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Nutritional status, chronic diseases and healthy food choices

Chronic diseases are usually associated with weight loss (cachexia) which can be adduced to various reasons. Of interest in this issue of the journal is the article by Ogunkunle and others highlighting an unusually high frequency of stunting and malnutrition in over 70% of the 151 children diagnosed with acyanotic and cyanotic congenital heart diseases based on echocardiography with Doppler and color flow mapping. Almost two thirds of the children were underweight with 57% stunted and 37.7% had wasting.

When such children develop heart failure, the reasons for stunting include: abdominal distension resulting in early satiety, fatigue associated with feeding, liver congestion with impaired metabolism and nutrient absorption. It thus becomes important that nutritional assessment should be included in the management of children with congenital heart diseases even before they decompensate and present in heart failure followed by counseling to ensure early corrective interventions as advocated by the authors. An appropriate next step would be to determine changes in muscle mass in these individuals and relate this to stunting to either sarcopenia which is more commonly associated with aging [1] or loss of subcutaneous fat. This would be an interesting line of research as well as a search for other biomarkers.

Dietary advice is an essential component in the prevention and management of chronic diseases including cerebrovascular diseases, hypertension, chronic kidney disease and diabetes mellitus. In this issue of the journal, Akinlade and others reported that the consumption of wheat meal was associated with low glycaemic and insulin index when compared with yam flour paste (amala) and cooked cowpea. This has translational implication because whole, unprocessed wheat is available as staple meal in many homes and it would seem to be an appropriate choice in the prevention and management of diabetes mellitus. The time is right for us to have a table of the glycaemic indices, insulin indices and glycaemic load of most of the traditional meals available in Nigeria so that patients can make informed choices on what to eat to prevent chronic diseases.

Reference.

1. Di Tano G, Fulle S, Pietrangelo T, Bellomo R and Fanò G. Sarcopenia: char-acteristics, genesis, remedies. *Sport Sci Health*. 2005;1(2): 69–74.

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Knowledge and attitude of doctors in a Nigerian tertiary hospital to the health effects of climate change: Policy implications

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Abstract

Introduction: Globally, physicians are expected to understand the threats posed by climate change and advocate for strong mitigation and adaptation strategies. This study was therefore designed to assess the knowledge and attitude of doctors at the University College Hospital Ibadan to the health effects of climate change as well as the policy implications of the findings.

Methods: A descriptive cross-sectional survey was conducted among 423 physicians who were grouped by cadre into house officers (82), medical officers (28), resident doctors (275) and consultants (38). A stratified random sampling technique was used to select respondents from the different physician cadres in the hospital. A semi-structured self-administered questionnaire was used to collect information. Knowledge and attitude were assessed using 44-point and 40-point scales respectively. Knowledge score <22 was regarded as poor. Similarly, attitude scores <25 were regarded as poor. Chi-square test and t-test were used to analyse the data at 5% level of significance.

Results: Mean age of respondents was 35.8±9.3 years, 59.3% were female. More than half (54.1%) had practiced for ≥6 years. Almost all (97.0%) were aware of climate change and the commonest source of information was television (81.1%). More than half of respondents (55.8%) had good knowledge of climate change; similarly, about half (53.7%) had good attitude towards climate change. Respondents' characteristics that were significantly associated with good knowledge and attitude included age ≤29 years, male gender, being currently unmarried, being a resident doctor and practice for ≤5 years.

Conclusion: Climate change issues should be integrated more into the continuing medical education of physicians in order to improve their knowledge and attitude to climate change matters as well as their capacity to participate in its policy analysis.

Keywords: *Climate change, Physicians, Policy analysis, Nigeria*

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Résumé

Contexte: Globalement, on s'attend à ce que les médecins comprennent les menaces posées par le changement climatique et préconisent des stratégies d'atténuation et d'adaptation fortes. Cette étude a donc été conçue pour évaluer les connaissances et l'attitude des médecins du Collège Hospitalier Universitaire Ibadan sur les effets sur la santé des changements climatiques ainsi que sur les implications politiques des résultats.

Méthodes: Une étude transversale descriptive a été menée auprès de 423 médecins qui ont été regroupés en officiers de maison par des cadres (72), des médecins (28), des médecins résidents (275) et des consultants (38). Une technique d'échantillonnage aléatoire stratifié a été utilisée pour sélectionner les répondants des différents cadres médicaux de l'hôpital. Un questionnaire semi-structuré auto-administré a été utilisé pour recueillir des informations. La connaissance et l'attitude ont été évaluées en utilisant des échelles de 44 points et 40 points respectivement. Le score de connaissance <22 était considéré comme médiocre. De même, les scores d'attitude <25 ont été considérés comme médiocres. Le test du Chi-carré et le test t ont été utilisés pour analyser les données à un niveau de signification de 5%.

Résultats: L'âge moyen des répondants était de 35,8 ± 9,3 ans, 59,3% étaient des femmes. Plus de la moitié (54,1%) avaient pratiqué pendant ≥6 ans. Presque tous (97,0%) connaissaient bien le changement climatique et la source d'information la plus commune était la télévision (81,1%). Plus de la moitié des répondants (55,8%) connaissaient bien le changement climatique; De même, environ la moitié (53,7%) avait une bonne attitude face au changement climatique. Les caractéristiques des répondants qui étaient significativement associés à une bonne connaissance et à une attitude incluaient l'âge ≤29 ans, le sexe masculin, n'étant pas célibataire, étant un médecin résident et pratiquaient pendant plus ou moins de 5 ans.

Conclusion: Les problèmes de changement climatique devraient être davantage intégrés à la formation médicale continue des médecins afin d'améliorer leur connaissance et leur attitude à l'égard des questions liées au changement climatique ainsi que leur capacité à participer à l'analyse de leurs politiques.

Mots-clés: *Changement climatique, Médecins, Analyse des politiques, Nigéria*

Introduction

Climate change is long-term alteration in global weather patterns, characterised by increase in temperature and storm activity, and also regarded as a potential consequence of the greenhouse effect. Climate is the average weather over a long period of time while climate change is long-term alteration in global weather patterns, characterised by increase in temperature and storm activity, and also regarded as a potential consequence of the greenhouse effect. [1].

Climate change is increasing the burden of climate-sensitive health determinants and outcomes worldwide. Acting through increasing temperature, changes in the hydrologic cycle, and sea level rise, climate change is projected to increase the frequency and intensity of heat events and extreme events - floods and droughts [2]. It is also predicted that climate change will change the geographic range and incidence of climate-sensitive vector-, food-, and waterborne diseases, and increase diseases associated with air pollution and aeroallergens [2].

Health care professionals should understand these threats, anticipate their effects on public health, and participate as advocates for strong mitigation and adaptation strategies now. Any solutions that address climate change must be developed within the context of overall sustainability (the use of resources by the current generation to meet current needs while ensuring that the survival of future generations is not jeopardised in terms of their needs being met). Health care professionals can be leaders in a move away from a traditional focus on disease prevention and instituting appropriate therapies to a broad, integrated focus on sustainability as synonymous with health [3].

Studies have shown that health care professionals have an important role in preparing for and responding to these climate change related threats to human health particularly in participating in the policy process [3, 4].Very few studies have been done in developing countries to assess the perception of health workers about the health effects of climate change especially in the developing countries. It is therefore important to assess the perception of the Nigerian health workers to the health effects of climate change since they are well positioned to affect public attitude on these issues [5, 6]. Thus, this study was designed to assess the perception of doctors at the University College Hospital Ibadan on the health effect of climate change. Hence, this study provides preliminary local data which could be used to strengthen the capacity of health workers in Nigeria to manage the climate-change-related health effects.

Materials and method

The study employed a descriptive cross-sectional design involving a questionnaire survey. The study took place in Oyo State, one of the 36 States of the Federal Republic of Nigeria, located in the South-western part of the country. The state has a population of 5,591,589 with an annual growth rate of 3.2% [7].

The survey was conducted at the University College Hospital, Ibadan, the capital city of Oyo State. The hospital was established in 1952 in response to the need for the training of medical students following the establishment of the then Faculty of Medicine (now College of Medicine) in the University College, Ibadan in 1948 (currently the University of Ibadan). It serves as a major referral centre for Nigeria as a whole and many parts of the Sub-Saharan Africa.

In addition to undergraduate medical training (based in the College of Medicine of the University of Ibadan), the University College Hospital also provides facilities for residency training and other postgraduate medical and health trainings.

The study population included doctors currently in the employment of UCH, Ibadan for at least 6 months. These comprised house officers (82), medical officers (28), resident doctors (275) and consultants (38). There are about 1000 doctors practicing at various levels in the hospital. This includes about 200 Consultants, about 350 Resident doctors and Medical Officers and about 450 House Officers. Proportionate sampling of respondents was carried out. Employee list generated from the Finance Department was used to stratify doctors by cadres into house officers, medical officers, resident doctors and consultants. Using a computer generated table of random numbers, a proportionate fraction of each group was sampled. Knowledge and attitude were assessed using 44-point and 40-point scales respectively. Knowledge score <22 was regarded as poor while ≥ 22 was regarded as good. Similarly, attitude scores <25 and ≥ 25 were regarded as poor and good respectively.

Approval for this study was obtained from the University of Ibadan/University College Hospital (UI/UCH) Institutional review board (No. UI/EC/14/0152). Participation was voluntary and each respondent received detailed information on the purpose of the study. Informed verbal consent

was obtained from participants before the administration of questionnaires.

Results

A total of 423 doctors participated in the study. The mean age was 35.8±9.3 years while majority, 174 (41.1%), were between 30 to 39 years. There were 251 (59.3%) females and slightly above three-fifth, 264 (62.4%), of respondents were currently married. Majority of respondents, 340 (80.4%), were Yoruba. The highest proportion, 275 (65.0%), were resident doctors while more than half of the respondents, 229 (54.1%), had practiced for 6 or more years. The mean duration of practice was 10.6±9.6 years with a range of 1 – 36 years (see Table 1).

Table 1: Socio – demographic characteristics of respondents (N = 423)

Variable	Frequency (n)	Proportion (%)
Age group (years)		
≤ 29	117	27.7
30-39	174	41.1
≥ 40	132	31.2
Mean age (±SD)	35.8±9.3	
Sex		
Male	172	40.7
Female	251	59.3
Marital status		
Currently married	264	62.4
Not currently married	159	37.6
Ethnic groups		
Yoruba	340	80.4
Igbo	41	9.7
Hausa	7	1.7
Others*	35	8.3
Designation		
House officers	82	19.4
Medical officers	28	6.6
Resident doctors	275	65.0
Consultants	38	9.0
Completed years of practice		
≤ 5	194	45.9
≥ 6	229	54.1
Mean duration of practice (±SD)	10.6±9.6	

*Urhobo, Idoma, Isoko, Ishan, Ebira, Fulani, Ijaw, Ogori, Egun

Respondents' knowledge of climate change

Almost all, 410 (96.9%), of participants had heard about climate change. However, only about half, 236 (55.8%) had good knowledge of all aspects of climate change. Slightly less than three-fifth, 243 (57.4%) of respondents had good knowledge of the possible health effects of climate change while about two-third, 276 (65.2%) had good knowledge of adaptation to climate change (Table 2).

Table 2: Composite knowledge scores on various aspects of climate change (N=423)

Knowledge variables	Frequency (n)	Proportion (%)
General knowledge		
Good	221	52.2
Poor	202	47.8
Knowledge on health effects		
Good	243	57.4
Poor	180	42.6
Knowledge on adaptation		
Good	276	65.2
Poor	147	34.8
Total knowledge		
Good	236	55.8
Poor	187	44.2

Source of information on climate change

The television was reported as the commonest (81.1%) source of information on climate change while information from colleagues (30.0%) was the least common (Figure 1).

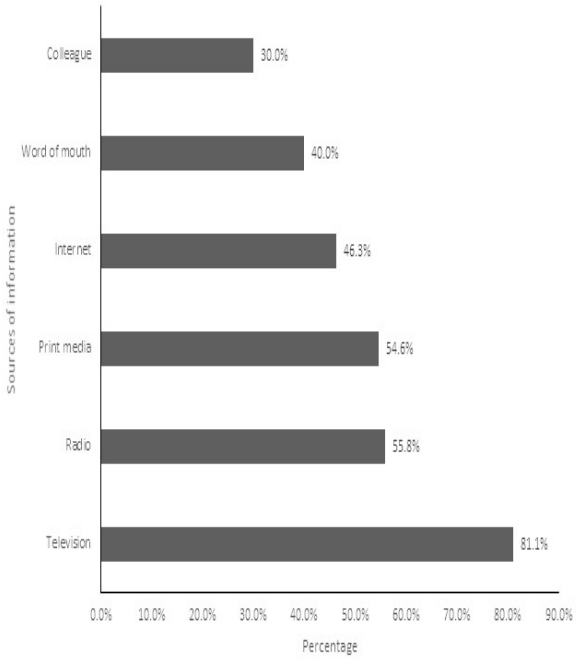

Fig. 1: Respondents' source(s) of information about climate change

Knowledge of the health effects of climate change

Slightly more than a third, 159 (37.6%), of respondents knew that climate change is not the same as weather change while about the same proportion, 154 (36.4%), alluded to the fact that climate change is

not entirely a natural occurrence. More than half, 248 (58.6%), of participants agreed that climate change poses a serious problem for Nigeria (Table 3).

Table 3: Positive responses to general questions on climate change (N=423)

Climate change issues	Frequency (n)	Proportion (%)
Climate change is not the same as weather change	159	37.6
Climate change is not synonymous to global warming	267	63.1
Global warming results in climate change	335	79.2
The world's climate is changing	372	87.9
Climate change is the result of human behavior	0	0.0
Climate change is not entirely a natural occurrence	154	36.4
Climate change poses a serious problem for Nigeria	248	58.6
Greenhouse gas (GHG) emissions are very important causes of climate change	272	64.3
Methane is not the most important greenhouse gas	331	78.3
Water vapor is one of the greenhouse gases	85	20.1
Industrialization & urbanization are important causes of climate change	344	81.3
Agriculture has an impact on climate change	285	67.4
Climate change is not entirely man-made	98	23.2

Less than a fifth, 66 (15.6%), of the study's respondents knew that climate change could result in some positive health effects although majority, 362 (85.6%), were aware that climate change could result in important health effects. Table 4 summarises the knowledge of respondents about possible health effects of climate change.

Table 4: Positive responses to issues on health effects of climate change (N=423)

Issues on health effects of climate change	Frequency (n)	Proportion (%)
Climate change has some positive health effects	66	15.6
Climate change can result in important health effects	362	85.6
Lives are being lost globally due to the effect of climate change	329	77.8
Children and the aged are the age groups most affected by climate change	148	35.0
Climate change increases the frequency and intensity of severe weather events	359	84.9
Climate change may result in flooding of the coastal regions	327	77.3
Levels of UV radiation reaching the earth's surface may increase as a result of climate change	325	76.8
Diseases whose incidence could increase as a result of climate change		
Malaria	303	71.6
Tuberculosis	241	57.0
Diarrhoea	240	56.7
Food poisoning	163	38.5
HIV/AIDS	242	57.2
Onchocerciasis	174	41.1
Schistosomiasis	176	41.6
Leprosy	158	37.4
Dengue fever	134	31.7
Yellow fever	151	35.7
Encephalitis	152	35.9
Hypertension	170	40.2
Diseases that could be aggravated by climate change		
Cardiovascular diseases	173	40.9
Diabetes mellitus	180	42.6
Asthma	338	79.9
Malnutrition	255	60.3

Table 5: Positive responses to adaptation issues on Climate Change (N=423)

Adaptation to climate change	Frequency (n)	Proportion (%)
Improving access to cooler or air-conditioned environments is an important adaptation strategy	209	49.4
Surveillance and control of infectious diseases are climate change adaptation strategies	273	64.5
Immunization campaigns for existing and emerging health risks that target vulnerable populations are useful	317	74.9
Provision of protective shelter and clothing are important in climate change adaptation	344	81.3
Clearing of drainages and construction of floodways are important	283	66.9
Health promotion campaigns that link energies use to behavior are beneficial	297	70.2

Knowledge about adaptation to climate change

Slightly less than two-third, 273 (64.5%), of respondents opined that surveillance and control of infectious diseases are climate change adaptation strategies while about the same proportion, 283 (66.9%) agreed that clearing of drainages and construction of flood channels are also important. Table 5 sums up the knowledge of respondents on specific aspects of climate change adaptation.

agreed that healthcare professionals should understand and anticipate the health effects of climate change. Likewise, only a small proportion (6.8%) agreed that health workers should be advocates of climate change issues. Similarly, only 3.0% of respondents agreed that climate change initiatives should be integrated into MDG-based sustainable development plans. Overall, slightly above half of respondents,

Table 6: Attitudes toward the Health Effects of Climate Change

Attitude	Agree n (%)	Undecided n (%)	Disagree n (%)
Healthcare professionals have no business with climate change issues	41 (10.0)	34 (8.0)	347 (82.0)
I personally can help to limit the effects of climate change	93 (22.0)	60 (14.2)	270 (63.8)
Healthcare professionals should understand the threats and anticipate the effects of climate change on health	9 (2.1)	40 (9.5)	374 (88.4)
Health workers should participate as advocates in climate change issues	29 (6.8)	36 (8.5)	358 (84.6)
I should not be concerned about climate change issues	30 (7.1)	44 (10.4)	349 (32.4)
I have a role to play in mitigating climate change effect	36 (8.5)	79 (18.7)	308 (72.8)
Climate change initiatives should be integrated into the national MDG-based sustainable development plans	13 (3.0)	76 (18.0)	334 (79.0)
Turning appliances off and reducing waste may help mitigate the effects of climate change	34 (8.0)	73 (17.3)	316 (74.7)

Respondents' attitude to climate change and its health effect

The respondents' attitudes to various aspects of climate change are shown in Table 6. Majority of respondents (82.0%) agreed that health professionals have something to do with climate change issues. Only about one-fifth of respondents (22.0%) believed they can personally help to limit the effects of climate change. Only nine participants (2.1%)

227 (53.7%), have positive attitude score to climate change issues.

Socio-demographic characteristics and knowledge of climate change

Associations between demographic characteristics of respondents and mean knowledge of climate change are summarised in Table 7. Doctors aged 29 years and below had the highest mean knowledge

Table 7: Respondents' socio-demographic characteristics and knowledge of climate change

Characteristics	Mean knowledge score (±SD)	ANOVA	t-test	p-value
Age group (years)				
≤ 29	26.5±7.1			
30-39	23.9±7.7	12.919		<0.001
≥ 40	21.8±6.9			
Sex Male	26.4±6.7		5.631	<0.001
Female	22.3±7.6			
Marital status				
Currently married	22.9±7.3		-3.815	<0.001
Not currently married	25.7±7.5			
Ethnic groups				
Yoruba	23.9±7.6			
Igbo	23.5±8.3	0.897		0.443
Hausa	21.9±5.9			
Others*	24.0±7.5			
Designation				
House officers	27.8±5.6			
Medical officers	26.2±6.0	11.679		<0.001
Resident doctors	23.0±7.8			
Consultants	21.5±6.8			
Completed years of practice				
≤ 5	25.9±7.8		5.096	<0.001
> 6	22.3±6.8			

*Urhobo, Idoma, Isoko, Ishan, Ebira, Fulani, Ijaw, Ogori, Egun

Table 8: Respondents' socio-demographic characteristics and knowledge of the health effects of climate change

Characteristics	Knowledge score		χ^2	p-value
	Good n (%)	Poor n (%)		
Age group (years)				
≤ 29	78 (32.1)	39 (21.7)		
30-39	96 (39.5)	78 (43.3)	5.882	0.053
≥ 40	69 (28.4)	63 (35.0)		
Sex: Male	109 (44.9)	63 (35.0)	4.163	0.041
Female	134 (55.1)	117 (65.0)		
Marital status				
Currently married	147 (60.5)	117 (65.0)	0.895	0.334
Not currently married	96 (39.5)	63 (35.0)		
Ethnic groups				
Yoruba	195 (80.2)	145 (80.6)		
Igbo	23 (9.5)	18 (10.0)	3.398	0.334
Hausa	2 (0.8)	5 (2.8)		
Others*	23 (9.5)	12 (6.7)		
Designation				
House officers	58 (23.9)	24 (13.3)		
Medical officers	17 (7.0)	11 (6.1)	8.696	0.034
Resident doctors	145 (59.7)	130 (72.2)		
Consultants	23 (9.5)	15 (8.3)		
Completed years of practice				
≤ 5	123 (50.6)	71 (39.4)	5.199	0.023
≥ 6	120 (49.4)	109 (60.6)		

*Urhobo, Idoma, Isoko, Ishan, Ebira, Fulani, Ijaw, Ogori, Egun

Table 9: Respondents' socio-demographic characteristics and mean attitude scores to climate change

Characteristics	Mean attitude score (±SD)	ANOVA	t-test	p-value
Age group (years)				
≤ 29	1.7±0.5			
30-39	1.6±0.5	15.394		<0.001
≥ 40	1.4±0.5			
Sex				
Male	34.4±4.8		6.027	<0.001
Female	31.4±5.1			
Marital status				
Currently married	31.8±5.1		-4.448	<0.001
Not currently married	34.0±5.0			
Ethnic groups				
Yoruba	1.5±0.5			
Igbo	1.6±0.5	1.225		0.300
Hausa	1.7±0.5			
Others*	1.6±0.5			
Designation				
House officers	1.8±0.4			
Medical officers	1.6±0.5	8.109		<0.001
Resident doctors	1.5±0.5			
Consultants	1.4±0.5			
Completed years of practice				
≤ 5	34.2±4.8		5.85	<0.001
≥ 6	31.3±5.1			

*Urhobo, Idoma, Isoko, Ishan, Ebira, Fulani, Ijaw, Ogori, Egun

score (26.5±7.14) among the study participants (p<0.001). Also, male physicians had significantly higher (p<0.001) climate change knowledge score (26.4±6.6) compared with the females (22.3±7.6). Marital status, designation and duration of practice of respondents were all significantly associated with higher mean climate change knowledge score (p<0.001).

Socio-demographic characteristics and knowledge of health effects of climate change

Table 8 shows cross tabulations of demographic variables and the knowledge of the health effects of climate change. The highest proportion of those with good knowledge, 96 (39.5%), were aged 30 – 39 years (p=0.053). Other characteristics significantly associated with good knowledge of the health effects of climate change include female gender, being resident doctors and length of practice ≤ 5 years (p<0.05). Marital status and ethnic affiliation were not significantly associated with knowledge of the health effects of climate change (p>0.05).

Socio-demographic variables, knowledge and attitude to climate change

The association between socio-demographic characteristics and mean attitude scores to climate change are illustrated in Table 9. Respondents aged 29 years and below, males, not currently married, house officers and those who have practiced for 6 or more years had significantly higher mean attitude scores (p<0.001). Mean attitude scores among ethnic groups were not significantly different (p>0.05).

Table 10 also revealed that younger age group, male gender, marital status, being a resident doctor and duration of practise were associated with positive attitude to climate change. Similarly, a greater proportion of participants with good climate change knowledge, 167 (73.6%), also had positive attitude to climate change (p<0.001).

Predictors of good knowledge of climate change among respondents

Table 11demonstrated that only sex was the significant predictor of good knowledge in this study. Male respondents were two times more likely to have good knowledge of climate change compared with the female respondents [OR: 2.1; 95%CI: 1.4 – 3.3].

Predictors of positive attitude towards climate change among respondents

The significant predictors of positive attitude to climate change following logistic regression is

Table 10: Respondents' socio-demographic characteristics and attitude to climate change

Characteristics	Attitude Positive n (%)	Negative n (%)	χ^2	p-value
Age group (years)				
≤ 29	78 (34.4)	39 (19.9)		
30-39	103 (45.4)	71 (36.2)	28.890	<0.001
≥ 40	46 (20.3)	86 (43.9)		
Sex				
Male	120 (52.9)	52 (26.5)	30.228	<0.001
Female	107 (47.1)	144 (73.5)		
Marital status				
Currently married	122 (53.7)	142 (72.4)	15.686	<0.001
Not currently married	105 (46.3)	54 (27.6)		
Ethnic groups				
Yoruba	175 (77.1)	165 (84.2)		
Igbo	26 (11.5)	15 (7.7)	3.746**	0.290
Hausa	5 (2.2)	2 (1.0)		
Others*	21 (9.3)	14 (7.1)		
Designation				
House officers	62 (27.3)	20 (10.2)		
Medical officers	18 (7.9)	10 (5.1)	23.213	<0.001
Resident doctors	131 (57.7)	144 (73.5)		
Consultants	16 (7.0)	22 (11.2)		
Completed years of practice				
≤ 5	131 (57.7)	63 (32.1)	27.690	<0.001
≥ 6	96 (42.3)	133 (67.9)		

*Urhobo, Idoma, Isoko, Ishan, Ebira, Fulani, Ijaw, Ogori, Egun
**Likelihood ratio

presented in Table 12. Male respondents were two times more likely to have positive attitude towards climate change issues compared to their female counterparts [OR: 1.9; 95%CI: 1.2 – 3.1].

Respondents with good climate change knowledge were four times more likely to have positive attitude towards climate change issues [OR: 4.0; 95%CI: 2.6 – 6.3].

Table 11: Logistic regression model for predicting good knowledge of climate change among respondents

Characteristics	Odds ratio	95% Confidence Interval	p-value
Sex			
Male	2.122	1.351 – 3.334	0.001
Female	1		
Age			
≤35	1.717	0.963 – 3.064	0.064
≥36	1		
Marital status			
Currently married	1.125	0.633 – 1.998	0.688
Not currently married	1		
Years of practice			
≤5	1.014	0.554 – 1.859	0.963
≥6	1		

Variables not retained – ethnicity, designation

Table 12: Logistic regression model for predicting positive attitude towards climate change issues among respondents

Characteristics	Odds ratio	95% Confidence Interval	p-value
Sex			
Male	1.953	1.211 – 3.151	0.006
Female	1		
Age			
≤35	1.803	0.968 – 3.358	0.063
≥36	1		
Marital status			
Currently married	1.086	0.588 – 2.008	0.792
Not currently married	1		
Years of practice			
≤5	1.353	0.712 – 2.572	0.356
≥6	1		
Total knowledge score			
Good	4.032	2.597 – 6.250	<0.001
Poor	1		

Variables not retained – ethnicity, designation

Discussion

It has been documented that the perception of a problem may show the knowledge and the readiness to act towards preventing it [8]. The result of this study shows that majority of respondents were aware of climate change. This finding is similar to results of studies done in the United States of America and the United Kingdom which showed that the populations demonstrated a high level of awareness for global climate change [9, 10]. The high awareness about climate change among physicians in this study is anticipated as they are regarded as and looked up to as role models and well-informed people in the community [11-14]. Although awareness about climate change was high, knowledge of the various aspects of climate change (especially the health effects of climate change) was much lower. With such level of knowledge, it is doubtful if the physicians will be able to effectively discharge their expected social responsibility of climate change prevention and mitigation and to contribute meaningfully to policies meant to promote human health in the face of climate change realities [15-18].

In this study, the commonest source of information about climate change was the television followed by the radio, print media and the internet. Similar to this finding, Wilson [19], in a study carried out in the United States, showed that the mass media was the commonest source of scientific information especially climate change issues. This fact has been corroborated by other studies [17, 20]. However, Bell [21], in a New Zealand study reported that over 80% of climate change stories were slightly inaccurate while one in six of the stories contained significant misreporting. Hence, Bell emphasized the need for scientists to work together with journalists to better inform the public about climate change. Also, about two-thirds of this study's respondents had good knowledge of adaptation to climate change. In a study on adaptation to climate change and sustainable development, Smit and Pilifosova[22], argued that the current knowledge of adaptation to climate change is insufficient.

Males were more knowledgeable about climate change in this study. However, Denton [23], reasoned that it is important to ensure that women who are more likely to suffer from the adverse effects of climate change understand the threats so as to ensure sustainable environmental development. This study also discovered that younger physicians in practice were more knowledgeable. In contrast, a Canadian qualitative study done among the general population reported that more of the older respondents were informed about climate change issues [24]. A possible explanation for this observed scenario is that these younger physicians study more, especially for their professional examinations and thus more likely to be in tune with contemporary public health issues. However, since doctors and scientists are often the source of information for journalists who in turn educate the population, it is imperative therefore that doctors as respected scientists update their knowledge on climate change [20, 25].

In this study, about half of the respondents had a positive or favourable attitude towards climate change and what their response should be. Physicians' attitude towards climate change is very important as it has been documented that physicians are well positioned to affect public attitude [5, 6]. Gill [26], was of the opinion that health professionals have not developed the attitude needed to make it easy to tackle climate change. As said by Gill and colleagues [16], doctors have a duty to inform their professional colleagues and the larger public, including policy makers, about the health costs of climate change. Gill et al [16] also opined that doctors, in addition to advocating on climate change issues, should also use their many networks to promote these issues.

Conclusion

This study revealed that awareness of climate change was high but in-depth knowledge of climate change and its health effects was only average among physicians at the University College Hospital, Ibadan. The mass media (television and radio) were the commonest sources of information on climate change. Respondents' attitude to climate change was fair as about half of them had positive attitude scores. This study showed younger age, male gender and being a resident doctor to be significantly associated with good knowledge about climate change and its health effect. In addition to the preceding factors, being currently married was also found to be significantly associated with positive attitude towards climate change issues.

Given the findings in this study, we recommend that climate change issues should be inculcated more into the continuing medical education of doctors and other health workers in order to improve their knowledge and attitude to prevention, mitigation and adaptation to climate change. This will go a long way to strengthen the capacity of the Nigerian health workers to manage the climate-change-related health effects and to participate in climate change policy discourse. Also, there is need for medical professionals to take more leadership roles in advocacy on climate change issues since they have a great influence on the public's knowledge and attitude to public health issues. Health workers should see this as an important social responsibility.

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Nutritional status of children with congenital heart diseases at the University College Hospital, Ibadan, Nigeria

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Abstract
Background: Congenital Heart Disease (CHD) is an important cause of childhood morbidity and mortality. Poor nutritional status is one of the clinical manifestations. The extent of deviation from the normal weight and/or height has not been adequately documented in Nigeria.
*Study design***:** This cross-sectional study, conducted between April 2011 and June 2012 (fourteen months), involved151 Nigerian children comprising 96 males and 55 females aged between one and 60 months. Nutritional status of the children was assessed using weight, height, mid-upper arm circumference (where appropriate), triceps and subscapular skin fold thicknesses. The z-scores of the anthropometric data were compared with the World Health Organisation (WHO) Standard reference charts.
*Results***:** The mean age of the children was 17.3±15.7 months. The male: female ratio was 1.7:1. The overall frequency of malnutrition was 72.8%.Severe malnutrition was found in 36.4% of the children, with the frequency of underweight being 64.2%, that of wasting 37.7% and of stunting 57.0%.
Conclusion: The prevalence of malnutrition in Nigerian children with CHD is very high. Appropriate management of the heart disease together with routine nutritional screening and counselling are likely to benefit children with CHD in order to improve management outcomes. Early corrective interventions, including surgery, need to be intensified.

Keywords: *Congenital heart disease; anthropometry; nutritional status; malnutrition; children.*

Résumé
Contexte: La Maladie Cardiaque Congénitale (MCC) est une cause importante de morbidité et de mortalité infantile. Le mauvais état nutritionnel est l'une des manifestations cliniques. L'étendue de l'écart par rapport au poids et / ou à la taille normale n'a pas été suffisamment documentée au Nigeria.

Conception de l'étude: Cette étude transversale, menée entre avril 2011 et juin 2012 (quatorze mois), impliquait 151 enfants nigérians comprenant 96 garçons et 55 filles âgés entre l'intervalle de 1 à 60 mois. L'état nutritionnel des enfants a été évalué à l'aide du poids, de la taille, de la circonférence du milieu de la partie supérieure du bras (le cas échéant), du triceps et de l'épaisseur de plis de la peau sous-calcique. Les scores-z des données anthropométriques étaient comparés aux tableaux de référence standard de l'Organisation Mondiale de la Santé (OMS).
Résultats: L'âge moyen des enfants était de 17,3 ± 15,7 mois. Le rapport garçon : fille était de 1,7: 1. La fréquence globale de la malnutrition était de 72,8%. Une malnutrition sévère était trouvée dans 36,4% des enfants, avec la fréquence de sous-pondération étant 64,2%, celle du gaspillage 37,7% et de rabougri 57,0%.
Conclusion: La prévalence de la malnutrition chez les enfants nigérians atteints de MCC est très élevée. Une gestion appropriée de la maladie cardiaque, ainsi que le dépistage et le conseil nutritionnels de routine, sont susceptibles de bénéficier aux enfants atteints de MCC afin d'améliorer les résultats de la gestion. Les interventions correctives précoces, y compris la chirurgie, doivent être intensifiées.

Mots-clés: *Maladie cardiaque congénitale; anthropométrie; l'état nutritionnel; malnutrition; les enfants.*

Introduction

Congenital Heart Disease (CHD accounts for about a third of all congenital birth defects [1] and remains an important cause of childhood morbidity and mortality. The clinical presentation of CHD varies according to the type and severity of the cardiac defect, and whether the defect is symptomatic or asymptomatic.

Children with CHD are prone to varying degrees of malnutrition for several reasons, including: decreased energy intake, disturbances in gastrointestinal function, disturbances in energy metabolism and prenatal factors [2]. Using anthropometry as an index of nutritional assessment,

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studies from Europe [3, 4] and North America [5, 6] have reported significant growth retardation among children with CHD; studies from developing countries including Nigeria [7-12], have also reported similar findings. The problem of malnutrition is worse in the developing countries, where it is common even among the general population [13].

Poor body nutritional reserves may be a significant factor militating against a successful outcome after corrective surgery [14,15] which is becoming more available in developing countries. Clinicians practising in such countries therefore have the additional responsibility of nutritional management in addition to treatment of heart failure and other associated conditions while awaiting definitive treatment. Maintenance of good nutritional status however, has received very little attention in the management of such children in developing countries.

Previous studies have demonstrated the predictive value of anthropometry to the outcome of therapeutic interventions [16,17]. Nutritional assessment by anthropometry would therefore be useful in planning management strategies which will ensure that patients achieve adequate nutritional supplementation in early life and prevent deviation from normal growth centiles.

Children with CHD are known to deviate from their birth percentiles for weight or length/height [9, 18]. The extent of this deviation has however not been adequately documented in Nigeria. The present study was therefore undertaken to determine the nutritional status of children with CHD using anthropometry, with a view to addressing this gap in knowledge. It is expected that the study outcome would provide a basis for evolving appropriate nutritional management strategies as part of the overall management of children with CHD.

Materials and methods

The study, conducted between April 2011 and June 2012(fourteen months), involved a cross-sectional survey of all children with CHD aged one month to 60 months presenting consecutively at the Cardiology clinic and the Otunba Tunwase Children's Emergency Ward (OTCHEW) of the Department of Paediatrics, University College Hospital (UCH), Ibadan. The diagnosis of acyanotic or cyanotic CHD was based on clinical parameters, chest radiograph (CXR), electrocardiographic (ECG) and 2-Dimensional (2-D) echocardiographic data with Doppler and colour flow mapping. Patients with co-existent chronic morbidities associated with malnutrition, such as tuberculosis, HIV/AIDS, sickle cell disease, diabetes mellitus, and chronic kidney diseases were excluded.

Each child underwent a full physical examination, with emphasis on the cardiovascular system. The following anthropometric measurements were taken by the same investigator, using standard WHO procedures [16].

1. Weight was measured to the nearest 10gm, using a battery-powered SECA® digital baby scale for children under 24 months and the SECA® Model 220 stadiometer for older children (*Medical Scales and Measuring Systems, SECA® limited, UK*). 2.The height of children >24 months old was measured to the nearest 1mm using a SECA® Model 220 stadiometer (*Medical Scales and Measuring Systems, SECA® limited, UK*) and the length of children ≤24 months was measured with a standard measuring board placed on a flat surface. 3.Mid-Upper Arm Circumference (MUAC) was measured to the nearest 1mm with a non-stretchable tape measure round the upper arm at a point midway between the olecranon and the acromion while Subscapular skinfold (SSF) thickness were measured to the nearest 0.2 mm using the "Holtain" skinfold calliper (*Holtain Limited, UK*). All the measurements were taken on the left side of the body. Two measurements were taken and an average calculated.

Derived parameters

The z-scores of weight-for-age (WAZ), weight-for-height (WHZ) and height-for-age (HAZ) were computed using the WHO Anthro-calculator v 3.0.1. Underweight, wasting, stunting and overall nutritional status (ONS) - a composite of WAZ, WHZ, HAZ - were defined at cut-off points of z-scores < -2SD below the median of the reference population based on the WHO Child Growth Standards [19].

Data management

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (*SPSS Inc. Chicago, USA*). Overall malnutrition was defined as the proportion of patients who had a z-score less than 2.0 for any of the WAZ, WHZ and HAZ estimates or their combination. Categorical and discrete variables were analysed using the Chi squared test and Fisher's exact test or Yates corrected Chi squared test (for cells with counts less than five) as applicable.

Values were expressed as a mean (±SD) and proportions expressed as percentages. Statistical significance was set at < 0.05.

Ethical considerations

Participation in the study was completely voluntary and based on informed consent. The study proposal was reviewed by, and approval was obtained from the Joint University of Ibadan/University College Hospital, Ibadan Ethics Committee.

Results

Characteristics of the participants

One hundred and fifty one children, comprising 96 (63.6%) males and 55 (36.4%) females (M:F= 1.7:1) met the inclusion criteria for the study. The ages ranged from one to 59 (mean = 17.3±15.7) months. Infants constituted 51.6% of the study participants. There was no statistically significant difference in the distribution of patients by age group between males and females (p = 0.251).

Distribution of the cardiac defects

The distribution of the cardiac defects is shown in Table 1. One hundred and seventeen (77.5%) of the 151 study participants had acyanotic lesions, while 34 (22.5%) had cyanotic lesions. Overall, ventricular septal defect (VSD) was the single most common isolated acyanotic cardiac lesion, accounting for nearly half of all cardiac lesions, followed by atrial septal defect (ASD)and patent ductus arteriosus (PDA). Fallot's tetralogy (TOF) was the most common cyanotic congenital heart lesion encountered, followed by tricuspid atresia (TA) combined with VSD and transposition of the great arteries (TGA) with ASD. The four most common lesions seen among the study participants were VSD (46.3%), TOF (15.9%), ASD (9.3%), and PDA (7.2%). There was no significant gender difference in the distribution of the defects.

Nutritional status of the study participants

The frequency and pattern of malnutrition observed among the study participants are as shown in Tables 2 and 3. Overall, the proportion of participants who had z-score less than 2.0 for any of the WAZ, WHZ and HAZ estimates, thereby being adjudged to be malnourished, was 72.8%, with the majority (64.2%) being underweight,while 57.0% were stunted and 37.7% were wasted (Table 2). There was no significant difference in the nutritional status with respect to gender of the study participants. Table 3 similarly shows that the differences in proportions of under nutrition, wasting and stunting across age groups were not statistically significant.

Table 1: Distribution of cardiac defects according to genderof participants

Cardiac Diagnosis	Gender			
	Male n(%)	Female n(%)	Total n(%)	X^2 (p-value)
Acyanotic lesions	71(60.9)	46(39.1)	117(77.5)	
Ventricular septal defect(VSD)	43(61.4)	27(38.6)	70(46.3)	
VSD + Dextrocardia	1(100)	0(0.0)	1 (0.7)	
Multiple VSD	0(0.0)	1(0.0)	1(0.7)	
Atrial septal defect(ASD)	7(50.0)	7(50.0)	14(9.3)	
Patent ductusarteriosus(PDA)	5(45.5)	6(55.5)	11(7.2)	
PDA +VSD	3(60.0)	2(40.0)	5(3.3)	11.46$^+$
ASD +VSD	5(100)	0(0.0)	5(3.3)	(0.405)
Atrioventricularseptal defect (AVSD)	2(66.7)	1(33.3)	3(2.0)	
Pulmonary stenosis(PS)	3(100)	0(0.0)	3(2.0)	
PS + VSD	1(50.0)	1(50.0)	2(1.3)	
PS + ASD	0(0.0)	1(100)	1(0.7)	
Coarctation of aorta (CoA)	1(100)	0(0)	1(0.7)	
Cyanotic lesions	25(73.5)	9(26.5)	34(22.5)	
Tetralogy of Fallot (TOF)	19(79.2)	5(20.8)	24(15.9)	
Tricuspid atresia (TA) + VSD	2(50.0)	2(50.0)	4(2.6)	
Transposition of great arteries (TGA)+ASD	2(66.7)	1(33.3)	3(2.0)	2.60$^+$
Hypoplastic left heart syndrome (HLHS)	1(50.0)	1(50.0)	2(1.3)	(0.627)
TGA + VSD	1(100)	0(0.0)	1(0.7)	
Grand Total	96(63.6)	55(36.4)	151(100)	

$^+$Likelihood ratio

Yates corrected Chi squared test was used for comparison where values were less than 5

Table 2: Nutritional status of study participants according to gender

*Nutritional Indices	Male n (%)	Female n (%)	Total n (%)	LR	p-value
Overall Nutritional Status					
Normal	25(26.0)	16(29.1)	41(27.2)		
Malnourished	71(74.0)	39(70.9)	110(72.8)	0.13	0.723
Total	96(63.6)	55(36.4)	151(100)		
WAZ					
Normal	36(37.5)	18(32.7)	54(35.8)		
Malnourished	60(62.5)	37(67.3)	97(64.2)	0.35	0.556
Total	96(63.6)	55(36.4)	151(100)		
WHZ					
Normal	59(61.5)	35(63.6)	94(62.3)		
Malnourished	37(38.5)	20(36.4)	57(37.7)	0.07	0.790
Total	96(63.6)	55(36.4)	151(100)		
HAZ					
Normal	38(39.6)	27(49.1)	65(43.0)		
Malnourished	58(60.4)	28(50.9)	86(57.0)	1.29	0.256
Total	96(63.6)	55(36.4)	151(100)		
MUACZ					
Normal	43(51.2)	24(52.2)	67(51.5)		
Malnourished	41(48.8)	22(47.8)	63(48.5)	0.01	0.915
Total	84(64.6)	46(35.4)	130(100)		
SSFZ					
Normal	45(53.6)	28(60.9)	73(56.1)		
Malnourished	39(46.4)	18(39.1)	57(43.9)	0.64	0.423
Total	84(64.6)	46(35.4)	130(100)		

**Z-scores for weight-for-age (WAZ); weight-for-height (WHZ); height-for-age (HAZ); mid-upper arm circumference-for-age (MUACZ); subscapular skinfold thickness-for-age (SSFZ) LR-Likelihood ratio*
Yates corrected Chi squared test was used for comparison where values were less than 5

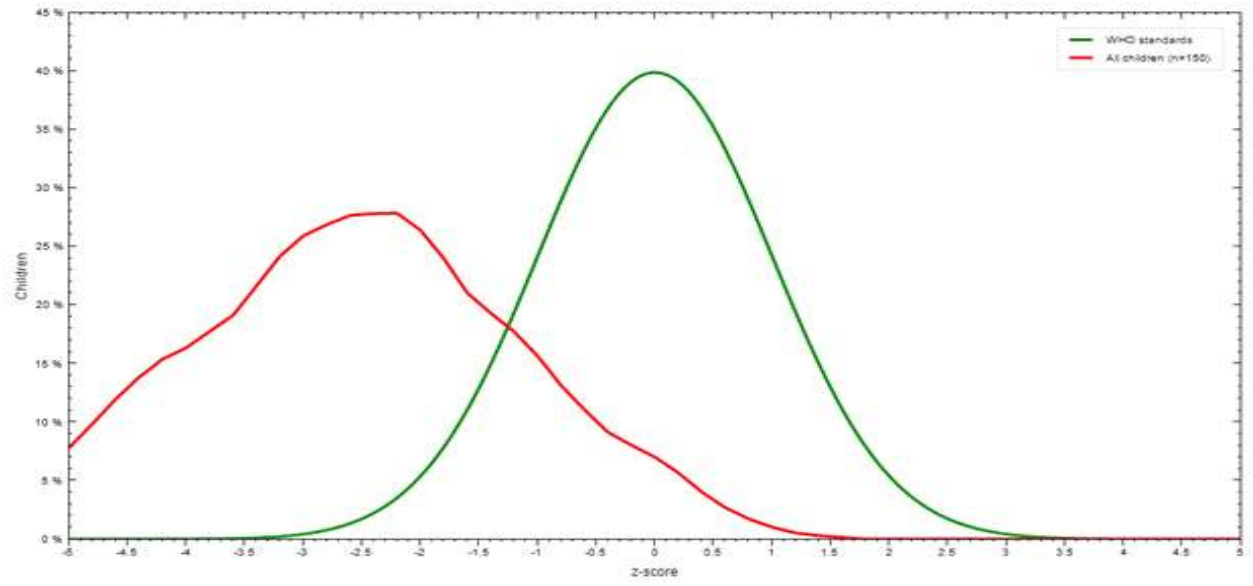

Fig. 1: Distribution of weight-for-age of study population compared with the WHO standard.

Table 3: Nutritional status of study participants according to age

Nutritional Indices	Age (months)						
	1-12 n (%)	13-24 n (%)	25-36 n (%)	37-48 n (%)	49-60 n (%)	X^2	p-value
Overall Nutritional Status							
Normal	23(29.5)	6(20.0)	4(22.2)	4(28.6)	4(36.4)		
Malnourished	55(70.5)	24(80.0)	14(77.8)	10(71.4)	7(63.6)	1.73	0.786
Total	78(100)	30(100)	18(100)	14(100)	11(100)		
WAZ							
Normal	29(62.8)	8(26.7)	7(38.9)	4(28.6)	6(54.5)		
Malnourished	49(37.2)	22(73.3)	11(61.1)	10(71.4)	5(45.6)	3.23	0.520
Total	78(100)	30(100)	18(100)	14(100)	11(100)		
WHZ							
Normal	52(66.7)	14(46.7)	11(61.1)	9(64.3)	8(72.7)		
Malnourished	26(33.3)	16(53.3)	7(38.9)	5(35.7)	3(27.3)	5.43	0.246
Total	78(100)	30(100)	18(100)	14(100)	11(100)		
HAZ							
Normal	36(46.2)	11(36.7)	7(38.9)	7(50.0)	4(36.4)		
Malnourished	42(53.8)	19(63.3)	11(61.1)	7(50.0)	7(63.6)	1.41	0.843
Total	78(100)	30(100)	18(100)	14(100)	11(100)		
MUACZ							
Normal	29(50.9)	16(53.3)	7(38.9)	6(42.9)	9(81.8)		
Malnourished	28(49.1)	14(46.7)	11(61.1)	8(57.1)	2(18.9)	5.66	0.226
Total	57(100)	30(100)	18(100)	14(100)	11(100)		
SSFZ							
Normal	28(49.1)	17(56.7)	10(55.6)	9(64.3)	9(81.8)		
Malnourished	29(50.9)	13(43.3)	8(45.4)	5(35.7)	2(18.2)	4.47	0.346
Total	57(100)	30(100)	18(100)	14(100)	11(100)		

**Z-scores for weight-for-age (WAZ); weight-for-height (WHZ); height-for-age (HAZ); mid-upper arm circumference (MUACZ); subscapular skinfold (SSFZ)*

Yates corrected Chi squared test was used for comparison where values were less than 5

Table 4: Severity of malnutrition according to age and gender

*Severity of malnutrition	Normal Nutrition n (%)	Moderate Malnutrition n (%)	Severe Malnutrition n (%)	Total n (%)	X^2 (p-value)
Age (months)					
1-12	31(39.7)	17(21.8)	30(38.5)	78(51.6)	
13-24	5(16.7)	11(36.7)	14(46.6)	30(20.0)	
25-36	7(38.9)	8(44.4)	3(16.7)	18(11.9)	8.89^{+}(0.352)
37-48	5(35.7)	4(28.6)	5(35.7)	14(9.2)	
49-60	6(54.5)	2(18.2)	3(27.3)	11(7.3)	
Total	54(35.8)	42(27.8)	55(36.4)	151(100)	
Gender					
Male	35(36.4)	26(27.1)	35(36.5)	96(63.6)	
Female	19(34.5)	16(29.1)	20(36.4)	55(36.4)	0.35 (0.841)
Total	54(35.8)	42(27.8)	55(36.4)	151(100)	

**Severe malnutrition = z-scores for weight-for-age (WAZ) d" -3*

**Moderate malnutrition = z-scores for weight-for-age (WAZ) >-3.0 - d"-2.0*

**Normal nutrition = z-scores for weight-for-age (WAZ) > -2.0*

$^{+}$ Likelihood ratio

Yates corrected Chi squared test was used for comparison where values were less than 5

Severity of malnutrition among the study participants

Table 4 shows the severity of malnutrition (computed by weight-for-age) according to age and gender. Normal nutritional status was found in only 54 (35.8%) of the study population. Severe malnutrition (WAZ $\leq$ -3) occurred in 55(36.4%) of the study participants and moderate malnutrition (WAZ >-3.0 to $\leq$-2.0) in 42(27.8%). Severe malnutrition was more common among participants in their second year of life, while moderate malnutrition was more common in the 25-36-month age group. These differences, however, did not reach statistical significance. Similarly, there was no significant gender difference in the severity of malnutrition among the study participants.

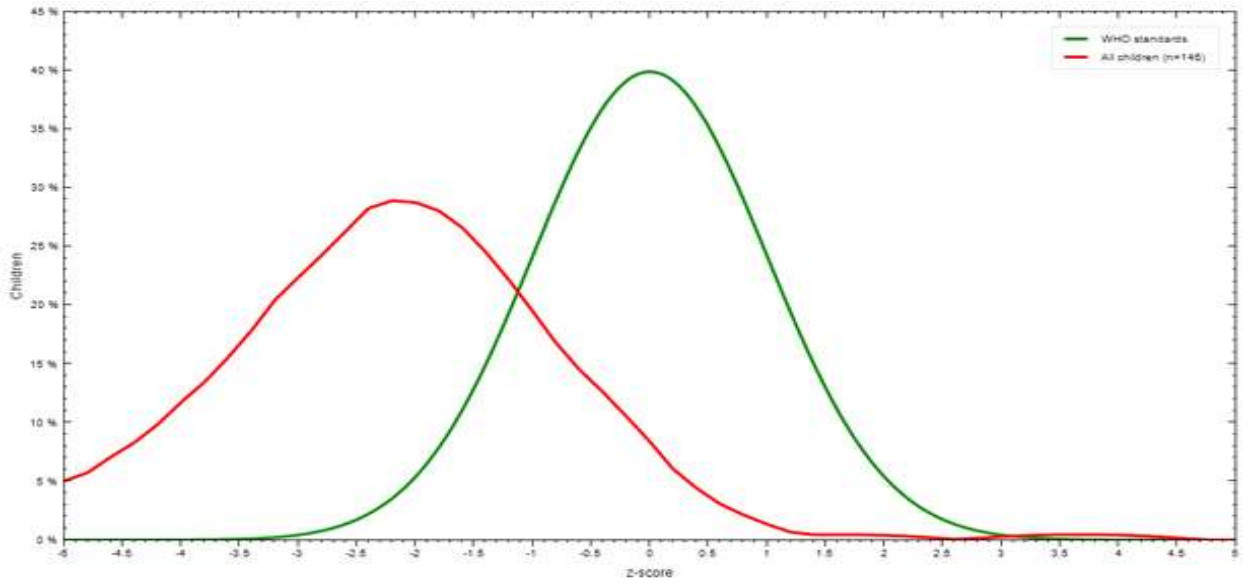

Fig. 2: Distribution of height-for-age of study population compared with WHO standard.

Nutritional status of study subjects compared with WHO reference values

Figures 1 and 2 show that the distributions of the study population by weight-for-age and height-for-age in the study population were markedly lower than the WHO reference standards. The distribution curve for weight-for-age appears skewed to the right, while the curve for height-for-age relatively conformed to the Gaussian distribution.

Discussion

The present study has identified a high frequency of malnutrition (72.8% overall) among children with CHD, the prevalence obtained for underweight (64.2%), stunting (57.0%) and wasting (37.7%) being well above the population-based prevalence of underweight (23%), stunting (41%) and wasting (14%) in the WHO national survey estimates for growth deficiency in Nigeria [20].Severe malnutrition occurred in 36.4% of cases. This means that the nutritional status of Nigerian children with CHD is generally poor, with a marked deviation from the WHO reference standards [19]. The frequency of severe malnutrition was highest among children aged 13-24 months compared with other age groups, a finding that agrees with the observations from the 2008 NDHS record on the general population [21]. Regarding specific forms of malnutrition, underweight was the commonest form of malnutrition encountered, the 64.2% frequency obtained being much higher that of the national prevalence of underweight among the general population, quoted in the 2008 NDHS report [21], which identified 23% of children as underweight. Similarly, the frequency of wasting (37.7%) recorded in the present study is far higher than the 14% reported for the general population in Nigeria [21].

Okoromah *et al* [10] in Lagos, Nigeria, estimated the overall prevalence of malnutrition in children with CHD to be as high as 90.4%, with the prevalence of severe malnutrition being 61.2%, both figures being even higher than those observed in the present study, but similar to those obtained by Hassan *et al* [8] from Egypt (84.0% overall,71.4% with severe malnutrition).It is however to be noted that while under-fives constituted the target population in the present study, Okoromah *et al*'s [10] results were based on data obtained from children with a much wider age range. On the other hand, figures

obtained from the present study are higher than those reported earlier from south India by Vaidyanathan *et al* [15] (59.0% overall and 27.7% severe malnutrition, respectively) and Sjarif *et al* [9] in Indonesia (51.1% and 22.3%, respectively).

Even though the proportion of severely malnourished participants in this study was very high, it is still much lower than the 61.1% reported by Okoromah *et al* [10].This might be because, whereas our subjects with CHD were recruited whether or not they were symptomatic, those in Okoromah's [10] series involved only patients with symptomatic CHD and associated complications, and were therefore more likely to include a greater proportion of those whose haemodynamic disturbances would have affected their nutritional status.

The frequency of underweight in the present study is also relatively high compared with the findings in previous studies on children with CHD [10,18]. However, comparable values with those of the present study had earlier been reported by Vaidyanathan *et al* [15]in South India. On the other hand, lower prevalence values of underweight than those of the present study were reported from Lagos, Nigeria [10], Oman[18]and Turkey [3]. The differences could be explained by differences in the populations from which the reference data were generated.

The frequency of wasting (37.7%) recorded in the present study is apparently much higher that of the corresponding value for the general population in Nigeria[21]but is consistent with the recorded prevalence of 41.7% from Lagos, Nigeria [10] and similar to other reports emanating from Turkey [3] and America [5, 6], Earlier reports from India [13, 22] however, suggested higher figures than those obtained in the present study. The import of this observation is that despite the similar socioeconomic indices in India and Nigeria, it would appear that wasting in children with CHD poses a relatively greater morbidity burden in India.

The frequency of stunting (57.0%) among the participants in the present study, is considerably higher than the 41% reported for under-fives in the 2008 NDHS record[21]. Stunting, which represents the long-term effects of malnutrition is poorly sensitive to recent short-term changes in dietary intake. Therefore, it is apparent that CHD, being a chronic illness (with its attendant increased metabolic demands, despite the prevailing inadequate calorie intake), is likely to worsen considerably the magnitude of stunting among the affected children. The high frequency of stunting in children with CHD in the present study is close to the value reported in an earlier cross-sectional study in Michigan, USA [5].While the similarities in the methodology of these two studies may partly explain the similar values obtained, it is equally remarkable that these values are higher than those from Glasgow [4], Turkey [3], Oman [11,18], India [22] and Lagos, Nigeria [10].

Using the MUAC and SSFT, the frequencies of malnutrition among the study population of children with CHD were found to be 48.5% and 43.9% respectively, suggesting a generally poor body fat store among the study participants. The values obtained are higher than those of a comparable cohort in an earlier report by Mitchell *et al*[4]who recorded figures of 20.1%, and 18.8% for MUAC and SSFT, respectively in a UK based population of children with CHD. The poor body fat (as suggested by the findings in this study) might be ascribed to the background of a poorer nutritional status in the general population in Nigeria, with comparably poorer socio-economic indices than those in the UK.

The present study failed to show a significant association between the age of children with CHD and the severity/degree of malnutrition. While this may be at variance with the identification of an increasing age as a significant predictor of malnutrition in children with CHD by earlier workers [5, 10],the logistic limitation of the study subjects to only the under-five children may partly explain the apparent discordance with respect to the significance of age.

The findings of the present study would suggest that CHD bestows a significant additional adverse effect on the nutritional status of children in Nigeria. This has implications for the outcome of surgical intervention, whenever this should be undertaken. It has been shown that delayed cardiac surgery in children with CHD aggravated growth deficit and caused slow and incomplete postoperative growth catch-up [23]. This implies that the earlier a defect is corrected, the better the outcome is likely to be. In Nigeria, there are often delays between diagnosis of CHD and surgical intervention, majorly for economic or logistic reasons, local facilities for surgical correction being few and far between [24].

Vaidyanathan *et al* [22]in South India had earlier shown that severe malnutrition was not always reversed by corrective intervention. They suggested that the occurrence of persistent malnutrition after corrective intervention for CHD was predicated by the nutritional status of the children at presentation,

in contrast to an earlier report by Cheung *et al* [25]which demonstrated satisfactory recovery in somatic growth after surgery. It is to be noted however that Cheung *et al's* [25] patients were all patients with Fallot's tetralogy, a cyanotic condition. It is therefore not clear how much the correction of the chronic hypoxia associated with this condition, contributed to the improvement in the nutritional status after surgery.

Acceptable explanations for the relatively high morbidity burden and severity of CHD-related malnutrition in the present study may include the study setting, the distribution of cardiac lesions in the study cohort, and expectedly, the fact that this study was conducted in a tertiary hospital where cases of CHD associated with complications are likely to be referred for evaluation.

Conclusions

This study has shown that the overall frequency of malnutrition, as well as the relative frequencies of underweight, wasting and stunting in children with CHD in Ibadan, are well above the reported prevalence among the general population in Nigeria. These findings could have significant implications, not only for physicians caring for children with CHD and policymakers in Nigeria and other developing countries, where resources are limited, but also for future research.

We recommend that cardiac evaluation should be performed at birth and in postnatal clinics and in immunization centres, in order to facilitate early detection and treatment of CHD.A careful assessment of all children with significant malnutrition should be carried out to rule out any underlying CHD. Also, greater attention should be paid to routine growth monitoring and proper documentation right from the first presentation of any child with CHD through the period of definitive surgery and thereafter. Nutrition counselling should be introduced/intensified and appropriate nutritional strategies employed as part of the routine care for children with CHD in order to prevent permanent growth disturbances while intensifying efforts to ensure early definitive corrective interventions including surgery in order to improve the nutritional status of children with CHD.

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Chebulinic acid potentiates antidepressant-like properties through monoaminergic systems and adult hippocampal neurogenesis in laboratory mice

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Abstract

Background: Chebulinic acid, an *ellagitannin*, present in the fruits of *Terminalia chebula* has been reported to show antioxidant, anti-nociceptive and antidepressant properties. Pre-clinical and clinical studies have suggested the involvement of monoamines, particularly the serotonergic and noradrenergic systems in the molecular mechanism of depression. Research has also suggested that the mode of action of antidepressants may be via hippocampal neurogenesis. There is no report elucidating how chebulinic acid potentiates its antidepressant properties. This study was therefore carried out to investigate its probable antidepressant mechanisms of action.

Methods: Involvement of serotonergic, cholinergic, dopaminergic and adrenergic systems were studied using Forced Swimming Test, Tail Suspension Test (as models of depression) and Open Field Test which measures ambulatory behaviours. Antagonists Prazosin, an alpha1-adrenergic receptor blocker (62.5 µg/kg, *i.p.*), metergoline, a $5HT_2$ receptor blocker (4 mg/kg, *i.p.*), atropine, a -muscarinic cholinergic receptor blocker (1 mg/kg *i.p.*) and sulpiride, a dopaminergic$_3$ receptor blocker (50 mg/kg *i.p*) were administered before chebulinic acid (20 mg/kg). Animals were also treated with chebulinic acid for 7, 21 and 28 days to see the effect of continuous administration, after which neuronal cell proliferation in the hippocampus was determined by Immunohistochemistry using Ki-67 antibody.

Results: Results showed that mobility was reversed in animals pre-treated with metergoline significantly ($p < 0.05$), but not sulpiride, prazosin and atropine, showing a possible involvement of serotoninergic system. There is a marked increase in the number of proliferating neuronal cells as observed after chronic treatment with chebulinic acid.

Conclusion: Chebulinic acid probably exhibits its antidepressant-like activity via serotonergic systems. The presence of proliferating cells in the chronic-treated groups indicates antidepressant-like activities, which is consistent with the time course for the therapeutic action of antidepressants. Chebulinic acid may serve as a template in the development of an alternative antidepressant drug.

Keywords: *Terminalia chebula, hippocampus, antidepressant-like activities, serotonergic systems.*

Résumé

Contexte: L'acide *chebulinique*, une *ellagitannine*, présent dans les fruits de *Terminalia chebula*, a révélé des propriétés neurologiques anti oxydantes, anti-nociceptif et antidépresseur. Des études précliniques et cliniques ont suggéré l'implication de monoamines, en particulier les systèmes sérotonergiques et noradrénergiques dans le mécanisme moléculaire de la dépression. La recherche a également suggéré que le mode d'action des antidépresseurs peut se faire à travers la neurogenèse de l'hippocampe. Il n'existe aucun rapport expliquant comment l'acide chebulinique potentialise ses propriétés antidépresseur. Cette étude a donc été réalisée pour étudier son (ses) mécanisme (s) d'action antidépresseur (s) probable (s).

Méthodes: l'implication des systèmes sérotonergiques, cholinergiques, dopaminergiques et adrénergiques a été étudiée en utilisant le test de natation forcée, le test de suspension de la queue (en tant que modèles de dépression) et le test de terrain ouvert qui mesure les comportements ambulatoires. Les antagonistes Prazosin, un bloqueur des récepteurs alpha1-adrénergiques (62,5 µg / kg, ip), la métérergine, un bloqueur de récepteur 5HT2 (4 mg / kg, ip), l'atropine, un inhibiteur de récepteur cholinergique a -muscarinique (1 mg / kg ip) et le sulpiride, un bloqueur de récepteur D2 et D3 (50 mg / kg ip) a été administré avant l'acide chebulinique (20 mg / kg). Les animaux ont également été traités avec de l'acide chebulinique pendant 7, 21 et 28 jours pour voir l'effet de l'administration continue, après

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quoi la prolifération des cellules neuronales dans l'hippocampe a été déterminée par l'immunohistochimie en utilisant l'anticorps Ki-67. *Résultats:* Les résultats ont montré que la mobilité a été inversée chez les animaux prétraités avec de la méthergoline de manière significative (p Â 0,05), mais pas le sulpiride, la prazosine et l'atropine, ce qui montre une implication possible du système sérotoninergique. Il y a une augmentation marquée du nombre de cellules neuronales proliférantes observées après un traitement chronique avec de l'acide chebulinique.
Conclusion: l'acide chebulinique présente probablement son fonctionnement antidépresseur par des systèmes sérotonergiques. La présence de cellules proliférantes dans les groupes traités par voie chronique indique des activités semblables aux antidépresseurs, ce qui correspond au cours de l'action thérapeutique des antidépresseurs. L'acide chebulinique peut servir de modèle dans le développement d'un autre médicament antidépresseur.

Mots-clés: *Terminalia chebula, hippocampe, activités antidépresseurs, systèmes sérotonergiques.*

Introduction

Depression is defined by as a mental disorder, presenting with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness and poor concentration, and at worst, suicide [1] Over 20% of the adult population suffers from depression at some time during their life [2]. Depression is a highly disabling condition associated with significant morbidity and mortality [3, 4] with a lifetime prevalence approaching 17% [5]. It is recognized as a major health problem [6] and the World Health Organization predicted that depression will become the second leading cause of premature death or disability worldwide by the year 2020 [7].

Conventionally available antidepressants are inadequate for many individuals, and may have frequent and persistent side effects [8]. Another drawback is that available treatments remain sub-optimal, with a delay of 3–6 weeks before their clinical effects can be achieved, and a lack of efficacy is also observed in many cases [9, 10]. For these reasons, the discovery of new drugs or innovative compounds that could further improve current depression therapies should be encouraged [4]. Although the molecular alterations underlying the pathogenesis of depression remain to be clearly established, pre-clinical and clinical studies have suggested the involvement of monoamines, particularly the serotonergic and noradrenergic systems [11]. There are several lines of evidence indicating that serotonergic and noradrenergic neurotransmissions are involved in the expression of an antidepressant-like effect in the behavioural despair models of depression [12]. In addition, there is a considerable amount of pharmacological evidence regarding the efficacy of antidepressants with dopaminergic effects in the treatment of depression [13] and studies that suggest an involvement of this system with antidepressant-like responses in pre-clinical models of depression [14]. Recently, research has suggested that the mode of action of antidepressants may be through hippocampal neurogenesis [15]. Two regions of the adult brain, the sub ventricular zone (SVZ) and sub granular zone (SGZ) of dentate gyrus of the hippocampus, are assumed to be involved in neurogenesis [16, 17].

People for many years have sought therapeutics gains from herbal sources, including ones to alleviate affective disorders. In addition, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the last decade. An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines, and also there are a large number of herbal medicines whose therapeutic potential has been assessed in a variety of animal models [18]. However, it may seem paradoxical to use animals to study the one organ that most clearly distinguishes humans from animals. Animals, after all, are not capable of many of the more complex functions found in humans, such as advanced language, moral reasoning, or complex learning skills. But many of the basic structures and functions of the brain are common to all animals. Since complex human thoughts are built on a foundation of simpler mental processes that are evident in animals, animal studies can shed light on uniquely human behaviors. Animal research involving the brain has already produced dramatic improvements in human health and well-being, and it promises many more [19]

The plant *Terminalia chebula* also known as *Haritaki* has an esteemed origin in Indian mythology. Its fruits have been reported to treat many diseases such as digestive problems, diabetes, colic pain, chronic cough, sore throat, asthma, bleeding piles, vomiting, gout, etc. Scientifically, it has been proven to have numerous therapeutic effects such as antibacterial activity [20], antioxidant activity [21] antinociceptive activity [22], and anti-inflammatory activity [23]

Chebulinic acid, an *ellagitannin*, isolated from the fruits of *Terminalia chebula* by high-speed counter chromatography however has its own unique properties. Chebulinic acid has therapeutic activities which include antihypertensive activity [24] and antiulcerogenic activity [25]. It has been shown that chebulinic acid has antidepressant-like properties in laboratory mice [26]. However, the mechanisms of action are yet to be elucidated.

So, the primary aim of the present study was to elucidate potential mechanism of action of antidepressant-like activity of chebulinic acid. The specific objectives were to investigate involvement of monoaminergic neurotransmission in the antidepressant-like property of chebulinic acid and to assess its effect on neurogenesis in the hippocampus.

Materials and methods

Animals

Adult Swiss mice of either sex (25-30 g) obtained from Central Animal House, University of Ibadan, Ibadan were used in this study. Each mouse was used once in the study. They were housed in a well-ventilated and a raised mesh-bottom galvanized cage to prevent coprophagy at 12 hours light and dark cycle, at room temperature. They were allowed to have free access to water, and standard diet (Ladokun Feeds, Nigeria) was given *ad libitum*. All procedures here conformed to the Guiding Principle of Research involving laboratory animals, recommended by the Declaration of Helsinki and National Institute of Health [27].

Animals were divided into two main groups for the three behavioural models to be used: monoaminergic system evaluation and adult hippocampal neurogenesis. Each sub-group consists of 6 mice.

Drugs and treatment schedule

The following drugs were used: Sulpiride, a dopaminergic antagonist (50 mg/kg, *i.p*); prazosin, an alpha-1-adrenergic receptor blocker (62.5 µg/kg, *i.p*); atropine, a muscarinic cholinergic receptor blocker (1 mg/kg, *i.p.*); and metergoline a $5HT_2$ receptor blocker (4 mg/kg, *i.p.*). All the drugs used were purchased from Sigma-Aldrich, St. Louis, MO, U.S.A.

All the blockers used were administered 15 minutes before administration of chebulinic acid (20 mg/kg), and animals were subjected to the corresponding tests 45 minutes later. All drugs were freshly prepared before use.

Plant material

The dried fruits of *Terminalia chebula* were purchased from the local market in Lucknow, India, and the authentication was done by the Botany Division, Central Drug Research Institute, Lucknow, Uttar Pradesh, India (Chem. Reg. No. 109).

Extraction of Terminalia chebula

Dried fruits of *Terminalia chebula* after removing the seeds were hammered into small pieces and were placed in glass percolator with 5 litre of ethanol: distilled water (1:1) and allowed to stand at room temperature for 24 hours. The percolate was collected and this process was repeated four times. The combined percolate was concentrated under vacuum using rotary evaporator at 40ºC and weighed. The weight of extract was found to be 430 grams.

Isolation of Chebulinic acid

Size exclusion column chromatography of the n-butanol fraction has been done over sephadex LH 20 using triple distilled water and methanol in varying polarity as mobile phase. Chebulinic acid was isolated from the 40% methanol water fractions with little impurities visible in reverse TLC which was then recrystallized using acetonitrile water (20:80) to afford a pure compound (8 g). The compound gave the phenolic test with ferric chloride solution. Structure elucidation was performed by spectroscopic technique [25]. On the basis of these data, this compound was identified as chebulinic acid which was confirmed by comparison of its physiochemical data with that reported in the literature [28].

Forced swimming test

The Forced Swimming Test (FST) is the most widely used pharmacological *in-vivo* model for assessing antidepressant activity and was performed according to the method of Porsolt *et al.*, [29]. The apparatus consists of a clear plexiglass cylinder (20 cm by 12 cm) filled to a 15 cm depth with water. Water to be used is kept to a temperature (34 ± 1°C). Pre-test: all the animals to be used for the FST were allowed to swim individually for fifteen minutes each, 24 hours before the test day as a pre-test to expose the animals to the new swimming environment, and also to screen-out incompetent ones.

Tail suspension test

The tail suspension test (TST) was performed according to the method described by Steru *et al*, [30]. The mice were individually suspended 60cm above the surface of a table with an adhesive tape placed 1 cm away from the tip of the tail. Immobility duration was recorded for the last 5 minutes during 6-minute test. Mice were considered immobile only

when they hung passively and were completely motionless.

Open Field Test

The assessment of locomotor activity and exploration in a novel environment was done in open filed apparatus. Procedure was same as described earlier [31, 32, 33], with slight modification. It was made of well-painted wood 50 cm by 50 cm by 25 cm. The plain floor of the box was divided into 8 cm, with 16 squares on it. A 20 W white bulb illuminated the apparatus. To determine the activity, mice were placed in the centre square of the open field. Latency to move (in sec) and numbers of square crossed with all four paws were recorded for 5 minutes.

After the 5-minute test, mice were returned to their home cages and the open field was cleaned with 10% ethanol and permitted to dry between tests.

Evaluation of the involvement of Adult Hippocampal Neurogenesis

Pre-treatment:

Animals were divided into six groups of six animals each. Animals were treated with either vehicle or chebulinic acid (20 mg/kg) for 7, 21 or 28 days. In each group animals were classified into two (2) subgroups: one for behavioural studies and the other for immunohistochemical studies. Forced swimming test (FST) and Open field test (OFT) were used to assess the behavioural activities.

Immunohistochemistry (IHC) for paraffin-embedded tissue

Animals were anaesthetized with ketamine at dose 80 mg/kg. Immediately after the animal losing its righting reflexes, we laid mouse on its back and opened the thorax carefully to avoid bleeding. We cut quickly through rib cage and removed diaphragm to access the heart. Syringe filled with normal saline was injected into the left ventricle and at the same time we cut open the right atrium for drainage, allowing the normal saline to be slowly but constantly perfused into heart. After most of the blood had been flushed out, we removed the syringe with normal saline and we inserted the syringe filled with 4% PBF into the same puncture in the left ventricle. The onset of fixation is noted by the "formalin dance", in which the body of the animal moved uncontrollably. A signal of proper fixation is when the body part feels harder than normal and paleness of the body. After proper fixation, the animal skull was cut open, brain harvested and put in vial containing 4% PBF for 72 hours before slide preparation. Brain tissues were further processed into paraffin blocks and 40 µm section were cut and made into slides for immunohistochemistry.

Slides were baked in the oven at 50-60 degrees for 20-30 minutes. This is to warm the paraffin wax on the slides, so that it will remove easily. Slides were later de-paraffinised using xylene twice for 5 minutes each, and afterwards in equal parts xylene and alcohol. Tissues were hydrated by putting in the following solution 5minutes each 100% ethanol, 90% ethanol, 70% ethanol, 50% ethanol and Distilled water. The tissues were further put in microwave to open the binding sites. The tissues were blocked using methanol/ 3% H_2O_2 for 20 minutes. This is to block the background for proper staining. This process blocks endogenous peroxidases. Tissues were later incubated in primary antibody (Thermo Fisher Scientific Ki-67 Monoclonal Antibody) in a humidity chamber overnight, at a dilution of 1: 200

On the next day, the slides were rinsed off the antibody and incubated with the secondary antibody using the (Thermo Fisher Scientific, Quanto Detection System protocols) and other steps were followed according to the manufacturer's instructions. The slides were covered with permount on the bottom in the fume cupboard and slides prepared for viewing under the microscope.

Statistical analysis

The results obtained were expressed as means ± S.E.M. Variance was analysed using One-way Analysis of Variance (ANOVA), followed by Newman-Keuls' post hoc tests. $P < 0.05$ was considered to be statistically significant. All statistical analyses were done by using software Prism, Version 5 (Graph-Pad Software Inc., San Diego, CA, USA).

Results

Involvement of monoaminergic system on immobility in antidepressant-like activities of chebulinic acid in forced swimming test

The effects of the antagonists and imipramine (60 mg/kg) on immobility in the forced swimming test of mice when compared to chebulinic acid are shown in figure 1. ` 20 mg/kg of chebulinic acid significantly reduced immobility (74.20 ± 6.42, $^{\#}p < 0.05$) in comparison with control (132.80 ± 18.75). However, pre-treatment with the antagonists: prazosin (62.5 µg/kg *i.p.*), an alpha-1-adrenergic receptor blocker; atropine (1 mg/kg), a muscarinic cholinergic receptor blocker; and sulpiride (50 mg/kg), a dopamine-D_2 receptor blocker did not significantly reverse decreased immobility as observed with group treated

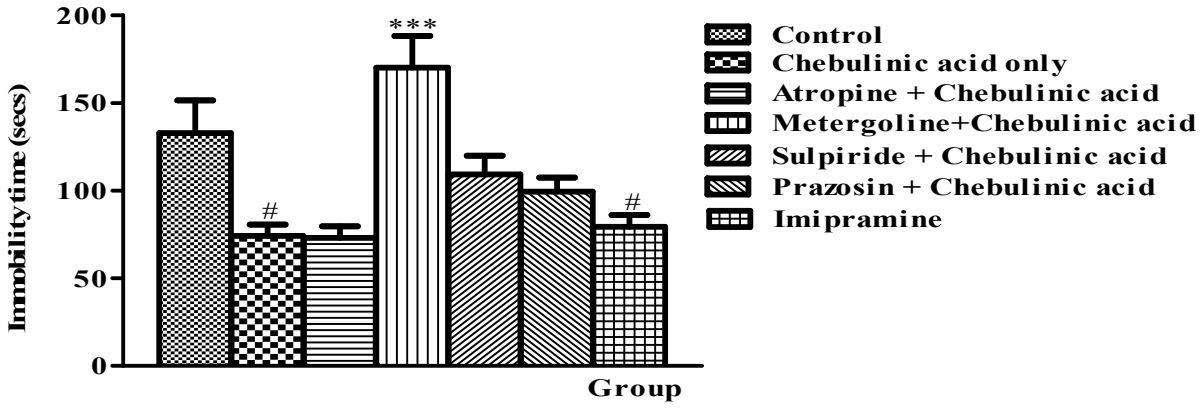

Fig. 1: *Effect of chebulinic acid, monoamine antagonists and imipramine on immobility in forced swimming test in mice. Data represent mean ± S.E.M. of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test. Statistical significant at ***p < 0.001, in comparison to chebulinic acid only (20 mg/kg), #p < 0.05 when compared to control.*

Fig. 2: *Effect of chebulinic acid, monoamine antagonist and imipramine on climbing in forced swimming test in mice. Data represent mean ± S.E.M of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test. **P < 0.01 in comparison to chebulinic acid (20 mg/kg).*

with 20 mg/kg of chebulinic acid only. Metergoline (4 mg/kg), 5-HT_2 receptor blocker significantly increased immobility (170.20 ± 18.10) when compared with chebulinic acid only-treated group. Imipramine (60 mg/kg) significantly reduced immobility (79.40 ± 6.72, $^{\#}p < 0.05$) when compared to the control group.

Involvement of monoaminergic system on climbing in antidepressant-like activities of chebulinic acid in forced swimming test

The effects of antagonists and imipramine on climbing in the forced swimming test of mice are shown in figure 2. There was no significant change in the climbing time of animals pre-treated with antagonists and imipramine when compared with chebulinic acid (20 mg/kg) only. However, there was a significant increase on climbing time ($p < 0.01$) in vehicle treated animals when compared to chebulinic acid only treated animal.

Involvement of monoaminergic system on swimming in antidepressant-like activities of chebulinic acid in forced swimming test

The effects of antagonist and imipramine on swimming in the forced swimming test of mice are shown in Figure 3. There was a significant reduction in swimming time of control group animals when compared to chebulinic acid (20 mg/kg)-treated animals. Similarly, intraperitoneal injection of metergoline (4 mg/kg, *i.p.*); a 5-HT_2 receptor blocker

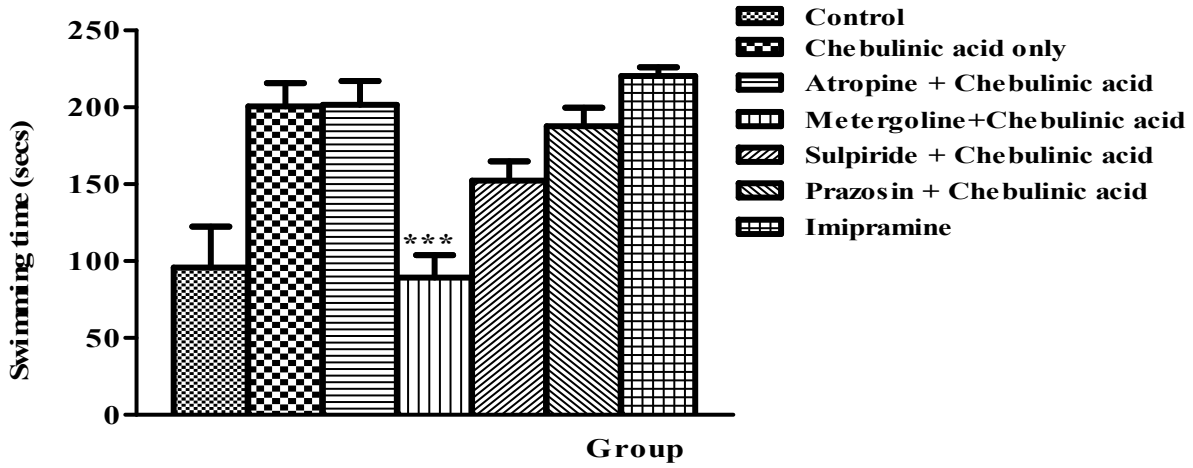

Fig. 3: *Effects of chebulinic acid, monoamine antagonist and imipramine on swimming in forced swimming test in mice. Data represent mean ± S.E.M of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test. ***P < 0.001 in comparison with chebulinic acid (20 mg/kg).*

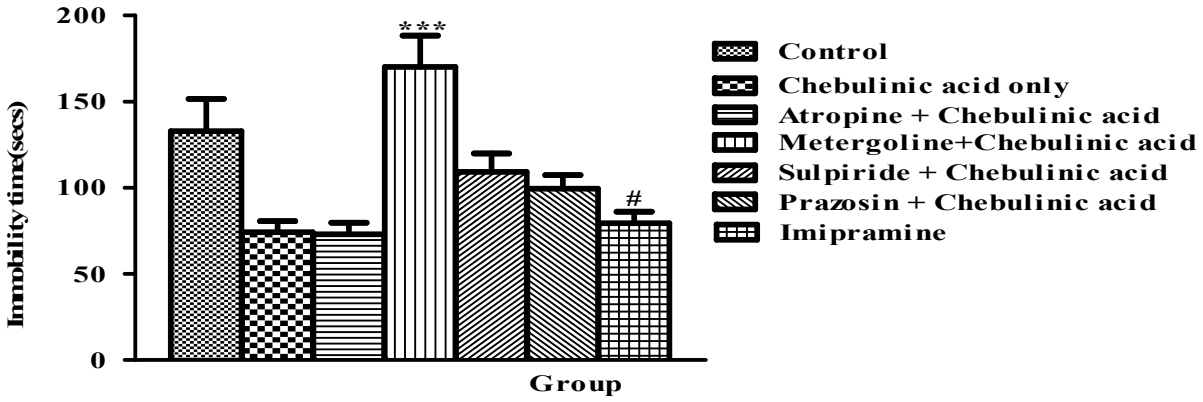

Fig. 4: *Effects of chebulinic acid, monoamine antagonist and imipramine on immobility time in tail suspension test in mice. Data represent mean ± S.E.M of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test, ***p < 0.001 in comparison with chebulinic acid (20 mg/kg). #P < 0.05 in comparison with control.*

significantly (***$p < 0.001$) reduced swimming (89.33 ± 14.47) when compared to chebulinic acid only group (200.70 ± 15.12).

Involvement of the monoaminergic system in antidepressant-like activities of chebulinic acid in tail suspension test.

The results showed the effects of antagonist and imipramine on immobility time in the tail suspension test (Figure 4); imipramine significantly reduced immobility (79.40 ± 6.77) when compared to control ($^{\#}p < 0.05$). Animals pre-treated with metergoline (antagonist) reversed mobility (170.20 ± 18.10) significantly (***$p < 0.001$) when compared with animals treated with 20 mg/kg of chebulinic acid only, while animals pre-treated with other antagonists did not produce any significant changes.

Involvement of the monoaminergic system on line crossing in antidepressant-like activities of chebulinic acid in an open field test.

The results as shown in figure 5 showed the effects of antagonists and imipramine on line crossing in an open field test. One-way ANOVA revealed that there is a significant decrease in the number of line crossed in animals pre-treated with antagonists when compared to chebulinic acid (20 mg/kg) only treated group.

Fig. 5: *Effects of chebulinic acid, monoamine antagonists and imipramine on line crossing in an open field test in mice. Data represent mean ± S.E.M of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test. **P < 0.01, ***p < 0.001 in comparison with chebulinic acid (20 mg/kg) only.*

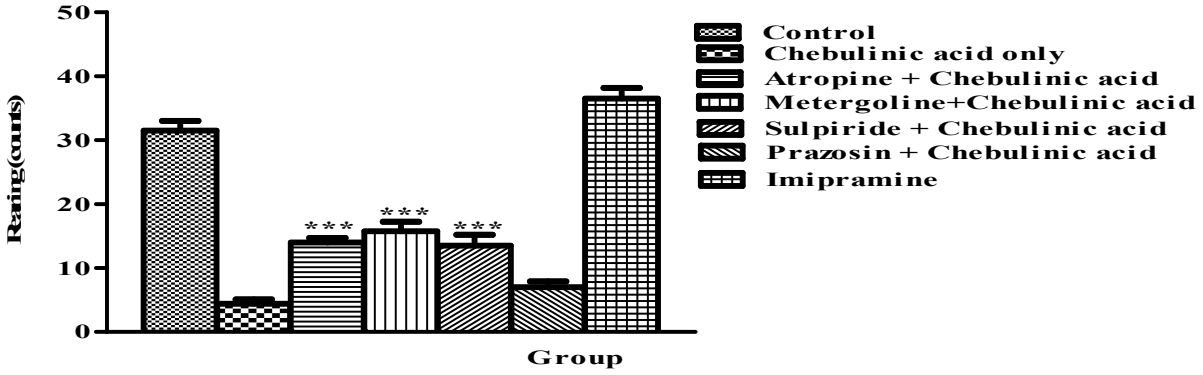

Fig. 6: *Effects of chebulinic acid, monoamine antagonists and imipramine on rearing in an open field test in mice. Data represent mean ± S.E.M of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test. ***P < 0.001 in comparison with chebulinic acid (20 mg/kg).*

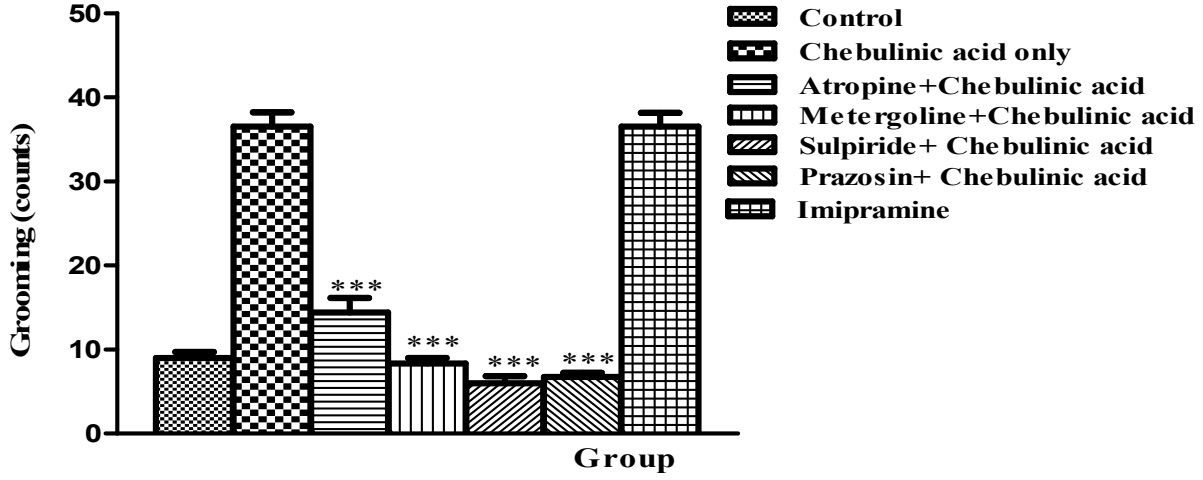

Fig. 7: *Effects of chebulinic acid, monoamine antagonist and imipramine on grooming in an open field test in mice. Data represent mean ± S.E.M of 6 mice during the 5-minute test session. Comparisons were made using one-way ANOVA followed by Newman-Keuls' post hoc test. Statistical significance was at ***p < 0.001 in comparison to chebulinic acid (20 mg/kg).*

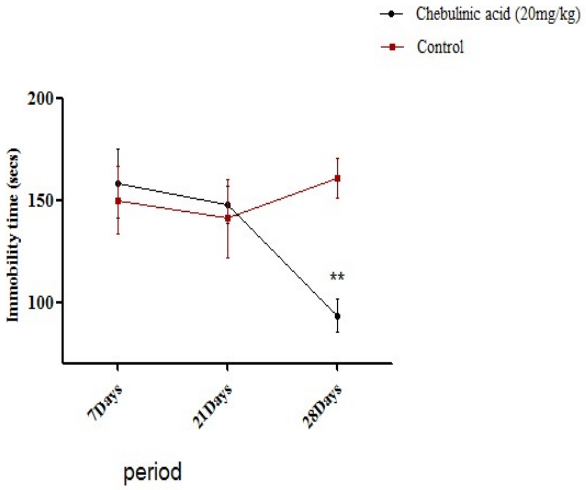

Fig. 8: *Effects of chronic administration of chebulinic acid on immobility time in forced swimming test. Data represent mean ± S.E.M. Comparisons were made using Two-way ANOVA followed by Bonferroni post hoc test. **p < 0.01 when compared to control.*

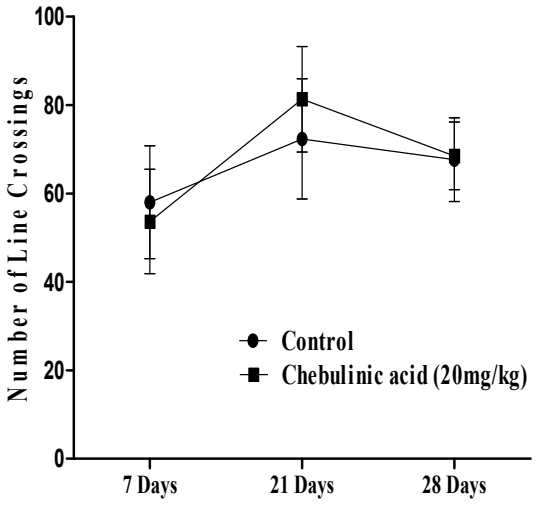

Fig. 9: *Effects of chronic administration of chebulinic acid on the number of line crossing in an open field test. Data represent mean ± S.E.M. Comparisons were made using two-way ANOVA followed by Bonferroni post hoc test.*

Involvement of the monoaminergic system on rearing in antidepressant-like activities of chebulinic acid in an open field test

Figure 6 showed the effects of antagonists (sulpiride, 50 mg/kg, *i.p.*; prazosin, 62.5 µg/kg, *i.p*; atropine, 1 mg/kg, *i.p.*; and metergoline, 4 mg/kg, *i.p.*) and imipramine on rearing frequency in an open field test. Pre-treatment with all monoamine antagonists, except prazosin significantly increased (***p < 0.001) rearing frequency, when compared with chebulinic acid (20 mg/kg) group.

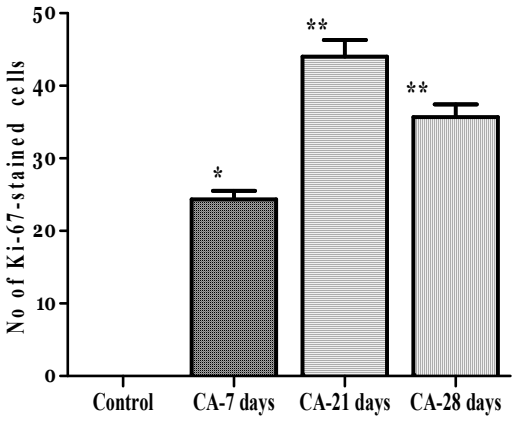

Fig. 10: *Effect of chronic administration of vehicle and Chebulinic acid (20 mg/kg) on the number of antibody Ki-67 positively-stained nuclei of proliferating cells in the dentate gyrus of hippocampus of mice. Data represent mean ± S.E.M. Comparisons were made using One-way ANOVA followed by Newman-keuls' post hoc test. *p < 0.05, **p < 0.01 when compared to control.*

Involvement of the monoaminergic system on grooming in antidepressant-like activities of chebulinic acid on open field test.

The results showed the effects of antagonists sulpiride (50 mg/kg, *i.p.*); prazosin (62.5 µg/kg, *i.p.*); atropine (1 mg/kg, *i.p.*) and metergoline (4 mg/kg, *i.p.*) and imipramine on grooming time in an open field test (Figure 7), there was a significant reduction ($p<0.001$) between animals pre-treated with monoamine antagonist when compared to chebulinic acid 20 mg/kg.

Involvement of chronic administration in the antidepressant potentials of chebulinic acid in forced swimming test

The results show the effects of chronic administration of chebulinic acid for 7, 21 and 28 days (Figure 8). There was a significant reduction in immobility (**p < 0.01) in the group treated with chebulinic acid at 28 days when compared with its respective control treated groups.

Involvement of chronic administration in the antidepressant potentials of chebulinic acid in an open field test

The results show the effects of chronic administration of chebulinic acid for 7, 21 and 28 days (Figure 9). There was no significant reduction in the number of line crossing in the groups pre-treated for 7, 21 and 28 when compared with their respective control groups.

Immunohistochemistry

In the immunohistochemistry analysis, by labelling with Ki-67 antibody; the morphology of the Ki-67-positive nuclei in the hippocampal area sections was examined.

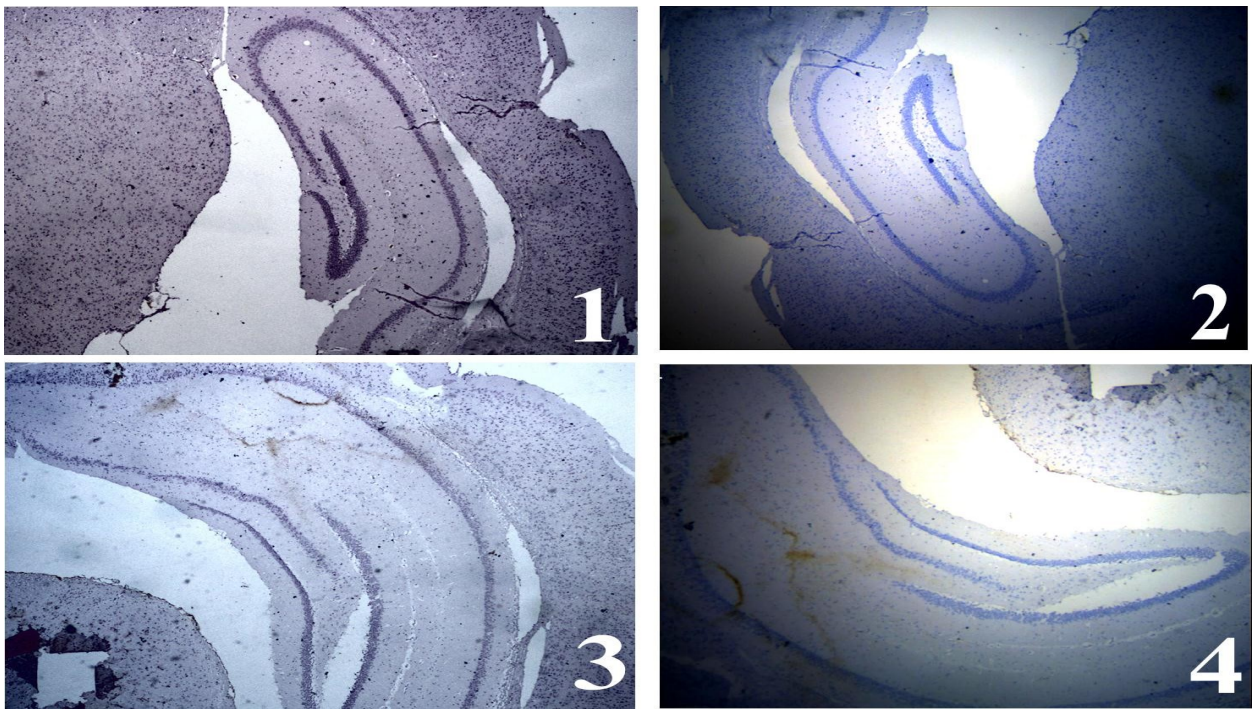

Fig.11: ***Fig. 1*** *Immunohistochemistry of brain tissue stained with Ki-67 antibody showing areas of the hippocampus of mouse treated with vehicle, Magnification (x40).* ***Fig. 2-4:*** *Immunohistochemistry of brain tissues stained with Ki-67 antibody, showing areas of the hippocampus of mice treated with chebulinic acid for 7, 21 and 28 days respectively, Magnification (x40).*

Discussion and conclusion

Depression is a serious mental illness with a high global occurrence, and the pathophysiology of depression is linked to insufficiency in the availability of monoamines in affected individuals. Available antidepressants show their effects by regulating the monoamines at the synaptic levels [12].

The possible mechanisms of antidepressant effect were investigated using 5-hydroxytryptamine 5-HT_2, alpha-1-adrenergic, dopaminergic, and muscarinic cholinergic receptor antagonists. Hence, this study explored the impact of monoaminergic antagonists on the antidepressant-like effect of chebulinic acid. The effective dose of chebulinic acid in Forced Swimming Test (FST) and Tail Suspension Test (TST) was 20 mg/kg [26].

The Forced Swimming Test (FST) and Tail Suspension Test (TST) were used in mechanistic studies due to its increased sensitivity [34, 35, 36]. The mobility time of mice in the FST and TST is increased by the majority of anti-depressants including tricyclic and atypical anti-depressants, Monoamine oxidase (MAO) inhibitors and selective serotonin reuptake inhibitors (SSRI) [29, 37], and their effectiveness correlates significantly with clinical potency [38]. The modified Porsolt's forced swimming test was developed [36], which had good sensitivity to detect the effects of antidepressants in rodents. This test involves measuring the immobility, swimming behaviour and climbing behaviour of rodents upon subsequent exposure to swimming.

A large number of evidence indicates that the serotonergic system is closely implicated in the pathogenesis of depression and in the mechanism of action of antidepressants [39, 40]. Most of the prescribed antidepressants directly affect serotonin turnover in the brain [41], inhibits serotonin reuptake and also interact with 5-HT_{1A} and 5-HT_2 receptors [35]. Our earlier study shows that chebulinic acid at 20 mg/kg dose reduced immobility in laboratory mice which suggests its antidepressant activities. Chebulinic acid also increased swimming suggesting an involvement of 5-hydroxytryptaminergic neurotransmission in its antidepressant-like activity. However, this reduced immobility was reversed when metergoline, a selective $5HT_2$ receptor antagonist was administered to the animals before

treatment with chebulinic acid. Therefore, it may possibly produce its antidepressant effects through interaction with serotonergic system.

Experimental and clinical studies indicate that the noradrenergic system is strongly implicated in the pathophysiology of depression [42, 43]. Pre-clinically, the alpha-1-, and alpha-2-adrenoceptors have been shown to underlie some of the antidepressant-like responses of drugs in behavioural models of depression [44, 45, 46]. In this study, pre-treatment with prazosin (4 mg/kg), an alpha-1-adrenergic receptor antagonist did not reverse the reduced immobility that was observed when 20 mg/kg of chebulinic acid was administered only in Tail Suspension Test (TST) paradigms. This result indicates that chebulinic acid may not exert its effect in TST paradigms by interacting with alpha-1-adrenoceptors.

The dopaminergic system is also implicated in regulation of mood. Since dopamine is the main neurotransmitter involved in the mesolimbic reward pathway, it has been proposed that an increase in dopaminergic neurotransmission might counteract the anhedonic effect, which is a prominent symptom of depression [14]. In this work, we observed that sulpiride; (a dopaminergic antagonist) did not reverse the decrease in immobility time observed when chebulinic acid was administered alone in TST. This suggests that the dopaminergic system may not be involved in the potentiation of the antidepressant-like activities of chebulinic acid.

The cholinergic system plays a major role in the regulation of various central nervous system functions, such as arousal, attention, cognition and memory. Cholinergic dysfunctions may account for the development of cognitive symptoms during the course of depression. Administration of atropine, (a muscarinic cholinergic antagonist) could not reverse the reduced immobility time induced by chebulinic acid in the TST, suggesting that it is not involved in its antidepressant effects.

"Emotional" rats, so termed, [47, 48, 49] show high defecation, little motor activity and low levels of grooming and rearing. They avoid the centre of the field. Their engagement in their immediate surroundings may be termed passive and limited. The frequency of line crossing and rearing was lower in the monoamine antagonist pre-treated groups when compared with the control and this was in accordance with [50] which stated that high frequency of line crossing indicates increased locomotion and exploration or a lower level of anxiety.

Long-term administration of chebulinic acid also increased mobility time in animals. This may be in agreement with the time course required for the therapeutic efficacy of conventional antidepressant. It is established that antidepressants stimulate the expression of neurotropic factors, increase the density, the length and the arborization of the dendrites [51] and enhance the synaptic plasticity [51, 52, 53]. Also, chronic administration of chebulinic acid did not alter locomotor activities of the animals, ruling out the possibility that a locomotor impairment might have accounted for the increase in immobility in the FST that was observed in the chebulinic acid-treated rats.

The involvement of adult hippocampal neurogenesis was also studied in the brain. Neurogenesis in the adult dentate gyrus is a process involving many stages. It can broadly be defined as the cell proliferation, migration, differentiation and ultimately the maturation of granule cells. By using an antibody, this study aimed to characterize this phenomenon in the adult hippocampus. Cell proliferation is a pre-requisite for adult neurogenesis, and Ki67 protein; a marker for cell proliferation was expressed in the hippocampal cells. In this study, it was observed that there was an increase in the number of proliferating cells in the dentate gyri of the hippocampi (Figure 10) of mice treated with chebulinic acid for 7, 21 and 28 days. It can then be suggested that chronic administration of chebulinic acid may lead to the production of new neurons in the hippocampus of adult mice, there by indicating a probable antidepressant effect.

In conclusion, antidepressant potential of chebulinic acid may be mediated by serotoninergic systems, and partly by hippocampal neurogenesis.

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Attitude and practice of medical professionals towards prenatal ultrasound screening for congenital anomalies

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Abstract

Background: Congenital anomalies are among the leading causes of perinatal morbidity and mortality worldwide. The advent of prenatal ultrasound screening for anomalies has profoundly affected perinatal care positively. This study was aimed at obtaining the views of the practitioners which usually constitute an integral part of the success of any practice, including screening for congenital anomalies.

Method: This is a cross-sectional descriptive survey conducted during a practical ultrasound training course. It involved the use of a self-administered semi-structured questionnaire. The responses were codified and relevant statistical analysis was performed.

Results: Seventy-seven medical professionals participated in the workshop and filled the questionnaires. 71% of the respondents were Consultant Obstetricians. Only few (13%) respondents refer patients for routine fetal anomaly scans while 70.1% reserved referral to only cases at high risk of congenital malformations. Informed consent-mostly verbal (90%)-for fetal anomaly scan was routinely obtained by less than half (46%) of the respondents. Protocols to guide practice, diagnosis, decision and management of congenital anomalies *in utero* existed in only few centers. 75% of the respondents will advocate pregnancy termination for lethal fetal anomalies, while 27.3% will advocate pregnancy termination for anomalies that will reduce quality of life.

Conclusion: Since congenital anomalies is one of the leading causes of perinatal morbidity and mortality, there is need for established protocols to guide the practice, diagnosis, decision and management of these congenital anomalies found in pregnancy so as to improve the quality of care.

Keywords: *Congenital anomalies, prenatal ultrasound, medical professionals*

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Résumé

Contexte: Les anomalies congénitales sont parmi les principales causes de morbidité et de mortalité périnatales dans le monde entier. L'avènement du dépistage par ultrasons prénatal pour les anomalies a profondément affecté les soins périnatals positivement. Cette étude visait à obtenir les points de vue des praticiens qui constituent généralement une partie intégrante du succès de toute pratique, y compris le dépistage d'anomalies congénitales.

Méthode: Il s'agit d'une enquête descriptive transversale réalisée lors d'un cours pratique d'échographie. Il s'agissait de l'utilisation d'un questionnaire semi-structuré auto-administré. Les réponses ont été codifiées et des analyses statistiques pertinentes ont été effectuées.

Résultats: soixante-dix-sept professionnels de la santé ont participé à l'atelier et ont rempli les questionnaires. 71% des répondants étaient des obstétriciens consultants. Seuls quelques répondants (13%) se réfèrent aux patients pour les analyses d'anomalie fœtale de routine, tandis que 70,1% ont réservé la recommandation uniquement aux cas à risque élevé de malformations congénitales. Le consentement éclairé - principalement verbal (90%) - pour l'analyse de l'anomalie fœtale a été habituellement obtenu par moins de la moitié (46%) des répondants. Les protocoles pour guider la pratique, le diagnostic, la décision et la gestion des anomalies congénitales dans l'utérus n'existaient que dans quelques centres. 75% des répondants préconiseront la fin de la grossesse pour les anomalies fatales mortelles, tandis que 27,3% préconiseront la fin de la grossesse pour des anomalies qui réduiront la qualité de vie.

Conclusion: Étant donné que les anomalies congénitales sont l'une des principales causes de morbidité et de mortalité périnatales, il est nécessaire de disposer de protocoles établis pour guider la pratique, le diagnostic, la décision et la prise en charge de ces anomalies congénitales découvertes pendant la grossesse afin d'améliorer la qualité des soins.

Mots-clés: *Anomalies congénitales, échographie prénatale, professionnels de la santé*

Introduction

Congenital anomalies can be defined as structural or functional anomalies (e.g. metabolic disorders) that occur during intrauterine life and can be identified prenatally, at birth or later in life [1]. Congenital anomalies are among the leading causes of perinatal morbidity and mortality worldwide [2]. According to the WHO, worldwide "around 1% of infants have a major congenital anomaly" with a greater proportion occurring in developing countries [1]. It is estimated that about 10% of neonatal deaths could be attributed to congenital anomalies and about 94% of severe congenital anomalies occur in the developing world [1, 3].

The development of prenatal screening for anomalies has profoundly affected perinatal care positively. In the developed world, ultrasound has been the preferred method of imaging fetal abnormalities for several decades. This is because of its advantages, including safety for the mother and fetus, cost-effectiveness, easy accessibility and real time imaging [4,5]. For many anomalies, early prenatal diagnosis provides the opportunity to influence perinatal management favorably by changing the site of delivery for immediate postnatal treatment; altering the mode of delivery to prevent hemorrhage or dystocia; early delivery to prevent ongoing fetal organ damage; or treatment *in utero* to prevent, reverse, or minimize fetal organ injury as a result of a structural defect [6,7].

The prenatal diagnosis and announcement of a fetal anomaly to a couple has far reaching implications. Skillful counseling of such a couple is therefore crucial toward obtaining the best possible outcome in the given circumstance. The multidisciplinary team has the responsibility to provide sufficient information about the anomaly to permit the parents to make informed decision [8]. While many have argued that counseling should be reserved for genetic counselors in the case of fetal anomalies, this role is being increasingly assumed by clinicians generally, especially in regions with dearth of qualified genetic counselors [9].

Interruption of pregnancy is one of the options the clinician-counsellor can offer a couple when severe foetal anomaly is diagnosed during prenatal sonographic screening. The benefits of such an extreme intervention- like lower perinatal mortality rate and huge cost savings by avoidance of long-term care for major malformations- outweigh any other consideration significantly [10,11].

However, uncertainties about the full nature or extent and exact prognosis of any detected anomaly coupled with limited facilities for genetic diagnostics cast doubt on the clinical utility of prenatal sonographic screening for congenital anomalies in low resource countries. These perceived inadequacies also pose a challenge to evidence-based counseling by the practitioner.

Ultimately, the view of the referring medical practitioner is integral to the success of any practice including sonographic screening for congenital anomalies. While prenatal diagnosis is gaining traction in our environment, the attitude of the doctors towards the practice is crucial for its widespread adoption in order to improve quality of care and aid design of relevant programs for necessary interventions. Therefore, this article aims at assessing the attitude of medical professionals to prenatal ultrasound screening for congenital anomalies.

Materials and methods

This cross-sectional descriptive survey was conducted during a practical obstetric ultrasound training course organized by the Fetal Medicine Unit of the University of Benin Teaching Hospital, Benin City, Nigeria. The ISUOG [International Society for Ultrasound in Obstetrics and Gynaecology] approved course which covered basic and advanced obstetric ultrasound scan including fetal anatomical survey and Doppler velocimetry attracted participants from Nigeria and Ghana in West Africa.

The survey involved the use of a self-administered semi-structured questionnaire (appendix I) which was applied on the participants of the training programme. The questionnaire sought information on the biodata, clinical practice level and scope of sonographic practice of the respondents. It then sought information on the attitude and current practice of the respondents as it pertains to fetal anomaly ultrasound scans as well as termination of pregnancies following ultrasound diagnosis of different severities of fetal anomalies. The responses were then coded into the computer and relevant analysis carried out.

Results

Seventy-seven medical professionals participated in the workshop and filled the questionnaire. 58(75.3%) were males while 19(24.7%) were females. The age range was between 20 and 60 years, with the highest number 32(41.6%) of participants in the 40-50years age group.

Sixty-three participants (81.8%) worked in tertiary hospitals while the others worked in General or private hospitals. Consultant Obstetricians constituted 71% of the participants while the

remaining 21% included resident doctors, general practitioners, sonographer and nurses.

Figure 1 is a graphical representation of the years of experience since graduation of the respondents. About half of the respondents were between 5 and 15 years post-graduation. The geographical spread of the respondents as presented in figure 2 shows that all the geopolitical zones of Nigeria were represented at the course with majority from the South-South and North-Central geopolitical zones of Nigeria while a handful came from Ghana.

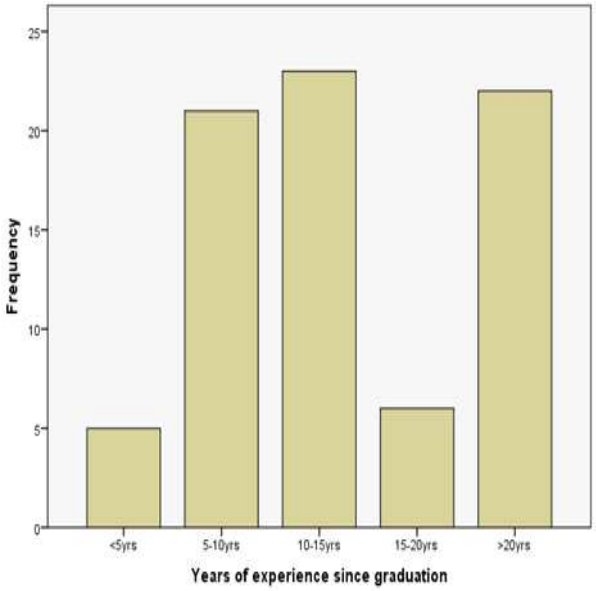

Fig. 1: Distribution of the years of experience of the participants

Table 1 shows the attitudes of respondents toward prenatal diagnosis of congenital anomaly by ultrasound scan. A very high proportion (88%) of respondents feel that fetal anomaly scans should be done between 18 and 22 weeks gestational age while about one in ten respondents feel it should be done earlier. Eighty-eight percent (88%) of respondent did not consider expertise in 3D and 4D ultrasound scans to be a sure proof of high proficiency in fetal anomaly scan. All respondents believe fetal anomaly scan is inadequate to rule out congenital malformations in all cases even in the best hands.

The perspectives of the respondents on termination of pregnancy for fetal anomalies and awareness of existing abortion law are seen in table 2. While 79.2% of the respondents will advocate pregnancy termination for lethal fetal anomalies, 27.3% will advocate pregnancy termination for anomalies which may reduce the quality of life of the baby. Almost all respondents were aware of the existence of an abortion law in their country.

Table 3 shows the current practices of respondents with regards to preparation, procedure, and communication as it pertains to fetal anomaly scans. Only few (13%) respondents refer patients for routine fetal anomaly scans while more than two-thirds (70.1%) reserve referral for fetal anomaly scans to only cases at high risk of congenital malformations. Informed consent for fetal anomaly

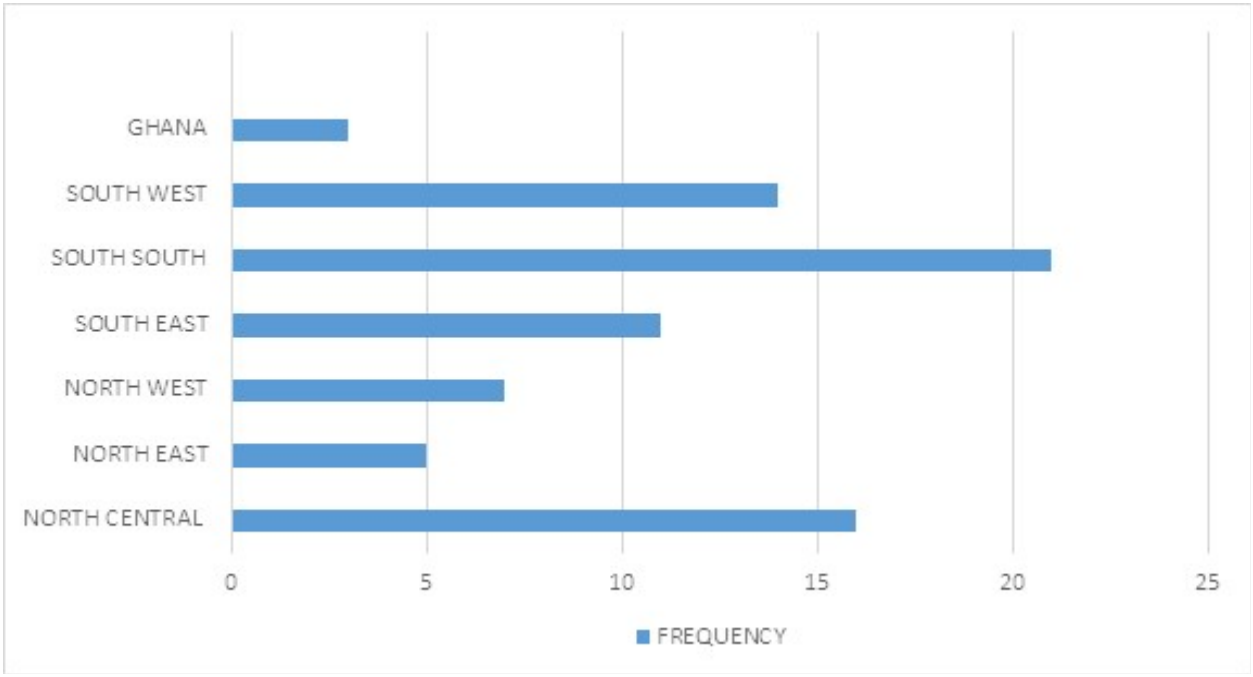

Fig. 2: Composition of participants by geographical zones

Table 1: Attitude of respondents to fetal anomaly scan

Question	n (%)
At what GA do you believe fetal anomaly scan is best done?	
<18wk	8(10.4)
18-22wk	68(88.3)
>22wk	1(1.3)
Is expertise in 3D or 4D ultrasound a sure proof of competence in FAS?	
Yes	9(11.7)
No	68(88.3)
Does fetal anomaly scan rule out presence of all fetal anomalies?	
Yes	0(0)
No	77(100)

GA: Gestational age
FAS: Fetal anomaly scan

Table 2: Perspectives of respondents on termination of pregnancies with fetal anomalies

Question	n(%)
Are fetal anomalies common in Nigeria?	
Yes	58(75.3)
No	19(24.7)
TOP for lethal anomalies?	
Yes	61(79.2)
No	16(20.8)
TOP for anomalies that may reduce quality of life?	
Yes	21(27.3)
No	56(72.2)
TOP for confirmed Down syndrome?	
Yes	27(35.1)
No	50(64.9)
Knowledge about abortion law?	
Yes	73(94.8)
No	4(5.2)

TOP: Termination of pregnancy

scan was routinely obtained by less than half (46%) of the respondents. However, verbal consent was the usual practice by over 90% of those who claim to routinely obtain informed consent for fetal anomaly scan. Majority (65%) of the respondents practice where there is lack of established protocol for communicating results of fetal anomaly scan to the patient and referring physician. Similarly, established protocol for managing pregnancies with ultrasound diagnosed fetal anomaly or expertise for prenatal therapeutic intervention were not available in the practice of most of the respondents.

Table 3: Practice of fetal anomaly scan by respondents

Question	n (%)
Is patient referral for FAS routine for all pregnancies	
Yes	10(13)
No	67(87)
Is patient referral for FAS routine for only high risk pregnancies	
Yes	54(70.1)
No	23 (29.9)
What type of informed consent do you routinely obtain for FAS?	
Written	3(3.9)
Verbal	32(41.6)
None	42(54.5)
Is there established protocol for communicating result of FAS?	
Yes	12(15.6)
No	65(84.4)
Is there established protocol for managing pregnancies with fetal anomalies?	
Yes	8(10.4)
No	69(89.6)
Is there expertise for therapeutic prenatal intervention?	
Yes	8(10.4)
No	69(89.6)

FAS: Fetal anomaly scan

Discussion

This study captures the views of a cross-section of medical professionals as it pertains to the practice of prenatal sonographic screening for fetal anomalies in Nigeria. The sample represents high level manpower which provides prenatal and perinatal care in the country. The findings of this study therefore reflect some of the perceptions which shape the practice of fetal anomaly scans, and are worthy of consideration in order to curb the growing mortality attributable to congenital anomalies in Nigeria.

Fetal anomaly scans are normally done between 18 and 22 weeks of gestation, usually with 2D grey scale ultrasound. The use of 3D and 4D ultrasound has additional utility for better detection of facial abnormalities [12,13] . Fetal anomaly scan, like all ultrasound studies, is however observer dependent and may not be able to detect all possible fetal structural abnormalities. The perspectives of a high proportion of the respondents in this study align with the aforementioned facts. This result is not unexpected given the high representation of

specialist obstetricians in the study sample. Further studies may be required to find out the perspectives of middle and lower level obstetric practitioners, such as nurses, midwives and community health workers, toward fetal anomaly scans.

The low rate of referral for routine prenatal sonographic screening service found in this study may be related to the level of availability of such service to the population. Bulas DI [2] opined that "regional differences in the availability of prenatal diagnosis can influence mortality rates". Data from this study suggests that fetal anomaly scan is not yet routinely done in Nigeria whereas nearly every pregnant woman in the US undergoes at least one such examination in the second trimester [13]. Lack of access to advanced obstetric ultrasound service may therefore account for the increasing perinatal manifestation of congenital anomalies in developing nations. Furthermore, this study identified areas in need of improvement with regards to the current practice of the respondents offering fetal anomaly scan. For instance, less than half of the respondents obtain informed consent, mostly verbal, for fetal anomaly scan. Also, lack of established protocol guiding the conduct and management of fetal anomaly scans in this survey was the norm in the practice of majority of the respondents, with only few exceptions. This suggests wide variations in quality of practice which has no place in modern clinical practice and tends to produce suboptimal outcomes for all involved [12,13]. Future studies with more robust design are therefore necessary to perform a root cause analysis on the inferred low quality of antenatal care in order to improve upon the local practice of prenatal diagnosis in Nigeria.

It has been reported that prenatal diagnosis and pregnancy termination for lethal congenital anomalies is associated with reduced infant mortality [14]. This study has shown a predominantly liberal attitude among the respondents to termination of pregnancy for diagnosed lethal fetal anomalies. This favorable disposition is highly encouraging in this environment with restrictive abortion laws even for lethal fetal anomalies. The large percentage (75%) in support of termination of pregnancy in this survey is in consonance with previous reports from the high income countries where the abortion laws are more liberal [15,16]. The reported attitude of respondents toward termination of pregnancy however depends on the presumed severity of the anomaly detected, being more restrictive with less severe anomalies. Almost 75% of the respondents will not offer pregnancy termination if the detected anomaly is severe but non-lethal. This finding is in line with the findings of previous studies [15-17]. These studies also found that the gestational age at diagnosis also has significant effect on this attitude. Despite the attitude of the respondents, it was reported that the majority however will comply with the patients' wishes on management including surgical obstetric interventions [15,17].

The abortion law is generally well known by most practitioners. The abortion law in Nigeria is highly restrictive and only allows termination of pregnancy to save the life of the mother [18]. In this strict context, fetal factors are not considered. While at present most centers are not practicing fetal diagnosis, as practice and training in Fetal Medicine improves, more of the unfavorable diagnosis of fetal anomalies will be made. Based on the attitude expressed by the respondents, there may be need for spirited advocacy to review the abortion law for fetal reasons.

This study has only performed and reported a baseline survey of attitudes of practitioners towards termination of pregnancy for fetal reasons. This is expected to be a prelude to a more detailed survey to assess factors responsible for the attitudes. However, previous studies reported the influence of religious beliefs on the attitudes. Garell *et al* discussed extensively the ethical dilemma and moral conflict involved in counseling and dealing with issues of congenital fetal anomaly, as reported in a qualitative survey of maternal health practitioners. They also raised the issues of the cost of caring for the child with fetal anomalies and the emotional, social and financial stress it imposes on the family and society [19]. This is worse in the low income countries where there is lack of social services even for the children with handicaps.

Overall, the findings from this study highlight the need for improvement of training in Fetal Medicine and prenatal diagnosis. It also reiterates the need for review of the abortion laws in Nigeria as detailed in a recent editorial commentary [20] especially on issues of lethal fetal anomalies. The ISUOG approved training offered by the Fetal diagnostic center in Benin City, Nigeria, attempts to contribute in a modest way to fill the gap in training and experience [21]. There is however need for an in-depth survey of the knowledge, attitude and practice of medical professionals at all levels of care toward prenatal ultrasound diagnosis in order to improve pregnancy outcomes in Nigeria, and this is underway.

Conclusion

Since congenital anomalies is one of the leading causes of perinatal morbidity and mortality, there is need for established protocols to guide the practice,

diagnosis, decision and management of these congenital anomalies found in pregnancy so as to improve the quality of care.

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Appendix 1

Fetal anomaly knowledge, attitude and practice survey

Dear Respondent,

Kindly spare a few minutes to complete this questionnaire on fetal anomaly, your answer would be treated in strict confidence, Thank you.

A

Demographic Data

Study Number_____

Age (YRS) (1) <20(2)20-30(3)30-40(4)40-50(5)50-60(6)>60

Sex :(1) M (2) F

Marital Status (1) Married (2) Single (3) Widowed (4) Divorced

Occupation _____

Place of Work (1) Public (2) Private

Level of Practice (1) Primary (2) Secondary (3) Tertiary

Religion _____

Ethnicity _____

Years of experience since graduation: (1)< 5 yrs (2) 5-10 yrs (3) 10-15 yrs (4) 15 -20 yrs (5)>20yrs

B

Knowledge of fetal anomaly

1 Do you think fetal anomalies are common in this country (1) Yes (2) No

2 In which of the following conditions do you think fetal anomalies are commonly detected

A Elderly primigravida

B Previous baby with congenital anomaly

C Diabetic mellitus

D Hypertension

E Genetic abnormality

F Sickle cell diseases

G No known predisposing factor

3 Which system do you think is most commonly affected by anomalies

A Central nervous system

B Cardiovascular system

C Musculoskeletal

D Genitourinary

E Gastrointestinal system

F Maxillofacial system

4 Are you aware of lethal anomalies? (1) Yes (2) No

5 If yes, do you think termination should be offered for a lethal anomaly? (1) Yes (2) No

6 Do you think termination should be offered for other anomalies that are not lethal but may reduce quality of life? (1) Yes (2) No

7 Do you think termination should be offered for Down syndrome? (1) Yes (2) No

8 Give reasons for your answers

_____

_____

_____

9 Are you aware of any abortion laws in your country? (1) Yes (2) No

10 If yes, state your country and the abortion law

_____

_____

_____

C Fetal anomaly ultrasonography

1 Are you aware of fetal anomaly sonography? (1) Yes (2) No

2 How did you become aware, if yes to above? (1) Media (2) colleague (3) seminars (4) Others (pls state)_____

3 What GA do you consider as most appropriate to offer prenatal ultrasound screening scan for detection of fetal anomaly? (1) 9-12 wks (2) 18-22 wks (3) 24- 26 wks (4) > 28 wks

4 Give reason for your answer

_____

_____

5 Do fetal anomaly scan rule out presence of all fetal anomaly (1) Yes (2) No

6 Expertise in 3D and 4D scan is sure proof of competence for fetal anomaly scans (1) Yes (2) No

7 Patient referral for fetal anomaly scan in my practice is routine for all pregnancies (1) Yes (2) No

8 Referral for fetal anomaly scan is restricted to high risk pregnancies in my practice (1) Yes (2) No

9 Do you routinely seek and obtain informed consent for fetal anomaly scan? (1) Yes (2) No

10 If yes to above, what form of informed consent do you obtain? (1) Verbal (2) Written

11 Is there an established protocol in your practice for communicating results of fetal anomaly scan to patient and source of referral (1) Yes (2) No
12 Is there an established protocol in your practice for management of pregnancies diagnose with fetal anomalies (1) Yes (2) No
13 Is there expertise for prenatal therapeutic interventions in my practice (1) Yes (2) No
14 Do you think there is need for specialist training on genetic counseling? (1) Yes (2) No

Thank you for your time

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Prevalence of extended spectrum βeta-lactamase producing *Escherichia coli* from patients diagnosed with urinary tract infections in Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State.

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Abstract

Background: Extended spectrum Beta-lactamases (ESβLs) are variants of beta lactamase enzymes that are capable of hydrolyzing broader spectrum of beta-lactams antibiotics. The enzymes have mutation in the gene at the active site that is believed to be the cause of high Beta lactamase activity. ESβL mediate resistance to all third generation cephalosporins, including monobactams. This study was carried out to determine the prevalence of ESBL producing *Escherichia coli* from patients presenting with cases of urinary tract infection at Olabisi Onabanjo University Teaching Hospital between April and June 2016.

Method: Urine samples from cases of UTI were centrifuged and the subnatants were diluted serially up to 10^5 with sterile distilled water. A loopful of each of the last two dilutions was streaked on a plate of sterile Eosin Methylene Blue (EMB) agar. The plates were incubated at 37^0C for 24 hrs. Plates that elicited growth were sub-cultured and stored for further use. Gram staining and conventional biochemical tests including indole, citrate utilization, hydrogen sulphide utilization, nitrate, catalase and urease tests were conducted on selected distinct colonies with green metallic sheen on the EMB culture plate. Antimicrobial susceptibility was determined by disc-diffusion method. ESBL detection was done by using the double-disc synergy test. An antibiotic disc of amoxicillin-clavulanic acid (Oxoid, UK) was placed at the center of the plate and discs containing Ceftazidime (CAZ - 30µg) (Oxoid, UK), Ceftriaxone (CRO - 30µg) Aztreonam (ATM - 30µg) were sited 0.2cm equidistant from the amoxicillin-clavulanic acid disc. After aerobic incubation at 37ºC for 18 hours, a clear extension of the edge of the growth inhibition zone of the cephalosporins towards amoxicillin-clavulanic acid disc was measured and used as positive index of ESβL production.

Results: Of the 100 urine samples examined, 79 (79%) isolates of *Escherichia coli* were detected by conventional biochemical tests of which 30 (38%) isolates were found to exhibit ESβL production. The antibiotic susceptibility profile of the isolates elicited highest susceptibility to ofloxacin (90%), gentamicin (87%) and amoxicillin-clavulanate (53%). A progressive decrease in sensitivity to cefixime (60%) and cefuroxime (27%) – a cephalosporinase effect was recorded.

Conclusion: Judicious use of antibiotics is more important to prevent infections by these resistant organisms in the community coupled with awareness by microbiologists and clinicians serving the community as key to early detection and appropriate treatment of patients affected by ESβL producing *Escherichia coli.*

Keywords: *Urinary tract infection, ESâL , Escherichia coli*

Résumé

Contexte: Les bêta-lactamases à spectre étendu (ESBL) sont des variantes des enzymes bêta-lactamase qui sont capables d'hydrolyser un plus large spectre d'antibiotiques bêta-lactames. Les enzymes ont une mutation dans le gène sur le site actif qui est considéré comme la cause d'une activité bêta-lactamase élevée. L'ESβL opère une résistance à toutes les céphalosporines de troisième génération, y compris les mono-bactames. Cette étude a été réalisée pour déterminer la prévalence d'Escherichia coli produisant des ESβL chez des patients présentant des cas d'infection urinaire à l'Hôpital d'Enseignement Universitaire Olabisi Onabanjo entre avril et juin 2016.

Méthode: Des échantillons d'urine provenant de cas d'UTI ont été centrifugés et les sous-produits ont été dilués en série jusqu'à 10^5 avec de l'eau distillée stérile. Une boucle de chacune des deux dernières dilutions a été striée sur une plaque d'agar stérile Éosine Méthylène Blue (EMB). Les plaques ont été incubées à 37 ° C pendant 24 heures. Les plaques qui ont suscité une croissance ont été sous-cultivées

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et stockées pour une utilisation ultérieure. La coloration à Gram et les tests biochimiques classiques, y compris l'utilisation d'indole, de citrate, d'utilisation de sulfure d'hydrogène, d'essais de nitrate, de catalase et d'uease ont été réalisés sur des colonies différentes avec un brillant métallique vert sur la plaque de culture EMB. La susceptibilité aux antimicrobiens a été déterminée par la méthode de diffusion de disque. La détection ESβL a été effectuée en utilisant le test de synergie à double disque. Un disque antibiotique d'amoxicilline-acide clavulanique (Oxoid, UK) a été placé au centre de la plaque et des disques contenant du Ceftazidime (CAZ-30µg) (Oxoid, UK), Ceftriaxone (CRO - 30µg) Aztreonam (ATM - 30µg) ont été installés 0,2 cm équidistant du disque acide amoxicilline-clavulanique. Après une incubation aérobique à 37 ° C pendant 18 heures, on a mesuré un prolongement clair du bord de la zone d'inhibition de la croissance des céphalosporines par rapport au disque acide amoxicilline-clavulanique et utilisé comme indice positif de la production ESβL.
Résultats: Sur les 100 échantillons d'urine examinés, 79 (79%) des isolats d'Escherichia coli ont été détectés par des tests biochimiques classiques dont 30 (38%) isolés ont été exposés à la production ESBL. Le profil de sensibilité aux antibiotiques des isolats a suscité la plus forte susceptibilité à l'ofloxacine (90%), à la gentamicine (87%) et à l'amoxicilline-clavulanate (53%). Une diminution progressive de la sensibilité au cefixime (60%) et à la cefuroxime (27%) - un effet céphalosporinase a été enregistré.
Conclusion: l'utilisation judicieuse des antibiotiques est plus importante pour prévenir les infections par ces organismes résistants dans la communauté, associée à la prise de conscience par les microbiologistes et les cliniciens qui servent la communauté comme élément clé du dépistage précoce et du traitement approprié des patients atteints d'ESβL produisant *Escherichia coli*.

Mots-clés: *Infection des voies urinaires, ESβL, Escherichia coli*

Introduction

Urinary tract infection (UTI) is a common problem diagnosed and treated in urgent care medical practice. An estimated eight million episodes of UTI occur in the United State of America each year with one out of three women requiring treatment for UTI before age 24 years of age. There is no statistical data or accurate facts on the numbers of infected individuals in Nigeria. This could be due to the self-management of infections as a result of high cost of medical care in the country. Urinalysis including Gram staining and culture may assist with diagnosis, but also add to the cost of care. UTIs can affect the lower urinary tract (cystitis) or upper tract (pyelonephritis) [1].

Varieties of antibiotics are available for treating UTIs, but changing antibiotic sensitivities make appropriate empiric treatment a moving target over time. Antibiotic chemotherapy for UTIs has been found to have a profound effect on the urethral microbiota, for example, 4-6 weeks after the administration of amoxicillin or bacampicillin, the urethral microbiota were found to be dominated by *Escherichia coli* and *Staphylococcus epidermidis* respectively [2]. The urethral microbiota of healthy individuals is dominated by lactobacilli, and these organisms are important in preventing UTIs. Administration of amoxicillin or bacampicillin, therefore, results in urethral communities that would have a reduced ability to prevent subsequent colonization by uropathogens and possible re-infection [2].

UTIs which occur in men, pregnant women, and patients with immunosuppression or urinary tract abnormalities, such as congenital malformations, urinary calculi, recent urologic instrumentation, indwelling catheters, neurogenic bladder and kidney transplant, are considered complicated and require more complex decision-making [2].

There is an increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections [3]. Of particular interest are the multidrug resistant pathogens, e.g. *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*. Administration of co-trimoxazole appears to have little effect on the urethral microbiota. *Pseudomonas aeruginosa,* an organism often detected in the urethra, may be isolated from the urethra of individuals with recurrent UTIs that are frequently treated with antibiotics [3].

Extended-spectrum β-lactamases (ESβLs) are enzymes produced by many Gram-negative bacteria which have ability to change the susceptibility of different antibiotics. They are plasmid-mediated enzymes with the capability to hydrolyze and inactivate a broad spectrum β-Lactam antimicrobials, including third-generation cephalosporins, penicillins and aztreonam but their action is inhibited by clavulanic acid [4].

ESβLs are usually inhibited by β-lactamase-inhibitors such as clavulanic acid and tazobactam, which makes a difference between ESBL- and Amp-C (aminopenicillin hydrolysing cephalosporinase)-β-lactamases producing bacteria. ESβLs have been widely reported in several Gram-negative bacteria,

but they are usually linked to the family Enterobacteriaceae, including *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. and *Escherichia coli.* The increase of ESβL producing *E. coli* among humans is worrisome since their mechanism of resistance is involved in the failure of the pharmacological treatment of diseases. Majority of community-acquired ESβL-producing *Escherichia coli* infections present as urinary tract infections (UTIs), sometimes complicated by pyelonephritis or bacteraemia [5].

Previous use of antibiotics (especially fluoroquinolones) has been constantly identified as an independent risk factor for urinary infections. Treatment options may be limited depending on the degree of multidrug resistance. Frequently, ESβL-producing *Escherichia coli* exhibits co-resistance to antibiotic agents such as trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin. A major complicating factor is the possibility of horizontal gene transfer, which can disseminate resistance to multiple antibiotics in a single step [6]. Thus, few treatment options remain. Fosfomycin or amoxicillin/clavulanate may be the treatment options for UTI, although not all ESβL producing organisms are susceptible. On the other hand, carbapenems are considered the first line agents for more severe infections like pyelonephritis and bacteremia caused by ESβL producers, given the data on favourable clinical outcome with this class. Though still rare, severe sepsis has been reported with community-acquired ESβL-producing *Escherichia coli*. Of concern is horizontal transmission of ESβL - producing *Escherichia coli* from a mother to a newborn causing bacteremia which has also been reported. The progressive reduction in the efficacy of ESβL antibiotics in the family of cephalosporins as a result of evolution of resistance is becoming alarming and this must be taken into consideration in the management of infections caused by ESβL producing *Escherichia coli.*. Therefore, knowledge of the local epidemiology and risk factors for these infections is crucial in choosing appropriate empiric therapy for severe *Escherichia coli* infections that originate in the community [7].

This study was an attempt to evaluate the phenotypic prevalence of ESβL from isolates of *Escherichia coli* obtained from urinary tract infection patients and assess their antimicrobial resistance profiles.

Materials and methods

Collection of samples

Mid-stream urine samples voided into clean sterile specimen bottles were obtained from patients visiting the outpatient department of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state, Nigeria.

Isolation and Identification *Esch. coli*

Urine samples from cases of UTI were centrifuged and the subnatants were diluted serially up to 10^{5} with sterile distilled water. A loopful of each of the last two dilutions was streaked on a plate of sterile Eosin Methylene Blue (EMB) agar. The plates were incubated at 37^{0}C for 24 hrs. Plates that elicited growth were sub-cultured on agar slants and stored for further use.

Gram staining and conventional biochemical tests including indole, citrate utilization, hydrogen sulphide utilization, nitrate, catalase and urease tests were conducted on selected distinct colonies with green metallic sheen to confirm the presence of *Escherichia coli.*

Antibiogram

Antimicrobial susceptibility was determined using disc diffusion method. The confirmed *Escherichia coli* isolates were sub-cultured into 5ml sterile nutrient broth and incubated overnight. Three-fold serial dilution of the overnight culture which was then adjusted to 0.5 McFarland standard (10^{8}CFU/ml) was streaked on Mueller Hinton Agar. The following antibiotic discs for Gram negative bacteria were used; Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacin (5µg), Amoxicillin/clauvulanic (30µg), Nitrofurantoin (300µg) and Ciprofloxacin (5µg). The discs were placed firmly on the inoculated culture plates. The plates were incubated at 37°C for 24 hours. The diameter of zones of growth inhibition was measured in millimeter and was interpreted as resistant or sensitive using CLSI standard (2014).

Detection of extended spectrum β- *lactamase (ESβL)*

ESBL detection was done using the double-disc synergy test. This was done on all the isolates which was sub-cultured into 5ml sterile nutrient broth and incubated overnight. A disc impregnated with amoxicillin-clavulanic acid (AMC – 20/10 µg) (Oxoid, UK) was placed at the center of the culture plate and discs containing ceftazidime (CAZ - 30µg) (Oxoid,UK), ceftriaxone (CRX - 30µg) and aztreonam (ATM - 30µg) were sited 0.2cm equidistant from the amoxicillin-clavulanic acid disc. After aerobic incubation at 37°C for 18 hours, a clear extension of the edge of the growth inhibition zone of cephalosporins towards amoxicillin-clavulanic acid disc was measured as positive index of ESBL production.

Results

In this study, 100 samples of urine were collected from 100 patients (40 male and 60 female). Of the total number of samples examined, seventy-nine(79) isolates of *Escherichia coli* were obtained, The gender distribution of the isolates was twenty nine 29(72.5%) from males and fifty 50 (83.3%) from females. The highest prevalence of UTI was found within the 31—40 years age range with prevalence of (85%).However, the variation observed in the prevalence of the infection in both sexes was not statistically significant (p>0.005).(Table 1.0)

Table 1: Age and Gender Distribution of the UTI isolates.

	Urinary Tract Infection		
Age (in years)	Number Examined	Number Positive	(%) Positive
0-10	5	3	60
11-20	15	10	66.7
21-30	20	15	75
31-40	20	17	85
41-50	20	18	90
51-60	10	8	40
61-70	10	8	40
Gender			
Male	40	29	72.5
Female	60	50	83.3
Total	100	79	

Tables 2 and 3 show the susceptibility pattern and numbers that were positive for ESBL tests. Thirty (30) out of the 79 E*sherichia coli* isolates were positive for ESβL. The total percentage was found to be the highest in ceftazidime (93%) resistance while resistance gentamicin and ofloxacin were recorded to tables 4 and 5 which show the numbers and percentage of the isolated strains that were resistant to two or more different classes of antibiotics. The multiple antibiotic resistance index (MARI) of varied values were recorded. MARI of 46.67% were recorded for 14 numbers of *Esch. Coli* while isolate *E24*with (MARI) 0.75 was the only one with multiple resistance to six antibiotics.

Plate 1.0: is a selected culture plate of the pictorial representation of the ESβL producing *Escherichia coli* patterns of growth towards amoxicillin-clavulanate placed at the center of culture plate.

Discussion

The gender distribution of the isolates was found to be the highest in female (50) in comparison to male (29), which could be attributed to the closeness of female urethra to the anus, sexual activities associated with the use of diaphragm and hygiene practice which can vary from one individual to another. The age gender distribution of the samples collected within the period of two months (April to May, 2016) shows variation in percentage which could be due to underlying factors associated with the immune status of the visiting patients at that very period coupled with self-management with un-prescribed herbal formulae. The prevalence of 38% of the extended spectrum beta lactamase *Esch. coli* were obtained from the total numbers of patients that visited the teaching hospital within the period of two months and can be said to be significant with the reports of the trend of the gradual upsurge of ESβ L *Escherichia coli* in many information compendia and are mostly community acquired [8].

A total percentage (93%) of the isolates studied were resistant to ceftazidime, 53% to cefuroxime and 30% to cefixime. This can be suggestive of the decreasing potency of these ESBL antibiotics on the spectra of *Esch. coli* obtained from UTI cases. This portends an impending danger in the management of ESβL associated with the infection. The resistance of 60% and 53% recorded against ciprofloxacin and amoxicillin-clavulanic respectively could be attributed to strain differentiations and probably frequent exposure of these drugs (at a lesser concentration) in the management of UTI infection, which is corroborated with the findings of Ellner (1987) on the epidemiological factors affecting the antimicrobial resistance of common bacterial isolates [9].

The susceptibility of the isolates to ofloxacin (90%), gentamicin (87%) and cefixime (60%) invitrobially could be attributed to the potency of the concentration of these antibiotics on the isolates, which could make them to be considered drugs of choice in the treatment of ESβL *Esch. coli* associated urinary tract infection. Nitrofurantoin was recorded to be totally ineffective on any of the isolates tested, an indication of absolute resistance of the isolates to this amino-furan derivative antibiotics.

The multiple antibiotic resistance index obtained in this study is 46.7% against 14 isolates could be due to many factors such as genetic make-up, selective pressure from the environment and abuse of antibiotic use in the management of UTI infection, which buttressed the findings of David (2005) on rational antibiotic treatment of outpatient genitourinary infection in a changing environment [10].

Table 2: Antibiogram and ESBL determination of isolate of *Escherchia coli* isolate

Isolates Codes	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	ESBL
	S≥21 R≤ 15 1 = 18-20	S≥23 R≤ 14 1= 15-22	S≥15 R≤ 12 1= 13-15	S≥ 19 R≤ 15 1= 16-18	S≥ 16 R≤ 12 1=13-15	S≥ 18 R≤ 13 1=14-17	S≥ 17 R≤ 14 1= 15-16	S≥ 17 R≤ 15 1=16-20	
E1	10 R	26 S	24S	22 S	26 S	10R	0R	38S	+
E3	0 R	30 S	18 S	28 S	23 S	0 R	0 R	34 S	+
E7	0 R	30 S	10 R	30 S	26 S	40 S	0 R	30 S	+
E9	0 R	0 R	24 S	12 R	24 S	16 I	0 R	24 S	+
E10	0 R	0 R	17 S	20 S	21 S	0 R	0 R	30 S	+
E12	0 R	0 R	22 S	20 S	30 S	21 S	0 R	0 R	+
E13	10 R	18 I	18 S	30 S	28 S	12 R	0 R	0 R	+
E17	12 R	0 R	26 S	20 S	27 S	25 S	0 R	0 R	+
E19	10 R	0 R	24 S	10 R	36 S	27 S	0 R	10 R	+
E21	0 R	0 R	22 S	12 R	26 S	36 S	0 R	30 S	+
E23	0 R	18 I	14 I	30 S	32 S	27 S	0 R	0 R	+
E24	0R	20 I	0 R	10 R	40 S	0 R	0 R	10 R	+
E27	10 R	0 R	20 S	20 S	27 S	36 S	0 R	0 R	+
E28	0 R	0 R	16 S	32 S	26 S	36 S	0 R	20 I	+
E35	10 R	0 R	14 I	36 S	12 R	18 S	0 R	10 R	+
E38	0 R	0 R	20 R	16 I	25 S	0 R	0 R	0 R	+
E41	0 R	0 R	28 S	24 S	38 S	25 S	10 R	0 R	+
E42	0 R	16 I	24 S	27 S	31 S	28 S	10 R	0 R	+
E45	0 R	18 I	26 S	22 S	32 S	27 S	0 R	0 R	+
E49	0 R	12 R	30 S	10 R	30 S	18 S	0 R	20 I	+
E50	15 R	0 R	18 S	10 R	30 S	21 S	0 R	20 I	+
E52	20 I	0R	16 S	27 S	26 S	36 S	0 R	10 R	+
E54	10 R	0 R	22 S	28 S	27 S	15 I	0 R	15 R	+
E55	10 R	0 R	26 S	30 S	30 S	17 I	0 R	0 R	+
E60	0 R	24 S	22 S	20 S	0 R	0 R	0 R	10 R	+
E62	19 I	26 S	16 S	16 I	16 S	12 R	0 R	0 R	+
E68	0 R	32 S	16 S	14 R	18 S	12 R	0 R	40 S	+
E70	0 R	28 S	16 S	12 R	16 S	18 S	0 R	30 S	+
E75	12 R	24 S	20 S	10 R	20 S	0 R	0 R	21 S	+
E79	0 R	20 I	22 S	16 I	14 I	12 R	0 R	0 R	+

Table 3: Percentage (susceptibility/resistance) profiles of the isolates of *Escherichia coli* exposed to selected antibiotics.

Antibiotics	% Resistance	% Susceptibility	% Intermediate
Ceftazidime	93%	-	7%
Cefuroxime	53%	27%	20%
Gentamicin	7%	87%	7%
Cefixime	30%	60%	10%
Ofloxacin	7%	90%	3%
Amoxicillin-Clavulanate	37%	53%	10%
Nitrofurantoin	100%	-	-
Ciprofloxacin	60%	30%	10%

Table 4: Multiple antibiotic resistance (MAR) index and resistance pattern of isolated *Escherichia coli*

Isolate Code	Resistance pattern	MAR index	Resistance category
E1	CAZ, AUG, NIT	0.375	MDR
E3	CAZ, AUG, NIT	0.375	MDR
E7	CAZ, GEN, NIT	0.375	MDR
E9	CAZ, CRX, CXM, NIT	0.5	MDR
E10	CAZ, CRX, AUG, NIT	0.5	MDR
E12	CAZ, CRX, NIT, CPR	0.5	MDR
E13	CAZ, AUG, NIT, CPR	0.5	MDR
E17	CAZ, CRX, NIT, CPR	0.5	MDR
E19	CAZ, CRX, CXM, NIT, CPR	0.625	MDR
E21	CAZ, CRX, CXM, NIT	0.5	MDR
E23	CAZ, NIT, CPR	0.375	MDR
E24	CAZ, GEN, CXM, AUG, NIT, CPR	0.75	MDR
E27	CAZ, CRX, NIT, CPR	0.5	MDR
E28	CAZ, CRX, NIT	0.375	MDR
E35	CAZ, CRX, OFL, NIT, CPR	0.625	MDR
E38	CAZ, CRX, AUG, NIT, CPR	0.625	MDR
E41	CAZ, CRX, NIT, CPR	0.5	MDR
E42	CAZ, NIT, CPR	0.375	MDR
E45	CAZ, NIT, CPR	0.375	MDR
E49	CAZ, CXM, NIT	0.375	MDR
E50	CAZ, CRX, CXM, NIT	0.5	MDR
E52	CRX, NIT, CPR	0.375	MDR
E54	CAZ, CRX, NIT, CPR	0.5	MDR
E55	CAZ, CRX, NIT, CPR	0.5	MDR
E60	CAZ OFL, AUG, NIT, CPR	0.625	MDR
E62	AUG, NIT, CPR	0.375	MDR
E68	CAZ, CXM, AUG, NIT	0.5	MDR
E70	CAZ, CXM, NIT	0.375	MDR
E75	CAZ, CXM, AUG, NIT	0.5	MDR
E79	CAZ, AUG, NIT, CPR	0.5	MDR

MAR= Number of antibiotic resistance ÷ Number of antibiotics used

Table 5: Percentage Multiple Antibiotic Resistance Index of *Escherichia coli* isolates

MAR Index	No. of *Escherichia coli* isolates	Percentage (%)
0.375	11	36.67
0.5	14	46.67
0.625	4	13.33
0.75	1	3.33
Total	30	100%

Percentage MARI = MARI ÷ Total number of isolates multiplied by 100

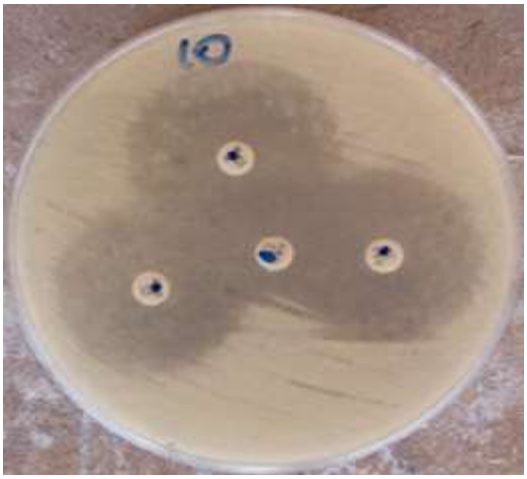

Fig. 1: Pictorial representation of ESβLproducing isolates of *Escherichia coli*

The recent global spread of ESBL -producing *E. coli* in the community has been explosive. They are likely already part of the flora in communities worldwide, making elimination impossible, just as we have seen with community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) [11].

Judicious use of antibiotics is highly imperative to prevent infections by these resistant organisms in the community. On the local level, awareness by microbiologists and clinicians serving the community is key to early detection and appropriate treatment of patients affected by ESāL producing *Escherichia coli.* [12]. Rational use of antibiotics should be encouraged. Antibiotics should

not be available for sale without prescription from a physician or a health care professional; also patients to whom antibiotics are prescribed should be monitored closely to ensure that they adhere strictly to the dosage regimen.

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Neonatal surgery in Sub Saharan Africa: challenges and solutions

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Abstract
Background: The last two decades have witnessed a gradual attempt at solving the manpower problems facing neonatal surgical care in many parts of Sub-Saharan Africa (SSA). Unfortunately this has not translated to a reduction in the very high morbidity and mortality facing neonatal surgical care as there are other challenges bedeviling the practice of neonatal surgery in the region.
Purpose: This review examined these challenges which include poor obstetric care leading to late presentation, lack of appropriate equipment and facilities, poor funding and lack of awareness about availability of neonatal surgical services as well as proffer solutions to overcome the challenges in order to minimize the high morbidity and mortality.
Conclusion: Adequate funding should be provided by the government and other funding agencies for proper training of professionals involved in the care of the surgical neonate, provision of requisite equipment and facilities, good obstetric care and advocacy to improve awareness as well as encourage early presentation.

Keywords: *Surgical neonate, care, challenges, Sub Saharan Africa, mortality*

Résumé
Contexte: Les deux dernières décennies ont été témoins d'une tentative progressive à résoudre les problèmes de main-d'œuvre rencontrés dans les soins chirurgicaux néonatals dans de nombreuses régions de l'Afrique subsaharienne (ASS).
Malheureusement, cela ne s'est pas traduit à une réduction de morbidité et de mortalité très élevées auxquelles sont confrontés les soins chirurgicaux néonatals, vu qu'il existe d'autres défis qui endommagent la pratique de la chirurgie néonatale dans la région.
Objectif: Cette revue a examiné ces défis qui incluent des soins obstétriques pitoyables conduisant à une présentation tardive, un manque de matériel et de facilités appropriés, un financement insuffisant et un manque de sensibilisation à la disponibilité des services chirurgicaux néonatals ainsi que des solutions proposées pour surmonter les défis afin de minimiser la morbidité et la mortalité élevée.
Conclusion: Un financement adéquat devraient être fourni par le gouvernement et autres agences de financement pour la formation appropriée des professionnels impliqués dans le soin chirurgical des nouveau-nés, la fourniture d'équipements et de facilités nécessaires, de bons soins obstétricaux et défense pour améliorer la sensibilisation et encourager une présentation précoce.

Mots-clés: *Chirurgie néonatal, soins, défis, Afrique subsaharienne, mortalité*

Introduction

The understanding of the fact that "children are not small adults" has impacted on the care of children with surgical problems with consequent improvement in their survival. The consequences of paediatric surgical conditions may be life long since they affect children at critical times during development. Thus, they present with lifelong burden both in human and financial terms [1,2]. Among children, neonates develop distinct surgical conditions, present unique anaesthetic challenges and have special perioperative needs that may interfere with their survival [1]. The recognition of these challenges and significant advances in neonatal surgical care have resulted in improved survival with children that had many types of congenital malformations that were once considered lethal and incompatible with life are now living normally [1,3].

In the developed world, improvement in obstetric care, neonatal anaesthesia, well organized neonatal intensive care unit, surgical techniques, availability of facilities for perinatal support, perinatal pathological diagnoses and appropriate management of associated abnormalities have all contributed to improved survival of the surgical neonate [3-5]. Interhospital transfer, a current practice in neonatal surgical care in the developed countries is often hazardous and may result in the clinical deterioration of critically ill neonates. This has

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necessitated the development of transport medicine as a subspecialty by neonatologists, anaesthetists and intensivists in order to reduce the mortality associated with transfer of these neonates [6,7].

There are still major gaps in the surgical care of children in developing countries and indeed sub Saharan Africa (SSA). The care is often regarded as too expensive and, as such, unnecessary [1]. The outcome of neonatal surgery is still very poor [6,8,9] with unacceptably high morbidity and mortality rates as up to 98% of all neonatal deaths worldwide occur in the developing countries and this is not unconnected with poor interhospital transfer, delay in making diagnosis, exclusion of paediatric surgery from most child health programmes in these countries and defective healthcare financing system.

This article seeks to highlight the challenges of neonatal surgery and examines the various solutions that have been put in place to overcome the various challenges towards providing a well-established paediatric surgical care in SSA.

Burden of the disease

The burden of surgical disease in children in many parts of SSA is unknown and this is worse in the newborns. Population based research and publications are scarce in addition to non-availability of proper epidemiological data and absence of birth defects registry that will provide information on these diseases. The available records are mostly hospital-based which sometimes may not be reliable consequently, this has negatively affected adequate planning and delivery of neonatal surgical services in most SSA countries. Also, the paucity of epidemiological studies on the prevalence of congenital malformations in addition to absence of birth defect registry in these countries often make the job of convincing the government, non-governmental organisations and other stakeholders to provide adequate funding for the care of these neonates a herculean task [8,10,11]. Congenital anomalies account for the majority of the diseases encountered in neonatal surgical practice the management of which is often problematic especially in emergency settings [1,6,12,13]. Of the congenital anomalies, gastrointestinal anomalies causing acute intestinal obstruction account for the majority of the problems in the emergency setting, the management of which is challenging. Sowande *et al* [13] in a 10 year review of the pattern of neonatal emergencies observed that 89% of cases were due to gastrointestinal anomalies. Over the same period, a similar study from northern part of Nigeria [4] reported that gastrointestinal anomalies accounted for over 50% of cases.

Problems of neonatal surgical care

Neonatal surgical care in SSA is still highly challenging, problematic and associated with high morbidity and mortality. For example, in Nigeria the reported mortality varies between 30.5% and 53.6% [6, 12-14] whereas this has decreased to about 5% in high income countries [15]. This has been due largely to improvements in anaesthesia and neonatal intensive care, advances in technology and availability of more paediatric surgeons [8].

Obstetric issues and late referral

Antenatal or prenatal diagnosis is important and key to reducing morbidity and mortality in neonatal surgery. However, prenatal diagnostic services are restricted to a few centres in most parts of SSA. Ultrasonography is most commonly used in making prenatal diagnosis and the diagnoses are mostly incidental findings during routine ultrasound scanning of pregnant women.

Studies showed that 75%-95% of neonates with congenital anomalies were delivered at home and these are sometimes supervised by traditional birth attendants who may not be able to recognize the congenital anomalies and when they are able to recognize them, they may not be able to appreciate the urgency involved in early referral [12,16]. Also, they are not competent enough to give the initial treatment needed by the patients as they may even be too scared by the sight of the anomaly. Such neonates may develop sepsis, electrolyte and metabolic problems which become worsened by poor transportation to the nearest surgical centre. Similarly, when they are delivered in the hospital, the initial resuscitation and management more often than not are inadequate before they are referred and transported in suboptimal conditions over bad roads to the specialist centres. The problem of late presentation is often made worse by the fact that families have to pay out of pocket to cater for them as the current health insurance policy in the country does not cover the management of congenital malformations and health care delivery services is not free even for children. The consequence of these is that majority of the neonates have developed hypothermia, hypoglycaemia, sepsis and haemodynamic instability. Incidences of complex anomalies requiring more specialized treatment in paediatric surgery centres have been reported to be lower than expected, consequently, most surgical neonates born in Africa and other developing countries of the world may not have access to surgical care [17].

Poor and inadequate facilities

In Nigeria, paediatric surgery specialty has evolved rapidly over the last two decades with the numbers of specialized centres increasing rapidly in all the geopolitical zones of the country. In other parts of SSA, the specialized centres are fewer due to lack of funds consequently most countries depend on foreign aids from high income countries and missionary hospitals who also supply manpower. Unfortunately, the centres have been predominantly located in the major cities, they are poorly funded, ill equipped with no dedicated neonatal intensive care unit. These hospitals were established principally to cater for adult patients even though various governments at the state and national levels have recently tried unsuccessfully to establish Children's hospital to cater for the health needs of the children, this has not enjoyed the goodwill of the people in government as they tend to lose focus and turn these hospitals to another of the various hospitals taking care of adults [16].

Neonates with surgical problems are often nursed in uncontrolled environments in most centres in the country because of non-availability of Neonatal intensive care unit (NICU) and its basic facilities like incubators, monitoring equipment, mechanical ventilators for neonates and facilities for parenteral nutrition. The consequence of this is that the requisite ventilatory support and cardiopulmonary monitoring often required postoperatively following neonatal surgery in some clinical conditions like oesophageal atresia and anterior abdominal wall defect are usually not available as these children are often nursed in adult intensive care units with inappropriate equipment. Also, neonatal surgical procedures are made difficult and inept with prolongation of the operating time because paediatric surgeons in most of these centres are made to use inappropriate and improvised instruments that are unduly big on these relatively small sized surgical neonates [8].

Anaesthetic challenges

The peculiar anatomic, physiologic and pharmacologic features of the neonate pose serious challenges to providing safe anaesthesia for the neonates. This is made worse by the fact that they tend to present with life threatening anomalies that often require emergency surgery [18]. Additionally, prematurity a commonly encountered condition among the neonates may predispose them to hypoglycaemia, hypothermia, bleeding problems and frequent apnoeic attacks requiring special attention to the airway perioperatively. The care of these requires special knowledge and great skills for successful surgical outcome. Gaining vascular access is often difficult in the neonate requiring establishment of arterial and central venous cannulation which require special skills. Unfortunately, these are not performed regularly in most centres in SSA and Nigeria because of non-availability of materials to carry out the procedure.

Consequently, most anaesthetists do not have this skill because of lack of opportunity and infrequency of practice. The relatively large head in relation to the rest of the baby's body, the presence of high hanging epiglottis and the presence of cranio-facial abnormalities may cause difficult or failed intubation in the neonate even in the hands of experienced anaesthetists [19]. Regional anaesthetic techniques such as caudal anaesthesia which can be used to provide intra-operative as well as postoperative analgesia are not practiced widely. This is due to lack of experience and the frequent shortages of local anaesthetic drugs. This situation means that the anaesthetists and the trainees do not acquire these necessary skills [19].

Trained anaesthetists are in short supply in SSA, and worse still, those with training, experience, and interest in neonatal/paediatric anaesthesia are very few [20]. General anaesthesia for the sick newborn is often turbulent, prolonged, and practiced in a state of lack of appropriate anaesthetic equipment, inappropriate drugs, and by inexperienced non-physician anaesthetists in most centres in Africa. Hence, most of these neonates fail to recover adequately or end up with brain damage from prolonged hypoxia [11].

Shortage of manpower

Chirdan *et al* [11] observed that there is acute shortage of health manpower all over Africa with SSA being the worst hit. This shortage cuts across all cadres of health workers. The total care of the surgical neonate requires input and support from the paediatric surgeon, neonatologist, the neonatal anaesthetist and the neonatal intensivists. These specialists are not readily available in most centres for consultation and participation in the team approach to the management of these neonates [19]. The paediatric surgical manpower need has been calculated for some African countries, which showed serious deficit in most of these countries, and in one center, there was only one paediatric surgeon to a population of more than 13 million people [21]. For instance, Amponsah in 2010, reported that there were only seven paediatric surgeons in Ghana with a population of over 20 million [19] whereas a ratio of one paediatric surgeon per 2.2 million population

was reported in Nigeria in 2006 [22]. This is a clear improvement in the earlier report from Nigeria in which there were only 26 paediatric surgeons to a population of about 120 million people [8]. Some other African countries have fewer or none. Similarly, there is dearth of anaesthetists in most countries in SSA and most anaesthetics are provided by non-physicians, sometimes under the direct supervision of the surgeon [23]. Also, the few paediatric surgeons available are located in tertiary hospitals and many neonates with surgical problems are treated by general surgeons, or worse still, general duty doctors or 'quacks' without the support of skilled and experienced anaesthetist in referral and other peripheral centres [8,19]. The consequence of this inadequate health manpower is an unduly high postoperative morbidity and mortality observed in the surgical management of the neonate in these countries.

Inadequate documentation and research/ Lack of Awareness

The awareness level of neonatal surgical problems is generally low. This low awareness may be attributed to lack of adequate information about the epidemiology of congenital malformations and other neonatal surgical problems in the region. This gives an erroneous impression that neonatal surgical problems are not common and significant enough as a public health problem. Thus, the funding agencies and the government do not pay adequate attention and support for neonatal surgical management and research. A typical situation is the exclusion of congenital malformations as one of the diseases that most health insurance organizations treat in Nigeria.

Lack of awareness of the care of neonatal surgical problems may also be responsible for delayed presentation in the hospital and high rate of infanticide in the region [1]. Culturally, the presence of any anomaly leads to neglect and rejection by the mother and family members and sometimes the traditional birth attendant. The baby may be left unprotected and not fed leading to hypothermia and hypoglycaemia on arrival at the health facility. This is done hoping that the baby may die. Outright infanticide may be practiced. It is however known that many of the neonates with congenital anomaly, who were once thought to be incompatible with life, are now living normally [1,19].

Tackling the challenges

Although, neonatal surgical care in Africa is bedeviled by very high morbidity and mortality, the responsibility of reducing them is beyond the paediatric surgeons alone as everybody involved in the care of the surgical neonate, the community, the government and all the funding agencies must be prepared to provide workable solutions towards reducing the mortality as most of them are preventable.

Obstetrics issues and early referral

Prenatal diagnosis ensures that antenatal care is well supervised and adequate plans made to deliver babies with congenital anomalies including in utero transfer to the nearest surgical centre where specialized services will be rendered. Prenatal diagnostic centres should be established where other diagnostic services in addition to ultrasonography can be done for early diagnosis of congenital anomalies and in utero transfer of such babies when indicated to obstetric centre nearest to the paediatric surgical centre. It also ensures proper planning of delivery of the baby in order to reduce the morbidity and mortality associated with neonatal surgery. Hospital delivery should be encouraged in order to reduce the number of babies born at home with congenital anomalies as this will ensure early presentation in the hospital. The traditional birth attendants in the health centres, mission houses and homes should be trained adequately to empower them to be able to provide initial management like minimal resuscitation for the neonates immediately after birth, recognize congenital malformations when they are present and refer them early to hospitals with facilities to cater for neonatal surgical diseases. This will reduce the high morbidity and mortality associated with neonatal surgical care.

Efforts should be made to provide good road networks and repair bad roads linking the peripheral areas to the referral hospitals while well-equipped ambulance services should also be provided to transport these babies over long distances in order to prevent the neonates from developing the metabolic and cardiopulmonary instability they are often prone to.

Provision of equipment and facilities

Appropriately sized surgical instruments is key to successful outcome of neonatal surgical procedures as the use of fine instruments would give less tissue destruction, allow for precision during surgical procedures, reduce perioperative blood loss and enhance wound healing with consequent reduction in morbidity and mortality of these procedures. Therefore, the provision of appropriately sized surgical instruments, equipment and other relevant facilities will enhance neonatal surgical care.

Neonatal intensive care is critical to survival of the ill neonate and the provision of well-equipped

NICU with trained staff is mandatory for improved morbidity and mortality rate of neonatal surgery. Neonatal intensive care units are non-existent in most hospitals in Africa because they are very expensive to maintain. Where they exist, running the facilities efficiently is constrained by shortage of personnel, essential drugs and non-drug consumables. These constraints make the running of these units far from satisfactory [19]. The absence [24,25] or poor neonatal intensive care [4] facilities is a contributing factor to the poor outcome. Therefore, the establishment of NICU can be regionalized for effective utilization of resources and for easy collation of data necessary for the purpose of research. Hospitals dedicated to the care of children can also be established with good compliment of adequately staffed and well equipped NICU to enhance the perioperative management of these neonates.

Provision of safe anaesthesia

Anaesthetists should be encouraged to take up interest in paediatric anaesthesia and general duty doctors to take up anesthesia as a career by giving them incentives [8,11]. This will improve their knowledge of the anatomical, physiological and pharmacological make-up of the surgical neonate thus enhancing improved neonatal anaesthetic services. They should also be exposed to the use of regional anaesthesia on the surgical neonate because of its advantage of providing effective perioperative analgesia that precludes respiratory depressant effect and to also reduce the incidence of anaesthesia related problems often associated with general anaesthesia.

Adequate analgesia is essential to the outcome of neonatal surgical procedures and it has been suggested that the appropriate use of correct doses of opioids together with multimodal approaches such as rectal paracetamol and wound infiltration with local anaesthetic agents such as bupivacaine would give better quality analgesia in the neonate without respiratory depression [19].

Training of relevant personnel

The training and retraining of paediatric surgeons should be encouraged and neonatal surgery training should form a major portion of the paediatric surgical curriculum in order to improve the outcome of neonatal surgical care. Apart from ensuring adequate exposure to paediatric surgery during general surgical training, general surgeons can also be made to undergo short training or courses in paediatric surgery in order to take care of minor to moderate neonatal surgical problems while the major ones can be referred to the tertiary centres for paediatric surgeons to handle [11]. Similarly, efforts should be made to train other specialists like the neonatologist, neonatal intensivist and neonatal nurses who will support the paediatric surgeon. They should also be made to go for periodic workshops and retraining in order to improve outcome.

Increasing awareness and research

The host communities should be informed through public enlightenment campaign on the availability of paediatric surgical services in their respective hospitals. The hospitals can achieve this by sponsoring talks and interviews in both prints and electronic media to educate the members of the public that neonatal surgical problems which they hitherto thought cannot be treated can be treated if they come to the hospital, sensitize them about the availability of paediatric surgery service in the hospital and the need to come early for treatment.

There are a few published reports on various aspects of neonatal surgery from SSA. To improve this, paediatric surgeons have a duty to generate/ spawn awareness about the availability and problems of neonatal surgical services in the region through regular publication of their experiences in relevant journals and through presentations of their experiences at symposia and meetings of the various associations of paediatric surgery in SSA [8]. It is also important for these associations to work together with the various paediatric associations to formulate policies in the various aspects of the management of medical and surgical problems of the neonate for the government and other stakeholders to work with while providing funds for their care.

Adequate healthcare financing and proper planning

The provision of neonatal surgical services is expensive. The regular maintenance of the facilities required to cater for the neonates is capital intensive and often beyond the reach of most SSA countries. Therefore, adequate awareness about the epidemiology of neonatal surgical health problems would assist the government to provide adequate funds for the care of the surgical neonate, plan properly on the effective utilization of the funds made available and formulate appropriate policies that would improve the outcome of care. It will also provide a platform for non-governmental organisations and other donor agencies to examine the various areas where they can function and provide funds to assist in the management of the surgical neonate. For example, the last two decades

have witnessed entrance of the Smile Train foundation into Nigeria and they have been providing free surgical care for neonates and infants with cleft lip and palate in the country and other parts of Africa. The government should also establish well-staffed Children's hospitals that are adequately equipped in the various regions of the country in order to concentrate the care of the neonate and use available resources judiciously to obtain a very good outcome of care. This will also improve data collection method and research output for proper planning and policy formulation.

Conclusion

Neonatal surgery is a highly specialized service. Improving the economy, public health, sanitation and provision of social amenities like electricity and good roads are fundamental to improving child health generally and neonatal health in particular. The quality of neonatal surgical services is a measure of the quality of paediatric surgical services provided in any centre [8,11]. In reducing the neonatal surgical morbidity and mortality, there should be increase in the number of paediatric surgeons and other professionals needed to achieve good outcome. These professionals should be involved in health advocacy and enlightenment of the members of the public on the need to shun harmful cultural beliefs and practices and present early to the hospital for better outcome. The government should embark on appropriate allocation of resources with proper accountability for effective use. The NGOs, funding organisations and donor agencies should also be encouraged to assist in providing funds, facilities and equipment for the care of the surgical neonate. They should also assist in advocacy and provision of training updates or retraining programmes for professionals whose clinical practices converge on the critically ill surgical neonate.

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Paediatric Endocrinology in University College Hospital, Ibadan, Nigeria: Past, Present, Future

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Abstract
Paediatric Endocrinology is a relatively new sub-speciality of Paediatrics in Africa especially in Nigeria. In the past eight years the European Society for Paediatric Endocrinology has laboured to build capacity among health professionals in Africa. This kind gesture has started off a chain of events that has led to the emergence of Nigerian and African Societies for Paediatric and Adolescent Endocrinology as well as a progressive increase in the number of Paediatric Endocrinologists on the continent. It has also led to increased awareness, improved referrals and enhanced clinical care of children with endocrine disorders in Nigeria. This is an attempt to give a historic account of Paediatric Endocrinology at the University College Hospital, Ibadan, a major referral teaching hospital in Nigeria.

Keywords: *Paediatrics, endocrine, multi-disciplinary teams, University College Hospital (Ibadan)*

Résumé
L'endocrinologie pédiatrique est une sous-spécialité relativement nouvelle de la pédiatrie en Afrique, en particulier au Nigeria. Au cours des huit dernières années, la Société Européenne pour l'Endocrinologie Pédiatrique a travaillé à renforcer les capacités des professionnels de la santé en Afrique. Ce geste généreux a débuté une chaîne d'événements qui a conduit à l'émergence de la société nigériane et africaine pour l'endocrinologie pédiatrique et adolescente, ainsi qu'une augmentation progressive du nombre d'endocrinologues pédiatriques sur le continent. Cela a également permis une prise de conscience accrue, des références améliorées et des soins cliniques améliorés pour les enfants souffrant des maux endocriniens au Nigeria. Il s'agit d'une tentative de donner un compte rendu historique de l'endocrinologie pédiatrique au Collège Hospitalier Universitaire, Ibadan, un hôpital d'enseignement de référence majeure au Nigéria.

Mots-clés: *Pédiatrie, endocrine, équipes multidisciplinaires Collège Hospitalier Universitaire (Ibadan)*

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Introduction

Paediatric Endocrinology is a sub-specialty that deals with the diagnosis and management of diseases of the hormonal system in children and adolescents. The diseases include diabetes and disorders of growth and puberty, sex development, thyroid function, adrenals, calcium and bone metabolism and obesity and its complications [1, 2]. In the past, Paediatric Endocrinology as a sub-specialty was under-developed in Nigeria and its importance was majorly under recognized as caregivers focused on communicable and infectious diseases [3]. It was rated very low in the educational curriculum at the University level and the Residency Training Programme also did not lay much emphasis on the acquisition of the relevant knowledge and skills in Paediatric Endocrinology. There were only two internationally recognized practising Paediatric Endocrinologists in Nigeria; Dr. Oduwole at the Lagos University Teaching Hospital (LUTH), Lagos and Dr. Ayoola at the University College Hospital (UCH), Ibadan. They operated in isolation with no support from the Federal government or any international agency. The national emphasis was on infectious and other communicable diseases and hence funding opportunities were limited for endocrine diseases. There were practically little or no national data on the prevalence of paediatric endocrine disorders while very little was going on in terms of research [3 - 5]. There was little or no collaboration with national or international bodies in the field of paediatric endocrinology.

Paediatric endocrinology in the immediate past Nigeria (2007 – 2015)

In 2005 the initial attempt by European Society of Paediatric Endocrinology (ESPE) to collaborate with Nigerian doctors for capacity development was futile until, the emergence of the Paediatric Endocrinology Training Centre for Africa (PETCA) in 2007, which led to the attainment of the desired goals. The PETCA is a capacity-building initiative developed to fill the urgent need for paediatric endocrinology sub-specialists across Africa [5]. The Centre is located in Nairobi, Kenya and is sponsored by the World Diabetes Foundation (WDF) as well as the ESPE and the International Society for Paediatric

and Adolescent Diabetes (ISPAD). The Fellowship training consists of didactic sessions, lectures, journal reviews, clinics and research [5]. The stakeholders in the establishment of the Centre were Profs Zeev Hochberg, Martin Ritzen, Kerstin Albertsson-Wikland, Faisal Ahmed, John Gregory, Ragnar Hanas and others. The PETCA between 2007 and 2013 trained 12 Nigerians who were then mandated to establish good endocrine units in Nigeria. In 2012, the Paediatric Endocrinology Training Centre for West Africa (PETCWA) was established at the LUTH to serve the West African countries [2]. More Nigerians have been trained at this Centre as well.

Between 2010 and 2013 a lot has taken place: the African Society for Paediatric and Adolescent Endocrinology (ASPAE) was founded in 2010 and held scientific conferences in Kenya, Nigeria and South Africa. In 2011, Jarrett facilitated the formation of the Society for Paediatric and Adolescent Endocrinology in Nigeria (SPAEN). The ESPE decided to sponsor the initial PETCA graduates for a 3-6 months ESPE clinical Fellowship (for better exposure in the sub-specialty) to good centres in the United Kingdom and other parts of Europe which hosted the trainees. Update courses on diabetes as well as on growth were held in South Africa, Kenya and Nigeria with International European facilitators (Figure 1). Figure 1 shows some of the patients with diabetes mellitus attending a session during the Diabetes-In –Practise (DIP) course, Ibadan, Nigeria.

The ASPAE also gained recognition as a Paediatric Endocrine Society in the endocrine world as it was listed alongside the other societies during the 9th joint paediatric endocrine conference in Milan, Italy in 2013. There was a huge attendance by members at the ESPE conferences with members presenting their posters as well as being involved in abstract review.

The mandate received from ESPE was to build up a functional paediatric endocrine units at various centres and offer education through the training of medical personnel especially in the area of early detection and prompt referral of suspected endocrine diseases. The trainees were also to collect relevant data for determining the prevalence of different disorders as well as acquire skills through international exposure to established centres.

Paediatric Endocrinology in the past at the UCH Ibadan (before 2011)

Paediatric Endocrinology at the UCH evolved from the practice of Paediatrics with Dr.Olabiwoninu being the first Paediatrician to care for children with endocrine disorders in the hospital. Other Paediatric Endocrinologists who have worked in the Hospital include Dr. Akindele and Dr. Ayoola. There was a joint Endocrine/ Gastroenterology (GIT) clinic which held once a week. Attendance at the weekly clinic was very poor with predominant endocrine disorder being rickets, which constituted 56.4% of all cases seen in the Children's outpatient clinic in a

Fig. I. Diabetes-In-Practise (DIP) course Ibadan, Nigeria 2013 hosted by the Unit and facilitated by ISPAD.

review by Jarrett *et al* [6]. There was no definite unit and so patients requiring inpatient care were admitted and seen alongside neurology patients. A proper endocrine unit was established in April 2012 and then allowed for bed assignment and with the adoption of a functional sub-specialty standard operating procedures.

What were the challenges?

Poor referral practices

Lack of knowledge and skills in the sub-specialty in Nigeria and Africa in general were responsible for the high level of ignorance leading to missed diagnosis and late or poor referral practices [7]. To change the poor referral practices there is a need for education of health workers through periodic organization of growth in practice (GIP) and other training courses in endocrinology. Well baby clinics, schools and primary health care facilities should be equipped to take measurements of both height and weight with provisions for referrals if these parameters are found to be abnormal based on growth standards.

Poor Laboratory support

The intense need for laboratory support has not been met up until the time of this report. Most hospitals, UCH inclusive, do not have sufficient infrastructure required to back the practice of paediatric endocrinology. The available services are most of the time, not within the reach of most patients because of the exorbitant costs of these investigations.

Unavailability of necessary hormones and other endocrine medicines for the treatment of endocrine disorders is a great challenge to the practice. Some cannot be brought into the country because of lack of authorization by the regulatory bodies while those medicines that eventually get approval to be sold in the country cost so much that potential buyers who have no medical Insurance scheme in place and would have to pay out of pocket are unable to buy them. The long duration of treatment required in some endocrine disorders also contribute to the high level of defaults and poor compliance to treatment with the resultant poor outcome. Improved diagnostic and treatment facilities are required to develop adequate skills for clinical diagnosis when resources are limited [1].

Multidisciplinary approach needing a network of specialists

Specific expertise in specialized areas requires practitioner education at all levels, with access to rapidly available and accurate radio diagnostic and genetic testing in centres of excellence [1]. The treatment requires a team of experts in different fields working together. This multidisciplinary approach to the management of affected children has not been available in various hospitals in the country. Additionally, unstable power supply makes it difficult to maintain the potency of hormones like insulin.

Research development

There is a need for standard registries and database for paediatric endocrine disorders. There should be good information technology systems and skilled staff to set up these registries. There has been very minimal funding within the country geared towards research in paediatric endocrinology.

The practice of Paediatric Endocrinology in the present in UCH (2012 – 2015)

The Paediatric Endocrine Unit of UCH developed a patient record keeping system in which the patient's vital contact information was recorded for the

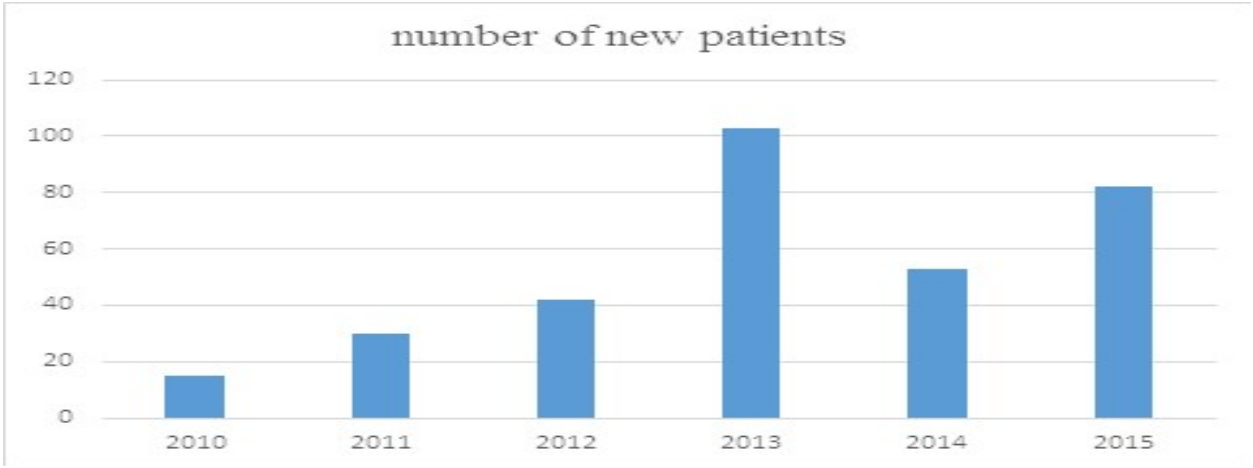

Fig. 2. New cases seen in the Endocrine Clinic of UCH from 2010 – 2015.

purpose of easy appointment scheduling and effective phone call reminders thus ensuring appointments for follow up visits were kept. Patients were also granted access to the doctor's phone number with which they could call in the event of emergencies or when there was a need for clarification in the course of patient's treatment. Figure 2 shows the progressive increase in the number of new cases seen in the endocrine clinic. In 2014 and 2015 the hospital experienced frequent and prolonged industrial strikes by different health professional associations which expectedly affected the number of patients seen during those years. Table I shows available data of old cases that presented in clinic for follow up.

Table I. Follow up cases seen at the Endocrine clinic from 2013 – 2015.

	Male	Female	Total
2013	109	118	227
2014	102	102	204
2015	126	134	260

The team developed different sub-teams with other sub-specialties and health professionals with the hope to improve clinical care and also boost research. It has good working relationship with other departments, organises case conferences, and conducts joint clinics periodically.

Health workers that have shown interest in the subspecialty have been sponsored to attend update courses and workshops.

Well-structured clinics

Before 2012, the Endocrine and gastroenterology out-patient cases were seen in the same clinic by the same set of doctors; but since a unit system was put in place in the department, different sub-specialty clinics have evolved. An integrated approach to the management of different common and uncommon endocrine conditions has been adopted by the Unit to ensure improved child care services.

Formation of Multidisciplinary teams

The team has formed the following teams for improved service delivery.

(1) Diabetes Mellitus (DM) Team: Diabetes clinic holds once a month (figure 3). The DM team is made up of the Diabetologists, Child Psychiatrists, Diabetic Educators, Dieticians and Paediatric Social workers. Every member of the DM team attends to these children in their own capacities and area of specialty.

Fig. 3: Paediatric Diabetes Clinic established during the DIP course in UCH Ibadan. Present are from the right to the left: Magaret Zacharin, Kathryn Hamilton, Carine de Beaufort, Bolanle Fetuga (at the door entrance), Tokunbo Jarrett and Abiola Oduwole

(2) Disorders of sex development (DSD) team: The Unit conducts a joint (with the Paediatric Surgeon and Paediatric Endocrinologist in attendance) DSD clinic bimonthly. The DSD team is made up of the Paediatric Endocrinologists, Paediatric Surgeons, Gynecologists, Child Psychiatrists and Paediatric Radiologists. Case conferences are held with all members of the team in attendance as well as parents/caregivers for improved service delivery. Interesting cases managed include Congenital Adrenal Hyperplasia (CAH), Ovotestis DSD, sex chromosome DSD, partial androgen insensitivity syndrome (PAIS), 5α reductase deficiency, micropenis and cryptochidism.
(3) Genetic team: Genetic clinic now runs once a month. The Unit designed a standard operating procedure (SOP) for every case of dysmorphism. Down syndrome cases constitute the largest number of patients seen with an average of one new case a week. In collaboration with the Down Syndrome Foundation of Nigeria (DSFN) in Lagos, an Ibadan branch of the Foundation, was formed. The Down syndrome Protocol of the Foundation for managing the affected children was adapted for local use thus allowing for an integrated approach to their management with impact on their quality of life. The Unit had a total of 120 patients with Down syndrome as of December 2015. Other syndromes managed in the Unit include: Edwards, Turners, Noonan, CHARGE, Treacher Collins, Klipper-weber-trenauny, Genoa, Ectodermal dysplasia, Osteogenesis imperfecta, Achondroplasia and Hemihypertrophy.

Dynamic testings

Despite the limitations, the unit is still able to carry out some tests for the patients. Some private laboratories process the samples collected during the hormone stimulation tests to complement the services offered in the Hospital's Laboratories.

Stimulation tests carried out to evaluate growth and glucose metabolism include: insulin tolerance tests (ITT) and prolong fasting tests. Glucagon, clonidine and arginine are not available in Nigeria for tests, hence insulin, being readily available, is used. The ITT is still considered the 'gold standard' practice though previous convulsions are an absolute contraindication [4]. Oral glucose tolerance test (OGTT) and assays for insulin, c peptide and HbA1c can be readily carried out on the patients attending the endocrine clinics. Thyroid function tests are the most frequently requested set of tests in the Unit and can be carried out readily within the Hospital. Auto antibodies testing are less frequently requested because of costs. Gonadotropin releasing hormone stimulation (GnRH) test and Human chorionic gonadotropin (HCG) stimulation test are also performed readily by the Unit. Similarly, adrenal function evaluation tests like dexamethasone test are also done.

Even though the requests for calcium, phosphate and alkaline phosphate assays are quite frequent, assays for parathyroid hormone and 25-hydroxycholicalciferol levels are too expensive, and hence are not done routinely.

Research focus in recent times has been the gathering of data to determine the pattern and burden of Paediatric endoctrine disorders in Africa. Presently, graduates of the PETCA and PETCWA schools have over 90 publications in reputable journals.

The Unit enjoyed free supplies of insulin, glucometers and strips for the DM patients for a period of one year (2013) as part of the 'Life for a Child' Programme of the International Diabetes Federation (IDF); other donors included pharmaceutical companies such as:- Eli Lilly and Company; and Roche Holding AG pharmaceutical company.

The team has been able to establish collaborations with foreign centres to boost its laboratory exemplified by its ability to send samples to Sweden for genetic testings in DSD children.
Unit Protocols have been developed for the management of different endocrine conditions such as diabetic ketoacidosis (DKA), short stature, congenital disorders, DSD and rickets. These have gone a long way in guiding Paediatric Residents and other health professionals that rotate through the Unit in the management of these conditions.

The practice of Paediatric Endocrinology in UCH: Future

The Paediatric Endocrine/ Genetic Unit should eventually develop into a department with different sub-specialists: Diabetologists, Experts in thyroid, DSD, pituitary, adrenal, growth, bone, as well as experts working alongside the Paediatric Oncologists for managing children with cancer especially post therapy, since cancer treatment is a well-known cause of long term endocrine disease [8].

The Outpatients Department is expected to grow to the extent that more clinics would then run weekly like the Growth Clinic, Thyroid Clinic, Adrenal Clinic, Joint Orthopaedic/Bone Clinic, and Young Oncology/ Late Endocrine Effects Clinic.

The Unit hopes to establish a vibrant community outreach programme which will include a functional School Health Programme.

We hope to establish a well-structured growth monitoring system (skillful staff, good equipment, growth charts etc) and also to develop different growth charts based on local data. Small-for-gestational age (SGA) infants from the neonatal units could be followed up for growth problems.

The establishment of a National Genetic centre where mutational analysis and other genetic tests would be carried out as well as a Hormone/ Endocrine Laboratory where all the assays can be carried out will further enhance the practice of Paediatric Endocrinology in Nigeria. This centre would serve not only the UCH, but the whole of Nigeria and the West Africa. We also hope to establish a well-equipped Pharmacy with links to international centres where medicines/hormones needed are brought into the country promptly at and when needed.

We hope to engage in more international collaborations in the area of research, especially in molecular genetic studies. We look forward to becoming a center where other health professionals like paediatric endocrine nurses, diabetic educators etc could receive formal training. We also hope to establish a vibrant centre for newborn screening for congenital hypothyroidism and other metabolic disorders to help in early detection and intervention thus reducing morbidity [9].

Conclusion

The practice of Paediatric Endocrinology in the UCH, Ibadan has improved with greater awareness among health professionals but there is still need for growth. There is a need for changes in the health care system as regards referral criteria, laboratory and pharmaceutical development, as well as improved acquisition of skills and knowledge amongst other disciplines, forming teams responsible for taking care of children with chronic endocrine diseases and syndromes.

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Neuroprotective effects of ethanol extract of *Ocimum gratissimum* leaf on monosodium glutamate-induced oxidative stress in developing Wistar rat cerebellum

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Abstract

Background: The safety of monosodium glutamate (MSG) as a flavour enhancer has generated much controversy. This research investigated the neuroprotective effect of ethanolic leaf extract of *Ocimum gratissimum* (OG) against MSG-induced oxidative stress in developing Wistar rat brains.
Methods: Thirty pregnant rats were divided randomly into six groups of five animals per group. Control; MSG (4 g/kg); MSG + Vitamin E (500 mg/kg); OG (300 mg/kg dissolved in corn oil) + MSG; OG; Corn oil, CO (vehicle). The pregnant rats received oral administration from day 7 of gestation to postnatal day 28, while the pups received oral administration from the 21st to the 28th postnatal day. Behavioural test was done on day 21 and the pups were killed. The cerebella of pups of days 1, 7, 14, 21 and 28 were dissected out for histological and histomorphometric studies, while biochemical evaluation was done on day 21*postpartum*.
Results: In the behavioural assessment (open field test), only the rats in the MSG + Vit. E (5.00±1.41) and CO (4.75±1.50) groups showed a significant reduction at $p<0.05$ compared to the control (19.00±8.71) in longitudinal movement and number of grid lines crossed with four paws within five minutes. Rats in all the treatment groups showed a reduced tendency for rearing compared with the control group at $p<0.05$. Biochemical analysis showed a decreased glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in the MSG group compared with the control pups, and that OG significantly ($P<0.05$) increased the GSH and GHS-Px activity compared with MSG animals. Superoxide dismutase (SOD) activity was significantly increased in the control and OG+MSG rats compared with the MSG, OG, MSG + Vit E and cornoil groups at $p<0.05$. Histological alterations were minimal, with a thicker external granular layer seen in the MSG and MSG+Vit E pups on day 14, and decrwased molecular layer thickness on day 28.
Conclusion: These results suggest that the adverse effects of MSG are mild and OG possesses bioactive phytochemicals capable of preventing and/or reversing MSG-induced oxidative stress. Further studies are recommended to clearly define the mechanism of OG action

Keywords: *Ocimum gratissimum, monosodium glutamate, oxidative stress, developing cerebellum, neuroprotection*

Résumé

Contexte: La sécurité du glutamate mono-sodique (MSG) comme stimulateur de saveur a suscité beaucoup de controverse. Cette recherche a étudié l'effet neuro-protecteur de l'extrait éthanoïque de feuilles d'Ocimum gratissimum (OG) contre le stress oxydatif induit par MSG dans le développement des cerveaux de rats Wistar.
Méthodes: Trente rats en gestations ont été divisées de façon aléatoire en six groupes de cinq animaux par groupe. Contrôle; MSG (4 g / kg); MSG + vitamine E (500 mg / kg); OG (300 mg / kg dissous dans de l'huile de maïs) + MSG; OG; Huile de maïs (véhicule). Les rats en gestations ont reçu une administration par voie orale entre le jour 7 de la gestation et le jour 28 postnatal, tandis que les souriceaux ont reçu une administration orale du 21ème au 28ème jour postnatal. Le test de comportement a été effectué le jour 21 et les souriceaux ont été tués. Les cervelets des souriceaux du jour 1, 7, 14, 21 et 28 ont été disséqués pour les études histologiques et histomorphométriques, alors que l'évaluation biochimique a été effectuée le jour 21 après le départ.
Résultats: Dans l'évaluation comportementale (test en champ ouvert), seuls les rats du MSG + Vit. E (5,00 ± 1,41) et les groupes CO (4,75 ± 1,50) ont montré une réduction significative à $p<0,05$ par rapport au contrôle (19,00 ± 8,71) dans le mouvement longitudinal et le nombre de lignes de grille croisées avec quatre pattes dans les cinq minutes. Les rats dans tous les groupes de traitement ont montré une tendance réduite à l'élevage par rapport au groupe témoin à $p<0,05$. L'analyse biochimique a montré une activité diminuée de glutathion (GSH) et de

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glutathion peroxydase (GSH-Px) dans le groupe MSG par rapport aux chats témoins et que OG a significativement (P <0,05) a augmenté l'activité GSH et GHS-Px par rapport aux animaux MSG. L'activité du super-oxyde dismutase (SOD) a été significativement augmentée dans le contrôle et les rats OG + MSG par rapport aux groupes MSG, OG, MSG + Vit E et control à p <0,05. Les altérations histologiques ont été minimes, avec une couche granulaire externe plus épaisse observée chez les souriceaux MSG et MSG + Vit E le jour 14 et une diminution de l'épaisseur de la couche moléculaire au jour 28.
Conclusion: Ces résultats suggèrent que les effets indésirables du MSG sont doux et le OG possède des phyto-chimiques bioactifs capables de prévenir et / ou inverser le stress oxydatif induit par MSG. D'autres études sont recommandées pour définir clairement le mécanisme de l'action OG

Mots-clés: *Ocimum gratissimum, glutamate monosodique, stress oxydatif, développement du cervelet, neuro-protection*

Introduction

Monosodium Glutamate (MSG) is one of the world's most extensively used food additive ingested as part of commercially processed foods. As a flavour enhancer, it increases the palatability of food and produces a flavour that cannot be provided by other foods. The safety of MSG usage has generated much controversy locally and globally. In Nigeria, some communities and individuals often use MSG as a bleaching agent for the removal of tough stains from clothes. There is a growing apprehension that its bleaching properties could be injurious to health [1].

In spite of its ubiquitous role as a neurotransmitter, glutamate is highly toxic to neurons, a phenomenon dubbed '*excitotoxicity'* [2]. This neurotoxicity of L-glutamate has been implicated both in the acute degenerative changes that occur after status epilepticus, hypoglycemia, ischemia, and trauma, and in chronic neurodegenerative disorders as Huntington's disease, olivopontocerebellar atrophy, Alzheimer's dementia, Parkinsonism, and amyotrophic lateral sclerosis (ALS) [3,4]. Although the detailed mechanisms are still not fully clarified, growing evidence points to the role of receptor-mediated intracellular Ca^{2+} overload and increased reactive oxygen species (ROS) production in glutamate-mediated neurotoxicity. This type of over excitation-induced oxidative stress and intracellular Ca^{2+} metabolism disorders have been identified to execute cell death via distinct downstream signalling cascades, including activation of potentially lethal second messengers and enzymes, disturbance of mitochondrial function, and inhibition of anti-apoptotic pathways [5,6]. Studies providing the evidence of MSG toxic effects have generated increasing interest in MSG intake as flavour enhancer. Neurotoxic effects in the brain, detrimental effects on sex organs, obesity and metabolic defects are the most discussed in connection with MSG intake.

Contrary arguments have pointed to the quantity and routes of MSG administration as incomparable to human intake. Although the detailed mechanisms are still not fully clarified, growing evidence points to the role of receptor-mediated intracellular Ca^{2} overload and increased reactive oxygen species (ROS) production in glutamate-mediated neurotoxicity [5,6]. Oxidative stress associated with MSG administration in rats have been prevented or ameliorated by the use of antioxidant complexes like vitamins C, E, and Quercetin [7]. Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases [8].

Ocimum gratissimum (OG) is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, pubescent and woody at the base [9], known here in Nigeria as 'scent leaf' and consumed by most people especially pregnant and nursing mothers in pepper soup. When extracts from the leaves of OG were investigated for phytochemical constituent and anti-oxidant activity, tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in both methanolic and aqueous extracts [10]. The neuroprotection against glutamate-induced neurotoxicity will be a therapeutic strategy for preventing and/or treating both acute and chronic forms of neurodegeneration [11]. We therefore explored the antioxidant and neuroprotective properties of OG against MSG-induced oxidative stress in developing rat brain.

Materials and methods

Plant extraction

Fresh leaves of *Ocimum gratissimum* was obtained at Bodija in Ibadan, Oyo state. Identification and authentication was done at the Department of Botany, University of Ibadan. A voucher specimen (UIH-22411) was deposited at the herbarium. The dried leaves was then blended, 1.8 kg of pulverized leaves was obtained and defatted in N-hexane. Extraction

of plant constituent was by cold maceration in ethanol at room temperature over a period of 72 hours. The solvent was evaporated in a rotary vacuum evaporator until a solid residue was formed. This residue was observed to be insoluble in distilled water hence was re-constituted into a fine suspension using pure corn oil.

Animals management

Thirty sexually matured female Wistar rats weighing between 170 g and 220 g were obtained from the animal house of the College of Medicine, University of Ibadan and housed in plastic cages at room temperature with a 12 hour light/dark cycle. The rats were divided into six groups of five animals each, mated and pregnancy confirmed by the presence of vaginal plug and smear. They were fed with mice cubes obtained from Ladokun Feeds, Ibadan, Oyo State and drinking water provided *ad libitum*. Sawdust beddings were changed every three days to maintain a hygienic environment. As the pregnancy advanced, each rat was then placed in a separate plastic cage.

Grouping and treatment of animals

Group 1:	Received 4 g/kg of MSG
Group 2:	Received 4 g/kg of MSG + 500 mg/kg of Vitamin E (MSG + Vit. E)
Group 3:	Received 300 mg/kg of OG
Group 4:	Received 4 g/kg of MSG + 300 mg/kg of OG (OG + MSG)
Group 5:	Received Corn Oil (CO) (vehicle for OG)
Group 6:	Served as normal Control group, received clean tap water

All the interventions were orally administered with an oral gavage and under hygienic conditions from day 7 of pregnancy to day 28 after birth. However, the pups, after weaning on day 21, received direct oral administration to day 28.

MSG preparation and administration

Four hundred and fifty-four (454) grams of 99.9% monosodium glutamate with the brand name 'Vedan' was purchased from the local market in Bodija, Ibadan. The crystals were dissolved in distilled water at a concentration of 0.5 g/ml and 4 g/kg was orally administered using an oral gavage to experimental animals in groups 1, 2 and 4. Phytochemical analysis of OG leave was done in the Department of Pharmacognosy, University of Ibadan and the following phytochemicals were screened for; Alkaloids, Cardenolides, Saponins, Tannins, Flavonoids and Anthraquinones.

Preparation and administration of α-tocopherol (Vitamin E)

Each soft gelatin capsule containing 100 mg of DL-α-tocopheryl acetate as 100 mg vitamin E acetate was punctured with a new size 21G needle attached to a new 1mL hypothermic syringe (Becton Dickinson, La Portde- Clair, France). The oily formulation of vitamin E was then neatly and completely aspirated out with the syringe. Each aspirate measured approximately 0.2 mL containing 100 mg of DL- α-tocopherol. The insulin syringe was thereafter attached to a clean intra-gastric gavage through which each rat was administered orally the measured dose of 500 mg/kg/daily throughout gestation and weaning (lactation).

Sacrifice

The day 21 pups of all the groups were weighed, behavioural assessment done and sacrificed, with their brains dissected out on days 1, 7, 14, 21 and 28. Brains of day 21 pups were preserved in phosphate buffered saline at 4°C and pH 7.4 for biochemical analysis. Cerebellar tissues were fixed in 10% formol-saline, processed employing routine paraffin embedding and stained in H and E for histological and histomorphometric studies.

Statistical analysis

The data obtained (mean±SD) was further analyzed using one way analysis of variance (ANOVA) followed by Tukey-Kramer posthoc test analysis and $p<0.05$ was considered to be statistically significant. Statistical analysis was performed with Graph Pad Prism Software (version 5.04), San Diego, California, USA.

Results

Phytochemical analysis

The phytochemical analysis of OG leave revealed the presence of Alkaloids, Cardenolides, Saponins, Tannins, Flavonoids and absence of Anthraquinones.

Brain weight

A progressive increase was observed in the brain weight from Day 1 through Day 28. A slight decrease was observed in MSG + Vit. E, OG only and OG + MSG rats compared with the control rats at $p>0.05$ (Table 2).

Behavioural studies

Open field test of pups at day 21

In the open field test conducted, only the rats in the MSG, MSG + Vit. E (5.00±1.41) and CO (4.75±1.50)

Table 1: Phytochemical Analysis of *Ocimum gratissimum Linn*

Phytochemicals	Result
Alkaloids	+
Cardenolides	+
Anthraquinones	-
Saponins	+
Tannins	+
Flavonoids	+

– negative (absent), + positive (present)

groups showed a significant difference at $p<0.05$ compared to the control (19.00±8.71) in longitudinal movement and number of grid lines crossed with four paws within five minutes (Figure 2). Rats in all the treatment groups showed a reduced tendency for rearing compared with the control group at $p<0.05$. There was no significant difference between the treatment groups when compared with the Control in every other parameter studied in the open field test at $p>0.05$ (frequency of grooming, centre square entry, centre square duration, number of faecal bolus and urine drops).

Table 2: Mean brain weight (grams) of the control and treated pups on days 1, 7, 14, 21 and 28

Group	Day 1	Day 7	Day 14	Day 21	Day 28
Control	0.37±0.04	0.76±0.05	1.27±0.06	1.45±0.13	1.48±0.10
MSG	0.29±0.01	0.77±0.06	1.26±0.07	1.27±0.04	1.40±0.07
Vit. E+MSG	0.33±0.06	0.74±0.03	1.01±0.10	1.37±0.06	1.18±0.04
OG	0.32±0.04	0.75±0.07	1.22±0.05	1.37±0.03	1.32±0.02
OG+MSG	0.28±0.04	0.75±0.04	1.45±0.44	1.46±0.15	1.44±0.09
CO	0.32±0.07	0.71±0.11	1.10±0.05	1.24±0.09	1.87±0.09

Values are presented as Mean±SD at $p>0.05$, n=5. MSG-Monosodium Glutamate; Vit. E- Vitamin E; OG- *Ocimum gratissimum*; CO- Corn Oil (vehicle).

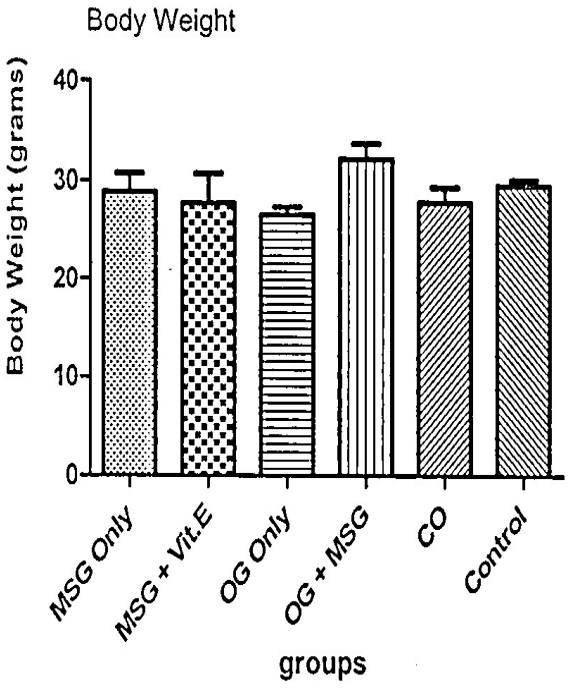

Fig. 1: Body weight of pups in grams on day 21. No significant difference between the control and all the other experimental groups at $p>0.05$. MSG- Monosodium Glutamate; Vit. E- Vitamin E; OG- *Ocimum gratissimum*; CO- Corn Oil (vehicle). Valuesare presented as Mean±SD at $p>0.05$, n=5.

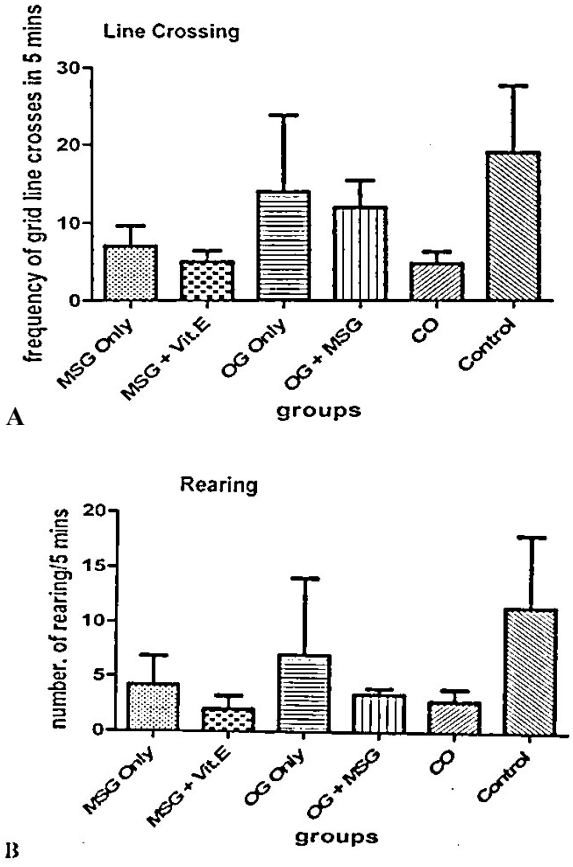

Fig. 2: Open Field Test of pups at day 21. A) Line crossing B) Rearing. MSG- Monosodium Glutamate; Vit. E- Vitamin E; OG- *Ocimum gratissimum*; CO- Corn Oil (vehicle). Values are presented as Mean±SD at $p<0.05$, n=5.

Table 3: Biochemical evaluation of the brains of pups on Day 21

Group	CAT µmol/mg	SOD µg/mg	GSH µg/ml	`LPO µmol/mg	GSH-Px µg/mg
Control	0.02±0.00	0.85±0.11	0.49±0.13	1.66±0.17	108.40± 9.11
MSG	0.02±0.01	0.49±0.04	0.19±0.06	1.62±0.52	86.01±12.79
MSG+Vit E	0.01±0.00	0.37±0.14	0.40±0.15	1.06±0.06	101.10±19.17
OG	0.03±0.01	0.40±0.08	0.40±0.18	1.17±0.21	115.40±21.27
OG+MSG	0.02±0.01	0.75±0.24	0.51±0.11	1.85±0.53	118.20± 9.05
CO	0.02±0.00	0.33±0.16	0.39±0.17	1.32±0.25	135.60±15.30

Values are presented Mean±SD, n=5. MSG- Monosodium glutamate, Vit. E- Vitamin E, OG - *Ocimum gratissimum*, CO- Corn Oil (vehicle). CAT- Catalase, GSH- Glutathione, SOD- Superoxide dismutase, LPO- Lipid Peroxidation, GSH-Px- Glutathione Peroxidase.

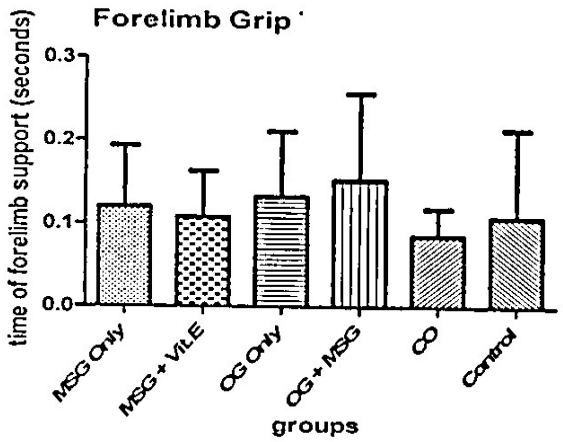

Fig. 3: Forelimb grip test of pups at day 21 showed no significant difference between the experimental groups and the control at p>0.05. MSG- Monosodium Glutamate; Vit. E- Vitamin E; OG- *Ocimum gratissimum*; CO- Corn Oil (vehicle). Values are presented as Mean±SD at p>0.05, n=5.

Table 4: Thickness of external granular layer (EGL) of pups on day 14 and molecular layer (ML) on day 28

Group	EGL day 14 (µm)	ML day 28 (µm)
Control	7.80±0.80	131.43±1.30
MSG	11.29±1.40	109.30±6.29
MSG+Vit E	10.64±0.65	123.40±3.80
OG	8.49±1.33	121.20±4.79
OG+MSG	9.96±1.74	148.60±6.18
CO	7.91±1.88	141.50±7.04

Values are presented as Mean±SD at p<0.05, n=5. MSG- Monosodium Glutamate; Vit.E- Vitamin E; OG- *Ocimum gratissimum*; CO- Corn Oil (vehicle).

Biochemical analysis

A significantly decreased levels of reduced GSH and GSH-Px was observed in MSG group compared with the control and other treated groups at p<0.05. Superoxide dismutase levels was significantly increased in the control and OG+MSG rats compared with other treated groups at p<0.05 (Table 3).

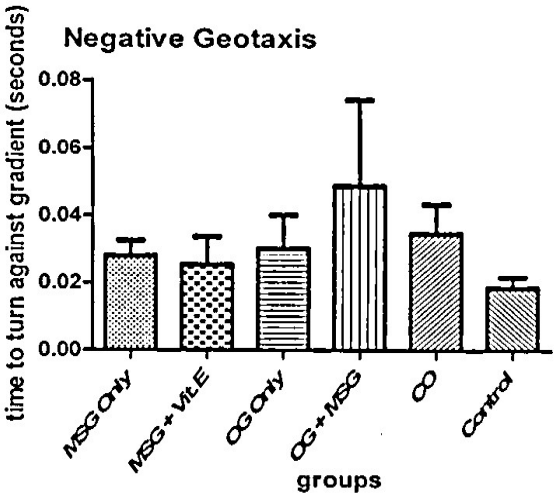

Fig. 4: Negative Geotaxis of pups at day 21**.** No significant difference at p>0.05 in the treatment groups when compared with the control except for pups of rats that received OG + MSG at p<0.05. MSG- Monosodium Glutamate; Vit. E- Vitamin E; OG- *Ocimum gratissimum*; CO- Corn Oil (vehicle). Values are presented as Mean±SD at pÂ0.05, n=5.

Histomorphometric evaluation

Thickness of Layers of Cerebellar Cortex in Micrometres on Day 14 and 28 Postpartum

A thicker external granular layer (EGL) was seen in the MSG and MSG+Vit E compared with the control (p<0.05) and other treated pups on day 14 (Table 4, figure 5). The molecular layer was thicker in the control, OG+MSG and CO pups on day 28 compared with the MSG and MSG+Vit E pups at p<0.05 (Table 4, figure 6).

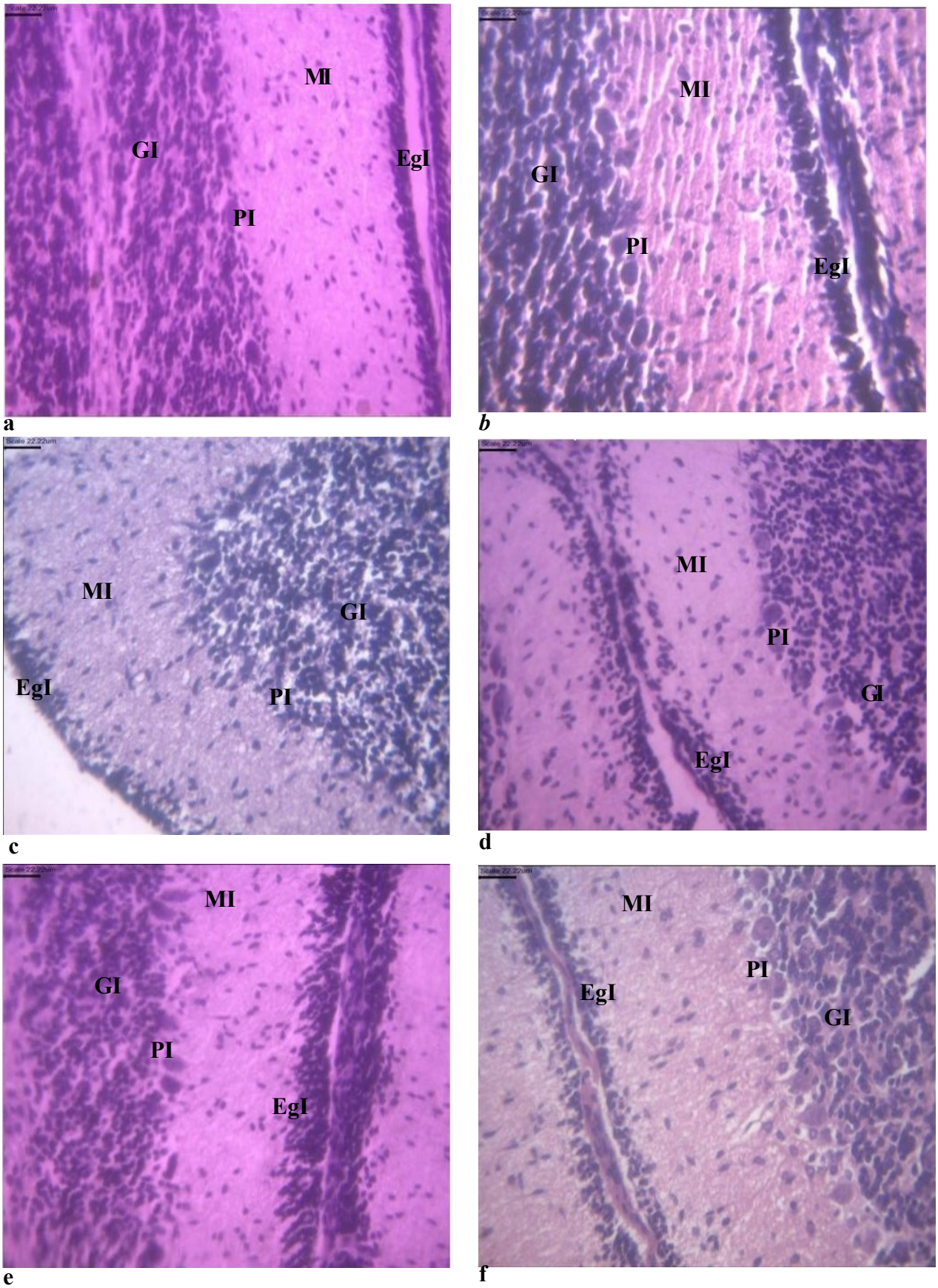

Fig. 5: Photomicrograph of the cerebellar cortex of pups of day 14. a) control b) MSG with thicker EGL c) OG+MSG d) OG only e) MSG+Vit E with thicker EGL, f) cornoil. EGL- External granular layer, ML- Molecular layer, PL- Purkinje layer, GL- Granule layer. H & E 400.

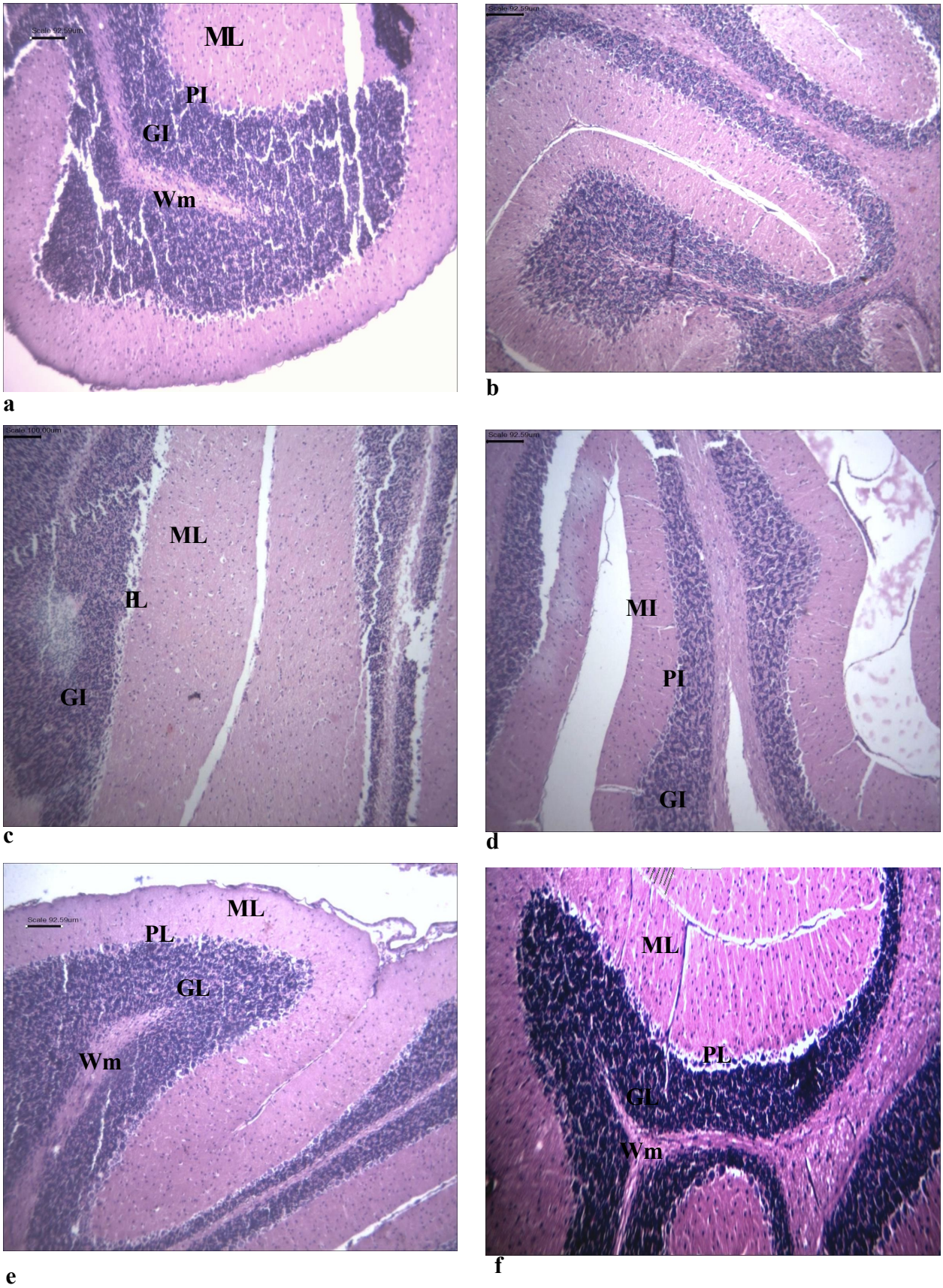

Fig. 6: Photomicrograph of the cerebellar cortex of pups of day 28. a) control b) MSG with thin ML c) OG+MSG d) OG only with thin ML e) MSG+Vit E with thin ML, f) cornoil. ML- Molecular layer, PL- Purkinje layer, GL- Granule layer. H & E 100.

Discussion

Monosodium glutamate is frequently used as a flavour enhancer and this is the reason for its widespread use as a food additive all over the world [12]. In this current study, MSG was administered via the oral route to mimic how it is consumed by humans. Pregnant rats were used in the study to examine the possible effects of MSG on the developing brain and the protective role of ethanol extracts of *Ocimum gratissimum* in the prevention and repair of MSG-induced neurodegeneration.

In this study, no significant reduction in body weight and brain weight was observed between the MSG treated rats and the control. This contradicts the results of previous research that MSG administration leads to obesity [13, 14], in which the parenteral route of administration of MSG in rats was employed. Nevertheless, the current study corroborates the work of Von Diemen and Trinidade [15] where no significant change was observed between the pups of MSG treated rats and the control.

The open field test that was designed to measure the locomotor, hyperactivity, anxiety and exploratory behaviour in experimental rats and it has been shown that other tests like negative geotaxis and forelimb grip test have been used to demonstrate that MSG has dose-dependent negative influences on various centres of the brain involved in the control of learning, memory, motor coordination and response to a novel environment [16]. In the present study, slight changes in the line crossing was observed in this study and a significant decrease in rearing of only pups whose mothers received MSG only but no significant difference was observed in all the other parameters measured in the open field test when compared to the control. This may be due to the mitigating effect of ethanol extract of *Ocimum gratissimum* and vitamin E that was orally administered along with MSG.

It has been reported that rats with degenerating axons in the periventricular white mater such as tracts that project to the spinal cord performed poorly in the forelimb grip test designed to measure muscular strength in the limbs [17]. Also, poor motor coordination in animals injected subcutaneously with MSG has been reported [16]. However, the present study showed no significant relationship between MSG and performance during the forelimb grip test. This may probably be due to the oral route of administration employed in the study in an attempt to mimic how MSG is consumed by humans in food. In most studies that show marked effects of MSG, subcutaneous [18] and intraperitoneal [19] routes of administration was used hence by-passed enteral and hepatic metabolism. Reeds et al., [20] had earlier stated from a study with pigs that only about 5% of ingested MSG was absorbed in the gut.

The large amount of polyunsaturated fatty acids associated with the brain and the fact that the entire CNS accumulate redox metal ions, consumes large amount of oxygen and is composed of non-mitotic highly differentiated cells that are hard to repair when damaged makes it particularly vulnerable to oxidative stress [21]. Also, several neurodegenerative diseases have been linked to the increased production of reactive oxygen species (ROS) within the brain and other neuronal tissues [22, 23]. Oxidative glutamate toxicity is initiated by high concentrations of extracellular glutamate that prevents cysteine uptake into the cells via the cysteine/glutamate anti-portal system [19]. This results in the depletion of intracellular cysteine and glutathione (GSH). Glutathione depletion induces cellular accumulation of ROS leading to cellular injury. GSH is one of the most important tripeptides that along with SOD, GSH-Px, CAT and Glutathione reductase, play a role in maintaining cellular antioxidant homeostasis by detoxifying ROS [24].

In the present study, MSG caused oxidative stress as evidenced by significantly decreased levels of GSH and GSH-Px in the MSG only group when compared to the control. This is in line with the findings of Rajagopal et al., [19] where MSG administered intraperitoneally brought about decreased levels of GSH, GSH-Px, SOD, CAT and Na-K ATPase and elevated malondialdehyde and nitrite content in the brain as compared with the control. Previous studies have demonstrated that *O. gratissimum* has a potent antioxidant activity by scavenging free radicals because of the presence of tannins, steroids, flavonoids, terpenoids and cardiac glycosides [10]. In this study, GSH, GSH-Px and SOD were significantly higher in brains of pups that received 300 mg/kg OG + MSG when compared to those that received only 4 g/kg MSG orally. This is likely due to the antioxidant properties of the plant extract which was observed to be more potent in comparison with vitamin E.

The balance of ROS can be termed the "balance of creation and destruction". Under normal circumstances, there is an appropriate balance between pro-oxidant and antioxidant. A shift in the levels of ROS towards prooxidants in developing brain can generate free radicals which in turn can induce an oxidative stress on the cerebellum and may result in cerebellar dysfunction [25]. The absence of widespread neuronal death in the toxicant group

can be attributed to the ability of the plant extract to attenuate neuronal loss (decrease in the ML thickness in post weaned MSG-treated pups) and promote neuroregeneration and the protection offered by enteral and hepatic metabolism [20].

Conclusion

This study has shown that oral monosodium glutamate administration caused mild but significant damages in the brain of rats in-utero which continued during weaning in post natal life and the antioxidant properties of ethanol extracts of *Ocimum gratissimum* was able to moderately mitigate the effect of MSG on the developing rat brain, which was found to be better than Vitamin E supplementation. The present study also showed that, it is likely that the oral route offers some protection to the body because MSG per oral was subjected to enteral and hepatic metabolism which probably reduced the quantity of MSG released into systemic circulation that caused severe damages in previous works.

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External genital warts in HIV-infected patients with sexually transmitted infections in Ibadan, Nigeria

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Abstract

*Background***:** Human Papilloma Virus (HPV) infection in the genital area is usually asymptomatic, and when symptomatic, manifests in either benign or malignant forms. This study aims at providing information on the prevalence of external genital warts (EGWs) among people living with HIV (PLWHIVs) attending Antiretroviral Treatment (ART) clinic at the University College Hospital, Ibadan, Nigeria.

Methods: This is a descriptive cross-sectional survey of PLWHIVs attending ART clinic between January 2006 and December 2007. Diagnosis of genital warts was based on the findings of typical lesions on the external genitalia, vaginal, cervix or perianal region after clinical examination and informed consent from each participant. Antibodies against Herpes and HPV were measured using, Enzyme-linked immunosorbent Assay (ELISA).

Results: A total of 5,207 patients, 3519 female and 1688 males attended the ART clinic during the period. The mean age of the patients was 34.67 yrs (± 9.16). Five hundred and forty-two (10.0%) had various sexually transmitted infections (STIs). The prevalence of anogenital warts was 3.65% among the HIV-infected patients and 35.0 % among the subset of HIV- infected patients with STIs. The prevalence of genital warts was 1.5 times higher in treatment experienced patients (OR =1.46; 95%CI: 1.02, 2.10). Genital wart was found to be associated with low CD4 count, high viral load, treatment-experience and non-use of condom during sexual intercourse. (P = 0.002).

Conclusions: External genital warts are common among people living with HIV infection. According to the appropriate guidelines, HPV vaccine should also be offered to HIV-infected adolescents that are non-reactive to the virus.

Keywords: *External Genital warts, Human papilloma virus, HIV, Sexually transmitted infections*

Résumé

Contexte: L'infection par le virus du papillome humain (VPH) dans la région génitale est généralement asymptomatique et, lorsqu'elle est symptomatique, se manifeste sous forme bénigne ou maligne. Cette étude vise à fournir des informations sur la prévalence des verrues génitales externes (EGW) chez les personnes vivant avec le VIH (PVVIH) qui fréquentent une clinique de traitement antirétroviral (ART) à l'hôpital collège universitaire, Ibadan, au Nigeria.

Méthodes: Il s'agit d'une étude descriptive transversale des PVVIH fréquentant la clinique ART entre janvier 2006 et décembre 2007. Le diagnostic des verrues génitales était basé sur les résultats de lésions typiques sur l'organe génital externe, vaginal, cervical ou péri-anal après examen clinique et consentement informé de chaque participant. Les anticorps contre l'herpès et le VPH ont été mesurés en utilisant un dosage immunosorbant enzymatique (ELISA).

Résultats: Au total, 5 207 patients, 3519 femmes et 1688 hommes ont assisté à la clinique ART pendant la période. L'âge moyen des patients était de 34,67 ans (± 9,16). Cinq cent quarante-deux (10,0%) avaient des diverses infections sexuellement transmissibles (IST). La prévalence des verrues ano-génitales était de 3,65% parmi les patients infectés par le VIH et de 35,0% parmi le sous-groupe de patients infectés par le VIH atteints d'IST. La prévalence des verrues génitales était 1,5 fois plus élevée dans le traitement expérimentés des patients (OR = 1,46; IC à 95%: 1,02, 2,10). On a constaté que la verrue génitale était associée à un faible taux de CD4, à une charge virale élevée, à une expérience de traitement et à la non-utilisation du préservatif pendant les rapports sexuels. (P = 0,002).

Conclusions: Les verrues génitales externes sont fréquentes chez les personnes vivant avec l'infection VIH. Selon les directives appropriées, le vaccin contre le VPH devrait également être offert aux adolescents infectés par le VIH qui ne sont pas réactifs au virus.

Mots-clés: *Verrues génitales externes, virus du papillome humain, VIH, infections sexuellement transmissibles*

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Introduction

Human papilloma virus (HPV) infection is one of the commonest sexually transmitted infections (STIs) worldwide among both men and women [1,2] with increasing incidence among men who have sex with men (MSM) (3). Genital Human Papilloma Virus (HPV) infection genital is frequently recognized as external genital warts (EGWs) in benign forms otherwise known as *condyloma acuminata* when symptoms are present [1,4]. EGWs may be fleshy, pedunculated, papular or macular lesions of the anal and/or genital mucosa and its adjoining areas of the body [4]. The lesions are filiform or sessile shaped. The warts can occur individually, in grape-like clusters, or form a cauliflower-like mass [4]

Majority of HPV infections are asymptomatic, transient, and usually resolve without causing clinical disease [1]. External genital warts are mostly linked to low risk HPV types 6 and 11 which have not been associated with anogenital cancers [4.5], though in rare occasions they are associated with Bushke-Lowenstein malignant tumours [6]. However, persistent infection with high-risk oncogenic types can progress to anogenital and oral cancers [7].. It has also been shown that women with previous history of EGWs have increased risk of cervical intraepithelial neoplasia (CIN) and cancer [2]. The true prevalence of HPV anogenital infection in the adult population has been found to be high (8, 9). Current evidence suggests that over 50 per cent of sexually active adults (15–25years of age) have been infected with one or more HPV types [7,10-12].. HPV is highly transmissible and transmission occurs most commonly through vaginal and anal intercourse [10,12-14]. First HPV infection is commonly acquired soon after sexual debut, even if exposure is limited to one sex partner [10, 11, 13] . The median time of development of the lesion after exposure has been estimated to be 5-6 months among women [15] and 11-12 months among men [16].

HIV infection and its associated immunosuppression are known to alter the course of HPV infection and its associated diseases [17,18]. The incidence of ano-genital and oral HPV infections among HIV – infected patients has been documented to increase progressively with the lowering of CD4 count [9, 19]. HIV infection among women has been found to be associated with an increased prevalence of cervical cancer precursors [19,20]. However, the co-infection with HPV could occur but this might not be sufficient to induce genital lesions [21]. In HIV-infected patients, external genital warts are more common, usually resistant to available treatment and more likely to recur than in general population [7, 9, 17, 22-24]. If the immune status of most of these patients are restored through effective therapy with Highly Active Retroviral Therapy (HAART), management of external genital warts are effective [24].

According to the meta-analysis conducted by Banura *et al* , the prevalence of EGWs varies from different regions of Africa [9]. Among sex workers and women with STIs, the prevalence ranges from 3.5-10.5% in West Africa, 3.3-10.7% in the East and 2.4-14.0% in Central and southern Africa [9]. The prevalence is lower among sexually active men in all regions(9). However, HIV positivity has been found to be a risk factor for acquisition of HPV infections and the prevalence is significantly higher in HIV – infected adult population [25]. The clinical evaluation of the burden of genital warts especially among women and men with the advent of HIV infection in the tropics needs to be reviewed regularly.

The objective of this study was to determine the prevalence and burden of anogenital warts among the subset of HIV-infected patients attending ART clinic at the University College Hospital, Ibadan, Nigeria.

Methods

This is a descriptive cross-sectional survey of 5,207 patients attending the antiretroviral clinic of the University College Hospital (UCH), Ibadan between the periods of January 2006 and December 2007. UCH is a tertiary health facility in Ibadan, southwestern, Nigeria. The adult ART clinic is one of the Government of Nigeria HIV clinics with support, through AIDS Prevention Initiative in Nigeria (APIN) and Harvard University, by President's Emergency Plan for AIDS Relief (PEPFAR) Program.

As part of the evaluation at enrolment and under aseptic conditions, physicians inspect the perineum /genitalia for signs of STIs. Diagnosis of genital warts was based on the clinical findings of typical lesions as previously described by Okesola & Fawole, Ekweozor *et al* and Lacy *et al* [4, 12, 26] on the external genitalia, penile shaft, vaginal, cervix or perianal region. Urethral swabs were taken from males as well as High vaginal swabs (HVS) and endocervical swabs (ECS) from females for microscopy and culture to establish diagnosis of other associated STIs from the patients with suspected STIs. About 5-10ml of venous blood was collected aseptically from each patient into EDTA bottle and tested for HPV IgG antibody and herpes simplex virus (HSV) IgG antibody. The detection of

HSV IgG was done using the 3rd generation enzyme-linked immunosorbent assay (ELISA) while ELISA test kits by DIAPRO Diagnostics Bioprobes Milano-Italy were used to tests the plasma samples for HPV IgG antibodies according to the manufacturer's instruction. The measurement of CD4 + T lymphocyte (CD4) count was carried out by flow cytometry. Roche Ampiclor RNA PCR assay was used for the measurement of plasma HIV RNA (viral load). Risky sexual behavior was defined as non-use or inconsistent use of condom.

Ethical considerations

The Antiretroviral Treatment program was approved by the University of Ibadan/University College Hospital Ibadan Joint Institutional Review Board. Informed consent forms were signed by all patients during enrollment procedures. Pictures were also taken on few occasions after informed consent.

Data analysis

Statistical Analysis was performed with the Statistical Package for Social Sciences (SPSS) version 20. Continuous variables were presented as mean and standard deviation while categorical variables were presented as frequencies / percentages. Associations between categorical variables were investigated using Chi square test, while student's t-test was used to test for significant difference in continuous variables. P value was set at 5%.

Results:

Demographic and clinico-pathological presentations

A total of 5,207 patients, 3519 female and 1688 males attended the ARV clinic during the period with mean age of 34.67 years (SD= 9.16; range 19-77years). Two thousand five hundred and sixty-seven (2,567, 49.3%) were in the 30-39 year age range. Three thousand and seventy-two (3,072, 59.0%) of the patients evaluated were married. Table 1 is a summary of the socio-demographic status of the patients. Five hundred and forty two patients had at least one STI based on history and examination at baseline. The prevalence of STIs among the patients evaluated was 10.4%, 12.42% (437 of 3519) in females and 6.22% (105 of 1688) among the males. The male to female ratio of those patients with various STIs was 1: 4.

External genital warts were diagnosed in 3.65% (190 of 5,207) of the HIV-infected patients. The prevalence of external genital warts among the subset of patients with STIs was 35.1% (190 of 542).

Table 1: Socio-demographic characteristics and treatment status of the patients

Characteristics	Frequency (N=542)	Percentage (%)
Age categories		
10-19	1	0.2
20-29	143	26.4
30-39	267	49.2
40-49	87	16.0
50-59	28	5.2
60-69	15	2.8
70-79	1	0.2
Level of Education		
None	38	7.0
Primary	160	29.5
Secondary	215	39.7
Tertiary	129	23.8
Marital Status		
Single	86	15.9
Married	320	59.0
Separated	51	9.4
Divorced	25	4.6
Widowed	60	11.1
Sex		
Male	105	19.4
Female	437	80.6
Treatment status		
Naïve	241	44.5
Experienced	301	55.5

All the patients diagnosed with EGW were positive to HPV IgG antibody. Of the 190 cases of external genital warts, one hundred and fifty-two (80.0%) were females while 38 (20.0%) were males giving male to female ratio of 1: 4.2. The external genital wart lesions were found on both adolescents and adults. The sites of the EGWs in females included the vulva, vagina, perineum and perianal region (Figures 1 and 2). In males, warts were mostly found on the glans penis, shaft of the penis, perineum, suprapubic and anal regions (Figure 3). Two of the male patients with EGWs had florid anal warts. The mean duration of the external genital warts prior to presentation was nine months (Range: 3months-2 years). Four hundred and sixty one patients (85.0%) usually engage in heterosexual relationship while two of the males admitted having sex with males.

Two hundred and twenty-three women who had STIs at presentation (223/437, 41.1%) had vulvo-vaginal candidiasis, 115 (21.3%) had herpes genitalis, two (0.4%) had gonorrhoea while one

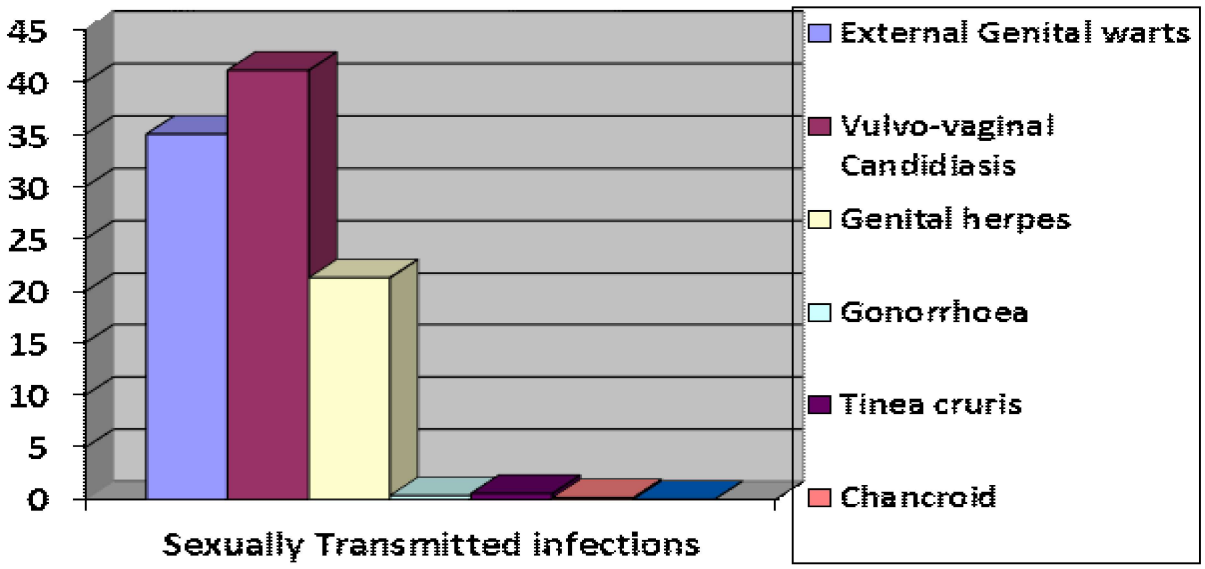

Fig. 1: Distribution of sexually transmitted infections among the PLWHIV in Ibadan

Fig. 2: Giant Vulva warts

Fig. 4: Genital warts in male

Fig. 3: Intra-vaginal warts

(0.2%) had *Molluscum contagiosum* virus infection as shown in Figure 4. Twenty (3.69%) of them who presented with STIs had EGWs co-infection. The common co-infections in the evaluated population of patients were vaginal candidosis and EGWs (9, 1.66%), as well as genital herpes and EGWs (7, 1.29 %). Two hundred and forty-seven (45.6 %) of those with STIs were HAART naive. Prevalence of EGW was found to be higher among the married (63.2%), and secondary school leavers (42.1%).

Most of the patients who were unwilling to have protected sexual intercourse (140/392, 35.7 %) had higher prevalence of genital warts compared to those who reported condom use (50/150, 33.3%), though not significantly associated (P>0.05).

Table 2: Association of social demographic and clinical correlates with prevalence of genital warts

Characteristics	Genital Warts Yes (n=190)	No (n=352)	P values	OR (95% CI)
Sex				
Male	38 (20.0%)	67 (19.0%)	0.786	1.06 (0.68 -1.66)
Female	152 (80.0%)	285 (81.0%)		
Condom Use				
Yes	50 (26.3%)	100 (28.4%)	0.603	0.9 (0.61-1.34)
No	140 (73.7%)	252 (71.6%)		
Treatment status				
Naive	73 (38.4%)	168 (47.7%)	0.037	1.46 (1.02-2.10)
Experienced	117 (61.6%)	184 (52.3%)		
Age				
10-19	1 (0.5%)	0 (0.0%)	0.185	
20-29	53 (27.9%)	90 (25.6%)		
30-39	99 (52.1%)	168 (47.7%)		
40-49	25 (13.2%)	62 (17.6%)		
50-59	10 (5.3%)	18 (5.1%)		
60-69	2 (1.0%)	13 (3.7%)		
70-79	0 (0.0%)	1 (0.3%)		
Level of Education				
None	8 (4.2%)	30 (8.5%)	0.46*	
Primary	59 (31.1%)	101(28.7%)		
Secondary	80 (42.1%)	135 (38.4%)		
Tertiary	43 (22.6%)	86 (24.4%)		
Marital status				
Single	30 (15.8%)	56 (15.9%)	0.361	
Married	120 (63.2%)	200 (56.8%)		
Divorced	10 (5.3%)	15 (4.3%)		
Separated	13 (6.8%)	13 (6.8%)		
Widowed	17 (8.9%)	43 (12.2%)		-
CD4 group	N=145	N=266		
<200	89 (61.4%)	183(68.8%)	0.164	
201-350	27(18.6%)	48(18.0%)		
>350	29(20.0%)	35(13.2%)		

**P value obtained from Chi square test for trend.*

Analysis of viral load and CD4 counts

Out of the 542 PLWHAs with STIs, one hundred and fifty (27.7%) reported condom use, Eighteen (3.3 %) had viral load < 200 copies/ ml while 272 (50.1 %) had low CD4 count (< 200 cells / mm3.) The mean log viral load was 5.02 $\pm$ 0.94. The prevalence of external genital warts was 1.5 times higher in treatment experienced patients compared to those that are HAART naïve. (OR =1.46; 95%CI: 1.02, 2.10) as shown in Table 2.

External genital wart was also found to be higher in patients with low CD4 count (89/190, 61.9%), high viral load (129/136, 94.9%) and unwillingness to have protected sexual intercourse (140/190, 73.7%) (P >0.05), though not significantly associated (Table 2).

Discussion

HIV infection and its associated immunosuppression are known to alter the course of HPV infection and its associated diseases [17, 18] . In the early 1990s, the rate of reported cases of genital warts was found to be scanty [12]. Our study has demonstrated a lower rate (3.65%) of external genital warts among the patients enrolled in antiretroviral clinic, University College Hospital, Ibadan, Nigeria. Previous studies

have confirmed rates ranging from 8-42% depending on methods of diagnosis that includes visible anogenital warts, cytopathology and viral DNA for the molecular diagnosis of HPV [12, 23, 26]. Our finding is also much lower than 7.0 % obtained in a previous study conducted in Burkina Faso [23]. However, the prevalence of EGW was found to be 35.0% among the subsets of patients diagnosed with sexually transmitted infections. This is within the range of the findings of the previous studies [24, 27]. The increased prevalence observed among these cohorts of patients might not be unconnected with the recrudescence of previously latent HPV infection in those who are sexually active, while others may have been as a result of new infections.

External genital wart has been found to be common among young sexually active female gender with multiple sexual partners [7, 10-12]. This has been demonstrated in our study that shows female to male infection ratio to be 4:1. In a study by Thompson *et al*, the incidence of EGW was found to be higher in females than males prior to year 2000 [27]. However, the incidence was found to be increasing steadily among males from 2000 to 2011(27). Contrary to disproportionately higher genital warts in MSM in the study conducted by Jiamton *et al* [3], anogenital warts were higher in females living with HIV / AIDS in our centre from our study. Young adults especially the married women within the age bracket of 30-39 years were noted to be the highest risk group with the rate of 49.3%. This is closely followed by the most sexually active group aged between 20-29years with the rate of 26.4%. This is contrary to what was obtained in similar studies among sexually active adults where the peak age incidence of adults with EGW was between 20-29 years [12, 26]. These findings from our study may not be unconnected with the late presentations of young women at the ART clinic who might have been infected when they were younger. The various sites of EGW as noted on the vulva, mons pubis, inguinal folds, glans penis, shaft of the penis, scrotum and coronal sulcus in this study are similar to the findings in other studies [14, 28].

Genital herpes (21.3%) was found to be the most sexually transmitted co-infection in both male and female patients in our study while vulvo-vaginal candidiasis (41.1%) is the most common sexually transmissible disease co-infection among women especially in middle-aged group. This observation is in agreement with the findings in similar studies [12, 23, 26, 28, 29], though the rate of co-infections was lower than what we have from our findings.

Since anogenital HPV related disease is related to immunosuppression, it could be expected that the clinical epidemiology of HPV infection may change as a result of HAART-associated reversals in immunosuppression. Prevalence of genital warts was 1.5 times higher among the treatment experienced patients than HAART naïve patients. This might be explained by the fact that at the time the study was conducted; only those with significant immunosuppression were placed on treatment while those with high CD4 counts were monitored serially until their CD4 count dropped below a certain threshold (350cells/mm^3). This meant that in general, being on ART meant that the patient had a lower CD4 count and thus more immunosuppressed. This result is contrary to the previous findings and reviews which believed that clinical management of genital warts would be easier with effective HAART [24]. However, our finding is in agreement with what was obtained by Low *et al* who reported that antiretroviral therapy was not protective against the persistence of genital warts in HIV-infected patients [23].

Majority of the infected patients who reported non-use of condom during sexual intercourse were noted to have higher prevalence of genital warts (73.7%) than those that reported condom use. The finding is in agreement with the finding of meta-analysis conducted by Manhart and Koutsky, [30] who reported that the available data could not provide a precise estimate of prevention of genital HPV infection due to inconsistency, but they however suggested that condom may not prevent HPV infection but could protect genital warts [30]. Risky sexual behavior was found to be significantly associated with low level of education ($P<0.0001$). This result is in agreement with the findings of Soori *et al,* 2013 [29] and more often transmission has been reported from female partners to male partners than from male to female [31]. In this study, external genital wart was also associated with low CD4 count, high viral load, treatment-experienced and non-use of condom during sexual intercourse. ($P = 0.002$).

Most of our patients (133/190, 73.7%) were treated with 20% podophyllin in a tincture of benzoin with complete resolution of their lesions. However, there are many treatment options that are currently being adopted for external genital warts in different part of the world [12, 26, 28, 32], with wide variation across many sites depending on the budget and policies [28]. Some of the treatment options include 5% imiquimod, cryotherapy, electrosurgical resection, interferon alpha-2b and electro fulguration [28]. Combination of interferon alpha-2b and electro

fulguration has been found to be highly effective for children with massive genital warts [28]. Those with extensive intravaginal warts were referred to the gynecological clinic for further management. Two of our patients who were offered surgical resection had a complete resolution of their lesions.

Conclusions and recommendation

Preventive program is mandatory for anogenital warts, especially among this group of patients. Full screening for CIN remains necessary in HIV-positive women and it is likely that screening for AIN will be beneficial as well to prevent invasive anogenital cancer in long-term AIDS survivors(20). Diagnosis and treatment of genital warts as well as follow-up of HIV-infected patients should be performed routinely in the HIV clinics. PLWHIVs should be counseled on practices that may reduce the risk of acquiring HPV infection, including safe sexual practices and reduction in number of sexual partners. Screening for genital warts and follow-up of HIV-infected patients should be performed routinely. According to the appropriate guidelines, HPV vaccine should also be offered to HIV-infected adolescents using appropriate guidelines.

HIV-infected persons in care may be an important target group in which to conduct regular screening for Sexually Transmitted Infections (STIs) to prevent enhanced transmission of HIV.(1) Pap smear screening for cervical dysplasia and cancer in women with HIV infection should be more frequent than routinely recommended for the general population and requires careful follow-up. HPV testing of pap smear samples where facilities exist should be performed to detect infection even before it has been present long enough to cause cervical or other anogenital abnormalities. Widespread HPV vaccination could make an important reduction to morbidity and mortality related to HPV infection in developing countries like Nigeria. The quadrivalent HPV types 6, 11, 16 and 18 recombinant vaccine is highly immunogenic and is highly recommended. The possibility of making the vaccine available to adolescent boys and men should also be considered to help break the cycle of widespread HPV infection.

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Congenital solitary functioning kidney; an incidental cadaveric dissection finding in an adult male Nigerian: Case report.

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Abstract
Background: Congenital solitary functioning kidney is one of the congenital anomalies of the kidney and the urinary tract. It is very rare and has a male predominance. An incidental case of a congenital solitary functioning kidney in an apparently healthy adult Nigerian male was discovered during cadaveric dissection for a study of kidney dimensions in Nigerians.
Conclusion: This report was premised on the rarity of this congenital anomaly, its existence as the only anomaly in an adult and its compatibility with normal adult life.This case report further strengthened the crucial role and benefit of cadaveric dissection in the teaching and learning of human anatomy.

Keywords: *Congenital solitary kidney, adult male, Nigerian, Cadaveric dissection*

Résumé
Contexte: le fonctionnement solitaire congénital du rein est l'une des anomalies congénitales du rein et des voies urinaires. C'est très rare et a une prédominance masculine. Un cas incident d'un rein de fonctionnement solitaire congénital chez un homme nigérian adulte apparemment en bonne santé a été découvert lors d'une dissection cadavérique pour une étude des dimensions rénales chez les Nigérians.
Conclusion: Ce rapport était fondé sur la rareté de cette anomalie congénitale, son existence étant la seule anomalie chez un adulte et sa compatibilité avec la vie adulte normale. Ce rapport de cas a renforcé le rôle crucial et le bénéfice de la dissection cadavérique dans l'enseignement et l'apprentissage d'anatomie humaine.

Mots-clés: *Rein congénital solitaire, Homme adulte, Nigérian, dissection cadavérique*

Introduction

Congenital solitary functioning kidney (CSFK) is one of the anomalies of the genitourinary organs and tract. It is characterized by the presence of only one kidney either due to failure of development of one kidney i.e unilateral renal agenesis or regression and complete disappearance of a malformed kidney before birth. In children, diagnosis is usually made at abdominal ultrasonography in the course of investigation for renal and urinary tract pathologies. In adults, its diagnosis may be incidental finding at surgery, postmortem examination, cadaveric dissection or radiologic investigations for either renal or non-renal indications. The exact incidence of CSFK is unknown, however, a postnatal autopsy series put it at 1: 1000 [1], an ultrasonographic study reported an incidence rate of 1:500 [2] while another ultrasonographic study put it at 1 per 1500-3200 live births [3]. In an ultrasound screening study of 4000 neonates, the incidence of CSFK was found to be 1: 1300 [4]. The incidence of CSFK in Nigerians is unknown. It may be an isolated anomaly as in the index case. It may be syndromic; associated syndromes and anomalies with which CSFK had been reported included acro-renal syndrome [5-8]; VACTREL anomalies [9]; MURCS syndrome [10], Poland syndrome [11] and Ear, Nose and Throat anomalies (ENT) [12]. It has been reported to be commoner in males than females [3,12].

A review of literature revealed that only one case, a 38 year old Nigerian male with unilateral renal agenesis coexisting with bilateral cryptorchidism had been reported [13]. Thus to this best of our knowledge, this is the second case of CSFK in an adult Nigerian that will be reported. It is worthy of note that this case being reported was an incidental finding in the course of cadaveric dissection for a study on assessment of kidney dimensions in Nigerians by us.

Case report

The index case was one of the cadavers sourced from Lagos, Nigeria for the purpose of supervised gross anatomy dissection by undergraduate medical students. Unconfirmed source had it that he was involved in deadly clash between rival cult groups at which dangerous weapons were freely deployed.

The body was that of a young negroloid, healthy looking man of medium to heavy habitus.

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Fig. 1a: Anterior view of the Index Case.Note the facial appearance

Fig. 1b: Posterior view: The sutured deep lacerations are indicated by **** While **§§§** indicate an extensive ragged-edge gaped laceration.

He had a stylish hair cut with dark full grown and groomed beard (Figure 1a). Physical examination of the body did not reveal any scar that would have been suggestive of previous abdominal surgery however, about forty eight deep matchet cuts in all the regions of the body were noted. They were mostly sited in the head, neck, back (posterior trunk) and the upper limbs. Prominent amongst the lacerations were a ragged- edge one approximately 10.0 cm in length and 3.0 cm deep at the level of posterior hair line and another one over the left parotid region with a length of about 6.0 cm. (Fig. 1b).

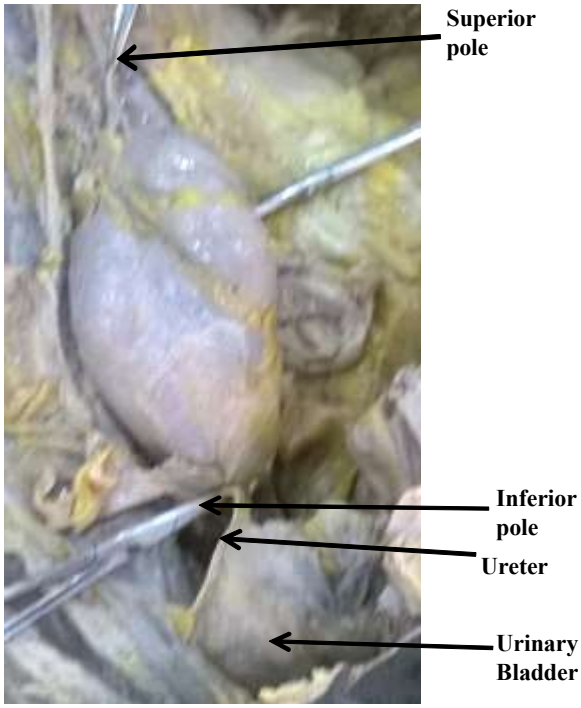

Fig. 2: Anterior view of the Solitary Kidney (In-situ) The superior pole is conical while the inferior pole

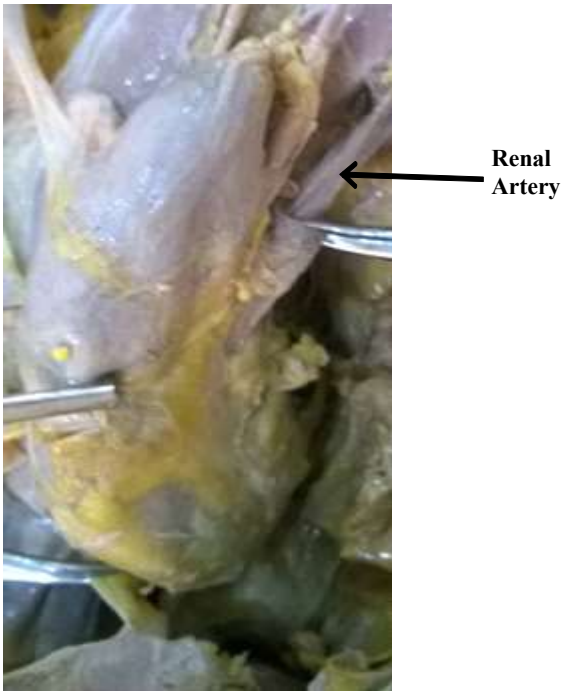

Fig. 3: Side view showing the single appears roundish. Renal artery

During the dissection of the abdominopelvic cavity, both lumbar (paravertebral) regions were explored for the respective kidneys but none was

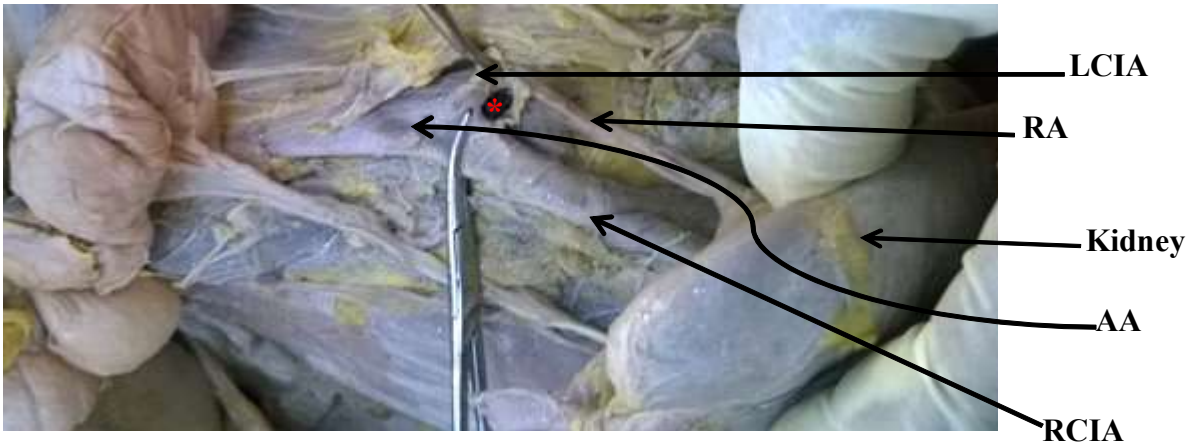

Fig. 4: The renal artery and its origin**.** *The small rent in the wall of the of the LCIA just at its origin was iatrogenic.LCIA=Left common iliac artery. RA = Renal artery. AA = Abdominal aorta. RCIA=Right common iliac artery.

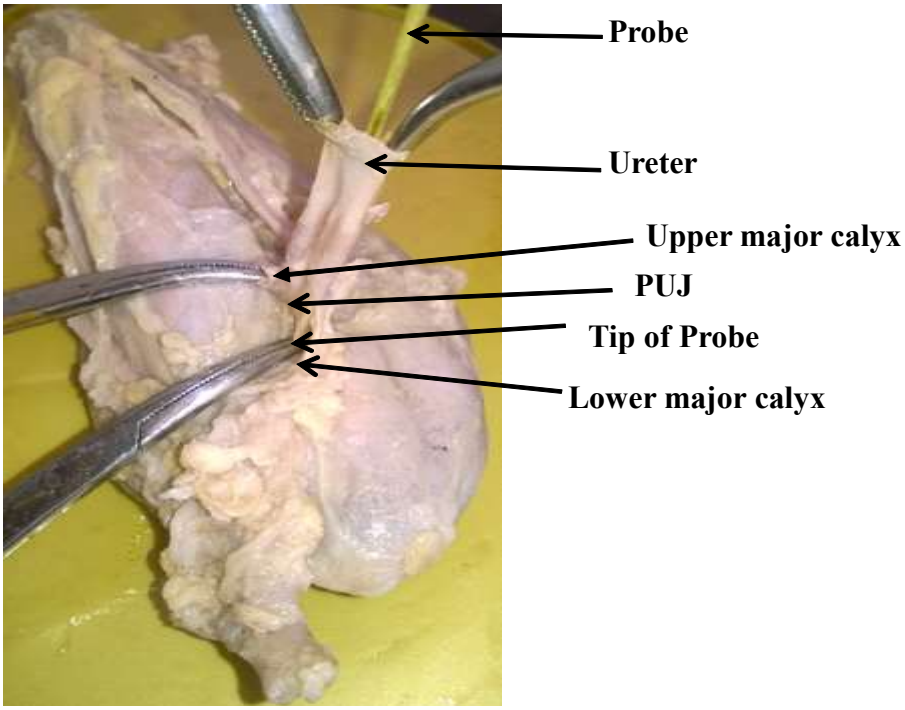

Fig. 5: Posterior view of the solitary kidney. Showing the ureter, the pelvicureteric junction (PUJ) and two major calices

found. It is pertinent to note that there was no evidence of previous exploration of the lumbar regions i.e both lumbar regions were 'virgin'. A solitary kidney with the following description was however found in the lower midline. The kidney was ovoid in shape extending from the upper border of the 4th lumbar vertebra down to the sacral promontory (figure 2). It had a bipolar dimension (length) of 10.4 cm, transverse dimension (width) of 5.1 cm, a thickness of 3.0 cm and weighed 96.0 g. There were two veins on the anterior surface and one on the posterior surface. The two anterior veins were 3.0cm and 3.5 cm distal from the upper pole respectively. They merged to form the proper anterior renal vein. The posterior vein arose from its posterior aspect approximately 5.0 cm from the upper pole. Both the anterior and posterior renal veins unite at the upper pole to form the main renal vein which emptied into the inferior vena cava at about 3 cm proximal to the formation of the inferior vena cava by the merging of the right and left common iliac veins. The main renal vein was 3.2 cm long, the proper anterior vein was 2.9 cm in length and the posterior renal vein was 3.9 cm long.The kidney had a single artery which

was a branch of the left common iliac artery and it pierced the parenchyma of the kidney on its posterior surface about 1.0 cm distal to the origin of the posterior renal vein (Figures 3&4). The ureter arose from the posterior surface and was about 6.0 cm long (figure 5). The kidney was in alignment with the urinary bladder along the vertical axis.All the other abdominopelvic structures and organs were grossly normal and intact. Examination of the head and neck, thoracic cavity, limbs and the perineum including the gonads did not reveal any gross congenital anomaly.

Discussion

The kidneys start to develop at the beginning of fourth week of gestation and they become functional by the beginning of the 9th week, nephrogenesis however continues till the 36th week.

The precursors of the paired human kidneys are the pronephroi, mesonephroi and metanephroi. The pronephroi found in the cervical region, degenerate after giving rise to the mesonephric ducts. The mesonephroi which are located in the thoraco-lumbar region give rise to the initial glomeruli and tubules (both constitute the mesonephric kidneys).These tubules open into the mesonephric ducts. The mesonephric kidneys will subsequently degenerate. The metanephroi located in the sacral region become the permanent kidneys. Each metanephros consists of metanephric diverticulum (MD) (an outgrowth of the mesonephric duct) and metanephrogenic blastema (MB) which is a mass of intermediate mesoderm. The MD forms the ureter, renal pelvis, calices and collecting tubules. .

The glomerulus and its capsule, proximal convoluted tubule, loop of Henle and distal convoluted tubule are derived from the metanephrogenic blastema. Both MD and MB interact and induce each other this is known as reciprocal induction. It is this process that gives rise to the permanent kidney [14]. Certain molecules known as renal developmental genes (RDG) regulate this reciprocal induction. These RDG include glial derived neurotropic factor (GDNF), fibroblast growth factor-2 (FGF-2), bone morphogenetic protein 7 (BMP7), hepatocyte nuclear factor-1β (HNF1β), Pax2, BMP4, FRAS1, FREM2, Six2 and Ret which is a receptor tyrosine kinase for GDNF [15-18]. Thus mutations of the RDG or defects in the transcription factors may result in congenital anomalies of the kidney and urinary tract.

The metanephric kidneys lie closely to each other ventral to the sacrum, as the embryo grows; they progressively ascend and become further apart. The normal adult position of 11th thoracic vertebra to the 3rd lumbar vertebra (T11- L 3) is reached by the 9th week. The hilium is initially anterior but with subsequent 90^0 medial rotation it becomes anteromedial. The origin of the renal artery changes with the accession of the kidney [14].

Congenital anomalies of the kidney may be in form of non-formation of one or both kidneys (renal agenesis), abnormal location (ectopic kidney), disruption of rotation (malrotated kidney) and polar fusion of the kidneys (horseshoe kidney). With serial ultrasound scanning, cases of renal aplasia have been observed to regress to renal agenesis [4].

In the case being reported, only one kidney was found, thus it is a case of unilateral agenesis. This most probably arose from failure of reciprocal induction between the mesonephric diverticulum and metanephrogenic blastema. The origin of the renal artery of the index case was the left common iliac artery thus the reciprocal induction failure must have involved the right metanephric kidney. The normal outgrowth of the MD requires transcription factors such as WT1gene and signaling molecules such as GDNF and its epithelial receptor ret. Thus non - expression or under expression of these molecules will hinder the reciprocal induction and result in agenesis. The expected location of the kindney was the left lumbar region however, the index case was located anterior to the lower lumbar vertebrae (4th and 5th) and the sacral promontory (midline) ; this is a form of ectopia. This ectopia could have resulted from etopic ureteric budding, failure of vascularization or abnormal migration [19]. The single artery that supplied this index arose from the left common iliac artery. Thus the most probable factor responsible for its ectopic position was the arrest of its accession. Though, no anatomical structure that could have been responsible for the arrest of the accession was observed; this must have been atrophic and subsequently disappeared since the index case was an adult.

The exact causes of renal agenesis are unknown but certain genetic and environmental factors either maternal or inherent in the developing embryo may be responsible. Such implicated factors include foetal alcohol syndrome, maternal diabetics [20] , maternal usage of thalidomide [21], chromosomal abnormalities such as trisomies 10, 21 and 22; 45 X mosaicism and 22q11 microdeletion [22]. Certain drugs if administered during pregnancy may interfere with renal development. Such drugs include angiotensin converting enzyme inhibitors, dexamethasone, anticonvulsants and amino

glycosides [23]. Individuals with CFSK are at increased risk of renal injury. This injury includes hypertension, proteinuria and progression to chronic kidney disease [22]. This renal injury is precipitated by glomerular hyperfiltration which results into glomerular hypertension. No medical history was available in the index case; hence the presence or absence of renal injury in him could not be ascertained.

Urinary tract infections (UTI) and vesicoureteral reflux have been documented to retard kidney growth in children with CSFK [24], thus it is pertinent to treat UTI promptly and adequately in such patients.

At least one kidney is required for sustenance of life thus in patients with solitary functioning kidney (SFK) with renal tumours total nephrectomy is contraindicated. Nephron sparing surgery (NSS) is the gold standard for patients with SFK [25]. Operative modalities for NSS include open partial nephrectomy (OPN), laparoscopic partial nephrectomy (LPN) and robotic partial nephrectomy (RPN). Both LPN and RPN procedures have better perioperative outcomes [25] however, they require more expertise and equipment than OPN. These additional requirements (expertise and equipment) place a greater professional burden on the Urological Surgeon. Other procedures for SFK with tumours are cryotherapy and radiofrequency ablations [26, 27] both however have inferior outcomes to the previously mentioned procedures [28].

The definitive management for patients with chronic renal failure is replacement of only one kidney at one transplant surgery. The case being reported further strengthened this age-long tested transplant surgical procedure of replacing only one kidney and not the two kidneys in patients with end stage kidney disease. Also, this case report further attested to the previously held view that cadaveric dissection is crucial to the teaching and learning of gross anatomy and should not be replaced rather, available alternatives should be complementary [29].

Conclusion

From circumstantial evidences, it might be inferred that the index case being reported was apparently healthy. If his renal status was compromised, he would not have had the strength to live a violent life style as evidenced by the findings on physical examination and the multiplicity and the depth of the lacerations. Thus this case highlights the rarity of congenital solitary functioning kidney in adult Nigerians.

This case also attests to the fact that congenital solitary functioning kidney may be the only anomaly in an individual and such person may live a normal life with death being unrelated to the kidney.

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Isolated left common iliac aneurysm – a case report

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Abstract
Background: Isolated iliac artery aneurysms (IAAs) are rare and comprise 1% of all intra-abdominal aneurysms. They tend to be asymptomatic or incidentally diagnosed at imaging. A 40-year-old hypertensive male who presented with a left abdominal swelling of 4-year duration and 6-month history of left lower limb swelling and pain is reviewed. Abdominal ultrasound with colour Doppler and Computed Tomography (with Angiography) confirmed the presence of a slow leaking left common iliac aneurysm. The patient had open repair of the aneurysm. He had a smooth postoperative recovery and was discharged 15 days postoperatively.
Conclusion: An unusual presentation of this rare aneurysm in our environment is reported, using appropriate imaging modalities.

Keywords: *Isolated iliac artery aneurysms, hypertension, open repair*

Résumé
Contexte: Les anévrismes de l'artère iliaque isolée (AAI) sont rares et représentent 1% de tous les anévrismes intra-abdominaux. Ils ont tendance à être asymptomatiques ou éventuellement diagnostiqués lors de l'imagerie. Un homme hypertendu de 40 ans qui a présenté un gonflement abdominal gauche de 4 ans et une histoire de 6 mois d'enflure et de douleur des membres inférieurs gauche est revu. L'échographie abdominale avec couleur Doppler et tomodensitométrie (avec angiographie) a confirmé la présence d'un anévrisme iliaque commun à fuite lente. Le patient a été, de manière ouverte, traité de l'anévrisme. Il a eu une récupération postopératoire lisse et a été déchargé 15 jours après la chirurgie.
Conclusion: Une présentation inhabituelle de cet anévrisme rare dans notre environnement est signalée, en utilisant les modalités d'imagerie appropriées.

Mots-clés: *Anévrismes de l'artère iliaque isolée, hypertension, réparation ouverte*

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Introduction

Isolated iliac artery aneurysms (IAAs) are either single or multiple aneurysms located only within the iliac artery system without a concurrent abdominal aortic aneurysm (AAA) [1,2]. Most iliac artery aneurysms (IAA) occur in association with abdominal aortic aneurysms (AAA). This could be either as an extension of the aortic aneurysm into the common iliac artery or a coexisting AAA. However, isolated IAAs are rare and comprise 1% of all intra-abdominal aneurysms. Several reports have suggested that isolated IAAs have a high risk of rupture with an associated high mortality rate. They tend to be asymptomatic or incidentally diagnosed at imaging [2]. The signs and symptoms of these aneurysms are influenced by their concealed location within the abdomen and bony pelvis [3]. Its rarity justifies that its natural history and management be documented. Awareness of the special characteristics of this aneurysm improves the chances of early diagnosis and proper treatment before possible rupture**.** This case is being presented because of the rarity of an isolated common iliac aneurysm in the population.

Case Report

A 40-year-old hypertensive male presented to our surgical outpatient department with a left abdominal swelling of 4 years duration and 6-month history of left lower limb swelling and pain. The abdominal swelling has been progressively increasing with associated severe back pain. The patient initially presented two years earlier with abdominal discomfort, left sided pulsatile abdominal swelling and low back pain. A clinical diagnosis of a probably vascular intra abdominal mass was made. Patient however absconded from clinic before representing 2 years later.

Physical examination at presentation revealed a middle aged man in painful distress, not pale and afebrile. The blood pressure was 180/100 mmHg while the pulse rate was 88 per minute. Examination of the abdomen revealed a left sided abdominal swelling which measured about 12 by 15cm. Scarification marks were seen on the skin over this mass. It was warm, pulsatile and tender to touch and tense on palpation. We made a clinical diagnosis of a ruptured intra abdominal aneurysm.

Abdominal Ultrasound (US) (fig 1) showed huge enlargement of the left common iliac artery about 6cm in its transverse diameter. There was associated thickening of its wall which was about 4.5cm thick. Colour Doppler (fig 2) showed turbulent flow in this portion.

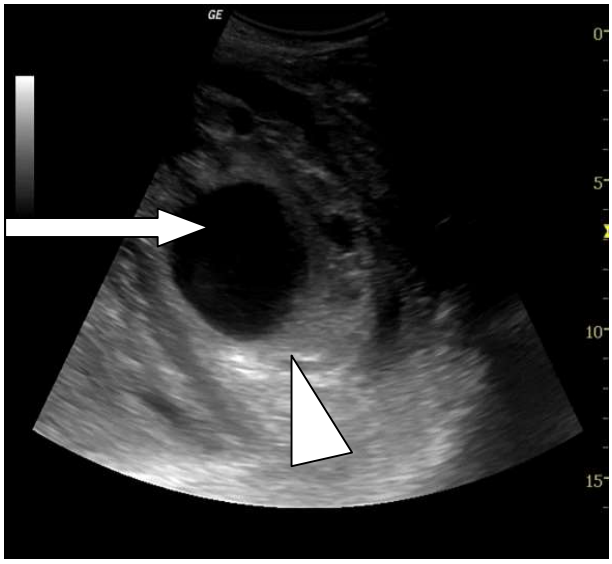

Fig. 1: Transverse view of the Abdominal US showing the dilated left common iliac artery (arrow) with marked thickening of its wall (arrow head).

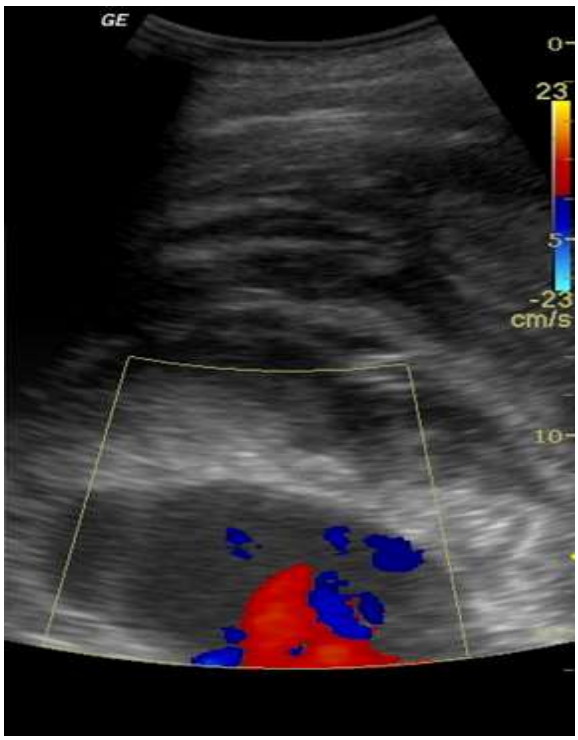

Fig. 2: Doppler USS showing colour flow within the aneurysm as well as the thickened wall

Abdominal Computed Tomography (CT) Axial images (fig 3a & 3b) showed gross dilatation of the proximal portion of the left common iliac artery (CIA) with marked thickening of its wall. Some calcifications were seen within its wall. There was associated erosion of the related vertebral body (fig 3). Volume rendered CT Angiographic study (fig 4) showed the dilated CIA with pooling of contrast within its lumen. It also showed reduction in the caliber of the distal left CIA.

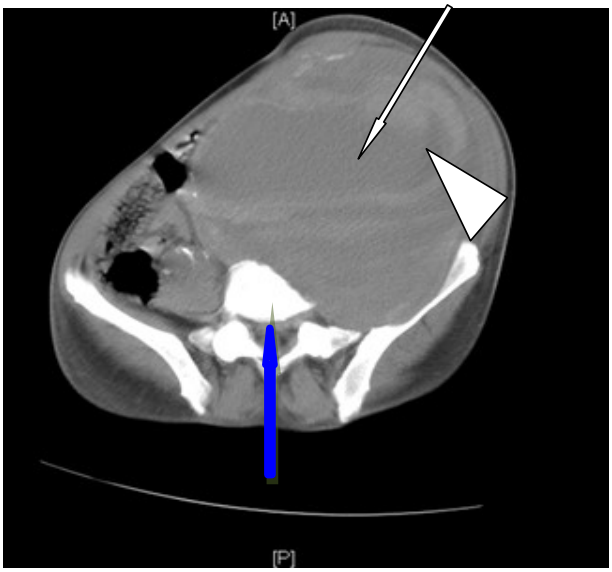

Fig. 3a: Serial pre and post contrast axial images showing the aneurysmal left common iliac artery with pooling of contrast within its lumen (white arrow) in the post contrast phase

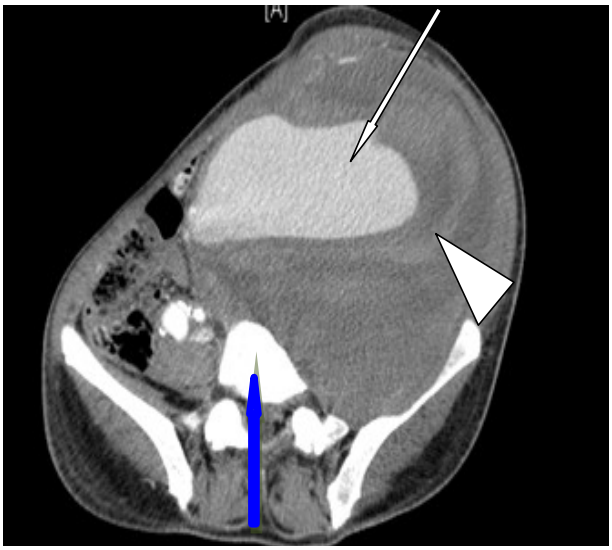

Fig. 3b: The markedly thickened wall (arrow head). There is also erosion of the related vertebral body(blue arrow).

A diagnosis of a left common iliac aneurysm was made. The patient had open repair of the aneurysm - aneurysmorrhaphy. Surgical approach was via a midline laparotomy with findings of a large aneurysmal sac measuring 24 x 16 cm rising from the pelvis to the left subcostal area. The aorta was

dissected free with a loop passed around it just above the bifurcation into the common iliacs. The aorta was clamped below the inferior mesenteric artery and the external iliac also clamped. The aneurysm was opened into and thrombus evacuated. There was an area of leak noted in the left common iliac aneurysm from where some back leak was noted. Attempt at distal embolectomy through the left external iliac was futile as the artery was completely thrombosed. Endarterectomy was done at the orifice of the left internal iliac and towards the left common iliac. The area around the left internal iliac was dissected free and the opening closed with 3-0 prolene. The aneurysmal sac was closed over the repair. The patient had a smooth postoperative recovery and was discharged 15 days postoperatively.

Fig. 4: Volume rendered CT Angiographic image showing the grossly dilated left common iliac artery (small arrow) with reduced caliber of its distal portion.

Discussion

An aneurysm is a permanent localized dilation of an artery having at least a 50% increased diameter compared with the expected normal diameter of the vessel. The term ectasia is used when the dilatation is less than 50%. A common iliac artery aneurysm (IAA) is defined as any permanent, localized dilatation of the common iliac artery larger than 2.5cm in diameter [3]. The definition of an iliac artery aneurysm is however controversial as some authors consider the use of a diameter >2.5cm described by Khosa *et al* [3] as being too large as several authors indicate that the normal diameter of the common iliac artery is 1.0cm [4]. It is therefore argued that a left common iliac artery greater than 1.5cm is aneurysmal by other authors [2]. Although iliac aneurysms co-exist with aortic aneurysms in around 10-20% of cases, isolated iliac aneurysms are rare, with a prevalence of around 2% in patients with aorto-iliac aneurysms [5]. Over 80% of IAAs occur in males with a mean age of 69 years, and almost all are due to atherosclerosis [5-6]. The patient presented however is a 40-year-old male, an age not noted for remarkable atherosclerotic disease.

The position of iliac artery aneurysms makes clinical diagnosis difficult with approximately 75 percent of iliac aneurysms said to be asymptomatic. Some patients present with symptoms resulting from aneurysm rupture or local compression of adjacent structures, but the majority were incidentally discovered on imaging [7-9]. Symptomatic aneurysms often appear as a pulsatile abdominal mass, and cause pain in the back, testicles, or groin, and sometimes haemorrhagic shock from rupture. Aneurysms tend to become symptomatic when they rapidly expand, leak, or rupture. Clinical history and examination, while reliable in the case of aneurysm rupture, is frequently misleading in the non-ruptured aneurysm [10] as was in the case presented.

The anatomic site and morphology of an aneurysm can be preoperatively determined by radiologic means as was done in the case presented on both ultrasound and Computed Tomography Angiography (CTA). Our patient appeared to have had a type B common iliac artery aneurysm which has an adequate proximal neck (i.e. $\geq$1.5 cm of non-aneurysmal artery) without a distal landing zone [11]. This classification allows selection of appropriate candidates for endovascular or surgical therapy. Our patient was better suited to open repair.

Plain radiographs indicate the diagnosis in 35% of cases [12]. It may show curvilinear calcification in the abdomen or pelvis and have been described as showing erosion of either the vertebral body or sacroiliac joint. The scanogram in this patient showed marked scoliosis but the bony erosion of the related vertebral body was only seen on axial CT.

Ultrasonography (US) remains the initial imaging modality when an asymptomatic, pulsatile abdominal mass is palpated; because of portability, lack of ionizing radiation, cost, and availability. However, in urgent situations in which the clinical diagnosis is fairly certain or rupture is imminent or suspected and in which the patient's condition is stable, CT and/or CTA may be the initial and only examination required. CT is positive in almost all cases and can demonstrate both the site and true size of every

aneurysm as was seen in the case presented. Conventional CT will not demonstrate all the feeding vessels of an aneurysm. Angiography remains the gold standard imaging modality [13].

Most aneurysms progressively enlarge as seen in this patient who was first diagnosed two years earlier after which the patient defaulted. The diameter of an aneurysm is directly related to its risk of rupture. Santili *et al* demonstrated that small aneurysms (< 3 cm) expand at a slower rate, averaging 1.1 mm/year, whereas the growth rate of aneurysms larger than 3 cm increased more rapidly, at an average rate of 2.6 mm/year [2].

Once an aneurysm is identified, it should be repaired or followed up with imaging, depending on the clinical scenario and the size of the aneurysm at the time of diagnosis. Endovascular treatment is also becoming popular with guidelines published for interventional treatment which is indicated with IAAs > 3.5 cm [11]. The advantages of endovascular treatment over open repair include reduced transfusion requirements, the time of the procedure and the mean hospital stay. However, the superiority of this option at long-term has not yet been established

Conclusion: An unusual presentation of an isolated iliac artery aneurysm in our environment is reported, using appropriate imaging modalities. The patient successfully had aneurysmorapphy.

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Correlation between Gastric mucosal histology and Faecal antigen test for the detection of Helicobacter pylori infection among dyspeptic patients in a tertiary hospital in south-west Nigeria.

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Abstract

Background and objectives: Dyspepsia is a term used to describe upper abdominal pain or discomfort often associated with eating, early satiety, postprandial abdominal bloating or distention, and nausea. Many of these patients are infected with *Helicobacter pylori*. A simple method of diagnosis becomes imperative, to reduce the burden on endoscopy services. This study was aimed at determining the correlation between faecal antigen test and gastric mucosal histology in the diagnosis of Helicobacter *pylori* infection amongst patients undergoing upper gastrointestinal endoscopy.

Methods: A cross-sectional study of consecutive, consenting patients with dyspepsia presenting for upper gastrointestinal endoscopy. Fresh stool samples (rectal smears) were obtained from patients for *Helicobacter pylori* stool antigen test. Bio-data and results were recorded. Upper gastrointestinal endoscopy was performed using Pentax FG-29W gastroscope with a video monitor and detailed findings noted. Gastric biopsies (3 corpus and 3 antrum) were taken for histological diagnosis of *Helicobacter pylori*. Data were entered into SPSS (version 20) and analyzed using descriptive and inferential statistics.

Results: There were 150 patients comprising 61(40.7%) males and 89 (59.3%) females. The mean age (±SD) was 45.5 (±14.7) years with a range of 18 – 79 years. *Helicobacter pylori* was present in 93(62%) of patients on histology, and in 80 (53.3%) of them using Helicobacter pylori Stool Antigen Test (p = 0.000). The sensitivity, specificity, diagnostic accuracy, Positive predictive value, Negative predictive value and Area under the receiver operating characteristic curve for Helicobacter pylori stool antigen test were 79.6%, 89.5%, 83.3%, 92.5, 72.9% and 0.827 respectively. Positive and negative likelihood ratios were 7.58 and 0.228 respectively.

Conclusion: *Helicobacter pylori* stool antigen test compared favourably with histology and could possibly be used to complement the detection of *H. pylori* where Urea Breath Test and endoscopy are not available.

Keywords: *Faecal antigen test, H. pylori, histology, gastric biopsy, correlation.*

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Résumé

Contexte et objectifs: La dyspepsie est un terme utilisé pour décrire les douleurs abdominales supérieures ou les inconvénients souvent associés à la consommation, la satiété précoce, les ballonnements abdominaux postprandial ou la distension et la nausée. Beaucoup de ces patients sont infectés par *Helicobacter pylori*. Une simple méthode de diagnostic devient impérative, afin de réduire le fardeau des services d'endoscopie. Cette étude visait à déterminer la corrélation entre le test de l'antigène fécal et l'histologie de la muqueuse gastrique dans le diagnostic de l'infection à Helicobacter *pylori* chez les patients soumis à une endoscopie gastro-intestinale supérieure.

Méthodes: étude transversale de patients consécutifs consentements à la dyspepsie présentant une endoscopie gastro-intestinale supérieure. Des échantillons de selles fraîches (frottis rectaux) ont été obtenus chez des patients pour un test d'antigène des selles *Helicobacter pylori*. Les données biologiques et les résultats ont été enregistrés. L'endoscopie gastro-intestinale supérieure a été effectuée en utilisant un gastroscope Pentax FG-29W avec un moniteur vidéo et des résultats détaillés constatés. Des biopsies gastriques (3 corpus et 3 antres) ont été prises pour le diagnostic histologique d'*Helicobacter pylori*. Les données ont été entrées dans SPSS (version 20) et analysées à l'aide de statistiques descriptives et par inférences.

Résultats: 150 patients comptant 61 (40,7%) hommes et 89 (59,3%) des femmes. L'âge moyen (± DE) était de 45,5 (± 14,7) ans avec une étendue de 18 à 79 ans. *Helicobacter pylori* était présent dans 93 (62%) des patients sur histologie et dans 80 (53,3%) d'entre eux faisant usage du Test Helicobacter pylori Stool Antigen (p = 0,000). La sensibilité, la spécificité, la précision diagnostique,

la valeur prédictive positive, la valeur prédictive négative et la zone sous la courbe caractéristique de fonctionnement du récepteur pour le test antigénique des selles Helicobacter pylori étaient respectivement de 79,6%, 89,5%, 83,3%, 92,5, 72,9% et 0,827. Les rapports de vraisemblance positive et négative étaient respectivement de 7,58 et 0,228.
Conclusion: Le test d'antigène des selles de *Helicobacter pylori* a été comparé favorablement à l'histologie et pourrait éventuellement être utilisé pour compléter la détection de H. *pylori* où le test de respiration d'urée et l'endoscopie ne sont pas disponibles.

Mots-clés: *Test d'antigène fécal, H. pylori, histologie, biopsie gastrique, corrélation.*

Introduction

Dyspepsia according to the Rome III working group, is the presence of symptoms considered by the physician to originate from the gastroduodenal region and only for symptoms consisting of bothersome postprandial fullness, early satiation, epigastric pain and epigastric burning [1]. Dyspepsia has a worldwide distribution with a prevalence rate of 41% reported among the British population in 1990 [2]. Studies in the United States found prevalence values of approximately 25% [3]. However, a study carried out in Lagos, Nigeria recorded a prevalence rate of 29% [4], while another report from the North-Eastern part of Nigeria found a prevalence of 26% [5]. Organic dyspepsia may be caused by food intolerance, medications, systemic disorders, and diseases of the gastro-intestinal tract such as peptic ulcer disease(PUD), gastroduodenitis, oesophagitis, pancreatitis, gastric cancer and many others including infection with the organism *Helicobacter pylori* (*H. pylori*) which has been recognized as a key factor [6].

Helicobacter *pylori* is a gram-negative bacterium that selectively colonizes the gastric epithelium. The bacterium is urease, catalase, and oxidase positive. It is spiral shaped, and possesses 3 to 5 polar flagellae that are used for motility [7]. It has evolved the ability to colonize the highly acidic environment found within the stomach by metabolizing urea to ammonia using its enzyme, urease, which generates a neutral environment enveloping the bacterium [7]. Diagnostic methods for this infection are either invasive or non-invasive. Invasive methods used for the diagnosis of *H. pylori* infection require endoscopic examination which is expensive and inconvenient and may cause complications. Non-invasive methods such as urea breath test (UBT), serology and stool antigen tests are more readily preferable in the absence of compelling indications for endoscopy. Several studies have reported that a high percentage of dyspeptic patients in Nigeria are infected with *H. pylori* [8-11], it is therefore imperative to evaluate an alternative means of diagnosing this infection as *H. pylori* is mostly found among people from the low socio-economic status. Histological diagnosis of this infection is relatively expensive and has with it its attendant complications arising from pre-medication and the endoscopy procedure itself. A commonly used non-invasive test with high performance in diagnosing *H. pylori* infection is the UBT, which is equally expensive.

H. pylori stool antigen tests (HpSAT) are relatively simple and cheaper than the UBT. Human stool specimen is very easy to obtain as it is part of the physiological processes of the human body. Though studies on HpSAT have been done in Nigeria [8-11], most of these studies were based on very small sample sizes and as such there is need for more studies on its usefulness in evaluating patients with dyspepsia and possibly compare such with the existing standard means of diagnosing *H. pylori* infection.

This study was aimed at determining the correlation between faecal antigen test and gastric mucosal histology in the diagnosis of H. *pylori* infection amongst patients undergoing upper gastrointestinal endoscopy. This study therefore, looked at the usefulness of HpSAT in comparison with histological diagnosis of *H. pylori* which is commonly used in Nigeria.

Methods

This was a comparative study comparing two methods of diagnosing *H. pylori* infection; faecal antigen test and histology. The study was carried out at the Gastroenterology Unit of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) from May 2014 to Aug 2015. The study population comprised 150 adult patients (18yrs and above), with dyspepsia who were recruited consecutively from the Gastroenterology and Endoscopy Unit of the hospital after an informed consent. Patients who had taken any antibiotics within one month or a proton pump inhibitor within two weeks of the procedure, [12] or were pregnant or breastfeeding were excluded from the study. Also, patients who, on endoscopic examination, had features suggestive of gastric cancer were excluded.

The study was approved by the hospital's Ethics and Research Committee with approval number: IRB/IEC/0004553.

A structured interviewer-administered questionnaire was used to obtain information from every participant. Patients were fasted overnight, for a minimum of 8 – 10hours before the morning of the test. Fresh stool samples were obtained from the participants on the morning of endoscopy. The stool was processed and tested for *H. pylori* using one step H.pylori immuno-chromatographic antigen rapid test kit by SD (Standard Diagnosis) BIOLINE (Batch number: 04FK20-02-4). The procedure was carried out in accordance with the manufacturer's instructions.

All cases thereafter underwent an oesophagogastroduodenoscopy. Pre-medication given were, 10% xylocaine throat spray, intravenous midazolam of between (2.5 – 5.0mg) as conscious sedation and intravenous 20mg of buscopan (hyocine) as a smooth muscle relaxant. Midazolam was avoided in patients with chronic liver disease. Endoscopy was performed using the Pentax FG-29W forward viewing flexible oesophagogastroduodenoscope. Details of gastric mucosal pattern were recorded and gastric mucosa biopsies were obtained from the gastric corpus (from 2 separate areas) and antrum (from 3 separate areas including the incisura angularis).

The gastric tissue biopsies were fixed in 10% buffered formalin from the endoscopy room. Biopsies were placed on filter paper, into tissue cassettes and processed in an automatic tissue processor (Leica TP1020) to produce formalin-fixed, paraffin-embedded tissue blocks. These paraffin-embedded tissue blocks were subsequently cut into thin sections of less than 3μm thick using the semi-automatic rotary microtome (Leica RM2125RT). The sections were then picked up onto glass slides and allowed to dry at room temperature. One set of tissue sections was stained with hematoxylin and eosin, while the other set with Giemsa stain for histopathological examination. The stained slides of the gastric tissue biopsies were viewed and evaluated using the conventional light microscope.

The haematoxylin and eosin stained slides were evaluated using the updated Sydney system of classification and grading of gastritis,[13] while the modified Giemsa stained slides were evaluated to identify presence of H. *pylori.*(figure 3).

Data obtained were analyzed using SPSS-version 20. Frequency distribution tables of variables were generated. Measure of central tendency and dispersion for quantitative variables as well as proportions for quantitative variables were determined. Continuous variables were presented as means $\pm$ standard deviations while categorical variables were presented as frequency tables, bar and pie charts. Discrete variables were analyzed using Chi square test. Specificity, sensitivity, positive and negative predictive values, positive and negative likelihood ratios, diagnostic accuracy and area under the receiver operating characteristic (AUROC) curve for stool antigen test in diagnosing H. pylori infection were calculated, compared to histological diagnosis used as the gold standard [14,15]. Area under the ROC curve was used to compare the usefulness of the tests, with larger areas corresponding to greater usefulness. A p-value of less than or equal to 0.05 was considered as statistically significant at 95% confidence level.

Results

A total of 150 cases completed the study with a mean age (±SD) of 45.5 (±14.7) years and a range of 18 – 79years. The modal age group was 40 – 59 years with a median age of 45years. There were 89 (59.3%) females and 61(40.7%) males. Thirty-eight (25%) were civil servants and 116 (77.3%) of the patients were married. Seventy-six (50.7%) had up to a secondary school education and 57(38%) had post-secondary education (Table 1).

Table 1. Socio-demographic characteristics of study population

Demographic characteristics	No of patients (N=150)	Percentage (100%)
Age group		
<20	3	2
20 – 39	51	34.0
40 – 59	67	44.7
60 – 79	29	19.3
Gender		
Male	61	40.7
Female	89	59.3
Occupation		
Civil servants	38	25.3
Artisans	12	8.0
Retired	16	10.7
Farmers	13	8.7
Business	14	9.3
Trader	26	17.3
Unemployed/students	31	20.7

Risk factors for dyspepsia in the study patients were non-steroidal anti-inflammatory drugs (NSAIDS) in 21(14%); 14(9.3%) of the patients took alcohol and 41(27.3%) had a positive family history of dyspepsia while 53(35.3%) had used herbal mixtures (Table 2). Majority of those who took alcohol,12(8%), took between 20 – 50g of alcohol

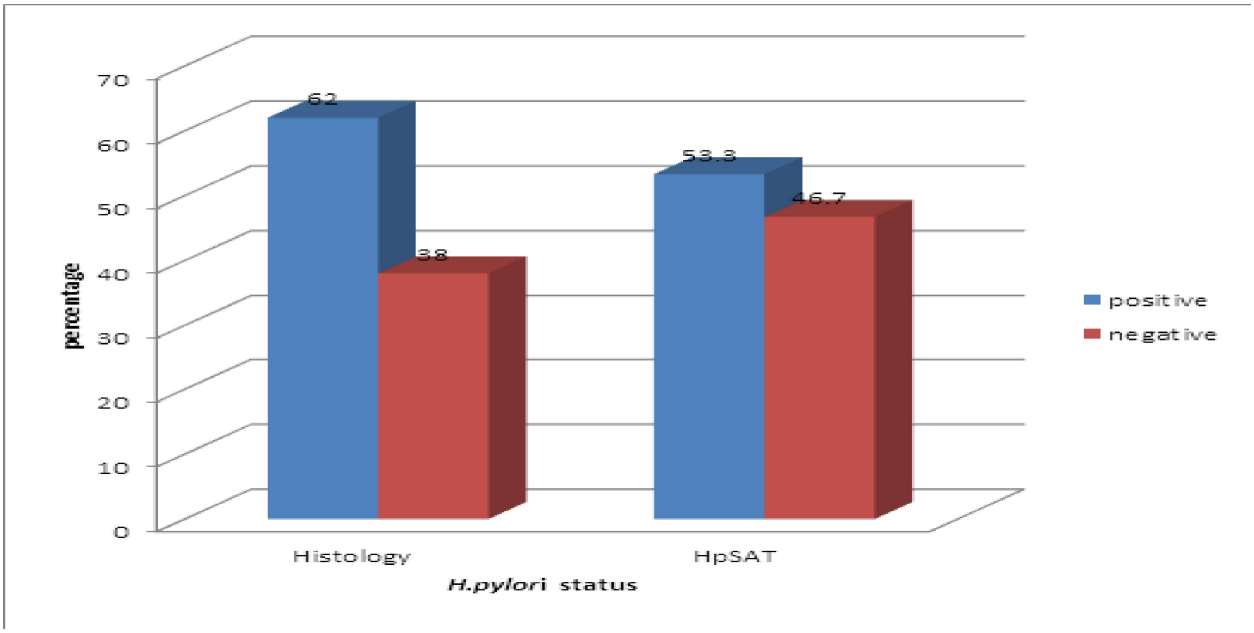
Table 2. Risk factors for Dyspepsia

Risk factors		Frequency(N=150)n(%)	χ^2	df	p-value
NSAIDs	Yes	21(14)	77.76	1	0.000
	No	129(86)			
Smoking	Yes	6(4)	126.96	1	0.000
	No	144(96)			
Alcohol	Yes	14(9.3)	99.227	1	0.000
	No	136(90.7)			
Steroids	Yes	2(1.3)	142.107	1	0.000
	No	148(98.7)			
CCB	Yes	13(8.7)	102.507	1	0.000
	No	137(91.3)			
DM	Yes	2(1.3)	142.107	1	0.000
	No	148(98.7)			
Herbal mixtures	Yes	53(35.3)	12.907	1	0.000
	No	97(64.7)			
FHOD	Yes	41(27.3)			
	No	92(61.4)	58.680	2	0.000
	Don't know	17(11.3)			

*p-value < 0.05 is statistically significant
NSAIDs = Non-Steroidal Anti-Inflammatory Drugs; CCB = Calcium Channel Blockers; FHOD = Family History of Dyspepsia; DM = Diabetes Mellitus; df = degree of freedom.

per day for more than 5 years while those that smoked cigarette,4(2.7%), consumed between 1 - 5 pack years of cigarette as at the time of this study.

of *H. pylori* was highest amongst the age group 20-39 years using histology (p > 0.05). (Table 3) With respect to gender, 64% of males and 61% of females



HpSAT = *H. pylori* Stool Antigen Test; P-value = 0.0000.
Fig. 1: Prevalence of *H. pylori* using Histology

Helicobacter *pylori* was detected on histology of the gastric mucosa in 93 (62%) of the 150 patients studied. HpSAT test was positive in 80 patients (53.3%), p < 0.05. (Fig.1). The prevalence

tested positive to *H. pylori* on histology while 59% and 49% of males and females respectively were positive on HpSAT. However, there was no statistically significant difference in the rates

Table 3. Prevalence of *H. pylori* according to socio-demography

Variables	N(150)	Histology n (%+ve)	HpSAT n (%+ve)	p-value
Age groups(yrs)				
<20	3	2(66.7%)	2(66.7%)	0.083
20 - 39	51	34(66.7%)	30(58.8%)	0.000
40 - 59	67	39(58.2%)	32(47.8%)	0.000
60 - 79	29	18(62.1%)	16(55.2%)	0.002
Gender				
Male	61	39(64%)	36(59%)	0.000
Female	89	54(61%)	44(49%)	0.000
Occupation				
Civil servants	38	22(58%)	15(39%)	0.000
Farmers	13	4(30.7%)	4(30.7%)	0.048
Artisans	12	9(75%)	8(66.7%)	0.028
Traders	26	21(80.7%)	18(69%)	0.001
Business	14	8(57%)	9(64%)	0.036
Retired	16	10(62.5%)	9(56%)	0.000
Unemployed	31	19(61%)	17(55%)	0.000

Table 4. *H. pylori* diagnostic test evaluation for HpSAT

	Histology		
HpSAT	Positive	Negative	Total
Positive	74(a)	6(c)	80(a + c)
Negative	19(b)	51(d)	70(b + d)
Total	93(a + b)	57(c + d)	150(a+b+c+d)

a = True positive (TP); b = False negative (FN); c = False positive (FP); d = True negative(TN)
CI = Confidence Interval.
Sensitivity = 74/93×100% = 79.6% (95% CI = 73.15% to 86.05%)
Specificity = 51/57×100% = 89.5% (95% CI = 84.59% to 94%)
Positive Likelihood Ratio (LR+) [sensitivity/ (100 - specificity)] =7.58 (95% CI =3.34 to 11.82)
Negative Likelihood Ratio (LR-) [(100 – sensitivity)/specificity] =0.228(95%CI= -0.54 to 0.99)
**The HpSAT kit Manufacturer's sensitivity and specificity were both reported as 100% compared with a combination of Rapid urease test (RUT) and Urea breath test(UBT) used as gold standard of diag*nosis.

according to gender using both histology and HpSAT (p-value > 0.05). The *H. pylori* infection rates were highest amongst traders and artisans (80.7% and 75% respectively) on histology and (69% and 66.7% respectively) on HpSAT.

The relationship between *H. pylori* status and some risk factors for dyspepsia showed that the rates were higher amongst those who took alcohol and smoked tobacco product; 71.4% and 83.3% on histology and 57.1% and 66.7% on HpSAT respectively.

A vast majority of those who presented with a history of haematemesis tested positive to *H. pylori*; moreso on histology (80%) as compared with HpSAT (60%). The sensitivity and specificity of HpSAT were 79.6% and 89.5% respectively. The likelihood diagnostic evaluation showed that HpSAT has about 8-fold [Positive likelihood ratio (LR+) = 7.58] increase in the chance of detecting *H. pylori* infection when the test is positive in a patient. In the same vein, there was about 23% [Negative likelihood ratio (LR-) = 0.228] chance of the presence of H. pylori infection in a patient when the test is negative (Table 4).

The precision level for HpSAT to detect truly positive *H. pylori* infection was as high as 92.5% while that for detecting truly negative *H. pylori* infection was 72.9% using histology as the gold standard. (Table 5) The diagnostic accuracy for HpSAT is 83.3%. (Table 5) It has a high diagnostic

Table 5. Other Diagnostic Evaluation Tests For HpSAT

HpSAT	Histology Positive	Negative	Total
Positive	74(a)	6(c)	80(a + c)
Negative	19(b)	51(d)	70(b + d)
Total	93(a + b)	57(c + d)	150(a+b+c+d)

a = True positive (TP); b = False negative (FN); c = False positive (FP); d = True negative (TN)
CI = Confidence Interval.
Positive Predictive Value (PPV) = 74/80 × 100% = 92.5% (95% CI = 88.28% to 96.72%)
Negative Predictive Value (NPV) = 51/70 × 100% = 72.9% (95% CI = 65.79% to 80.01%)
Diagnostic Accuracy (DA) = (74+51)/150 × 100% = 83.3% (95% CI=77.33% to 89.27%)
Diagnostic Odds Ratio (DOR) = (TP/FN)/ (FP/TN) = (74/19)/(6/51)=33.1(95%CI=25.7 to 40.63)

Odds Ratio (LR+/LR-) of 33.1, HpSAT has a very high positive likelihood compared with negative likelihood when applied in the diagnosis of *H. pylori* infection. (Table 5)

Table 6. H. pylori status in the various endoscopic findings

Endoscopic Findings	Freq (N=150) n(%)	n (% +ve) (Histology)	n (% +ve) (HpSAT)	(Histology Vs HpSAT) p-value
Normal Finding	47(31.3)	29(61.7)	20(42.6)	0.000*
Antral Gastritis	51(34)	36(70.6)	23(45.1)	0.000*
Gastroduodenitis	18(12)	16(88.9)	15(83.3)	0.314
Gastric Erosion	14(9.3)	10(71.4)	8(57.1)	0.580
Duodenal ulcer	18(12)	14(80.0)	13(75.0)	0.032*
Erosive GERD	24(16)	12(50.0)	7(29.0)	0.193
Gastric ulcer	6(4)	5(83.3)	3(50.0)	1.000
Gastric mucosal thinning	34(22.6)	18(52.9)	15(44.1)	0.000*
PHG with Oesophageal varices	3(2)	2(66.7)	2(66.7)	0.333
Hiatus hernia	11(7.3)	5(45.5)	3(27.3)	0.015*

P-value < 0.05 is statistically significant; * = statistically significant
PHG = Portal Hypertensive Gastropathy
% positive on Histology = Percentage of patients positive for *H. pylori* on Histology
% positive on HpSAT = Percentage of patients positive for *H. pylori* on HpSAT

At endoscopy, *H. pylori* positivity was 88.9%, 83.3% and 80% of patients with gastroduodenitis, gastric ulcer and duodenal ulcer using histology while 83%, 50% and 75% of those with gastroduodenitis, gastric ulcer and duodenal ulcer respectively were positive using HpSAT (Table 6). The Receiver operating characteristic (ROC) curve and the area under the curve (AUROC) for HpSAT was 0.827. (Figure 2).

Discussion

In this study comparing the usefulness of HpSAT with histology, the mean age of the study subjects was comparable with that by Syam *et al* [15] in Indonesia and that by Jemilohun *et al* [16] in Nigeria. These studies, however, had small sample sizes of 63 and 86 respectively. The mean ages in these studies probably reflect the population of patients who usually seek health care in our health facilities as well as the mean age of individuals with dyspepsia in the population.

The gender distribution of predominantly female of 59.3% is also similar to the work done by Jemilohun *et al* [16] where they also found female

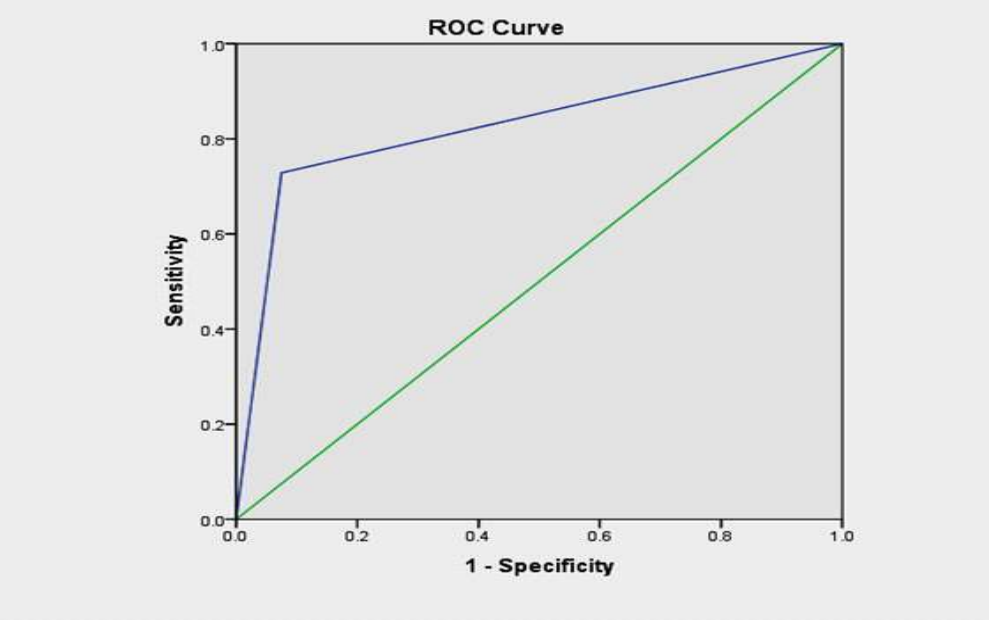


Fig. 2: Area Under The Receiver Operating Characteristic (AUROC) Curve of HpSAT
_____ HpSAT ROC Area: 0.827
_____ Reference line

The interpretation of AUROC is as follows; a value of;-
0.9 – 1.0 is excellent; **0.8 – 0.9** is very good; **0.7 – 0.8** is good; **0.6 – 0.7** is sufficient; **0.5 – 0.6** is bad; **< 0.5** means the test is not useful.

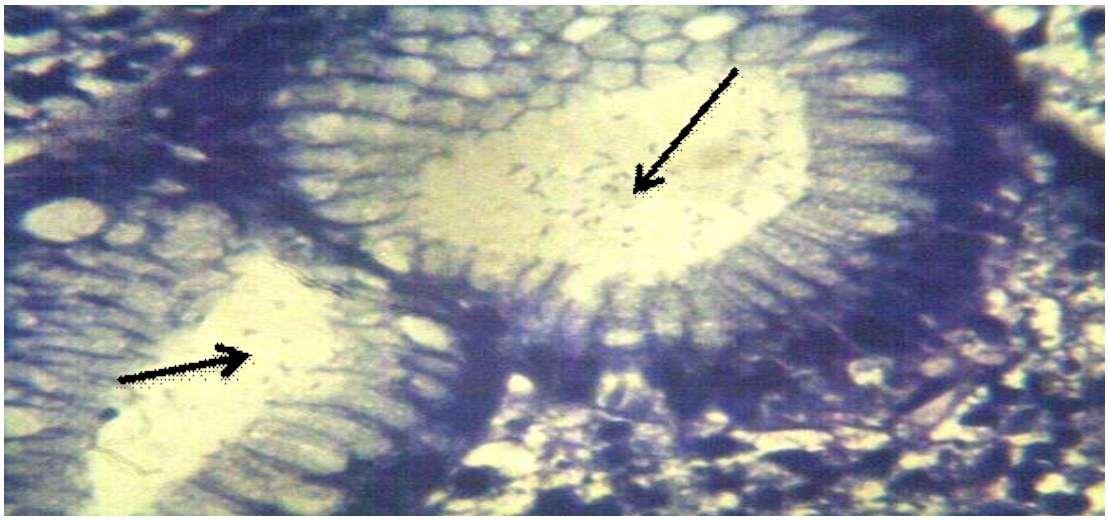


Fig. 3: Histology slide of a study patient (Giemsa Stain ×40) showing *H. Pylori* (Thick Arrows)

preponderance of 54.7%. In another study by Aje *et al* [9] in south-west, Nigeria, they also reported a slight female preponderance of (20 males vs 26 females). The female predominance in these studies among dyspeptic patients may be due to the fact that females have lower threshold for pain as was also opined by Aje *et al* [9] they may also be more conscious of issues of health than males and it is also possible that psychosomatic and emotional disorders are likely more common in female, than male hence the female preponderance.

In this study, the majority of participants were civil servants, followed by students/the unemployed and traders. About fifty-percent of them had up to a secondary school education and 30% had post-secondary education. This pattern is similar to another study by Hameed *et al* [4] in Lagos, Nigeria, where the participants consisted of traders/ business men and women, students, and civil servants. They also found that approximately half of their respondents had post-secondary education, while 38.2% had up to secondary education only.

Majority of our patients were civil servants and had up to secondary education. This could be a reflection of information on health issues and consequently on health seeking behaviour. Health seeking behaviour is a reflection of educational background.

The prevalence of *H. pylori* in this study was 62% on histology and 53.3% on faecal antigen test. A study carried out by Ndububa *et al* [17] in 2001, about 14yrs before now, in the same hospital reported a prevalence rate of 73% on histology. The drop in the prevalence rate perhaps reflects an increasing awareness and possible over-the-counter use of antibiotics and anti-ulcer drugs. Another reason could be that good hygiene, as reflected in their educational background, may also have impacted on their *H. pylori* infection rate. Nevertheless, the prevalence rate of 62% is still high. Prevalence rates similar to the findings in this study have also been reported in other parts of the world. Jemilohun *et al* [16] in 2011 in Ibadan, Southwest Nigeria, reported a prevalence rate of 64% from their study of 86 patients using histology and rapid urease test (RUT). Abo-Shadi *et al* [18] in 2013 reported a prevalence of 64.7% in a study of 68 patients using histology in Egypt. However, Olokoba *et al* [19] in 2013 reported H. pylori prevalence of 80% using histology from their study in the Northern Nigeria. Mustapha *et al* [20] in a prospective study carried out in the same North-Eastern Nigeria from 2003 to 2006 had reported a similar prevalence of 78.5% using the same histology. Tijani *et al* [21] in Kano, North-western Nigeria found a prevalence rate of 81% amongst patients studied using histology. Hameed *et al* [4] in Lagos using histology reported a prevalence rate of 41% for which the authors were of the opinion that inadequate biopsy samples due to the cost of processing samples may be a factor and also that the patients' ability to remember the details of antibiotics used over the previous six weeks might not be completely reliable and could have affected the results of the study. It thus seems that geographical variations and socio- demographics of the population studied and the socio-economic status of people possibly influences *H. pylori* prevalence. Bani-Hani *et al* [22] in their work in Jordan demonstrated that environmental factors such as barometric pressure and seasonal temperature variation and not necessarily genetic background played a role in the prevalence of *H. pylori* in different populations further strengthening this postulate.

In this study, the prevalence of *H pylori* with HpSAT was 53.3% which is similar to that reported by Naji *et al* [23] in Yemen. Higher values were reported by Aje *et al* [9] and Smith *et al* .[8] The difference in rates may have reflected the general prevalence of *H. pylori* infection in these regions of Nigeria and perhaps, the season of the study.

The sensitivity, specificity, diagnostic accuracy, positive predictive value and negative predictive value for HpSAT were 79.6%, 89.5%, 83.3%, 92.5%, and 72.9% respectively. Positive and Negative likelihood ratios (LR+ and LR-) were 7.58 and 0.228 respectively. This is similar to the report by Falaknazi *et al* [24]. Findings in this study also compare with, though, slightly lower than what was obtained by Asfeldt *et al* [25] in 2003 where they reported sensitivity and specificity of 98% and 94%, with positive and negative likelihood ratios of 16.7 and 0.02, respectively. Gisbert *et al* [26] in their systematic review of 22 studies reported pooled sensitivity, specificity, LR+, and LR- as; 95%, 97%, 24, and 0.07 respectively.

The rates of *H. pylori* infection using HpSAT in the various endoscopic findings like; normal stomach, antral gastritis, DU, GU and gastroduodenitis were similar to those obtained using histology. These findings are also similar to the works by Mohammed *et al* [27] and Syam *et al* [15].

The area under the receiver operating characteristic curve (AUROC) for HpSAT was 0.827 which according to Youden *et al* [28] implies that this test is very good compared to the gold standard used in this study which is histology. The value for the ROC is also similar to the work by Kazemi *et al* [29] with AUROC value of 0.897 and Farazi *et al* [30] with an AUROC value of 0.889.

Conclusion

This study has shown a clear relationship between *H. pylori* infection and occurrence of dyspepsia. There is still a high prevalence of *H. pylori* infection amongst patients with dyspepsia. *H. pylori* infection is highest amongst the young age group of 20-39years. The diagnostic accuracy tests for HpSAT shows that it is comparable to gastric histology in diagnosing *H. pylori* infection and may be used to complement the diagnosis of H. pylori where UBT is not available. However, histology remains a superior method of H. pylori diagnosis especially in the event of compelling indications for upper gastrointestinal endoscopy.

Limitation of the study

It is a hospital-based study and it therefore may not have captured the true prevalence of *H. pylori* in the community. Possibility of inadequate drug history as it may have been underestimated by the study participants

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Routine clinical biochemical investigations in a Nigerian tertiary hospital- a 3-year analysis

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Abstract
*Background***:** University College Hospital (UCH) Ibadan, established in 1957 to offer teaching, research and routine medical services to the College of Medicine of the then University College Ibadan is the foremost medical centre in Nigeria. The Department of Clinical Biochemistry was one of the foundation departments meant to provide clinical biochemical investigations, a specialty applying basic biochemical techniques in the determination of biochemical changes occurring in the human system. The fact that all metabolic processes either in health or disease has a biochemical basis informed this 3-year (2010-2012) longitudinal analysis of requests for biochemical investigations in UCH, Ibadan, Nigeria.
Method: Data on requests for biochemical investigations for basic biochemical investigations like electrolyte and urea, liver and renal function tests, lipids profile, plasma glucose estimations, hormonal level determinations and urinalysis including patients' attendance at the hospital were obtained after due process from the Department of Clinical Biochemistry and the Central Medical Records of the hospital respectively. The data covered total number of patients that attended the hospital in the years 1958-2012 and laboratory requests within 2010 and 2012.
Results: A total of 243,652.00, 240,136.00 and 284,027.00 patients were recorded out of which 102,832 (42.2%), 89,122 (37.1%) and 82,242 patients (29.0%) had biochemical investigation requests in the years 2010, 2011 and 2012 respectively. The preponderance of these requests which averaged 36.1% annually for the periods under consideration were for electrolyte and urea, plasma glucose and basic liver function investigations in that descending order.
Conclusion: Aside from underscoring the possible prevalence of non-communicable diseases, the possible impact of this on the quality of medical care and the economic implication on the finances of this hospital were discussed in this study.

Keywords: *Biochemical investigation, Non-communicable diseases, University College Hospital, Nigeria.*

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Résumé
Contexte: Le Collège Hospitalier Universitaire (CHU) Ibadan créé en 1957 pour offrir de l'enseignement, de la recherche et des services médicaux de routine au Collège de Médicine a l'auparavant Collège Universitaire Ibadan est le centre médical principal du Nigeria. Le Département de la biochimie clinique était l'un des départements fondateurs destinés à fournir des recherches cliniques biochimiques, une spécialité appliquant des techniques biochimiques de base dans la détermination des changements biochimiques qui se produisent dans le système humain. Le fait que tous les processus métaboliques, soit en santé, soit en matière de maladie a une base biochimique, a informé cette analyse longitudinale de trois ans (2010-2012) des demandes d'études biochimiques dans UCH, Ibadan, Nigeria.
Méthode: Les données sur les demandes d'études biochimiques pour les études biochimiques de base, telles que l'électrolyte et l'urée, les tests de la fonction hépatique et rénale, le profil des lipides, les estimations du glucose plasmatique, les déterminations du niveau hormonal et l'analyse d'urine, y compris la fréquentation des patients à l'hôpital, ont été obtenues après une procédure régulière du Département de la Biochimie clinique et des dossiers médicaux centraux de l'hôpital respectivement. Les données couvraient le nombre total de patients qui ont fréquenté l'hôpital dans les années 1958-2012 et les demandes de laboratoire dans les années 2010 et 2012.
Résultats: Au total, 243 652,00, 240,166 et 284 027,00 patients ont été enregistrés, dont 102 832 (42,2%), 89,122 (37,1%) et 82 242 patients (29,0%) avaient des demandes d'enquête biochimique respectivement en 2010, 2011 et 2012. La prépondérance de ces demandes, qui était en moyenne de 36,1% par an pour les périodes considérées, était l'électrolyte et l'urée, la glycémie plasmatique et les fonctions basiques de la fonction hépatique dans cet ordre décroissant.
Conclusion: En plus de souligner la prévalence possible des maladies non transmissibles, l'impact possible sur la qualité des soins médicaux et l'implication économique sur les finances de cet hôpital ont été discutés dans cette étude.

Mots-clés: *Enquête biochimique, Maladies non transmissibles, hôpital collège universitaire, Nigeria.*

Introduction

The University College Hospital (UCH) is strategically located in Ibadan, the largest city in West Africa which is also the seat of the first University in Nigeria. The institution was established by an act of Parliament primarily as a teaching hospital to serve the then new College of Medicine of the University of Ibadan and secondarily, as a fall out of the primary reason, to carry out teaching, research and routine medical services towards fulfilment of the mandate of the hospital. The hospital commenced services at the State General Hospital located at Adeoyo before the permanent site was built in 1957. The University College Hospital, Ibadan was initially commissioned with 800 bed spaces [1]. Currently the hospital has over 1000 bed spaces while current bed occupancy rates ranges from 55-60%. At inception in 1948 and prior to the enactment of the Act of parliament heralding its birth, the hospital had two Clinical Departments (Medicine and Surgery). However, the Hospital has evolved to accommodate about 60 departments, among which is the first Department of Nuclear Medicine in Nigeria. The hospital is primarily a tertiary institution with appendages of community based outreach activities at Igbo Ora, Abedo, Okuku, Sepeteri, Elesu, and Jago (located quite distally to the hospital) where it offers primary and secondary health care services.

The Hospital has 60 service and clinical departments and runs 96 consultative out-patient clinics a week in 50 specialty and sub-specialty disciplines aside from an accident and emergency unit. The consultative clinics are the specialist clinics in virtually all disciplines of medicine (medicine, surgery, paediatrics, Obstetrics and Gynaecology, Psychiatry, Haematology, Metabolic unit etc) meant to provide specialist medicare ideally on referral, while the non-consultative clinic (General Out-Patient) operates like a secondary health facility in a general hospital.

In the area of offering medical services, patients' turn out in the Accident and Emergency (A & E) Department of the University College Hospital alone averages 6000 annually and about 150,000 new patients are seen in the various Out-Patient Clinics (consultative and non-consultative) every year. In 2001, the million clientele mark was attained. Due to the aforementioned facilities, manpower and track records, the Hospital enjoy wide patronage of both local and international clientele including attending to referral cases from far and near [2].

Early at its inception, patients' patronage was over 200,000 with more than 90% attending the general clinics. By 1964/65, patient patronage was still about 270,000; however, attendance at the consultative clinic rose to 44% indicating the gradual emergence of the hospital as a specialist tertiary hospital. This trend continued till date and in the year 2010 and 2011, total patient patronage stood at about 242,000 and 241,000 per annum respectively which later rose to 284,000 in 2012. Although, the recent patient patronage figure might not be significantly different from what it was at inception, this may not be unconnected with increased availability of medical facilities from both government and private interests over the years. This is clearly in conformity with increasing rate of urbanisation which has allowed for greater and better accessibility to medical services thus relieving government centres like UCH from the pressure of clients. However, one factor that stood out clearly and significantly is that the steady rise in attendance at the consultative clinics of the hospital stood at 72% of the total patronage in 2010. This was a sharp rise from what it was within the first decade of the birth of the hospital between 1957 and 1967. The attendance/patronage at the consultative clinic was 68% and 64% in years 2011 and 2012 respectively clearly confirming the specialist status of the hospital.

Just as it was elsewhere, the objective of setting up laboratory arms for the hospital was to provide correct diagnoses for monitoring patient's health and provide avenue for medical research and training of future medical staff. Hence, the practice of clinical biochemical analysis as applied biochemistry in the diagnosis and management of diseases started as one of the earliest laboratory departments in the hospital. Historically, it started as a room laboratory at Adeoyo between 1948 and 1951 before it moved to its present site where it assumed a departmental status. The hospital as at 1958 to 1961 had a total patient patronage of approximately 270,000 (including in-patient, consultative and non-consultative patient's attendance) [2].

As at that time, more than 70% of this number attended non-consultative clinic indicating the non-development of the specialist focus of the hospital at inception. Since 1979 when the first Auto analyzer (AAII) was procured for the analysis of basic tests like urea, creatinine etc signalling the advent of automation in this laboratory, the department has graduated into acquiring and using the best in automated equipments for virtually every aspect of her work – from ISE for Electrolytes to Vitrous machine for hormones analysis.

In the area of service, the clinical biochemistry laboratory which handled about fifty thousand investigations from about thirty-five

thousand specimens in 1970 now handled an average of ninety thousand biochemical investigations between 2010 and 2012. Averagely, one might be assuming that there has been a numerical reduction in the number of single investigations done in the laboratory. However, a closer look will show that most of the less sensitive/specific investigations of yester-years (like thymol turbidity/flocculation tests, investigation of mal-absorption syndrome etc) have now been replaced with single but more specific, sensitive and sophisticated test with better result outcome for proper patient management. Also, with advances in medical sciences, the spectrum of diagnostic investigations that patients undergo has widened to include x-ray and ultra-scans, electromagnetic and audio-thermal investigations. All these which were not available at the inception of the hospital might have contributed to a reduction in requests for more invasive investigations like those in clinical biochemistry.

Analysis of requests for biochemical investigations in the laboratory can be grouped under the following: requests for Electrolyte and Urea, Renal Function Tests, Lipids profile, Liver function tests, and tests of Carbohydrate metabolism, hormones and urine analysis. Aside from above, the depth of biochemical investigations in the department has placed it as a referral centre for clinical biochemical analysis to virtually all hospitals and laboratories in Nigeria and sometimes beyond. This unique role of the laboratory in such a diverse setting in a developing country like Nigeria (the largest black nation in the whole world) is the focus of this presentation [3].

Materials and methods

All relevant and necessary information was obtained from the medical records and data bank of clinical biochemistry departments of the hospital after proper ethical clearance following due process. The two units are well organised and have an up-to- date data on all patients attending the hospital from 1957. Hence, the number of patients that attended different clinics of the hospital in the year 2010, 2011 and 2012 was retrieved from the Medical Records Department of the hospital. Along with these, data on patients' attendance in the hospital in its early years were also obtained while the number and type of biochemical investigation carried out were retrieved from the records of clinical biochemistry department. That of the clinical biochemistry department was made easier because routinely, the laboratory prepares a monthly return of requests and results which is properly kept in its records unit. Data were obtained on the number of requests for Electrolyte and Urea (E and U), Liver Function Tests (LFTs), Lipids Profile, Renal Function analysis, Thyroid Function Tests (TFTs), Carbohydrate (CHO) metabolism, hormone and urine analysis over the periods 2010-2012. Aside from these, the average monthly requests for these investigations were also obtained while the number of both inpatients and outpatients (consultative and non-consultative) attendees at the clinics was also obtained.

The data were analysed statistically using histogram, Student T-test with P values ≤ 0.05 as significant. 2-tailed Pearson Correlation analysis with P values ≤ 0.05 being considered as significant.

Result

From the data, it was apparent that apart from in year 2010 when request for electrolyte and urea estimation, liver function investigations and request for pregnancy tests were unusually high relative to subsequent years under review, requests for basic renal function investigations, plasma glucose as an index of carbohydrate metabolism, lipid profile, hormone analysis and common pregnancy test continue to increase over the years under reference. An analysis of the data also revealed that requests

Table 1: The number and percentage of patients' attending UCH between 1958 and 2012

Year	In patients	Consultative Clinics (Specialist)	Non–consultative Clinics	TOTAL
1958-59	7,637 (3.47%)	72,313 (32.90%)*	139,839 (63.62%)	219,789
1959-60	12,056 (4.46%)	81,187 (34.99%)*	138,780 (59.81%)	232,023
1960-61	13,122 (4.86%)	86,052 (31.87%)*	170,862 (63.27%)	270,040
1989	10,756 (4.14%)	176,744 (67.99%)	72,505 (27.89%)	260,005
1990	11,635 (4.73%)	166,729 (67.81%)	67,515 (27.46%)	245,879
1991	10,852 (4.43%)	172,974 (70.63%)	61,057 (24.93%)	244,882
2010	16,057 (6.59%)	175,871 (72.18%)**	51,724 (21.23%)	243,651
2011	16,260 (6.77%)	164,540 (68.52%)**	59,336 (24.71%)	240,136
2012	16,626 (5.85%)	181,540 (63.92%)**	85,861 (30.23%)	284,027

Table 2: The number of Biochemical request for years 2010-2012

Assay	2010	2011	2012	Mean
Electrolytes and Urea	52,691	23,784	22,538	33,004
Renal function tests	14,044	22,824	21,542	19470
Lipid profile assay	5,513	10,895	5,155	7188
Liver function tests	13,645	6,180	5,575	8467
Glucose analysis	13,443	15,398	64,512	31118
Urinalysis	2,328	8,839	8,485	6551
Pregnancy tests	558	295	375	409
Hormonal assays	589	907	842	779
Total	102,841	89,125	85,997	92654

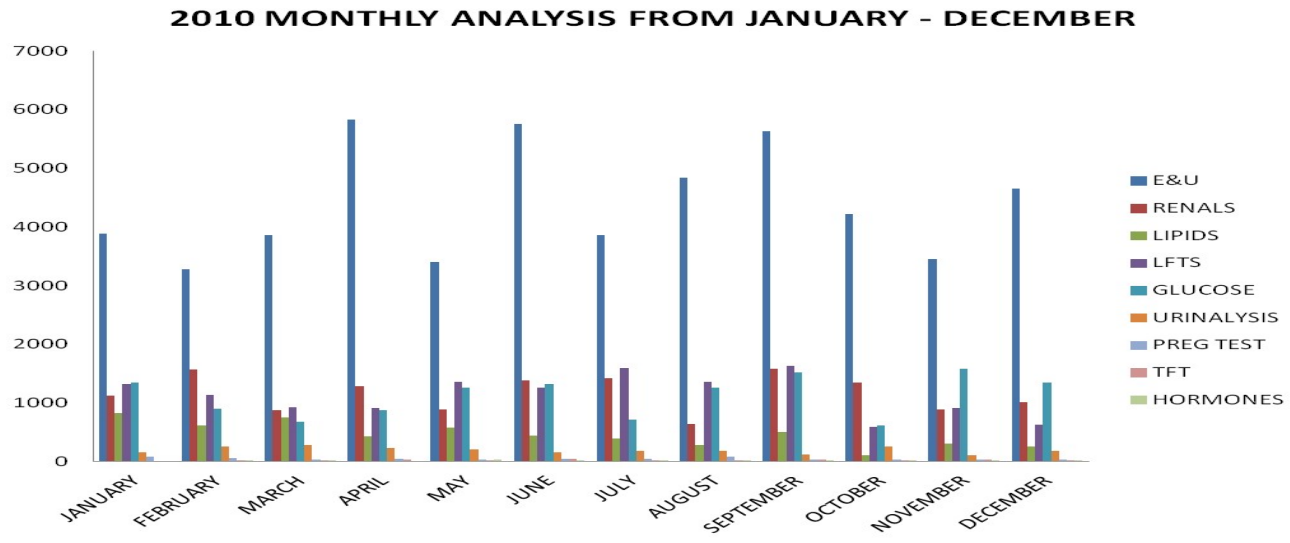

Fig. 1: 2010 Monthly analysis (January- December)

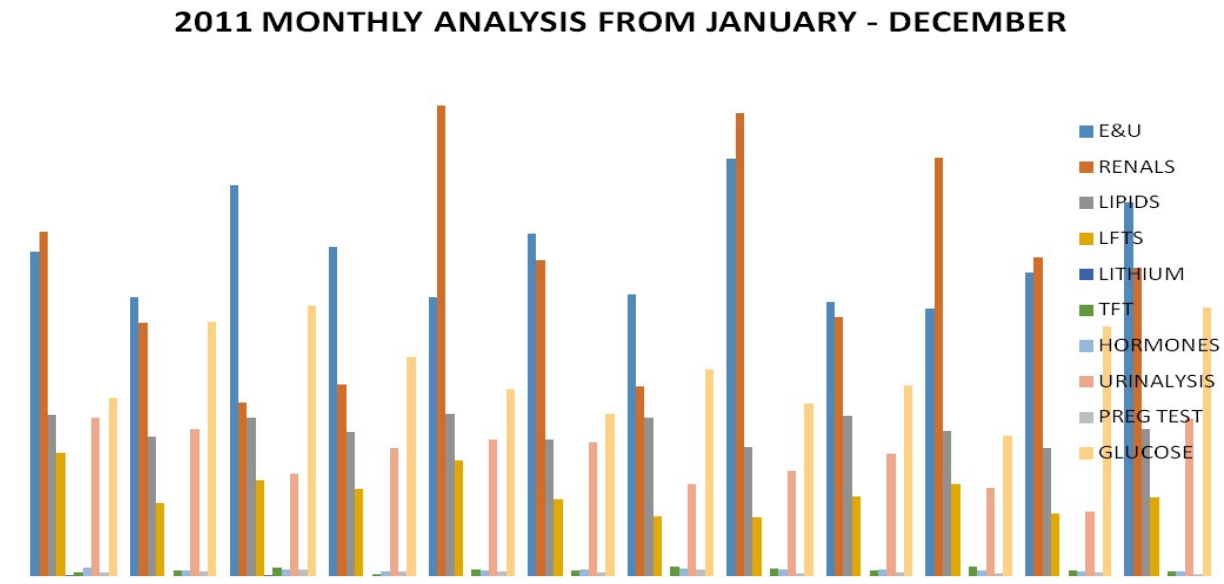

Fig 2: 2011 Monthly Analysis (January-December)

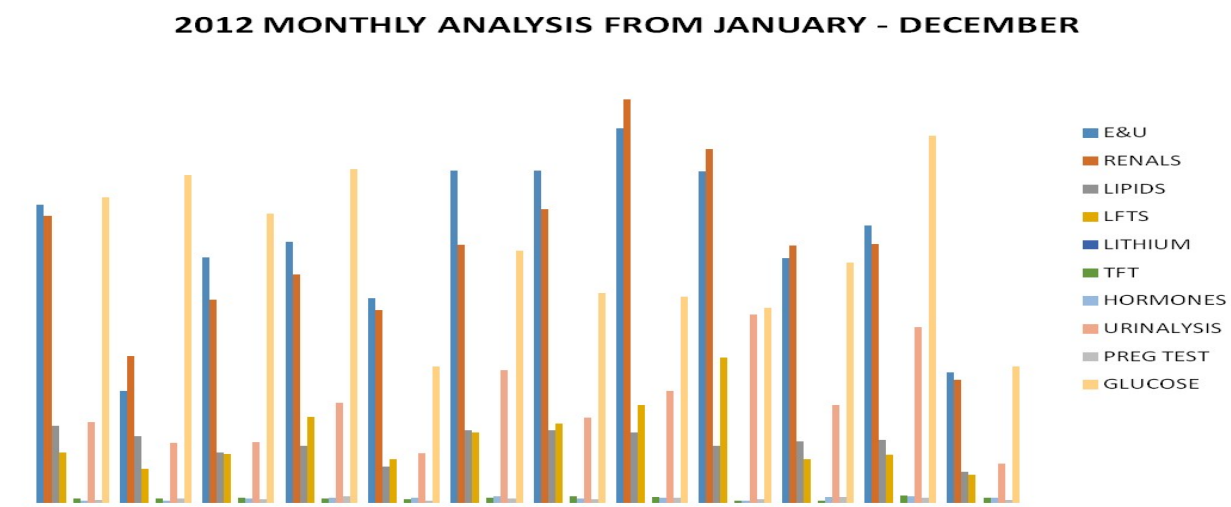

Fig 3: 2012 Monthly Analysis (January- December)

Fig 4: Chart Relating Laboratory Requests to Patients Flow (2010-2012)

for electrolyte and urea along with that for plasma glucose estimation were averagely highest over the years under review.

When these figures were related to percentage of patients' attendance in the three broad clinics and wards, the % attendance at the specialist clinics varied between 32.9% and 31.9% in the early days of the hospital (1958-1961); this figure stood at between 72.2% and 64% during the period 2010 – 2012 clearly indicating the emergence of the hospital as a tertiary (specialist) centre.

The average requests sent to the clinical biochemistry laboratory during these periods (2010-2012) constituted about 42%, 37% and 30% respectively when the requests were all corrected and harmonized with patients' attendance in the hospital.

The distribution of various biochemical requests during the years under review and during each month of the period were graphically represented in figures 1-6.

The preponderance of requests for electrolyte, urea and plasma glucose estimations was also highlighted in the graphs.

Analysis of the results showed that requests for investigations like E&U, LFT and CHO metabolic dysfunction were highest not only overall but also between April and October of the years under review.

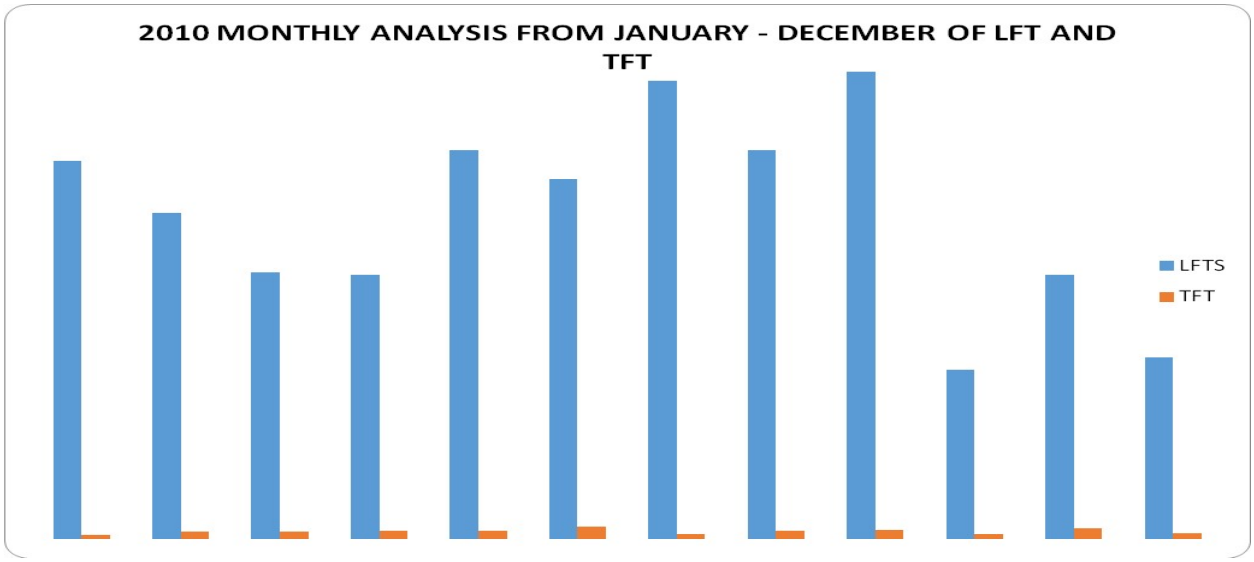

Fig. 5

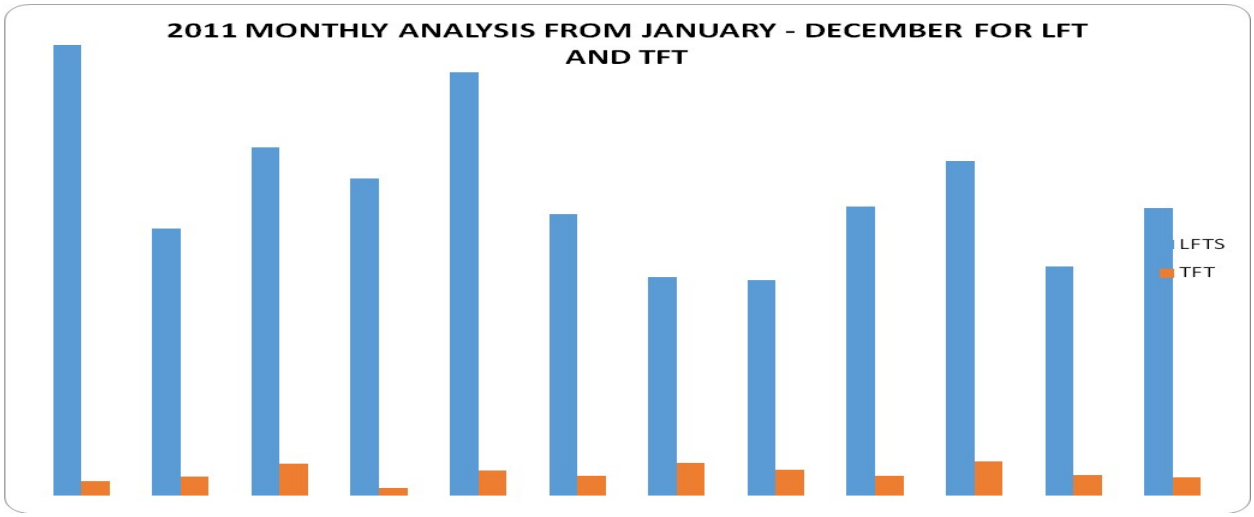

Fig. 6: 2011 Monthly Record for LFT and TFT Requests

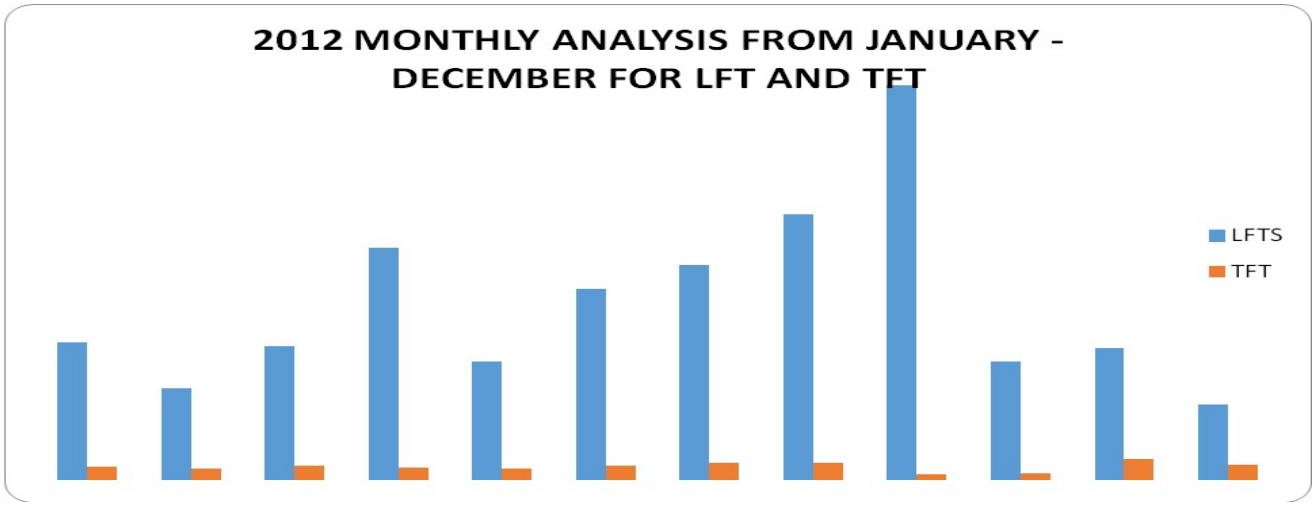

Fig 7: 2012 Monthly Record for LFT and TFT requests

Discussion

The focus of this study is to highlight the frequency and type of various biochemical investigations carried out in the Department of Clinical Biochemistry (Chemical Pathology) of University College Hospital Ibadan, Nigeria in relation to patients' attendance in the clinics of the hospital with a view to establishing the rate and mode of utilization of biochemical requests in patients' management. This could also be used to infer the possible contribution of this arm of pathology to the diagnosis and management of patients in this hospital. Information from this could also help in estimating the financial commitment into this area of diagnostic medicine in the overall finances of the hospital.

In making the deductions it will be assumed that there was a direct relationship between patients' rate (days and times) of attendance in the hospital and the number of times laboratory investigations were requested. This is because just as the number and days/times of attendance vary, the rate, type and frequency of investigation also vary especially in a tertiary hospital setting where traditionally, patients' care is often derived from research and teaching. Based on above, it may therefore be deduced from this exercise that over the years, there has been an increase in attendance/patronage of this hospital from its inception up to date.

As a tertiary hospital, one of the core mandates of the hospital was to serve as referral centre where specialist services in various arms of medical practice will be rendered to other secondary hospitals. This informed the basis for the specialist clinics that were set up to offer rather advanced medicare in specialties like nephrology, neurology, cardiology and others in medicine or cardiothoracic , orthopaedic, oncology, neurosurgery among others in surgery. The essence of these clinics is therefore to offer better and advanced medical care based on wider knowledge of these core areas in medicine to ensure better and ultimately cheaper medicare to the needy.

At the inception of the hospital, expectedly, emphasis was on general medical practice since that was what was in practice where the hospital started from; besides, the needed highly trained specialists to render the envisaged specialist service were not fully on ground. Hence, between 1958 and 1961 attendance at the few specialist clinics was about 32% of the total hospital attendees (Tables 1 and 2). However, as years rolled by, attendance at the consultative clinics increased to 64%-72% of patients attending the hospital within the period under review (2010-2012). This was a clear departure from the early days of the hospital (1958-1961) when patronage of the specialist clinics was averagely 32% ($P<0.05$) thus underscoring the recognition and prowes of the hospital as a tertiary specialist hospital.

However, between 30% and 42% of patients attending the specialist clinics had clinical biochemical investigation, this underscores the depth and contribution of laboratory requests to research and by implication depth of clinical management of various diseases/complaints brought to the hospital by the patients. This is significant because adequate, proper and concise diagnosis (the focus of the various investigations) remain the acknowledged basis for sound and proper management of diseases especially in a tertiary hospital setting [4,5]. Although the upsurge in laboratory requests is significant when compared to the number of requests at the inception of the hospital, however, in terms of adequate use of biochemical facility, it still falls short of expectations in a tertiary hospital setting almost bothering on underutilisation of the facilities. This can be seen from the various plots comparing attendance at the various specialist clinics showing less than 30% requests for diagnostic investigations.

In a laboratory request/cost analysis by Tahir in a South African Hospital in Cape Town where the emphasis was on effectiveness of the requested tests in comparison with the clinical state of the patients [6], the number of laboratory requests by this primary health care centre in comparison to this study clearly shows the underutilisation of laboratory facilities especially in this tertiary hospital facility. If diagnosis will be the bedrock of good clinical treatment and by extension, good health care practice, then the number of investigation per hospital visit should be higher than observed; of course this is without prejudice to cost effectiveness of investigations.

A number of factors could be responsible for the relative underutilisation; the issue of cost implication is a strong factor especially in a developing setting like ours where income per capital is abysmally low and budgetary allocation to health is insufficient to subsidize some of these services [7]. The physician will therefore have to strike a balance between ordering investigations and the cost of treating the outcome in terms of drugs, other medical procedures and possibly counselling. These considerations are quite minimal in the developed economy where the burden of medical cost is largely borne by well organised health insurance coverage.

The type and frequency of the various biochemical investigations is another area of interest in this publication. Investigating electrolyte imbalance and carbohydrate metabolism as reflected by the blood electrolyte and glucose levels were the

most frequently requested investigations during the period under review. Averagely, there were more requests for electrolyte and urea, other renal function and plasma glucose analysis than for other investigations routinely carried out in the laboratory within the period under review. Electrolyte and urea estimations remain a major biochemical hallmark investigation of hypertension, hence the preponderance of requests for this may also be an indirect indication of the incidence of hypertension in this environment. Although the number of requests for electrolyte and urea investigations was highest (13.1%), however, investigation of carbohydrate metabolism as reflected by request for plasma glucose assay was next with a frequency of 11.5% over the 3year period under review. It is a known fact that hypertension and diabetes are like Siamese twins with one predisposing to the other; thus possible complication of one with the other is a common occurrence in most patients especially when poorly controlled [8,9]. Based on this finding, maintaining a strong diagnostic setting for the management of the two diseases will not be wasteful investment by the hospital management.

The import of this may also be an indirect reference to the preponderance of non-communicable diseases in our environment. As at 1992, Akinkugbe in an edited monograph on "Non-Communicable Diseases in Nigeria" put the prevalence of hypertension as 10-12% in Nigerian adults [10]. Recently, a monograph on "Mortality Statistics in UCH over the period August 2012 to September 2013" reported that death due to circulatory diseases (largely hypertension) constituted about 13.27% of the mortality within the period [11]. This figure is very similar to what was reported in 1992. A further analysis of this in relation to requests for biochemical investigations showed that about 65.6% of requests to the biochemistry laboratory were largely for non-communicable diseases while investigating hypertension alone constituted 20.2% of these requests. It may therefore be said that generally renal dysfunction, glucose metabolic dysfunction and organ toxicity (either primarily or secondarily which could have precipitated electrolyte imbalance) probably constituted one of the major medical challenges handled in the hospital within this period. From the Mortality Statistics of UCH (2012-2013), the pattern might not have changed. The need to therefore develop expertise in the management of hypertension and diabetes and their complications in this environment is largely underscored by findings in this analysis.

Another area of interest is in biochemical indices of Metabolic Syndrome (MS). Amongst indicators of metabolic syndrome are the presence of the triad of carbohydrate metabolic dysfunction, dyslipidaemia and obesity [12]; although metabolic syndrome is a medical challenge worldwide, development of dyslipidaemia in patients having this condition might not have been a serious medical challenge in patients attending the hospital within the period under review. However, it is equally very probable that presence of dyslipidaemia might have been under diagnosed because request for lipid metabolic profiles was amongst the least in terms of biochemical requests in the period under review. Reasons for this might not be unconnected with the generally low incidence of obesity in this part of the world and the fact that lipid metabolic studies has gradually been gaining momentum in this environment on the advent of westernised life style in the last decade or so [13,14]. Therefore, investigating MS in this environment might require drawing up other criteria than the traditional ones generally applied in Europe and other developed places. This becomes imperative in the face of a high prevalence of carbohydrate metabolic dysfunction and the observed preponderance electrolyte studies which might have been necessitated by covert metabolic syndrome.

Investigating liver and hormonal dysfunction was also not as frequent as the others previously mentioned. This again may be a further indication of underutilisation of biochemical investigations by the various consulting physicians in the clinics because hepatic diseases including hepatic cancer are amongst the leading causes of death in this environment [15, 11]. This was also attested to from the UCH mortality report which placed death due to liver diseases as significant amongst others. However, the cultural practice of our people might be responsible for the low patronage of some of the services because most patients would not patronise hospitals early and when they do, lack of money to pay for most of the investigations may also drive them off from doing the tests. This may be quite important because liver and hormonal investigations are amongst the costliest of most routine tests in the biochemical laboratory.

One of the issues that need to be addressed as outcome of this report is to determine whether there is under or overutilization of diagnostic services in this tertiary centre and also whether there is appropriate utilization of laboratory requests by the physician. This in my opinion can be better tackled

by a committee involving all parties that will evaluate the issues in all ramifications.

Conclusion

In spite of all odds, request for biochemical investigations has been on the increase since the inception of this hospital as a tertiary hospital; although there might have been underutilisation of biochemical investigations in comparison to other centres of similar status in the developed world. The need to build on the gains in terms of strengthening the services by way of diversification and better utilisation for better, quicker and precise patient management is underscored by findings in this study.

Acknowledgements

We want to acknowledge the permission and support of UCH management in carrying out this exercise as part of the Department of Chemical Pathology UCH presentation during the 40th Anniversary celebration of the Ibadan College of Medicine. We also thank and acknowledge the Chief Medical Director of University College Hospital for permitting us to use data from the UCH Mortality Statistics (August 2012- July 2013) in this paper. Our thanks also go to the former head of UCH Medical Records and the entire staff of Medical records/ Information unit for the data used in this paper.

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Glycaemic response and insulin index in apparently healthy male adults following ingestion of some Nigerian meals

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Abstract

Background: There are available reports on the glycaemic indices (GI) of some local Nigerian diets but there is the dearth of information on insulinogenic responses to the diets which are also vital in chronic diseases prediction and management. Therefore, this study was carried out to determine the GI, glycaemic load (GL) and insulin index (II) of selected commonly consumed Nigerian diets.

Materials and Methods: Twelve apparently healthy males were recruited into this randomized cross over study. Meal tolerance testing (MTT) was carried out on each participant, on separate days, after an overnight fast using 50g available carbohydrate of each of yam flour paste (amala), wheat paste and cooked cowpea with 50g of glucose serving as the reference meal. Venous blood was collected at 0 minute and then postprandially at 30, 60, 90 and 120 minutes to determine the plasma levels of glucose and insulin. Thereafter, the area under the curve (AUC) of each meal was calculated using trapezoidal rule and then, GI, GL and II were calculated using appropriate formula. $P<0.05$ was considered as statistically significant.

Results: Wheat meal had the lowest GI, GL and II amongst the three meals. Also, the glucose mean area under the curve (AUC_G) of wheat was significantly lower compared with the reference meal. There were no significant differences in AUC_G of amala and cowpea meal when compared with the reference meal. Furthermore, the insulin mean AUC (AUC_I) of wheat meal was significantly lower when compared with the reference meal and cowpea meal.

Conclusion: Wheat meal had the lowest GI, lowest glycaemic response and provoked less insulin release compared with amala and cowpea meal. Therefore, consumption of whole, unprocessed wheat meal might be helpful in the prevention and management of diabetes mellitus.

Keywords: *Glycamic response, insulin index, Nigerian meals, wheat.*

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Résumé

Contexte: Il existe des rapports disponibles sur les indices glycémiques (IG) de certains régimes locaux nigérians, mais il y a une pénurie d'informations sur les réponses insulinogeniques aux régimes, qui sont également essentielles dans la prévision et la gestion des maladies chroniques. Par conséquent, cette étude a été réalisée pour déterminer l'IG, la charge glycémique (CG) et l'index d'insuline (II) de certains régimes nigérians consommés couramment.

Matériels et méthodes: Douze mâles apparemment en bonne santé ont été recrutés dans cette étude croisée randomisée. Un test de tolérance au repas (TTR) a été effectué sur chaque participant, après un jeûne de nuit, en utilisant 50g de glucides disponibles de pâte de farine d'igname (amala), de pâte de blé et de niébé cuite avec 50g de glucose comme repas de référence. Le sang veineux a été recueilli à 0 minute, puis de manière postprandiale à 30, 60, 90 et 120 minutes pour déterminer les taux plasmatiques de glucose et d'insuline. Par la suite, l'aire sous la courbe (ASC) de chaque repas a été calculée en utilisant la règle trapézoïdale et ensuite, GI, CG et II ont été calculés en utilisant la formule appropriée. $P<0,05$ a été considéré comme statistiquement significatif.

Résultats: Le repas de blé avait l'IG, CG et II plus bas parmi les trois repas. De plus, la surface moyenne du glucose sous la courbe (ASC_G) du blé était significativement plus faible par rapport au repas de référence. Il n'y avait pas de différences significatives dans l'ASC_G de l'amala et de la farine de niébé par rapport au repas de référence. De plus, l'ASC moyenne (AUC_I) de l'insuline de la farine de blé était significativement plus faible que celle du repas de référence et de la farine de niébé.

Conclusion: Le repas de blé a présenté la plus faible IG, la réponse glycémique la plus faible et a provoqué moins de libération d'insuline comparativement à l'amala et au niébé. Par conséquent, la consommation de farine de blé entière non transformée pourrait être utile dans la prévention et la gestion du diabète sucré.

Mots-clés: *Réponse glycémique, indice d'insuline, repas nigérian, blé*

Introduction

Nutrition is an intricate issue with myriads of factors and variables which impact on metabolic health and incidence of disease [1]. It is therefore not surprising that medical nutrition therapy remains a cornerstone in the prevention and management of chronic diseases such as diabetes. Almost four decades ago, Burkitt and Trowell [2] propounded the dietary fiber hypothesis which showed that foods that are high in fiber are protective against certain metabolic diseases due to the ability of fiber to reduce the rate of nutrient influx from the gut. Observational studies have also shown that high intake of whole grains, is associated with lower body mass index (BMI), improved insulin sensitivity, reduced risk of type 2 diabetes mellitus (T2DM) and of premature death [3-5].

An extension of the dietary fiber hypothesis, known as glycaemic index (GI) concept, was reported in 1980 [6]. It is a measure used to assess the blood glucose raising potential of the available carbohydrate in high carbohydrate foods [1]. Invariably, it is an index of glycaemic response of a fixed amount of available carbohydrate from a test food to the same amount of available carbohydrate from a standard food (glucose or white bread) consumed by the same person [7-9]. Epidemiological and dietary intervention studies suggest that a low GI diet is beneficial for blood glucose control especially, in patients with T2DM. In contrast, foods with high GI contribute to insulin resistance, increased T2DM risk, obesity, cardiovascular disease and cancer [10,11].

Another concept which quantifies the impact of a carbohydrate-containing meal or a single food eaten in a "normal" portion has on blood glucose level is called glycaemic load (GL) [12]. It is a measure of the potency of a carbohydrate food on glycaemia and is calculated using the GI and the grams of available carbohydrate in the portion size [1]. Although there have been controversies over the clinical applications of GI and GL especially, in nutritional epidemiology, these concepts still remain important in planning for many dietary interventions. However, the GI and GL concepts do not reflect, in its entirety, the relationship between dietary factors and physiological insulin secretion provoked by foods. Therefore, comprehensive understanding of this relationship could be important in the dietary management and prevention of chronic diseases [13]. The quest for this understanding led to the development of insulin index concept which is used to classify foods according to their postprandial insulin response [14]. The index is similar to GI and GL, but rather than relying on blood glucose levels, it is based on blood insulin levels. As it is well known that carbohydrate is the primary stimulus for insulin secretion, protein rich foods are also known insulin secretagogue and, when combined with carbohydrate, act synergistically to raise insulin concentrations and reduce glycaemia [15,16]. This concept thus has an advantage of taking care of all food contents and not the carbohydrate content only as all dietary components and their metabolic interactions need to be considered in order to allow for an all-inclusive approach to determining insulin demand [13].

Insulin index concept has been shown to be useful in the management of type 1 diabetes mellitus and hyperlipidaemia [17]. It has been shown that consumption of low GL foods results in a reduced postprandial rise of insulin [18]. Furthermore, slow gastric emptying, decreased hunger, increased satiety, and decreased voluntary food intake are associated with low GI/GL meals [19-21]. Although there are available reports on GI of some commonly consumed Nigerian diets, there is paucity of information on the postprandial insulin response to the diets which could be vital in energy recommendation. This study was therefore carried out to provide information on this gap in knowledge with the hypothesis that there are potential differences in GI and insulin responses following the ingestion of selected Nigerian diets.

Methodology

Study participants

Twelve apparently healthy males with regular eating habits were enrolled into this randomized cross-over study. They were adults (25 – 35 years), non-smokers and were neither underweight nor overweight. All the participants had no thyroid disease, metabolic disorders such as diabetes mellitus and were not allergic to any of the selected local diets. Also, the participants had no gastrointestinal disease and had no history of abdominal surgery or family history of diabetes.

Ethical consideration

This study was reviewed and approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Committee. Also, written informed consent was obtained from each study participant.

Preparation of study participants

The purpose of the investigation was explained to all the study participants before meal testing day in order to ensure compliance. They were asked to refrain from unusual physical activity, avoid alcohol consumption and eat their regular meals. On the morning of each meal testing, they were instructed to report at the Metabolic Research Ward of UCH after an overnight fast of about 8 to 10 hours.

Data collection

History of diabetes mellitus, hypertension and other metabolic diseases was obtained using a standard questionnaire. Anthropometric indices were obtained using standard procedures. Body weight of each subject was measured to the nearest kilogram in light clothing using a weighing scale and the height was measured to the nearest centimeter using a stadiometer. The body mass index (BMI) was calculated as the ratio of the body weight in kilogram to the square of the height in meter.

Food preparation

Three different meals; yam flour paste (amala), wheat flour paste and boiled cowpea that are commonly consumed in the South Western part of Nigeria were prepared from yam flour, wheat flour and brown cowpea obtained from the Bodija market, Ibadan, Nigeria. Yam flour tubers and whole wheat grains were milled and sieved into fine flour before using them to prepare the meals. The meals were prepared in the Department of Human Nutrition kitchen, University of Ibadan. Each serving of the meal provided 50g of available carbohydrate and was prepared using 74g, 69g and 125g of yam flour, wheat flour and cowpea respectively. The quantity of each raw food that gives the required 50g of available carbohydrate was arrived at using simple calculation. From the West Africa food composition Table [22], 100g of dry brown cowpea contains 40g of available carbohydrate therefore; 125g of dry brown cowpea will give 50g of available carbohydrate. Similarly, 100g of wheat flour contains 72.5g of available carbohydrate; therefore 69g of wheat flour will give 50g of available carbohydrate. Also, 100g of yam flour contains 67.9g of available carbohydrate therefore; 74g of yam flour will give 50g of available carbohydrate. Measurement of the raw foods was done using the Ohaus Scout Pro Portable Electronic Balance 4000g Capacity, 0.1g Readability (Ohaus Corporation, USA). The reference meal with which the meals were compared was 50g of glucose. Therefore, our glycaemic index is on glucose scale and not the bread scale.

Meal preparation

Flour of each of yam or wheat was added to boiling water and stirred until thick and consistent paste was obtained. Paste was allowed to cook properly for additional 2 to 3 minutes.

Raw dry cowpea was cooked for about 45 min and allowed to soften properly. The cowpea was well cooked and contained no additive but a little quantity of salt to enhance palatability.

Amala and wheat meals were served with plain ewedu soup (without stew) using the same number of servings. The ewedu soup was prepared by adding ewedu leaves (*Corchorus olitorius*) into boiling water and mashed continuously inside the pot until blended into smooth paste.

Meal tolerance testing

Meal tolerance testing (MTT) was done using meal servings of amala, wheat and cooked cowpea with each containing 50g of available carbohydrate. Each participant consumed the entire meal serving within 15 minutes and each meal serving was served with 50 cl of water. Throughout the 120 min of MTT, all the participants were sedentary. Each meal, including the reference meal, was tested on separate days with a week interval between each meal as wash-out period.

Sample collection

Five milliliter of venous blood was collected from the antecubital fossa at 0 minute and then post-prandially at 30, 60, 90 and 120 minutes using cannula. The samples were dispensed into fluoride oxalate bottle and lithium heparin containing sample bottles and thereafter, plasma was obtained and stored at -20°C until analysis.

Laboratory analysis

Plasma levels of glucose and insulin were determined using glucose oxidase method and ELISA (GenWay Biotech Inc., USA) respectively.

Calculation of glycaemic index, glycaemic load and insulin index

$$\text{Glycemic index} = \frac{AUC_{\text{test food}}}{AUC_G} \times 100$$

$$\text{Insulin index} = \frac{AUC_{\text{test food}}}{AUC_I} \times 100$$

$$\text{Glycemic load} = \frac{\text{Carbohydrate content (g)} \times \text{GI of the food}}{100}$$

Statistical analysis

The incremental area under the 120 min curve (AUC) for both glucose (AUC_G) and insulin (AUC_I) response to each test meal was calculated using the trapezoidal rule. Thereafter, Student's t-test was used to determine differences in means and p-values less than 0.05 were considered as statistically significant.

Result

The general characteristics of the study participants are shown in Table 1. The participants were male adults, normotensive and had normal body weight with no central obesity.

Table 1: Characteristics of the study participants

Parameters	Mean ± SD
Age (years)	29.0 ± 3.1
Body weight (kg)	64.0 ± 10.8
Height (m)	1.7 ± 0.0
Body Mass Index (kg/m^2)	22.0 ± 3.1
Waist Circumference (cm)	78 .0 ± 8.4
Hip Circumference (cm)	90.0 ± 4.4
Waist-Hip Ratio	0.9 ± 0.1
SBP (mmHg)	108.0 ± 14.0
DBP (mmHg)	75.0 ± 10.00

concentration started after 30 min. In wheat and cowpea however, there were progressive rises in the plasma glucose levels until about 60 min when the fall in concentrations began. At 120 min, the post-meal plasma glucose levels mirrored that of the fasting state except with amala whose fasting level was reached at about 90 min post ingestion.

As for insulin, the insulin level post glucose ingestion rose steadily until about 30 min when the progressive sharp fall in concentration commenced. Cowpea meal induced a similar insulin response except that the insulin level rose steadily until about 60 min when it maintained an apparently steady concentration until about 90 min before its level started to fall. The pattern of changes in insulin levels post ingestion of amala and wheat meal was irregular. Following amala ingestion, there was rise in insulin level up until about 30 min when the level started to fall. However, at about 90 min, the level began to rise again. Similarly, there was a zigzag-like insulin pattern following wheat meal ingestion. The insulin level rose post wheat meal ingestion and at about 30 min, its level started declining until about 60 min when it started rising again. The rise in concentration continues till about 90 min when it started falling again (Figure 2).

Table 3 shows the glycaemic index (GI), Glycaemic load (GL) and insulin index (II) of each meal. It was observed that all the meals are of high

Table 2: The moisture content of the meals after preparation

Meal type	Raw weight (g)	Weight after preparation (g)	Moisture content after preparation (%)*
Wheat	69.0	209.5	66.0
Amala	74.0	215.0	29.4
Cowpea	125.0	325.0	20.4

*% Moisture content = $\frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$ [23]

The total weight of all the meals increased after preparation. This is a reflection of their moisture content which is shown in Table 2.

The pattern of glucose changes during the meal tolerance test had some variations as shown in Figure 1. The response curve following ingestion of glucose showed that there was a rise in plasma glucose level after ingestion up until 30 min when it assumed a steady concentration till about 60 min after which it started falling to parallel the fasting concentration. Amala had a similar response curve to that of glucose but the fall in plasma glucose

GI ($\geq$70). Wheat meal had the lowest GI, GL and II compared with the other 2 meals. In contrast, amala had the highest GI and GL compared with the other 2 meals. However, cowpea meal had the highest insulin index among the 3 meals.

Comparing the means area under the curve of glucose (AUC_G) and insulin (AUC_I) of the 3 meals with the reference meal, AUC_G of wheat meal was significantly lower compared with the reference meal. There were no significant differences in AUC_G of amala and cowpea meal when compared with the reference meal. Comparing the 3 meals with one

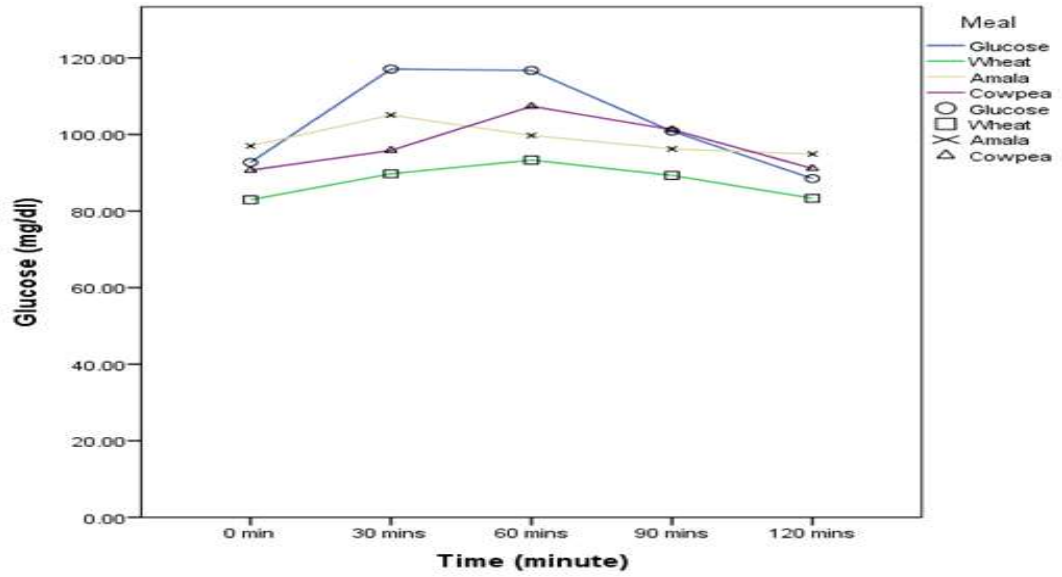

Fig.1: Glucose response curv

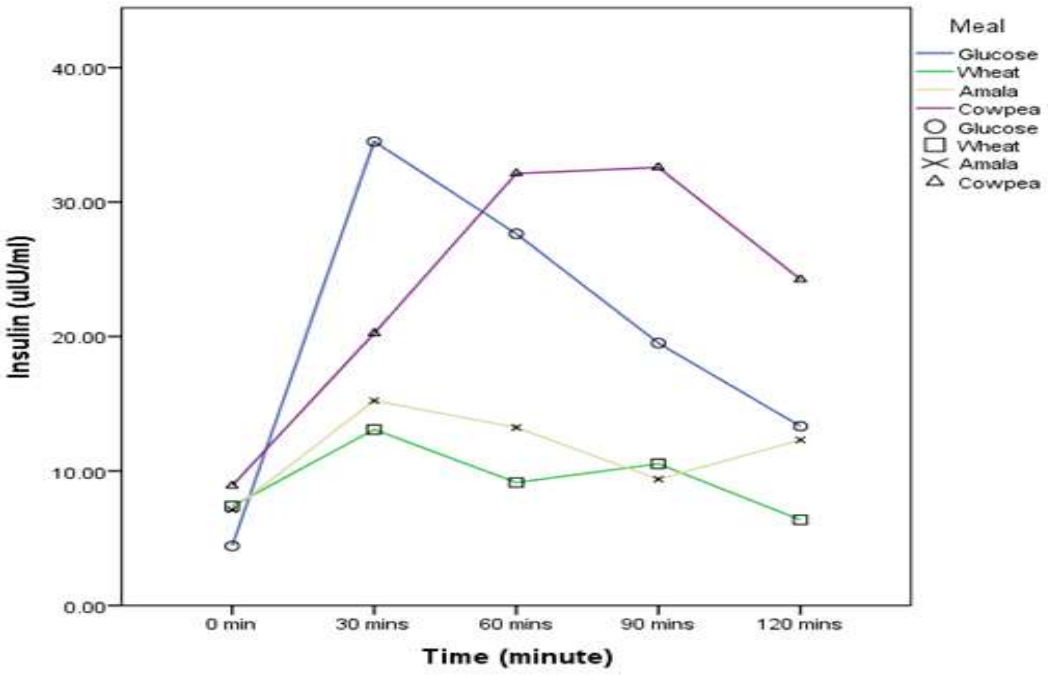

Fig. 2: Insulin response curve

Table 3: Glycemic indices, glycemic loads, insulin indices and AUC_G and AUC_I of wheat, amala and cowpea

	Wheat	Amala	Cowpea
Glycaemic Index (%)	77.3	87.2	86.2
Glycaemic Load (g/serving)	38.7	43.8	43.6
Insulin Index (%)	47.6	56.2	110.2
AUC_G (mg x min/dL)	10649.42 ± 377.90	12005.42 ± 330.97	16867.09 ± 360.01
AUC_I (µIU x min/ml)	1292.29 ± 179.23	1443.92 ± 208.60	3005.92 ± 344.39

another however, AUC_G of wheat meal was significantly lower when compared with amala and cowpea meal (Table 4). Also in Table 4, the AUC_I of wheat meal and amala were significantly lower compared with the reference meal. Similarly, the AUC_I of wheat meal and amala were significantly lower compared with cowpea meal.

Table 4: Areas under the curve for glucose and insulin following ingestion of the test meals and the reference meal

	AUC_G (mg x min/dL)	AUC_I (µIU x min/ml)
Glucose	13769.63 ± 1168.28	2715.42 ± 271.49
Wheat	10649.42 ± 377.90	1292.29 ± 179.23
p-value	0.019*	0.000*
Glucose	13769.63 ± 1168.28	2715.42 ± 271.49
Amala	12005.42 ± 330.97	1443.92 ± 208.60
p-value	0.160	0.001*
Glucose	13769.63 ± 1168.28	2715.42 ± 271.49
Cowpea	16867.09 ± 360.01	3005.92 ± 344.39
p-value	0.134	0.515
Wheat	10649.42 ± 377.90	1292.29 ± 179.93
Amala	12005.42 ± 330.97	1443.92 ± 208.60
p-value	0.013*	0.588
Wheat	10649.42 ± 377.90	1292.29 ± 179.93
Cowpea	11867.09 ± 360.01	3005.92 ± 344.39
p-value	0.029*	0.000*
Amala	12005.42 ± 330.97	1443.92 ± 208.60
Cowpea	1247.09 ± 360.01	3005.92 ± 344.39
p-value	0.780	0.001*

*Significant at $p<0.05$

Discussion

The concept of glycaemic index (GI) remains an important tool in the dietary management of chronic diseases. However, its use is limited by its inability to provide a detailed information on insulin production and responses which are also vital in chronic diseases management.

Several reports have shown that diets with low GI have possible potential in preventing chronic diseases such as coronary heart disease, diabetes and obesity [24,25]. In this study, all the 3 tested meals are of high GI ($\geq$70). This might suggest that consumption of any of the three meals should be regulated so as to forestall possible development of metabolic diseases associated with consumption of high GI foods. The observed GI and GL for amala were in line with the report of Omoregie and Osagie [26] who reported a relatively similar GI of 84.35 and GL of 42.18 for amala. However, the GI and GL observed for wheat meal in this study were lower compared with GI of 95.28 and GL of 47.64 for wheat meal reported in their study. In their study, semolina and semovita brands of wheat were used whereas whole, unprocessed wheat was used in this study. The differences in the brands of wheat used could be responsible for the discrepancies in GI and GL. In addition, Foster-Powell *et al.* [27] reported that increased processing of meals can affect GI. Therefore, the differences in GI and GL could also be due to the mode of processing.

The observed GI and GL for cowpea meal were higher than the GI (65.01) and GL (15.12) reported by Akinlua *et al.* [28]. The differences in observations could also be due to the mode of preparation and the available carbohydrate content of the cowpea used. In this study, the cowpea was consumed without stew whereas in their study, the cowpea was served with stew. This could introduce some disparity as components of the stew might have some effects on GI. Also, they reported a total available carbohydrate of 15g in their cowpea meal whereas; our cowpea meal was prepared to contain 50g of available carbohydrate.

Foods with low GI have been shown to have metabolic benefits because they are slowly digested, absorbed and metabolized [1]. Based on the observed GI and GL of wheat meal compared with the other 2 meals, consumption of whole, unprocessed wheat meal may be relatively beneficial in preventing and managing metabolic diseases such as type 2 diabetes.

Diets that provoke less insulin secretion may be helpful in the prevention and management of diabetes and indirectly, of cancer [29]. The observed low insulin index for wheat meal might not be surprising as its GI was also low. This observation further buttresses the suggested relative benefit of wheat meal in the management of metabolic diseases. Interestingly, cowpea meal had the highest II amongst the three meals. This is unexpected as its GI and GL were slightly lower than that of amala whose II was lower than that of cowpea. This elevated II in cowpea meal could be as a result of the protein content of cowpea which can act synergistically to stimulate insulin release. Therefore, insulin index of a food is not directly proportional to the glycaemic index of the food but could be dependent on the food contents and their insulin stimulatory effect. It might be suggested thus, that information on GI of foods cannot be directly inferred from the II of the foods.

The observed significantly lower AUC_G of wheat meal compared with the reference meal and the other test meals may indicate that the amount of glucose released per unit time following wheat meal ingestion is lower than that of the reference meal

and the other test meals. These observations are in line with the observed low GI and GL of wheat meal. It is known that there is slow gastric emptying, digestion, absorption and metabolism following ingestion of low GI foods [1].

The observed significantly lower AUC_I of wheat meal and amala compared with the reference meal might mean that the amount of insulin produced per unit time following ingestion of the two meals were lower compared with the reference meal. This might not be untrue as amala and wheat meal, upon swallowing, will undergo normal digestion while the reference meal is rapidly absorbed as it requires no digestion.

Carbohydrate is the primary stimulus for insulin secretion however, protein rich foods, such as cowpea, also elicit a significant insulin response and, when combined with carbohydrate, act synergistically to raise insulin concentrations and reduce glycaemia [29,30]. This might explain the observed significantly lower mean AUC_I of wheat meal and amala when compared with cowpea meal. This could mean that the amount of insulin produced per unit time following ingestion of the two meals were lower compared with cowpea meal which is richer in protein content than the other test meals. Indetermination of the fiber content of the meals is a notable limitation of this study.

It could be concluded from this study that whole, unprocessed wheat meal had low GI, glycaemic response and provoked less insulin release relative to amala and cowpea meal. Therefore, consumption of whole, unprocessed wheat meal might be helpful in the prevention and management of diabetes mellitus. Also, the insulin index of a food appears not to be a direct reflection of its glycaemic index hence, direct deduction of information from each other should be done with some caution.

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