronic conditions (e.g. diabetes)	338 (79.3)
Assessment for potential pregnancy risks in the future	337 (79.1)
Premarital Counseling	331 (77.7)
Vaccination against rubella virus, hepatitis B, tetanus or diphtheria	331 (77.7)
Screening for sexually transmitted infections (e.g. syphilis, gonorrhea)	330 (77.5)
Advice on intimate partner violence	307 (72.1)
Mental health care	303 (71.1)
Advice on psychoactive substance use/tobacco use	292 (68.5)

**Multiple response*

who had one pregnancy (44%) and those who had 1 child (26.1%) had good practice of preconception care. A higher proportion of respondents who had good knowledge of preconception care had good practice. This was statistically significant.

On logistic regression, older age, 25-30years, was significantly associated with good knowledge of preconception care (OR: 1.5, 95% CI.04-2.27). Good knowledge of preconception care was a predictor of good practice. (OR: 1.8, 95% CI: 1.10-3.06). No other variable showed significant association with good practice.

Discussion

The study assessed knowledge, perception and practice of preconception care among young female graduates. Respondents' preferred components of preconception care were also identified.

Majority of respondents had poor knowledge of preconception care even though these young females are graduates of higher institutions and should be

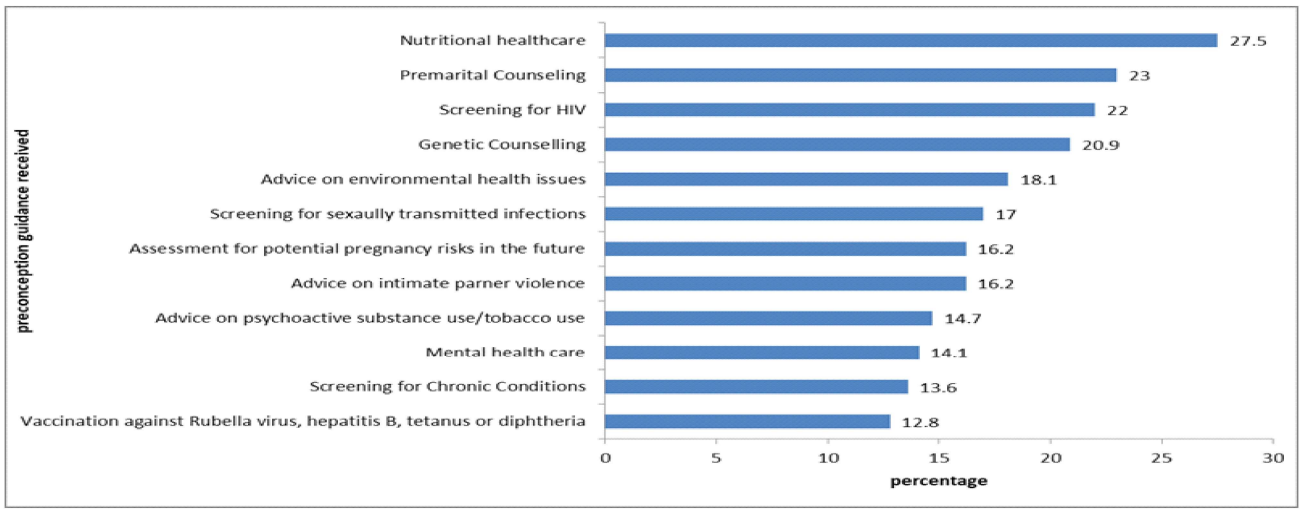

Fig. 1: Components of preconception care received

Table 5: Bivariate analysis of Respondents'socio-demographic characteristics and knowledge and practice of preconception care

Variables	Knowledge of preconception care		Total (n=426)	Chi-square	p-value
	Good	Poor			
Age group (years)					
19 -24	91 (37.3)*	153 (62.7)	244	8.874	0.031*
25 - 30	87 (47.8)	95 (52.2)	182		
Marital status					
Not married	147 (40.9)	212 (59.1)	359	0.657	0.418
Married	31 (46.3)	36 (53.7)	67		
Religion					
Christianity	131 (43.7)	169 (56.3)	300	1.478	0.224
Islam	47 (37.3)	79 (62.7)	126		
Tribe					
Yoruba	125 (42.1)	172 (57.9)	297	0.314	0.855
Igbo	29 (43.3)	38 (56.7)	67		
Hausa / others	24 (38.7)	38 (61.3)	62		
Highest level of education					
University	116 (39.3)	179 (60.7)	295	2.390	0.122
Polytechnic	62 (47.3)	69 (52.7)	131		
Number of pregnancies					
0	145 (41.0)	209 (59.0)	354	1.045	0.593
1	11 (40.7)	16 (59.3)	27		
≥2	22 (48.9)	23 (51.1)	45		
Number of children					
0	148(40.8)	215 (59.2)	363	1.037	0.595
1	12(48.0)	13 (52.0)	25		
≥2	18(47.4)	20 (52.6)	38		

knowledgeable of preconception care. This is similar to findings from studies carried out among women receiving antenatal care in Enugu [11], Kaduna [13], Bayelsa [12] communities and Port Harcourt [15], all in South South and South East Nigeria. This shows that knowledge of preconception care among women in Nigeria is still very poor. Efforts should be made to improve the knowledge of preconception care among

young women. Contrary to the findings of this study, a study conducted among women attending selected Primary Care centers for annual well woman examination in the United States reported that almost all participants understood the importance of knowledge of preconception care. Older graduates are likely to have been pregnant and had children and could have received information on preconception care during antenatal and postnatal care. There was no association between educational status and knowledge of

Table 6: Bivariate analysis of Respondents'socio-demographic characteristics andpractice of preconception care

Variables	Practice of preconception care		Total (n=382)	Chi-square	p-value
	Good	Poor			
Age group (years)					
19-24	41 (36.0)	184 (64.0)	225	2.949	0.400
25 - 30	40 (51.3)	117 (148.7)	157		
Marital status					
Not married	63 (19.0)	268 (81.0)	331	6.994	0.008*
Married	18 (35.3)	33 (64.7)	51		
Religion					
Christianity	61 (22.3)	212 (77.7)	273	0.744	0.388
Islam	20 (18.3)	89 (81.7)	109		
Tribe					
Yoruba	60 (21.8)	215 (78.2)	275	1.311	0.519
Igbo	13 (23.6)	42 (76.4)	55		
Hausa / others	8 (15.4)	44 (84.6)	52		
Highest level of education					
University	55 (20.5)	213 (79.5)	268	0.250	0.617
Polytechnic	26 (22.8)	88 (77.2)	114		
Number of pregnancies					
0	62 (18.8)	268(81.2)	330	10.076	0.006*
1	11 (44.0)	14 (56.0)	25		
$\geq$2	8 (29.6)	19 (70.4)	27		
Number of children					
0	64 (19.0)	273 (81.0)	337	10.383	0.006*
1	11 (45.8)	13 (54.2)	24		
$\geq$2	6 (28.6)	16 (76.2)	21		

*$p<0.05$

preconception healthcare and knew that it should be obtained prior to conception [20]. Fifty percent of the women knew that preconceptional use of folic acid could prevent birth defects. This is however higher than that reported among mothers in early pregnancy in Ibadan, South West Nigeria [14].

This is probably due to the fact that all respondents had tertiary education and were therefore aware of the importance of use of folic acid in pregnancy. Very few women knew that women planning to conceive could consume any kind of fish showing that their knowledge of preconception care is inadequate. Health education on preconception care should be provided to these young women to improve their knowledge. Older age was a predictor of good preconception care. This is probably because all respondents had tertiary education. Contrary to findings in this study, it has been reported that educational status was associated with knowledge of preconception care [11,15].

All respondents had a positive perception towards preconception care and this can improve the chances of having a healthy pregnancy and a healthy baby. Similarly, positive perception to preconception care was reported among university students in Egypt [21] and women attending ante natal clinic [11]. Preconception care is a well-accepted health intervention among women. The practice of preconception care will ultimately reduce high maternal mortality ratio in Nigeria. Majority of respondents were willing to receive and advice others to receive preconception care. This

is similar to findings from a study conducted among Fayoum University medical students in Egypt [22] where almost all the students were willing to receive and advice others to receive preconception care. Female graduates could serve as change agents in encouraging women to access preconception care.

Respondents preferred some components of preconception care. Nutritional health care (such as advice on folic acid use), genetic counseling, advice on environmental health and HIV screening were most preferred. There are limited studies that assessed women's preferred component of preconception care in Nigeria which will guide intervention programme or services. Health education intervention programmes could focus on these components and provide information geared towards provision of preconception care and their benefits. Nutritional health care especially preconceptional usage of folic acid is beneficial in preventing birth defect. Genetic counseling will provide information on genetic inheritance especially the sickle cell disease or sickle cell gene, which is prevalent among Nigerians, and the medical, psychological and familial implications of genetic contributions to disease [23]. High educational status of respondents could have enabled respondents to be knowledgeable of the benefits of these preferred components. The least preferred component was advice on psychoactive substance / tobacco use. This may be due to the fact that psychoactive substance and tobacco use is low among women in Nigeria.

Preconception care was poorly practiced among respondents. Only one fifth of those with pregnancy intention had good practice of preconception care. This is lower than one third of women attending antenatal clinic in a study conducted in the United Kingdom who took folic acid prior to conceiving [24]. Poorer practice of preconception care among female graduates compared to women attending antenatal clinic could be because they were younger with a higher proportion of them being single and nulliparous. Education may also explain the difference in the findings. A quarter of the women attending antenatal clinic had postgraduate education increasing the likelihood of exposure to information on and practice of preconception care. The proportion of female graduates who received preconception care was however higher than 5% of mothers attending immunization clinics in Ibadan [14] and 2.7% of mothers in Kaduna [13] who received preconception care. This could be because of their low educational status as only 23% of mothers in Ibadan had university degree and 48.7% of women in Kaduna had formal education. Findings from this study were comparable to a similar study among women attending antenatal clinic in the southeastern part of Nigeria where less than half of them consulted medical practitioners prior to conception [11]. Doctors and nurses were the highest source of preconception care received by respondents in this study. Health care workers should therefore take advantage of their contact with young women to provide and reinforce information on preconception care. Respondents who had good knowledge of preconception care were twice as likely to have good practice of preconception care. It is important that women receive preconception care. Researches have shown that women who received preconception care had improved pregnancy outcome [25,26].

Findings suggest that there is a need for intervention programmes especially health education on preconception care and its benefits to female graduates during their one-year youth corps service programme. Higher institutions of learning should include preconception care as part of their curriculum. Intervention programmes incorporating some aspects on preconception care which would target young reproductive aged women is advocated. Healthcare workers should make use of opportunities they have with female clients especially women of reproductive age to provide information on preconception care.

Strength of the study

The study assessed knowledge and practice of preconception care among a population of multiethnic young female graduates in the community and identified poor knowledge of PC among them. The study provides useful information that will guide preconception care intervention programs for young women to improve their knowledge and encourage practice. Preconception care services can be provided to female graduates during their youth corps programs through the Primary Health Care centers at the Local Government Area.

Limitation of the study

The study was cross-sectional in design; a longitudinal study would be useful to assess impact of preconception care on birth outcomes among these young women. In addition, the study had information bias as some female graduates were not comfortable answering questions related to parity or gynecological history. In order to remove this limitation, confidentiality and privacy were

ensured during data collection. The study only focused on knowledge, perception and practice of preconception care among female youth corpers. Barriers to practice of preconception care need to be explored. Further studies can also be carried out to investigate role of family, community and government in improving the practice of preconception care.

Conclusion

Young female graduates had poor knowledge and practice of preconception care. However, their perception towards preconception care was positive. Nutritional health care, genetic counseling, advice on environmental health and HIV screening were most preferred components. Knowledge of preconception care is the single predictor of its practice. Future research such as an intervention study to determine the effect of preconception care on maternal mortality among these young females should be explored.

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References

1. WHO. Trends in maternal mortality:1990 to 2015 [Internet]. Available from: https://www.afro.who.int/sites/default/files/2017-05/trends-in-maternal-mortality-1990-to-2015.pdf
2. Meeting to Develop a Global Consensus on Preconception Care to Reduce Maternal and Childhood Mortality and Morbidity [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/78067/9789241505000_eng.pdf;jsessionid=6FA5A2FF62EE6FD14B5708630EB321C2?sequence=1
3. Genuis RA and Genuis SJ. Preconception care: the next frontier for improving maternal-child health care. Public Health. 2017 Aug 1;149:57–9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0033350617301294?via%3Dihub
4. Dean S, Rudan I, Althabe F, *et al.* Setting research priorities for preconception care in low- and middle-income countries: aiming to reduce maternal and child mortality and morbidity. PLoS Med. 2013;10(9):e1001508. Available from: http://www.ncbi.nlm.nih.gov/ pubmed/24019762
5. Fuehrer L, Buckler E, Bowman E, Gregory T and McDaniel J. Promoting preconception health in primary care. J Am Acad Physician Assist. 2015 Aug;28(8):27–32. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP :landingpage &an= 01720610-201508000-00004
6. Nypaver C, Arbour M and Niederegger E. Preconception Care: Improving the Health of Women and Families. J Midwifery Womens Health. 2016 May;61(3):356–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27218593
7. Johnson K, Posner S, Biermann J, *et al.* Recommendations to Improve Preconception Health and Health Care — United States: A Report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preconception Care [Internet]. CDC/ATSDR 55(6). 2006. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5506a1.htm
8. Mumford SL, Michels KA, Salaria N, Valanzasca P and Belizán JM. Preconception care: it's never too early. Reprod Health. 2014 Oct 2;11:73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25273543
9. Delgado CEF. Undergraduate Student Awareness of Issues Related to Preconception Health and Pregnancy. Matern Child Health J. 2008 Nov 2;12(6):774–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17975718
10. Charafeddine L, El Rafei R, Azizi S, *et al*. Improving awareness of preconception health among adolescents: experience of a school-based intervention in Lebanon. BMC Public Health. 2014 Dec 31;14(1):774. Available from: http://bmcpublichealth.biomedcentral .com/articles/10.1186/1471-2458-14-774
11. Ezegwui HU, Dim C, Dim N and Ikeme AC. Preconception care in South Eastern Nigeria. J Obstet Gynaecol (Lahore). 2008 Jan 2;28(8):765–8. Available from: http://www.ncbi.nlm.nih. gov/pubmed/19085540
12. Olayinka OA, Achi OT, Amos AO and Chiedu EM. International Journal of Nursing and Midwifery Awareness and barriers to utilization of maternal health care services among reproductive women in Amassoma community, Bayelsa State. 2014;6(1):10–5. Available from: http://www.academicjournals.org/IJNM
13. Idris S, Sambo M and Ibrahim M. Barriers to utilisation of maternal health services in a semi-urban community in northern Nigeria: The clients2

perspective. Niger Med J. 2013;54(1):27. Available from: http://www.nigeriamedj.com/text.asp?2013/54/1/27/108890

14. Lawal TA and Adeleye AO. Determinants of folic acid intake during preconception and in early pregnancy by mothers in Ibadan, Nigeria. Pan Afr Med J. 2014;19. Available from: http://www.panafrican-med-journal.com/content/article/19/113/full/
15. Oranu EO, Ojule JD and Nnah EW. Preconception care in a southern Nigeria tertiary institution. Niger J Med. 24(1):58–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25807676
16. Janz NK and Becker MH. The Health Belief Model: A Decade Later. Health Educ Q. 1984 Mar 4;11(1):1–47. Available from: http://journals.sagepub.com/doi/10.1177/10901981 8401100101
17. Charron-Prochownik D, Wang SL, Sereika SM, Kim Y and Janz NK. A theory-based reproductive health and diabetes instrument. Am J Health Behav. 2006;30(2):208–220.
18. Preconception/Prenatal Family Health History Questionnaire [Internet]. 2008. Available from: http://marchofdimes.com/gyponline
19. Corbet E. Preconception health and wellness: knowledge and attitudes of undergraduate women [Internet]. Colorado State University; 2011. Available from: pdfs.semanticscholar.org/ 88dd/8e5ac 2eab65ca3205888160bfd60b8fdca3a.pdf
20. Frey KA and Files JA. Preconception healthcare: what women know and believe. Matern Child Health J. 2006 Sep;10(5 Suppl):S73-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16775757
21. Mohaseb MM, Farahat T, Shaheen HE and Mohamed H. Knowledge and attitude of students in Menoufia University, Shebin Elkom city toward premarital care in 2012. Menoufia Med J. 2014;27(2):347. Available from: http://www.mmj.eg.net/text.asp?2014/27/2/347/141706
22. Tawfik S, Al Azeem A, Taher Elsayed E, *et al*. Promotion of knowledge and attitude towards premarital care: An interventional study among medical student in Fayoum University. J Public Heal Epidemiol. 2011;3(3):121–128. Available from: http://www. academicjournals.org/jphe
23. Adeyemo O, Oyenike A, Omidiji O, *et al* Level of awareness of genetic counselling in Lagos, Nigeria: its advocacy on the inheritance of sickle cell disease. African J Biotechnol. 2007 Dec 31;6(24):2758–2765. Available from: http://academicjournals.org/journal/AJB/ article-abstract/D7A18D58694
24. Lane IR. Preventing neural tube defects with folic acid: Nearly 20 years on, the majority of women remain unprotected. J Obstet Gynaecol (Lahore). 2011 Oct 5;31(7):581–5. Available from: http://www.tandfonline.com/doi/full/10.3109/01443615.2011.594917
25. Saravelos S and Regan L. The Importance of Preconception Counseling and Early Pregnancy Monitoring. Semin Reprod Med. 2011 Nov 8;29(06):557–68. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0031-1293209
26. Ajayi GO, Popoola AT, Dina T and Okorie N. Pre-pregnancy counseling in Lagos: a report on the first 1,000 cases. Clin Exp Obstet Gynecol. 2013;40(3):359–360. Available from: http://www.ncbi.nlm .nih.gov/pubmed/24283165

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Serum total antioxidant capacity and hydrogen peroxide in pregnant women using skin lightening creams and cord serum of their babies at birth

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Abstract
Background: Skin lightening creams have been reported to have deleterious effects on the users, but no such report was encountered on pregnant women and their fetuses. Also, there is paucity of information on the mechanisms of these effects.
Method: Spectrophotometer was used to determine the levels of total antioxidant capacity (an antioxidant) and H_2O_2 (an oxidant) in sera of pregnant women using skin lightening creams and cord sera of their babies compared with pregnant women not using skin lightening creams.
Results: The mean level of H_2O_2 in sera of mothers using skin lightening cream or the sera from cord blood of their babies was significantly higher compared with non-skin lightening cream users ($p< 0.05$ respectively). However, the mean level of total antioxidant capacity (TAC) in cord sera of babies from mothers using skin lightening cream was significantly reduced compared with cord sera of babies from mothers not using skin lightening cream. There was significant positive correlation between levels of maternal TAC and maternal H_2O_2 ($r=0.408$; $p=0.048$) or levels of maternal serum H_2O_2 level and babies' cord H_2O_2 ($r=0.399$; $p=0.049$) in pregnant women using lightening creams. This study suggests that skin lightening creams might be harmful to pregnant women and their babies.

Keywords: *Oxidant-antioxidants, Pregnancy, Skin lightening.*

Abstrait
Contexte: Il a été rapporté que les crèmes cutanées éclaircissantes ont des effets délétères sur les utilisatrices, mais aucun rapport de ce type n'a été rencontré sur les femmes enceintes et leurs fœtus. De plus, il y a une pénurie d'information sur les mécanismes de ces effets.
Méthode : Un spectrophotomètre a été utilisé pour déterminer les niveaux de capacité antioxydante totale (un antioxydant) et de H_2O_2 (un oxydant) dans les sérums de femmes enceintes utilisant des crèmes cutanées éclaircissantes et des sérums de cordon de leurs bébés par rapport aux femmes enceintes n'utilisant pas de crèmes cutanées éclaircissantes.
Résultats: Le niveau moyen de H_2O_2 dans les sérums des mères utilisant une crème cutanée éclaircissante ou les sérums de sang de cordon de leurs bébés était significativement plus élevé par rapport aux utilisatrices de crèmes cutanées non-éclaircissantes ($p <0,05$ respectivement). Cependant, le niveau moyen de capacité antioxydante totale (CAT) dans les sérums de cordon de bébés de mères utilisant une crème cutanée éclaircissante était significativement réduit par rapport aux sérums de cordon de bébés de mères n'utilisant pas de crème cutanée éclaircissante. Il y avait une corrélation positive significative entre les niveaux de CAT maternelle et H_2O_2 maternelle ($r = 0,408$; $p = 0,048$) ou les niveaux de sérum maternel niveau d'H_2O_2 et le cordon de bébés H_2O_2 ($r = 0,399$; $p = 0,049$) chez les femmes enceintes utilisant des crèmes éclaircissantes. Cette étude suggère que les crèmes cutanées éclaircissantes pourraient être nuisibles pour les femmes enceintes et leurs bébés.

Mots clés: *Oxydant-antioxydants, Grossesse, Éclaircissement de la peau.*

Introduction

This is a follow-up to a study on immunoglobulin levels in maternal blood, cord blood and breast milk of Nigerian pregnant women using hydroquinone and non-hydroquinone containing skin lightening creams, which reported raised IgG and IgM in mothers and babies of skin lightening creams users that might lead to autoimmune disease later in life [1]. Skin lightening (bleaching) cosmetic is widely used in most African countries and hydroquinone, mercury and corticosteroids have been identified as the major ingredients in these cosmetic products [2]. Prolonged use of these products and the hot humid conditions that characterize sub-Saharan Africa has been reported to enhance

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percutaneous absorption of the components of these products which leads to serious and sometimes fatal complications [3]. Complications associated with the use of skin lightening products include impaired wound healing and wound dehiscence, nephropathy, predisposition to infections and broad spectrum of cutaneous disorders and suppression of hypothalamic-pituitary-adrenal axis [2, 3]. All these complications in skin lightening cream users have been previously associated with oxidative stress [2, 3]. None of these studies was carried out on pregnant women and their babies. Hence, the need for present study.

Pregnancy is associated with high metabolic demand and elevated requirements for tissue oxygen which leads to an increased tendency towards reactive oxygen species production with a counteractive increase in antioxidant activity in pregnancy [4, 5].

However, problems arise when there is an imbalance between pro-oxidants and anti-oxidants leading to inadequate oxidant scavenging capacity. This imbalance could also be induced by exposure to harmful agents in the skin lightening cream especially mercury [5-8].This pro-oxidant/anti-oxidant imbalance is proposed to affect maternal health and birth outcomes. During pregnancy, because of the physiological changes that occur on the skin, some pregnant women use skin lightening creams in a bid to look more attractive, go with existing fashion trend, treat skin blemishes like acne or melisma, cleanse or 'tone' the face and body, or to satisfy the taste of their spouses [2, 3]. Exposure to some of the active ingredients of skin lightening products was found to have deleterious effects to both the pregnant mother and the fetus as a result of mitochondrial dysfunction, depolarization and autoxidation of the inner mitochondrial membrane [7, 8].

Total antioxidant capacity (TAC) is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced. Low total antioxidant capacity could be indicative of oxidative stress or increased susceptibility to oxidative damage [9]. Hydrogen peroxide (H_2O_2) is widely regarded as a cytotoxic agent whose levels must be minimized by the action of antioxidant defense systems [10].

This study determined the total antioxidant capacity and hydrogen peroxide in babies'cord blood and maternal blood of pregnant women using skin lightening creams compared with pregnant women not using skin lightening creams.

Materials and method

Subjects

This study was a case control study involving pregnant women attending Adeoyo Maternity Hospital, Ibadan, Oyo State, Nigeria from March 2016 to December 2017. A total of 48 participants were recruited for this study comprising 24 pregnant women who were regular users of skin lightening creams (7 months to 7 years) and 24 pregnant women who were non users of skin lightening creams. All participants were age matched. Inclusion criteria included consenting pregnant women using skin lightening creams and pregnant women with no pregnancy associated complications. Exclusion criteria were non consenting pregnant women, pregnant women with pregnancy associated complications, pregnant women with HIV/AIDS, pregnant women with skin disorders and non-pregnant women. Ethical approval was obtained from the Ethics board committee for Oyo State Ministry of Health, Secretariat, Ibadan and written informed consent was obtained from each of the participants prior to specimen collection. A short structured interviewer administered questionnaire was used as an instrument for demographic data collection. Participants age, cream used, frequency of cream use and duration of cream use were obtained. Bleaching cream users were determined based on the cream type reported.

Sample collection, processing and storage

Prepartum blood samples were obtained from pregnant women in late third trimester of pregnancy (8 months of gestation). Five mls of blood was drawn from the ante cubital vein and dispensed into plain tubes. Samples were allowed to clot and centrifuged at 5000rpm for 5minutes. Serum was obtained and stored at -20^{o}C for two weeks before analysis.

Cord blood samples were obtained from new born babies immediately after delivery. Immediately after delivery, the umbilical cord was clamped at two points. Sterile needle was inserted into a superficial umbilical artery between the two clamps and 5mls of blood was drawn. This was dispensed into a plain specimen bottles and left to clot. Samples were centrifuged at 5000rpm for 5minutes and cord serum was transferred into another plain screw cap specimen bottle. Cord serum was kept frozen at -20^{o}C until laboratory analysis.

Determination of plasma total antioxidant status (TAS)

Plasma total antioxidant status was determined by the method as previously described by Rahamon *et al* [9]. Standardization solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction leading to the formation of hydroxyl radicals ($HO^{\cdot}$). These reactive oxygen species degrade benzoate resulting in the release of thiobarbituric acid reactive substance (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrometrically at 532nm and the rate of inhibition of colour is proportional to the concentration of antioxidant status.

Determination of hydrogen peroxide concentration

H_2O_2 was determined as previously carried out by Edem *et al* [10]. Serial dilution of H_2O_2 was made by adding 0.95 ml, 0.90 ml, 0.85 ml, 0.80 ml, 0.70 ml, 0.60 ml, 0.50 ml of distilled water to 0.05 ml, 0.10 ml, 0.15 ml, 0.20 ml, 0.30 ml, 0.40 ml, 0.50 ml of H_2O_2 to yield 10, 20, 30, 40, 60, 80, and 100 µmoles of H_2O_2 respectively. 2.5 ml buffer, 250 µL AFS, 100 µL sorbitol, 100 µL xylenol orange and 25 µL of H_2SO_4 were dispensed into all the test tubes. 50 µL of sample/standard was dispensed into the tubes appropriately. The samples were mixed by vortexing and incubated at room temperature for 30 minutes. Absorbance was read at 560 nm. Standard curve was plotted and concentrations of the samples were extrapolated from the standard curve.

Statistical analysis

Data were analysed using statistical package for social sciences (SPSS) version 22.0. Quantitative data was presented was as mean ± SD while qualitative data was presented as frequency (percentage). Student t-test was used to compare the means of the study groups while Chi-square test was used to compare proportions and test for associations. Pearson's correlation test was used to examine the correlation between continuous variables. *P*-value less than 0.05 was considered statistically significant.

Results

A total of 48 pregnant women were recruited for this study comprising: 24 lightening cream users with mean age 27.58(3.51) years and 24 non- lightening cream users with mean age 26.95(5.27) years. Among lightening cream users, 9 (37.5%) reported <3years duration of lightening cream use while 15 (62.5%) reported 37years duration

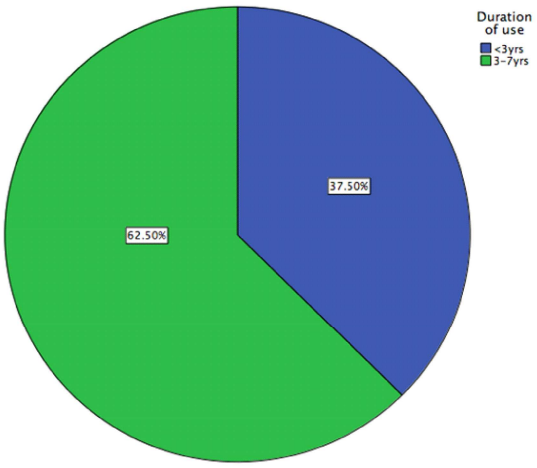

Fig.1: Duration of use of skin lightening cream by pregnant mothers

of lightening cream use (Figure 1). Also, 8 (33.3%) reported use of lightening cream once daily while 16 (66.7%) reported use of lightening cream twice daily. The types of lightening cream used by the participants included; Caro white (12, 50.0%), Perfect white (3, 12.5%), Skin light (1, 4.2%), Pure skin (6, 25.0%), Clear essence (1, 4.2%) and Make me white (1, 4.2%) (Figure 2).

When compared with non- lightening cream users, there was no significant difference in age, body weight, height and BMI of lightening cream users (27±3.51 vs 26.95±5.27yrs, 69.47±15.33 vs 64.24±10.01kg, 1.60±0.07 vs 1.59±0.05m, 27.22±5.59 vs 25.41±3.58kg/m^2 respectively). Maternal and babies' cord serum levels of H_2O_2 were significantly higher in lightening cream users compared to non- lightening cream users (28.84±8.41 vs 22.55±7.68µmol/l, 28.81±6.81 vs 23.67±8.84 µmol/l respectively). However, babies' cord serum TAC was significantly lower in lightening cream users (0.05±0.24mmol/l) compared to non- lightening cream users (0.68±0.30mmol/l). There was no difference in maternal serum TAC between the two groups (Table 1).

There were statistically significant positive correlations between maternal serum TAC with maternal serum H_2O_2 levels (r=0.408; p=0.048) as well as maternal serum H_2O_2 level and babies' cord H_2O_2 level (r=0.399; p=0.049) in pregnant women using lightening creams but not non-users (Table 2). Correlations of age, body weight and BMI with maternal and babies' cord blood serum H_2O_2 and TAC were not statistically significant in both lightening cream users

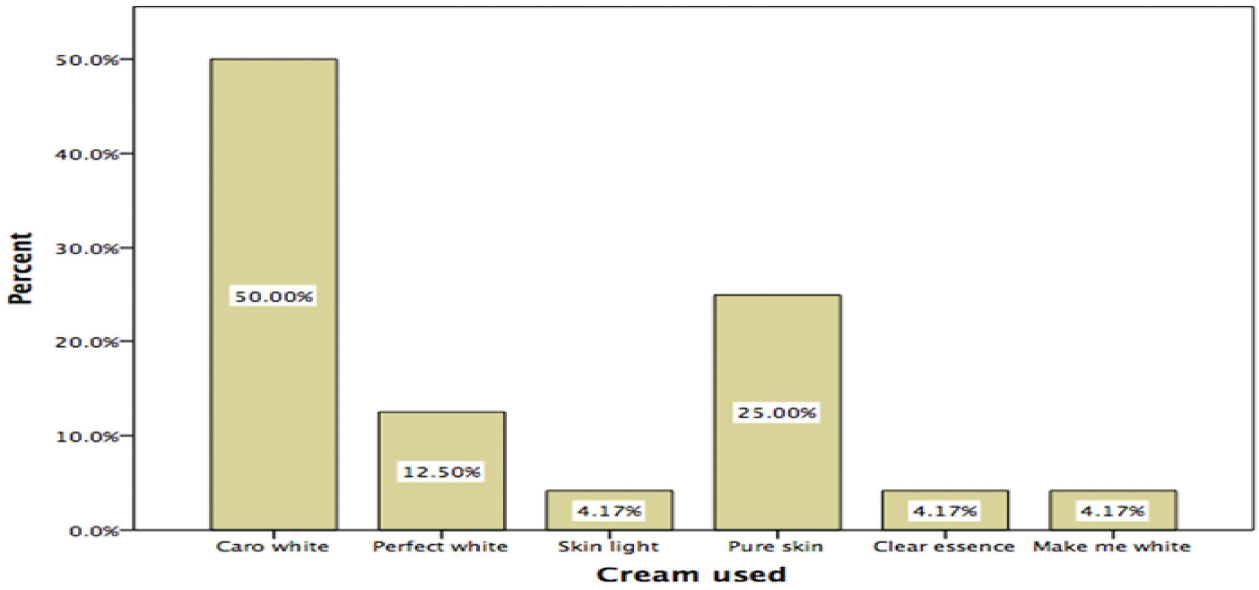

Fig. 2: Types of skin lighteningcreams used by pregnant women

and non-users ($p>0.05$ in each case). There was no significant correlation between maternal serum TAC with cord TAC ($r= -0.344$; $p=0.099$) and H_2O_2 ($r=0.027$; $p=0.899$) level. Correlations between maternal serum H_2O_2 level and babies' cord TAC as well as babies' cord TAC and H_2O_2 were not statistically significant ($p>0.05$). There was no association between duration of lightening cream use, frequency of bleaching cream use and type of lightening cream with maternal and babies' cord serum TAC as well as H_2O_2 levels (Tables 3, 4, 5 and 6).

Table 1 shows age, anthropometric indices, cord and maternal serum TAC and H_2O_2 levels in skin lightening and non- lightening pregnant women. The mean serum H_2O_2, cord TAC, cord H_2O_2 of lightening cream users were significantly higher when compared to non-lightening cream users. There was no significant difference in the mean age, body weight, height and BMI between lightening cream users and non users. Table 2 shows the correlation of variables in lightening and non- lightening pregnant women. Correlations of age, body weight and BMI with maternal and babies' cord blood serum H_2O_2 and TAC were not statistically significant in both lightening cream users and non-users ($p>0.05$ in each case). There was no significant correlation between maternal serum TAC with cord TAC and H_2O_2 level. Correlations between maternal serum H_2O_2 level and babies' cord TAC as well as babies' cord TAC and H_2O_2 were not statistically significant.

Table 3 shows the test of association between maternal serum TAC with duration, frequency and type of lightening cream. There was no association between duration of lightening cream use, frequency of lightening cream use and type of lightening cream with maternal serum TAC. Also there was no association between duration of lightening cream use, frequency of lightening cream use and type of lightening cream with maternal H_2O_2 level (Table 4). There were no association between cord TAC with duration, frequency and type of lightening cream (Table 5). Table 6 shows test of association between cord H_2O_2 with duration, frequency and type of lightening cream. There was no association between cord H_2O_2 with duration, frequency and type of lightening cream.

Discussion

Earlier studies have focused on the effect of skin lightening creams on innate immune functions of maternal skin [11, 12]. However information on the effects of skin lightening cream use during pregnancy on both babies and mothers are scarce. A recent study determined immunoglobulin levels in maternal blood, cord blood and breast milk of Nigerian pregnant women using hydroquinone and non-hydroquinone containing

Table 1: Age, anthropometric indices, cord and maternal serum TAC and H_2O_2 levels in pregnant womenusing skin lightening and non- lightening cream users.

Variables	Skin lightening users	Non skin lightening users	t-values	p-values
Age (years)	27.58±3.51	26.95±5.27	0.478	0.635
Body weight (kg)	69.42±15.33	64.24±10.01	1.320	0.194
Height (m)	1.60±0.07	1.59±0.05	0.400	0.691
BMI (kg/m^2)	27.22±5.59	25.41±3.58	1.274	0.209
MaternalTAC (mmol/l)	0.73±0.21	0.77±0.27	0.701	0.487
MaternalH_2O_2 (µmol/l)	28.84±8.41	22.55±7.68	2.705	0.010*
Cord TAC (mmol/l)	0.50±0.24	0.68±0.30	2.171	0.035*
Cord H_2O_2 (µmol/l)	28.81±6.81	23.67±8.84	2.255	0.029*

**Significant at p<0.05*
t = Student- t test

Table 2: Correlation of variables in pregnant womenusing skin lightening and non- lightening cream users.

Correlating pair		Skin lightening mothers		Non skin lightening mothers	
		r	p	r	p
Maternal TAC	Age	0.171,	0.425	0.049,	0.833
	Body weight	-0.103,	0.631	-0.235,	0.304
	BMI	-0.005,	0.980	-0.364,	0.080
	Serum H_2O_2	0.408,	0.048*	-0.236,	0.267
	Cord TAC	-0.344,	0.099	0.109,	0.613
	Cord H_2O_2	0.027,	0.899	0.146,	0.496
Maternal H_2O_2	Age	0.263,	0.215	-0.032,	0.892
	Body weight	0.082,	0.704	0.222,	0.334
	BMI	0.193,	0.367	0.088,	0.705
	Cord TAC	-0.376,	0.080	0.096,	0.654
	Cord H_2O_2	0.399,	0.049*	0.324,	0.123
Cord TAC	Age	-0.153,	0.474	-0.192,	0.404
	Body weight	0.011,	0.959	-0.259,	0.257
	BMI	0.108,	0.614	-0.239,	0.296
	Cord H_2O_2	0.008,	0.969	0.041,	0.848
Cord H_2O_2	Age	0.203,	0.342	0.038,	0.871
	Body weight	0.177,	0.407	-0.124,	0.591
	BMI	0.210,	0.324	-0.159,	0.491

**Significant at p<0.05*

skin lightening creams [1]. Due to the physiological changes that occur on the skin during pregnancy, some pregnant women use skin lightening creams in a bid to look more attractive, go with existing fashion trend, treat skin blemishes like acne or melisma, cleanse or 'tone' the face and body, or to satisfy the taste of their spouses [2,3,7,8]. Use of skin lightening creams during pregnancy could pose a threat to both the pregnant mother and fetus or eventually lead to adverse birth outcomes in pregnant women who use skin lightening products. Result of the present study shows that 66.67% of pregnant women used skin lightening creams twice daily and 62.50% have used the creams for 3-7years. This is a confirmation of a previous study [2] that skin lightening act is an old and continuous practices. Also the belief is that the creams must be applied in the morning and night to produce desired skin lightening effect.

Table 3: Test of association between maternal serum TAC with duration, frequency and type of skin lightening cream

Variable	Response	Serum TAC ≤0.77mmol/l	Serum TAC >0.77mmol/l	$\chi 2$	P
Duration of cream use.	<3years	6 (46.2)	3 (27.3)	0.906	0.341
	3-7years	7 (53.8)	8 (72.7)		
Frequency of cream use.	Once daily	6 (46.2)	2 (18.2)	2.098	0.148
	Twice daily	7 (53.8)	9 (81.8)		
Type of cream.	Caro white	7 (53.8)	5 (45.5)	3.524	0.620
	Perfect white	1 (7.7)	2 (18.2)		
	Skin light	1 (7.7)	0 (0.0)		
	Pure skin	3 (23.1)	3 (27.3)		
	Clear essence	0 (0.0)	1 (9.1)		
	Make me white	1 (7.7)	0 (0.0)		

Table 4: Test of association between maternal serum H_2O_2 with duration, frequency and type of skin lightening cream

Variable	Response	Serum H_2O_2 ≤22.5µmol/l	Serum H_2O_2 >22.5µmol/l	$\chi 2$	P
Duration of cream use.	<3years	2 (50.0)	7 (35.0)	0.320	0.572
	3-7years	2 (50.0)	13 (65.0)		
Frequency of cream use.	Once daily	1 (25.0)	7 (35.0)	0.150	0.699
	Twice daily	3 (75.0)	13 (65.0)		
Type of cream.	Caro white	3 (75.0)	9 (45.0)	1.800	0.876
	Perfect white	0 (0.0)	3 (15.0)		
	Skin light	0 (0.0)	1 (5.0)		
	Pure skin	1 (25.0)	5 (25.0)		
	Clear essence	0 (0.0)	1 (5.0)		
	Make me white	0 (0.0)	1 (5.0)		

Table 5: Association between cord TAC with duration, frequency and type of skin lightening cream

Variable	Response	Cord TAC ≤0.68mmol/l	Cord TAC >0.68mmol/l	$\chi 2$	P
Duration of cream use.	<3years	8 (42.1)	1 (20.0)	0.825	0.364
	3-7years	11 (57.9)	4 (80.0)		
Frequency of cream use.	Once daily	7 (36.8)	1 (20.0)	0.505	0.477
	Twice daily	12 (63.2)	4 (80.0)		
Type of cream.	Caro white	10 (52.6)	1 (40.0)	8.842	0.116
	Perfect white	3 (15.8)	0 (0.0)		
	Skin light	0 (0.0)	1 (20.0)		
	Pure skin	5 (26.3)	1 (20.0)		
	Clear essence	0 (0.0)	1 (20.0)		
	Make me white	1 (5.3)	0 (0.0)		

Anthropometric measurements are associated with health status and socioeconomic conditions in population groups, as they reflect the exposition to deprivation or excessive food, insufficient physical activity and occurrence of diseases. Insignificant

Table 6: Association between cord H_2O_2 with duration, frequency and type of skin lightening cream

Variable	Response	Cord H_2O_2		$\chi 2$	P
		≤23.67μmol/l	>23.67μmol/l		
Duration of cream use.	<3years	3 (60.0)	6 (31.6)	1.364	0.243
	3-7years	2 (40.0)	13 (68.4)		
Frequency of cream use.	Once daily	1 (20.0)	7 (36.8)	0.505	0.477
	Twice daily	4 (80.0)	12 (63.2)		
Type of cream.	Caro white	3 (60.0)	9 (47.4)	2.274	0.810
	Perfect white	0 (0.0)	3 (15.8)		
	Skin light	0 (0.0)	1 (5.3)		
	Pure skin	2 (40.0)	4 (21.1)		
	Clear essence	0 (0.0)	1 (5.3)		
	Make me white	0 (0.0)	1 (5.3)		

differences in anthropometric indices of skin lightening cream users and non-users indicated that all participants might have similar nutrition status, lifestyles and physical activity, and that anthropometric measurements are not affected by skin lightening cream use. Moreso, there was no significant association between duration of cream use, frequency of cream use and type of cream use with the levels of TAC and H2O2. This is an indication that short term use, type and frequency of cream used might have no effect on anti-oxidant/oxidant balance. Complications of chronic use of skin bleaching cosmetics on the users were previously highlighted [2]. Some of these complications are exogenous ochronosis, impaired wound healing and wound dehiscence, the fish odor syndrome, nephropathy, steroid addiction syndrome, predisposition to infections, a broad spectrum of cutaneous and endocrinologic complications of corticosteroids, including suppression of hypothalamic-pituitary-adrenal axis [2]. Therefore, there will be need to analyse the compositions of skin lightening creams.

In the process of lightening the skin, bleaching agents have been reported to disrupt the primary innate immune function of the epidermal layer of the skin thereby making the users prone to various infections including bacterial, fungal, parasitic and viral infection [11, 12]. Erosion of top layer of the skin apart from disruption of dermal innate immunity prone the skin to infection of the epidermal layer which leads to activation of phagocytes, production of cytokines and mobilization of circulating neutrophils [13]. The activation of phagocytes further leads to production of free radicals which could produce local and systemic oxidative stress [14]. Also, components of many skin lightening products, has been reported to induce oxidative stress by decreasing the activity of two major antioxidant enzymes, namely glutathione peroxidase and superoxide dismutase and increased production of H2O2 [15, 16].

Previous studies indicated that repeated application of skin-lightening creams could induce permanent damage to the skin, kidneys, brain and liver [17, 18, 19] while exposure of placental cells to mercury, an active ingredient in most skin lightening creams leads to accumulation in the placental membrane and lowers the membrane fluidity [6, 8]. Thus, affecting membrane function and cause damage to the developing foetus.

Hydrogen peroxide at higher concentrations cause membrane damage to cells as detected by increased lactate dehydrogenase leakage, indicating a change in membrane permeability or cell necrosis [21]. Therefore, a change in membrane permeability disturbs structural integrity, which could lead to the increased entry of toxins to cells, and in cell death at a later stage. Also, hydrogen peroxide has been reported to inactivate superoxide dismutase which is an antioxidant hence leading to oxidative stress [21]. In this study, serum level of hydrogen peroxide was significantly higher in babies'cord serum and serum of pregnant women using skin lightening creams compared with non-users. This is indicative of cream induced oxidative stress in pregnant women using skin lightening creams. Oxidative stress in these women has the potential to lead to adverse pregnancy outcomes, pregnancy complications and reduced immunity. Wisdom et al [22] also reported an association between oxidative stress and pregnancy induced hypertension while Myatt and Cui [23] demonstrated that increase in reactive oxygen species such as hydrogen peroxide and others could be linked to gestational diabetes mellitus. Oxidative stress in pregnancy has also been associated with future diseases

in adulthood such as obesity, diabetes mellitus, and hypertension [24].

The placenta in normal pregnancy exhibits intense cellular activity and is the major source of pro-oxidant agents and oxidative stress in normal human pregnancy [25]. However, studies have shown that the placenta of pregnant women with gestational diabetes have a reduced capacity to respond to exogenous oxidative stress [26]. This study observed a significant reduction in the total antioxidant capacity of babies' cord blood from mothers using bleaching cream compared to non users. We hypothesize that hydrogen peroxide produced by exposure to harmful components of bleaching creams may leak through layers of the skin into circulation and through placenta to the babies with continuous use in pregnancy. Hence, the reduction of total antioxidant capacity might be due to its neutralization by hydrogen peroxide.

This study was limited by use of mixtures/ multiples of skin lightening creams and not knowing actual duration of the cream use. These groups of participants were excluded.

In conclusion, pregnant women using skin lightening creams and their babies are at higher risk of oxidative stress induced by the exposure to contents of these lightening creams. The recommendation from this study is that women should desist from the use of skin lightening creams or be advised to suspend the use of skin lightening creams during pregnancy as part of instructions given during pre-natal care, if it is compulsory for them to use skin lightening creams.

References

1. Nwosu AO and Arinola OG. Immunoglobulin levels in maternal blood, cord blood and breast milk of Nigerian pregnant women using hydroquinone and non-hydroquinone containing skin lightening creams. Our Dermatol Online. 2019;10 (2):131-137.
2. Olumide YM, Akinkugbe AO, Altraide D, *et al*. Complications of chronic use of skin lightening cosmetics. Int'l. J. of Dermatol. 2008; 47(4): 344-353.
3. Mahe A, Keita S and Bobin P. Dermatologic complications of the cosmetic use of bleaching agents in Bamako (Mali). Ann. Dermatol. Venereol. 1994;121:142-146.
4. Fialova L, Malhoban I, Kalousova M, *et al*. Oxidative stress and inflammation in pregnancy. Scand. J Clin Lab Invest. 2006;66:121-127.
5. Stark JM. Inadequate reducing system in pre-eclampsia: a complementary role for vitamin C and E with thioredoxin-related activities. Brit. J Obs.Gyn. 2001;108:339-343.
6. Wells EM, Herbstman JB and Lin YH, Methyl mercury, but not inorganic mercury, associated with higher blood pressure during pregnancy. Environ Res 2017;154: 247-252.
7. Genchi G, Sinicropi MS, Carocci A, *et al*. Mercury Exposure and Heart Diseases. Intl. J Environ Res Public Health. 2017;14:74.
8. El-Badry A, Rezk M and El-Sayed H. Mercury-induced Oxidative Stress May Adversely Affect Pregnancy Outcome among Dental Staff: A Cohort Study. Intl J. Occup. and Enviro. Med. 2018; 9(3): 113-119.
9. Rahamon SK, Arinola OG and Akiibinu MO. Total antioxidant potential and essential trace metals in the breast milk and plasma of Nigerian human immunodeficiency virus-infected lactating mothers. J. Res.Med.Sci. 2013; 18(1): 27–30.
10. Edem VF, Kosoko A, Akinyoola SB, *et al*. Plasma antioxidant enzymes, lipid peroxidation and hydrogen peroxide in wistar rats exposed to Dichlorvos insecticide. Arch Applied Sci Res. 2012;. 4: 1778-1781
11. Arinola, OG. Akiibinu, MO and Afolabi, KA. Assessment of acute phase proteins and oxidative stress status of Nigerians using bleaching agents. Pak. J. of Med. Scs. 2010;26(4), 860–863.
12. Arinola OG, Afolabi K and Olopade C.O. Immunological skin tests and hematological indices in Nigerian users of skin lightening creams. Egyptian Journal of Dermatology. 2012. 2: 3-6
13. Janeway CA Jr and Medzhitov R. Innate immune recognition. Ann. Rev. Immunol. 2002;20:197-216.
14. Belikov AV, Schraven B and Simeoni L. T cells and reactive oxygen species. J. Biomed. Sci. 2015; 22: 85-90.
15. Samir AM and Aref WM. Impact of occupational exposure to elemental mercury on some antioxidative enzymes among dental staff. Toxicol. and Health. 2011; 27: 779-786.
16. Derikvand F, Bigi F, Maggi P, Piscopo G and Sartori G. Oxidation of hydroquinones to benzoquinones with hydrogen peroxide using catalytic amount of silver oxide under batch and continuous-flow conditions. J. of Catalysis. 2010; 271 (1): 99-103
17. Al-Saleh, I., El-Doush. I., Shinwari, N., *et al*. M. Slow mercury containing skin lightening creams. Cutan. Ocul. Toxicol. 2005; 24:11-29.

18. Petit A, Cohen-Ludman C, Clevenbergh P, Bergmann J F and Dubertret L. Skin lightening and its complications among African people living in Paris. J. Amer. Ass. Dermatol. 2006; 55 (5): 873–878
19. Al-Saleh, I. and Shinwari, N. Urinary mercury levels in females: Influence of skin lightening creams and dental amalgam fillings. Biometals. 2009;10:315-323.
20. Valen G, Sondén A, Vaage J, Malm E and Kjellström T. Hydrogen peroxide induces endothelial cell atypia and cytoskeleton depolymerisation. Free Radical Biology and Medicine. 1999; 26: 1480-1488
21. Burton G.J and Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol. 2011; 25(3): 287–299.
22. Wisdom SJ, Wilson R, McKillop JH and Walker JJ. Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. Amer. J. Obstet. Gynecol. 1991; 165: 170–174.
23. Myatt L. Review: Reactive oxygen and nitrogen species and functional adaptation of the placenta. Placenta. 2010; 31(Suppl): S66–569.
24. Pereira AC and Martel F. Oxidative stress in pregnancy and fertility pathologies. Cell Biology and Toxicology. 2014; 30(5): 301-312
25. Coughlan MT, Vervaart PP, Permezel M, Georgiou HM and Rice GE. Altered placental oxidative stress status in gestational diabetes mellitus. Placenta. 2004b;25:78–84.
26. Lappas M, Mittion A and Permezel M. In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in placenta, but not adipose tissue, of women with gestational diabetes. J. Endocrinol. 2010;204:75–84.

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Total hip replacement for acetabular protrusio. Challenges of a deep acetabulum

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Abstract
Protrusio deformity is an uncommon presentation in our environment and presents a problem of a deep acetabulum due to medial displacement of the medial wall. Management must involve reconstitution of the medial wall and therefore the biomechanics of the hip for long term survival. We present a review, discussing the pathomechanics and surgical management, with emphasis on the central role of the femoral head autograft in reconstructing the deformity.

Keywords: *Protrusio deformity, acetabulum, biomechanics, pathomechanics*

Abstrait
La déformation protrusio est une présentation rare dans notre environnement et présente un problème d'acétabulum profond dû au déplacement médial de la paroi médiale. La prise en charge doit comprendre la reconstitution de la paroi médiale et donc la biomécanique de la hanche pour une survie à long terme. Nous présentons une revue, discutant la pathomécanique et la gestion chirurgicale, en mettant l'accent sur le rôle central de l'autogreffe de la tête fémorale dans la reconstruction de la déformation.

Mots clés: *déformation protrusio . acétabulum , biomécanique, pathomécanique , reconstruction*

Introduction

Acetabular protrusio is a pathological hip condition characterised by medial protrusion of the acetabulum into the pelvis. This can involve the native hip joint or the prosthetic hip replacement.

The primary form of the disease is rare and known as arthrokatadysis. Dr William Otto, a German pathologist first described it in 1824 [1]. It is commoner in young adult to middle aged women between the second and fifth decades of life. It is frequently bilateral.

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Aetiology

Protrusio can occasionally be secondary to other pathologies. The final common pathway of these conditions involves softening or weakening, and failure of the medial wall of the acetabulum with resultant failure to resist medially directed joint reaction force.

Secondary causes [1] include Infections-bacterial and tuberculous, inflammatory joint diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, juvenile rheumatoid arthritis, Reiter's disease. Others include avascular necrosis from sickle cell disease, primary/idiopathic, steroid use or alcohol. Metabolic bone diseases include osteomalacia, osteoporosis, parathyroid disorders, renal osteodystrophy and Pagets disease, fibrous dysplasia. Connective tissue diseases such as Marfan syndrome, Erhler-Danlos syndrome and osteogenesis imperfecta. Post-traumatic causes can be from malunited acetabular fractures as well as iatrogenic acetabular fractures during primary total hip replacement. Prosthetic hip joint failure, Including acetabular erosion from hemiarthroplasty as well as failed THR.

Acetabular protrusio secondary to these pathologies is uncommon in patients undergoing total hip replacement in our environment. Rather, they are often due to post infectious acetabular wall damage, inflammatory arthritis, post-traumatic acetabular protusio due to untreated central fracture dislocation, protrusion resulting from advanced AVN. Failure to recognise and address this medial cavitary defect and restore the hip joint biomechanics can result in early failure of the arthroplasty.

Pathomechanics of protrusio

Acetabular protrusio occurs due to weakness or failure of the medial buttressing wall of the acetabulum. The 65-degree angle that protrusion subtends from the horizontal was noted by McCollum *et al* [2] to be identical to the angle of the joint reaction force in single leg stance. The joint reaction force is directed medially and superiorly and causes displacement of the medial wall into the pelvic cavity. McCollum *et al* [2] noted that protrusio is a progressive deformity and only stops

when the trochanter abuts the pelvic sidewall. The progression of the deformity is due to the increasing joint reaction force resulting from the progressively increasing abductor force as the abductor moment arm reduces with superior-medial displacement of the greater trochanter.

The tunnelling effect of the femoral head leads to a change in acetabular shape from the hemispheric acetabulum to a cylindrical tunnel shaped deformity. The medial displacement of the femoral head and neck results in stiffness and loss of motion because of the entrapment of the femoral head and neck in the acetabulum.

In summary, there is progressive superior-medial displacement of the femoral head and medial acetabular wall into the pelvis with elevation and medialisation of the hip centre driven by the joint reaction force. There is a change in shape from the normal hemispherical acetabulum to the cylinder noted in protrusio. There is also a medial bone loss with variable medial cavitary defect. There may be abutment of the proximal femur against the pelvic side wall in severe cases. There is usually an associated varus deformity of the femoral neck. Periarticular soft tissue contractures may occur.

Presentation

Total hip replacement is the treatment of choice for arthritis secondary to acetabular protrusio. The history can provide an indication of the underlying pathology and this should be sought in the history of presenting complaint, review of systems, as well as family history, all of which may point to underlying heritable diseases and syndromes such as haemoglobinopathy, Marfan syndrome, inflammatory arthritis etc. The impairment of function and quality of life should be documented and quantified. If it is prosthetic protrusion, then information about the indication for the primary surgery, the operation record and post-operative course should be sought. The time of onset of symptoms post operation should also be sought.

In cases of post-traumatic protrusion information regarding the initial trauma and its treatment is important as operative treatment may point to potential problems with surgery such as heterotopic ossification and the need to remove metalwork may be necessary. In our environment these are more likely to be treated non-operatively as pelvic/acetabular fixation surgery is complex and experience with this type of surgery is lacking.

In cases of post-traumatic deformity, post-infectious cases and cases with underlying haemoglobinopathy, or in patients with rapidly progressive destruction of the hip joint, blood screen with inflammatory markers are mandatory and if elevated, hip aspiration should be carried out. This can be done with image intensifier if available or with USS guidance under strict aseptic precautions.

Clinical examination may reveal features of the underlying pathology such as polyarticular involvement in inflammatory joint disease, sickle disease, connective tissue disorders such as Marfans syndrome and Paget disease.

There is usually shortening of the involved extremity and stiffness of the involved joint. Peri-articular soft tissue contractures may be present. Investigation and treatment of other underlying pathology responsible for native protrusio should be carried out if possible.

Radiographic definiton of acetabular protrusio

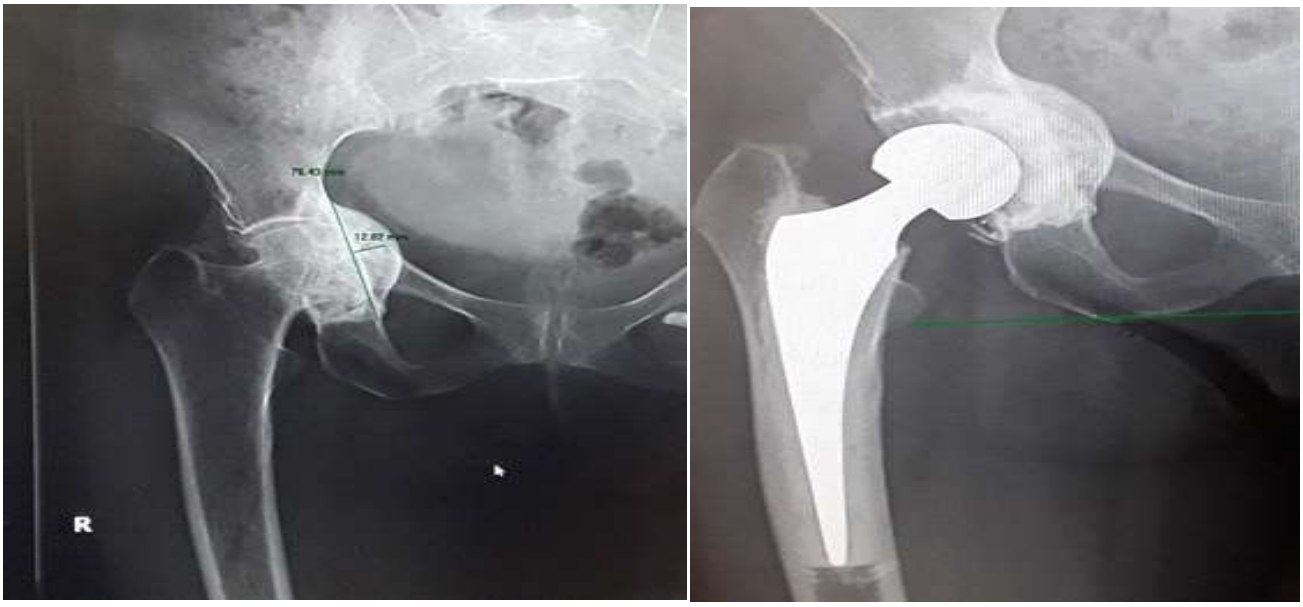
Displacement of the medial wall beyond the Kohler's line on AP radiograph by more than 3mm in males and more than 6 mm in females is defined by Armbuster *et al* [3] as diagnostic of protrusio. Displacement of the medial wall beyond the Kohler's line on AP radiograph is further graded by Charnley and Sotelo-Garza as mild (1-5mm) Moderate (6-15mm) and severe (>15mm) medial to Kohlers line [4]. Centre-edge angle of Wirberg measurement above 40 degrees [5] is also diagnostic of protrusio.

Radiographic assessment and templating

Pre-operative radiographic templating is important as it helps in deciding where the joint centre should be reconstructed to, while revealing the size of the medial defect behind the templated acetabular component. There is often femoral neck varus and femoral templating will help to determine the level of neck cut and the required offset. Generally, a low femoral neck cut and high offset will help in optimising abductor tension, reduce the likelihood of bony impingement with the acetabular side wall and minimize leg length discrepancy.

In the presence of post-traumatic protrusio, Judet view will provide information about the walls and columns as these may have displaced and their new positions are important as this may affect component placement as well as hip stability. Computed tomography scan with 3 D reconstruction is helpful in providing further information about the acetabular walls

and columns and 3D reconstruction can be obtained in severe or complex cases.



Fig, 1: Preoperative estimate of medial protrusion and post-operative cemented reconstruction using femoral autograft

Operative management

The hip should be exposed by the approach with which the surgeon is familiar. Hip dislocation can be difficult and the neck may need to be osteotomised in-situ to prevent iatrogenic femoral shaft fracture with attempts at dislocation. The head is removed and preserved as it is crucially important for the reconstruction of the medial buttress. It must not be discarded. The acetabulum should be properly exposed widely and the rim and walls clearly identified and thickness assessed. The medial wall can be reamed lightly or a curette used to remove any soft tissues and freshen the bone but there should be no attempt at medial bone removal. The rim should be sequentially reamed with the aim of converting the cylinder back to a hemisphere, thus bringing the cup downwards and laterally. Once good rim fit is obtained the trial cup can be impacted at the rim. Next the medial cavitary defect is grafted with morselised bone derived from the femoral head autograft. The size of the bone chip should be between 6mm and 8 mm to enable impaction. Impaction is carried out with tamp or reverse reaming.

The rim fitting trial shell is next impacted to ensure stable rim fit and to ensure that excessive medial grating has not occurred as this can displace the cup laterally with risk of cup failure. The cup should be multi-hole and adjunctive screw fixation should be carried out to augment primary stability. The inferior and lateral position of the cup should correct the leg length discrepancy with care being taken not to overlengthen the leg. There is often femoral neck varus. A low femoral neck cut and high offset stem will help in optimising abductor tension, reduce the likelihood of bony impingement with the acetabular side wall, and minimize leg length discrepancy.

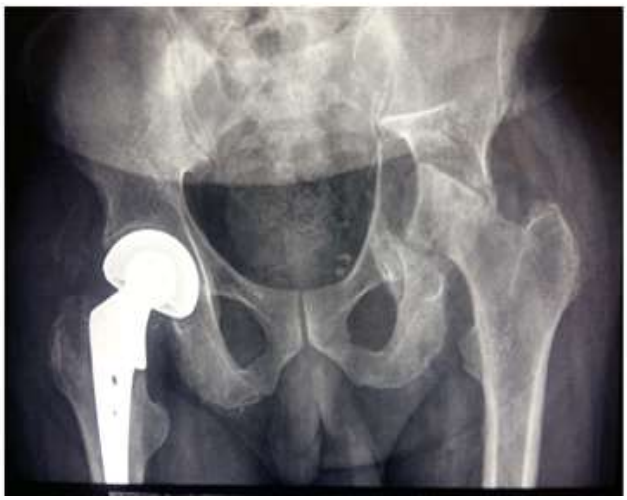


Fig.2a. Preoperative radiograph of central fracture-dislocation with secondary OA

The usual assessment of proper implant positioning and stability testing should be carried out to ensure that the hip is stable. Leg lengths should be checked to ensure satisfactory equalisation without excessive tension. Definitive components are placed and further trials to assure stability and equal leg lengths. Soft tissue repairs and surgical wound are closed in layers. Post operatively, mobilization can be full or partial depending on the complexity of the reconstruction. Bone incorporation takes about 6 weeks but continues to mature over the following few years.

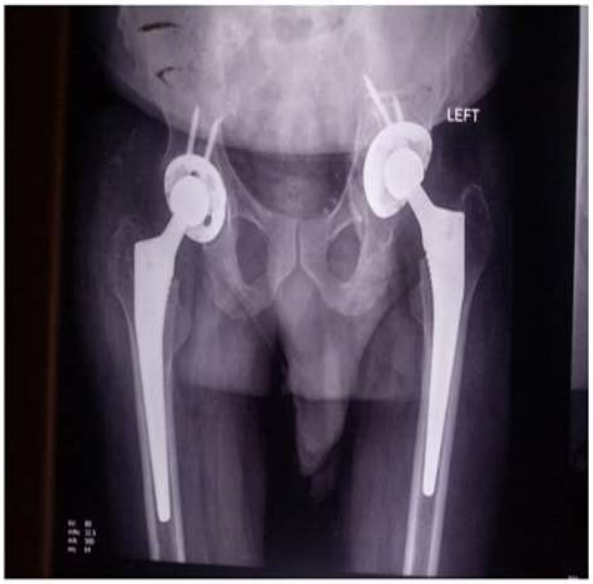


Fig.2b. At 3 months post op showing healing and incorporation of medial morselised femoral head autograft.

Discussion

Cemented hip reconstruction with impacted morselised medial autograft has resulted in midterm to long-term survival of about 85% to 90% in some series [6,7]. There are however other series where cemented reconstruction with medial grafting has not fared so well with failures at mid-term follow-up. Furthermore, cemented acetabular reconstruction is a more exacting technique and care must be taken not to displace the graft during the cementing procedure.

The uncemented reconstruction is the recommended technique in our environment. A rim fit acetabular shell is optimal as this best engages the rim. Under-reaming and cup impaction also works as well. A multi-hole cup is preferred with fixation augmented with multiple screws.

The medial buttress must be reconstructed using the resected femoral head as impacted morselised medial autograft. This restores bone stock and acts as a medial buttress that resists the joint reaction force and by lateralizing and distalizing the acetabular component, restores the anatomic hip centre, thus optimizing the hip biomechanics and ensuring long-term survival. [8,9]. Uncemented acetabular reconstruction with medial autograft has resulted in 90% survival at mid to long term follow-up. It is relatively straightforward technique and results in consistently good outcome

When the acetabular rim is deficient, or the protrusion is very large, an antiprotrusio cage may be necessary to span the defect and protect the grafted cavity.

Surgical pearls and pitfalls

. Identify and address underlying cause of protrusion.
. Understand the pathoanatomy in protrusion.
. Plain radiograph for pre-operative assessment, templating and surgical planning is adequate. For more complex cases including protrusion secondary to acetabular fracture, CT scan including 3-D reconstruction is helpful in better defining the bony anatomy.
. In-situ neck osteotomy should be carried out if hip dislocation is difficult as femoral shaft fracture may occur with persistent attempts to dislocate a deep-seated femoral head.
. The acetabular exposure must be adequate with clear identification of rim, and walls including the displaced medial wall. Freshen the medial wall to accept bone graft but do not ream medially as intrapelvic breach may occur. The patients femoral head autograft must not be discarded and must be retained. It is crucial for reconstruction of the medial cavitary defect as failure is almost inevitable if the medial buttress is not restored. Rim fitting cup is optimal for this condition, but press-fit cup is also satisfactory. Multi-hole cup is preferred as primary stability should be augmented with screws
. Be mindful of leg length discrepancy and risk of iatrogenic sciatic nerve injury.

References

1. Van de Velde S, Fillman R and Yandow S. The aetiology of protusio acetabuli: literature review from 1824 to 2006. Acta Orthop Belg. 2006; 72:524-529.

2. McCollum DE, Nunley JA and Harrelson JM. Bone grafting in total hip replacement for acetabular protrusion. J Bone Joint Surg AM 1980; 62: 1065-1073.
3. Armbuster TG, Guerra J Jr, Resnick D, *et al*. The adult hip: an anatomic study. Part I: the bony landmarks. Radiology. 1978; 128(1):1-10.
4. Sotelo-Garza A and Charnley J. The results of Charnley arthroplasty of hip performed for protrusion acetabuli. Clin Orthop Relat Res. 1978; (132):12-18
5 Hooper JC and Jones EW. Primary protrusion of the acetabulum. J Bone Joint Surg Br. 1971; 53 (10:23-29.
6. Rosenberg WW, Schreurs BW, de Waal Malefijt MC, Veth RP and Slooff TJ. Impacted morsellized bone grafting and cemented primary total hip arthroplasty for acetabular protrusion in patients with rheumatoid arthritis: an 8- to 18-year follow-up study of 36 hips. Acta Orthop Scand.2000; 71:143–146. 17.
7. Schutzer SFand Harris WH. High placement of porous-coated acetabular components in complex total hip arthroplasty. J. Arthroplasty.1994 Aug; 9(4): 359-367.
8. Baghdadi YM, Larson N A, Sierra RJ. Restoration of the Hip Center During THA Performed for Protrusio Acetabuli Is Associated With Better Implant Survival. Clin Orthop Rel Res**.** 2013 Oct 471(10):3251-3259.
9. Garcia-Cimbrelo E, Diaz-Martin A, Madero R, Munera L. Loosening of the cup after low-friction arthroplasty in patients with acetabular protrusion. The importance of the position of the cup. J Bone Joint Surg Br. 2000 Jan; 82(1):108-115.

HIV/AIDS stigma in Nigeria: Exploring the nexus of social processes and social policies

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Abstract
Background: Stigma is an outcome of an 'identity' which is rooted in the complex meanings of social reality. Culture as the underlying structure of social meanings, is largely invested in the epidemiological narrative of disease conditions. Hence, the cultural interpretations of HIV/AIDS' causes and symptoms and outcomes, inform the structure of the social networks, social reference and social process of interactions with People Living with HIV/AIDS.
Method: The study reviews literature primarily originating from Medical Subject Headings (MeSH)- stigma, social stigma, stigmatization, Epidemiology of HIV/AIDS', 'social aspects of HIV/AIDS', 'social support for people living with HIV/AIDS (PLWHA)', 'knowledge of prevention about HIV/AIDS', 'community support for PLWHA', 'Religion and HIV/AIDS', 'Prevalence of HIV/AIDS', 'Acceptance index of Antiretroviral therapy (ART)', 'family support for PLWHA', 'Ethnicity in HIV/AIDS Epidemiology'. A review of the selected databases – PubMed, AJOL, JSTOR, Google scholar and the National Agency for the Control of AIDS (NACA) Library was done.
Result: The epidemiological narrative of stigma in Nigeria, the nature of stigmatization as it subsists in Nigerian communities, and a compendium of government's programmes and policies for stigma reduction – a response to social processes as 'stimulus' were identified in relation to policy outcomes.
Conclusion: Policies are although a response to stigmatizing social processes, both are synchronous and are potentially effective for building stronger social institutions for stigma reduction.

Keywords: *HIV/AIDS, Stigma, Epidemiology, Social Processes, Nigeria.*

Abstrait
Contexte : La stigmatisation est le résultat d'une 'identité' qui est enracinée dans les sens complexes de la réalité sociale. La culture, en tant que structure sous-jacente des sens sociales, est largement investie dans le récit épidémiologique des maladies. Par conséquent, les interprétations culturelles des causes, des symptômes et des résultats du VIH/SIDA informent la structure des réseaux sociaux, la référence sociale et le processus social des interactions avec les personnes vivant avec le VIH/SIDA.
Méthode : L'étude passe en revue la littérature, principalement issue des Titres de Sujets Médicaux (MeSH) - stigma, stigma sociale, stigmatisation, épidémiologie du VIH/SIDA', 'aspects sociaux du VIH/SIDA', ' soutien social aux personnes vivant avec le VIH/SIDA (PVVIH)', ' connaissance de la prévention du VIH/SIDA', 'soutien communautaire aux PVVIH', 'Religion et VIH/SIDA', 'Prévalence du VIH/SIDA', ' Indice d'acceptation de la thérapie antirétrovirale (ART)', 'soutien familial aux PVVIH', 'Ethnicité dans l' épidémiologie du VIH/SIDA'. Une revue des bases de données sélectionnées - PubMed, AJOL, JSTOR, Google Scolaire et la bibliothèque de l'Agence Nationale du Control du SIDA (NACA) a été effectué.
Résultat : Le récit épidémiologique du stigma au Nigéria, la nature de la stigmatisation telle qu'elle existe dans les communautés nigérianes et un recueil des programmes et des politiques du gouvernement pour la réduction de la stigmatisation - une réponse aux processus sociaux comme 'stimulus' ont été identifiés en relation avec les résultats politiques.
Conclusion : Les politiques sont bien qu'une réponse aux processus sociaux stigmatisants, les deux sont synchrones et sont potentiellement efficaces pour construire des institutions sociales plus solides pour la réduction de la stigmatisation.

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Mots clés : *VIH/SIDA, stigma, épidémiologie, processus sociaux, Nigeria.*

Introduction

Across societies, human beings react to health problems with feelings of compassion, sympathy or even empathy with special privileges for the ill. However, an uncommonly infectious problem such as HIV/AIDs which is socially 'constructed' to be dishonouring or shameful, elicits unfavourable reactions from members of society in the form of stigmatization [1]. Goffman asserts that stigma is a social construct by human beings against other human beings; hence, a stigma is any characteristic or attribute that sets a person or group of persons apart from most of the population with the result that the person or group so stigmatized is treated with suspicion or hostility. In the view of [2], a stigma is a "powerfully negative label that radically changes a person's self-concept and social identity." Stigmatization overwhelms other dimensions of a person's identity by discrediting him or her in the minds of others through a 'degradation ceremony' [3], which eventually makes the so *labeled* socially isolated. Stigmas are usually not based on a valid understanding of the actual situation or, but on erroneous beliefs about a phenomenon [4]; and this is true in the case of HIV/AIDS for which the National HIV/AIDS Strategic Plan 2010-2015 asserts that there is low comprehensive knowledge of HIV transmission (24.2%) (National Agency for Control of AIDS [5]. Comprehensive knowledge here refers to – consistent and correct condom use during sexual intercourse; having one-uninfected and stable sexual partner; healthy-looking persons can be living with the virus; and rejecting the idea that the virus can be contacted through mosquito bite and sharing food.

Hence, beyond social isolation, stigmatization impacts every stage of the illness course of PLWHA, including Anti-Retroviral drugs (ART) utilization and other aspects of HIV/AIDS treatment. This therefore has great implications, not only for treatment outcomes, but also the ability of the country to successfully negotiate the challenge posed by the disease. Due to the stigma that accompanies their condition, PLWHA suffer discrimination, marginalization and oppression in their different shades [6]. In a 2013 report, UNAIDS noted that HIV-related stigma and discrimination persist as major obstacles to an effective HIV response in all parts of the world, including segregation in employment, access to education, family planning, dental and other health services.

People living with HIV/AIDS (PLWHA) are stigmatized due to the erroneous beliefs about the mode of transmission of the disease, and the perception that such persons must have lived a promiscuous or sexually irresponsible life, which 'earned' them the suffering that they now bear [7,8]. In the 2016 Country Factsheet, in Nigeria, only 22.3% of young women (aged 15-24) and 27% of young men (aged 15-24) had adequate knowledge about HIV prevention and mode of transmission [9]. A stigma survey among HIV positive persons in Nigeria showed that 34% of affected persons were excluded from family events, 35% were verbally assaulted, 28% were physically assaulted, while 29% suffered the loss of job or income; and beyond their immediate community, 21% reported being denied health services generally, and 8% Sexual and Reproductive Health – SRH services [10]. Thus, stigmatization of PLWHA begins from their immediate family where they should have had a haven for themselves in the face of hostile treatment from members of society. It is in view of this and associated issues that the Nigerian Government enacted the HIV/AIDS *anti-stigma and discrimination bill* in 2014, with a view to curbing the stigmatization of and discrimination against PLWHA in Nigeria as well as people who are merely affected and not infected by the disease. The National AIDS Spending Assessment (2011-2013) identified HIV stigma, among other issues, as a key driver of the HIV/AIDS epidemic in Nigeria.

This review paper focuses generally on the social processes of HIV/AIDS stigma in Nigeria, and specifically explores the social processes and policy nexus in Nigeria. It focuses on the sociological issues in the stigmatization of people living with HIV/AIDS (PLWHA); policies that emanated as a response to areas of societal interest as well as policies and programmes that focus on reducing the stigmatization of PLWHA within the context of community support programmes/ policies aimed at reducing the stigmatization of PLWHA.

Methodology

The review was based on empirical as well as theoretical literature on the social aspects of HIV/AIDS stigma, published before November 2017, with no specific timeline, but effort was made not to go beyond year 2000. Search terms and strings were built from the concept paper developed for the review. Thus, the words and concepts that informed database searches included stigma, social stigma, stigmatization, Epidemiology of HIV/AIDS', 'social aspects of HIV/AIDS', 'social support for people living with HIV/AIDS (PLWHA)', 'knowledge of prevention about

HIV/AIDS', 'community support for PLWHA', 'Religion and HIV/AIDS', 'Prevalence of HIV/AIDS', 'Acceptance index of Antiretroviral therapy (ART)', 'family support for PLWHA', 'Ethnicity in HIV/AIDS Epidemiology'. Boolean operators (AND, OR, NOT) and wild card symbols (*, !, ?, or #) were used to guide and structure the search process, making it as inclusive as possible, with emphasis on the Nigerian situation, and selected states that are of interest to the study.

The search terms and strings were applied to PubMed, AJOL, JSTOR, Google scholar and the National Agency for the control of AIDS (NACA) Library – the databases that constituted the literature sources for the review. The Medical Subject Headings (MeSH) was used to explore/develop alternative concepts on PubMed which were later utilized in carrying out literature searches in other data bases. Aside from scholarly academic literature, the review further included relevant technical reports, statistics, and policy/programmes documents on Nigeria, that were published during the period under review.

The Abstracts of the articles were screened strictly based on currency and relevance. Currency was purposively defined in terms of articles that were published from January 2000, the beginning of the new millennium when renewed vigour was introduced to the pursuit of developmental goals, particularly those related to containing global menaces like HIV/AIDS. The relevance of articles was adjudged based on their ability to address key themes in the brief *vis-à-vis* the Nigerian situation. Articles that made it through the screening process were then compiled for the review, a process which covered the entirety of each of the articles included.

Cultural epidemiological narrative of HIV/AIDS stigma in Nigeria

The HIV and AIDS epidemic cuts across all the demographic categories and socio-cultural divides of Nigeria. Consequently, the disease burden is borne by all Nigerians either as infected persons or people who are impacted through the infection of other people. The National HIV/AIDS Epidemiology and Impact Analysis (NHEIA) [11]. National HIV/AIDS Epidemiology and Impact,[11] reported that deaths due to AIDS rose from 141,225 in 2000 to 209,340 in 2006, to 218,996 in 2012, and 233,604 in 2013. The astronomical rise in fatality due to HIV/AIDS is attributable to ignorance, poor access to health and social services, including ART stigma and discrimination, gender issues and poverty [12]. The analysis of data on HIV prevalence among key populations, [11]. further revealed that infection rates are still very high among Female Sex Workers (48.1%) compared to 17.2% for men who have sex with men (MSM) and 4.2% for injecting drug users (IDU); and that HIV/AIDs knowledge, though increasing, is low across the general population (35.6% for male and 23.6% female).

Although gender, social class and geographical area are identified as some of the factors related to stigma and discrimination, arguing that, in the Nigerian context, all geographical zones are affected in the same way, education, information and awareness about HIV/AIDS are major factors that influence developments related to HIV/AIDS and associated perceptions, misconceptions and related issues [11]. These factors vary across ethnic groups, and geographical and political divides in Nigeria, and they affect prevalent cultural reactions to HIV/AIDS vis-à-vis the social processes of stigmatization. It follows that areas and/or people with more educated persons and higher awareness about HIV/AIDS are likely to be more liberal to HIV/AIDS than their counterparts in other places, since education is a major determinant of knowledge and its application [113].

HIV infections in Nigeria result mostly from heterosexual relationship [12] accounting for more than 80% of HIV/AIDS infections [5], with a more pronounced epidemic proportion among females than males, while young people have a high prevalence of the disease. Socio-cultural practices such as sexual behaviour constitute some of the major factors influencing the incidence, prevalence and patterning of HIV/AIDS in population groups. These include sexual behaviour (multiple sexual partners), early and forced marriage, female genital cutting, sexual cleansing rites for widows, gender inequality, invasive practices by traditional medical practitioners, tribal marks and other bodily scarification practices as well as other structural factors influencing HIV/AIDS vulnerabilities[10,12]. It follows therefore, that people and cultures among whom these practices are prevalent, are more likely to be more exposed to and infected by HIV/AIDS, with a higher burden of the disease. This largely accounts for the variation across cultures about HIV/AIDS, and the various dimensions to its distribution and patterning. Although there is paucity of information on the variation of HIV/AIDS across Nigerian cultures and ethnic groups, some populations are more 'at risk' than others. The low status of women

in most of Africa's societies promotes gender-based violence, including rape which can contribute to HIV transmission due to resultant the tears and lacerations [15]. People in highly mobile occupations like drivers, military and police officers, and commercial sex workers [16,17] are particularly at disadvantage.

The 2013 report indicated that more than 10% of the global burden of HIV/AIDS abounds in Nigeria [18]. Four states (Rivers, Taraba, Nassarawa and Kaduna) were identified as bearing the highest portion of the burden (over 8%), while Yobe, Benue, Cross River, Akwa Ibom, Abia, Ondo, Oyo, Sokoto, Gombe and the FCT had a prevalence rate ranging between 3% and 8%. Certain population categories are 'key' to HIV/AIDS prevalence, due to the higher exposure that characterizes them in relation to the disease [11,14]. These include female sex workers (FSW), men who have sex with men (MSM) and injecting drug users (IDU). In a report by,[11]. Gombe, Adamawa, Nasarawa, FCT, Benue, Cross River, Abia and Ondo states were noted to have the highest density of key populations greater than 5000, and a consequently increasing rate of HIV infection. Although the prevalence rate has been fluctuating among FSW and IDU, with howbeit little reductions, the rate is consistently on the increase among the MSM (having increased from 13.5% to 17.2%) between 2007 and 2012. In terms of the hotspot of key populations, Lagos had the highest percentage (38%) followed by Abuja (14%) and Nassarawa (12%). Whereas this calls for intensified attention to be directed to the key population groups, the refusal of the federal government to enact the same-sex marriage bill and the criminalization of prostitution by some states in Nigeria, including the FCT has driven such key populations beyond the reach of interventions [11].

The Social Processes involved in HIV/AIDS stigma

Stigmatization is a major issue in the struggle to negotiate the challenge of HIV/AIDs in Nigeria [16]. It affects PLWHA right from the private sphere of their immediate family to every public domain where their HIV status is known. PLWHA are stigmatized in their everyday interaction with people around them, largely due to misconceptions about the mode of transmission of the disease. The stereotyping and stigmatization of, and discrimination against PLWHA are recurring decimals in most African countries [19]. Stigmatization– the devaluation of a spoiled social identity- which occurs in everyday interaction is reproduced by social inequalities in modern societies. HIV evokes moral judgement which attaches the responsibility of infection on PLWHA.

In recognizing the social processes that accentuate HIV/AIDS stigma. Social actors and 'agency' are significant. Discriminatory practices are embedded in relationship-centred outcomes. These outcomes are processes (verbal or non-verbal) that occur in the workplace, communities and family. Hence, given its association with death and 'wasting' and the highly transmittable nature, HIV/AIDS generates anxiety and fear about potential infection. Stigmatization of PLWHA occurs within the family, at the workplace and in communities [20]. The predominant stigma and discrimination against PLWHAs in Nigeria are described in terms of: negative attitudes to PLWHAs, reluctance by health workers to treat PLWHAs, and the perceived stigma of PLWHAs [21]. Incidentally, PLWHA also experience stigmatization from health workers who have the responsibility of providing them with healthcare services [22]. Such unethical behaviour undermines the quality of confidence that PLWHAs should have in the health system, leaving patients with the fear that the confidentiality, which they cherish very much, will be compromised. Consequently, this portends negative implications for the decision to get tested, seek counseling or even present for treatment [22]. Hence, it has been substantiated[23] that one-third of PLWHA are uncomfortable with receiving ART services in health facilities close to where they live. This is for reasons ranging from disclosure of their status (80.4%), fear of being discriminated against (74.3%), and satisfactory services enjoyed at their current facility (74.3%).

In a survey of 254 journalists in Ibadan, it was established that the knowledge of HIV/AIDS was generally low, and laden with the challenge of misconceptions about the cause and management of HIV/AIDS [24]. This consequently induces negative attitudes towards PLWHA bordering often on stigmatization. According to their report, 46.9% of the journalists thought that PLWHA should be detained in the hospital, to avoid spreading the disease which they perceived was transmittable through mosquito bite and other means, erroneously conceived – a tendency which challenges the capacity of opinion moulders. Thus, people avoid having physical contact with PLWHA-hand shaking, talking or eating together. This results in various forms of discrimination against PLWHA,

which generally affect their ability to live through the disease. It has been found [16] that the fear of stigma (70.8%) and concern over disclosing status to spouse or partner were major barriers to testing for HIV. The persistence of stigma and discrimination against PLWHA contributes directly and significantly to the spread of the infection [25]. Research has shown that interventions to reduce stigma in the national response is rather very poor and palliative at most, poorly coordinated and inadequate. This is an important area in which more concerted effort is required as the national response is being scaled up under the president's Comprehensive Response Plan 2014-2014 [11].

The range of discriminatory practices against PLWHA due to stigmatization within the health system includes awkward social interactions; avoidance; physical distance from patients; unnecessary referral to other healthcare providers; neglect; discouragement of treatment regimen; contempt; blaming; labeling of charts, beds, rooms and abuse of confidentiality; isolation; and verbal abuse [26,27,20]. An unethical behaviour such as the stigmatization of PLWHA by people who have the responsibility of helping patients live through the disease legitimizes other discriminatory practices and violations of the human rights of PLWHA. Ultimately, this has disrupting implications, not only for PLWHA's quality of life, [28] but also for the success of any intervention or development efforts regarding PLWHA. Some of the problems, due to perceived or real stigmatization and discriminatory practices within the one setting where patients should comfortably disclose their status, portend serious problems, including treatment delays, avoidance of health services, and poor-quality care due to non-disclosure to healthcare professionals [26,29]. Beyond these, such a situation could generate the consequences of psychological problems, low-self-esteem, feelings of rejection, and diminished health seeking behaviour, among others.

In a study by the Global Network of People Living with HIV/AIDS, many patients had their status disclosed to their employers or co-workers, without their consent [30]. In such cases, the report noted that PLWHA were discriminated against, in terms of employment, promotion and capacity building opportunities, once their status was disclosed to employers or colleagues. Nigeria was rated highest in terms of discrimination in access to employment (27%) for PLWHA; and this is despite the anti-stigmatization law in force in the country. Similarly, PLWHA were precluded from attending educational institutions, in which case, Nigeria rated highest with 14%, compared to Mexico's 1%. These might have informed the position that stigma and discrimination against PLWHA are negative factors for the decentralization of ART, [18] which, if unabated, will undermine prevention of HIV/AIDS, and treatment and care for PLWHA in Nigeria. There is a link between HIV-related stigma with delayed HIV testing, as well as non-disclosure to partners and poor engagement with HIV services [23]. PLWHA experience judgmental attitudes even from care providers, a phenomenon that sometimes leads to the refusal of services and involuntary sterilization of women infected by the virus.

Failed Social Capital: The emergence of PLWHA-centered programmes/policies in Nigeria

The health systems of developing countries generally lack the arrangement for long-term care and support for PLWHA, outside of the hospital. Hence, they discharge patients without contact with family and care givers [31]. This approach draws from the concept of social capital [32], there is an implicit assumption that family members will be available to provide care and support. Care-giving and support constitutes the social capital the family offers. Social capital thrives on trust, co-operation, reciprocity and sociability [33]. In traditional African societies, the family is a major source of social capital for individuals who are ill. However, within the context of HIV/AIDS care in Africa, the preceding assumption has been faulted. This is because family members are mostly not integrated into the care delivery structure of HIV/AIDS. They usually do not receive special education/trainings on their expected roles towards the dependent ill. The family being a part of the larger society, has a vertical relationship with other forms of 'agency'. Therefore, the role of the family as a source of social capital is an undercurrent for the entrenched tradition of hostility towards PLWHA [34]. Thus, PLWHA are left to confront stigmatization, discrimination and marginalization. PLWHA experience rejection, not only from the public, but also from their own family, proximate groups and at the community level [35].

The vulnerability of PLWHA to discrimination, rejection, social isolation and low access to coping resources necessitates the provision of quality social support at all levels. This is more imperative, given the transition of HIV/AIDS from an acute fatal disease

to a chronic illness. The emergence of HAART provokes a shift in the priority of households from the imminent death of PLWHA, to the construction of a life-encompassing function and well-being [36]. Given the toll that stigmatization takes on PLWHA and its tendency to undermine efforts at containing the disease, it is pertinent to develop commensurate strategies towards addressing the problem, particularly at the grassroots where proximate impact can be made on PLWHA. Studies [19,23,37,38] have pointed to the fact that community support is a potent approach to overcoming the dilemma of the stigmatization of PLWHA. Proper management of HIV patients can be realized by effective community support in form of counselling, treatment and improved access to facilities and services [39].

Community support for PLWHA is possible at the family, group and community levels. However, there is a correlation between effective family support for PLWHA and proper adherence to Highly Active Antiretroviral Therapy [HAART] [35]. A proactive response by communities is indispensable to sustainable interventions for the disease, especially in resource-constrained zones where funds have been flat-lined in HIV/AIDS [40]. In this regard, it has been contended that the household is a viable structure to which recourse must be made in relation to community support for PLWHA [36]. The household is indispensable in building an 'AIDS-competent community'. It is defined as a social setting in which people are most likely to work collaboratively to optimise HIV/AIDS prevention, care and treatment, leveraging on local norms, relationships and networks between the sexes and among generations, which constitute assets that even the most resource-poor settings can exploit. The community and religious strategies can be engaged to minimize oppositions and barriers to HIV/AIDS interventions with a view to filling in the gaps in programmes and addressing the negative beliefs. This will require the participation of various actors and stakeholders: Parents/family members, teachers, religious institutions/leaders, support groups, extension workers, and community health practitioners. The role of CBOs in bridging the ART uptake gap is notable [41]. This is particularly with respect to improving access to ART drugs, which though are becoming affordable; require the agency of institutional blocs to facilitate PLWHA's access. Although the National policy on HIV includes specific directives for the reduction of stigma and discrimination, it has not been fully implemented or utilized. In response to the wide-ranging social processes that constitute the stigmatization of PLWHA, programmes which have been developed to address/ reduce the stigma embedded in support structures are herein examined.

Some selected programmes/policies that respond to the social processes of stigma among PLWHA and/or to reduce stigmatization in Nigeria include:

National workplace policy on HIV/AIDS

This policy was developed by the Federal Government of Nigeria through the Ministry of Labour and Productivity in 2013. The policy emphasized the need to provide care and support to PLWHA, since the disease increased rapidly between 2010 and 2012, mostly affecting the youths, persons within the productive age brackets. Hence, HIV/AIDS impacts workplaces by reducing labour supply, causing absenteeism, increasing medical expenditure and production cost, while reducing productivity and occupational health and safety. To address the situation, the policy emphasizes the need for the effective implementation of workplace strategies and programmes geared towards prevention, treatment, care and support for employees infected by HIV/AIDS. Due to the changing epidemiology of the disease, the need for the periodic revision of HIV/AIDS regulations for workplaces is also emphasized, with recourse to other relevant policies and legislations. The scope of the policy includes persons in any employment or occupation; those in training (including interns and apprentices); volunteers; job seekers and applicants as well as laid-off and suspended workers, across all sectors of economic activities in private and public organizations, armed forces and uniformed services. The policy posits that HIV/AIDS is a workplace issue; it affects the work place, which, being part of the community, has serious roles to play in containing the disease. The policy argued that HIV screening; testing or questions about tests already taken or about medication should not be required of job seekers.

Gender and HIV/AIDS Work Place Policy

Developed in 2009 by the Civil Society Legislative Advocacy Center (CISLAC), the policy emerged as part of the Center's mission, hinged on various national and international frameworks for a national response to HIV/AIDS and gender mainstreaming in Nigeria. It is hinged on the: Universal Declaration of Human Rights (1948), the Convention on Economic, Social and

Cultural Rights (1976), the Convention on the Elimination of All Forms of Discrimination against Women (1979), the Millennium Development goals 3 and 5, the National HIV/AIDS Workplace Policy, Beijing Conference Declaration (1995) and the constitution of the Federal Republic of Nigeria (1999), all of which lay the foundations for and maintain strong prohibitions against all forms of discrimination against human population categories. The policy stems from the epidemic nature of HIV/AIDS in Nigeria and the need to ensure gender mainstreaming in all decisions making processes within organizations that control the spread of the disease, and its social and economic impact. Recognizing that prevention, treatment, care, support, and impact mitigation are mutually reinforcing elements of a comprehensive response to HIV/AIDS in a workplace, the policy advocates for the development of specific objectives and for the definition of strategic and annual plans for programme implementation. It also maintains that HIV/AIDS-related stigma remains pervasive and that people infected or affected by HIV/AIDS are discriminated against and denied access to compassion, care and support, and social services.

Nigerian Labour Congress (NLC) National AIDS Policy document

The policy is a product of collaboration between the NLC and 29 other industrial unions that constitute it. It was developed by the union's HIV/AID's committee. Committed to the provision of a healthy and safe work environment for employees, based on current medical standards, the policy aims at regulating industrial practices as it affects HIV/AIDs and employees infected by the disease. In the policy, NLC aims at providing education, training and research. This is with the goal of arming members with quality information, promoting multi-sectoral collaboration to guarantee access to quality care and support for infected and affected members; support for treatment for affected workers and their families. This way, the protection of the fundamental human rights of PLWHA would be guaranteed while eliminating discrimination and stigmatization and promoting partnership, advocacy and support for the fight against HIV/AIDS.

National Agency for the Control of AIDS. 2016. National Plan of Action for the Removal of Legal and Human Rights' Barriers to HIV Response in Nigeria

Arising from the implication of HIV/AIDS for human rights (and vice versa), the policy acknowledges the huge burden of HIV/AIDS that Nigeria bears with an adult prevalence rate of 3.4% which is second only to South Africa's in the comity of nations. With its disproportionate incidence and spread across social, legal and economic divides, the policy emphasizes the role of the law in promoting change and protecting vulnerable persons. The policy took inspiration from the UN General Assembly's special session of 2001 and the political declarations of 2006 and 2011 which identified the law as a veritable tool in any response to HIV/AIDS; a position which encouraged countries to commit resources to the creation of enabling legal, social and policy frameworks at the national level. This was with a view to eliminating stigma, discrimination and violence due to HIV/AIDS, while promoting access to prevention, treatment, care and support as well as non-discriminatory access to education, healthcare, employment and social services; and legal protection, inheritance rights, confidentiality and privacy, and fundamental freedoms for PLWHA and those vulnerable to the disease. Based on the legal environment assessment in Nigeria, the report noted the high rate of human rights abuse of PLWHA and key populations (Gays, Lesbians, female sex workers and injecting drug users), especially by criminalizing law enforcement agents without direct legal backing. With all efforts at providing effective prevention, treatment, care and support being threatened by discrimination, the 2016 plan has as its overall goal, the removal of legal and human rights barriers to HIV/AIDS response in Nigeria.

Society for family health: Strengthening HIV Prevention services for most-at-risk populations (Ships For Marps) May 2012-May 2017

Managed by a consortium of partners focused primarily on populations vulnerable to HIV/AIDS, the programme ran for 5 years, between May 2012 and May, 2017. The SHIPS for MARPS programme was purely a preventive approach to providing support for PLWHA. The project's scope was consequently expanded to include a 'support, care and treatment services' component, following directives and support from the sponsor, USAID. The project partnered with government at all levels, civil society organizations/ networks and MARPS' communities, in order to deliver on its objectives. With the overarching goal of reducing new HIV infections, the specific 5 objectives that guided the project were: an improved continuum of community and family-based prevention services; improved use of

data for targeted MARPS HIV programme interventions; increased organizational capacity of local stakeholders to implement HIV interventions; increased access to comprehensive package of HIV sexual prevention activities; and finally, improvement in the health of people in Nigeria by reducing the prevalence of HIV among MARPS. Aside from the 4 consortiums, Society for Family Health (SFH), Population Services International (PSI), Population Council (PC), and Centre for the Right to Health (CRH) with SFH as the managing partner, there was collaboration with other partners, including the National Agency for the Control of AIDS (NACA), the World Bank, Global Fund, the University of Manitoba, MARPs' Networks and Civil Society Organizations (CSO). They employed the following activities as the strategy for achieving the stated objectives: promotion of appropriate behaviour conducive for safer sexual practices; capacity strengthening of government institutions towards a holistic coordinating and oversight role; strengthening of CSOs/CBOs for evidence-informed intervention programmes; creation of enabling environment for sustained behaviour change. The project utilized the nationally approved minimum prevention packaged that best met the prevention need of the target population. Interventions in the project included, biomedical interventions, behavioural interventions and structural interventions.

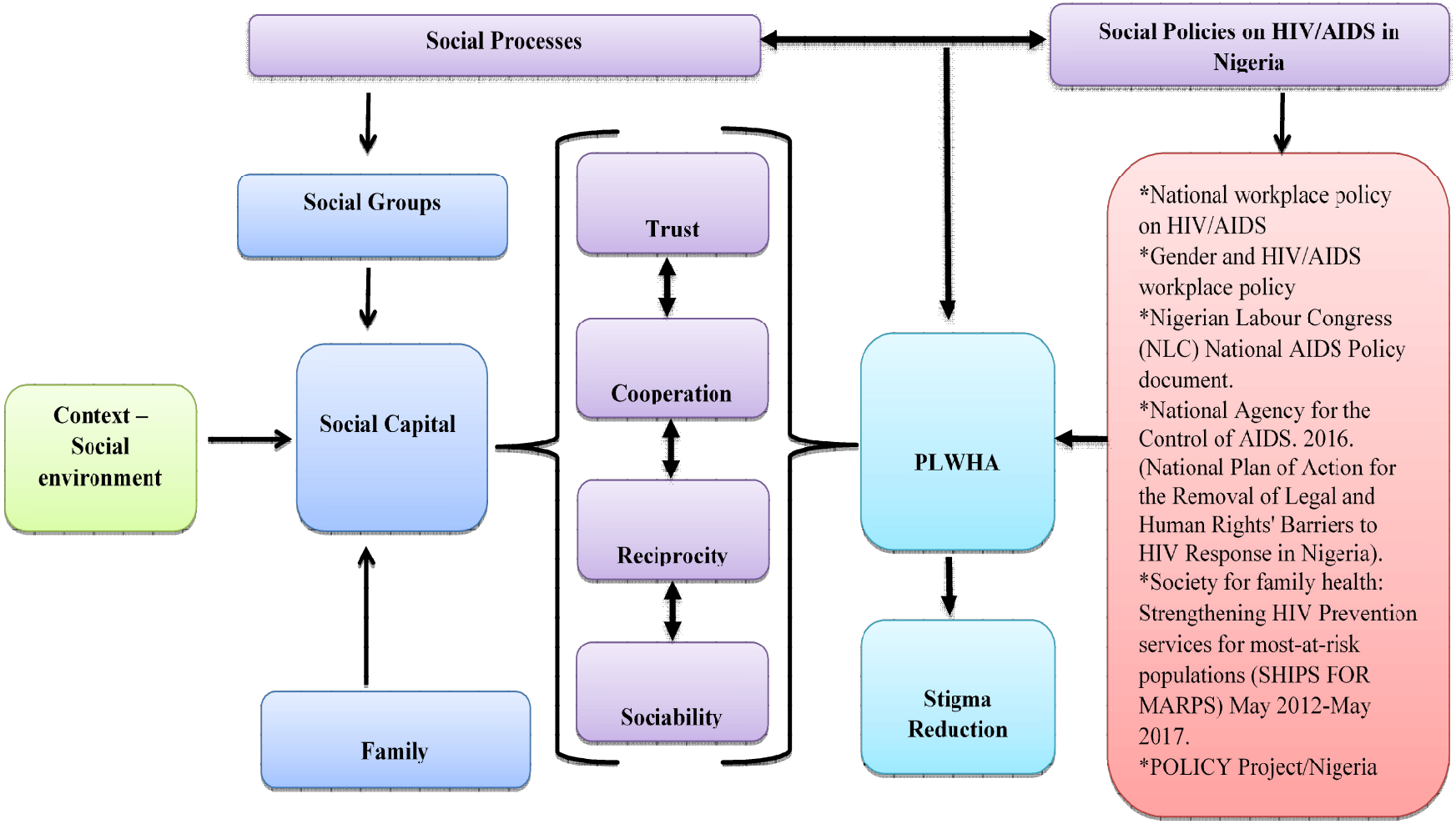
Policy Project/Nigeria

Policy is a five-year project funded by the US agency for International development and implemented by the Futures Group International in collaboration with Research Triangle Institute (RTI) and the Centre for Development and Population Activities (CEDPA). The aim of the project was to promote access to drugs for HIV/AIDS and related opportunistic infections in Nigeria by harnessing the socio-political, economic and policy climate on drug availability. Like the WHO standard, the policy maintains that access should be universal, covering therapeutic, physical and financial aspects of prioritizing health problems, and within easy physical and affordable reach. The project conducted various studies across Nigeria with a view to understanding the actual, understanding the dynamics of drug distribution and barriers within the system, for the eventual development of strategies vis-à-vis recommendations towards a more effective drug distribution system for HIV/AIDS and other opportunistic infections. The project report noted that more than one-third of the world's population and more than one-half of Africa's poorest peoples lacked access to essential drugs, and given the vulnerability of Nigeria's 3.5 Million PLWHA to opportunistic infections, there was the need to improve access to quality antiretroviral drugs through a number of strategies in the areas of: law and policy making on drugs and healthcare accessibility which must be considered as fundamental human rights; effective communication and synergy between the three tiers of government; quality assurance of drugs, regulation of the market and effective drug distribution. The project suggested the need to develop other strategies to improve access to drugs rather than reliance on donated drugs, which was not sustainable anymore. Emphasis was placed on local drug manufacturing and improved distribution channels. The report further stated the need to monitor drug stock for expiration date, price, patent status, long term subvention; and effective usage of traditional medicine.

The nexus of social processes and social policies

The nexus between social processes and policies on HIV/AIDS is crucial to the context in which the policy is being implemented. It is the context that defines how social capital is being utilised. As seen in the framework on Figure 1, social capital is drawn from the family as a social unit and from social groups. The family is expected to provide social support and constitute an important element of social capital. Also, social groups contribute to existing social capital found in communities from which PLWHA derive support. These factors make up the social processes in which care and support for PLWHA takes place. In Nigeria, the context various from one community unto another but the nature of social capital remains the same. Families and social groups remain an important sub-set that PLWHA look unto. Although in some communities, the value of social capital is high based on the communal nature of social relationships and the strong mechanical solidarity that thrives there. But in some others these social relations have been flawed by renewed reconstruction of social reality hinged on individualistic philosophies.

Social capital is composed on trust, cooperation, reciprocity and sociability. These influence how social policies are formed for PLWHA. In the Nigerian context, the trust shared between the family and PLWHA determines the level of social support being offered. The trust is a guarantee against any form of social stigma that PLWHA may experience in their families or immediate environment. The nature of trust


Social Processes
Social Policies on HIV/AIDS in Nigeria
Social Groups
Trust
Cooperation
Reciprocity
Sociability
Context – Social environment
Social Capital
Family
PLWHA
Stigma Reduction
*National workplace policy on HIV/AIDS
*Gender and HIV/AIDS workplace policy
*Nigerian Labour Congress (NLC) National AIDS Policy document.
*National Agency for the Control of AIDS. 2016. (National Plan of Action for the Removal of Legal and Human Rights' Barriers to HIV Response in Nigeria).
*Society for family health: Strengthening HIV Prevention services for most-at-risk populations (SHIPS FOR MARPS) May 2012-May 2017.
*POLICY Project/Nigeria

influences PLWHA to adhere to treatment as they feel safe within the family and social group in which they live. This form of social capital is crucial in the formulation of social policies on HIV/AIDS. Trust safeguard PLWHA and reduces all forms of social stigma they may experience. Cooperation is equally important in the care and management of PLWHA. Cooperation is two-sided as a form of social capital. Within the context of HIV/AIDS, cooperation as a social process, places responsibility in the hands of the family members, social groups and the PLWHA. Cooperation is essential as a social process in the care and management of HIV/AIDS. Reciprocity and Sociability is also an important element of social capital from which PLWHA can derive security and safety, support, sense of purpose and care. Reciprocity is fused on the dyad nature of social relations. As a social capital, PLWHA benefit from the commitment of family members and social groups. While the nature of social interaction between family members and the PLWHA promotes their social relations. As seen in Figure 1, the connection between social process and the social policies on HIV/AIDS is hinged on the various components explained. More so, the various types of *social policies on HIV/AIDS* in Nigeria namely: the National workplace policy on HIV/AIDS, Gender and HIV/AIDS workplace policy, Nigerian Labour Congress (NLC) National AIDS Policy document, National Agency for the Control of AIDS. 2016. (National Plan of Action for the Removal of Legal and Human Rights' Barriers to HIV Response in Nigeria), Society for family health: Strengthening HIV Prevention services for most-at-risk populations (SHIPS FOR MARPS) May 2012-May 2017 and POLICY Project/Nigeria were identified. The attainment of the goals of each policy rest on existing social capital within the context of HIV/AIDS. More so, to reduce stigma and safeguard PLWHA, these policies must draw from existing social processes in the Nigerian context of their implementation. For *stigma reduction* to be achieved, social processes play a crucial part and must be taken into serious consideration.

Conclusion

The epidemic of HIV/AIDS is one that has not only persisted, but defied solutions despite concerted efforts to contain it. The epidemic has expanded in large proportions leveraging on the existing as well as emerging flux of social processes to perpetuate its scourge in resource-constraint Nigeria. Although government, development partners, civil society groups and the organized private sector have all developed strategies for containing the menace, little success has been recorded. Literature is replete with evidence demonstrating stigmatization as a major cog in the wheel of progress of the war against HIV/AIDS, first, by compounding the survival challenges of PLWHA, and second, by adversely affecting the uptake of ART by patients. Stigmatisation is also a major factor impeding community support, by undermining the social network upon which PLWHA can leverage during their illness. If the tide and trend of HIV/AIDS must be stemmed in its progressive dimensions, there is the need to, first, strengthen campaigns and public enlightenment against stigmatisation and its various consequences, and second, prioritise key populations, their hotspots and states with higher prevalence in the direction of interventions. Finally, effort must be made to not politicise the war against HIV/AIDS, by making interventions based on empirical evidence, and not sentiments or mere 'arm-chair' theorising.

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References

1. Goffman E. Stigma: Notes on the management of spoiled identity. New Jersey: Prentice-Hall; 1963.
2. Macionis JJ. Society: The basics. New Jersey: Prentice Hall; 2000.
3. Garfinkel H. Conditions for successful degradation ceremonies. Amer J of Soc. 1956; 61(5):420-424.
4. Giddens A, Duneier M and Appelbaum R. Introduction to Sociology. New York: W.W. Norton and Company; 2005.
5. National Agency for Control of AIDS [NACA]. National HIV/AIDS Strategic Plan 2010-2015.

National Agency for Control of AIDS (NACA), Federal Republic of Nigeria, Abuja, Nigeria; 2009.
6. Kontomanolis EN, Michalopoulos S, Gkasdaris G and Fasoulakis Z. The social stigma of HIV–AIDS: society's role. HIV/AIDS-Res and Pall Care. 2017; 9:111–118.
7. Mbonu NC, Borne B and Vries NK. Stigma of people with HIV/AIDS in sub-Saharan Africa: A literature review. J of Trop Med. 2009; Article ID 145891. doi:10.1155/2009/145891.
8. Fatoki B. Understanding the causes and effects of stigma and discrimination in the lives of People Living with HIV/AIDS: Qualitative study. J AIDS and Clin Res. 2016; 7:635. doi:10.4172/2155-6113.1000635.
9. United Nations Programme on HIV/AIDS [UNAIDS]. UNAIDS AIDS Info: Country factsheets for Nigeria. UNAIDS, Geneva, Switzerland; 2016.
10. National AIDS Spending Assessment [NASA]. National AIDS Spending Assessment 2013-2014. NASA Steering Committee. Federal Republic of Nigeria, Abuja, Nigeria; 2014.
11. National Agency for the Control of AIDS [NACA]. National HIV/AIDS Epidemiology and Impact Analysis (NHEIA) Report. Federal Republic of Nigeria, Abuja, Nigeria; 2014.
12. Amzat J and Razum O. Medical Sociology in Africa. Switzerland: Springer; 2014.
13. Awofala AA and Ogundele OE. HIV epidemiology in Nigeria. Saudi J Bio Sci. 2018; 25(4):697-703. http://dx.doi.org/10.1016/j.sjbs.2016.03.006.
14. National Bureau of Statistics [NBS]. The Nigeria Poverty Profile 2012 Report. National Bureau of Statistics, Abuja, Nigeria; 2012.
15. World Health Organization [WHO]. Integrating gender into HIV/AIDS programmes in the health sector: Tool to improve responsiveness to women's needs. Geneva, Switzerland; 2009.
16. Research Alliance to Combat HIV and AIDS [REACH]. Social dimensions of HIV and AIDS prevention in Nigeria: A report by the Research Alliance to combat HIV and AIDS (REACH); 2010.
17. Amzat J. Social correlates of HIV among youths in Nigeria. Hemispheres–Stud on Cul and Soc. 2010; 25:1–13.
18. Joint United Nations Programme on HIV/AIDS [UNAIDS]. Joint United Nations Programme on HIV/AIDS [UNAIDS]: Report on the global AIDS epidemic. www.unaids.org. Geneva. Switzerland; 2013.
19. Chikwendu E. AIDS/HIV–when the state fails: NGOs in grassroot AIDS care. Dialectical Anthro. 2004; 28:245-259.
20. Stutterheim SE, Sicking L, Brands R, *et al*. Patient and provider perspectives on HIV and HIV-related stigma in Dutch health care settings. AIDS Patient Care and STDs. 2014;28(12):652-665.
21. Monjok E, Smesny A and Essien EJ. HIV/AIDS-related stigma and discrimination in Nigeria: Review of research studies and future directions for prevention strategies. Afri J Repro Health. 2009; 13(3):21-35.
22. Center for the Right to Health [CRH]. Human rights and HIV/AIDS: Experiences of people living with HIV/AIDS in Nigeria. 2001; http://www.crhonline.org. [Online]. http://www.crhonline.org/pubdetail.php?pubid¼2. Retrieved on November 19, 2017.
23. Onwujekwe O, Chikezie I, Mbachu C, *et al*. Investigating client perception and attitude to decentralization of HIV/AIDS treatment services to primary health centres in three Nigerian states. Health Expectations. 2015;19(5):1111–1120.
24. Isibor MD and Ajuwon A. Journalists' knowledge of AIDS and attitude to Persons living with HIV in Ibadan, Nigeria. Afri J Repro Health. 2004;8(2):101-110.
25. National Agency for the Control of AIDS [NACA]. Stigma and Discrimination Reduction in the National HIV/AIDS Response. National Agency for the Control of AIDS (NACA), Federal Republic of Nigeria, Abuja, Nigeria; 2016.
26. Rintamaki LS, Scott AM, Kosenko KA and Jensen RE. Male patient perceptions of HIV stigma in health care contexts. AIDS Patient Care. 2007; 21:956–969.
27. Pickles D, King L and Belan I. Attitudes of nursing students towards caring for people with HIV/AIDS: Thematic literature review. Advanced Nurs. 2009; 65:2262–2273.
28. Davtyan M, Brown B and Folayan MO. Addressing Ebola-related stigma: Lessons learned from HIV/AIDS. Global Health Action. 2014;7:26058. http://dx.doi.org/10.3402/gha.v7.26058.
29. Adetola OB, Adedeji IA and Popoola O. Systems theory analysis of Ebola virus disease and nursing needs in the West African Sub-Region. Inter J

Healthcare Mgt. 2017; 10(4). doi: 10.1080/20479700.2017.1418278.
30. Global Network of People Living with HIV/AIDS [GNP+, ILO]. The PLHIV Stigma Index - Evidence Brief: Stigma and Discrimination at Work. Findings from the PLHIV Stigma Index. GNP+, ILO, Amsterdam; 2012.
31. Asuquo EF, Etowa JB and Akpan, MI. Assessing women's caregiving role to People Living with HIV/AIDS in Nigeria, West Africa. SAGE Open. 2017; 1–10. doi: 10.1177/215824401769201.
32. Bourdieu P. The forms of capital. In: Richardson JE, editor. Handbook of theory of research for the sociology of education. New York: Greenwood Press. 1986. pp. 241–258.
33. Ware NC, Idoko J, Kaaya S, *et al*. Explaining Adherence Success in Sub-Saharan Africa: An Ethnographic Study. PLoS Med 6(1), 2009: e1000011. https://doi.org/10.1371/journal.pmed.1000011
34. Ehsan A, Klaas HS, Bastianen A and Spini D. Social capital and health: A systematic review of systematic reviews. SSM - Population Health. Volume 8. 2019. 100425. S2352827319301144.35.
35. Afolabi BA, Afolabi MO, Afolabi AA, Odewale MA and Olowookere SA. Roles of family dynamics in adherence to highly active antiretroviral therapy among people living with HIV/AIDS at a tertiary hospital in Osogbo, South-West Nigeria. Afri Health Sci. 2013; 13(4): 920–926. http://dx.doi.org/10.4314/ahs.v13i4.9.
36. Masquillier C, Wouters E, Mortelmans D, Wyk BV. On the road to HIV/AIDS competence in the household: Building a health-enabling environment for People Living with HIV/AIDS. Inter J Environ Res in Pub Health. 2015; 12: 3264-3292; doi:10.3390/ijerph120303264.
37. Adedimeji AA, Alawode OO, Odutolu O. Impact of care and social support on wellbeing among people living with HIV/AIDS in Nigeria. Iran J Pub Health. 2010; 39(2): 30-38.
38. Shodimu MA, Yusuf OB, Akinyemi JO, Fagbamigbe AF, Bamgboye EA, Ngige E, et al. Determinants of perceived stigmatizing and discriminating attitudes towards people living with HIV/AIDS among women of reproductive age in Nigeria. J AIDS and HIV Res. 2017; 9(6):139-151.
39. Ajagu N, Anetoh MU, and Nduka SO. Expanding HIV/AIDS care service sites: A cross sectional survey of community pharmacists' views in South-East, Nigeria. J Pharm Policy and Prac. 2017; 10(34). doi:10.1186/s40545-017-0122-x.
40. Rodriguez-García R, and Bonnel R. Increasing the evidence base on the role of the community in response to HIV/AIDS. J Epidemiol and Comm Health. 2012; 66(S2), ii7-ii8.
41. Joint United Nations Programme on HIV/AIDS [UNAIDS] and the World Health Organization [WHO]. Expanding access to HIV treatment through community-based organizations: A 2005 joint publication of Sidaction. UNAIDS/WHO, Geneva, Switzerland; 2005.

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Mophometry of the mandibular ramus: a Nigerian study

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Abstract
Background: The mandibular ramus has been termed the most dimorphic, strongest and well preserved component of the skull**.** Its dimensions will help in locating the mandibular foramen which will further prevent neurovascular complications during various orthognatic surgeries. The study investigated the mophometry of the mandibular ramus in a Nigerian population.
Methodology: The study was carried out among 20 dry mandibles of unknown age and gender. Parameters were measured with a digital vernier caliper. The condylar (BH) and base to notch height (BN) were measured. The minimum breadth (AP), mandibular base and notch to the mandibular foramen (BF, NF) distance were also taken. T-test compared means of either sides. Statistical evaluation was done with a statistical package for social sciences (SPSS) version 23.Significance was accepted at $p< 0.05$.
Result: Findings showed that the mean condylar, base to notch height was 68.95±4.38mm; 51.67± 4.12mm while the mandibular base and notch to the foramen was 32.60±5.40mm; 20.67 ±5.19mm.The minimum breadth of the ramus was 38.76±4.71mm. Findings also showed that there was no significant association between the right and left sides of BH, BN, AP,BF and NF (t= 0.49;-0.77;-1.02;-0.53;-0.19.p=0.63; 0.46;0.33; 0.61;0.85) respectively.
Conclusion: The mandibular ramus from this study, showed bilateral symmetry. There were also variations observed when it was compared with other populations.

Keywords: *Mandibles; mandibular ramus; Nigerian; population.*

Abstrait
Contexte: La ramure mandibulaire a été appelé la composante la plus dimorphe, la plus ferme et mieux conservée du crâne **.** Ses dimensions aideront à localiser le foramen mandibulaire, ce qui permettra de prévenir devantage les complications neurovasculaires lors de diverses chirurgies ortho-gnathiques. L'étude a investigué la morphométrie de la ramure mandibulaire dans une population nigériane.
Méthodologie: L'étude a été réalisée sur 20 mandibules sèches d'âge et de sexe inconnus. Les paramètres ont été mesurés avec un calliper vernier numérique. Le condylien (BH) et la hauteur de la base à l'entaille (BN) ont été mesurés. La largeur minimale (AP), la base mandibulaire et la distance de l'entaille au foramen mandibulaire (BF, NF) ont également été prises. Le test T a comparé les moyennes des deux côtés. L'évaluation statistique a été réalisée avec un progiciel statistique pour les sciences sociales (SPSS) version 23. La signification a été acceptée à $p <0,05$.
Résultat: Les résultats ont montré que la moyenne condylienne, la hauteur de la base à l'entaille était de 68,95 ± 4,38 mm; 51,67 ± 4,12 mm tandis que la base mandibulaire et l'entaille du foramen étaient de 32,60 ± 5,40 mm; 20,67 ± 5,19 mm. La largeur minimale de la ramure était de 38,76 ± 4,71 mm. Les résultats ont également montré qu'il n'y avait pas d'association significative entre les côtés droit et gauche de BH, BN, AP, BF et NF (t = 0,49; -0,77; -1,02; -0,53; -0,19.p = 0,63; 0,46; 0,33; 0,61; 0,85) respectivement.
Conclusion: La ramure mandibulaire de cette étude a montré une symétrie bilatérale. Il avait aussi une variation observée quand elle a été comparée à d'autres populations.

Mots-clés: *Mandibules; ramure mandibulaire; Nigérian; population.*

Introduction

The mandibular ramus has been described as the perpendicular part of the mandible, quadrilateral in shape with two surfaces and processes alongside four boarders [1]. Several authors defined it as the most dimorphic, more preserved and strongest component of the skull [2-3]. They further described it as a vital bone for gender and age determination, especially in cases were an intact skull is not available, hence useful

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in the identification of skeletal remains and for medico-legal investigation [2-3]. However metric measurements of the mandibular ramus have also been very useful to orthognatic surgeons because basic values from landmarks had help to locate the position of the mandibular foramen which further lead to a proper nerve block [4]. It was documented that inaccurate localization of the foramen is one of the factors that may lead to an improper inferior alveolar nerve block during various types of orthognatic surgeries [6]. This nerve block has been a common anesthetic technique prior to surgeries and the success depends on the tip of the needle to the mandibular foramen [6]. The anatomy of the ramus also has its significance in sagital split osteotomy [7]. Hence knowledge of the mandibular ramus is of immense value.

Metric studies had been carried out on several population [8-10] but there is limited information in a Nigerian Population. Hence this study was carried out in a Nigerian population and compared with other populations previously studied.

Materials and methods

The study was a crossectional study that involved 20 dry mandibles of unknown age and gender from the Anatomy Musuem of Delta State University, Abraka, Delta State. Parameters were taken with a digital vernier caliper calibrated to 0.1mm. Measurements were obtained twice with the average taken to improve validity and avoid errors. Mandibles that were included in the study possessed complete dentation with complete body, ramus and coronoid process. Those excluded were partially dentate, with fractures, wear and tear. Approval for this research work was obtained from the Research and Ethic Committee of the Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Science, Delta State University, Abraka (DELSU/CHS/ANA/18/68). Anthropometric measurements of the mandibles obtained were the condylar height which was referred as the distance between the base of the mandible to the highest point on the mandibular condyle (BH), distance between the base of the mandible to the mandibular notch (BN), width of the mandibular ramus taken from the anterior border to the posterior border (AP), distance from the base of the mandible to the mandibular foramen (BF) and the distance from mandibular notch to the mandibular foramen (NF).

Descriptive statistics were represented in tables, mean and standard deviation. T test was used to compare means between the right and left sides of the mandibular ramus. Statistical evaluation was with a statistical package for social sciences (SPSS) version 23 and $P<0.05$ was considered statistically significant.

Fig 1: Mandibular Base to Notch (BN)

Fig 2: Breadth of mandible (AB-PB)

Fig 3: Mandibular Base to Foramen (BF)

Fig 4. Mandibular Notch to Foramen (NF)

was seen as 69.20± 4.22mm. The mean AP was recorded as 38.53±4.69mm and that of BF was 32.05±5.78mm (table 1). Further findings showed that the distance of the right mandibular notch to foramen (NF) was 20.5± 4.85mm.

Parameters for the left mandibular ramus were also obtained as shown in table 2. Findings showed that the mean BN distance was 52.09± 4.73mm, BH was 68.69± 4.53mm, while AP was seen as 38.98±4.69mm. The study also showed that the left BF distance was 32.60± 5.40mm while that of NF was 20.67±5.19mm.

Table 3 reports a combined data for the mandibular ramus. Findings from this study discovered that the mean BN and BH dimension for the mandibular ramus was seen as 51.67± 4.12mm and 68.95± 4.38mm. Further findings from table 4 showed that the

Table I: Descriptive Statistics of Right Mandibular Ramus

Parameter (mm)	N	Range	Minimum	Maximum	Mean± SD
BN	20	14.01	45.22	59.23	51.24±3.50
BH	20	14.42	63.48	77.90	69.20±4.22
AP	20	17.55	33.05	50.60	38.53±4.69
BF	20	21.14	23.82	44.96	32.05±5.78
NF	20	18.97	15.09	34.06	20.56±4.85

Table 2: Descriptive Statistics of Left Mandibular Ramus

Parameter (mm)	N	Minimum	Maximum	Mean±SD
BN	20	44.91	64.54	52.09±4.73
BH	20	62.32	80.19	68.69±4.53
AP	20	32.72	50.36	38.98±4.69
BF	20	24.31	46.46	32.60±5.40
NF	20	14.70	33.28	20.67±5.19

Findings from table 1 showed that the right mean BN dimension was 51.24± 3.50mm while that of BH

Table 3. Combined Statistics of Mandibular Ramus

Parameter (mm)	N	Minimum	Maximum	Mean±SD
BN	20	44.91	64.54	51.67±4.12
BH	20	62.32	80.19	68.95±4.38
AP	20	32.72	50.60	38.76±4.71
BF	20	23.82	46.46	32.33±5.59
NF	20	14.70	34.06	20.62±5.02

bilateral difference of measured parameters were statiscally not significant at $p<0.05$.

The study showed that the values obtained for the right and left sides of the mandibular notch to the

Table 4: Comparison of Parameters of the Right and Left Mandibular Ramus

Parameter (mm)	N	Right	Left	T-test	P-value	Inference
BN	20	51.24	52.09	-0.765	0.457	Insignificant
BH	20	69.20	68.67	0.490	0.632	Insignificant
AP	20	38.53	38.98	-1.016	0.327	Insignificant
BF	20	32.05	32.60	-0.525	0.608	Insignificant
NF	20	20.56	20.67	-0.190	0.852	Insignificant

Discussion

The mandibular ramus which maintains proximity with the skull by forming the temporomandibular joint has been reported to vary among different races [11]. The present study on Nigerian mandibles was compared with studies on other populations. Findings from this study showed that the dimension of the condylar height was lesser than those from Zimbabwe and Egypt [9,12]. According to Mbajiorgu et al. and Noha et al. the Zimbabwe and Egypt male condylar height was seen as 77.8mm and 84mm [9,12]. This shows that despite Negriods having similar skull pattern, there are still some morphological differences among various skeletal components of the skull. It also differed from those of North Indian male (60.67mm), Iraq (51.41mm), Andhrapradesh (61.98mm) and Telangana (60.31mm) population [2,8,10,11] which shows that the mandibular ramus varies among different populations. The condyle has been considered an important component of the mandibular ramus because it provides the appearance of the mandibular growth [13]. it has also been referred to the site linked with the greatest structural changes in dimensions and remodeling in the course of growth [13].

The study also showed that the dimension from the mandibular base to notch was higher than values obtained among Northern Croatia (44.79mm) but was lower than that of the Mangalore male population (59.20mm) [14-15]. Further findings from this study showed that the minimum breadth of the mandibular ramus was quite different from those of Saini et al. (31.29mm); Punnarjeevan and Keros et al.(30.50mm,24.87mm) [2, 8]. Apart from locating the position of the mandibular foramen, dimensions of minimum breadth of the mandibular ramus was used by Rajat et al. in evaluating the structure of the mandible in dissimilar facial types[16].

foramen was lower than that of Oguz et al ; Lavanya et al and Padmavathi et al. studies in Turkey and South Indians [4,17-18]. However values obtained were higher than those of the Brazilian mandibles [5].

Findings from this investigation showed that measurements of the mandibular ramus of either side were almost the same, establishing bilateral symmetry. It was also observed that there was no significant association between the right and left sides of measured parameters. Findings were similar to Nagaraj et al.[11] who also discovered no association between either sides of the Telengana mandibular ramus. However it differed from a study carried out among mandibles in Dehradun India which reported a significant difference obtained in mean right and left ramus height respectively [19].

Conclusion

The mandibular ramus on either side from this study showed bilateral symmetry. However it has been established from this study that the mandibular ramus varies among different population hence vital in investigating skeletal remains.

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References

1. Richard LD, Wayne V and Adam WM. Head and neck: Mandible: Gray's Anatomy for students (39th ed). 2005; pp 764.
2. Saini V, Srivastava R, Rai RK, *et al*. Shamal SN, Singh TB, Tripathi SK. Mandibular ramus: An indicator for sex in fragmentary mandible. J Forensic Sci. 2011;56:S13-16.

3. Hu KS, Koh KS, Han SH, Shin KJ and Kim HJ. Sex determination using non-metric characteristics of the mandible in Koreans. J Forensic Sci. 2006;51:1376-1382
4. Oguz, O1 and Bozkir MG. Evaluation of location of mandibular and mental foramina in dry, young, adult human male dentulous mandibles, West Ind. Med J. 2005; 51(1) 14-16.
5. Ennes JP and Medeiros RM. Localization of Mandibular Foramen and Clinical Implications. Int. J. Morphol., 2009; 27(4)1305-1311.
6. Heasman PA. Variation in the position of the inferior dental canal and its significance to restorative dentistry. J Dent 1987;16:363-9
7. Daw JL, Jr, de la Paz MG, Han H, Aitken ME and Patel PK. The mandibular foramen: an anatomic study and its relevance to the sagittal ramus osteotomy. J Craniofac Surg. 1999;10:475–479
8. Punarjeevan KM and Lokanadham S. Sex determination& morphometric parameters of human mandible. Int. J .Research in Med. Sc. 2013; 1(2); 93-96
9. Mbajiorgu FE, Zivanovic S, Asala SA and Mawera GA. Pilot study of the mandibular angle in black Zimbabweans. Cent Afr J Med. 1996; 42(10):285-287
10. Yassir AY. Ramus height and its relationship with skeletal and dental measurements. Journal of Oral and Dental Research. 2013; 1(1): 4-8
11. Nagaraj S, Gayatri N and Anil RS. Study of Mandibular Ramus by metric parameters. Int.J Anat.Reserch 2017;5(1) 3358-3361
12. Noha S Abu-Taleb and D Mohamed El Beshlawy. Mandibular Ramus and Gonial Angle Measurements as Predictors of Sex and Age in an Egyptian Population Sample: A Digital Panoramic Study. J Forensic Res. 2015; 6(5): 308.
13. Indira AP, Markande A and Davis MP. Mandibular ramus: An indicator for sex determination- A digital radiographic study.J Forensic Dent Sci 2012;4:58-62.
14. Keros NJ, Panduric J and Buntak KD. Some anatomical and anthopological measures of mandibular ramus in our population. Coll.Antropol 1997; 21(1):203-210.
15. Rupa KR, Laxmikanth C, Prashanth S, *et al.* Gonial angle and ramus height as sex determinants. A radiographic pilot study. 2015; 4(2): 111-116.
16. Rajat M, Navjot S, Vinay D, Prajeesh P and Mannu K. Evaluation of mandibular morphology in different fascial types. Contemp Clin Dent. 2011; 2(3): 200–206.
17. Lavanya CV, Imtiazul H and Rajeshwari T. Position of Mandibular Foramen in South Indian Mandibles. Anat. Karnat., 2011; Vol 5, Pp 53–56.
18. Padmavathi G, Suman T, Varalakshmi KL and Roopashree R. An anatomical study of the mandibular foramen and accessory mandibular foramen in Dry Adult Human Mandibles of South Indians Origin. J of Dent. Med. Sc. 2014; Vol 13(4). pp 83-88.
19. Kishore CT, Alok KC, Sanjeev KJ and Lalit K. "Racial architecture of Human Mandible- an Anthropological Study". J Evol. Med. Den. Sc., 2013; 2(23):4177-4188.

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Distinct Difference between microbiome of rural and urban population in Lagos State, Nigeria

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Abstract

Background: The human gut microbiome differs among populations and also varies with diet, genetic and geographic locations. The diet in Nigeria is changing to a more western diet with extra sugar and processed food. There is a need to investigate dietary pattern and impact on gut microbiome in health and in diseases.

Aim: We determined the impact of diet on the microbiome of Yoruba ethnic group living in rural and urban areas.

Methodology: We characterized bacterial species present in faecal samples obtained from 20 Yoruba; Ten from each site, matched by age and sex. A universal primer set was used to amplify the V3–V4 region for faecal microbial 16S rRNA sequences. The resultant data was compared between the rural and urban diet and to those from other nations.

Results: The composition of the Yoruba gut microbiome of both rural and urban were mainly organisms from the phylum Bacteroidetes Actinobacteria, and Firmicutes, particularly the genus *Prevotella, Bifidobacteria,* and *Faecalibacterium.* In the urban population, *Bifidobacterium, Prevotella and Faecalibacterium* species were the predominant organisms while *Prevotella and Faecalibacterium* species predominated in the rural population. There were similarities between the microbiomes found in rural Nigerians with those from Africans with similar diets. The diversity of core gut microbiome in Nigeria differs between diets.

Conclusion: The urban region of Lagos seems to have transitioned towards a diet pattern typical of 'Westernized' societies and this may have contributed to the variations in microbiomes of those of similar ethnicity, living in the rural region. Further studies needs to be conducted in a larger population to fully ascertain this relationship.

Keywords: *Prevotella*, *Bifidobacterium*, *Faecalibacterium, Gut microbiome, Urban, Rural, Next Generation Sequencing, Nigeria*

Abstrait

Contexte : Le microbiome intestinal humain diffère d'une population à l'autre et varie également en fonction du régime alimentaire, des emplacements génétiques et géographiques. Le régime au Nigéria est en train de passer à un régime plus occidental avec un supplément de sucre et des aliments transformés. Il est nécessaire d'étudier les habitudes alimentaires et l'impact sur le microbiome intestinal dans la santé et les maladies.

Objectif : Nous avons détermines l'impact de l'alimentation sur le microbiome du groupe ethnique Yorouba vivant dans les zones rurales et urbaines.

Méthodologie : Nous avons caractérisé les espèces bactériennes présentes dans les échantillons fécaux obtenus à partir de 20 Yoroubas ; Dix de chaque site, apparié par âge et sexe. Un ensemble d'amorce universel a été utilisé pour amplifier la région V3 – V4 pour les séquences 16S ARNr microbiennes fécales. Les données résultantes ont été comparées entre le régime alimentaire rural et urbain et celui des autres pays.

Résultats : La composition de l'intestin microbiome Yorouba des localités rurale et urbaine étaient principalement des organismes du phylum Bacteroidetes Actinobactéries et Firmicutes,

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en particulier du genre *Prevotella, bifidobactéries,* et *Faecalibacterium*. Dans la population urbaine, les espèces *Bifidobacterium , Prevotella et Faecalibacterium* étaient les organismes prédominants tandis que les espèces *Prevotella et Faecalibacterium* prédominaient dans la population rurale. Il y avait des similitudes entre les microbiomes trouvés dans les Nigérians ruraux avec ceux des Africains avec régimes similaires. La diversité du microbiome intestinal de base au Nigéria diffère selon les régimes.

Conclusion : Les régions urbaines de Lagos semble avoir fait la transition vers un modèle de régime typique des sociétés 'Occidentalisés' et cela peut avoir contribué aux variations des microbiomes de ceux d'origine ethnique similaire, vivant dans la région rurale. Des études complémentaires doivent être menées dans une population plus large pour vérifier pleinement cette relation .

Mots clés: *Prevotella* **,** *Bifidobacterium* **,** *Faecalibacterium, Microbiome intestinal, Urbain, Rural, Séquençage de nouvelle génération, Nigéria*

Introduction

The relationship between the gut microbiome, diet, genetic and lifestyle has been extensively studied [1-4]. These characteristics are major factors justifying variation in gut microbiome that is reported globally [5-9]. Distinct differences have been reported between the gut microbiomes of indigenous populations in Africa, Asia, South America and western populations in Europe and the United States [9-17]. In addition to other benefits, microbiome studies set out to characterize the combined genomes (metagenome) of all microbes living in healthy people so as to identify and preserve the beneficial microbes and develop ways to prevent the proliferation of pathogenic ones [5].

There is good evidence that beneficial intestinal microbiome plays an important role in predicting immune status of human diseases [4-6]. They have protective role during inflammatory bowel diseases, prevent pathogenic infection and reduce intestinal concentrations of certain carcinogenic enzymes [5,6]. Studies on gut microbiomes and diet have revealed species implicated in the development and management of depression, obesity as well as cardiovascular diseases, type 2 diabetes and colorectal cancer [18,19]. Moreso, diversity, increased proportions and decreased relative abundance of certain species that predispose to high blood pressure [18]. The major groups of bacteria found in the gut are Firmicutes, Bacteroidetes, Enterobacteriaceae, and Proteobacteria phyla [1-5].The genera found in the gut of adults belong to *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, *Prevotella*, *Escherichia*, *Streptococcus* and *Ruminococcus species* [1-5]. The presence, level and functionality of any of these species in the human intestine is dependent on diet, antibiotics, toxins and stress [20,21]. Any of these factors is capable of causing an imbalance of the core gut microbiomes and subsequently may result into disease conditions. Among these factors, diet remains one of the major determinants aligned with daily lifestyle. Vegetable-rich foods increased both the abundance of fiber-degrading bacteria responsible for synthesis of short-chain fatty acids (SCFAs) and other metabolites beneficial to humans [22]. It also support generation of host factors that modulated host response and expression of Toll-like receptor 5, lectin RegIIIα, interleukins (IL-8, IL-22 and IL-23). This immune booster is said to prevent and alleviate the risk of acquiring a disease [20]. On the contrary, westernized diet rich in animal proteins, high fats and low fibres is associated with abundance of harmful microbial metabolites, such as phenolic and indole derivatives, and trimethylamine N-oxide (TMAO) [20]. Thus, westernized diet alters the composition of guts microbiomes away from that found in a healthy one. These alterations have been associated with increased rate of inflammatory bowel diseases (IBD) especially Crohn's disease and ulcerative colitis [23].

The core phyla, family, genus and species found in the gut are shown to be dependent on urbanization [5]. Thus, in the African setting, it is important to consider human development, dietary lifestyle, biological and functional variations, and the impact of westernization on the microbiome [13]. Nigeria has the largest population in Africa and is made up of over 250 ethnic groups with different diets. These diets have become more varied with the introduction of western type diets especially in urban cities [24]. Defining the microbiome of Nigerians will give more insight into the effect of diet on the microbiome as well as the associated species of bacteria. This study analysed the variability of bacterial species in the gut of young healthy Nigerian adults of same ethnic groups living on traditional diets and on western diets.

Methods

Study population

Study Design

This observational study was conducted between June 2016 – June 2017. The volunteers were matched "age for sex"; male (10) and female (10) adults aged 18 - 32 years.

Data collection

Volunteers were recruited and demographic data as well as dietary habits obtained by structured questionnaire.

Study population

The Yoruba ethnic group is found in South Western Nigeria and other parts of Africa. Their population is estimated at ~36 million. It is one of the largest ethnic groups in Africa. The rural population, was drawn from Ikoga-Zebbe a small village in coastal Badagry Local Government Area of Lagos State whose latitude longitude coordinates were 6°24'54.07"N, 2°52'52.75"E. Indigenes of Ikoga-Zebbe are the Egun (Ogu) speaking people. They are mainly farmers basically involved in fishing and cultivation of maize. The urban population was drawn from undergraduate students of the College of Medicine of University the University of Lagos, Idi-Araba, Lagos located at Latitude: 6° 27' 11.0016" and Longitude: 3° 23' 44.9988" who had stayed, in the University for > 3 years. They were adapted to the same living environmental conditions, such as the same living spaces, dining hall, and drinking water.

Exclusion criteria included anyone with a body mass index, $\geq$ 35 or $\leq$ 18; blood pressure >160/100, Oral temperature >100°F, Pulse >100, history of major GI tract surgery, with the exception of cholecystectomy and appendectomy, in the past five years. Any major bowel resection at any time. Those with history of active uncontrolled gastrointestinal disorders or diseases including: inflammatory bowel disease (IBD), ulcerative colitis Crohn's disease, indeterminate colitis; irritable bowel syndrome (IBS), persistent, infectious gastroenteritis, colitis or gastritis, persistent or chronic diarrhoea of unknown etiology, *Clostridium difficile* infection (recurrent) or *Helicobacter pylori* infection (untreated) and chronic constipation were also excluded. Other exclusion criteria included the use of any of the following drugs within the last 6 months: systemic antibiotics (intravenous, intramuscular, or oral), oral, intravenous, cytokines, immunosuppressive cytotoxic agents. Others were those who may have consumed large doses of commercial probiotics (greater than or equal to 10^8 cfu or organisms per day) that is; tablets, capsules, lozenges, chewing gum or powders in which probiotic was the primary component.

Ethical approval

This study was approved by the Human Research Ethics Committee of Lagos University Teaching Hospital with Ethical Approval Ref No: ADM/DCST/HREC/752.Written informed consent was obtained from all volunteers.

Sample collection and gut bacterial genotyping

Sample collection

A total of 20 faecal samples were collected. 150 mg of sample from a single bowel movement from each volunteer was placed into Stool Nucleic Acid Collection and Preservation Tube (NORGEN Item No: 45630) and stored at 28^{O}C. DNA was extracted within 7-12 days of sample collection.

Bacterial DNA isolation from faecal samples

Bacterial DNA was extracted from approximately 2 ml of the faecal sample using the standard protocol for ZR Fecal DNA MiniPrep™ D6010 (Zymo Research, USA) according to the manufacturer's instructions. The concentration of the extracted DNA was determined using a UV- visible spectrophotometer (NanoDrop, Mode l3300, Thermo Fisher Scientific, USA). Bacterial DNA was stored in DNA Elution Buffer at "20°C prior to further analysis. Only specimens with DNA concentration of > 300 µg/µl or the equivalent of 60-100 ng were further analysed.

DNA Amplification

DNA amplification and sequencing was conducted at Inqaba Biotechnical (Pty) limited Pretoria, South Africa. Our target was the V3-V4 hyper-variable region of the bacterial 16S rRNA genes present in each sample.Two universal bacterial 16S rRNA gene amplicon PCR primers TruSeq tailed 341F TGACTGGAGTTCAGACGTGTGCTCTTCCGATCT CCT ACGGGNGGCWGCAG and TruSeq tailed 785R CACTCTTTCCCCACACGACGCTCTTCCGATCT GACTACHVGGGTATCTAATCC were used [11]. The reaction mixture was set up as follows: microbial DNA (5 ng/µl)2.5 µl; amplicon PCR forward primer (1 µM) 5 µl; PCR reverse primer (1 µM) 5 µl; 2× KAPA HiFi Hot Start Ready Mix 12.5 µl (total 25 µl).The PCR was performed in a thermal cycler (Applied Biosystems

9700, USA) with: 1 cycle of denaturingat 98 °C for 3 min, followed by 30 cycles of denaturing at 95 °C for 30s, annealing at 55 °C for 30s, elongation at 72 °C for 30s, and a final extension at 72 °C for 5s. The PCR products were checked using electrophoresis in 1 % (w/v) agarosegel in TBE buffer (Tris, boric acid, EDTA) stained with EZ vision and visualized under UV light (E box). Fast DNA ladder (BioLab N3238) was used as the Standard DNA standard marker. The 341 bp and 785 bp fragments were purified using Gel DNA recovery kit (A bioanalyzer, Agilent Tech. 2100, USA) and LAB-ON-A-CHIP was used to verify the size of the PCR product.

Microbiome sequencing of fecal DNA

Sequencing libraries was generated using NEBNext® Ultra DNA Library Prep Kit for Illumina® (IlluminaInc, San Diego, California USA) following the manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina MiSeq system (Illumina Inc, San Diego, California USA) to define the phylogenetic types (phylotypes) present. Species-level bacterial phylotypeswas defined as organisms sharing $\geq$ 97% nucleotide sequence identity (% ID) in the V4 regions of their 16S rRNA genes.

Bioinformatic analysis

Sequence data was generated in order to differentiate faecal community composition of individuals from the rural and urban communities. The data was edited by trimming low-quality ends from reads in addition to adapter removal. Fast Length Adjustment of Short reads (FLASH) to improve genome assemblies was used to merge paired-end reads that were generated from DNA fragments whose lengths were shorter than length of reads [25]. Datasets obtained from all 20 individuals were identified to bacterial species-level operational taxonomic units (OTUs) using the Quantitative Insight into Microbial Ecology (QIIME) Package version 1.70 as described by Kuczynski *et al*. in 2011 [26]. Samples with <1000 counts were discarded and only reads of sufficient Q scores (>q20) and lengths were used in the analysis. Closed-reference OTU picking was used and OTUs with a number of sequences <0.005% of the total number of sequences were discarded Taxa information for every BLAST hit was recorded, the focus was on analysing the OTUs with 97% similarity cut-off value. In addition, SPSS window version 20.0 was used for descriptive and inferential analyses of demographic and clinical information.

Results

Sociodemographic and clinical characterization of study participants

The study characterized and compared the gut microbiota of 20 volunteers from the same ethnic group divided into two groups by residence in urban and rural communities. The volunteers were within the age range of 20-32 years, with an average of 24.7 years for urban population and 25.5 years for rural population. The average body mass index for the urban population was 23.94kg/m (SD 6.06), weight 72.6kg (SD 22.17), height 5.43ft (SD 0.36), blood pressure of 117/70mmHg and oral temperatures of 36.4 (SD 0.50).Volunteers from the rural community had an average body mass index of 20.04kg/m(SD 2.35), weight 50.6kg(SD 10.02), height 5.08ft(SD 0.54), blood pressure 117/80mmHg and oral temperatures of 35.6°C (SD 1.16)(Table 1). Results obtained showed that the study population did not have high blood pressure and were not obese, The diet for the rural Ikoga-Zebbe community was"bush meat"(wild animals such as antelope) (60%), fish (80%), Maize flour (90%); yam (100%); cassava flour (80%) eaten with traditional "ewedu" soup(a vegetable soup made by blending the leaves of *Corchorus olitorius*);water leaves (*Talinum triangulare*), and African spinach, "Green" (*Amaranthus hybridus*)(100%) obtained from their farms.

Their food was seasoned with red chilipepper (*Capsicum frutescent s*and *Capsicum annuum*). They snacked on raw coconut. All volunteers drank herbal natural products made from root and stem of Neem or dogoyaro (*Azadirachta indica*), mango (*Mangifera indica*) and "monkey leaves". Their source of drinking water was the village well (shovel or backhoe dug pits that tap into naturally occurring underground water). The medical students, on the other hand, ate mainly animal protein (beef 80 %, chicken, fish, or egg) rice (100%), sweet potatoes(100%) pastries (60%) vegetables (10%). In addition, the diet included: rice, beans, yam, steamed bean pudding (moin-moin), (cassava root flour *(Manihot* esculenta)) and "amala" (made from dried yam flour), eaten with soup made from ewedu, okro (*Abelmoschus esculentus*), eforiro (*Parkiabi globosa*) and egusi (Melon seed). Others were processed foods (pizza, doughnut, egg roll, shawarma, fish roll, biscuits, corn flakes and soft drinks) that were high in sugar. The fruits consumed were

orange, pineapple, watermelon and apple. They consumed on a daily basis, processed milk and yogurt. Their source of water from a machine drilled deep narrow well that accesses naturally occurring ground *water)*. Similar to the rural population, there was less than 20% alcohol consumption. They also consumed some traditional foods slightly seasoned with chili pepper.

Table 1: Participant Characteristics

S/N	Gender	Tribe/Site	Body Mass Index(BMI)	Age	Marital Status	Weight (kg)	Height (ft)	Blood pressure (mmHg)	Oral Temp. (°C)
1	F	YOR/URB	18.52	28	SINGLE	54	5.4	119/70	35.1
2	F	YOR/URB	26.8	24	SINGLE	67	5	117/72	36.4
3	F	YOR/URB	19.55	20	SINGLE	57	5.4	120/75	36.7
4	M	YOR/URB	22.8	25	SINGLE	69	5.5	118/70	36.6
5	M	YOR/URB	18.13	21	SINGLE	61	5.8	121/70	36.5
6	F	YOR/URB	20.81	29	SINGLE	70	5.8	119/75	36.3
7	F	YOR/URB	18.52	23	SINGLE	54	5.4	110/70	36
8	M	YOR/URB	27.2	20	SINGLE	68	5	110/65	36.7
9	M	YOR/URB	32.22	28	SINGLE	116	6	115/60	36.5
10	M	YOR/URB	34.89	29	SINGLE	110	5	119/70	36.8
Average			23.94	24.7		72.6	5.43	117/70	36.4
11	M	YOR/RUR	18.75	26	SINGLE	30	4	110/75	32.8
12	M	YOR/RUR	18.01	29	SINGLE	45	5	115/70	36
13	F	YOR/RUR	18.86	22	SINGLE	55	5.4	110/71	36.4
14	M	YOR/RUR	20.17	28	SINGLE	61	5.5	110/68	36.3
15	M	YOR/RUR	18.05	22	SINGLE	54	5.5	120/75	36.2
16	F	YOR/RUR	20.58	30	MARRIED	60	5.4	120/80	36.7
17	M	YOR/RUR	23.63	20	SINGLE	50	4.6	118/70	36.7
18	F	YOR/RUR	19.45	25	MARRIED	61	5.6	125/80	36
19	F	YOR/RUR	18.24	27	MARRIED	40	5.3	130/87	35.5
20	F	YOR/RUR	24.69	26	SINGLE	50	4.5	120/80	36.7
Average			20.04	25.5		50.6	5.08	117/80	35.9

Key: F: Female, M: Male, YOR: Yoruba, RUR, Rural, URB, Urban

Taxonomic Composition and Diversity of Faecal Microbiota

The taxonomic composition of the faecal microbiomes was investigated at the phylum, class, order, genus and species level. OTUs with a number of sequences <0.005% of the total number of sequences were discarded. We found 17 prokaryotic phyla and 84 genera in the 20 samples. Eight (47%) Phyla were present in both urban and rural population. Four additional phyla (Spirochates, Fibrobacterea, Euryacuales and Acidobacteria) were observed only in the urban population while five (Traches, Mycetozo, Chloroflexi, Ascomyceter, Basidomycetes) were only found in the rural communities though at low levels (d" 0.01% respectively). There were 50 tax of families common to urban and rural populations and 34 peculiar to rural population.

We found that the urban population had significantly higher numbers of Actinobacteria with 17.1% of the gut microbial population versus 1.5% for rural population (Figure 1a, Table 2a). However, they had less Bacteroidetes (34.7% versus 41.2% for rural participants), Firmicutes (43.7% versus 51.7%) and Proteobateria (1.4% versus 5.0%). At the genus level, in the Actinobacteria group, we found that *Bifidobacterium* were significantly higher in urban guts at an average of 14.4% versus low level abundance of 0.4% from rural guts. In the rural population, the most common phylum was Bacteroidetes and families were Prevotellaceae, Ruminococcaceae, Clostridiaceae, Lachnospiraceae in decreasing order. At the genus level *Prevotella* spp. predominated in this population

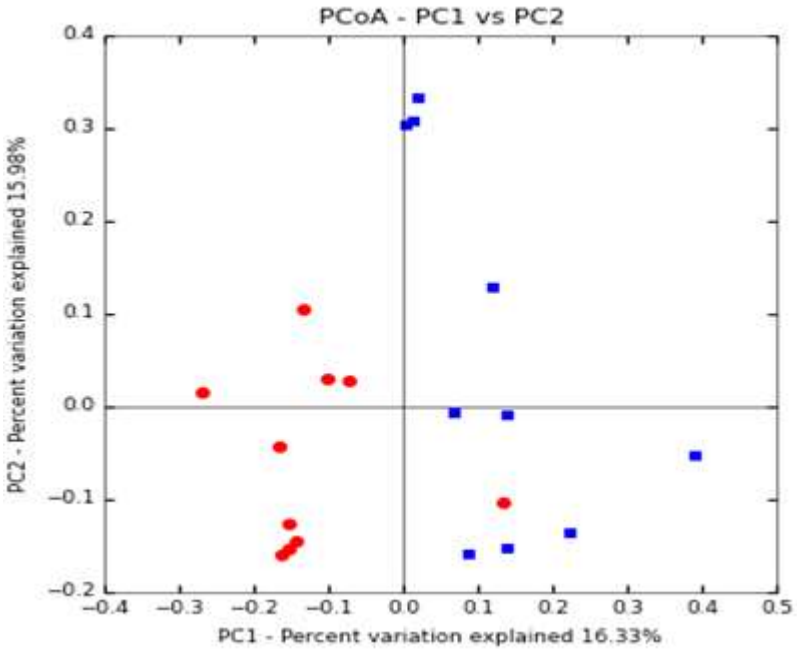

Fig. 1a: Differences in the microbiome between urban and rural Nigerians

(37.3%) as against the urban population (20.7%). We also observed the presence of the genus *Faecalibacterium*in 15% of rural and 9.6% of the urban population. There was no difference between the bacterial families by gender or age group.

Dietary Impact on gut microbiota in Lagos, Nigeria

Our data showed a lower diversity of the gut microbiome associated with the urban Western type diet (Figure 1a). The mean Alpha diversity (chao1) was 513.56 ± 85.95 and 444.07 ± 100.70 (n ± standard deviation)

Table 2a: Biodiversity of Microbiome between Healthy Urban and Rural Nigerians at Genus Level

OTU ID	Rural	Urban
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanosphaera	0.00%	0.02%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	0.44%	14.41%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	0.18%	0.12%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Adlercreutzia	0.00%	0.02%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella	0.90%	2.55%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Eggerthella	0.00%	0.02%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Slackia	0.03%	0.02%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__;g__	1.36%	0.11%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides	0.14%	9.49%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	0.03%	0.43%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella	37.31%	20.66%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__	0.03%	0.31%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes	0.00%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;g__	0.80%	2.72%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Barnesiellaceae];g__	0.08%	0.35%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Butyricimonas	0.00%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Odoribacter	0.00%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__CF231	0.02%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__[Prevotella]	1.30%	0.57%
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;f__;g__	0.22%	0.04%

k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__;g__	0.02%	0.00%
k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__Elusimicrobiales;f__Elusimicrobiaceae;g__	0.02%	0.02%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	0.02%	1.67%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	0.04%	0.63%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter	0.04%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__	3.13%	2.68%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;g__	0.09%	0.23%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__	1.47%	0.52%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.35%	0.22%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__SMB53	0.94%	0.62%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__	6.95%	5.56%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia	0.70%	0.75%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Butyrivibrio	0.04%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus	0.48%	0.55%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea	0.12%	0.20%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnobacterium	0.07%	0.08%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospira	0.87%	1.48%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Roseburia	0.43%	0.53%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]	0.06%	1.27%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	0.05%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__	13.59%	11.46%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Faecalibacterium	15.55%	9.58%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira	0.52%	0.56%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus	0.89%	1.53%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Anaerovibrio	0.06%	0.02%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Dialister	2.07%	0.61%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megamonas	0.42%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megasphaera	0.01%	1.60%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Mitsuokella	0.03%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Phascolarctobacterium	0.67%	1.04%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella	0.05%	0.06%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__	0.21%	0.11%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__	1.58%	0.01%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Bulleidia	0.01%	0.02%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Catenibacterium	0.08%	0.00%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Eubacterium]	0.17%	0.04%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae;g__	0.16%	0.04%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae;g__Victivallis	0.01%	0.07%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__RF32;f__;g__	0.03%	0.00%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella	0.40%	0.62%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Bilophila	0.01%	0.06%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	0.03%	0.04%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter	0.00%	0.01%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae;g__Succinivibrio	3.77%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__	0.42%	0.44%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Enterobacter	0.00%	0.01%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Erwinia	0.01%	0.03%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus	0.31%	0.23%
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__	0.01%	0.01%
k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__;g__	0.02%	0.00%
k__Bacteria;p__Tenericutes;c__RF3;o__ML615J-28;f__;g__	0.01%	0.00%
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__[Cerasicoccales];f__[Cerasicoccaceae];g__	0.02%	0.00%
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia	0.15%	2.80%

with a P value of 0.14. Our principal coordinates analysis (PCoA) and heatmap showed a clear clustering for the urban and rural populations studied. The identity of most of the microbiota were unknown. The urban population were on diet made up of animal protein, processed foods and soft drinks that were high in sugar in addition to traditional foods, fruits as well as vegetables and the rural population were primarily consuming plant-rich diets.

volunteers from Malawi (8.7%), Mali (11.90%), and Nigeria (9.5%) (Table 2b). In addition to the three distinct phyla, the study observed members of the group Preobacteria in sequences from Malawi (8.28%) than in those from Mali (0.15%) and Nigeria (2.82%) and they were mainly of the genus *Succinivibrio.*

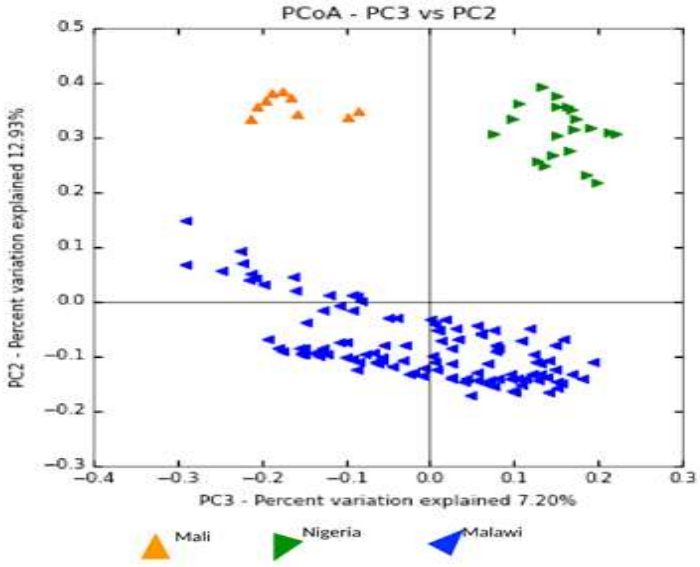

Fig. 1b: Differences in microbiome between Nigerians and other African countries

Comparison of Nigerians gut microbiota to other African countries

When compared with results from two other West African countries, our clustering analysis (PCoA) showed a similar diversity/composition trend between Mali and Nigeria, which was different from Malawi although all three groups clustered separately (Figure 1b). Three most distinct and abundant Phyla identified were Firmicutes (Malawi 52.64%, Mali 73.22%, Nigeria 46.84%), Bacteriodetes (Malawi 24.66%, Mali 12.21% and Nigeria 37.16%) and Actinobacteria (Malawi 12.32%, Mali 10.13%, Nigeria 11.09%) respectively. The genus *Bifidobacterium* was present at different levels in gut of individuals from Nigeria (8.99%) Mali (5.5%) and Malawi (12.02%). On the other hand, the relative abundance of the genus *Prevotella* was Nigeria 27.11% Malawi 22.34%, but lesser for Mali 5.67%. Comparately, genus *Faecalibacterium* of the family Ruminococcaceae was found to be present at almost same level in gut of

Comparison of Nigerians gut microbiota to non-Africans

When we compared our data with non- Africans, Nigerian microbiomes were closer to other developing countries such as Venezuela and Columbia compared to the United States (Figure 1c).

The three most distinct and abundant Phyla identified were also Firmicutes (Columbia 75.24%, Nigeria 46.84%, USA 68.32%, Venezuella 55.97%), Bacteriodetes (Columbia 19.18%, Nigeria 37.16%, USA 22.43%, Venezuella 23.42%), Actinobacteria (Columbia 1.83%, Nigeria 11.09%, USA 5.65%, Venezuella 6.70%), and Preobacteria (Columbia 2.14%, Nigeria 2.82%, USA 2.74%, Venezuella 12.14%). The distribution of the phyla is as shown on Table 2c. The genus *Bifidobacterium* was present at similar levels in gut of individuals from Nigeria (8.99%) USA (5.28%) and Venezuela (6.04%) but very low in those from Columbia (0.05%). On the other hand, genus *Prevotella* was higher in guts of Nigeria (27.11%) as against 17.78%

Table 2b: Biodiversity of microbiome between Nigerians and other African Countries at Genus level

OTU ID	Malawi	Mali	Nigeria
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobrevibacter	0.01%	0.98%	0.00%
k__Archaea;p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanosphaera	0.01%	0.35%	0.01%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces	0.01%	0.09%	0.00%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Rothia	0.02%	0.00%	0.00%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__	0.00%	0.20%	0.00%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	12.02%	5.55%	8.99%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__	0.19%	1.56%	0.14%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Adlercreutzia	0.00%	0.08%	0.01%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Atopobium	0.00%	0.01%	0.00%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella	0.10%	2.52%	1.91%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Coriobacterium	0.00%	0.01%	0.00%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Eggerthella	0.00%	0.00%	0.01%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Slackia	0.01%	0.11%	0.02%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__;g__	0.29%	1.13%	0.60%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BS11;g__	0.00%	0.01%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides	0.83%	2.76%	5.86%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	0.10%	0.17%	0.28%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__	0.04%	0.20%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella	22.34%	5.67%	27.11%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__RF16;g__	0.03%	0.01%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__	0.06%	0.14%	0.20%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Alistipes	0.00%	0.01%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;g__	0.50%	1.02%	1.98%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Barnesiellaceae];g__	0.01%	0.02%	0.24%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Butyricimonas	0.01%	0.11%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Odoribacter	0.01%	0.02%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__	0.06%	0.05%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__CF231	0.10%	0.03%	0.01%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__Paraprevotella	0.00%	0.02%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__YRC22	0.02%	0.00%	0.00%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae];g__[Prevotella]	0.24%	0.50%	0.85%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__p-2534-18B5;g__	0.03%	0.33%	0.00%
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;f__;g__	0.66%	0.36%	0.11%
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__;g__	0.01%	0.00%	0.01%
k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__Elusimicrobiales;f__Elusimicrobiaceae;g__	0.16%	0.01%	0.02%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__;g__	0.03%	0.15%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__	0.00%	0.29%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Listeriaceae;g__Listeria	0.20%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus	0.02%	0.03%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__	0.01%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__;g__	0.00%	0.01%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Granulicatella	0.01%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus	0.00%	0.01%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Vagococcus	0.01%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	2.80%	0.17%	1.03%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__	0.00%	0.02%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Leuconostoc	0.02%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Weissella	0.07%	0.01%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus	0.02%	0.01%	0.00%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	5.83%	0.41%	0.40%

k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter	0.04%	0.91%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__;f__;g__	0.00%	0.02%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__	2.25%	8.14%	2.85%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;g__	0.07%	0.48%	0.18%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__	3.51%	2.38%	0.89%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__02d06	0.00%	0.02%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Candidatus Arthromitus	0.10%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	0.61%	2.06%	0.27%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__SMB53	0.16%	1.36%	0.74%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Sarcina	0.01%	0.02%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__	5.08%	7.45%	6.09%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes	0.02%	0.11%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia	2.79%	5.05%	0.73%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Butyrivibrio	0.00%	0.04%	0.02%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Clostridium	0.02%	0.01%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus	1.47%	1.98%	0.52%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea	0.31%	1.63%	0.17%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnobacterium	0.16%	0.29%	0.08%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospira	0.49%	0.59%	1.24%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Roseburia	0.37%	0.40%	0.49%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Shuttleworthia	0.00%	0.02%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]	0.45%	0.92%	0.80%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Peptococcus	0.00%	0.07%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	0.31%	0.65%	0.04%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__[Clostridium]	0.01%	0.02%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__	6.48%	#####	12.28%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Anaerofilum	0.00%	0.05%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Butyricicoccus	0.01%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Faecalibacterium	8.71%	9.57%	11.90%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira	0.39%	0.76%	0.54%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus	1.67%	5.56%	1.28%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__	0.18%	0.06%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Acidaminococcus	0.01%	0.00%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Anaerovibrio	0.13%	0.00%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Dialister	1.73%	1.54%	1.18%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megamonas	0.35%	0.00%	0.18%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megasphaera	2.03%	0.06%	0.98%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Mitsuokella	0.04%	0.17%	0.03%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Phascolarctobacterium	0.33%	0.47%	0.89%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Succiniclasticum	0.00%	0.01%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella	1.53%	0.03%	0.05%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae];g__Fusibacter	0.00%	0.04%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__	0.06%	0.21%	0.15%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__Mogibacterium	0.00%	0.07%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Halanaerobiales;f__Halanaerobiaceae;g__	0.00%	0.03%	0.00%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Natranaerobiales;f__Anaerobrancaceae;g__	0.00%	0.06%	0.00%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__	0.59%	0.69%	0.62%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Bulleidia	0.22%	0.21%	0.02%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Catenibacterium	0.49%	2.60%	0.03%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Coprobacillus	0.00%	0.08%	0.00%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Sharpea	0.00%	0.05%	0.00%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Eubacterium]	0.39%	2.82%	0.09%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__p-75-a5	0.07%	0.05%	0.00%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Cetobacterium	0.11%	0.00%	0.00%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium	0.07%	0.00%	0.00%

k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Leptotrichia	0.01%	0.00%	0.00%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae;g__	0.08%	0.02%	0.09%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae;g__Victivallis	0.00%	0.00%	0.05%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Z20;f__R4-45B;g__	0.00%	0.10%	0.00%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__	0.03%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__RF32;f__;g__	0.11%	0.00%	0.01%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella	0.39%	0.08%	0.53%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__	0.00%	0.06%	0.00%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Comamonas	0.00%	0.17%	0.00%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnohabitans	0.00%	0.05%	0.00%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	0.00%	0.02%	0.00%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Oxalobacter	0.01%	0.01%	0.00%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Bilophila	0.01%	0.00%	0.04%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio	0.04%	0.05%	0.04%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__GMD14H09;f__;g__	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter	0.77%	0.00%	0.01%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Flexispira	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Helicobacter	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae;g__Anaerobiospirillum	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae;g__Ruminobacter	1.10%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Succinivibrionaceae;g__Succinivibrio	2.62%	0.01%	1.46%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__	2.31%	0.54%	0.43%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Citrobacter	0.00%	0.02%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Enterobacter	0.23%	0.00%	0.01%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Erwinia	0.00%	0.00%	0.03%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Klebsiella	0.00%	0.01%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Pantoea	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Serratia	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__	0.00%	0.01%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Aggregatibacter	0.01%	0.00%	0.00%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus	0.57%	0.02%	0.26%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiohalorhabdales;f__;g__	0.01%	0.00%	0.00%
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__Treponema	0.65%	0.22%	0.00%
k__Bacteria;p__Spirochaetes;c__[Brachyspirae];o__[Brachyspirales];f__Brachyspiraceae;g__Brachyspira	0.00%	0.04%	0.00%
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae;g__	0.04%	0.00%	0.01%
k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__;g__	0.25%	0.82%	0.01%
k__Bacteria;p__Tenericutes;c__RF3;o__ML615J-28;f__;g__	0.01%	0.02%	0.01%
k__Bacteria;p__Verrucomicrobia;c__Opitutae;o__[Cerasicoccales];f__[Cerasicoccaceae];g__	0.00%	0.00%	0.01%
k__Bacteria;p__Verrucomicrobia;c__Verruco-5;o__WCHB1-41;f__RFP12;g__	0.01%	0.00%	0.00%
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia	0.00%	0.51%	1.77%

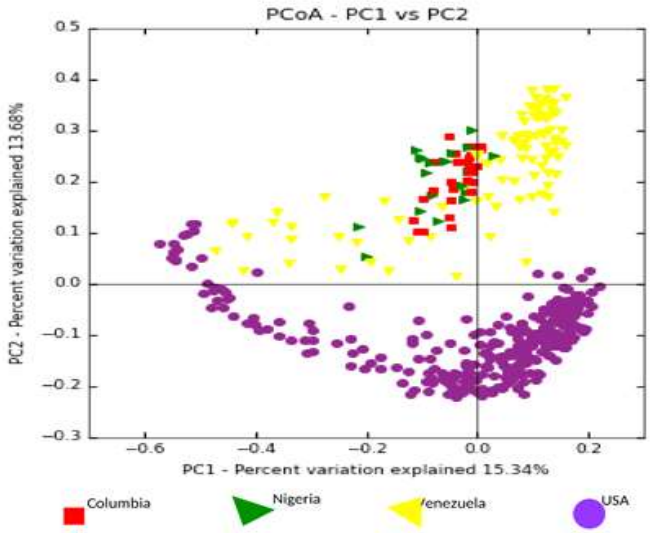

Fig. 1c. Differences in microbiome between Nigeria and non-African countries

for Venezuela, 6.08% for Columbia and 2.10% for USA. The prominent genus among the phyla Firmicutes was also found to be *Faecalibacterium* as follows; Columbia 8.24%, Nigeria 11.90%, USA 10.37% and Venezuella 8.46%, Others were members of the genus *Blautia*, which was seen in samples from Columbia 4.3%, USA 6.0% Venezuella 3,82%, and only 0.73% for Nigeria. Other Genus in this category with similar distribution were *Ruminococcus* and *Coprococcus*. The data from this project have been deposited with links to BioProject accession number PRJNA579839 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

Discussion

A high diversity of gut microbiome is usually considered beneficial and appears protective against many diseases. Certain life styles and dietary habits may predispose to a high or low diversity as well as specific microbiome patterns. To address this, we characterized and compared the gut microbiota of subjects from the same ethnic group. Overall findings revealed that the composition of the gut microbiome of the Yoruba population studied is primarily organisms from the phylum Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria. Members of the phyla are known as core gut microbes [4,5,27,28] and were detected in

Table 2c: Biodiversity of Microbiome between Nigerians and non-African countries at Phyla level

OTU ID	Columbia	Nigeria	USA	Venezuela
k__Archaea;p__Euryarchaeota	0.00%	0.01%	0.04%	0.02%
k__Bacteria;p__Actinobacteria	1.83%	11.09%	5.65%	6.70%
k__Bacteria;p__Bacteroidetes	19.18%	37.16%	22.43%	23.42%
k__Bacteria;p__Cyanobacteria	0.10%	0.12%	0.01%	0.38%
k__Bacteria;p__Elusimicrobia	0.00%	0.02%	0.00%	0.08%
k__Bacteria;p__Firmicutes	75.24%	46.84%	68.32%	55.97%
k__Bacteria;p__Fusobacteria	0.01%	0.00%	0.06%	0.02%
k__Bacteria;p__Lentisphaerae	0.00%	0.13%	0.00%	0.08%
k__Bacteria;p__Proteobacteria	2.14%	2.82%	2.74%	12.14%
k__Bacteria;p__Spirochaetes	0.00%	0.00%	0.00%	0.41%
k__Bacteria;p__Tenericutes	0.26%	0.02%	0.35%	0.69%
k__Bacteria;p__Verrucomicrobia	1.23%	1.78%	0.41%	0.04%
k__Bacteria;p__WPS-2	0.00%	0.00%	0.00%	0.03%

reasonable numbers in this healthy Nigerian population. Assessment of diversity showed that the Phylum Actinobacteria dominated the urban population and Bacteroidetes the rural population. Two phyla: Bacteroidetes and Firmicutes, were more dominant in a similar study in Nigeria [27]. Furthermore, the phyla found only in the urban population were Spirochaetes, Fibrobacterea, Euryacuales and acidobacteria. On the other hand, Traches, Mycetozo, Chloroflexi, Ascomyceter, Basidomycetes was only found in the rural community.

In African countries, urban areas are transitioning towards a lifestyle and fast-food diet typical of 'Westernized' societies [17,18,28]. It has been suggested that over time dietary habits will have an impact on the gut microbiota [2]. To support this claim, we observed differences between microbiome of those with western diets as opposed to those with more traditional diets. Analysis of stool samples showed that the healthy population studied in urban Yoruba community had more of the phylum Actinobacteria of the family Bifidobacteriaceae, Other families detected were those of Coriobacteriaceae, Lactobacillaceae Prevotellaceae and Bacteroidaceae.

Bifidobacterium spp. was the predominant genus found among volunteer medical students on Western diet. As observed, the diet of the urban university community was animal protein, processed foods and soft drinks that were high in sugar; these were similar to the western diet. Moreover, the students were usually from middle class families, consumed water from a machine drilled deep narrow well that accesses naturally occurring ground water and maintain a good hygiene. Such a westernized dietary life style could lead to a loss of the traditional gut microbiome. In addition, our data showed a lower diversity for urban Western type diet suggesting that diet may shape gut microbiota in a way that may have consequences on health and diseases. Few members of the Bifidobacteriaceae are known to have beneficial probiotic effects, in Western or more or less urban populations. Members of these species have been associated with the production of health promoting metabolites such as short chain fatty acids, conjugated linoleic acid and bacteriocins [29]. They are able to breakdown metabolic by-products generated through partial digestion of complex dietary carbohydrates. In addition, *Bifidobacterium* aids in the digestion of milk-based diets. Thus, the genus dominates the intestine of healthy breast-fed infants [26-30]. The genus was shown in this study to dominate the urban population of health young adult Yorubas.

The study recognised a shift from the level of *Prevotella* sp. found in the gut of volunteers from urban population and the presence of *Bifidobacterium.* In combination to diets made up of animal protein, processed foods and soft drinks, the urban population also consumed traditional foods and may have been on plant rich diets prior to urban lifestyle, hence the presence of substantial level of *Prevotella and Faecalibacterium* species in addition to *Bifidobacterium.* Comparatively, *Bifidobacterium* was seen at a very low abundance in the rural population. This was not unexpected because the study shows that the rural population primarily consume plant-rich diets. Conserving beneficial biodiversity and promoting diets that support proliferation of benefice species promote better health. Both *Bifidobacterium*, *Prevotella* and *Faecalibacterium* sp. are known to be present in healthy guts. Whether there would be a shift from good healthy to disease condition especially those found in western countries including obesity, diabetes, irritable bowel syndrome, inflammatory bowel disease, depression, and cardiovascular disease [21,23] while being on westernized diet calls for more investigation.

Analysis of stool samples showed that the healthy population studied in rural Yoruba community had more of the family Bacteroidales and Clostridiales especially the genus *Prevotella* and *Faecalibacterium*. Members of the Genus *Prevotella* are linked with non-western subjects and rural population [3,17]. This core microbiome is associated with plant-rich diets (high levels of complex carbohydrates and fruit and vegetable intake). This is in line with the diet in Ikoga community which is mainly tubers, maize and vegetable derived from their farmland. Our finding of the *Prevotella* driven microbiota is similar to results by Ayeni et al. [27] in rural Bassa community in Northern Nigeria who also consumed a traditional local diet. It was also the predominant genus among adult urban population from the Western region of India who were on local dietary that primarily comprised of fruits, vegetables, wheat, millet, sorghum, dairy products, sprouts, leafy vegetables, rice and pulses [12]. It has also been shown as a known discriminatory taxon, present in higher abundance in the fecal microbiota of children living in Burkina Faso, which is quite different when compared with those living in Italy [11]. Harbouring this beneficial microbes may have good effect in protecting the individuals from various health conditions and disorders.

The average body mass index of the participants in both population falls below that used in describing

obesity. Same as the level of blood pressure. On the other hand, the relation between gut microbiome, gender or age difference was not studied, apparently due to the small sample size. This study has highlighted the gut microbiomes of healthy youths in rural and urban environment. Further analysis is required to fully relate the distinct species identified with their metabolic functionality and to define the microbiomes in a specific communicable and non-communicable tropical diseases or disorder peculiar to West African region.

Prevotella is a beneficial bacterium but it has also been linked to chronic inflammatory conditions, such as arthritis [8] and mucosal and systemic T-cell activation in untreated human immunodeficiency virus type 1 (HIV-1) infection [31]. This should be considered in future research that may study populations with disease conditions. The genus *Faecalibacterium* associated with anti-inflammatory influences [32] were also found in significant amounts among our urban population volunteers. Our finding that two populations of same ethnicity living in different area have such distinct levels of microbiome diversity, supports the view that diet and lifestyle are driving forces behind microbiome development. It is important to conserve beneficial biodiversity and promote diets that support proliferation of beneficial species. Both population had beneficial microbiota. The data obtained, identifies possible biomarkers of the normal gut communities in Yoruba population.

The study investigated how Nigerians' microbiome compares to other populations in Africa or outside Africa. There were similar diversity/composition trends between Mali and Nigeria which was different from rural agricultural community in Malawi [13,33]. It was of interest to find that the Nigerians' microbiome in general was similar to other populations in Africa or outside Africa. We expected West Africans to have relatively similar diets that would differ from East Africans and even more differently from Non-Africans. When the data obtained was compared with that from non-African, the Nigerian microbiome was closer to other developing countries especially the amazon dwellers found in Venezuela and Columbia than the urban industrialized society found in United States [13].These patterns may be explained by various factors including diets, genetics, life style, environmental changes among others. However looking at the cohort studied, we observed that the urban regions of Lagos seems to have transitioned towards a diet pattern typical of 'Westernized' societies. The study focused on healthy volunteers thus, how this diet pattern may relate to disease conditions including obesity, diabetes, irritable bowel syndrome, inflammatory bowel disease, depression, and cardiovascular disease associated with westernized diets [21,23] needs further attention. Moreover, there maybe other predisposing conditions to the development of these disorders and disease conditions.

Limitations

First, a relatively small number of healthy Nigerian study subjects participated in the study, which would affect the comprehensive understanding of healthy human microbiota and generalizability of the data results. Second, only samples from these habitats at a single time point were included; samples from more ethnic tribes would verify the representative healthy human microbiota of Nigerians. In addition, we could not compare microbial taxa, diet with functional characteristic of the gut microbiome of young healthy Yoruba adults.

Conclusion

This finding reveals a distinct difference between the microbiome of healthy rural and urban population in Lagos State. Urban population were dominated by genus *Bifidobacterium, Prevotella and Faecalibacterium*, while rural population were by the *Prevotella and Faecalibacterium species.* The urban regions of Lagos seem to have transitioned towards a diet pattern typical of 'Westernized' societies and this may have contributed to the shift toward dominance by *Bifidobacterium* when compared with microbiome of similar ethnicity living in the rural region. Further studies need to be conducted in a larger population to fully ascertain this relationship. In addition, whether this shift will relate to development of diseases and disorders peculiar to 'Westernized' societies requires further investigation.

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References

1. Sender R, Fuchs S and Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoSBi. 2016: l14:e1002533.
2. Rinninella E, Raoul P, and Cintoni M. *et al.* What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms. 2019: 7:14.
3. Rothschild D, Weissbrod O, Barkan E. *et al.,* Environment dominates over host genetics in shaping human gut microbiota. Nature. 2018: 8(555):210-215.
4. Thomas S, Izard J, Walsh E. *et al*. The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists. Cancer Res. 2017: 77:1783-1812.
5. Round JL and Mazmanian Sk. The gut microbiota shapes intestinal immune responses during health and disease. Nature Rev Immunol. 2009: 313–323.
6. O'Callaghan A. van Sinderen D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. Fr Microbiol. 2016;7: 925.
7. Fukuda S, Toh H and Hase K. Bifidobacteria can protect from enteropathogenic infection through production of acetate Nature. 2011;469:543–547.
8. Pianta A, Arvikar S. Strle K *et al.* Evidence of the Immune Relevance of *Prevotella copri*, a Gut Microbe, in Patients with Rheumatoid Arthritis. Arth Rheumatol. 2017;69:964–975.
9. Nishijima1 S, Suda W. Oshima K. *et al.* The gut microbiome of healthy Japanese and its microbial and functional uniqueness. DNA Res. 2016;23: 125–133.
10. Qin J, Li R. Raes J. *et al*. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59–65.
11. De Filippo C, Cavalieri D, Di Paola M. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA. 2010; 107:14691-14696.
12. Yadav D, Ghosh TS, Mande SS. Global investigation of composition and interaction networks in gut microbiomes of individuals belonging to diverse geographies and age-groups. Gut Pathog. 2016; 8:17.
13. Yatsunenko T, Rey FE, Manary MJ. *et al.* Human gut microbiome viewed across age and geography. Nature. 2012; 9486: 222-227.
14. Jha AR, Davenport ER, Gautam Y1. *et al*. Gut microbiome transition across a lifestyle gradient in Himalaya. PLOS Biology. 2018; 16:e2005396.
15. Tito RY, Knights D, Metcalf J. Insights from characterizing extinct human gut microbiomes. PLoSOne. 2012;7: e51146-2210.
16. Liao M1, Xie Y, Mao Y. *et al*. Comparative analyses of fecal microbiota in Chinese isolated Yao population, minority Zhuang and rural Han by 16sRNA sequencing. Sci Rep. 2018; 18:1142.
17. Tandon D, Haque MM, Shaikh SPS. *et al*. Snapshot of gut microbiota of an adult urban population from Western region of India. *PloS one* 2018; 13; e0195643.
18. Silveira-Nunes G, Durso DF, Alves de Oliveira LR Jr *et al*. Hypertension is Associated with Intestinal Microbiota Dysbiosis and Inflammation in a Brazilian Population. Front. Pharmacol. 2020; 11:258.doi: 10.3389/fphar.2020.00258.18.
19. Brennan C.A. and Garrett, W.S. Gut microbiota, inflammation, and colorectal cancer. Annu. Rev. Microbiol. 2016; 70, 395–411.
20. De Angelis M., Ferrocino I, Calabrese F.M. *et al.* Diet influences the functions of the human intestinal microbiome. Sci Rep 2020; 10**,** 4247.
21. Hills RD, Pontefract BA, Mishcon HR. *et al.* Black CA, Sutton SC, and Cory R. Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. Nutrients. 2011; 19: 1613 29-40.
22. Oleskin AV and Shenderov BA. Neuromodulatory effects and targets of the SCFAs and gasotransmitters produced by the human symbiotic microbiota. Microb Ecol Health Dis 2016;

27:30971. https ://doi.org/10.3402/mehd.v27.30971[20]
23. Mohajeri, MH, Brummer, RJM and Rastall RA. 2018: 57 (1):S1 S14.https://doi.org/10.1007/s00394-018-1703-1704.
24. Kandala N and Stranges S. Geographic Variation of Overweight and Obesity among Women in Nigeria: A Case for Nutritional Transition in Sub-Saharan Africa. PLoS ONE. 2014; 9: e101103.
25. Magoc T and. Salzberg S. FLASH (Fast Length Adjustment of Short reads). Bioinformatics. 2011, 27:21 2957-2963.
26. Kuczynski J, Stombaugh J, Walters WA. *et al.* Using QIIME to analyze 16S rRNA gene sequences from microbial communities. *Curr. Protoc. Microbiol.* 2012; **C1:**1E 5.
27. Ayeni FA, Biagi E, Rampelli S *et al.* Infant and Adult Gut Microbiome and Metabolome in Rural Bassa and Urban Settlers from Nigeria. Cell Rep. 2018; 23:3056–3067.
28. Cockx L, Colen L, DeWeerdt J. From corn to popcorn? Urbanization and food consumption in sub-Saharan Africa: Evidence from rural-urban migrants in Tanzania. LICOS Discussion Paper Series. 2017; 390/2017.
29. Arboleya S, Watkins C and Stanton C. Gut Bifidobacteria Populations in Human Health and Aging. Front. Microbiol. 2016; 7:1204.
30. Rautava S. Early microbial contact, the breast milk microbiome and child health. J Dev Orig Health Dis. 2016; 7:5–14.
31. Dillon S. M., Lee EJ, Donovan AM. *et al*. Enhancement of HIV-1 infection and intestinal CD4+ T cell depletion *ex vivo* by gut microbes alered during chronic HIV-1 infection. Retrovirol. 2016; 13: 5.
32. Zhang M, Zhou L, Wang Y. *et al*. *Faecalibacterium prausnitzii* produces butyrate to decrease c-Myc-related metabolism and Th17 differentiation by inhibiting histone. deacetylase 3, Intern. Immunol. 2019;dxz022, https://doi.org/10.1093/intimm/dxz022.
33. Yooseph S, Kirkness EF, Tran TM. *et al*. Stool microbiota composition is associated with the prospective risk of Plasmodium falciparum infection. BMC Genom. 2015: 16631.

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Herbal remedy for treatment of HIV infection: a preliminary report

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Abstract

Background: Despite availability of antiretroviral therapy (ARV), side-effects and drug resistance had been major concern thus the need to evaluate efficacy of A-Zam traditional medicine used for HIV infections

*Patients and method***:** Nine HIV patients that consented and met the criteria were investigated, 3 sought herbal medicines as complementary while 6 as alternative therapy to HAART (4 patients discontinued HAART due to side-effects). Each HIV patient self- administered 27g of A Zam three times daily and monitored daily for possible side effects and effectiveness of the therapy, periodic CD4 count and viral load were measured.

*Result***:** Viral load in HIV patients on complementary therapy remained undetectable but CD4 significantly ($\alpha_{0.05}$) increased from 493±403 to 845±280 cells/µL after therapy. The viral load significantly decreased from 1,693±1691 to 55±55x10^3copies/ml and CD4 count increased from 564±334 to 639±280 cells/µL) in the 2 HIV patients using alternative therapy. There was significant gradual reduction in viral load from 758±584 to 24±34x10^3copies/ml while CD4 count increased from 572±185 to 679±243 cells/µL at 180th day in all the 4 HIV patients that discontinued HAART but commenced A-Zam herbal medicine as alternative therapy

Conclusion: The study concluded that A-Zam is a potential effective antiviral and immune-stimulatory agent and could be used when there is HIV resistance or serious side-effects to HAART.

Keywords: *Herbal medicine, Antiviral, Immune-stimulatory, HIV patients, HIV resistance*

Abstrait

Contexte: Malgré la disponibilité de la thérapie antirétrovirale (ARV), les effets secondaires et la résistance aux médicaments ont été une préoccupation majeure, d'où la nécessité d'évaluer l'efficacité de la médecine traditionnelle A-Zam utilisée pour les infections à VIH.

Patients et méthode: Neuf patients VIH qui ont consenti et ont répondu aux critères ont été étudiés, 3 ont recherché des médicaments à base de plantes comme complémentaires tandis que 6 comme thérapie alternative à l'HAART (4 patients ont interrompu l'HAART en raison d'effets secondaires). Chaque patient VIH s'est auto-administré 27 g de A Zam trois fois par jour et surveillé quotidiennement pour les effets secondaires possibles et l'efficacité de la thérapie, le nombre de CD4 périodique et la charge virale ont été mesurés.

Résultat: La charge virale chez les patients VIH sous thérapie complémentaire est restée indétectable mais CD4 a significativement (α0, 05) augmenté de 493 ± 403 à 845 ± 280 cellules / µL après la thérapie. La charge virale a diminué de manière significative de 1693 ± 1691 à 55 ± 55x10^3 copies / ml et le taux de CD4 a augmenté de 564 ± 334 à 639 ± 280 cellules / µL) chez les 2 patients VIH utilisant une thérapie alternative. Il y a eu une réduction graduelle significative de la charge virale de 758 ± 584 à 24 ± 34x10^3 copies / ml tandis que le nombre de CD4 a augmenté de 572 ± 185 à 679 ± 243 cellules / µL au 180ème jour chez les 4 patients VIH qui ont arrêté le traitement HAART mais ont commencé la phytothérapie A-Zam comme thérapie alternative.

Conclusion: L'étude a conclu qu'A-Zam est un potentiel agent efficace antiviral et immunostimulant, et pourrait être utilisé en cas de résistance au VIH ou d'effets secondaires graves à l'HAART.

Mots clés*: Phytothérapie, Antiviral, Immunostimulant, Patients VIH, Résistance au VIH*

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Introduction

Human immunodeficiency virus (HIV) infection is pandemic and has been ravaging for more than three

decades. HIV has claimed more lives than any other infectious agents in recent times. It was estimated that about 38 million people are still living with this dreadful virus while it claimed about 770,000 lives in 2018 alone [1]. Africa accounted for more than 700,000 deaths related to HIV infection in 2016 [2]. Although highly active antiretroviral therapy (HAART) is widely available, the burden of HIV infection is still high on patients, communities and governments with about 21 million HIV infected people accessed HAART in 2017 [3].

The fact that HIV patients had to be on long term therapy, with its associated burden made some to seek alternative solution. Herbal medicine therapy is very popular among Africans and HIV patients are not excluded from soliciting help from herbal therapists. Despite the rigorous media and medical practitioners' organizations campaign against use of traditional medicines, the herbal therapists' business continues to flourish in HIV infection treatment. World Health Organization (WHO) has classified herbal medicine as a substance as parts of plants, materials of plants or combination that are used to treat various illnesses throughout the World [4].

There are some herbal medicines that have been documented to be potent as anti- HIV activities [5,6]. Some of these herbal remedies like orthodox anti-retroviral agents inhibited some steps that were involved in HIV replication [7,8]. *Calophyllum lanigerum* obtained from Canolides (coumarins) of tropical forest tree had been documented to have equal potency with a non-nucleoside reverse transcriptase inhibitor [9]. Pentosan poly-sulphate derived from carbohydrates was reported to inhibit HIV tat regulatory protein (p14) that had been strongly associated with activation of transcription of proviral DNA [10]. Tropical liana plant derived *Ancistrocladus korupensis* inhibited reverse transcriptase and HIV induced cell fusion 11]. Although sero-reversion of HIV positive to negative rarely occurred with therapy, it was documented that some Chinese medicines induced this change in 8 HIV patients [12].

The usefulness of many traditional medicines in treatment of HIV infection had been documented [13]. Significant numbers of herbal medicines in Nigeria were documented to act as complementary therapy to orthodox anti-retroviral agents. It was documented that some Nigerian traditional medicines acted on the micro-organisms causing opportunistic infections in HIV/AIDS patients [14]. There are abundance *neem* leaves in Nigeria that was documented to increase CD4 count significantly while *Baissea axillaries Hua* is a common herbal medicines in Nigeria that had been used to treat many diseases related to diarrhea [14,15]. *Nigella sativa* is a popular seed that had been in use for many centuries for many therapeutic purposes (eg asthma, cancer, infection, diabetes mellitus etc) until recently HIV infection [16-18]. With the advent of resistance and serious side-effects to some components of HAART, there is need to source for other effective therapies for HIV infection. A-Zam contained *Nigella sativa* and honey as main constituents had been documented to have antimicrobial and immune-stimulatory effect in vitro [19,20].

A-Zam had been in used for HIV infection for over a decade because it had been found to increase the well-being, significantly reduce the viral load and increase CD4 count in HIV patients on it as complementary or alternative therapy [17,21]. It was documented that some patients on A-Zam therapy had sustained sero-reversion even after discontinuation of the therapy [22-24]. However, A-Zam classified therapeutic potentials had not been documented especially when HIV patients on HAART preferentially used it as complementary therapy or discontinued (HAART) and commenced this herbal medicine as alternative therapy thus need for this study.

Materials and methods

Ethical approval

Ethical approvals were obtained for this study from

1. Nigerian Institute of Medical Research (NIMR) (IRB/17/003)
2. University of Ibadan/University College Hospital Ethical Board (UI/EC/14/0277)
3. Osun State Ministry of Health, Osogbo (OSHRC/PRS/5691/23)

A-Zam

The herbal therapist declared that A-Zam contains black-seed (*Nigella sativa*) and honey as the major constituents. It was reported in earlier phytochemistry and acute toxicity studies that A-Zam is a safe herbal concoction and it contained low anthraquinones, titerpene glycosides (saponins), alkaloids, tannins and cardenolides[25].

HAART

These are 1st and 2nd line drugs used for HIV management in Nigeria. They are: Lamivudine, Nevirapine, Zidovudine, Efavirenz combinations.

Patients

HIV infected patients attending HIV clinic and seeking herbal medicine as alternative or complementary therapy to HAART at A-Zam therapist herbal centers and Center for Alternative and Complementary of Nigerian Institute of Medical Research (NIMR) were the patients of this study.

Patients' selection

Confirmed HIV patients from government secondary and tertiary health facilities were recruited and only those that gave full consent were enrolled as patients at HAART centre and able to repeat their periodic CD4 count and viral load at least on bi-monthly basis were considered for this study.

HIV patients on A-Zam only

These were confirmed 2 HIV patients awaiting commencement of HAART or were not qualified (high CD4 i.e $\geq$ 500cells/μL) before the advent of 'treat all' programme.

HIV patients on HAART and A-Zam

These were confirmed 3 HIV patients already commenced on HAART for minimum of 3 years before taking A-Zam as complementary therapy.

Post HAART - HIV patients on A Zam

These were 4 HIV patients already commenced on HAART for minimum of one year but discontinued due to side-effects or other reasons and commenced A-Zam immediately (after HAART discontinuation)

Procedure

All HIV patients were examined medically for anaemia, jaundice, skin lesions, peripheral lymphadenopathy, body mass index, organomegaly, chest, heart, including radiological investigations in order to determine the extent of involvement of the organs in HIV infection complications

Laboratory

Samples were taken every 2 months (60 days) for Immunologic and Virologic tests: HIV-DNA (confirmation), CD4 count using flow cytometer and viral (HIV-RNA) load using Polymerase Chain Reaction (PCR). Fresh 5mls of blood were collected in EDTA bottles and analysed within 2 hours following manufacturer's (Sysmex easy CD4 count kit) instructions for CD4 count. Roche COBAS Ampliprep /COBAS Taqman with limit detection of 20 (log 1.3) copies per ml was used to quantify HIV-RNA according to manufacturer's instruction on plasma samples

Haematologic tests: Full Blood Count (FBC) and blood film

Clinical chemistry**:** Electrolyte and Urea (E&U), Creatinine, Liver function test (LFT) and urinalysis.

Drug administration

A-Zam

Each of the patients self administered about 27g (1 adult tablespoon) three times daily of the A-Zam that was diluted with about 50ml of warm water. Each freshly constituted diluted medication was taken three times daily before food.

HAART

Each of the 3 patients took his/her HAART on daily basis as prescribed and described by physician and pharmacist respectively

Patient monitoring

Each patient was monitored daily (physically or phone) to ascertain possible problems associated with medication or HIV infection. Each patient presented on every 2 months basis for immunologic, virologic, haematologic and clinical chemistry laboratory assessment.

Statistical analysis- All data obtained were subjected to analysis of variance (ANOVA) and statistical significance was considered at $\alpha_{0.05}$

Result

The results of this study were categorized into: those 2 HIV patients on herbal medicine (A-Zam) alone, 3 HIV patients on HAART and A-Zam (complementary therapy) and 4 HIV patients that discontinued HAART and commenced A- Zam alone

General examination: There was no sign or symptom associated with infection, drug interaction (complementary therapy), side-effects or toxicity in all the 9 HIV infected patients that participated and took A-Zam therapy in this study

Clinical chemistry: There was no derangement in liver (bilirubin, Alanine and Aspartate transferases, gamma glutamyl transferases, albumin, total protein and alkaline phosphatase) and renal (electrolytes, urea and creatinine) functions tests throughout the study in all

the 9 HIV patients. No anomaly detected in urinalysis after the treatment in all 9 HIV patients.

Haematology:
The haematologic parameters (haematocrit, platelet and white blood cells counts) were within the normal reference interval in the 2 HIV patients on A-Zam only, 3 HIV patients on HAART and A Zam (complementary therapy) and 4 HIV patients that discontinued HAART and commenced A Zam as alternative therapy.

both G1 and G2 from 3,385,000 to 110,000 and 300 to 54 copies/ml respectively (figure 1). There were variable responses to CD4 count of these 2 patients on A Zam therapy alone. HIV patient G1 CD4 count significantly increased ($\alpha_{0.05}$) from 193 to 354 cells/µL but G2 CD4 count decreased non- significantly from 935 to 914 cells/µL (figure 2).

There was no difference in pre-treatment and post-treatment viral load in the 3 HIV patients taking HAART and A Zam (complementary therapy) because

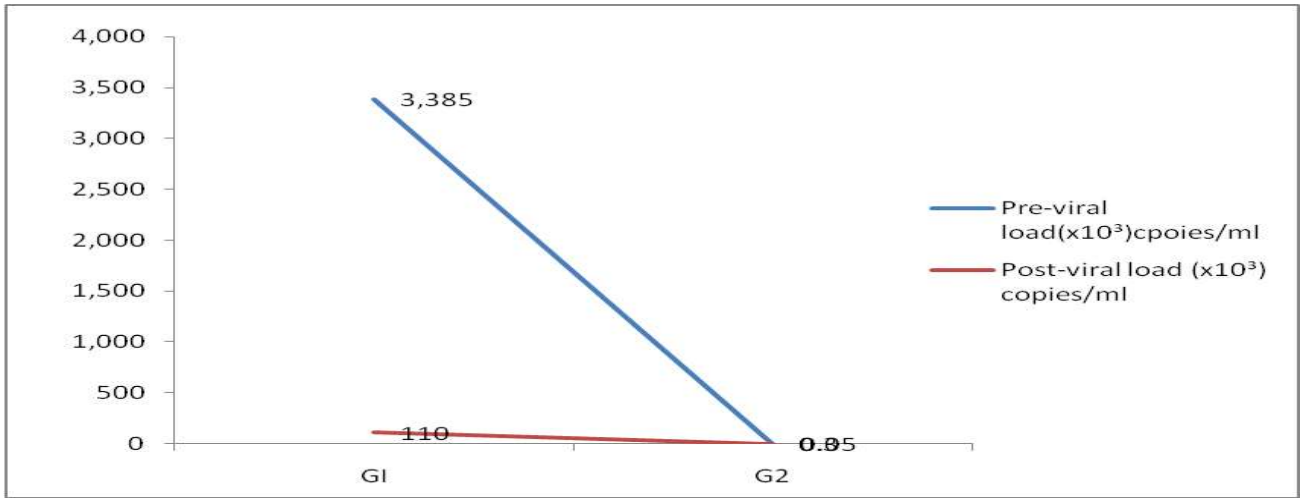

Fig.1: Viral load of 2 HIV patients on A Zam alone

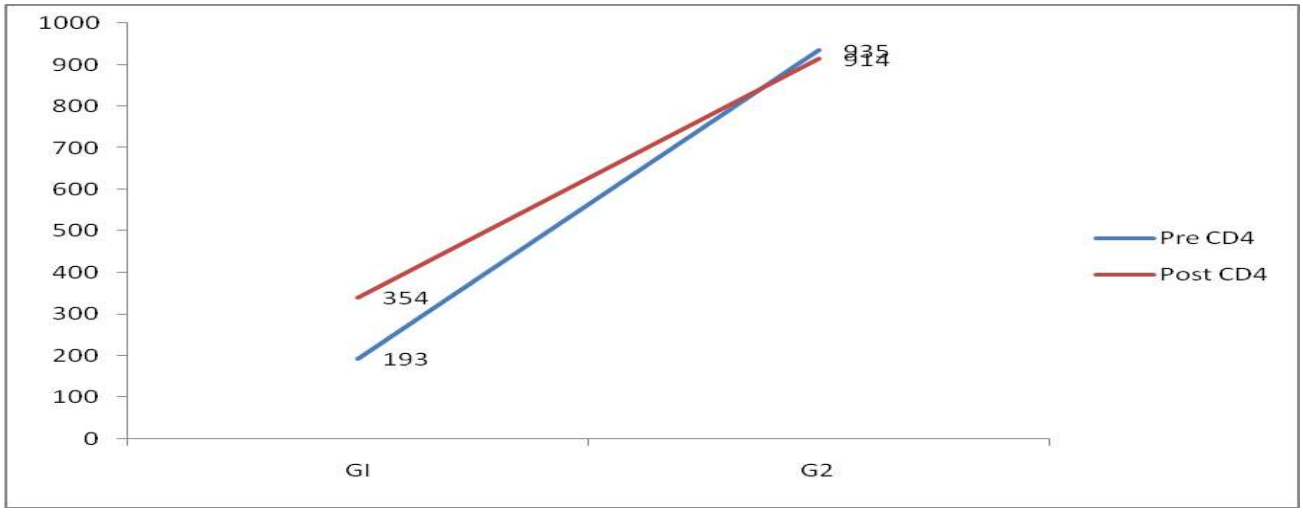

Fig. 2:CD4 count of 2 HIV patients taking A Zam alone

The viral load of HIV patients on A Zam alone as alternative therapy reduced significantly ($\alpha_{0.05}$) for the viral load remained undetectable but sero-positive to HIV antigen-antibody tests (ELISA, Unigold and

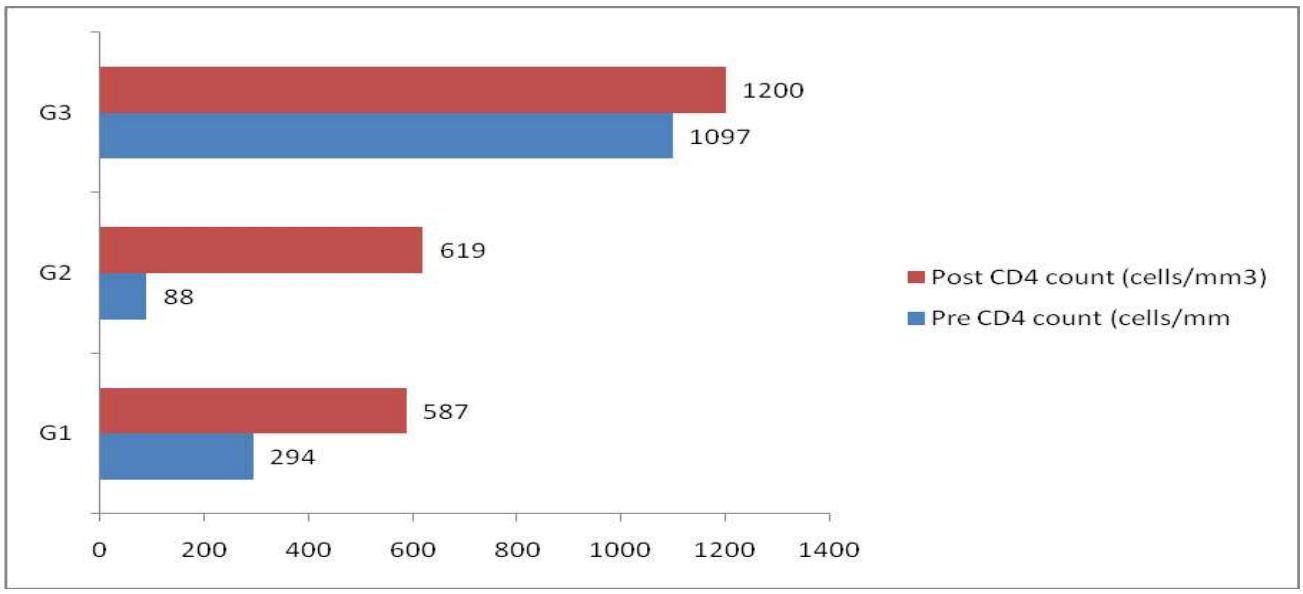

Fig.3: CD4 count of 3 HIV patients on Complementary therapy (HAART and A- Zam)

Determine). The CD4 count significantly increased ($\alpha_{0.05}$) in G2 (88 to 619) and G3 (294 to 587) cells/µL but not statistically significant increased in G1 (1097 to 1200) (figure 3).

There were variable responses in CD4 count and viral load of the 4 HIV patients that discontinued HAART and commenced A Zam therapy alone. There was gradual but not statistically significant ($P_{>0.05}$) decline in CD4 counts in all the 4 HIV patients at the 60th day (632.3±199) and 120th day (571.5±185) average CD4 counts before increasing at the 180th day (679 ±243) when compared with pre-treatment value (789.5±340).

The viral load in all the 4 HIV patients were significantly increased ($\alpha_{0.05}$) from undetectable (≤20) to average of 1±383x10^3 (60th day viral load after discontinuation of HAART and commencement of A -Zam therapy alone) and 4±754 x10^3 (120th day viral load after discontinuation of HAART and commencement of A Zam therapy alone). However, there was significant reduction ($\alpha_{0.05}$) at the 180th day viral load (0.5±24x 10^3) after discontinuation of HAART and commencement of A- Zam alone (table 1).

Table 1: 4 HIV patients that discontinued HAART with corresponding CD4counts and viral load

Pt ID	Pre CD4 (cells/ µL)	60th day CD4 (cells/ µL)	120th day CD4 (cells/ µL)	180th day CD4 (cells /µL)	Pre-Viral Load (copies/ml)	60th day Viral load (copies/ml) x10^3	120th day viral load (copies/ml) x10^3	180th day Viral Load (log copies /ml) x10^3
GI	1200±410	931±299	694±122	1165±486	20	85±299	8±75	1±23
G2	505± 285	363±269	202±370	346±333	20	101±283	2, 121±1363	4±21
G3	1059± 269	731±99	747±175	637±42	20	1,350± 966	900±142	92±68
G4	394±396	504±128	643±71	568±111	20	1±383	4±754	0.5±24
Mean	789.5±340	632.3±199	571.5±185	679±243	20	384±483	758±584	24±34

Discussion

The fact that HIV infection is a serious problem to the infected individual, relatives, community, nation and the World is not controversial. The problems associated with HIV infection are numerous with immense financial burden. It was estimated that about 19.1 billion dollars was donated as a grant to manage HIV infection in low and middle income countries in 2016 [2]. Each HIV infected patient contributes valuable time and resources to access health care delivery thus resulting in more

financial burden. Thus, average HIV patient is looking for a quick, effective and secretive way out of the HIV burden resulting in patronage to spiritual and herbal therapists.

Effectiveness of antiretroviral agents is based on suppression or inhibition of HIV replication in the host immune cells (CD4 antigen cells) and ability of the new un-infected CD4 antigen immune cells to perform expected normal immune functions. HAART effectiveness in HIV infection is therefore assessed by measuring significant reduction in viral load and increase in CD4 count. These two parameters (viral load and CD4 count) could significantly explain the magnitude of the HIV infection in patients although with limitations [26]. Although the mechanism of antiviral activities of A Zam has not been documented, it is evident that A-Zam reduced the viral load in HIV patients taking it alone as shown in figure 1. This means that A-Zam contained anti-HIV agents that may be similar in potency comparable with the components of HAART. The gradual reduction in viral load of these HIV patients can only be explained by effectiveness of active antiviral properties of the therapy that was commenced. This is in support of the earlier findings on A Zam that it is an effective anti-HIV agent [17,21,22,24].

HIV infection is associated with immune suppression because the virus infects, replicates and kills (lysis) the CD4 antigen expressing cells thus gradual reduction in immune cells that are involved in innate and adaptive immune responses. It is expected that with reduction in HIV replication, immunity should improve; which can be measured with circulating CD4 T cells (CD4 count). Despite sustained HIV replication suppression (undetectable viral load) with HAART, some patients had features of immune suppression with varying degree (eg plateau low CD4 count, skin lesions etc) because the agents (HAART) do not contain specific immune-stimulator [27,28]. Immune stimulatory potential of A Zam was manifested in HIV patients taking it with HAART (complementary therapy) as shown in Figure 3, where there was gradual significant increase in CD4 count. This might explain the wellness and increase in quality of life associated with A Zam when taking this therapy with HAART (complementary therapy). This is in support of the earlier findings that A-Zam demonstrated profound immune stimulatory effects in in-vitro immune cells [20].

There is no doubt that HAART is very effective in controlling HIV infection. However, the few reported cases of side-effects (e.g hepatotoxic, nephrotoxic etc) associated with HAART could lead to discontinuation of therapy. Discontinuation of HAART usually leads to progression of HIV replication cycle. Non inhibition of HIV replication in immune cells leads to immune cell lysis (immune cell reduction) and viraemia which may be associated with serious infections and disorders. Potential antiviral and immune-modulating effects of A-Zam in HIV patients that discontinued HAART was demonstrated in this study by absence of signs and symptoms that are normally associated with advanced HIV infection or when there is a sudden high viral load and low CD4 count (table 1).

Thus A- Zam antiviral potency manifested after discontinuation of HAART with significant reduction in viral load that was associated with increase in CD4 counts at 180th day after commencement of therapy. Because there was no evidence of infections during the period of drastic reduction in CD4 count and high level of viraemia in these four HIV patients in this study (table 1) confirmed the earlier studies that A Zam contained broad antimicrobial and immune stimulatory agents[19,20,29]. The results of this study in figure 3 and table 1 also confirmed the earlier reported studies that A-Zam probable mechanisms of actions are different form HAART thus accounting for the outcomes in HIV patients taking this herbal medicine therapy[17,19,20-24].

HIV resistance is one of the major problems with orthodox antiretroviral therapy. This led to the use of combination of antiretroviral agents termed HAART. Despite HIV patients' compliance with HAART, there have been reported cases of HIV resistance which has been a major concern. It is evident that both anti-HIV (Figures 1 and 2) and immune-stimulatory (Figure 3) agents are components of A Zam thus it is a potential therapeutic agent that could be used in HIV resistance and when there is discontinuation of HAART due to serious side effects (table 1).

Although few (9) HIV patients participated in this study, it is concluded that A- Zam herbal medicine is a potential antiviral and immune stimulatory agent for HIV infection and could be beneficial in patients with HIV resistance and serious side effects with HAART. The study is still in progress

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Reference

1. UNAIDS/WHO. ''UNAIDS fact sheet 2019'' pp1-6
2. UNAIDS/WHO. "UNAIDS Data 2017" pp. 1-3
3. UNAIDS/WHO. "UNAIDS fact sheet 2018" pp. 1-5.
4. WHO. Traditional Medicine; Growing Needs and Potential, WHO Policy Perspectives on Medicines. World Health Organization, Geneva. 2002; pp. 1–6.
5. Cos P, Maes L, Vlientinck A and Pieters L. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection- an update (1998-2007). Planta Medica 2008; 74: 1323-1337.
6. Onifade AA, Jewell AP and Okesina AB. Preliminary findings of are herbal remedies effective in HIV infection in Nigeria, Tropical Journal Health Sciences 2010; 17 (2): 51-55
7. De Clereq. Current lead natural products for the chemotherapy of human immunodeficiency virus infection. Medical Research Review 2000; 20: 323-349.
8. Kong JM, Goh NK, Chia LS and Chia TF. Recent advances in traditional plant drugs and orchids, Acta Pharmacologica Sinica 2003: 24: 7-21.
9. Dharmaratne HRW, Tan GT, Marasinghe GPK and Pezzuto JM. Inhibition of HIV-1 reverse transcriptase and HIV-1replicationbyCalophyllum coumarins and xanthones. Planta Medica 2002; 68: 86–87.
10. Watson K, Gooderham NJ, Davies DS, and Edwards RJ. Interaction of the transactivating protein HIV-1 tat with sulphated polysaccharides. Biochemica Pharmacologica 1999 ;57: 775–783.
11. Matthee G, Wright AD and König G. HIV reverse transcriptase inhibitors of natural origin. Planta Medica 1999; 65: 493–506.
12. Lu WB. A report on 8 seronegative converted HIV/AIDS patients with traditional Chinese medicine. Zhongguo Zhong Xi Yi Jie He Za Zhi Zhongguo Zhongxiyi Jiehe Zazhi 1997; 17 (5):271–273. Chinese.
13. Elujoba AA. Medicinal plants and herbal medicines in the management of opportunistic infections in people living with HIV/AIDS, Our experience so far, being a Guest lecture presented at the National Scientific Conference organized by the Nigeria Society of Pharmacognosy (NSP) at Zaria, Nigeria. 2005; pages: 11-12.
14. Abere TA and Agoreyo FO. Antimicrobial and toxicological evaluation of the leaves of Baissea axillaries Hua used in the management of HIV/AIDS. BMC Complementary Alternative Medicine 2006; 21; 6: 22.
15. Mbah AU, Udeinya IJ, Shu EN, *et al* Fractionated neem leaf extracts is safe and increases CD4+ cell levels in HIV/AIDS patients. American Journal Therapeutics 2007; 14 (4):369-374.
16. Ahmad A., Husain A., Mujeeb M., *et al*., A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific Journal Tropical Biomedicine 2013; *3*(5); 337–352.
17. Onifade AA, Jewell AP, and Okesina AB. Virologic and Immmunologic outcome of treatment of HIV infection with *herbal concoction, α-Zam*, among clients seeking herbal remedy in Nigeria. African Journal Traditional Complementary Alternative Medicine 2011; 8 (1): 37-44.
18. Yimer EM , Tuem KB, Karim A , Ur-Rehman N and Anwar F. Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Hindawi Evidence-Based Complementary and Alternative Medicine. Volume 2019, Article ID 1528635, 16 pages https://doi.org/10.1155/2019/1528635.
19. Oyero OG, Toyama M, Mitsuhiro N, *et al.* Selective inhibition of hepatitis C virus replication by alpha-zam, a *Nigella sativa* seed formulation. African Journal Traditional, Complementary & Alternative Medicine 2016; 13(6): 144-148.
20. Oyero OG, Onifade AA and Baba M. Immunomodulatory Potential of Herbal Formulations Containing Seeds of Nigella sativa Linn. African Journal Biomedical Research 2017; 20: 217- 221.
21. Onifade AA., Jewell AP, Ajadi TA, Rahamon SK, and Ogunrin OO. Effectiveness of herbal remedy in 6 HIV patients in Nigeria. Journal of Herbal Medicine 2013; 3 (3): 99- 103.
22. Onifade AA, Jewell AP, Okesina A B, *et al* 5-month herbal therapy and complete seroreversion with complete recovery in an adult HIV patient. Open Access Scientific reports 2012; 1; 124.
23. Onifade AA, Jewell A P and Adedeji WA *Nigella sativa* concoction induced sustained seroreversion in HIV patient. African Journal Traditional, Complementary & Alternative Medicine 2013; 10 (5): 332-335.
24. Onifade AA, Jewell A P, and Okesina AB. Seronegative conversion of an HIV positive subject

treated with *Nigella sativa* and honey. African Journal of Infectious diseases 2015; 9 (2):47 – 50

25. Onifade AA., Jewell AP, Okesina AB, *et al*. The Phytochemistry and Safety profiles of an herbal remedy α-zam used for treatment of HIV infection in Nigeria. Tropical Journal Health Sciences 2011; 18 (1): 40-45
26. Smurzynski M, Wu K, Benson CA, *et al*.. Relationship between CD4+ T-cell counts/HIV-1 RNA plasma viral load and AIDS-defining events among persons followed in the ACTG longitudinal linked randomized trials study. *Journal Acquired Immune Deficiency Syndrome* 2010;55(1):117-127
27. Engsig FN, Zangerle R, Katsarou O, *et al*. Long-term mortality in HIV-positive individuals virally suppressed for >3 years with incomplete CD4 recovery. Clinical Infectious Diseases 2014; 58(9):1312-1321
28. Moore RD and Keruly JC. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. Clinical Infectious Diseases 2007; 44(3):441-446
29. Onifade AA, Oladeinde. Antimirobial effects of *Nigella sativa* concoction: Herbal preparation for HIV infection in Nigeria. Archive Basic Applied Medicine 2014; 2 (3): 173 – 177

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Retarding progression of chronic kidney disease: a preliminary report of the use of oral bicarbonate therapy in a resource limited setting

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Abstract
Background; Chronic kidney disease is an established public health priority globally with gloomy outlook in developing countries due to constraints in resources. Acidosis is a major metabolic derangement in chronic kidney disease and its degree correlates with severity of renal failure and its amelioration has been shown to confer benefits by retarding the progression of the disease in a number of reports. We therefore set out to determine the effectiveness of sodium bicarbonate therapy in retarding progression to end-stage kidney failure.
Methodology: This was an open labelled, randomized prospective study of 75 patients with metabolic acidosis. They were randomly assigned to receive either thrice daily oral sodium bicarbonate tablets in the treatment group in addition to the routine medications, the controls were to have routine medications only. The patients' clinical and biochemical parameters were monitored monthly. The primary renal end points used were the rate of decline of the glomerular filtration and the presence of end stage renal disease.
Result: There were a total of 63 patients with completed results at the end of the study. They comprised of 32 subjects and 31 controls and they were aged between 18-68years. There was a modest increase in the creatinine clearance at the end of the study from 23.8 to 25.4ml/min for the treatment group.14 patients in the control arm progressed to end-stage renal disease while 4 patients amongst the subjects progressed to end stage kidney disease.
Conclusion: Sodium bicarbonate therapy is a potentially useful cost-effective medication in retarding progression to ESKD in developing countries.

Keywords:*Retardation, progression, pre-dialytic*

Abstrait
Contexte : L'insuffisance rénale chronique est une priorité de santé publique établie dans le monde avec des perspectives sombres dans les pays en voie de développement en raison de contraintes en ressources. L'acidose est un dérangement métabolique majeur dans la maladie rénale chronique et son degré est corrélé à la gravité de l'insuffisance rénale et son amélioration a été démontrée pour conférer des avantages en retardant la progression de la maladie dans un certain nombre d'articles. Nous avons donc cherché à déterminer l'efficacité de la thérapie au bicarbonate de sodium pour retarder la progression vers une insuffisance rénale terminale (IRT).
Méthodologie : Ceci était une étude étiqueté ouverte, prospective randomisée portant sur 75 patients atteints d'acidose métabolique. Ils ont été assignés au hasard pour recevoir trois fois par jour des comprimés de bicarbonate de sodium par voie orale dans le groupe de traitement en plus des médicaments de routine , les témoins devaient avoir des médicaments de routine uniquement. Les paramètres cliniques et biochimiques des patients ont été surveillés mensuellement. Les principaux paramètres rénaux utilisés étaient le taux de déclin de la filtration glomérulaire et la présence d'une maladie rénale en phase terminale.
Résultat : Il y avait un total de 63 patients avec des résultats complets à la fin de l'étude. Ils comprenaient 32 sujets et 31 témoins et ils étaient âgés de 18 à 68 ans. Il y avait une augmentation modeste de la clairance de la créatinine à la fin de l'étude de 23,8 à 25,4 ml / min pour le groupe de traitement. 14 patients du groupe témoin ont progressé vers une insuffisance rénale terminale tandis que 4 patients parmi les sujets ont progressé vers le stade terminal maladie du rein.
Conclusion : La thérapie au bicarbonate de sodium est un médicament potentiellement utile et rentable pour retarder la progression vers l'IRT dans les pays en voie de développement.

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Mots clés : *Retard, progression, pré- dialyse*

Introduction

Chronic kidney disease (CKD) is an established public health priority due to its global endemic nature [1]. Its prevalence in Nigerian adults has been reported to range between 10 and 18.8% [2,3]. Mortality rate in advanced clinical states in Nigeria, in the absence of an adequate renal replacement support, is very high, up to 100% in most cases [4,5].

Metabolic acidosis is a common complication of CKD and appears when the glomerular filtration rate falls below 25ml/min/1.73m^2 [6]. It manifests with a number of non-specific clinical features such as fatigue and exercise intolerance [7]. Acidosis approximately correlates with severity of renal failure and usually is more severe at lower glomerular filtration rate.

There are no local reports on the magnitude and actual prevalence of metabolic acidosis in pre-dialytic CKD patients in sub-Saharan Africa despite the increasing number of people with this condition. Administration of alkali either in the form of sodium bicarbonate, calcium citrate and sodium citrate have been found to reduce muscle degradation[8], improve albumin synthesis[9],reduce the progression of bone diseases[10] and retard progression to terminal stages in chronic kidney disease [11-15]. Delaying time to end stage renal disease is worth serious consideration and will certainly be of great benefit with the use of bicarbonate, even though the evidence level is low (2B).

The burden of renal replacement therapy in sub Saharan Africa is quite enormous, infrastructures are equally grossly inadequate and the cost is barely affordable by an insignificant proportion of people with end stage kidney failure. It is envisaged that with the wide use of low cost therapy such as sodium bicarbonate, most especially as an adjunct therapy to standard treatment in developing countries, will slow the rate of progression to end stage kidney failure in a majority of patients. We therefore set out to determine the capacity of sodium bicarbonate therapy in retarding progression of CKD.

Methodology

The study protocol was approved by the Ethics and Research committee of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria.*(International Registration number IRB/IEC/ 0004553 and RCT protocol number of ERC/2012/ 03/09)*. Ethical approval was given on 26/3/2012. The study was also adherent to the declaration of Helsinki.

This was a single centre, randomized, open labeled prospective study conducted over a time period of 24 months from April 2012 to April 2014.

The calculated minimum sample size for this study was determined using Fisher' formulae for sample size estimation. The statistical assumptions were a type 1 error rate of 5%, 14 % prevalence of the condition and a precision of ± 5 points. The calculated minimum sample size was 75 patients.

Using block randomization method in which every block of four alternate CKD stage 4 patients with metabolic acidosis were recruited into each group. They were further matched for age, sex and diagnosis.

The 75 consenting adults (38 treatment arm and 37 controls) above the age of 18years in stage 4 CKD with established metabolic acidosis with serum bicarbonate between 16mmol/l and 21mmol/l (measured on two consecutive occasions and at 2 weeks interval), and in stable clinical condition -were enlisted for the study.

The prevalent cause of CKD was established based on a set of general definitions in addition to some other established clinico-pathological indices as itemized below;

Hypertension induced CKD was diagnosed in patients 40years or older with history of hypertension in the previous 5 years, with cardiovascular evidence of long standing hypertension using clinical, bedside and ancillary parameters [16].

Chronic glomerulonephritis was diagnosed based on a history of age less than 35 years, with a history of acute or post infectious glomerulonephritis or nephrotic syndrome as evident by suggestive biochemical parameters [16]

Diabetic nephropathy was diagnosed based on history of diabetes mellitus for at least 5 years, diabetic retinopathy, proteinuria, hypertension, and anemia [16].

Autosomal dominant polycystic kidney disease was diagnosed on the basis of ultrasound confirmed bilateral multicystic kidneys (with more than 3 cysts in each kidneys)[16].

End stage kidney failure in this study was defined by the estimated glomerular filtration rate (eGFR) less than 15ml/min/1.73m^2 plus bilaterally shrunken kidneys,(except in cases of Autosomal Dominant Polycystic Kidney Disease) on ultrasound scan, presence of clinical features of uremia (such as hypertension, anemia and bone disease) and the need for dialysis [16].

Acute kidney insult in the course of the study was defined as an acute deterioration in renal function with a percentage increase in serum creatinine of > 300% from a stable three-month baseline in a patient with advanced CKD or commencement of acute renal replacement therapy.

Exclusion criteria included patients with resistant hypertension (Blood pressure > 150/100mmHg) despite the use of at least three anti-hypertensive (including a diuretic), individuals with biventricular heart failure, insulin dependent diabetic patients and pregnant patients. Patients with lupus nephritis, sickle cell nephropathy and HIV associated nephropathy were also excluded.

The first group of 38 treatment group participants were placed on a typical starting dosage of 0.5-1*Meq/kg/day* i.e 600mg thrice daily dose of sodium bicarbonate tablets (Zanza Labs, UK) in addition to other routine medications such as anti-diabetics (as indicated), hematinics, anti-hypertensives and anti-dyslipidemic agents, while the second group of 37 controls were on the same routine therapy, as above but without bicarbonate treatment.

Using a structured proforma, a detailed baseline biodata of the participants was noted and this included age, gender and the primary diagnosis. Their baseline anthropometric parameters were also documented.

They were followed up once every month with their heamogram and biochemical parameters (serum sodium, potassium, bicarbonate, creatinine, serum proteins; albumin and globulin- and 24 hours urine quantification of the protein levels and creatinine clearance estimation) in the pre-dialytic outpatient clinic over 24 months.

The serum creatinine was determined by the modified Jaffe kinetic reaction using the creatinase enzymatic method; the bicarbonate was determined using the ion selective electrode with direct pH measurement; urea with the diacetylmonoxime reaction, serum albumin with the dye binding method using bromocresolgreen, calcium with the 0- cresolphthalein-complexone method and the phosphate with the acidified molibdate method.

The subjects and the control arms were all placed on similar anti-hypertensive medications which included a combination of angiotensin converting enzyme inhibitors or angiotensin receptor blockers with a non dihydropyridine calcium channel blockers and loop diuretics. A further addition of minoxidil, for a limited period, was made in some cases.

Both groups were placed on a dietary regime of protein diet of 0.75-0.9g/kg body weight of high biological value, calorie diet of 30-40kcal/kg and sodium chloride (salt) of less than 2g/day.

The nutritional compliance was assessed by a renal dietician using a 24 hour dietary recall, by obtaining written dietary diaries every 3 months and by calculating the normalized protein equivalent of nitrogen appearance (*nPNA*), expressed in g/kg body weight/24hours[17]. Food models were also used to estimate sizes and proportions and to enhance compliance. A local adaptable standard food table consisting of peculiar local staples was used to estimate the nutrient composition.

Serial anthropometric parameters were measured over the same time period. These included the body weight (in kilogram), triceps skin fold thickness, and the mid-arm circumference using the Harpenden professional skin fold calipers (Health check systems Inc, NY).

The mid arm circumference was used as a measurement of the muscle mass and was measured to the nearest 0.1cm with a flexible steel tape which was placed gently but firmly around the arm. It was then measured at the mid point between the chromium and the olecranon process [18].

The mid arm muscle circumference was thus calculated as; MAMC (cm)= mid arm circumference (cm) -0.314 x triceps skinfold thickness (mm).

The tricep fold thickness was measured using a point on the posterior surface of the upper arm located the same area as the marked mid point for the upper arm circumference. A fold of skin and subcutaneous adipose tissue is grasped gently with the thumb and fingers approximately 2cm above the marked level with the skinfold parallel to the long axis of the arm. The jaws of the calipers were placed at the marked level, perpendicular to the length of the fold, and the skinfold thickness is measured to the nearest 0.1mm while the fingers continue to hold the skinfold [19].

The safety profile of sodium bicarbonate was periodically evaluated based on established adverse effects such as bloating, vomiting, peripheral oedema, metallic taste and worsening of hypertension.

The study was discontinued in participants in either group who had features of acute kidney failure or who manifested symptoms of uremia. They were further offered acute heamodialysis. The data collected were coded and later de-identified and analyzed using the Statistical Package of Social Statistics version 18.

Data was summarized using mean, range, standard deviation and proportions.

Both the mean initial and terminal creatinine clearance values were compared within group using the student's t-test; the evaluation of the rate of functional progression with a regression analysis of the relationship between the creatinine clearance over time. The estimate of the slope of the regression was further tested against a null hypothesis of a zero slope. The two slopes were then compared to each other. The numbers reaching end-stage renal disease as defined, at time points of 6, 12, 18, and 24 months respectively were also noted using the *Kaplan meir* statistics.

The nutritional parameters such as the serum albumin, the anthropometrics such as the mid upper arm circumference and biochemical parameters such as serum sodium, urea, potassium, bicarbonate and 24hour urinary protein excretion profile were compared before and after therapy and also between the subjects and the controls using the chi square for categorical variables and the students't test for the continuous variables.

The normalized protein nitrogen appearance (*n*PNA) was determined at every point serially and compared before and after and also between each groups. The level of significance was set at p value of 0.05.

The outcome measures used were the rate of decline of the glomerular filtration rate, presence of end-stage renal disease as earlier defined, changes in some markers of kidney damage such as urinary protein excretion, changes in the nutritional parameters such as the anthropometrics, serum albumin, and also mortality.

Results

A total of 750 patients in different stages of chronic kidney disease were seen at the out patient clinics (General medical practice, general nephrology, pre dialytic and pre transplant clinics) in the 2 year study period.

102 patients were in stage 4 using the Kidney Disease: Improving Global Outcomes (KDIGO) classification, while 90 stable patients had chronic metabolic acidosis (serum bicarbonate level of less than 22 mmol/l, repeated over a 2 week period).

75 of these were found eligible and recruited for this study over the study period, they were further divided into 2 groups of 38 treatment arm and 37 controls, 5 patients were lost to follow up in the course of the study, 6 patients had some other severe cardiovascular complications and could not complete the study, 1 patient became pregnant and was immediately withdrawn from the study.

We thus had completed data for 63 patients (31 Controls and 32 treatment arm) at the end of the study. Their age ranged between 18 and 68 years, with 37 males.19 patients had type 2 diabetic nephropathy as the primary aetiology, 19 had hypertensive nephropathy, 22 patients had chronic glomerulonephritis, 1 patient had autosomal polycystic kidney disease while 2 patients were unclassified.The baseline parameters are as depicted in table 1 & 2.

Table 1: Baseline Characteristics of study participants

Variable	Control	Treatment	P-value
Age (Mean's SD)	46.7(12.13)	50.7(13.4)	0.261
Gender (Frequency %)			
Male	18(72.0)	19(65.5)	0.609
Female	3(28.0)	13(34.5)	
Diagnosis			
ADPKD	10(0.0)	1(3.5)	
CGN – CKD	11(40.0)	11(37.9)	
DM – CKD	9(24.0)	11(24.1)	
HTN – CKN	10(36.0)	10(34.5)	

ADPKD - Autosomal dominant polycystic kidney disease
CGN - Chronic glomerulonephritis
DM - Diabetes Mellitus (Type 2)
HTN - Hypertensive nephropathy
CKD - Chronic glomerulonephritis

Table 2: The biochemical profile in both treatment arms and controls .

Mean parameters	Control		Subject		P value
	Initial	Terminal	Initial	Terminal	
Serum bicarbonate mmol/I	20.8	20.5	20.5	22.3	0.24
Serum urea mmol/I	14.2	12.2	12.6	12.7	0.34
Serum creatinine µmol/I	345.6	491.6	283.2	391.7	0.2
Serum sodium mmol/I	134	133	135	137.5	0.2
Serum potassium mmol/I	3.6	3.9	4.1	4.4	0.33
Serum albumin g/dl	31.8	31.3	33	35.1	0.06
24hour protein g/day	0.9	1.14	1.25	1.47	0.07
Creatinine clearance ml/ml	22.1	18.4	23.8	25.4	0.045

Table 3: Packed cell volume comparison between the subjects and the controls.

Study group		Mean	N	Standard deviation	Correlation	Significance
Subjects	PCV	Initial	25.78	3.22	0.846	0.000
	PCV	Final	26.81	4.18		
Controls	PCV	Initial	26.26	4.15	0.926	0.000
	PCV	Final	24.6	5.04		

Table 4: Sub group comparison and analysis of GFR between diabetic subjects and others on bicarbonate.

	Total	GFR <15ml/m	Percentage (%)
DM subjects on bicarb.	11	1	9.1
Other patients on bicarb.	21	3	14.28
Total on bicarb	32	4	23.38
Paired sample t test, P<0.05			

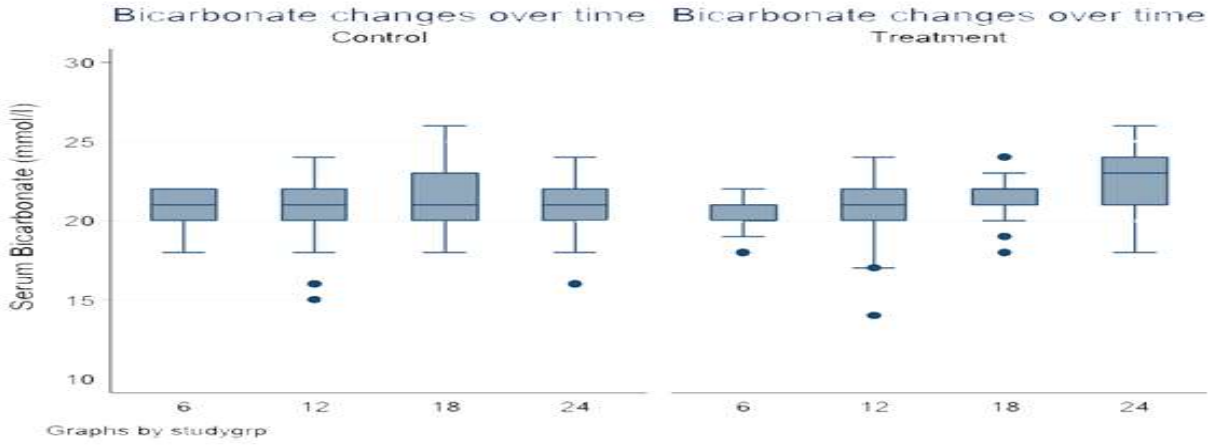


Fig. 1

There was a noticeable rise in the serum bicarbonate in the treatment group. From a mean value of 20.5mmol/l at commencement of the study to 22.3mmol/l at the end of the study amongst the treatment group as compared with 20.8mmol/l at commencement and 20.5mmol/l amongst the controls after 24 months. (Figure 1)

months, 18.7ml/min at 16 months, 18.4ml/min at 24 months. (P value of 0.028). (Figure 2).

The serum albumin level was used as a surrogate of nutritional level and there was a noticeable increase from a mean value of 33g/dl at baseline to 35.1g/dl for the treatment group at the end of the study. (p value of 0.047). (Figure 3)

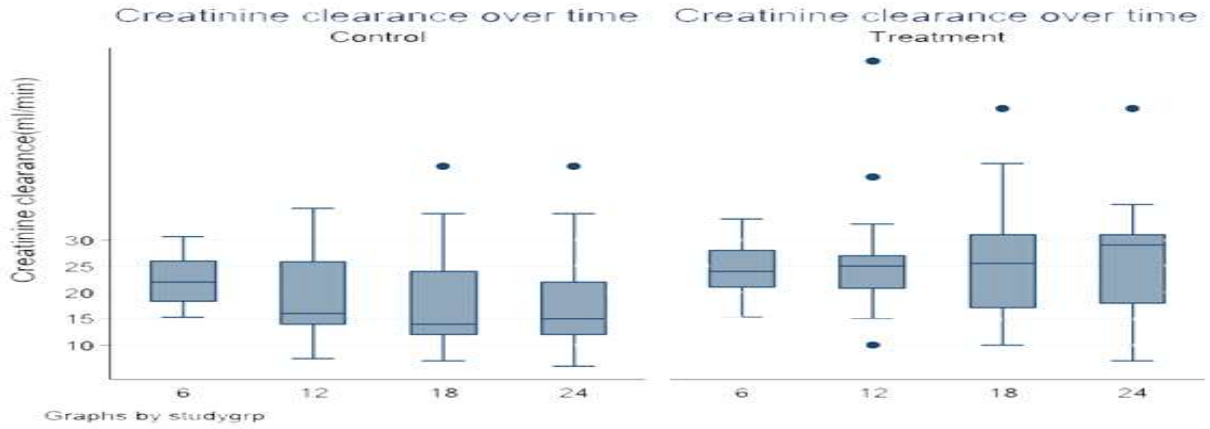


Fig. 2

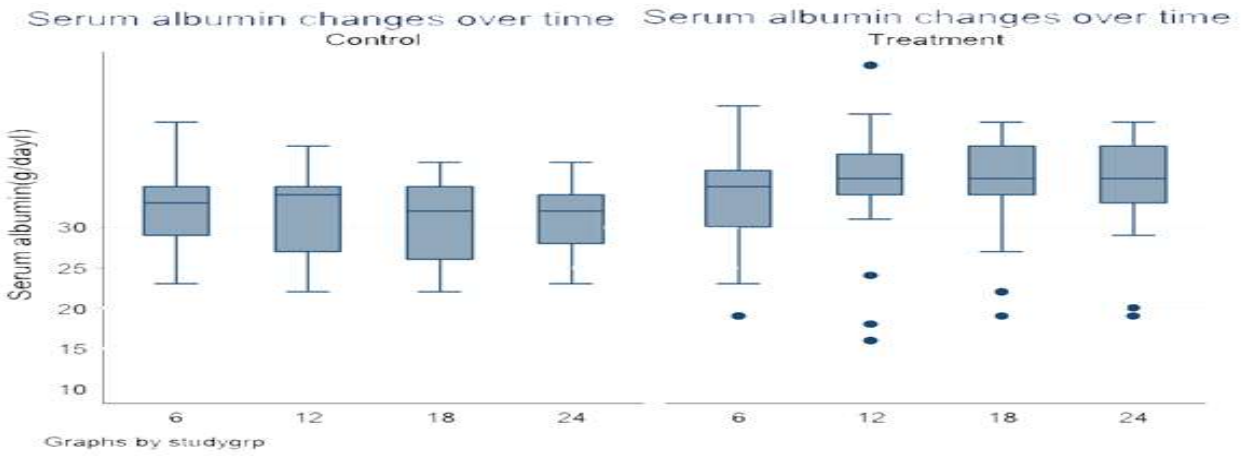


Fig. 3

There was a moderate increase in the mean creatinine clearance in the treatment arm, from 23.8ml/min at T_0, to 25.5ml/min at 8 months, 25.6ml/min at 16 months, and 25.4ml/min at 24 months as compared with the controls whose mean serum creatinine clearance dropped from 22.1ml/min at 8 months, 18.7ml/min at 8

Protein excretion levels using the 24 hour protein estimation was slightly increased among both the treatment group and the controls from 1.25g/24hours at the baseline to 1.47g/24 hours for the former and from 0.90g to 1.14g/24hours amongst the controls at the end of the study. (Figure 4).

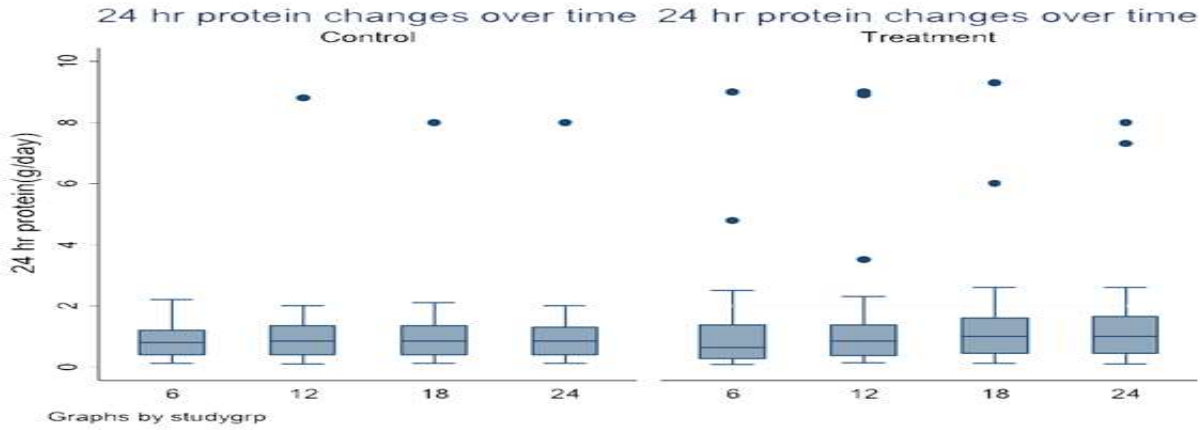
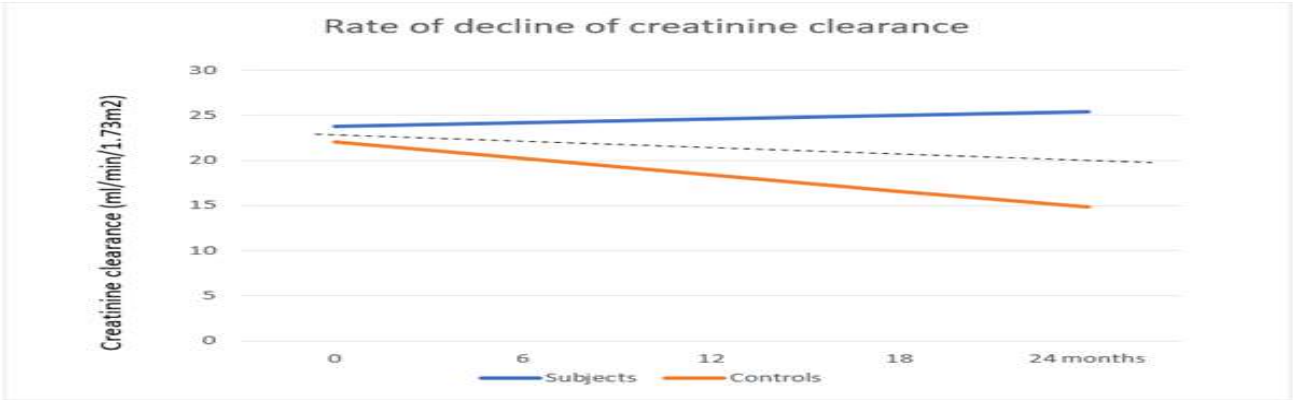


Fig. 4

Fig. 5



Patients at risk	0 month	6months	12months	18months	24months
Control group	37	36	32	28	20

Fig. 6

There was a slight significant improvement in the mean heamatocrit levels in the treatment group from an initial value of 25.7% to 26.8%, compared with the controls who had a reduction from 26.2% to 24.9% this was significant using paired sample t test analysis (Table 3).

Kaplan Meir analysis showed a higher summative probability of commencing dialysis in the control compared with the treatment group. Log rank test comparing the Kaplan Meier estimates: $chi^2 = 6.42$; p-value= 0.011. (Fig 5)

At the end of the study, it was observed that 14 patients out of 32 controls progressed to end stage kidney failure while 4 out of 31 in the treatment arm had progressed. (95% confidence interval of 0.03 to 1.12; P value of <0.05). (Fig. 6)

Only 1 patient amongst the treatment arm reported of a transient episode of severe bloating.
No other adverse drug reaction (to bicarbonate) was reported at each follow up.

Discussion

In this single center study done over a period of 24 months, the prevalence of metabolic acidosis in stage 4 pre-dialytic Chronic Kidney disease patients is 12%. This is much lower than the 19% cited in the United States NHANES study [20]; the difference can possibly be explained by the consumption of a typical *western* diet in the NHANES study population, a diet which is often high in animal protein with a rich sulfur containing amino acids [21]. The typical tropical diet which seems to be qualitatively less acidic with more leafy vegetables, fibres, and fruits might either be protective or the pattern of disease progression in our pre-dialytic patients runs an indolent course whereby a number of our patients present at the terminal stage.

Reduced albumin synthesis is often a typical feature seen in metabolic acidosis as a result of reduced intake, reduced protein synthesis, increased protein breakdown and enhanced amino acid oxidation. A post hoc analysis of the National Health and Nutritional Survey (NHANES III) has shown a graded relationship between serum bicarbonate and metabolic acidosis, and that the odds of having hypoalbuminemia increases substantially when serum bicarbonate was <22mmol/l[22]. There was, among the treatment group on bicarbonate tablet, a significant increase in serum albumin, which was used as a surrogate for nutritional status, over the study period from 33mg/dl to 35mg/dl (p<0.05). *De Bristo* had previously demonstrated that bicarbonate therapy reduces protein catabolism in parallel with increased serum albumin and lean body mass in non-dialysis patients with CKD [14].

Increased protein catabolism also leads to an activation of the adenosine triphosphate (ATP)-dependent ubiquitin-proteasome system due to increased gene transcription and loss of lean muscle mass specifically the skeletal muscles. This may be ameliorated by alkali therapy. In this study, there was a significant increase after 24 months in the mid arm muscle circumference (MAMC) among the treatment arm from mean of 22.3cm to 24.6 ±2.86 (p<0.05) compared to the controls of 20.4cm who had a reduction from an initial mean of 21.7cm. It should be noted that we do not have a standard means of comparing normal anthropometric standards for adults in our setting.

Using the normalizes protein equivalent of total nitrogen appearance (calculated using a kinetic modeling formula for CKD not on dialysis) [17] as a surrogate for nitrogen balance, there was a significant decrease in the nitrogen appearance from 1.04g/d to 1.00g/d in the treatment arm compared with the controls (0.833 to 0.822g/d) despite comparably similar dietary regime in both groups. As also reported by De-Bristo *et al*, nitrogen balance improved when the plasma bicarbonate concentration was corrected with alkali supplements [14].

As demonstrated in previous studies [12-14], bicarbonate therapy in this report improved survival over a time period of 2 years as fewer patients progressed to ESRD which was set at creatinine clearance of less than 15ml/min. One of the mechanisms thought to be contributory to this is the fact that sodium bicarbonate reduces both intra-tubular complement activation and tubulointerstitial injury and thus ameliorating further production of endothelin and ultimately slowing progression in chronic kidney disease [23]. In this study, 14 patients out of 32 controls progressed to end stage kidney failure while 4 out of 31 in the treatment group has progressed to end stage.

Contrary to reports in animal and human studies by Gadola, Nath and Phistikul respectively [13,23,24], showing reduction in urinary protein excretion rates with the use of alkali in metabolic acidosis. We observed that proteinuria in our patients at the end of the study did not improve in spite of the other benefits seen. The protein excretion levels using the 24 hour protein estimation was slightly increased in both groups from 1.25g/24 hours at baseline to 1.47g/24 hours for the treatment arm and from 0.90g to 1.14g/24hours amongst the controls even though the mean differences is not significant as also shown in a similar work by De-bristo.

It could be inferred that most of our patients had progressive nephropathies which typically manifest as glomerular proteinuria, also the use of bicarbonate is not to influence the nephritic process as the heamodynamics seems to be mostly affected. A longitudinal study over a longer period of time with a larger population using the albumin/creatinine ratio might offer a definitive assertion on the status of proteinuria in chronic kidney patients on alkaline therapy.

In a sub-analysis of the diabetic nephropathy subjects in this study (Table 4) , a sizable proportion of diabetic in the treatment group had improved creatinine clearance with the bicarbonate therapy compared with the non diabetics in the same group (9% vs 14.28%). There have been a suggestion of an efficient extra renal generation of buffers that exist in patients with diabetes mellitus. It is also plausible that keto acid anion might be the source of this extra renal bicarbonate generation further fueling the theory of an excess generation of bicarbonate blood buffers in diabetics [25]. This might be of some therapeutic value in delaying progression in pre-dialytic diabetics in view of the rising incidence of diabetic nephropathy in developing countries and further studies are warranted in this area.

The slight elevation in the haematocrit in the interventional arm could be due to the concomitant elevation in the GFR as a result of the use of recombinant human erythropoietin in addition to other standard therapy to retard progression and the test medication[26][27]. Finally there was not much change in the blood pressure and side effect profile as also reported in similar studies as the mean blood pressure was comparably the same in both groups over the study period [20]. Only a negligible proportion of the subjects had intolerable side effects.

In conclusion, in view of the cost effectiveness, the ability to improve anthropometrics and nutritional parameters and the limited side effect profile, sodium bicarbonate therapy in addition to other treatment modalities such as control of hypertension and diabetes and effective treatment of anemia and dyslipidemia, might be a veritable adjunct option to retard progression to end-stage renal disease in pre-dialytic CKD patients in developing countries.

Further studies including double blinded multi-centre studies with larger sample sizes are needed in resource-constrained settings to justify its wide spread use in addition to other adjunct treatment.

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References

1. El Nahas AM, Bello AK: Chronic kidney disease: the global challenge. Lancet 2005;365:331-340
2. Oluyombo R, Ayodele OE, Akinwusi PO *et al.* A community study of the prevalence, risk factors and pattern of chronic kidney disease in Osun State. Nigeria. WAJM 2013. 32(2):86-92
3. Akinsola W, Odesanmi WO, Oguniyi J *et al*. Disease causing renal failure in Nigerians- a prospective study of 100 cases. Afr J Med Med sci 1989; 18 : 131-137
4. Bamgboye EL. End stage renal disease in Sub Saharan Africa. Ethn Dis 2006; 16 (2 suppl 2) : S2-S9
5. Alebiosu CO, Ayodele OO and Abbas A. Chronic renal failure at the Olabisi Onabanjo University Teaching , Sagamu, Nigeria. African health sciences 2006; 6(3): 132-138.
6. Bailey JL. Metabolic acidosis: An unrecognized cause of morbidity in the patient with chronic kidney disease. Kid Int 2005: (S96); pp S15-S23
7. McSherry E and Morris RC Jr: Attainment of normal stature with alkali therapy in infants and chidren with classic renal tubular acidosis. J Clin Invest 1978; 61: 509-527.
8. Pickering WP, Price SR, Bircher G *et al*. Nutrition in CAPD: serum bicarbonate and the ubiquitin-proteasome system in muscle. Kidney Int. 2002; 61: 1286-1292.
9. Movilli E, Zani R, Carli O *et al*. Correction of metabolic acidosis increases serum albumin concentrations and reduces kinetically evaluated protein intake in heamodialysis patients: a prospective study. Nephrol Dial Transpl 1998; 13:1719-1722.
10. Bushinski DA; The contribution of acidosis to renal osteodystrophy. Kidney Int 1995;47: 1816-1832.
11. Lyon DM, Dunlop DM and Stewart CP: The alkaline treatment of chronic nephritis. Lancet 1931;2 :1009-1013.
12. Mahajan A, Simoni J, Sheather SJ *et al*. Daily oral sodium bicarbonate preserves glomerular filtration rate by slowing its decline in early hypertensive nephropathy. Kidney Int 2010; 78 (3): 303-309.

13. Gadola L Noboa O, Marquez MN *et al.* Calcium citrate ameliorates the progression of chronic renal injury. Kidney Int. 2004; 65:1224-1230.
14. Brito- AshurstId, Varagunam M and RafteryMJ. Bicarbonate supplementation slows progression of CKD and improves nutritional status. J Am Soc Nephrol 2009; 20:2075-2084.
15. Susantitaphong P, Sewaralthahab K, Balk EM *et al*. Short and long term effects of alkali therapy in Chronic Kidney Disease: A systematic Review. Am J Nephrol 2012; 35:540-547.
16. Alebiosu CO and Ayodele OE. The increasing prevalence of diabetic nephropathy as a cause of ESRD in Nigeria. Tropical Doctor 2006; 36:218-219.
17. McCain L. Pocket guide to the nutritional assessment of the patient with chronic kidney disease. 5th Ed. National kidney foundation, New York,NY
18. Blumenkrantz MJ, Kopple JD, Gutman RA *et al*. Methods for assessing nutritional status of patients with renal failure. The Am J of clinical Nutritional. 1980;33: 1567-1585.
19. Elhafiz EM, Osman EM, Elham G anthropometric evaluation of nutritional status for patients with end stage renal disease in Sudanese patients. SJMS 2011;6: 1;89-92.
20. Coresh J, Astor BC. Greene T *et al.* Prevalence of chronic kidney disease and decreased kidney function in the adult US population. Third National Health and Nutrition Examination Survey. Am J Kidney Dis 2003;41:1-12.
21. Di Iorio BR, Di micco L, Marzocco S *et al*. Very low protein (VLPD) reduce metabolic acidosis in subjects with CKD: The 'nutritional light signal' of the renal acid load. Nutrients. 2017 Jan 17;9(1)
22. Smith E, Nieto FJ and Crespo CJ. Estimates of animal and plant Intake in US Adults: Results from the Third National Health and Nutrition Examination Survey. 1988-1991. J Am Diet Assoc 1999; 99(7):813-820.
23. Nath KA, H ostetter MK and Hostetter TH: Pathophysiology of chronic tubulointerstitial disease in rats: Interactions of dietary acid load, ammonia, and complement component C3. J Clin Invest. 1985;76: 667-675.
24. Phisitkul S, Khanna A, Simoni J *et al*. Amelioration of metabolic acidosis reduces urine parameters of kidney injury including tubuinterstitial injury and slows estimated GFR preservation. Kidney Int 2010; 77 (7): 617-23
25. Cararaca F, Arrobas M, Pizaro JL and Esparrago JF. Metabolic acidosis in advanced renal failure: differences between diabetic and non diabetic patients. Am J Kidney Dis 1999; 33:892-898.
26. Kleinmanyk S, Schweitzer SU, Perdue ST *et al*. . The use of recombinant human erythropoietin in the correction of anemia in predialytic patients and its effect on renal functions; a double blind, placebo controlled trial. Am J Kidney dis 1989; 14:486-495.
27. Kuriyama S, Tomonari H, Yoshida H *et al*. Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in non diabetic patients. Nephron 1997; 77:176-185.

Erratum

The name of one of the authors in the paper: *Pain perception and patients satisfaction with pain management among Caesarean Section patients in Oyo State, Nigeria* in our publication Vol. 49, No. 1, 2020; 103-112 was wrongly written as E.O. Ojo **instead of I.O. Ojo.**

Also the name of one of the authors in the paper: *Serological markers of HBV infection: a community-based study of urban dwellers in Southwest Nigeria* in our publication Vol. 49, No.1, 2020; 207-213 was wrongly written as S.B. Bakarey in the Corresponding address **instead of A.S. Bakarey**.

The errors are regretted.

Editor-in-Chief