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The aims of *The African Journal of Medicine and Medical Sciences* are: (1) to provide a medium for wide dissemination of information resulting from biomedical research in Africa and elsewhere; (2) to furnish a means whereby appropriate international medical and health organisations may transmit information to medical scientists throughout Africa; (3) to serve as a medium for publication of proceedings of international conferences on medical sciences in Africa; (4) to serve as a medium for the exchange of information and opinion among medical scientists in medical institutions of Africa and elsewhere; (5) to promote inter-regional cooperation amongst medical scientists in Africa.

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The WHO list of Essential Diagnostic Tests and new dimensions…

Clinical details supplemented with results obtained from investigations are essential for arriving at the appropriate diagnosis for managing patients. In recent times, the use of biomarkers is taking centre stage for diagnostic specificity. Biomarkers are measurable indicators of normal biological, pathological or pharmacological responses that can lead to therapeutic intervention. Biomarkers have diagnostic and/or prognostic significance in clinical medicine.

The Strategic Advisory Group of Experts on In-Vitro Diagnostics (SAGE IVD) of the World Health Organization (WHO)[1] recently published a comprehensive list of essential diagnostic tests for communicable (malaria, tuberculosis, human immunodeficiency virus, hepatitis B and C, human papilloma virus etc) and non-communicable diseases (diabetes mellitus, ischemic heart disease, chronic kidney disease, cancer screening etc), which are applicable at all tiers of the health care system. The WHO has taken giant strides in ensuring optimal and cost-effective health care on a global basis. This is commendable.

Three articles in this issue of the journal focus on another potential biological material for testing and other biomarkers of diseases that hold some promise in diagnosis and treatment: The first is the manuscript by Lasisi et al which reported that the levels of biochemical abnormalities in the saliva of patients with chronic kidney disease correlated significantly with the serum levels. Thus saliva is being recommended as a useful alternative to blood for monitoring patients with chronic kidney injury undergoing dialysis. The ease of obtaining saliva and the avoidance of the pain of venesection are immediate reasons why the use of this biological specimen appears favourable. It is very likely that this specimen may be found useful in other clinical situations. The second manuscript by Ajani et al focused on leptin and its association with obesity and glycaemic control. Leptin is a gene product that regulates food intake and energy metabolism. In their study, serum leptin levels were significantly higher in obese type 2 diabetic patients when compared with non-obese diabetic patients and normal individuals. The authors also reported an inverse relationship between leptin levels and glycaemic control which may suggest a potential therapeutic role. The third manuscript is the study by Akinpelu and colleagues which compared adiponectin levels in pre-eclamptic and normotensive pregnant women. The authors observed that the pre-eclamptic group had significantly higher levels of adiponectin; again another useful diagnostic marker in obstetrics.

These innovative studies need to be acknowledged and their diagnostic/therapeutic values appraised. However, replication of these findings in more studies involving larger samples are needed before widespread use can be recommended. In addition, the psychometric properties must be such that the accuracy of diagnosis is not compromised. One can speculate at this stage that the addition of these biomarkers (adiponectin and leptin) will strengthen clinical use in pre-eclampsia and diabetes respectively while saliva becomes a specimen of choice for assessing biochemical changes after dialysis.

Reference

1. World Health Organization. First-ever WHO list of essential diagnostic tests. http://www.who.int/medical_devices/diagnostics/Selection_in-vitro_diagnostics/en/ (accessed May 18, 2018).

A. Ogunniyi
Editor-in-Chief

Contents

Afr. J. Med. Med. Sci. Vol. **47**, No. 2 June 2018

Publication details 103

Editorial Comment 104

Contents 105

Original Articles

Plantar dermatoglyphic traits of sub-Saharan African subjects.
P.S. Igbigbi 107-114

Gastric ulcer – healing promoting activity of cobalt chloride in rats.
A.T. Salami, P.A. James, T.O. Iyiola and S.B. Olaleye 115-122

Serum leptin in obese type 2 diabetic females in Southwestern Nigeria.
G.O.D Ajani, B.A. Kolawole, O. Okunola, A.A. Ajani, M.O. Ajala and R.T. Ikem 123-131

Antidepressant activity of ethanol extract of *Albizia adianthifolia* (Schumach) W.F. Wight leaf in mice.
N. Unumadu, A.O. Aderibigbe and O.A. Adeoluwa 133-140

Serum adiponectin levels in normotensive and pre-eclamptic women at the University College Hospital, Ibadan, Nigeria.
O.M. Akinpelu, F.A. Bello, O.A. Awolude, C.O. Adetayo, B.O. Akinwumi and A.A. Odukogbe 141-146

Onycgomycosis in patinets attending a dermatology outpatient clinic in Lagos, Nigeria.
R.O. Oladele and O.O. Ayanlowo 147-153

Internet use by orthopaedic patients: A survey of patients in three teaching hospitals in Southwestern Nigeria.
K.S. Oluwadiya, O. Esan, S.O. Popoola and E.O. Alabi 155-160

Effect of antacids on the pharmacokinetics of lumefantrine in healthy volunteers: a pilot study.
J.N. Nwogu, O.A. Kotila, O.S. Michael and C.P.Babalola 161-169

The International HIV dementia scale, a valuable screening instrument for HIV-Associated Neurocognitive Disorder (HAND) in HIV-Infected adults in North Central Nigeria.
O.G. Osaigbovo S. Ugoya and A. Ogunniyi 171-178

Saliva based analysis of biochemical factors in patients with chronic kidney disease undergoing haemodialysis.
T.J. lasisi, Y.R. Raji and B.L. Salako 179-184

Hepatotoxicity and clastogenicity of dichlorvos at high doses in Male Wistar rats.
M.A. Gbadegesin, E.C. Udegbubam, J.O. Olugbami, A.M. Adegoke and O.A. Odunola. 185-194

Sickle cell disease management in Nigeria: understanding the challenges from the Physicians' Perspectives.
T.S. Akingbola, O.O. Akinyemi, O.O. Amodu and B.O. Tayo 195-203

Afr. J. Med. Med. Sci. Vol. **47**, No. 2 June 2018

Metabolic alterations in Africans with prostate cancer before bilateral orchidectomy.
M.A. Charles-Davies, A.H. Oniye, E.O. Olapade-Olaopa, J.I. Anetor and E.O. Agbedana 205-213

Healthcare associated infections in a new born Unit in Nigeria.
A.E. Akindolire, O.O. Tongo, H. Dada-Adegbola and O.O. Akinyinka 215-221

Notes for Contributor 222

Afr. J. Med. Med. Sci. (2018) **47,** 115-122

Gastric ulcer - healing promoting activity of cobalt chloride in rats.

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Abstract
Background: Gastric ulcer develops when aggressive factors overcome protective factors in the gastrointestinal tract. Cobalt chloride is used in the manufacturing of vitamin B_{12}, essential for folate and fatty acid metabolism. Information regarding probable effects of Cobalt Chloride on Ulcer healing is void despite its vast use which this study addresses.
Method: 70 Male Wistar rats (150-180g, n=10) were used and induced with ulcer using acetic acid (excluding control) before grouping: Groups 1- Control, 2 - ulcer alone, 3 and 4 were ulcerated treated with 62 and 25mg/kg b.w of Cobalt chloride ($CoCl_2$); 5, 6 and 7 were ulcerated treated with 1, 40 and 30mg/kg b.w of Misoprostol, Cimetidine and Omeprazole respectively for 2 weeks. Gastric acid secretion, ulcer scores and histopathology of ulcerated areas were evaluated on days 7, 14 and 21. Data were analyzed using ANOVA and considered significant at $p<0.05$.
Result: This study revealed significant decrease in gastric acid secretion (and pH) of control (0.65±0.02) and treatment groups - high (0.86±0.01) and low (0.85±0.02) $CoCl_2$ compared to ulcer untreated (1.22±0.04) by day 7 post ulceration. A significant decrease in ulcer index of treatment group (high and low $CoCl_2$) by day 7(90.18% and 91.21%) respectively and complete healing day 14 was observed. Histological evaluations of $CoCl_2$ treated group revealed intact epithelium with normal glands by days 7 and post ulceration. There was no ulcer reformation in examined stomach, by day 21 (day 7 post 25mg/kg b.w $CoCl_2$ treatment).
Conclusion: Probably, Cobalt chloride exerts its anti-ulcerogenic property by stimulating gastric protective activities.

Keyword: *Cobalt chloride, gastric ulcer healing, gastric acidity*.

Résumé
Contexte: L'ulcère gastrique se développe lorsque des facteurs agressifs surmontent les facteurs protecteurs du tractus gastro-intestinal. Le chlorure de cobalt est utilisé dans la fabrication de la vitamine B_{12}, essentielle pour le métabolisme des acides foliques et gras. L'information concernant les effets probables du chlorure de cobalt sur la cicatrisation des ulcères est nulle en dépit de son vaste utilisation qui fait l'objet de cette étude.
Méthode : 70 rats Wistar (150-180g, n = 10) ont été utilisés et induits avec un ulcère en utilisant de l'acide acétique (excluant le contrôle) avant le regroupement: groupes 1- contrôle, 2 - ulcère seul, 3 et 4 ulcérés traités avec 62 et 25 mg / kg pc de chlorure de cobalt ($CoCl_2$); 5, 6 et 7 ont été traités avec 1, 40 et 30 mg / kg pc de misoprostole, de cimétidine et d'oméprazole respectivement pendant 2 semaines. La sécrétion d'acide gastrique, les scores d'ulcère et l'histopathologie des zones ulcérées ont été évalués aux jours 7, 14 et 21. Les données ont été analysées en utilisant ANOVA et considérées significatives à $p <0,05$.
Résultat : Cette étude a révélé la diminution significative de la sécrétion d'acide gastrique (et pH) des groupes de contrôle (0,65 ± 0,02) et de traitement - élevé (0,86 ± 0,01) et faible (0,85 ± 0,02) $CoCl_2$ par rapport au groupe d'ulcère non traité (1,22 ± 0,04) au jour 7 après l'ulcération. Une diminution significative de l'indice d'ulcère du groupe de traitement ($CoCl_2$ élevé et faible) au jour 7 (90,18% et 91,21%) respectivement et la cicatrisation complète jour 14 a été observée. Les évaluations histologiques du groupe traité par $CoCl_2$ ont révélé un épithélium intact avec des glandes normales au jour 7 et post-ulcération. Il n'y a pas eu de réformation d'ulcère dans les estomacs examinés, au jour 21 (au jour 7 après traitement avec 25 mg / kg pc de $CoCl_2$).
Conclusion: Probablement, le chlorure de cobalt exerce sa propriété d'anti-ulcération en stimulant des activités de protection gastrique.

Mot - clé : *Chlorure de cobalt, cicatrisation de l'ulcère gastrique, acidité gastrique* .

Introduction

In a normal scenario of a healthy stomach, there is usually a balance between protective factors (mucus and bicarbonate secretions) and aggressive factors (acid secretion and pepsin) [1]. Gastric ulcerations

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therefore develop when these aggressive factors overwhelm the protective mechanisms [2,3]. Gastric ulcers defined (peptic ulcers), as ulceration of the mucous membrane or stomach lining caused by hydrochloric acid (gastric digestive juice) action [4]. However, the major causative factor of peptic ulceration is a local decrease in the resistance of gastric mucosa to gastric juice digestive actions [5].

During gastric ulcer healing, a key factor is adequate blood flow and supply to ulcerated site in order to restore the damaged tissue components. This ensures oxygenation (red blood cells), removal of waste or necrotized tissues, prevention of infection (white blood cells) supply of nutrient and growth factors (platelets) to the ulcer site thus initiating angiogenesis [6].

Cobalt chloride has long been used for the treatment of anemia as it enhances erythropoiesis [7-10] especially those induced by hypoxia [11] or sickle cell [12]. It has been used by athletes to boost the endogenous erythropoietin levels [13]. It is inexpensive, readily available and very effective [14]. Various experiments have shown it to be effective in increasing physical performance (physical fitness) in rats [15], protect against ischemic injury and high altitude pulmonary edema [16] in rats.

Cobalt chloride has also been used as dietary supplements in ruminants [17] and observed to be protective against sodium thiosulfate during cyanide poisoning treatment [18]. Yildirim and Buyukbingol [19] observed that ascorbic acid enhanced the antioxidative effect of cobalt chloride by alleviating impaired oxidative stress in streptozotocin induced diabetes rats. Tephly and Hibbelin [20] observed that cobalt chloride (60mg/kg b.w) had an inhibitory effect on the synthesis of hepatic microsomal cytochrome P-450. It became a preferred choice [21] over uranium and tungsten alloys due to their toxicity [22] as well as its use (Cobalt-chromium alloys) in orthopedic hip replacement [23].

Cobalt has been found to cause several adverse effects when used in excess or prolonged exposure which might cause toxicity to various tissues and body system [24]. In man, ingestion of cobalt salts in excess for the treatment of certain refractory anemia produces nausea, vomiting, diarrhea, skin rashes and hot flushes in the short term [25] hence its none clinical prolong use [26, 24]. However its' over exposure leads to its toxicity [27] and uncontrolled adverse effects in the human tissue [28, 29]. Cobalt chloride has been reported to have varied effect on the respiratory tract, reproductive system, and bone tissue and heart- cardiomyopathy especially in beer drinkers [30] as it is used to stabilize foam in beer. There are however no reports on it being carcinogenic during oral exposure as it is poorly absorbed and excreted mostly through the faeces [31] (EFSA 2009). There has not been any beneficial report on its probable use in the gastrointestinal system despite its importance in the manufacturing of vitamin B12.

Vitamin B12 is important in the production of blood tonic or capsules generally referred to as hematinic which boosts hemoglobin production in anemia. Anemia has also been observed in some ulcer patients and has been treated with hematinic drug to boost their blood levels [32]. This study focuses on investigating the effect of cobalt chloride (in none lethal and sub lethal doses) [33] will exert on gastric ulcers healing.

Animal grouping

Seventy healthy adult male Wistar strain rats (150-170g) obtained from the Central Animal House, Department of Physiology, College of Medicine, University of Ibadan, Nigeria were used for this study. They were acclimatized for a period of 2 weeks and housed in cages at standard laboratory condition of room temperature (23±2°C), humidity (55±15%) with natural environmental 12 hours light and dark cycle. They were allowed free access to water and standard commercial rat pellets (Ladokun Feeds Nigeria Limited, Ibadan, Nigeria). The rats were handled according to the ethics of animal handling in compliance with the institution's guideline and criteria for human care (National institute of Health Guidelines for the care and Use of Laboratory Animals). The work was moderated by the Gastrointestinal Secretions and Inflammatory Research Unit, department of Physiology, University of Ibadan for thorough animal ethical humane compliance. These animals were then randomly divided into 7 groups of 10 animals each.

Group I (Normal control), Group II (Ulcer Untreated control), Group III ulcer+25mg/kg b.w $CoCl_2$, Group IV (ulcer + 62mg/kg b.w of $CoCl_2$, Group V (ulcer + 1mg/kg b.w Misoprostol), Group VI (ulcer + 40mg/kg b.w Cimetidine), Group VII (ulcer + 30mg/kg b.w Omeprazole)

Test and standard drugs

Cobalt chloride salts (manufactured and packed by Burgoyne, India Mumbai: batch number-24057) was purchased and used at two doses 25mg/kg b.w and 62mg/kg b.w.

Cimetidine capsule *(manufactured by Laborate Pharmaceutical (India) E-11,Ind. Area, Panipat – 132103: manufacturing date- 10-2012; expiry date- 09-2015)administered at* 40mg/kg b.w,
Mistoprotol tablets *(manufactured by JRP Co., Ltd. 34-40, Jeyakgongdan 2-gil, Hyangnam-eup, Hwaseong-si,Gyeonggi-do, Korea for Zolon healthcare limited, Isolo, lagos , Nigeria: manufacturing date-05-2013; expiry date-05-2016) administered at* 1mg/kg b.w and
Omeprazole tablets (*manufactured by Vee Excel Drugs and Pharmaceuticals Private Limited, Delhi, Ghaziabad No 703, Devika Tower, Ghaziabad – 201011, Uttar: manufacturing date- 09-2012; expiry date- 09-2015)* administered at 30mg/kg b.w respectively.

Gastric secretion

The gastric acid secretion was measured using the continuous perfusion method of Ghosh and Schild, [34], modified by Amure and Ginsburg, [35] at days 7 and 14 post ulceration after 24 hour fast but with access to clean drinking water alone.

Experimentally induced gastric ulceration

Gastric ulcer was produced by acetic acid via the release of histamine, which increases the capillary permeability and back diffusion of HCl as described by Okabe and Pfeifler [36]. Gastric ulcer were produced according to method of Jainu *et al.*, [37] and Okabe *et al* [38].

Measurement of ulcer index

The degree of ulceration was assessed by carrying out a microscopic examination with 2X magnification hand lens. Scoring of ulcerated area after gastric acid secretion was done by opening the stomach along the greater curvatures. The stomach was bathed in a normal saline and was then carefully spread out, pinned on a cock board for scoring. The ulcerated area was scored by plannimetry, represented in (mm^2), and then calculated according to the collection of the guiding principle of drug administration of ministry of health, Beijing using the equation $S=\pi (d_1/2) X (d_2/2)$ [39,40].

Statistical analysis

Data obtained were analysed by Graph pad prism statistical package using descriptive statistics, ANOVA and t-test at p=.05 were significant.

Results

Effects of cobalt chloride on gastric acid secretion basal secretion and ph for days 7 and 14

Effect of cobalt chloride on gastric acid secretion:

Results from this study show that at day 7, there was a significant decrease in gastric acid secretion of groups treated with: High cobalt chloride (0.86±0.001), low cobalt chloride (0.85±0.01), control group (0.65±0.02), Misoprostol (0.83±0.01) and Cimetidine (0.96±0.01) compared with ulcer alone (1.22±0.04). A significant decrease in the gastric acid secretion in omeprazole group (0.46±0.003) compared with control group (0.650+01) by day 7 was also observed. The gastric acid secretion was further decreased in the Cobalt chloride and omeprazole treated groups compared with the ulcerated untreated group by day 14 post ulceration (Table 1).

Effects of cobalt chloride on gastric pH

There was a significant decrease in pH of the high cobalt chloride (3.67±0.01), low cobalt chloride

Table 1: Effects of cobalt chloride on gastric acid secretion basal secretion, pH and acidity for days 7 and 14

Group	Basal Secretion		pH	
	Day 7	Day 14	Day 7	Day 14
Control	0.65±0.02	0.53±0.01	3.79±0.01	3.90±0.01
Ulcer untreated	1.23±0.04[a]	1.18±0.01[a]	3.52±0.02	3.54±0.01[a]
High $CoCl_2$	0.86±0.01[ab]	0.85±0.01[a]	3.67±0.01[ab]	3.67±0.01[a]
Low $CoCl_2$	0.85±0.02[ab]	0.77±0.03[ac]	3.68±0.01[ab]	3.72±0.01[abc]
Misoprostol	0.83±0.01[ab]	0.91±0.01[abd]	3.69±0.01[ab]	3.65±0.01[abe]
Cimetidine	0.96±0.01[ab]	0.91±0.01[abd]	3.62±0.01[ab]	3.78±0.01[abce]
Omeprazole	0.46±0.03[abcdef]	0.51±0.01[bcdef]	3.98±0.01[ab]	3.99±0.01[bcdef]

Values are represented as Mean ± SEM and significant at $p<0.05$

Keys for significance; [a]- compared with control, [b]- compared with ulcer untreated control, [c]- compared with high $CoCl_2$, [d]- compared with low $CoCl_2$, [e]- compared with Mistoprotol, [f]- compared with Cimetidine and [g] - compared with Omeprazole .

Table 2: Effect of cobalt chloride on body and stomach weight, ulcer index and percentage healing.

Group	Day 7				Day 14			
	Animal Weight (G)	Stomach Weight (G)	Ulcer Index	% Healing	Animal Weight (G)	Stomach Weight (G)	Ulcer Index	Percentage Healing
Control	170.8± 1.42	0.94 ± 0.02	0	100	171.8±1.59	0.93±0.07	0	100
Ulcer untreated	162.6 ± 1.75 [a]	1.00 ± 0.09	9.78± 0.89	0	167.0±0.95	1.09±0.06	2.39±0.49	0
High $CoCl_2$	162.4 ± 1.69 [a]	0.97 ± 0.06	0.96± 0.59	90.18	162.8 ±1.24 [a]	0.86±0.05	0	100
Low $CoCl_2$	162.4 ± 1.54 [a]	1.00±0.02	0.86± 0.53 [b]	91.21	164.2 ±1.16 [a]	1.26±0.05	0	100
Misoprostol	166.6± 2.60	0.95±0.05	0 [bcdfg]	100	169.2 ±1.02 [c]	1.10±0.05	0	100
Cimetidine	159.6 ± 1.33 [a]	0.90±0.09	0.47± 0.47 [bcd]	95.19	160 ±1.27 [abe]	1.08±0.03	0	100
Omeprazole	151.4 ± 0.75 [abcdef]	0.97±0.04	0.45±0.45 [bcd]	95.39	153.4 ±1.89 [abcdef]	1.48±0.07	0	100

Values are represented as Mean ± SEM and significant at $p<0.05$ *Keys for significance;* [a]*- compared with control,* [b]*- compared with ulcer untreated control,* [c]*- compared with high* $CoCl_2$ *,* [d]*- compared with low* $CoCl_2$*,* [e]*- compared with Mistoprotol,* [f]*- compared with Cimetidine and* [g] *- compared with Omeprazole .*

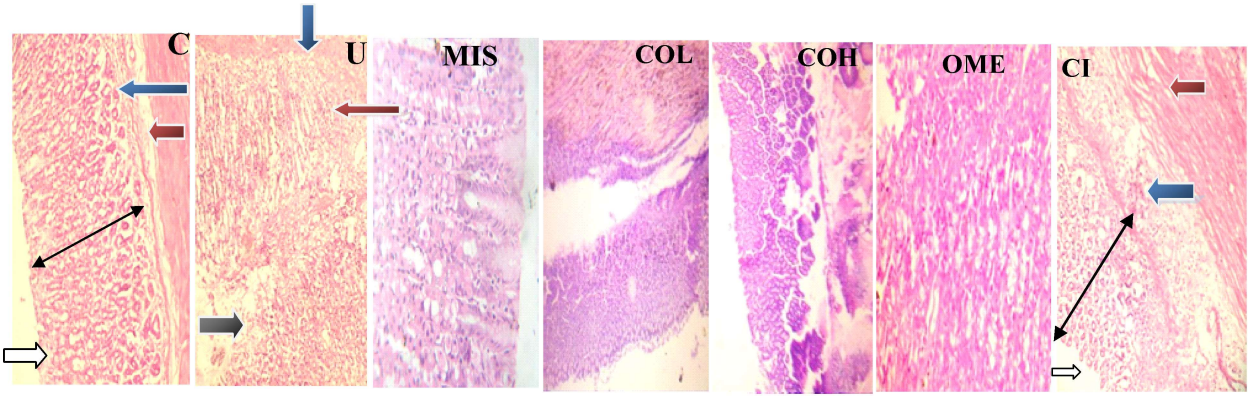

Plate 1: Photomicrograph of a stomach sections (MAG X 100) by day 7 showing **C (Control)**:- normal mucosa surface epithelial layer (white arrow). The mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal. The parietal cells appear normal (slender arrow). The circular muscle appears normal (red arrow); **U (ulcer untreated alone)**:- mucosa layer with mild ulcer (black arrow), the mucosa layer (spanned) shows severe infiltration of inflammatory cells.the gastric gland and lamina propria shows severe gastritis with severe infiltration (red arrow). The parietal cells appear normal but depleted. The submucosa layer shows moderate infiltration (blue arrow); **MIS (1 mg/kg b.w Mistoprotol)**:- intact surface epithelium, lamina muscularis mucosa, submucosa and muscularis externa. **COL (25 mg/kg b.w Cobalt chloride):-** intact Lamina muscularis mucosa and submucosal. Fairly intact surface epithelium and increased mucous cells in the lamina propia. There are congested blood vessel in the submucosa. **COH (62 mg/kg b.w Cobalt chloride):-** Intact surface epithelium, normal glands with mild amounts of resident neutrophils and macrophages at the base. Intact lamina muscularis mucosa, submucosa and muscularis externa. **CI (40 mg/kg b.w Cimetidine)**:- showing moderately preserved mucosa epithelial layer (white arrow), the mucosa layer (spanned) shows mild infiltration of inflammatory cells.the gastric gland and lamina propria shows moderate gastritis with mild infiltration (red arrow). There is no ulcer seen. The submucosa layer shows mild infiltration (blue arrow); **OME (30 mg/kg b.w Omeprazole)**:- widespread moderate erosion of the upper part of surface epithelium (slender black arrow), disrupted glands (☆), intact Lamina muscularis mucosa and widespread accumulation of neutrophils.

(3.68±0.01), misoprostol (3.69±0.01) and cimetidine (3.620±01) compared with the control group (3.79±0.01) day 7 as well as a significant increase in pH of the omeprazole treated group (3.98±0.01) compared with control group (3.79±0.01). A significant decrease in the gastric pH of ulcerated groups treated with high cobalt chloride, low cobalt chloride, misoprostol and cimetidine compared with control group was observed by day 14 (Table 1).

Effects of cobalt chloride on the body and stomach weight, ulcer index and percentage inhibition.Effect of cobalt chloride on body and stomach weight.

There was a significant decrease in the body weight of all the ulcerated treated and untreated groups compared with the control group by days 7 and 14 post ulceration (Table 2).

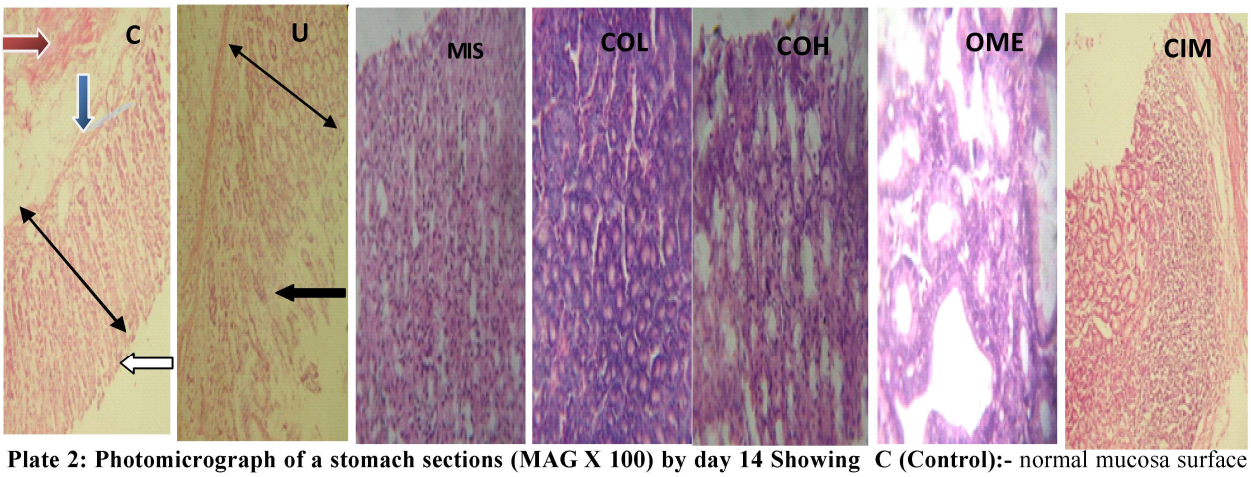

Plate 2: Photomicrograph of a stomach sections (MAG X 100) by day 14 Showing C (Control):- normal mucosa surface epithelial layer (white arrow). The mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal. The parietal cells appear normal. The circular muscle appears normal (red arrow). **U (Ulcer untreated alone):-** mucosa layer with moderate ulcer (black arrow), the mucosa layer (spanned) shows severe infiltration of inflammatory cells.the gastric gland and lamina propria shows severe gastritis with moderate infiltration (slender arrow).. The parietal cells appear normal but depleted. The submucosa layer shows moderate infiltration (blue arrow). **MIS (1 mg/kg b.w Mistoprotol)**:- fairly normal surface epithelium, moderatly congested blood vessel in the lamina propia with moderate amount of neutrophils. The submucosa is expanded with very loose connective tissue. **COL (25 mg/kg b.w Cobalt chloride):-** There were no visible lessions in all the tunics. **COH (62 mg/kg b.w Cobalt chloride):-** there are few foci of eroded lips in the surface epithelium and other tunics are normal. **OME (30 mg/kg b.w Omeprazole)**:- Mild atrophy of the muscle wall thickness, minimal inflammation **CI (40 mg/kg b.w Cimetidine):-** moderately preserved mucosa surface epithelium (white arrow) and normal mucosal layer (spanned) showing the gastric gland and lamina propria without infiltration. The eosinophilic parietal cells appear normal. There is no ulcer, no haemorrhage seen. The submucosa layer shows moderate inflammatory cells (blue arrow).

Effect of cobalt chloride on ulcer index

The ulcerated high cobalt chloride (0.96±0.59) and low dose (0.86±0.53) treated groups had a significantly low ulcer index compared with ulcerated untreated group (9.78±0.89) by day 7 post ulceration (Table 2).

All the treatment groups (high dose Cobalt chloride, low dose Cobalt chloride, Misoprostol, Cimetidine and Omeprazole) had no visible ulcer by day 14 post ulcer induction (i.e the ulcers had healed) compared with the ulcerated untreated groups (Table 2).

Discussion and conclusion

Ulcer etiology is linked to a decrease in the level of mucosal cell count or erosion of the gastric mucus layer as a result of imbalance between the aggressive factor (Gastric acid secretion and acidity) and defensive factors (mucous cell count, prostaglandin) [2,41]. The model of acetic acid induction of gastric ulcer [42] is well noted for studying ulcer healing as it has been proven to produce ulcers in close resemblance as those found in humans [43] hence its' use for screening potential anti-ulcer or healing activities of drugs or treatment.

The capacity of the stomach to secrete acid is almost linearly related to parietal cell number/count and its attacking effect on the mucous is inversely related to the mucous cell population [44]. Observations from this study revealed that cobalt chloride decreased gastric acid secretion. The pH of cobalt chloride treated group increased reflecting decreased acidity of gastric secretion in these groups. It might well be that cobalt chloride exerts anti-secretory activities among others in promoting gastric ulcer healing. Certain factors (gastric defense mechanism) have been proven to be beneficial in accelerating and ensuring proper healing of gastric ulceration [45]. These factors: increased blood flow, decreased gastric acid secretion, growth factors, and antioxidant system all have a role in the ulcer prophylaxis or healing. Cobalt chloride helped in ameliorating the adverse effect of acetic acid induced ulceration by accelerating healing in a manner comparable to the control drugs – omeprazole and cimentidine (i.e decreased gastric acid secretion).

Histological evaluations of the varied doses of cobalt chloride administered revealed intact epithelium and increased mucous cells at the *lamina propia* on both days unlike the ulcerated untreated groups. This was similar to findings in omeprazole treated groups. It is however worthy to note that there

was no reformation of ulcer at the lower dose (25 mg/kg b.w) day 21 post ulcer induction) after withdrawal of treatment (cobalt chloride) from day 14 post ulceration. The mucous gel (first layer of defense) is secreted by surface epithelium (second layer of defence) [46] which is formed by water and mucin glycoprotein [47]. This gel helps in acid neutralization, accelerated epithelial repair and maintenance of mucosal blood flow. It may well be that cobalt chloride accelerated the rate of ulcer healing by increasing the level of mucous cells present thereby increasing the production of mucous gel layer which increased gastric acidity (pH) neutralization, enhance epithelial repair and preventing re-occurrence of ulcer after treatment withdrawal. Domschke *et al*., [47] observed that decreased epithelial cell leads to a decreased mucous production and eventually ulceration. The intact epithelium of the cobalt chloride treated groups may also suggest another probable mechanism by which it (cobalt chloride) helped in mitigating the adverse effect of acid.

It may well be that cobalt chloride enhanced ulcer healing by conferring accelerated repair at the mucous gel and epithelial layer besides acting as an anti-secretory agent. Observations from the gastric tissue histology is also suggestive that the high dose though sub-lethal also conferred some level of gastric protection as observed during ulcer healing in this study. More work needs to be done to ascertain the probably mechanism by which cobalt chloride might be conferring this ulcer healing activities. It is also of interest as a result of the indiscriminate use of blood tonics (of which Cobalt chloride is an integral component) among many populaces – both old and young.

It can be concluded that cobalt has ulcer healing promoting potentials and work is ongoing to unravel its probable mechanism of action.

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Serum leptin in obese type 2 diabetic females in South-Western Nigeria

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Abstract

*Background***:** Obese people, especially females, are known to have high circulating levels of leptin, a hormone that increases energy expenditure and also regulates glucose metabolism. However, the link between obesity and type 2 diabetes (T2DM) through leptin is yet to be clearly defined.

Objectives: This study determined and compared the levels of serum leptin and HOMA-IR scores in obese and non-obese females with or without T2DM. We also determined the relationship between their serum leptin levels and glycaemic control.

Methodology: This was a cross sectional study involving 60 obese T2DM females, 60 non- obese T2DM females and 60 obese non-diabetic female adults who met selection criteria. Their demographic data and anthropometric parameters were obtained using standard methods. Fasting blood samples were collected aseptically from participants for determination of plasma glucose, serum leptin, HbA_{Ic} and HOMA-IR.

Results: Serum leptin levels in obese T2DM, obese non-diabetic and non-obese T2DM femaless were (15.61 $\pm$10.63), (11.33$\pm$14.22) and (5.92$\pm$3.68) ng/ml respectively. There were significantly much higher serum leptin levels in obese T2DM than in obese non-diabetic females (p = 0.035S). In the obese T2DM participants, serum leptin levels had strong negative correlation with HOMA-IR (r = -0.293, p = 0.023) and HbA_{IC} (r = -0.255, p = 0.049).

Conclusion: Serum leptin levels were much higher in obese females with diabetes than in those without diabetes. However, the strong negative correlation of serum leptin levels with improving glycaemic control may suggest a therapeutic potential of leptin for diabetes which needs to be further explored.

Keywords: *Obesity, Type 2 diabetes mellitus, Serum leptin, Insulin resistance, HOMA-IR, HbA_{1C}*

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Résumé

Contexte **:** Les personnes obèses, en particulier les femmes, sont connues pour leurs taux élevés de leptine en circulation, une hormone qui augmente la dépense énergétique et régule également le métabolisme du glucose. Cependant, le lien entre l'obésité et le diabète de type 2 (DT2) par le biais de la leptine n'est pas encore clairement défini.

Objectifs: Cette étude a déterminé et comparé les taux de leptine sérique et de scores HOMA-IR chez les femmes obèses et non obèses avec ou sans DT2. Nous avons également déterminé la relation entre leurs taux sériques de leptine et le contrôle glycémique .

Méthodologie: Il s'agissait d'une étude transversale portant sur 60 femelles DT2 obèses, 60 femelles DT2 non obèses et 60 femelles obèses non diabétiques qui répondaient aux critères de sélection. Leurs données démographiques et paramètres anthropométriques ont été obtenus en utilisant des méthodes standard. Des échantillons de sang à jeun ont été prélevés de manière aseptique chez les participants pour la détermination du glucose plasmatique, de la leptine sérique, de l'HbA_{1C} et de l'HOMA-IR.

Résultats: Les taux de leptine sérique des femelles DT2 obèses, obèses non diabétiques et non obèses DT2 étaient (15,61 $\pm$ 10,63), (11,33 $\pm$ 14,22) et (5,92 $\pm$ 3,68) ng/ml, respectivement. Les taux de leptine sérique étaient significativement plus élevés chez les femelles DT2 obèses que chez les femelles obèses non diabétiques (p = 0,035S). Chez les participantes DT2 obèses, les niveaux de leptine sérique avaient une forte corrélation négative avec l'HOMA-IR (r = -0,293, p = 0,023) et HbA_{1C} (r = -0,255, p = 0,049).

Conclusion: Les taux de leptine sérique étaient beaucoup plus élevés chez les femelles obèses diabétiques que chez celles non diabétiques. Cependant, la forte corrélation négative des niveaux sériques de leptine avec l'amélioration du contrôle glycémique peut suggérer un potentiel thérapeutique de la leptine pour le diabète qui doit être davantage exploré.

Mots clés: *Obésité, diabète sucré de type 2, leptine sérique, résistance à l'insuline, HOMA-IR, HbA_{1C}*

Introduction

Obesity is defined as an excess proportion of body fat relative to lean body mass of sufficient magnitude to produce adverse health consequences [1, 2]. It is associated with many chronic diseases including type 2 diabetes, cardiovascular disease and some cancers [3]. Type 2 diabetes is the most common metabolic disorder worldwide [4], and its prevalence is growing at an alarming rate in both developed and developing countries [5, 6]. This increase has been attributed to the rising prevalence of obesity which of itself, is also an independent health problem [6]. Worldwide, approximately 90% of people with diabetes are type 2, and of these, 44% are obese or overweight [7]. Globally, 23% of ischaemic heart disease burden and 7-41% of certain cancer burdens are also attributable to overweight and obesity [7].

The incidence of obesity is rapidly increasing in epidemic proportions all over the world [6, 8]. One billion of the approximately 6.5 billion people in the world are estimated to be overweight [body mass index (BMI) $\geq$ 25 kg/m^2] and, of these, at least 300 million are obese (BMI $S \geq$ 30 kg/m^2) [7]. In a population study in Ibadan, Nigeria, the prevalence of obesity and overweight were found to be comparable to rates seen in many industrialized countries, and rapidly emerging urbanized populations in Africa [9]. In that study, the prevalence of obesity among women was 17.27% and 2.75% among men [9].

A similar study in Ile-Ife, a semi urban region of Nigeria, also showed higher prevalence of obesity among women irrespective of the anthropometric indices of adiposity used [10]. It can be inferred from these findings that more Nigerian women than men are obese and at risk of obesity-related morbidity and mortality. There is therefore a need for better understanding of the physiological and pathological processes that balance energy intake and energy expenditure in order to help in combating the menace of obesity.

Leptin is the first obese gene product known to participate in many physiological processes such as: regulation of food intake and energy metabolism, cardiovascular function, glucose and lipid metabolism [11]. It is a protein hormone produced mainly by white adipocytes and it has structural similarities with the cytokine family [12]. In obesity, leptin looses the ability to inhibit energy intake and increase energy expenditure; this is termed leptin resistance [2]. There is also a suggestion that leptin could be a link between obesity and diabetes [13]. However, this link has not been clearly defined. It has been demonstrated that high serum leptin levels are associated with insulin resistance and the metabolic syndrome which is mediated by central obesity, independent of body mass index [14]. Studies also showed that plasma leptin levels are not affected by the presence of type 2 diabetes mellitus or by short-term treatment with diet or oral anti-diabetic drugs nor by the age of patients but rather related to glycaemic control in female patients with type 2 diabetes mellitus [15,16].

Other previous studies have also documented ethnic variations in serum leptin levels [17, 18], which may account for the variation in the relationship of circulating levels of leptin with the presence or absence of diabetes. In a study among non-obese Nigerian women with T2DM, Ajala et al., showed that plasma leptin levels in poorly-controlled diabetic patients were significantly increased compared to those obtained in well controlled diabetic subjects [19], though this study used HbA_{IC} value of less than 6% to determine subjects with controlled diabetes.

In Nigeria, there has not been a study designed purposely to determine the link between obesity, type 2 diabetes and serum leptin levels and it is plausible that a distinct relationship may exist. Therefore, this study determined and compared levels of serum leptin and HOMA-IR scores in three groups of participants i.e. obese female Nigerians with T2DM, obese female Nigerians without T2DM and non-obese female Nigerians with T2DM. We also determined the relationship between serum leptin and glycaemic control levels among the various groups.

Materials and methods

This was a cross-sectional, comparative hospital-based study carried out at the Endocrinology, Diabetes and Metabolism (EDM) Out-patient's Clinic of a tertiary hospital in Osun State, South-western Nigeria, between January and June 2012 following ethical approval from the institutional Ethics and Research Committee. In addition, signed informed consent was obtained from each participant after a discussion session explaining the required procedure.

Sixty obese and 60 non-obese females with type 2 diabetes mellitus and age comparable 60 obese apparently healthy female participants who met the inclusion criteria as stated below for each of the 3 groups were recruited consecutively from the EDM Unit outpatient's clinic, General Outpatient Department (GOPD) clinic and the hospital staff clinic. Inclusion criteria were obese females with BMI $\geq$ 30 kg/m^2 and non-obese female females with

BMI < 30 kg/m^2, type 2 diabetes mellitus was diagnosed based on the WHO criteria of 1998 [20], and participants were currently not on insulin treatment. Apparently healthy obese non-diabetic female Nigerians with fasting plasma glucose (FPG) less than 6.1 mmol/l as defined by WHO criteria of 1998 [20], and adult females aged between 30 years and 64 years based on the age range mostly affected by type 2 diabetes [21].

The exclusion criteria were unwilling participants, pregnancy, acute illness within a week before the study and participantts who were known or suspected to have chronic debilitating diseases. Females who were known or suspected to have other endocrine diseases related to diabetes mellitus or obesity such as Cushing's syndrome, hypothyroidism, polycystic ovarian syndrome, acromegaly, and those who were on long tern steroid use or currently on steroid therapy were also excluded.

Demographic data and clinical history were obtained with interviewer's administered structured questionnaire. Physical examination was performed on each eligible participant. Body weight, height, waist circumference (WC), hip circumference (HC), and blood pressure were measured in all participants according to standard protocol. BMI was calculated as weight in kilogrammes divided by square of height in metres and waist to hip ratio (WHR) was also calculated for each participant.

Fasting blood samples were collected aseptically from each participant after an overnight fast of between 8 to 12 hours for all laboratory blood tests. Glycosylated haemoglobin (HbA_{1C}) was measured only in participants with type 2 diabetes as a marker of glycaemic control. Based on HbA_{IC} results, type 2 diabetic participants were then categorized as controlled diabetic if HbA_{IC} was <7% or poorly controlled diabetics if $HbA_{IC} \geq 7\%$. All participants were assessed for insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR) as described by Matthews *et al*. [22, 23].

Fasting plasma glucose was measured with the spectrophotometer using the principle of Trinder reaction [24]. HbA_{1C} was measured from the blood samples by the principle based on boronate affinity chromatography with Biorad in-2-it HbA_{1C} auto-analyser and its test catridges after the initial standardization of the analyser with a system check cartridge.

Fasting serum leptin levels were measured by double assay from the sera of subjects as total serum leptin. This quantitative estimation of human serum leptin assay was done using the human leptin test kit on a Chemwell 2910 microwell enzyme linked immunosorbent assay (ELISA) analyser. Assay sensitivity was 0.3 ng/ml and specificity of antibodies for human leptin was 100%. Intra assay coefficient of variation (CV) was 6.42% while the inter assay coefficient of variation (CV) was 10.11 %. The test kit laboratory reference values for a normal weight male = 3.84 ± 1.79 ng/ml and for a normal weight female = 7.36 ± 3.73 ng/ml.

Double assay for serum insulin were also done by a quantitative method with microwell ELISA human insulin test kits on Chemwell 2910 Auto-analyser. The test kit laboratory reference values for normal adults range from 0.7 to 9.0 μIU/ml and values for adults with type 2 diabetes mellitus range from 0.7 to 25 μIU/ml. The sensitivity of this assay was 0.75 μIU/ml and the test has no cross reactivity with C-peptide, proinsulin and glucagon. The HOMA-IR estimate for insulin resistance is as follows: HOMA-IR = Fasting Glucose (mmol/l) x Fasting Insulin (μIU/ml)/22.5 [22]. HOMA-IR scores of ≥ 2 was used to define individuals with insulin resistance as previously described by Oli et al. for Nigerians [25].

Data analysis

This was done using statistical package for social sciences (SPSS) version 17.0 (SPSS Inc. Chicago Illinois). The data were tested for normality using Kolmogorov–Smirnov test. Except where otherwise stated, results were expressed as mean $\pm$ standard deviation (SD) and number count (N) with proportions (%). Median ± Interquartile Range (IQR) were used to express the result of serum leptin, serum insulin and HOMA-IR data which were not normally distributed. Serum leptin levels, insulin and HOMA-IR levels of the three groups were compared using Kruskal-Wallis-H- test while other normally distributed continuous variables were compared among the three groups with Analysis of Variance (ANOVA).

Serum leptin levels, insulin and HOMA-IR levels were also compared between two groups using Mann-Whitney-U test while other normally distributed continuous variables were compared between participants with controlled and poorly controlled diabetes using Student's t-test. Spearman's correlation coefficient was used to determine the relationship between serum leptin levels, HbA_{IC} and other continuous variables. Proportion of participants based on diabetes control status of the obese and non-obese type 2 diabetic groups and other categorical variables were also compared using Chi-

square test, Level of statistical significance was set as p-value $\leq$ 0.05.

Results

Table 1 presents the socio-demographic and clinical characteristics of study participants in each group. A total of 180 females participated in the study with 60 participants in each of the three groups. The age range for all participants was between 34 and 64 years with mean age of 52.0 ± 7.3 years. A large proportion (96.1%) of our participants were of Yoruba ethnicity.

In accordance to BMI grading by WHO [26], obesity class I, II and III were present in 41 (68.3%), 15 (25.0%) and 4 (6.7%) of the obese type 2 DM participants respectively. Among the obese non-diabetic participants, 27 (45.0%) had class I obesity, 18 (30.0%) had class II obesity and 15 (25.0%) had class III obesity. In the non-obese type 2 DM group, 21 (35.0%) participants had normal BMI and 39 (65.0%) participants were overweight. Central obesity as defined by waist circumference (WC) of at least 88 cm was present in all the obese T2DM participants, 59 (98.3 %) of the obese non-diabetic participants and in 43 (71.7%) of the non-obese T2DM participants. The anthropometric indices of participants are as shown in Table 2.

Table 1: Comparison of some clinical parameters of the study participants

Parameter	Obese T2DM	Obese Non- DM	Non-Obese T2DM	p-value
Age (Years)	52.8±7.3	50.7± 7.3	52.6±7.4	0.224
Family history of DM	25(41.7%)	17(28.3%)	16(26.7%)	0.036*
Family history of HTN	28 (46.7%)	21 (35.0%)	22 (36.7%)	0.286
Family history of obesity	50 (83.3%)	45 (75.0%)	25 (41.7%)	0.001*
Childhood history of obesity	27 (45.0%)	27 (45.0%)	14 (23.3%)	0.001*
Known HTN	46 (76.7%)	21 (35.0%)	38 (63.3%)	0.0001*
Antilipid drug use	19(31.7%)	0(0.0%)	13(21.7%)	0.0001*
DM Duration(Years)	3.8± 3.3	NA	5.5 ±4.3	0.017*

*HTN = Hypertension, DM – Diabetes Mellitus, T2DM = Type 2 DM, NA = Not Applicable, *p value <0.05 is statistically significant*

Table 2: Comparison of the anthropometric and biochemical parameters of the Study participants

			N = 180		
Parameter	Obese T2DM (a)	Obese Non-DM (b)	Non-Obese T2DM (c)	p-value (aVbVc)	p-value (aVb)
Ht(cm)	157.2±5.1	157.6±10.6	160.3±5.5	0.540	0.963
Wt (Kg)	85.6±10.1	92.1±14.0	66.1±7.6	0.0001*	0.014*
BMI (Kg/m^2)	34.5±3.4	36.5±5.1	25.9± 2.3	0.0001*	0.044*
WC(cm)	106.3±7.5	105.6±10.4	91.3±6.4	0.0001*	0.969
HC(cm)	113.7±8.9	19.9±10.4	97.9±5.5	0.0001*	0.003*
WHR	0.94±0.06	0.88±0.06	0.93±0.05	0.0001*	0.0001*
SBP(mmHg)	133.3 ±19.2	124.8±18.7	130.2±21.2	0.51	0.068
DBP(mmHg)	79.2 ±11.1	78.3±11.8	78.7±10.8	0.908	0.900
FPG (mmol/l)	8.1±2.9	5.4±0.5	8.3±2.9	0.0001*	0.0001*
HbA_1C (%)	8.3±2.9	NA	8.7±3.0	0.457	NA
Serum leptin(ng/ml)	15.61 ±10.63	11.33±14.22	5.92±3.68	0.0001*	0.035*
Serum Insulin (μIU/ml	13.37± 12.94	12.20± 2.37	6.72±1.42	0.0001 *	0.003*
HOMA-IR	5.23±4.38	2.90±0.86	2.25±1.18	0.0001*	0.0001*
Prevalence of IR	60 (100.0%)	59 (98.3%)	40 (66.7%)		

*WC = Waist Circumference, HC = Hip Circumference, Ht= Height, Wt – Weight, WHR = Waist to Hip Circumference Ratio, BMI = Body Mass Index, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, FPG =Fasting Plasmal Glucose, HbA_1C = Glycosylated Haemoglobin, HOMA-IR = Homeostasis Model Assessment of Insulin Resistance, NA = Not Applicable, IR = Insulin Resistance, *p value < 0.05 is statistically significant*

Comparison of biochemical parameters between the obese type 2 diabetic and obese non-diabetic female participants are also shown in Table 2. Serum leptin levels were significantly higher among obese T2DM participants than in obese non-diabetic participants (15.61± 10.63 ng/ml vs. 11.33±14.22 ng/ml, p = 0.035). In the obese T2DM participants, serum leptin levels had weak correlation with BMI, WC, and serum insulin levels but a strong negative correlation with HOMA-IR and HbA_{IC}. Among the obese non-diabetic participants, serum leptin levels had strong positive correlation with BMI, serum insulin and HOMA-IR but a weak positive correlation with WC. Among the non-obese type 2 DM participants, serum leptin levels had weak correlation with BMI, WC, serum insulin levels, HOMA-IR, and HbA_{IC} .Further detail on the relationship of serum leptin and HOMA-IR levels with BMI, WC, serum insulin levels and HbAlC in all the three study groups are as shown in Tables 3 and 4.

41.7% had controlled diabetes while 38.3% of the non-obese T2DM group had controlled diabetes. There were no statistically significant differences in the proportion of participants with controlled and poorly-controlled diabetes in both groups (X^2 =1.39, p = 0.709).

The serum leptin levels of the obese T2DM participants with controlled diabetes were not significantly higher than the serum leptin levels in those with poorly- controlled diabetes (16.41±22.85 ng/ml vs.15.11±9.50 ng/ml, p = 0.092). The serum insulin levels of obese T2DM participants with controlled diabetes were significantly higher than the serum insulin levels in those with poorly-controlled diabetes (14.03 ± 32.66 μIU/ml vs.12.93 ± 5.36 μIU/ml, p = 0.036). The HOMA-IR levels of the obese T2DM participants with controlled diabetes were not significantly lower than the HOMA-IR levels in those with poorly-controlled diabetes (5.01±8.71 vs. 5.51 ±2.81, p = 0.333S). There were no statistical significant differences

Table 3: Relationship of serum leptin levels with BMI, WC, serum insulin levels, HOMA-IR, and HbA_{IC} by group.

	Obese T2DM		Obese Non-DM		Non-ObeseT2DM	
Parameter	r-value	p- value	r-value	p- value	r-value	p- value
BMI	+0.038	0.776	+0.281	0.030*	+0.039	0.769
WC	0.025	-0.849	+0.237	0.068	+0.058	0.660
Serum insulin	-0.077	0.558	+0.446	0.0001*	+0.030	0.821
HOMA-IR	-0.293	0.023*	+0.385	0.002*	0.000	0.996
HbA_{IC}	-0.255	0.049*	NA	NA	-0.170	0.195

*BMI = Body Mass Index, WC= Waist Circumference, r= Spearman's simple correlation coefficient, *p < 0.05 is statistically significant, NA = Not Applicable.*

Table 4: Relationship of HOMA-IR with BMI, WC, serum insulin levels, and HbA1C by Group.

	Obese T2DM		Obese Non-DM		Non-Obese T2DM	
Parameter	r-value	p- value	r-value	p- value	r-value	p- value
BMI	-0.105	0.424	+0.432	0.001*	-0.011	0.932
WC	+0.008	0.951	+0.454	0.0001*	-0.007	0.956
Serum insulin	+0.483	0.0001*	+0.385	0.002*	+0.279	0.031*
HbA_{IC}	+0.196	0.134	NA	NA	+0.163	0.214

*BMI = Body Mass Index, WC= Waist Circumference, r = Spearman's simple correlation coefficient, *p<0.05 is statistically significant, NA = Not Applicable*

The total number of the diabetic participants was 120 of whom 40% were assessed to have controlled diabetes with a mean HbA_{IC} of 5.09 ± 0.7%. Among the obese T2DM participant group, between the non-obese diabetic females with controlled and poorly-controlled diabetes in their levels of serum leptin, insulin and HOMA-IR as shown inTable 5.

Table 5: Comparison of biochemical parameters between non-obese T2DM participants with controlled and poorly-controlled diabetes.

Parameters	Controlled DM n (%) = 23 (38.3%)	Poorly- Controlled DM n (%) = 37 (61.7%)	p-value
HbA_{IC} (%)	5.9±0.7	10.4±2.4	0.0001*
Serum leptin(ng/ml)	6.33±4.38	5.63±2.81	0.407
Serum Insulin(μIU/ml)	6.53±1.62	6.93±1.35	0.964
HOMA-IR	2.07±0.80	2.40 ±1.29	0.058

*T2DM = Type 2 Diabetes Mellitus, n = number of subjects, DM = Diabetes Mellitus, HOMA-IR = Homeostasis Model Assessment of Insulin Resistance, *p < 0.05 is statistically significant*

Discussion

The mean duration of T2DM was shorter in obese T2DM participants compared to non-obese T2DM participants. This may suggest that the non-obese T2DM participants had over time been subjected to life style modification and other diabetes treatment modalities that could have resulted in their present lower body mass index. More than 50% of all diabetic participants were known to be hypertensive while about one third of the obese non-diabetic participants were also found to be hypertensive. These finding are suggestive of the presence of metabolic syndrome in our participants. Hypertension, obesity and T2DM are essential components of metabolic syndrome [27], which is known to be associated with increased risk of cardiovascular morbidity and mortality.

There were many participants with combined family history of T2DM and obesity among the obese T2DM participants compared with non-obese T2DM participants. Similarly, participants with family history of obesity were more among the obese participants compared with non-obese participants. These findings give credence to the familial tendencies of these non-communicable diseases. Twin studies have demonstrated that familial aggregation of obesity has a genetic component and is not only due to cultural or environmental factors clustered in families [28]. In addition, linkage studies have also identified markers and genes related to obesity in virtually all human chromosomes [28]. Majority of the participants in each of the groups had documented evidence of central obesity by waist circumference irrespective of their BMI. The high prevalence of central obesity among diabetic participants was similar to that reported by Fasanmade et al. [29] in Lagos among Nigerian females with T2DM. Central obesity is particularly recognized as an independent risk factor for increased cardiovascular morbidity and mortality.

Serum leptin levels were significantly higher in both obese participants with or without type 2 diabetes mellitus than in non-obese type 2 diabetic participants. Higher serum leptin levels in obese participants have been previously reported [14, 16]. The higher levels of leptin in obese participants reflects the fact that leptin is produced by adipose tissue and in proportion to the amount of the adipose tissue in the body [30, 31]. The serum leptin levels in our participants were lower when compared to the levels reported in other populations [18, 32]. This could be due to ethnic variations in serum levels of leptin and possibly to variations in the severity of obesity [17, 18]. Luke et al. [18] have earlier demonstrated that serum leptin levels in Nigerians were lower when compared to that of Jamaicans and Americans respectively.

Our study showed that levels of serum leptin in obese T2DM participants were significantly much higher than the levels in the obese non-diabetic participants. Liuzzi et al. [16] found similar serum leptin levels in obese diabetic participants and obese non-diabetics, while Guler et al. [15] reported that leptin levels were not affected by the presence or absence of type 2 diabetes mellitus among Turkish women. However, Buyukbese et al. [33], in another study among Turkish obese women with and without type 2 diabetes mellitus demonstrated significantly higher serum levels of leptin in the group without type 2 diabetes mellitus. This disparity could be as a result of variation in insulin secretion and sensitivity in T2DM since insulin is also known to increase leptin production [34]. The leptin levels in our non-obese T2DM participants were also similar to the levels previously reported for non -obese females with type 2 diabetes mellitus in Nigeria [19], perhaps because of their common ethnic background.

Many investigators demonstrated that leptin had a significant correlation with BMI [33, 35, 36]. In this study, leptin correlated significantly with BMI

only in obese non-diabetic participants. This positive correlation was also observed in the relationship between their serum leptin and serum insulin. However among the obese diabetic participants, there were poor correlations between serum leptin levels, BMI and serum insulin levels. These may be because of ongoing therapeutic intervention such as lifestyle modifications and use of anti-diabetes agents for our diabetic participants which may modulate insulin secretion and also influence leptin secretion [15]. Serum leptin levels were inversely related to HOMA-IR in obese T2DM participants and it did not correlate with HOMA-IR in non-obese T2DM participants while it had had a significant positive correlation with HOMA-IR in obese non diabetic participants. This suggests that increase in endogenous serum leptin levels may reduce insulin resistance in obese T2DM patients and therefore be a potential therapeutic agent if it can be augmented from an exogenous source or by any another physiological means.

The potential therapeutic role of leptin for diabetes mellitus is further supported by higher serum leptin levels in the obese type 2 diabetic participants with controlled diabetes than the levels in those with poorly-controlled diabetes, though these differences in leptin levels had no statistical significance. There was also a significant negative correlation between serum leptin levels and HbA_{1C} levels among obese diabetic participants. The HbA_{1C} is an established marker of long term glycaemic control and its levels reduce with improving glycaemic control. The findings of this study are similar to that of Buyukbese *et al*. [33] who reported elevated levels of leptin in obese females with controlled diabetes. Another previous study similarly demonstrated a weak but significant negative correlation between serum levels of leptin and glycaemic control before and after a period of treatment of diabetes [15]. Even among our non-obese T2DM participants, those with controlled diabetes also had elevation in their serum leptin levels than their poorly-controlled diabetes counterparts. The elevated serum leptin levels in participants with controlled diabetes and the significant negative correlation of serum leptin levels with glycosylated haemoglobin levels among obese diabetic participants may be attributable to the known regulatory function of leptin on glucose metabolism [11, 15]. Elevated serum leptin levels therefore appear to be good for glycaemic control either as a therapeutic agent or as a biochemical marker of glycaemic control.

HOMA-IR is a surrogate marker of insulin resistance that has been found to be well correlated with the measure of insulin resistance determined by euglycaemic clamp which is the gold standard [22]. The higher the HOMA-IR score, the higher the severity of insulin resistance [22, 23]. In this study, HOMA-IR scores increased across the groups with the lowest scores recorded in non-obese T2DM participants and the highest scores recorded in obese T2DM participants. In addition, the proportions of participants with insulin resistance were 100% among obese T2DM participants, 98.3 % among obese non-diabetic participants, and 66.7% among non-obese T2DM participants. This finding further illustrates the fact that obesity is strongly associated with insulin resistance which is a known cause of type 2 diabetes mellitus. Oli et al. [25] in Enugu, Nigeria previously reported that insulin resistance estimated by HOMA-IR is a major feature of type 2 diabetes mellitus in Nigerians and that obesity consistently correlates with and predicts insulin resistance. The higher degree of insulin resistance among obese non -diabetic participants in this present study also suggests that obese individuals should be routinely investigated and treated for insulin resistance in order to prevent or delay future occurrence of T2DM in them.

There were no significant correlations between BMI or WC with HOMA~IR in both obese T2DM participants and non-obese T2DM participants unlike their statistically significant correlations in obese non-diabetic participants. Liuzzi et al. [16] similarly demonstrated a significant positive correlation between HOMA-IR and BMI in a population of obese non-diabetic Italians. The weak correlation between BMI and WC with HOMA-IR in all our diabetic participants may be due to the modulatory effect of therapy on insulin resistance and body weight control in diabetic patients.

Conclusion

Serum leptin levels were significantly higher in obese participants than in non-obese participants and there were significantly much higher serum leptin levels in obese women with T2DM than in those without T2DM. Serum levels of leptin appear to be higher in obese participants with controlled diabetes than in those with poorly-controlled diabetes. In particular, serum leptin levels had a significant negative correlation with HbA_{1C} levels among the obese diabetic participants thus suggesting a potential role for leptin either as a marker of glycaemic control and or as a therapeutic agent for diabetes mellitus.

HOMA-IR showed that the severity of insulin resistance worsened with obesity and more so when obesity and T2DM co-exist. This finding therefore gives additional evidence in support of the fact that both non pharmacological and pharmacological interventions that can reduce insulin resistance should continue to form part of the management plan for obese patients with or without T2DM.

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Antidepressant activity of ethanol extract of *Albizia adianthifolia* (Schumach) W. F. Wight leaf in mice

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Abstract
Background: *Albizia adianthifolia* (Mimosoideae) is a medicinal plant used in the management of infections and central nervous system disorders. The presented study evaluated the antidepressant properties of the ethanol extract of *Albizia adianthifolia* leaves (EEAAL) in mice.
Methods: Pulverised leaves of *Albizia adianthifolia* were extracted with 50% ethanol by cold maceration and concentrated to dryness. Swiss mice were divided into five groups and treated with distilled water (10 mL/kg), EEAAL (1.25, 2.50, 5.00 mg/kg, i.p.) and imipramine (12 mg/kg). Antidepressant activity was assessed by force swim test (FST), tail suspension test (TST), reserpine-induced depression model and yohimbine-induced lethality test. Open field paradigm was used to screen the false results in FST and TST.
Results: The EEAAL (1.25, 2.50 mg/kg) significantly reduced immobility time in FST at dose 1.25 mg/kg (40.8 ± 13.1) and 2.50 mg/kg (42.4 ± 9.7) compared to control (170.0 ± 10.1) [$p<0.05$]. Similarly, 1.25 mg/kg of the extract significantly reduced immobility time in TST (85.2 ± 8.9) compared to control (142.6 ± 3.9) [$p<0.05$] without causing changes in spontaneous motor activity in open field. EEAAL reversed diarrhoea, ptosis, and hypothermia induced by reserpine compared with control groups and did not potentiate yohimbine-induced lethality.
Conclusion: It was concluded that the extract has antidepressant like properties which supports its ethnomedicinal use in the treatment of depression.

Keywords: *Immobility, antidepressant, Albizia adiantifolia, imipramine, reserpine*

Résumé
Contexte: Albizia adianthifolia (Mimosoideae) est une plante médicinale utilisée dans la gestion des infections, et des troubles du système nerveux central. L'étude présentée a évalué les propriétés antidépressives de l'extrait à l'éthanol des feuilles d'*Albizia adianthifolia* (EEFAA) chez les souris.
Méthodes : Des feuilles pulvérisées d'*Albizia adianthifolia* ont été extraites avec de l'éthanol à 50% par macération froide et concentrées à sec. Des souris suisses ont été divisées en cinq groupes et traitées avec de l'eau distillée (10 ml / kg), de l'EEFAA (1,25 ; 2,50 ; 5,00 mg / kg, i.p.) et de l'imipramine (12 mg / kg). L'activité antidépressive a été évaluée par le test de nage à force (TNF), le test de suspension de la queue (TSQ), modèle de dépression induite par la réserpine et le test de létalité induite par la yohimbine. Le paradigme du champ ouvert a été utilisé pour filtrer les faux résultats dans TNF et TSQ.
Résultats : L'EEFAA (1,25 ; 2,50 mg / kg) a significativement réduit le temps d'immobilité dans le TNF à la dose de 1,25 mg / kg (40,8 ± 13,1) et de 2,50 mg / kg (42,4 ± 9,7) par rapport au témoin (170,0 ± 10,1) [$p<0,05$]. De même, 1,25 mg / kg de l'extrait réduit significativement le temps d'immobilité dans le TSQ (85,2 ± 8,9) par rapport au témoin (142,6 ± 3,9) [$p<0,05$] sans provoquer de modifications de l'activité motrice spontanée en champ ouvert. EEFAA a inversé la diarrhée, la ptose et l'hypothermie induites par la réserpine par rapport aux groupes témoins et n'a pas potentialisé la létalité induite par la yohimbine.
Conclusion: Il a été conclu que l'extrait a des propriétés antidépressives qui soutiennent son utilisation ethno-médicinale dans le traitement de la dépression.

Mots-clés: *Immobilité, antidépresseur, Albizia adiantifolia, imipramine, réserpine*

Introduction

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Sometimes, it also comes with symptoms of anxiety. These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. At its worst,

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depression can lead to suicide [1]. It is also associated with serious impairment of social, marital and occupational functioning as well as prominent and interpersonal distress [2]. According to Kessler *et al*. [2], depression is one of the most common psychiatric disorders with a life time prevalence of 10% - 20% in the general population. Women are twice at the risk of developing depression compared to men, and it is the leading cause of disease burden for women in most countries irrespective of the economic or income status [3].

Drugs used in management of depression such as monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitor, norepinephrine and dopamine reuptake inhibitors etc, act by blocking the reuptake or degradation of monoamine neurotransmitters. However, only 50-70% of the patients exhibit acceptable responses to treatment [4]. For those that do respond, therapeutic effect develops slowly (which is the major drawback in their usage), usually over several weeks of treatment [5, 6] constituting a major setback. Also, the adverse effects associated with antidepressant therapy frequently leads to discontinuation of treatment. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [7].

Albizia adianthifolia (Schumach) W. F. Wight (Mimosoideae), known as *ayinreta* or *igbabo* in Yoruba and *kawo* in Hausa, is a big tree found in moist and tropical forest zones as well as areas that are transitional to woodland [8]. It is used ethnomedicinally to treat mental illness, pain associated with labour, river blindness, conjunctivitis, arthritis, rheumatism, parasitic infection, toothache, stomachache, allergic reactions, diarrhoea, gonorrhoea, wounds and sore feet [9, 10, 11]. In addition, some of the pharmacological activities exhibited by *A. adianthifolia* have been documented. The root extract has been shown to possess antibacterial, anti-inflammatory and anticholinesterase effects [12]. Memory-enhancing activity of the aqueous leaf extract in the 6-hydroxydopamine-lesion rodent model of Parkinson's disease has also been documented [13]. Tamokou *et al*. [14] demonstrated the antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark. Previous studies have shown presence of certain phytochemicals such as alkaloids, glycosides, saponins, steroids, tannins, astringents [15] and three flavonoids: okanin, melanoxetin and dihydroflavonol [16]. The aim of this study was to evaluate the antidepressant effect of ethanol extract of *A. adianthifolia* leaves (EEAAL) in mice.

Materials and methods

Collection of plant materials

The leaves of *A. adianthifolia* were collected at the Botanical Garden of the University of Ibadan, Ibadan, Oyo state, Nigeria in April, 2014. The taxonomical identification and authentication of the plant was carried out at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number 109833 was deposited and compared with the reference specimen.

Preparation of extract

The fresh leaves were washed in clean water and air-dried under shade for five weeks. One hundred grammes (100 g) of the air-dried leaves were pulverized and soaked in 50% ethanol (2 L) for 48 hr. The filtrate was concentrated with a rotary evaporator to give a semisolid residue and evaporated to dryness to form solid residue (23 g). It was kept in the desiccator until use. The dried extract was dissolved in distilled water and administered intraperitoneally.

Experimental animals

One hundred and fifty female Swiss mice weighing between 20 – 25 g used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Ibadan, Nigeria. The animals were kept in hygienic and well-ventilated compartments, maintained under standard environmental conditions and fed with standard rodent pellet (Livestock Feed PLC, Lagos, Nigeria) and water ad *libitum*. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

Drugs and chemicals

Yohimbine (Sigma- Aldrich St.Louis, MO, USA), reserpine (Pfizer Inc., New York, NY, USA), imipramine (Shanghai Zhongxi Pharmaceutical Co., Ltd. Shanghai, China). The chemicals were purchased by the institution from respective companies.

Acute toxicity test

The method described by Lorke [17] was used to determine the LD_{50} using thirteen female mice (20 – 25 g). This method involved an initial dose finding

procedure, in which the animals were divided into three groups of three animals each. Doses of 10, 100 and 1000 mg/kg were administered intraperitoneally (i.p.), one dose for each group. The treated animals were monitored for 24 hr for mortality and general behaviour. From the results obtained, four different doses of (200, 400, 600 and 800 mg/kg) were chosen and administered i.p. respectively to four groups of one mouse each. The treated animals were monitored for 24 hr. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

Antidepressant assays

Force swimming test (FST)

The force swim test was carried out according to the method described by Porsolt *et al*. [18] and Matsuzaki *et al.* [19] with a minor modification. Female mice (20 - 25g) were assigned to five different groups of five animals each. Group 1 received distilled water (10 mL/kg), groups 2- 4 received EEAAL (1.25, 2.5 and 5 mg/kg, i.p.) respectively while group 5 which served as positive control received Imipramine (15 mg/kg, i.p.).The animals were forced to swim a day before the study in a Plexiglas cylinder (25 cm height, diameter 10 cm) containing water to a height of 10 cm maintained at a temperature of 25°C for 15 min (pre-session). On the following day (test session), thirty minutes after treatment, mice were placed back into the cylinder individually and forced to swim for 6 min. After an initial period of vigorous activity for two minutes, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling and making only minimum movements of its limbs necessary to keep its head above the water. The total duration of immobility was recorded during the last 4 min of the total test duration of 6 min.

Tail suspension test

The total duration of immobility following tail suspension was measured according to the method described for evaluating potential antidepressants [20]. Another set of female mice (20 - 25g) were assigned to five different groups (n = 5) and treated as in FST. Thirty min later mice were suspended on the edge of a table, 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during the last 4 min of the total duration of 6 min in different groups. Mice are considered to be immobile when they hang passively and completely motionless.

Open field test (OFT)

In order to rule out any nonspecific locomotor effect of *A. adianthifolia* on the observed antidepressant effect in the FST and TST, mice were evaluated in the open-field paradigm after pre-treatment with the same regimen as in the FST or TST. Their locomotor activities (crossing activity) were evaluated in the open field paradigm. Before each test, animals were kept in the test room at least 1 hr before the open-field test (OFT) for habituation. The ambulatory behaviour was assessed in open-field test as described by Rodrigues *et al*. [21]. The main apparatus consisted of square arena (50 cm × 50 cm × 40 cm) with grey surface covering every wall. The floor of the arena was divided equally into twenty-five squares (10 cm × 10 cm) marked by black lines. All animals were used only once in this test. Mice (20 - 25 g) were assigned to five different groups (n = 5) and treated as in FST. Thirty minutes after, each mouse was placed individually at the centre of the arena and allowed to explore freely. The number of squares crossed with all paws (crossing activity) were observed and counted in 5 min. The square arena was cleaned with a solution of 70% alcohol between tests and dried after occupancy by each mouse in order to hide animal clues and to prevent each mouse from being influenced by the odours present in the urine and faeces of the previous mouse.

Reserpine-induced depression

Five groups of animals were treated with reserpine (2.5 mg/kg, i.p.) 30 min after the respective drug and extract administration as stated previously in FST. The initial rectal temperature of all animals was determined before administration of reserpine. The acute effects of *A. adianthifolia* and Imipramine on reserpine-induced ptosis, hypothermia and diarrhoea were observed and recorded at 60, 120, 180 and 240 min after reserpine injection. The degree of ptosis was rated according to the following rating scale : 0, eyes open; 1, eyes one-quarter closed; 2, eyes half closed; 3, eyes three-quarters closed; 4, eyes completely closed [22]. The rectal temperature was determined by insertion of a digital thermometer to a constant depth of 2 cm into the anus of each animal. Diarrhea was assessed as previously described by Qing-Qiu [22]

Yohimbine-induced lethality test

To evaluate the involvement of noradrenergic system in the antidepressant-like effect of the extract, the yohimbine-induced lethality test was performed [23]. Mice were assigned to five different groups (n = 10) and treated as previously described for FST 30 min prior to yohimbine administration (35 mg/kg, i.p.).

The number of dead mice was recorded during a 24 h period after the injection of yohimbine and percentage mortality determined.

Statistical analysis

All data are presented as Mean ± SEM. The results were analyzed by One way analysis of variance (ANOVA), Chi square and post hoc tests (Student's-Newman-Keuls) were carried out to determine the source of significance using GraphPadInStat® Biostatistics software. The level of significance for all tests was set at $p < 0.05$.

Results

Acute toxicity test

The LD_{50} of the crude extract of *Albizia adianthifolia* was found to be 282 mg/kg i.p. body weight in mice.

Effect of EEAAL on immobility time in forced swim test (FST)

The EEAAL at 1.25 and 2.5 mg/kg significantly reduced ($p< 0.05$) immobility time of mice in FST compared to the negative control (distilled water) while 5 mg/kg did not reduce immobility time in mice. In addition, the anti-immobility effect of EEAAL (1.25 and 2.5 mg/kg) and that of imipramine are comparable ($p > 0.05$) [Fig. 1].

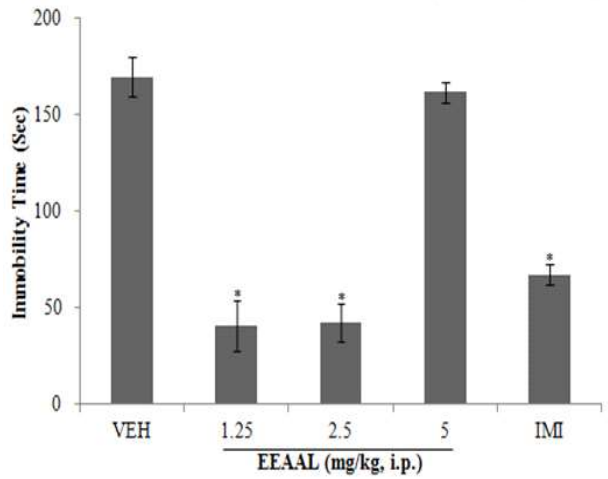

Fig. 1: Effect of *Albizia adianthifolia* on immobility time of Forced Swimming Test

* indicates significant difference from negative control ($p< 0.05$).**VEH**: Vehicle; **EEAAL**: Ethanol Extract of *Albizia adianthifolia;* **IMI**: imipramine (15 mg/kg, i.p.)

Effect of EEAAL on immobility time in tail suspension test (TST)

The EEAAL at 1.25 mg/kg significantly reduced ($p< 0.05$) immobility time of mice in TST compared to the negative control while doses at 2.5 mg/kg and 5 mg/kg did not reduce immobility time in mice. The antidepressant-like effects of EEAAL (1.25 mg/kg) and Imipramine are comparable ($p > 0.05$) [Fig. 2].

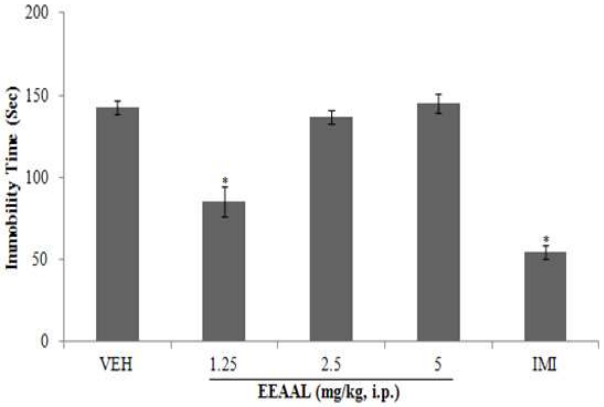

Fig. 2: Effect of *Albizia adianthifolia* on immobility time in Tail Suspension Test

* indicates significant difference from negative control ($p< 0.05$).**VEH**: Vehicle; **EEAAL**: Ethanol Extract of *Albizia adianthifolia;* **IMI**: imipramine (15 mg/kg, i.p.)

Effect of EEAAL on locomotor activity in open field test (OFT)

Treatment with EEAAL at 1.25 mg/kg and imipramine significantly reduced duration of immobility in FST and TST while 2.5 mg/kg which significantly reduced duration of immobility in FST produced no significant difference in number of crossing activity of mice in OFT ($p > 0.05$). However, the extract at 5 mg/kg significantly reduced number of line crossed ($p < 0.05$). This shows that the extract at 1.25 mg/kg and 2.5 mg/kg and imipramine did not affect locomotor activity of the animals (Fig. 3).

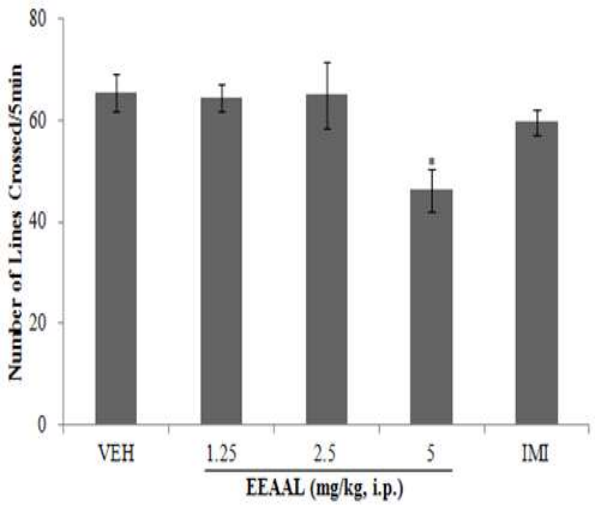

Fig. 3: Effect of EEAA on the locomotor activity of mice in open field test

* indicates significant difference from the negative control ($p< 0.05$).

VEH: Vehicle; **EEAAL**: Ethanol Extract of *Albizia adianthifolia;* **IMI**: imipramine (15 mg/kg, i.p.)

Effect of EEAAL in reserpine-induced depression

The EEAAL (1.25, 2.5, and 5 mg/kg) produced significant ($p< 0.05$) decrease in the mean watery faecal droppings (Table 1) and significantly antagonized reserpine-induced ptosis (Table 2) and hypothermia (Table 3) in all the groups compared to negative control.

Table 1: Effect of EEAAL on reserpine-induced diarrhoea

Groups	DOSE(mg/kg)	Number of droppings at various time interval 60 min	120 min	180 min	240 min
VEH	10mL/kg	3.20 ± 0.66	4.20 ± 0.58	5.60 ± 0.68	6.00 ± 0.71
	1.25	2.60 ± 0.40	2.20 ± 0.37*	1.00 ± 0.45*	0.80 ± 0.20*
EEAAL	2.5	0.80 ± 0.37*	1.80 ± 0.58*	1.60 ± 0.75*	1.40 ± 0.60*
	5	1.00 ± 0.32*	1.60 ± 0.68*	1.60 ± 0.81*	1.60 ± 0.51*
IMI	15	0.80 ± 0.37*	0.80 ± 0.20*	1.60 ± 0.24*	1.60 ± 0.24*

** indicates significant difference from the negative control ($p< 0.05$).*
***VEH**: Vehicle; **EEAAL**: Ethanol Extract of Albizia adianthifolia; **IMI**: imipramine (15 mg/kg, i.p.)*

Table 2: Effect of EEAAL on reserpine-induced ptosis

Groups	Dose (mg/kg)	Ptosis scores at various time interval 60 min	120 min	180 min	240 min
VEH	10mL/kg	3.20 ± 0.37	3.60 ± 0.24	3.60 ± 0.40	3.40 ± 0.40
	1.25	1.00 ± 0.55*	1.40 ± 0.60*	1.00 ± 0.45*	0.60 ± 0.40*
EEAAL	2.5	1.40 ± 0.60*	1.80 ± 0.37*	1.20 ± 0.58*	1.00 ± 0.63*
	5	1.00 ± 0.55*	1.40 ± 0.24*	1.20 ± 0.58*	1.40 ± 0.87*
IMI	15	1.00 ± 0.00*	1.00 ± 0.00*	1.00 ± 0.32*	1.00 ± 0.32*

** indicates significant difference from the negative control ($P < 0.05$).*
***VEH**: Vehicle; **EEAAL**: Ethanol Extract of Albizia adianthifolia; **IMI**: imipramine (15 mg/kg, i.p.)*

Table 3: Effect of EEAAL on reserpine-induced hypothermia

Groups	Dose (mg/kg)	Rectal Temperature (°C) at various time interval 0 min	60 min	120 min	180 min	240 min
VEH		38.26 ±0.22	38.6 6± 0.17	38.26 ± 0.22	38.52 ± 0.21	38.1S8 ±0.13
VEH+ Reserpine	10mL/kg	38.70 ± 0.30	37.46 ± 0.25*	37.02 ± 0.25*	36.48 ± 0.18*	35.96 ± 0.12*
	1.25	38.56± 0.19	38.30 ± 0.21	38.48 ± 0.12	38.50 ± 0.04	38.58 ± 0.28
EEAAL	2.5	38.52 ± 0.24	38.52 ± 0.15	38.28 ± 0.25	38.58 ± 0.29	38.24 ± 0.23
	5	38.46 ± 0.15	38.52 ± 0.16	38.52 ± 0.12	38.42 ± 0.17	38.30 ± 0.19
IMI	15	38.46 ± 0.20	38.46 ± 0.15	38.70 ± 0.11	38.56 ± 0.14	38.30 ± 0.22

** indicates significant difference from the negative control ($p< 0.05$).*
***VEH**: Vehicle; **EEAAL**: Ethanol Extract of Albizia adianthifolia; **IMI**: imipramine (15 mg/kg, i.p.)*

Effect of EEAAL on Yohimbine induced-lethality test

EEAAL (1.25, 2.5,5 mg/kg) did not significantly potentiate yohimbine-induced toxicity in mice compared to negative control. However, imipramine (15 mg/kg) produced marked significant increase in the number of death ($p< 0.05$) as compared to negative control (Table 4).

Discussion

Antidepressant- like effect of ethanol extract of *A. adianthifolia* leaves was investigated in this study using the behavioural despair tests [forced swimming test (FST) and tail suspension test, (TST)], reserpine-induced depression and yohimbine induced lethality.

The FST and TST are widely accepted behavioural models for screening antidepressants [18, 20, 21]. The characteristic behaviour evaluated in these tests, immobility reflects behavioural despair, similar to that seen in human depression and it is a well-established fact that antidepressants reduce the immobility time in rodents [18]. These tests are quite

Table 4: Effect of *Albizia adianthifolia* on Yohimbine-induced lethality Test

Group	Dose (mg/kg)	No of death	% mortality
VEH	10 mL/kg	2/10	20
	1.25	2/10	20
EEAAL	2.5	2/10	20
	5	0/10	0
IMI	15	7/10	70 *

** indicates significant difference from the negative control ($p< 0.05$)*
***VEH**: Vehicle; **EEAAL**: Ethanol Extract of A. adianthifolia; **IMI**: imipramine (15 mg/kg, i.p.)*

sensitive and relatively specific to all major classes of antidepressants including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors [21, 24].

In the FST, EEAAL reduced immobility compared to that of VEH, a negative control. This result shows that EEAAL by increasing duration of swimming has antidepressant effect. In the same vein, TST study shows that EEAAL treated animals showed significantly reduced immobility time, but at the lowest dose. This may not be unconnected with the sedation caused by the higher doses as shown in the open field test. The standard drug also reduced immobility time. The TST has been argued to be less stressful than FST and shows greater pharmacological sensitivity [25]. The observation of EEAAL treated animals demonstrating more mobility time suggests that it might have antidepressant property which is comparable to imipramine.

Drugs/agents which stimulate locomotor activity or cause hyperkinesia produce false positive results in FST and TST, it is therefore important to assess the influence of the test substance on the baseline spontaneous motor activity of the animals. Antidepressants would not cause general increase in locomotor activity as psychostimulants will cause hyperkinesia and produce false positive result [27]. Antidepressants such as TCAs, SSRIs and SNRIs have been shown to reduce immobility time without altering locomotor activity [26]. Therefore there is the need to carry out the open field test in order to eliminate the bias that EEAAL exerts psycho-stimulant like action on animals. The fact that EEAAL did not increase the number of line crossed in open field compared to the negative control suggests that the antidepressant action is specific. Consequent upon this observation, it can therefore be inferred that the decrease in immobility time at various doses in FST and TST are associated with the antidepressant like effect and not the locomotor enhancing or stimulant effect.

Reserpine is a vesicular re-uptake blocker which depletes catecholamines or lowers noradrenaline turnover in the brain to produce a depression like syndrome in animals [28]. Reserpine-induced depression is a model for assessing the mechanism of action of anti-depressants. Depletion of biogenic amines (noradrenaline, 5-hydroxytryptamine and dopamine) in the brain by reserpine produces effects such as ptosis, catalepsy, hypothermia and diarrhoea [29] which can be antagonized by antidepressants. Hypothermia induced by reserpine can also be antagonized by amphetamine like drugs, however, the time course is different: TCAs have a slow onset of action and a long lasting effect, whereas amphetamine like drugs have quick onset of action and a short lasting effect. In this study, reserpine-treated mice were observed for diarrhoea, ptosis and hypothermia. EAAL at all tested doses reversed these effects produced by reserpine over the period of 4 hr suggesting that its antidepressant action could be due to involvement of biogenic amines.

Potentiation of yohimbine toxicity has not only revealed antidepressant activity of compounds but also the participating system and has been reported for several antidepressants [30]. Yohimbine occupies central α_2-receptors and prevents noradrenaline from binding to these receptors leading to an increase in noradrenaline due to inhibition of the negative feedback mediated by α_2-receptors. Antagonism of α_2 receptors also causes an increase in the level of serotonin release which further contributes to the overall toxicity caused by yohimbine [31]. Simultaneous administration of yohimbine and antidepressant causes death of animal due to noradrenaline poisoning. The EAAL did not potentiate yohimbine action in this study thus precluding central adrenergic involvement in its antidepressant effect.

The antidepressant properties of the extract could be as a result of the presence of flavonoids. Though the phytochemistry of the extract was not done in the present study, previous studies have reported presence of three flavonoids: okanin, melanoxetin and dihydroflavonol [16].

Conclusion

The study has shown that the extract of the leaves of *A. Adianthiofolia* has antidepressant effect which could be due to involvement of biogenic amines. However, more studies are needed to identify the

bioactive compound and the exact mechanism of the antidepressant action.

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Serum adiponectin levels in normotensive and pre-eclamptic women at the University College Hospital, Ibadan, Nigeria

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Abstract

Background: Adiponectin is a hormone produced mainly by adipocytes. The levels of adiponectin are inversely related to insulin resistance, hypertension and obesity. Physiological insulin resistance is exaggerated in pre-eclamptic women. The objective of the present study was therefore to compare serum adiponectin levels in pre-eclamptic patients and normotensive patients.

Methods: This prospective study was conducted among one hundred and twenty women. Purposive sampling technique was used to select all consenting participants, consisting of sixty pre-eclamptic patients as cases and sixty normotensive pregnant women at comparable gestational age as control. Concentration of serum adiponectin was measured by using enzyme linked immunosorbent assay (ELISA) method.

Results: Serum levels of adiponectin were significantly higher in the pre-eclampticgroup(p<0.001). Body mass index was also significantly higher in women with pre eclampsia (p<0.01).In the pre-eclamptic patients, serum levels of adiponectin showed a weak negative correlation with BMI (r= -0.16, p= 0.22) and no correlation was found in normotensive patients.

Conclusion: This study showed a clear distinction in the serum adiponectin concentration between pre-eclamptic and normotensive pregnant women. The impact of BMI on serum adiponectin in both groups also differs. Therefore, serum adiponectin may be useful in predicting pre-eclampsia.

Keywords:*Serum adiponectin, Normotensive, pre-eclamptic, UCH, Pregnant woman*

Résumé

Contexte: Adiponectine est une hormone produite principalement par les adipocytes. Les niveaux d'adiponectine sont inversement liés à la résistance à l'insuline, à l'hypertension et à l'obésité. La résistance à l'insuline physiologique est exagérée chez les femmes pré-éclamptiques. L'objectif de la présente étude était donc de comparer les taux sériques d'adiponectine chez des patientes pré-éclamptiques et des patientes normo-tendues.

Méthodes: Cette étude prospective a été menée auprès de cent vingt femmes. La technique d'échantillonnage raisonné a été utilisée pour sélectionner tous les participants consentants, consistant soixante patientes pré-éclamptiques en tant que cas et soixante femmes enceintes normo-tendues à un âge gestationnel comparable en tant que témoin. La concentration d'adiponectine sérique a été mesurée en utilisant la méthode ELISA.

Résultats: Les taux sériques d'adiponectine étaient significativement plus élevés dans le groupe pré-éclamptique (p<0,001). L'indice de masse corporelle était également significativement plus élevé chez les femmes pré-éclamptiques (p <0,01). Chez les patientes pré-éclamptiques, les taux sériques d'adiponectine présentaient une faible corrélation négative avec l'IMC (r = -0,16 ; p = 0,22) et aucune corrélation chez les patientes normo-tendues.

Conclusion: Cette étude a montré une nette distinction dans la concentration sérique d'adiponectine entre les femmes enceintes pré-éclamptiques et normo-tendues. L'impact de l'IMC sur l'adiponectine sérique dans les deux groupes diffère également. Par conséquent, l'adiponectine sérique peut être utile pour prédire la pré-éclampsie.

Mots clés: *Sérum adiponectine, normo-tendus, pré-éclampsie, UCH, femme enceinte*

Introduction

Pre-eclampsia, one of the biggest causes of maternal and perinatal mortality and morbidity is a pregnancy complication which is associated with hypertension [1].Women with pre-eclampsia are at a higher risk of developing pulmonary edema, coagulation defects, blindness, cerebral hemorrhage, hepatic and/or renal failure, seizures and later in life, cardiovascular diseases while newborn babies of pre-eclamptic patients tend to be premature and are more prone to be small for gestational age [2,3].

The global estimate of complications of pregnancies due to pre-eclampsia is between 2 and 10% [2]. The World Health Organisation (WHO)

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reported that in developing countries,the incidence of pre-eclampsia is seven times higher than in developed countries[4].Various studies in Nigeria have revealed the prevalence of pre-eclampsia to be between 0.3% and 3.3% [5,6,7]. Many conditions have been linked to pre-eclampsia, including prior hypertension and insulin-dependent diabetes mellitus [8, 9]. The risk of pre-eclampsia is higher in primigravid women and it increases with greater interval between pregnancies, age greater than 40 years, pre-pregnancy obesity and pre-existing hypertension [9].

The etiology of pre-eclampsia remains obscure at the present [10]. In spite of the fact that researchers in the last century could not reveal the etiology of pre-eclampsia, much improvement was made in comprehending the pathophysiological changes related with its development [11]. Many hypotheses about etiology and pathogenesis of pre-eclampsia have been reported and they relate to angiogenesis, inflammation and endothelial dysfunction [11]. Adiponectin, a specific adipocyte-derived hormone has been thought to enhance insulin sensitivity, restrict atherogenesis and vascular inflammation [11].It has been suggested that adiponectin plays an important role in regulating metabolic adaptation during pregnancy as well as pathophysiology of pre-eclampsia [12]. Ali & Hassan concluded that insulin resistance indices correspond with circulating maternal adiponectin concentrations during pregnancy and that pre-eclampsia is linked with alterations in maternal adiponectin concentrations [12]. Several studies have reported elevated serum levels of adiponectin in pre-eclamptic women compared to normotensive women [11-16]. In contrast to this finding, some other studies have revealed decreased levels of serum adiponectin in women with pre-eclampsia [12]. Ramsey *et al* discovered that "serum adiponectin levels in the third trimester are higher in women with pre-eclampsia compared with controls". The reason for this elevation was stated to be due to exaggerated non-specific adipolysis and feedback mechanism to improve insulin sensitivity [17, 18]. Fasshauer *et al* also confirmed this finding in their study [18].

The relationship of adiponectin concentrations with BMI has also been examined by various researchers. For instance, one study attempting to determine "maternal serum levels of adiponectin in pre-eclampsia" found out that serum levels of adiponectin of pre-eclamptic patients revealed a significant negative correlation with BMI prior to pregnancy and third trimester [11].This finding has also been confirmed by Nien *et.al* [19].

There is no published research work done in Nigeria to determine serum levels of adiponectin, hence the need for this study. An understanding of the impact of pre-eclampsia on serum adiponectin levels in pregnancy will help to determine the usefulness of the protein as a valuable screening test for patients at risk, especially in our environment. This study therefore aimed to compare serum adiponectin levels between normotensive and pre-eclamptic women attending antenatal clinic at the University College Hospital, Ibadan.

Materials and method

This prospective case- control study was conducted at the University College Hospital, Ibadan, which is a tertiary health institution with eight hundred and fifty bed spaces. It is located in Ibadan, the capital of Oyo State. Its Obstetrics and Gynaecology department provides specialist care for antenatal, intrapartum and post natal patients. It is a study centre for many research works on pre-eclampsia.

One hundred and twenty pregnant women participated in the study. Purposive sampling technique was used to select all consenting participants. Sixty pre-eclamptic patients were cases while the other sixty were healthy normotensive patients at comparable gestational age served as the control group. Inclusion criteria were:Women diagnosed to have pre-eclampsia as cases and healthy normotensive pregnant women at a comparable gestational age as controls. Exclusion criteria included women with molar gestation, chronic hypertension, cardiac disease, Human Immunodeficiency Virus (HIV), renal disease, liver disease, diabetes mellitus, sickle cell disease and non-consenting patients. The diagnosis of pre-eclampsia was made using American College Of Obstetrician and Gynaecologists'(ACOG) guidelines [10].A proforma, designed based on existing literature findings, was used to collect patients' information and clinico-laboratory findings including maternal and gestational age, parity, height, weight, body mass index (BMI), blood pressure (BP), urinalysis, serum total adiponectin and high molecular weight adiponectin levels, maternal outcome(Glasgow coma scale score, need for intensive care unit, development of eclampsia and HELLP syndrome) and infant outcome (birth weight, Apgar score, need for admission into neonatal intensive care unit). Measurement of severity of the disease (mild or severe pre-eclampsia) was determined by the mean arterial blood pressure. The blood pressure was measured using a desk-type mercury sphygmomanometer with big cuff

(ACCOSON, Essex, England) calibrated in mmHg. The Korotkoff phase V (disappearance of sound) was used as the diastolic blood pressure. The mean arterial pressure (MAP) was calculated as DBP + (SBP – DBP)/3 mmHg.[SBP= Systolic blood pressure and DBP= Diastolic blood pressure]

The concentrations of adiponectin were measured using commercially available Human Adiponectinenzyme-linked immunosorbent assay (ELISA) by Biovendor®, Brno, Czech Republic. The procedures for the measurement were performed based on the instructions by the manufacturer.To measure the total Adiponectin concentrations,the sample was pre-treated with Sample Pretreatment Buffer (Citrate buffer + Sodium Dodecyl Sulphate [SDS]) which reduces multimeric adiponectin to dimers. Subsequent measurements by the ELISA quantified the amount of all multimers of adiponectin in the sample.

BMI was classified as normal (18.5-24.9)kg/m^2, overweight (25.0-30.0) kg/m^2 and >30kg/m^2 was regarded as obesity. Normal range of serum adiponectin is between 3.58 and 9.68µg/ml according to kit's manufacturer's manual. Apgar scores between 0 and 6 were considered to be low while 7 and above were considered normal.

All collected data were analysed using the Statistical Package for the Social Sciences (SPSS) version 17. The difference in mean serum adiponectin levels of the two groups was statistically tested using the Student t- test.

Spearman correlation coefficient was used to find a correlation between adiponectin and blood pressure, serum adiponectin and BMI. Level of significance was set at $p<0.05$.

Results

Table 1 shows the socio-demographic and clinical characteristics of normotensive and pre-eclamptic pregnant women. The mean ages of normotensive and pre-eclamptic pregnant women were similar. In the pre-eclampsia group, significant shorter length of gestation at delivery was observed ($p<0.0001$). Mean serum level of adiponectin and BMI was significantly higher in the pre-eclamptic group compared to normotensives ($p<0.01$).

The association between serum adiponectin levels and signs of severe pre-eclampsia among pre-eclamptic women is shown in Table 2. In the pre-eclamptic group, only four subjects developed seizures; out of these, three (75%) had significantly high levels of serum adiponectin ($p=0.02$). Development of headache was significantly associated with high serum adiponectin level

Table 1: Socio-demographic and clinical characeristics of normotensive and pre-eclamptic pregnant women

Variable	Normotensive (n=60)	Pre-eclamptic (n=60)	P-value
Age (years)	30.8±3.9	31.1 ±4.8	0.66
GA at delivery	38.55 ±1.11	36.93±2.84	<0.0001
SystolicBP	109.4 ±9.6	160.3 ±18.3	<0.0001
DiastolicBP	67.0 ±7.9	102.3±12.9	<0.0001
BMI	27.5 ±4.3	30.1 ±4.7	<0.01
Adiponectin (µg/ml)	6.3 ±1.8	9.9 ±5.4	<0.001

Table 2: Association between serum adiponectin levels and signs of severe pre-eclampsiaamong pre-eclamptic women

Variables	Serum adiponectin			
	Normal(%)	Abnormal(%)	Chi Square	p-value
Development of eclampsia (Seizures)				
Yes	1(25.0)	3(75.0)	5.83	0.02
No	90(77.6)	26(22.4)		
Development of headaches				
Yes	11(52.4)	10(47.6)	7.64	<0.01
No	80 (80.8)	19(19.2)		
Vomiting				
Yes	4(66.7)	2(33.3)	0.29	0.59
No	87(76.3)	27(23.7)		
Development of blurring of Vision				
Yes	3(75.0)	1(25.0)	0.002	0.97
No	88(75.9)	28(24.1)		
Development of epigastric pain				
Yes	5(50.0)	5(50.0)	3.97	0.05
No	86(78.2)	24(21.8)		

(p<0.01). Other severe pre-eclampsia symptoms such as vomiting (p=0.59) and epigastric pain(p=0.05) were associated with elevated serum adiponectin levels, but not significantly.

Table 4 shows serum adiponectin levels and neonatal outcomes in pre-eclamptic and normotensive women. There were more babies in the pre-eclamptic group with low Apgar scores. In the pre-eclamptic group, the participants whose babies had low apgar scores had higher mean serum adiponectin levels (p=0.02). In the same vein, mothers of babies with low birthweight in the pre-eclamptic group had higher mean serum adiponectin compared to those with low birthweight in the normotensive group. All six neonates that died were in the pre-eclamptic group.

Table 3: Relationship between BMI and serum adiponectin levels

BMI	Normotensive	Pre-eclamptic	T	p- value
Normal BMI (n=26)	6.2±1.8*	10.6±4.9*	-3.27	<0.01
Overweight (n=94)	6.4±1.9*	9.7±5.5*	-3.98	<0.0001

**mean+ SD serum adiponectin (in µg/ml)*

Table 4: Serum adiponectin levels and neonatal outcome among pre-eclamptic women

	n	Serum Adiponectin Normotensive (Mean±SD)	n	Serum Adiponectin Pre-eclamptic (Mean±SD)	p- value
Apgar score					
Low	6	6.6±2.4	46	10.0±5.4	0.02
Normal	54	6.3±1.8	14	9.4±5.8	0.07
Birthweight					
Low	4	7.2±1.0	22	10.6±6.1	0.03
Normal	56	6.3±1.9	38	9.4±5.0	<0.01
Need for SCBU admission					
Yes	0	0	16	9.9±6.7	-
No	60	4.4±0.3	44	10.7±5.5	<0.001
Neonatal condition at birth					
Alive	6	6.3±1.8	54	9.5±5.1	<0.01
Dead	00	0	6	11.5±6.6	-

Table 3 reveals relationship between BMI, blood pressure and serum adiponectin levels. Mean serum adiponectin was significantly higher in pre-eclamptic overweight women (p<0.0001). Similarly, among women with normal weight, the mean serum adiponectin level was significantly higher in the pre-eclamptic group (p<0.01). The relationship of adiponectin concentrations with BMI was further explored. In the pre-eclamptic patients, serum levels of adiponectin showed a weak negative correlation with BMI (r= -0.16, p= 0.22). In the normal pregnant women, no correlation was found between serum levels of adiponectin and BMI.

Also, a negative correlation of serum adiponectin levels with diastolic blood pressure (r=0.82, p=0.06) was observed and a positive correlation with systolic blood pressure (r=0.30, p<0.01) was found.

Discussion

The findings from the current study suggest that serum adiponectin levels are higher in pre-eclamptic pregnancy. This is also in line with results from studies by Ramsay *et al* [17], Nien *et al* [19], and Naruse *et al* [21] where these researchers indicated that plasma adiponectin concentrations were especially increased in women with pre-eclampsia compared with normotensive pregnant women. Ali and Hassan also confirmed that the most significant

finding of their study was an obvious increase in serum levels of adiponectin in women with pre-eclampsia when compared with pregnant women without pre-eclampsia [12]. Faussher *et al*, concluded that increased adiponectin serum levels in pre-eclamptic patients are positively associated with renal dysfunction [18], although this does not correlate with the creatinine levels of the women in their study which were within the normal range.Some of the authors likewise suggested that generation of adiponectin is increased due to physiological reaction by adipocytes in pre-eclamptic women to restrict atherogenesis and resultant vascular inflammation and also to enhance insulin sensitivity. However, contrary to these results, some other researchers have observed that pre-eclamptic women have reduced serum adiponectin concentrations than those of normal healthy pregnant women [12,16,18]. Some in the later group such as Mazaki-Tovi *et al* proposed that high and low levels of serum adiponectin in pre-eclamptic patients compared to normotensive pregnant women infers that adiponectin assumes a regulatory role in vascular and metabolic complications of pregnancy [22]. In the current study, serum levels were significantly associated with development of eclampsia.

Nienet al's study found out that adiponectin levels of normal pregnant women correlate negatively with BMI [19]. Dissimilar to their study, the findings from the present study have revealed a weak positive relationship between serum adiponectin level and BMI in the normotensive women, and very weak negative correlation in pre-eclamptic women [19]. This may be explained by the fact that BMI in pregnancy is impacted by the weight of the fetus, placenta and amniotic fluid and by plasma volume: this infers that maternal weight does not precisely reflect fat stores [27].

This study has shown a negative correlation of serum adiponectin levels with diastolic blood pressure and positive correlation with systolic blood pressure in normal healthy pregnant women. This is contrary to findings of Li *et al* where the authors reported a negative correlation between adiponectin and blood pressure in normotensive patients [25]. The authors concluded that this effect may be mediated through inflammatory pathway or lipid metabolism [25]. Abnormal serum adiponectin levels were found more in patients with signs of severe pre-eclampsia. This corroborates our earlier finding of higher mean adiponectin concentrations in pre-eclamptic patients.

Dalamangal *et al.*, in their case-control study, found that women in the pre-eclamptic group had a lower mean gestational age at delivery and smaller birth weights when compared with normal pregnant women [26].This finding was additionally affirmed in the current study. This may be partly explained by the fact that pre-eclamptic women may be delivered early to limit the progression of the disease.

Adiponectin levels were significantly higher in association with poor neonatal outcome (low birth weight and poor Apgar scores) in pre-eclamptic mothers. This again may highlight the usefulness of adiponectin in predicting poor outcomes of pregnancy.

The results of this study must be interpreted with caution as the findings may not be generalizable due to the small sample size. It would also have been desirable to take sequential samples to determine when Adiponectin levels begin to rise in pre-eclampsia and thus determine whether it can predict pre-eclampsia (rather than just be an association). The earlier gestational age at which normotensive women were recruited could be a confounder in the interpretation of results. Non-use of re-pregnancy body weight can be described as a limitation for the study.

Conclusion

This study revealed that there is a clear distinction in the serum adiponectin concentration between pre-eclamptic and normotensive pregnant women. The impact of BMI on serum adiponectin in both groups differs. Elevated serum adiponectin is associated with progression to eclampsia and poor neonatal outcome. Further longitudinal research is required to study these associations and to determine the usefulness of adiponectin as a predictor of severe pre-eclampsia, its progression and to determine the implications of weight loss on adiponectin and pregnancy outcome in pre-eclampsia.

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Onychomycosis in patients attending a dermatology outpatient clinic in Lagos, Nigeria

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Abstract

*Background***:** Onychomycosis refers to fungal infection of the nails either as a primary event or secondary infection of a previously diseased or traumatized nail. Some of the risk factors associated with onychomycosis include advancing age; smoking; peripheral arterial disease; diabetes mellitus (DM) and immunosuppression.

Aim: The work aims to determine the clinical characteristics; predisposing factors, causative organisms in patients with onychomycosis who attended the dermatology clinic in Lagos University Teaching Hospital between July 2013 and Jan 2014.

*Methods and Materials***:** This is a prospective observational study. All consenting patients with clinical features suggestive of superficial fungal infections were recruited. Data was collected using a structured questionnaire that contains bio- and socioeconomic data, clinical diagnosis, underlying disease condition and possible predisposing factors. Nail clippings and skin scrapings were taken for KOH microscopy and culture which were by standard methods.

Result: Onychomycosis was found in 19.0% of the patients recruited. The infection was most common between the ages of 30 and 39 (21.9%). Finger nail infections were more common affecting 16(61.5%) patients than toenails 6 (23.1%); and combined toe and fingernail infections were found in 4(15.4%). Dermatophytes accounted for 73.1% (19) of the isolates. The yeast *Candida albicans (17.5%)* and the dermatophyte *Trichophyton rubrum (17.5%)* were shown to be the most common pathogens of onychomycosis. Onychomycosis was more common in patients with HIV infection (25.0%).

Conclusion: Onychomycosis was most commonly seen in the adults in this study as opposed to the elderly in other climes and *T. rubrum* and *C albicans* were the most commonly cultured organisms.

Keywords: *Onychomycosis, fungal infections, risk factors, trichophyton rubrum, candida albicans*

Résumé

Contexte **:** L'onychomycose réfère à une infection fongique des ongles soit comme un événement primaire, ou comme une infection secondaire d'un ongle préalablement malade ou traumatisé. Certains des facteurs de risque associés à l'onychomycose comprennent l'âge avancé; le tabagisme; la maladie artérielle périphérique; le diabète sucré (DM) et l'immunosuppression.

But: Le travail vise à déterminer les caractéristiques cliniques; facteurs prédisposant, organismes causatifs chez les patients atteints d'onychomycose qui ont accédé à la clinique de dermatologie de l'Hôpital d'Enseignement Universitaire de Lagos entre Juillet 2013 et Janvier 2014.

Méthodes et matériaux **:** Ceci est une étude observationnelle prospective. Tous les patients consentants présentant des signes cliniques suggestifs d'infections fongiques superficielles ont été recrutés. Les données ont été recueillies à l'aide d'un questionnaire structuré qui contient des données bio- et socio-économiques, un diagnostic clinique, l'état pathologique sous étendu et d'éventuels facteurs prédisposant. Des coupures d'ongles et des raclures cutanées ont été prises pour la microscopie et la culture au KOH qui étaient par des méthodes standard.

Résultat: L'onychomycose a été retrouvée chez 19,0% des patients recrutés. L'infection était plus fréquente entre 30 et 39 ans (21,9%). Les infections des ongles des doigts étaient plus fréquentes touchant 16 (61,5%) patients que les ongles des orteils 6 (23,1%); et une infection combinée de l'orteil et de l'ongle ont été trouvées chez 4 (15,4%). Les dermatophytes représentaient 73,1% (19) des isolats. La levure *Candida albicans* (17,5%) et le dermatophyte *Trichophyton rubrum* (17,5%) se sont avérés être les pathogènes les plus communs de l'onychomycose. L'onychomycose était plus fréquente chez les patients infectés par le VIH (25,0%).

Conclusion: L'onychomycose était le plus souvent observée chez les adultes de cette étude, par opposition aux personnes âgées dans d'autres régions et *T. rubrum* et *C. albicans* étaient les organismes les plus couramment cultivés.

Mots - clés : *Onychomycose, infections fongiques, facteurs de risque, trichophyton rubrum, candida albicans*

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Introduction

Onychomycosis refers to fungal infection of the nails either as a primary event or secondary infection of a previously diseased or traumatized nail. Infection could be caused by a dermatophyte, yeast or non-dermatophyte mould species and the clinical appearance may indicate the nature of the causative organism.[1]The term 'Tinea unguium' which means dermatophyte infections of the nail has been used synonymously but erroneously with onychomycosis. Tinea unguium represents 90% of onychomycosis of the toe nails and 50% of that of the finger nails [2]. Onychomycosis, though not life threatening, causes symptoms such as pain, discomfort and disfigurement which impair activities of daily living and negatively impact the quality of life of affected individuals. [3-5] Onychomycosis constitutes 50% of all nail problems and about 30% of all dermatophyte infections [1].

Onychomycosis affects all races but there is wide geographical and ethnic variation in the causative species. Approximately 20% of individuals aged between 40 and 60 have onychomycosis [6,7]. A recent review on the global burden of onychomycosis revealed that the mean prevalence in Europe and North America was 4.3% in the population-based studies, but was 8.9% for the hospital-based studies [8]. Both population-based and hospital-based studies showed that onychomycosis is more common in toenails and is seen more frequently in males [8]. Studies from Finland and the UK also showed higher frequency in males than females [1,9]. Two previous hospital based studies done in Lagos, Nigeria revealed the frequency of onychomycosis to be 10.5 and 13.5%; higher than findings in Europe suggesting likelihood of higher prevalence in Africa than in Europe [1,10,11].

Some of the risk factors associated with onychomycosis include advancing age; smoking; peripheral arterial disease; diabetes mellitus (DM); immunosuppression from human immunodeficiency virus (HIV) infection, immunosuppressive therapy, chemotherapy and antibiotic use [12-16]. HIV is associated with extensive onychomycosis which is a marker of declining immunity in HIV infection.[17] Onychomycosis has also been documented as an occupational hazard in farmers who till the soil manually and those who walk bare- footed in developing countries [18]. A genetic basis for onychomycosis has been suspected in a study in which a familial pattern was found in patients with distal subungual onychomycosis [19]. Children of patients with onychomycosis were noted to harbor the pathogen (*T. rubrum*) on the soles of feet; and this spreads at a later date to the toe nails and other sites on the body [19]. The increased use of health clubs, commercial swimming pools and occlusive footwear have also been implicated in the increasing prevalence of onychomycosis [16].

Dermatophytes are the most commonly implicated group of fungi in onychomycosis, found all over the world. *Trichophyton rubrum* (*T. rubrum*) is the most common aetiological agent of nail infections worldwide and accounts for 70% of onychomycosis, followed by *T. mentagrophytes* (20% of onychomycosis) [1,20]. In a multi-centred study carried out in Australia and New Zealand, non-dermatophyte fungi (yeasts and moulds) were found in 64% of the patients either as a co-infection, secondary infection or contaminants.[21] The most common cause of yeast infection is *Candida albicans* [22].

This work aimed to determine the clinical characteristics; predisposing factors, and identify the prevalent causative organism in patients with onychomycosis who attended the dermatology clinic in Lagos University Teaching Hospital.

Materials and methods

This is a prospective observational study of adult patients who attended the dermatology clinic of the Lagos University Teaching Hospital between July 2013 and Jan 2014. All consenting patients were recruited.

Ethical consideration

Ethical approval was obtained from the research and ethics committee of the Lagos University Teaching Hospital. Each study participant or their next-of-kin gave written informed consent to participate in the study after the study procedure including possible benefits and risks had been explained to them.

All patients with suspected fungal infections who gave informed consent over the study period were recruited into the study. Patients unwilling to participate; children whose parents or guardian did not consent; and those on antifungal therapy and clinically responding to therapy were excluded. Onychomycosis was defined as fungal infection of nail. Tinea unguium was defined as dermatophyte infection of nail; tinea capitis as dermatophyte infection of scalp; tinea corporis as dermatophyte infection of skin; tinea mannum as dermatophyte infection of hands; and tinea pedis as dermatophyte infection of feet.

Data collection

A structured questionnaire was administered to each study participants by the investigators. Information requested included bio- and socioeconomic data, clinical diagnosis, underlying disease condition, hobbies such as swimming, gardening and playing with pets; previous history of dermatophyte infection (duration and drug therapy) and history of close contact with persons/animals that have dermatophyte infections.

Specimen collection

Scrapings or clippings of infected nails were collected after cleaning with 70% ethanol. All nails were scraped with a blunt scalpel from the proximal to the distal end of the nail. The first 4-5 scrapings were discarded (to reduce contaminants and get to viable tissue). All samples were collected in a folded square of paper and transported to the microbiology laboratory for analysis.

Microscopy

The specimen was divided into two parts. Potassium hydroxide solution (40% w/v) was added to a part of the specimen for 2 hours to dissolve the nail keratin and release fungal elements for observation by direct microscopy. Fungal elements were identified as hyphae or yeast cells

Culture

The second part of the specimen collected was inoculated onto Sabouraud's dextrose agar with chloramphenicol and gentamicin; plates were sealed with paraffin wax tape and incubated as duplicate cultures at 26^{o}C and 35^{o}C respectively. Yeasts were identified within 48hours, and germ tube test was used for presumptive diagnosis of *C.albicans*. Cultures were maintained for 4 weeks for molds. However, they were reviewed twice weekly for growth and colonial morphology, pigmentation and consistency.

Germ tube test

Principle: Strains of *C.albicans* produce germ tubes from their yeast cells when placed in a liquid nutrient environment and incubated at 35^{o}C for 2-3hours similar to the in vitro state.

Procedure: Using a sterile wire loop, a yeast colony from the Sabouraud agar was inoculated into 0.5mls of human serum in a test tube mixed properly and incubated at 35 – 37^{0}C for 2 – 3 hours. After which a drop of the mixture was transferred to a glass slide, covered with a cover slip and then examined under a light microscope using 10x and 40x objectives. Sprouting yeast cell that were tube-like outgrowth from the cells (germ tube) were reported as *C. albicans* and non-sprouting yeast cell as non-C. *albicans.*

Coccidiodes immitis was identified macroscopically and microscopically. Slides were prepared by tease mount stained with lactophenol blue, sealed with coverslips and examined microscopically. Hyphae seen were thin, hyaline and septate. Thicker side branches gave rise to unicellular, barrel-shaped arthroconidia that alternated with thin-walled, empty disjunctor cells. The culture demonstrated glistening off-white glabrous colonies.

All slides were examined and identified (slide preparation) in a class 2 biological safety cabinet.

Data analysis

Quantitative data generated from the questionnaire amongst all patients attending the dermatology clinic of Lagos University Teaching Hospital were entered into an excel spreadsheet. Data were analysed at univariate level using SPSS software.The analysis was done with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). Continuous variables were presented as the mean ± standard deviation. Categorical variables were presented as actual numbers and percentages or as bar charts.The univariate analysis involved the use of descriptive statistics and graph such as frequency distributions and bar chart.

Results

One hundred and thirty seven patients with superficial fungal infections attending the dermatology clinic of the Lagos University Teaching Hospital (LUTH) between July 2013 and February 2014 were recruited. Forty (29.2%) had abnormal looking finger and toe nails with 1:1 ratio of males/females. There were more abnormal finger nails 26 (65%) compared to toenails 10 (25%) with a ratio of 2.6:1. Four patients (10%) had both abnormal looking finger and toe nails. Onychomycosis accounted for 19.0% of the patients with superficial fungal infections at the dermatology outpatient clinic of LUTH.

Demographic characteristics and underlying diseases of patients recruited

One hundred and thirty seven participants were recruited into the study. Table 1 shows the demographic characteristics of these patients. More than half of the patients 103 (75.2%) were less than 50 years old, while only 35 (25.5%) were 50 years

Table 1-Demographic characteristics of patients studied and underlying diseases

Demographic characteristics	Frequency (n)	Percentages (%)
Age in years		
0 – 9	13	9.5
10 - 19	17	12.4
20 – 29	18	13.2
30 - 39	30	21.9
40 - 49	24	17.5
Above 50	35	25.5
Sex		
Male	67	48.9
Female	70	51.1
Marital status		
Single	37	72.9
Married	100	29.1
Tribe		
Yoruba	81	59.1
Igbo	33	24.1
Hausa	10	7.3
Others	13	9.5
Co existing medical diseases		
Diabetes mellitus	24	17.5
Hypertension	35	25.5
HIV/AIDS	26	18.9
Others	14	10.2
None	38	27.7

and above. The mean age was 36 ± 19.2 years (2SD). There were more females than males, with a ratio of approximately 1.04:1. The underlying diseases in the patients in this study include diabetes mellitus 24 (17.5%); hypertension 35 (25.6%) and HIV/AIDS 26 (18.9%). Others were side effects of use of over the counter topical and systemic steroid abuse.

Clinical assessment/diagnosis of patient were based on location of the lesion, with the highest frequency being dermatophyte infection of the nail (Tinea unguium) 35 (25.5%) followed by that of the glabrous skin (T. coporis) 34 (24.8%) and the least frequently occurring being simultaneous infections of various parts of the body accounting for 10 (7.0%) of studied population (Figure 1).

Laboratory diagnosis of all patients' specimen

Table 2 demonstrates the pattern of aetiological agents of keratinized tissue infections in the studied population. Of the 137 patients studied, 49 (35.8%) had no growth after 4 weeks incubation; *C. albicans* was isolated in 24(17.5%); *T. rubrum* in 29 (21.2%); *T. mentagrophyte* in 13(9.5%) and more than one isolate in 3 (2.2%).

Demographic characteristics of patients with onychomycosis

Nineteen (47.5%) of the participants with nail infections were in the 26 to 50 years age range; 14 (35.0%) were 50 years old and above, and only 1

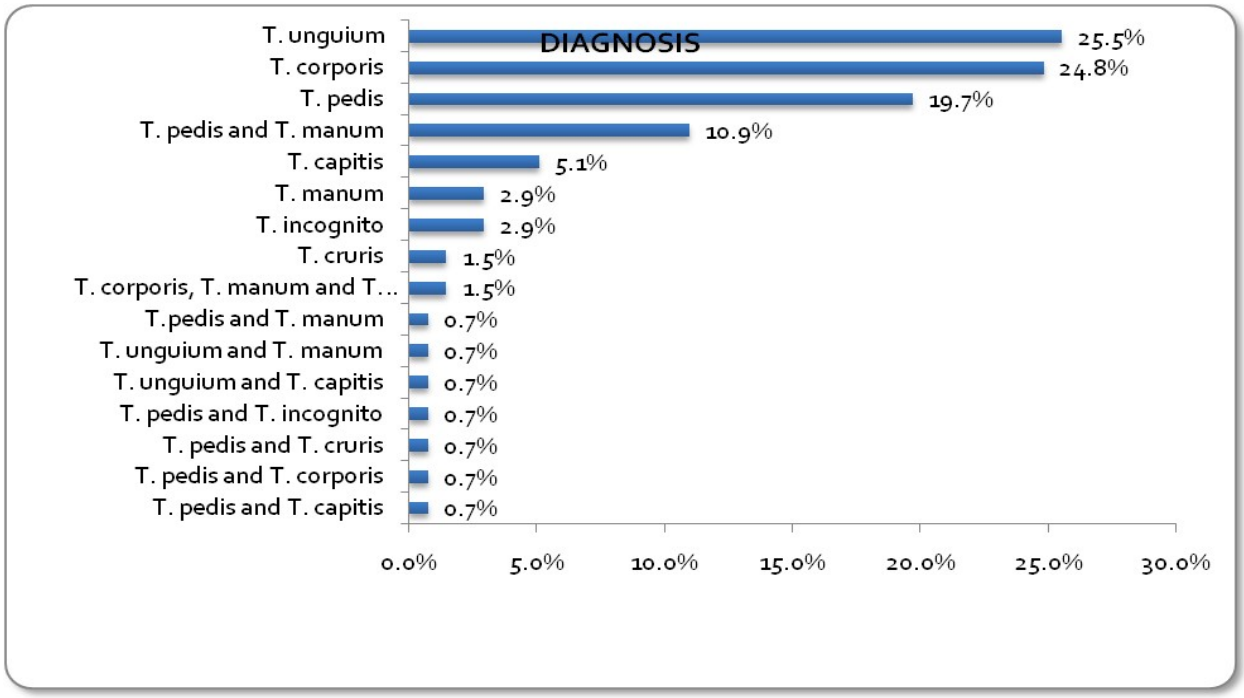

Fig.1: Distribution of clinical diagnosis of studied population

Table 2: Distribution of pathogens causing mycosis

Culture result Isolates	Frequency	Percentage
Coccidioides immitis	1	0.7
Microsporum manum and *C. albicans*	1	0.7
Microsporum spp	1	0.7
Penicillum notatum	1	0.7
T. rubrum and *T. soudanese*	1	0.7
T. verrucossa	1	0.7
T. mentagrophyte and *C. albicans*	2	1.5
T. rubrum and *C. albicans*	2	1.5
T. scholieni	2	1.5
Microsporum and *C. albicans*	3	2.2
T. verrucosa	3	2.2
T. soudanese	4	2.9
T. mentagrophytes	13	9.5
C. albicans	24	17.5
T. rubrum	29	21.2
No growth	49	35.8
Total	137	100.0

(7.5%) were between 1 and10 years of age (Table 1). Mean age was 31 years and male to female ratio was 1:1. There were 26 (19%) cases of onychomycosis. Sixteen (61.5%) were fingernail infections and 6(23.1%) were toenail infections; combined toe and fingernail infections were 4(15.3%). Six (15.0%) patients had DM, 4 (10.0%) had hypertension and 10 (25.0%) were HIV/AIDS patients. The patients with DM accounted for five cases of the toenail infections and one case of both toe and fingernail infection. The remaining cases of both finger and toe nail infections were in HIV positive patients.

Eighty five percent presented with only nail infections while the remaining fifteen percent had mixed infections with T. pedis, T. mannum and T. coporis.

Isolated causative pathogens of onychomychosis

Direct microscopy with 40% potassium hydroxide (KOH) demonstrated the presence of fungi element in 87% of the samples. Culture isolates demonstrated the most frequent isolates were *T.rubrum* 7 (17.5%) and *C.albicans* 7 (17.5%); 4 samples had more than one organism isolated, Non-dermatophyte moulds were not isolated in this study, (Table 3).

Candida albicans accounted for 10 (56.3%) of all fingernail onychomycosis in this study. The distribution of *C.albicans* was 7 (70%) as pure isolates and 3 (30%) isolated in the mixed growths. Dermatophytes accounted for 7 (43.7%) of cases of fingernail onychomycosis, with distribution of *T. rubrum* being identified in 4 (57.0) isolates and *T. mentagrophyte* in 3 (43.9%). Isolates from onychomycosis of the toenails were all dermatophytes, with *T. rubrum* accounting for 50% of all isolates.

Table 3: Distribution of aetiological agents of onychomycosis

Culture results Isolates	Frequency	Percentage
Microsporum and *C. albicans*	1	2.5
T. rubrum and *T. soudanese*	1	2.5
T. scholieni	1	2.5
T. soudanese	1	2.5
T. verrucossa	2	5.0
T. rubrum and *C. albicans*	2	5.0
T. mentagrophyte	4	10.0
C. albicans	7	17.5
T. rubrum	7	17.5
No growth	14	35.0
Total	40	100.0

Discussion

Onychomycosis accounted for 19.0% of the cases of dermatophyte infections seen in this study. This is higher than 10.5% and 13.6% obtained from previous reports from this same center; these were however retrospective studies [10,11]. This value is also higher compared to a study from neighboring Cameroun, which gave a prevalence of 8.8%, but lower than that from an Iranian study with a prevalence of 45.2% [23,24]. The variations among these studies may represent real differences in the geographic groups studied, climate, sampling variations, location, clinical type of the infection and other factors [25]. Onychomycosis was more common in those aged 26–50 years in this study. In contrast, Velez *et al*. 1997, and Mercantini et al. 1996, reported higher prevalence among adults who were over 50 years of age which was the second most frequent group in these studies [25,26]. This age-related increase of onychomycosis may have resulted from higher probability of nail microtrauma, exposure to pathogenic fungi, and venous insufficiency in older patients as described previously [12,27]. There was only one case of onychomycosis documented in the 1-10 years group in this study, and this is consistent with existing data that demonstrated very low prevalence in children [22,28].

The most common underlying disease in this study was HIV/AIDS. This is consistent with previous reports that demonstrated onychomycosis as a marker of declining immunity in HIV infection and occurs in the early stages of disease progression at $CD4^+$ count of > 400 cells/mm^3. In the late stages of HIV infection with $CD4^+$ count of <200 cells/mm^3, patients present with extensive disease: marked subungual hyperkeratosis, severe onychodystrophy, extensive involvement of all nails and profound physical discomfort from periungual inflammation [17]. We were unable to access the CD4 count of participants with HIV/AIDS. These group of patients also accounted for 75% of coexisting finger and toenail infections, which is in keeping with existing data.[17] Diabetes mellitus was also a common underlying condition amongst study participants and this is not surprising considering the fact that it has been estimated that at least one third of DM patients have onychomycosis.[15]

There was no difference amongst sexes in this study, the ratio was 1; 1. However more fingernail lesions were seen compared to toenails, this is not surprising since most times nail infections are viewed as more of a cosmetic problem and fingernails are more visible. Apart from the cosmetic nature of the disease, females in this environment may also present with more fingernail given the cultural background wherein women do more domestic work by hand, enabling chronic exposure of fingers to water.[29]

On direct microscopy, fungal elements were seen in 87% of the confirmed samples, which is relatively higher than documented in other studies; the use of 40% KOH (rather than 20% used in most studies) probably played a role in this. Larger studies will be needed to substantiate this. The results are however in accordance with the findings of a study which was carried by Mikaeili and Karimi, which showed positive results of 87.2% by direct microscopy. [24]

The most common organisms which were isolated in culture were dermatophytes (73.1%) and yeasts (17.5%). This finding was in accordance with those of many studies, which had demonstrated a greater prevalence of dermatophytes as the aetiological agents of onychomycosis but it was in contrast to some studies which found yeasts as the most common agents. [1,8,20,14,30] Fingernails accounted for 100% of all yeast infections in this study and most of these patients had concurrent fungal skin infections. A Libyan study also isolated *Candida spp* from 96% of fingernails in women with onychomycosis. [31] *Trichophyton rubrum* was the most common dermatophyte seen in our study, followed by *T. mentagrophytes*. Although some studies had reported T. *mentagrophytes* as the most common dermatophyte, our finding was in concordance with other studies which found *T. rubrum* as the most common dermatophyte responsible for onychomycosis.[1,8,20] The increased prevalence of *T. rubrum* could have been due to increased virulence and better adaptation to hard keratin of nails.

In conclusion, the most common fungal isolates from onychomycosis in this study were dermatophytes *followed by Candida albicans*. Clinicians, therefore, should inform the general population about onychomycosis prevention. The present findings suggest that clinic, community, and school based onychomycosis prevention programs may benefit the people by addressing the risk factors for onychomycosis infection.

The limitations of this study included the incessant industrial strike action by health care workers that impacted negatively on the sample size. The study was hospital based which might not be a true representation of the burden of the disease in the populace and finally some of the patients may have used herbal/local agents to treat their nails prior to hospital presentation.

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Internet use by orthopaedic patients: a survey of patients in three teaching hospitals in Southwestern Nigeria

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Abstract

Background: Patients use the Internet increasingly for information about their medical problems.

Objective: To evaluate Internet use among patients attending the orthopaedic clinics of three teaching hospitals in southwest Nigeria.

*Methodology***:** An anonymous survey was distributed to patients attending the orthopaedic clinics of the Obafemi Awolowo University Teaching hospitals Complex, Lagos University Teaching Hospital and Ekiti State University Teaching Hospital, Ado-Ekiti. The survey elicited information regarding demographics, health-related Internet use, and how the information obtained impacted their relationship with their orthopaedic surgeon.

Results: A total of 475 respondents returned the completed questionnaire out of which 16 did not meet the inclusion criteria. Of the 459 patients that met the inclusion criteria 69.5% has accessed the internet in the previous one year, and 39.2% sought health related information, but only 11.5% has ever e-mailed their health provider about health related problems. More of the patients were looking for information regarding their illnesses followed by information about health and nutrition. About 90% found the information useful. Those with post-secondary education, Christians, and patients who sought for treatment at LUTH, Lagos were more likely to seek health related information from the internet. Ethnicity and gender showed no statistically significant difference in predicting online health information seeking behavior among the patients.

Conclusion: While not as high as reported usage from the developed countries, the online health information seeking behaviour of orthopaedic patients in this study should justify investment into providing online, health information whose contents are targeted at orthopaedic patients in our environment.

Keywords: *Health- related internet use,*

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Résumé

Contexte: Les patients utilisent de plus en plus l'internet pour obtenir des informations sur leurs problèmes médicaux.

Objectif: Évaluer l'utilisation d'Internet chez les patients fréquentant les cliniques orthopédiques de trois hôpitaux universitaires du sud-ouest du Nigeria.

Méthodologie **:** Un sondage anonyme a été distribué aux patients fréquentant les cliniques orthopédiques des Complexes Hospitaliers d'Enseignement Universitaire d'Obafemi Awolowo, de l'Hôpital d'Enseignement Universitaire de Lagos et de l'Hôpital d'Enseignement Universitaire de l'Etat d'Ekiti, Ado- Ekiti. L'enquête a permis d'obtenir des informations sur la démographie, l'utilisation de l'internet liée à la santé et la manière dont les informations obtenues ont eu un impact sur leur relation avec leur chirurgien orthopédique.

Résultats: Au total, 475 répondants ont renvoyé le questionnaire rempli, dont 16 ne répondaient pas aux critères d'inclusion. Parmi les 459 patients qui répondaient aux critères d'inclusion, 69,5% avaient consulté l'internet au cours de l'année précédente et 39,2% cherchaient des informations sur la santé, mais seulement 11,5% ont auparavant envoyé un courrier de message électronique à leur fournisseur de soins pour des problèmes de santé. Un plus grand nombre de patients cherchaient des informations sur leurs maladies suivies d'informations sur la santé et la nutrition. Environ 90% ont trouvé l'information utile. Ceux qui ont une éducation postsecondaire, les chrétiens et les patients qui ont demandé un traitement à LUTH, Lagos étaient plus susceptibles de chercher des informations liées à la santé sur l'nternet. L'ethnicité et le sexe n'ont montré aucune différence statistiquement significative dans la prédiction du comportement de recherche d'information de santé en ligne parmi les patients.

Conclusion: Bien qu'il ne soit pas aussi élevé que celui des pays développés, le comportement de recherche d'information en ligne des patients orthopédiques dans cette étude devrait justifier un investissement dans la fourniture d'informations sanitaires en ligne dont le contenu cible les patients orthopédiques dans notre environnement.

Mots clés: *Utilisation de l'internet liée à la santé,*

Introduction

Patients acquire medical information from many sources, inclusive of which are; healthcare professionals, advertisements, the media, the internet and other lay persons. However, since the turn of the century, the internet has grown to be a major source of medical information for patients in both developed and developing countries. Studies have shown that the internet ranked second only to healthcare professionals as the preferred source of medical information for patients[1, 2].

In some instances, such as when patients sought for information on support for rare or chronic diseases or needed to know other people's experiences, the internet was the preferred source of medical information [1]. The Bupa Health Pulse 2010 international healthcare survey of online healthcare information seeking habit in twelve countries showed that 60% of the respondents had assessed the internet to search for medical information[2]. The survey also found that due to the relatively high cost of personal consultation with healthcare professionals, a significant proportion of the respondents have resorted to conducting online searches for health information[2].

At the turn of the century, access to online information in Africa was very poor. In 2000, the ratio of those with internet access to those without was 1 to 5000 in Africa, while in the United States and Europe the ratio was 1 to 6[3]. However, with the advent of cellular networks and cheap phones with internet capabilities, the situation is rapidly changing[4]. A recent study from Nigeria (In press) showed that more than 90% of orthopaedic surgeons and their trainees practicing across the country have encountered patients with internet medical information at consultations. However, there had been no study in Nigeria, and very few from Africa on the experience of patients seeking medical information from the Internet.

The present study was carried out to determine the nature of Nigerian orthopaedic patients' experience with internet medical information, and how this affects their subsequent interactions with their healthcare providers.

Patient and method

The setting was the orthopaedic outpatient clinic of three teaching hospitals in southwest Nigeria, namely the Obafemi Awolowo University Teaching Hospitals complex and Ekiti State University Teaching Hospital situated in Ile-Ife and Ado-Ekiti respectively. Both Ile-Ife and Ado-Ekiti are semi-urban university towns with a large population of students and civil servants. The main mode of Internet access in both towns is by cellular data. The third centre was the Lagos University Teaching Hospital in Lagos, commercial capital of Nigeria. This is a cosmopolitan city with broadband access to the internet. A convenient sampling technique was used and this was a questionnaire based study which lasted for 6 months.

The questionnaire

This had two sections: Section one contained socio-demographic questions. Section two is an 18-item questionnaire about patients' experience of using Internet derived medical information.

Procedure

During the waiting period, the research assistant (house officer, resident doctor or medical students) randomly selected a patient and enquired from the patient whether he or she had participated in the study in a previous visit. If the patient had not, the research assistant would then explain the purpose of the study to the patient. He or she would also be informed that he or she had the right to decline to be a part of the study, and that such decision would have no effect whatsoever on their subsequent treatment. If the patient agreed to be a part of the study, then he or she was served the questionnaire and pertinent explanations regarding the aims and objectives of the study given. After the patient had filled the questionnaire, the research assistant would scan the document for errors or missing variables. If any was discovered, the patient's attention would be brought to the item for it to be corrected. Thereafter, the patient was asked to sign the proforma, and this was taken as the signed consent.

All adult patients (15years and above) attending orthopaedic clinics of the participating hospital who had completed secondary education, and can read and write were eligible for inclusion in the study. The study was conducted in accordance with the principles of Helsinki declaration.

Statistical analysis

There were two outcome measures. One was whether or not the patient had used the internet to access medical information within the last one year, and the second was whether or not the patient has used e-mail to communicate with his healthcare professional within the last one year. We used Student's t-test and Pearson's chi-square to compare outcomes when the predictor variables were continuous and categorical respectively. We considered any P-value less than 0.05 as statistically significant. We used IBM SPSS version 20 for all analysis.

Results

We collected four hundred and seventy-five completed questionnaires from the patients. However, 16 (3.4%) were discarded because they had primary school educations (having less than secondary school education was an exclusion criteria). Of the remaining 459 respondents, 207 (45.1%) were from OAUTHC, Ile-Ife, 160 (34.9%) were from LUTH, Lagos while the remaining 92 (20.0%) were from EKSUTH, Ado-Ekiti. Table 1 summarizes the characteristics of the patients.

Table 1: Demographic characteristics of respondents

Variable	Frequency (%)
Gender	
Male	225 (50.2)
Female	223 (49.8)
Tribe	
Yoruba	353 (78.6)
Igbo	61 (13.6)
Others (Hausa,Benin,Idoma,Effik)	35 (7.8)
Marital status	
Single	183 (42.0)
Married	234 (53.7)
Widowed	19 (4.4)
Education	
Secondary School	134 (29.2)
University/Polytechnic	273 (59.5)
OND/Technical Education	25 (5.4)
Others	27 (5.9)

Table 2: Nature of internet use among medical information seekers

Variables	n(%)
[1]*Device Used*	
Phones	263 (58.1)
Personal Computers	118 (26.0)
Tablets	19 (4.2)
[2]Non-personal devices	53 (11.7)
How frequent was the Internet search for health information?	
At least once a day	75 (41.9)
At least once a week	63 (34.7)
At least once a month	14 (8.4)
At least once in the past one year	25 (15.0)
Who was the information for?	
Patient	206 (85.1)
Wife/Children	12 (4.9)
Parents	4 (1.7)
Others	20 (8.2)

[1]*Many respondents used multiple devices*
[2]*Includes office PC, library, cyber café and devices belonging to acquaintances*

Table 3: Respondents' characteristics and decision to use internet for medical information.

Variable	Search for health information		
	Yes n(%)	No n(%)	P-Value
Gender			
Male	85 (43.1)	112 (56.9)	0.184
Female	92 (50.0)	92 (50.0)	
Religion			
Christian	132 (46.6)	151 (53.4)	0.459
Muslim	22 (40.7)	32 (59.3)	
Ethnic group			
Yoruba	136 (44.0)	173 (56.0)	0.140
Igbo	31 (58.5)	22 (41.5)	
Others	10 (50.0)	10 (50.0)	
Education			
Secondary	22 (27.5)	58 (72.5)	<0.05
OND/Tech	9 (42.9)	12 (57.1)	
Univ/Poly	138 (56.1)	108 (43.9)	
Others	7 (29.2)	17 (70.8)	
Marital status			
Single	80 (48.2)	86 (51.8)	0.335
Married	88 (46.3)	102 (53.7)	
Widowed	5 (29.4)	12 (70.6)	
Hospital			
LUTH	76 (57.1)	57 (42.9)	<0.05
OAUTHC	72 (40.9)	104 (59.1)	
EKSUTH	32 (38.6)	51 (61.4)	

Internet use

Three hundred and nineteen (69.5%) patients have accessed the internet for information in the past one year preceding the study. Of these 180(56.4%) did it to obtain health information. As indicated in table 2 the majority of the patients accessed the information on their phones and most searches were personally by the patients. The table also showed that about 75% of the patients who used the internet for health information used it at least once a week in the past one year. Table 3 shows that those with university/ polytechnic education were significantly more likely to have accessed the Internet for medical information than other educated group (P<0.05). Similarly, the patients visiting LUTH used the Internet more frequently than those in EKSUTH and OAUTHC, which were located in semi-urban towns (P=0.006). However, demographic characteristics such as age, gender, religion and ethnicity were not significantly related to using the Internet to access medical information.

E-mail communication with healthcare professional

Fifty three patients (11.5%) had communicated with their healthcare providers via the e-mail one year

period prior to the study. Fifteen (29.4%) had done it only once, 25 (49.0%) had done it less than 5 times while the remaining 13 (21.2%) had done it more than 5 times. Most of the communications were initiated by the patients. Tables 3 and 4 show that patients with post-secondary education were significantly more likely to communicate with their doctors than patients with secondary school education (P=0.009). Similarly significantly larger proportion of patients in LUTH communicated with their doctors using e-mail compared their counterparts in OAUTHC, Ile-Ife and EKSUTH Ado-Ekiti (P=0.039). Age, gender, ethnicity or religion had no significant effect on whether or not patients communicated with their practitioner with e-mail (Table 4) .

Table 4: Respondents' characteristics and exchange of e-mails with healthcare providers.

Variable	Exchange e-mail with practitioner		
	Yes n(%)	No n(%)	P value
Gender			
Male	26 (13.3)	170 (86.7)	0.920
Female	26 (13.6)	165 (86.4)	
Religion			
Christian	35 (12.2)	253 (87.8)	0.266
Muslim	9 (17.6)	42 (82.4)	
Ethnic group			
Yoruba	34 (11.3)	267 (88,7)	0.111
Igbo	11 (19.6)	45 (80.4)	
Others	6 (20.7)	23 (79.3)	
Education			
Secondary	7 (10.8)	58 (89.2)	<0.05
OND/Tech	8 (38.1)	13 (61.9)	
Univ/Poly	34 (13.1)	225 (86.9)	
Others	2 (8.7)	21 (91.3)	
Marital status			
Single	24 (14.0)	148 (86.0)	0.720
Married	7 (14.1)	165 (85.9)	
Widowed	21 (6.7)	14 (93.3)	
Hospital			
LUTH	28 (19.0)	119 (81.0)	<0.05
OAUTHC	16 (10.2)	141 (89.8)	
EKSUTH	9 (9.8)	83 (90.2)	

Internet sites visited and type of health information obtained

One hundred and twenty (65.2%) visited internet sites providing general medical information, 63 (34.2%) visited disease specific sites maintained by individuals, universities, hospitals, support groups and other organizations, 28 (15.2%) visited retail sites offering or selling some kind of therapy while 19 (10.3%) visited online fora and chat rooms. One hundred and eighty four (70.8%) of the patients flagged off their internet search by using a general search engine such as Google, Yahoo and Bing. Only eight (3.1%) got their source from their healthcare provider. Table 5 shows the type of medical information obtained by the patients from the Internet.

Table 5: Types of health information obtained by patients

Type of health information	n(%)
Information about illness	132 (54.5)
Information on nutrition or fitness	67 (14.6)
Information about specific doctors, hospitals or drug	18 (3.9)
Information on alternative medicine	13 (2.8)
Information on experimental treatment	11 2.4)

* Multiple responses

Patients' assessment of the Internet Health information

Ninety percent of the patients found the information they obtained from the Internet either very useful or somewhat useful. In addition, 89.1% of the patients learnt something new about their illnesses when they accessed the internet for health information. However only 35.5% discussed the information obtained with their healthcare professionals while the remaining 64.5% never bothered to discuss their findings about their findings with their healthcare professional. One hundred and forty nine 149/459 (32.4%) patients came to a new decision as a result of their internet browsing for medical information (Table 6).

Table 6: Access to internet medical information and patients' subsequent actions regarding their illnesses

Decision based on internet medical information*	n(%)
Asked new questions about their illnesses or seek second opinions regarding the illness	70(36.3)
Change the way patient coped with illness	49(25.4)
Caused patient to see the doctor	40(20.7)
Modify treatment	34(17.6)
Did not take any new decision	31(17.2)

**Multiple responses*

Discussion

Historically, most patients would have consulted many sources including books, family members and friends before deciding on the need to consult a physician. The Internet has been added to the patients' domestic armamentariums, enabling them to seek more information about their illnesses. For

obvious reasons, this empowerment has been more pronounced in the developed world than in the developing world. However, as this study has shown, the Internet has become an important source of medical information among patients in the south western Nigeria[5]. In this study, about two out of every five patients in this study had accessed the internet for medical information within the past one year. We did not ask the respondents to compare the frequency with which they accessed the internet for medical information with their other online activities such as using e-mail, social media or checking for sport information. However, the fact that the patients who specifically used the Internet for seeking health information were more than 50% of those who accessed the internet showed that seeking health information is one of the frequent online activities among Nigerian orthopaedic patients.

The most common device for accessing the internet for medical information among the patients were smartphones. Internet penetration in Nigeria grew from a paltry 0.06% in 2000 to 33% in 2012[6 7]. The major driving force behind this growth are the mobile telecommunication companies. Most urban and semi-urban cities in Nigeria now have 3G services, while most rural communities have EDGE cellular technology. Broadband technology is still limited to urban centres like Lagos, Abuja and Port-Harcourt. 2013 data showed that about 74% of the 56 million active Internet users in Nigeria use their phone to access the Internet [4].

A significant proportion of patients accessed the internet for medical information and obtained useful significant information [8]. This is a cause for concern among health policy workers. Patients, unlike healthcare providers, are not trained to manage medical information. Consequently it is difficult to differentiate good and bad medical information [9].

Thus, the Internet information they assumed to be "useful" may in actuality be harmful. For example, Nadam et al. in 2005 evaluated the quality of information available on websites about clubfoot using a scoring system [10]. The average score of the websites was an abysmal 26 (maximum obtainable score was 100). The authors concluded that "This may result in misleading information and the real possibility that patients may be misinformed before they reach the consultation stage (e.g. web sites with poor individual experiences can adversely affect the decisions taken by patients)" [10].

In this study, more than four out of every five patients who had Internet medical information came to new decisions as a result. Such information empowered patients to take more active roles in decision making about access and treatment modality. On the other hand, patients are also in danger of being overwhelmed by the information deluge which does not pass through traditional editorial process [8]. The physician must play the role of a sheperd, guiding the patient through the information wilderness the Internet has turned out to be. Thus physicians themselves should know websites with appropriate medical information suitable for patient education. Many studies have shown academic websites to be the most reliable sources of medical information on the Internet. Physicians should inform patients about such sites [10-12].

Most repositories of acceptable medical information on the internet are maintained by institutions based in developed countries of North America and Europe, these are mainly focused on issues considered important to those countries. Currently, none of the medical websites maintained by Nigerian based organizations provide the same level of quality of information that institutions such as the Mayo Clinic or American Academy of Orthopaedic Science (AAOS) provide for the American patient. There is a need for academic institutions in Nigerian to start providing reliable, locally relevant internet medical information for patients in Nigeria.

As with similar studies conducted in other countries, patients with post-secondary school education were more likely than those with secondary school education to use the Internet for medical education as well as communicate with their healthcare providers through e-mail. Similarly patients in Lagos, the commercial capital of Nigeria were more likely to carry out these activities than their counterparts in Ado-Ekiti and Ile-Ife, where there are more limited access to internet.

In conclusion, this study has established that a sizable proportion of patients attending the orthopaedic clinics in urban and semi-urban cities in Nigeria access the internet for medical information consistently. There is a need for medical practitioners in the Nigeria and indeed, other developing countries to take advantage of this fact by directing the patients to reliable repositories of medical information on the Internet Furthermore, academic and medical institutions should also begin to provide medical information on the internet that is locally relevant and culturally acceptable to their clientele. For as stated Edejer: "the way forward (for healthcare providers) is to exploit the full interactivity of the internet, which allows rapid feedback and change to continuously mould information into useful knowledge"[13].

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Plantar dermatoglyphic traits of sub-Saharan African subjects

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Abstract
Introduction: Dermatoglyphic traits are important reliable tools for establishing and confirming population relationship, however not much has been documented in sub-Saharan Africa.
Materials and methods: The sole was mapped into 10 topographical zones based on Cummins and Midlo nomenclature and these were used to characterize the study. Bilateral plantar and digital prints of the soles were obtained by the inking procedure of Cummins and Midlo and the patterns of arches, loops and whorls were classified and counted using standard methods. Dankmeijer's (D1) and pattern intensity (PII) indices were determined and the frequencies of the ridge patterns compared in all subjects.
Results: In Nigerians, loop patterns were present in the proximal and distal plantar soles while whorls were limited to the distal zones I – V, but more prominent in zone I but least in zone V. the loop and whorl patterns were significantly higher in males than in females ($p<0.05$). In Kenyans and Tanzanians, loops and arches were present in the soles but loops occurred more than arches as was the case in Malawians and Zimbabweans. Whorls were however, restricted to the distal sole of zones I-V in men but only present in zones III & I in Tanzanian women. Whorls were only present in zones I-V with zone I having the highest occurrence in Malawians and Zimbabweans. Quantitative variables of DI and PII did not show any significant differences within the groups studied.
Conclusion: The study has shown that digital patterns are better indices for differentiation of population groups.

Keywords: *Plantar, Dermatoglyphics, Sub – Saharan, African Subjects.*

Résumé
Introduction: Les traits dermatoglyphiques sont d'importants outils fiables pour établir et confirmer les relations de population, mais peu de choses ont été documentées en Afrique subsaharienne.
Matériels et méthodes : La plante du pied a été cartographiée en 10 zones topographiques basées sur la nomenclature Cummins et Midlo et celles-ci ont été utilisées pour caractériser l'étude. Les empreintes plantaires et digitales bilatérales des plantes des pieds ont été obtenues par la procédure d'encrage de Cummins et Midlo et les modèles d'arches, de boucles et de verticilles ont été classés et comptés en utilisant des méthodes standard. Les indices de Dankmeijer (D1) et d'intensité de modèle (PII) ont été déterminés et les fréquences des profils de crête ont été comparées chez tous les sujets.
Résultats: Chez les Nigérians, les modèles de boucles étaient présents dans les zones plantaires proximales et distales des plantes des pieds, tandis que les verticilles étaient limitées aux zones distales I - V, mais plus proéminentes dans la zone I mais moins dans la zone V. Les modèles de boucle et de verticille étaient significativement plus élevés chez les mâles que chez femelles ($p<0,05$). Chez les Kenyans et les Tanzaniens, les boucles et des arches étaient présents dans les plantes des pieds, mais les boucles se sont produites plus que les arches, comme c'était le cas chez les Malawiens et les Zimbabwéens. Les verticilles étaient cependant limitées à la sole distale des zones I - V chez les hommes mais seulement présentes dans les zones III et I chez les femmes Tanzaniennes. Les verticilles n'étaient présents que dans les zones I – V avec la zone I étant la plus fréquente chez les Malawiens et les Zimbabwéens. Les variables quantitatives de DI et PII n'ont montré aucune différence significative au sein des groupes étudiés.
Conclusion: L'étude a montré que les modèles numériques sont de meilleurs indices pour la différenciation des groupes de population.

Mots clés: *Mots clés : Plantaire, Dermatoglyphiques, Sub - Saharienne, Sujets Africains.*

Introduction

Dermatoglyphic traits are reliable tools for establishing and confirming historical relationship between and within populations [1-3]. These traits are also genetically determined and the mode of inheritance is multifactorial [4].

The importance of plantar and digital dermatoglyphics in race studies has also been documented mostly on Caucasians. These studies [5-7] were carried out on Germans, European-Americans, Chinese, Japanese, Forest Nentsy, Nganasans and Chukchi and racial differences were shown in the frequency of pattern types in all the

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different groups. Despite the above, however, the dermatoglyphics of Chinese, Canadians and Charote Indians gave useful indications for the anthropological characterization of these groups [5].

In African populations, fewer plantar dermatoglyphic studies have been documented. In the Ibos of Nigeria, greater prominence of toe arches was found in males than in females, with the pattern intensity index being higher in females than in males and this appeared characteristics to the Ibos [8].Among the Moroccan Arabs, the prominence of digital arches was the most striking feature when compared to other Mediterranean groups [2]. The Urhobos of Southern Nigeria had the characteristic feature of less whorl distribution in the small toe of female subjects, and the complete absence of whorls in the male subjects [3]. These studies, however, highlighted the role of digital patterns in the characterization of ethnic groups. In a similar study involving Zimbabwean and Malawian subjects, the uniqueness of digital ridge patterns in differentiating population groups was further emphasized [9-10].

Despite these studies, which showed the importance of dermatoglyphics in general, not much work has been documented for African subjects especially those in sub-Saharan Africa. Jantz and colleagues [11] in 1982 grouped black Africans into two groups, namely Niger- Congo and Benue- Congo on linguistic grouping disparities. Could this linguistic grouping disparity be clearly demarcated on dermatoglyphic lineage? Could sub-Saharan African subjects be differentiated dermatoglyphically? Earlier studies have shown that Nationals of the same linguistic group do have certain dermatoglyphic traits that are similar and some that do differentiate them [3,5,10,12]. In the light of the above, and in an attempt to answer some of the questions raised above, this study was carried out on Nigerians (Yorubas, Hausas, Ibos and Urhobos), Malawians, Zimbabweans, Kenyans and Tanzanians.

Materials and methods

Nigerians

The sample consisted of 414 Ibos (260 males, 154 females) 408 Urhobos (212 males, 196 females) aged between 12 and 77 years. They were all physically healthy subjects with confirmed Urhobo and Ibo parents and grandparents respectively.

Kenyans and Tanzanians

The sample consisted of 304 Kenyans (164 males, 140 females) aged 12-14 years and 300 Tanzanians (180 males, 120 females) age 19-25 years. The subjects were all physically healthy with confirmed Kenyan and Tanzanian parents and grandparents, respectively.

Malawians and Zimbabweans

The sample consisted of 231 Malawians (142 females, 89 male) aged 11-39 years and 315 Zimbabweans (150 females) aged 11-47 years. The subjects were all physically healthy with confirmed Malawian and Zimbabwean parents and grandparents, respectively. The subjects representing the countries were selected from cosmopolitan cities, which gave a good mixture of social backgrounds to allow for the inclusion of quantitative plantar variables [13].

In the case of related individuals in the samples, only the print of one of them was included in the study to avoid analysis bias during the estimation of precision (e.g. standard error) for the population sample [14].

Topographical zones of the sole

The sole was mapped into 10 topographical zones based on Cummins and Midlo [15] nomenclature (1961), where zones I-V represented the distal plantar sole and zones VI-X represented the proximal plantar sole. These were used to describe the characteristics reported in the study (Table 1, Fig. 1).

Bilateral plantar and digital imprints (Fig. 2) of the right and left soles were made on paper, after application of ink to the sole with the aid of a hand lens, the prints were read and classified according to the method of Loesch and Skrinjaric (1979)[16]. The frequencies of these ridge patterns were recorded, expressed as percentages of the total pattern types and analyzed, each patterns being treated separately and by gender.

Table 1: Classification of the sole of the foot using Cummins and Midlo's (1961) nomenclature

Topographical Zones	Nomenclature	Topographical zone	Nomenclature
I	Hallucal	VI	Hypothenar
II	Second Interdigital	VII	Hypothenar Proximal
III	Third Interdigital	VIII	Calcar (heel)
IV	Forth Interdigital	IX	Thenar Proximal
V	Hypothenar distal	X	Thenar distal

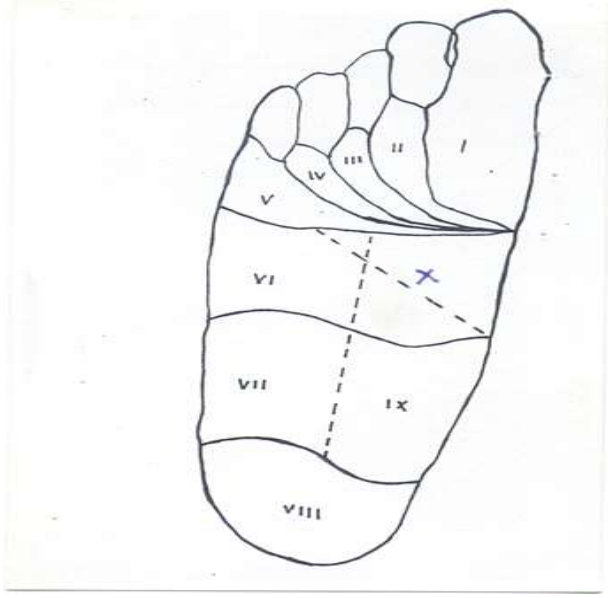

Fig. 1: *Classification of zones of the soleof foot by Cummins and Midlo's nomenclature*

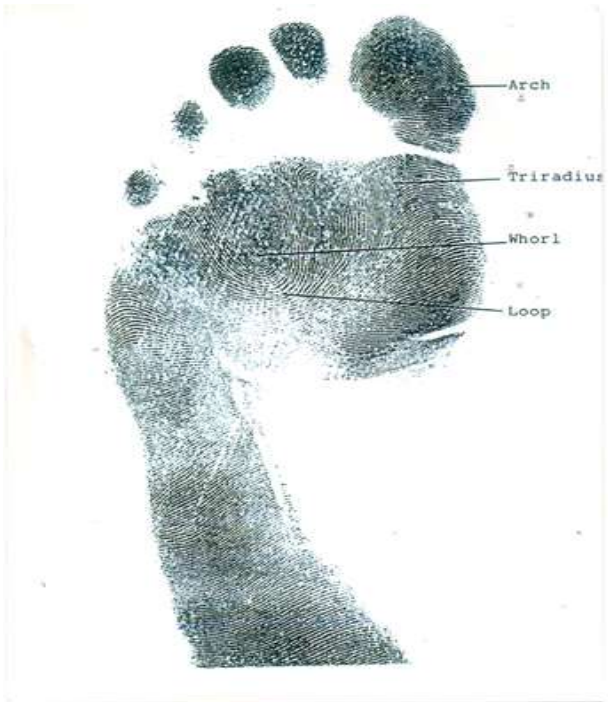

Fig. 2: *Foot print illustrating arches, whorts and triradii*

The Dankmeijer's [17] (DI) and pattern intensity (PII) Indices determined the digital variability of patterns. The DI was the total frequency of whorls ×100, while the PII was the mean number of triradii found per individual. Using these indices, the frequencies of ridge patterns were compared in all subjects. Inter observer variation in counting was eliminated as only one person examined all the prints. Chi-square tests were applied to discrete variables of arches, whorls and t-tests for quantitative variables of PII and DI.

Ethical considerations

All adult subjects used for the study gave informed consent while those subjects under the age of full legal responsibility had their parents to give consent after the study was explained to them.

Results

Topographical zones and the plantar ridge patterns

In Nigerians, loop patterns were present in the proximal and distal plantar soles while whorls were limited to the distal zones I-V and more predominant in zone I but least in zone V (Table 2a).

The loop and whorl patterns were significantly higher in males than in females ($P < 0.05$). In Kenyans and Tanzanians, loops and arches were present in the proximal and distal soles with loops occurring more than arches. Again whorls were restricted to the distal sole of zones I-V in all Kenyan subjects and Tanzanian men but only in zones III and I in Tanzanian women (Table 3b). Similarly, loops and arches were present in the proximal and distal plantar soles in Malawians and Zimbabweans with more loops than arches. These ridge patterns were, however, more in the proximal than distal plantar soles like in the Kenyan and Tanzanian subjects. Whorls were only present in zones I-V with zone I having the highest occurrence (Table 3a).

Digital pattern types

In the Ibos of Nigerian, arches and loops were abundant in all the toes with arches being more predominant. On the big toes, however, whorls were more than in the other digits but no whorls was present on the small toes. The Urhobos of Nigeria also showed similar features but had higher frequency of whorls in the big toes than the Ibos. Again whorls were absent only on the small toes of male Urhobo subjects (Table 2b). Whorl distribution was also more on the right small digit than on the left small digit in the female Urhobos (Table 2b).

In both Ibos and Urhobos, Dankmeijer's index (DI) was higher in males than females while the mean PII was higher in females than in males. The mean PII for Urhobo males and females were, however, lower than males for the Ibos but these values were not significantly different from each other ($P > 0.05$; Table 2c).

Arches were the most predominant ridge patterns found on the digits in Kenyan and Tanzanian subjects. Loops were, however, the most predominant features in the first digit. The percentage frequency of loops on the first digit was higher in Tanzanians than Kenyans. Loops were absent on the fifth digit in Tanzanian women but the

Table 2a: The mean frequency of whorls on the distal part of the sole (Zones I -V) expressed as a percentage in Nigerians

Gender	Limbs	Topographical zones- Ibo subjects						Topographical zones-Urhobo subjects					
		n	I	II	III	IV	V	n	I	II	III	IV	V
Male	Left	260	57.30	35.00	24.20	13.10	8.85	212	75.00	16.00	25.50	18.40	15.10
	Right	260	54.40	33.50	20.00	16.20	8.08	212	73.60	14.20	30.20	18.90	15.10
Female	Left	154	55.80	7.31	14.90	11.70	7.14	196	72.50	16.80	33.70	17.90	15.30
	Right	154	61.70	16.90	25.30	15.60	8.44	196	68.40	16.80	33.70	17.40	15.30
Male &	Left	414	58.90	26.60	20.80	12.60	8.21	408	73.80	16.80	29.40	18.10	15.20
Female	Right	414	57.70	27.30	21.90	15.90	8.21	408	71.40	15.40	31.90	18.10	15.20
Total for n and means for zones		828	58.30	26.95	21.35	14.25	8.21	816	72.60	16.10	30.65	18.10	15.20

Table 2b: The digital frequency of whorls, loops and arches on toes expressed as percentage in Nigerians

Sex	Limbs	Digital pattern types-Ibo						Digital pattern types-Urhobo					
		N	1	2	3	4	5	n	1	2	3	4	5
<u>Whorls</u>													
Male	Left	260	8.90	4.60	1.20	0.40	0.00	212	14.60	8.50	16.50	3.80	0.00
Female	Right	260	6.90	3.50	1.50	0.70	0.00	212	14.60	11.30	12.70	4.70	0.00
<u>Loops</u>													
Male	Left	154	9.70	1.90	0.60	0.00	0.00	196	10.20	16.30	19.90	3.60	0.50
	Right	154	11.00	5.80	1.30	0.00	0.00	196	8.67	16.80	19.38	7.14	2.04
Female	Left	260	75.80	40.80	32.70	21.90	12.00	212	45.80	16.00	6.10	3.30	3.30
	Right	260	58.80	36.80	24.60	18.50	11.10	212	46.70	14.50	14.62	1.89	5.20
<u>Arches</u>													
Male	Left	154	66.20	60.40	37.00	20.10	10.40	196	75.10	33.70	21.90	13.30	6.10
	Right	154	51.30	54.50	34.40	16.90	7.80	196	54.60	33.30	20.90	13.30	8.20
Female	Left	260	72.70	34.20	20.30	23.80	16.50	212	44.80	71.20	85.90	67.90	42.01
	Right	260	67.70	34.20	20.30	23.80	16.50	212	44.80	74.50	82.90	67.90	42.01

Table 2c: Comparison of digital patterns between Ibo and Urhobo subjects of Nigeria using the pattern intensity and Dankmeijer's indices

Variables	Ibo		Urhobo	
PII	11.60	11.90	11.20	11.40
DI	6.88	4.98	6.53	4.77

absence was confined to the left sole in Kenyan women (Table 3b). The percentage frequency of arches increased from the first to the fourth digit in all subjects but arches were absent in Tanzanian women in the first digit (Table 3b). Whorls were restricted to the first digit in Kenyan women but absent in Tanzanian men. Similarly, whorls were absent on the fourth and fifth digits in Tanzanians. Tanzanian women sampled lacked whorls on their first digit and this was their characteristic feature (Table 3b).

In Malawians, arches were again the most predominant ridge patterns found in the digits. Loops were next in predominance and whorls were absent on all digits. Loops were also absent on the small

Table 3a: The mean frequency of whorls on the distal part of the sole (Zone I -V) expressed as a percentage

Gender	Limbs	Digital Pattern types - Kenyans						Digital Pattern types - Tanzanians					
		n	I	II	III	IV	V	n	I	II	III	IV	V
Male	Left	164	56.10	1.22	4.88	6.10	2.44	180	80.00	11.00	13.33	4.44	4.44
	Right	164	59.76	3.66	4.88	7.32	2.44	180	80.00	8.89	13.33	8.89	2.22
Female	Left	140	64.29	1.43	10.00	7.14	4.29	120	83.33	0.00	8.33	0.00	0.00
	Right	140	68.57	2.86	2.86	11.43	4.29	120	83.33	0.00	0.00	0.00	0.00
Male and	Left	304	63.87	1.32	7.24	3.29	3.29	300	80.70	8.77	12.80	3.51	3.51
Female	Right	304	63.82	3.29	3.95	9.21	3.29	300	82.46	7.02	10.53	7.02	1.75
Total for n and **means for zones**		608	63.85	2.26	5.60	6.25	3.29	600	81.58	7.90	11.67	5.27	2.63

Table 3b: The digital frequency of whorls, loops and arches on toes expressed as percentage

Sex	Limbs	Digital pattern types-Kenyan						Digital pattern types-Tanzanian					
		n	1	2	3	4	5	n	1	2	3	4	5
a) Angels													
Male	Left	164	3.66	2.44	2.44	1.22	0.00	180	6.67	2.22	2.22	0.00	0.00
	Right	164	2.44	1.22	1.22	0.00	0.00	180	0.00	2.22	2.22	0.00	0.00
Female	Left	140	4.29	0.00	0.00	0.00	0.00	120	8.33	8.33	0.00	0.00	0.00
	Right	140	1.43	0.00	0.00	0.00	0.00	120	8.33	0.00	0.00	0.00	0.00
b) Loops													
Male	Left	164	53.66	14.63	10.98	7.32	2.44	180	91.11	26.67	6.67	6.67	6.67
	Right	164	48.78	19.51	13.42	8.54	2.44	180	97.78	26.67	6.67	6.67	6.67
Female	Left	140	58.57	14.29	15.17	5.71	0.00	120	100.00	25.00	8.33	16.67	0.00
	Right	140	45.75	21.43	21.43	10.00	1.43	120	100.00	25.00	25.00	16.67	0.00
c) Arches													
Male	Left	164	20.00	36.36	47.27	45.45	52.73	180	2.22	2.22	68.89	91.11	84.44
	Right	164	12.73	34.55	41.82	47.27	45.45	180	2.22	2.22	65.22	91.11	84.44
Female	Left	140	14.00	54.00	62.00	62.00	52.00	120	0.00	0.00	58.33	82.33	91.67
	Right	140	14.00	38.00	14.00	60.00	50.00	120	0.00	0.00	66.67	83.33	91.67

Table 3c: Comparison of digital patterns between Kenyans and Tanzanians using the pattern intensity and Dankmeijer's indices

Variables	Kenyans		Tanzanians	
PII	6.47	6.50; 11.90	8.44	8.72
DI	3.00	3.62	2.39	3.34

digits. The presence of loops on the big toe was significantly greater in males than in females ($P < 0.05$; Table 4b). In Zimbabweans, arches also predominated on the digits. However, loops were the most predominant in the first digit and next in overall predominance to arches. Loops were absent in digits 4 and 5 in men while whorls were very few and restricted to the first digit (Table 4b).

Table 4c compares the digital variables of PII and DI between Malawians and Zimbabweans. In both groups the mean PII was higher in males than females while DI was higher in females than males. However, for both gender the PII was significantly lower in Zimbabweans than Malawians ($P < 0.001$).

Discussion

In common with other sub- Saharan populations, the plantar ridge patterns exhibited in this study were consistent with those previously reported, an indication that plantar patterns may not differentiate population groups. The present study employed both qualitative traits (digital print pattern frequencies) and quantitative methods (DI and PII) and the results obtained were therefore comparable with previous work on African populations [18].

Table 4a: The mean frequency of whorls on the distal part of the sole (zones I -V) expressed as percentage

Sex	Limbs	Topographical Zone-s Malawians subjects						Topographical Zone-s Zimbabweans subjects					
		n	I	II	III	IV	V	n	I	II	III	IV	V
Male	Left	89	40.5	6.70	13.50	13.50	0.00	150	60.00	0.00	10.00	0.00	0.00
	Right	89	40.5	0.00	13.50	6.70	0.00	150	40.00	0.00	10.00	0.00	0.00
Female	Left	142	50.00	4.20	2.40	4.20	0.00	165	54.55	0.00	9.09	18.18	0.00
	Right	142	50.00	0.00	8.50	8.50	0.00	165	54.55	0.00	0.00	9.09	0.00
Male &	Left	231	56.60	4.30	10.00	10.00	0.00	315	57.14	0.00	9.52	9.25	0.00
Female	Right	231	56.60	2.60	11.30	11.30	0.00	315	47.62	0.00	4.76	4.76	0.00
Total		462	56.60	3.45	10.65	10.65	0.00	630	52.38	0.00	7.14	7.14	0.00

Table 4b: The digital frequency of whorls, loops and arches on toes expressed as percentage

Sex	Limbs	Digital pattern types-Malawians						Digital pattern types-Zimbabweans					
		n	1	2	3	4	5	n	1	2	3	4	5
a) Whorls													
Male	Left	89	0.00	0.00	1.20	0.40	0.00	212	14.60	8.50	16.50	3.80	0.00
	Right	89	0.00	0.00	1.50	0.70	0.00	212	14.60	11.30	12.70	4.70	0.00
Female	Left	142	0.00	0.00	0.60	0.00	0.00	196	10.20	16.30	19.90	3.60	0.50
	Right	142	0.00	0.00	1.30	0.00	0.00	196	8.67	16.80	19.38	7.14	2.04
b) Loops													
Male	Left	89	73.00	27.00	32.70	21.90	15.00	212	45.80	16.00	6.10	3.30	3.30
	Right	89	86.50	33.70	24.60	18.50	11.10	212	46.70	14.50	14.62	1.89	5.20
Female	Left	142	71.10	25.60	37.00	20.10	10.40	196	75.10	33.70	21.90	13.30	6.10
	Right	142	66.90	33.10	34.40	16.90	7.80	196	54.60	33.30	20.90	13.30	8.20
c) Arches													
Male	Left	89	100.00	100.00	20.30	23.80	16.50	212	44.80	74.50	85.90	67.90	42.01
	Right	89	100.00	100.00	25.00	22.70	13.10	212	27.80	74.50	55.20	62.30	49.50
Female	Left	142	66.90	100.00	23.40	26.00	14.90	196	31.10	47.50	34.10	71.40	43.40
	Right	142	92.30	100.00	24.70	24.00	21.40	196	33.20	46.40	54.10	71.40	46.40

Table 4c: Comparison of digital patterns between Malawians and Zimbabweans using the pattern intensity and Dankmeijer's

Variables	Malawians		Zimbabweans	
PII	7.65	6.66	11.67	11.56
DI	9.76	10.13	6.08	6.13

Table 5: Digital pattern variation in sub-Saharan African population

	West Africans	East Africans	South Africans
Males			
Arches	9.27 – 13.18	4..89 – 4.99	10.00
Radial Loops	1.83 – 3.22	6.34 – 6.86	5.55 – 7.61
Ulnar Loops	51.20 – 61.90	67.22 – 72.62	60.00 – 72.22
Whorls	23.30 – 32.40	16.05 – 19.40	12.32 – 22.23
Females			
Arches	10.90 – 14.89	2.89 – 3.33	10.00
Radial Loops	1.91 – 2.60	6.70 - 7.50	5.51 – 6.67
Ulnar Loops	52.42 – 62.90	69.65 – 75.00	39.59 – 78.33
Whorls	24.50 – 32.10	14.17 – 20.75	5.00 – 45.20

The plantar patterns shown in this study, demonstrated some similarities between Kenyan and Tanzanian subjects as indeed with Malawians [3,8]. This notwithstanding, there appeared to be some plantar patterns peculiar to the two groups. For instance, whereas whorls were absent in zones II, IV and V in Tanzanian women, this was not the case in Kenyan women. Digital whorls were absent in all digits except the first digits in women as against only the fifth digit in Kenyan men. Although the prominence of digital arches and loops in the present study were also observed in Malawian and Zimbabwean subjects [9-10], the digital features exhibited in the present study showed that digital whorls, arches and loops differed sufficiently to differentiate these population groups in the sample. They also showed greater affinity between Kenyans and Tanzanians than Malawians and Zimbabweans. However, if these features were compared with those of Nigerians[3,8], it could be seen that the East African subjects had greater dermatoglyphic affinity and were thus closer to Malawians and Zimbabweans than Nigerians.

The present study has also demonstrated lack of sexual dimorphism in PII and DI, as was the case with Malawians and Zimbabweans. Both PII and DI were higher in men than women in both Kenyan and Tanzanian subjects whereas in Malawians and Zimbabweans the PII was higher in men than women while DI was higher in women than men. Despite the foregoing, it was found that the PII indices for Kenyans were closer to those of Malawians, while those of Tanzanians lay midway between Kenyan, Malawian and those of Nigerian, Zimbabweans and Caucasians on the higher side. The DI values for Kenyans and Tanzanians, however, were the lowest so far recorded for sub-Saharan Africans.

The present study has demonstrated the normal plantar and digital dermatoglyphic features of Kenyan and Tanzanian subjects indicating affinities and anthropological relationship between them. However, their digital dermatoglyphic features differentiated them. Although East Africans were different from Malawians and Zimbabweans of Southern Africa, their dermatoglyphic features were closer to Malawians and Zimbabweans than Nigerians. These findings seem to correspond to the linguistic disparity between West and East Africans, confirming that dermatoglyphic features express affinities and differences between sub-Saharan African peoples.

Most of the plantar ridge patterns exhibited in this study were consistent with what had been shown in other races and ethnic groups, an indication that these patterns may not differentiate races. This not withstanding, there appeared to be some plantar ridge patterns that may be peculiar to Zimbabweans; although it must be pointed out that the frequency of whorls in zone II among Malawians was 3.5%. In the Urhobos of Nigeria, whorls being present in zone II could thus serve as a distinguishing feature between Zimbabweans, Malawians and Nigerians. This finding accorded the observation that Negroes exhibit low frequency of whorls [5].

The prominence of digital arches and loops in the sample was consistent with observation in Malawians [9], an indication of dermatoglyphic affinity. However, the prominence of loops in the first digit and their absence in digits 4 and 5 in Zimbabwean men are the opposite of what was found in Malawians were loops were absent in the small digits only [9]. These features were not shown in Nigerian studies earlier documented [3].

The present study has also demonstrated that PII and DI did not differentiate dermatoglyphic ridge patterns by gender, as indeed was the case with Malawians. These parameters, however, differentiated Zimbabweans from Malawians [9] and Caucasians [5,19,20] and the reverse was the case with Nigerians [8,9]. Despite the forgoing, Caucasians, Nigerians and Zimbabweans tended to have higher PII values than Malawians do. The DI values were nevertheless higher in females than males and this was consistent with most literature. In 1961, Holt [20] observed this when the showed that almost without exception women had higher frequency of arches and fewer whorls, thereby making the DI value to be higher in females.

The study has exhibited normal plantar and digital ridge patterns of Zimbabweans that indicate affinities with Malawians. These features were characterized better in digital than plantar ridge patterns, further proof that digital patterns are better indices for differentiation of population groups as shown by previous studies [3,8,9]. The affinities demonstrated in this study further indicate the close historical and anthropological relationship between Zimbabweans and Malawians, a point that becomes clearer if both groups are compared dermatoglyphically with Nigerians [3,8]. In this study their digital patterns types could differentiate Zimbabweans better from Malawians, further emphasizing the uniqueness of digital ridge patterns in differentiating population groups. When both plantar and digital features are considered, Zimbabweans are closer to Malawians than Nigerians are, hence dermatoglyphic traits could be used to explore affinities and differences between

population groups. This notwithstanding, there appeared to be some plantar ridge patterns that may be peculiar to Zimbabweans. In this study their digital pattern types could differentiate Zimbabweans better from Malawians, further emphasizing the uniqueness of digital ridge patterns in differentiating population groups.

When both plantar and digital features are considered, Zimbabweans are closer to Malawians than Nigerians are, hence dermatoglyphic traits could be used to explore affinities and differences between population groups.

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Effects of antacids on the pharmacokinetics of lumefantrine in healthy volunteers: A pilot study.

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Abstract

Background: Artemether-lumefantrine (A-L), an artemisinin-based combination therapy (ACT), is a widely used antimalarial drug and it could be prescribed together with antacids since malaria may co-exist with peptic ulcer. This study aimed to determine possible interaction following concurrent administration of A-L and commonly used antacids, and to monitor possible corrected-QT (QTc) interval prolongation.

*Methods***:** In a randomized crossover study, single oral dose of A-L (80/480 mg) tablet alone or in combination with antacid formulation (magnesium trisilicate, magnesium carbonate, sodium bicarbonate combination) were administered to 13 healthy volunteers after overnight fast. Blood samples were collected at predetermined time intervals and plasma samples for six volunteers were successfully assayed for lumefantrine using High performance Liquid Chromatography (HPLC). Electrocardiographic recording was carried out at predetermined times.

Results: The median lumefantrine AUC_{0-96} of 173 µg.hr/ml (IQR: 72.11-688.51) and 221.96 µg.hr/ml (IQR: 64.21-465.47) were obtained when A-L was taken alone and in combination with antacid formulation respectively. The median lumefantrine C_{max} for A-L alone and for A-L plus antacid formulation were 5.92 µg/ml (IQR: 2.08-14.44) and 4.42 µg/ml (IQR: 3.84-14.30) respectively. The mean QTc intervals obtained at pre-dose, 6, 72 and 504 hours post-dose were 390.08±19.84, 406.23±19.04, 394.60±19.91 and 396.33±23.94 ms respectively. The lengthening of the QTc interval at 6 hr post-dose compared to zero (0) hr was statistically significant ($P<0.05$).

Conclusion: In this preliminary study, antacids did not appear to alter the reported erratic bioavailability of lumefantrine in human. Also, the limited increase in QTc interval caused by lumefantrine was not clinically significant.

Keywords: *Artemether-lumefantrine, QTc interval, antacids, antimalarial, drug interaction.*

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Résumé

Contexte: L'artéméther-luméfantrine (A-L), une association thérapeutique à base d'artémisinine (ACT), est un médicament antipaludique largement utilisé qui peut être prescrit avec des antiacides, car le paludisme peut coexister avec l'ulcère peptique. Cette étude visait à déterminer l'interaction possible après l'administration concomitante d'A-L et d'antiacides couramment utilisés, et à surveiller l'allongement possible de l'intervalle QT corrigé (QTc).

Méthodes **:** Dans une étude croisée randomisée, une dose orale unique de comprimé A-L (80/480 mg) seul ou en combinaison avec une formulation antiacide (trisilicate de magnésium, carbonate de magnésium, bicarbonate de sodium) a été administrée à 13 volontaires sains après une nuit de jeûne. Des échantillons de sang ont été recueillis à des intervalles de temps prédéterminés et des échantillons de plasma pour six volontaires ont été testés avec succès pour la luméfantrine en utilisant une Chromatographie liquide à haute performance (HPLC). L'enregistrement électrocardiographique a été effectué à des moments prédéterminés.

Résultats: La luméfantrine médiane AUC_{0-96} de 173 µg.hr / ml (IQR: 72,11-688,51) et 221,96 µg.hr / ml (IQR: 64,21-465,47) ont été obtenues lorsque l'A-L était prise seule et en combinaison avec la formulation antiacide respectivement. La luméfantrine médiane Cmax pour A-L seul et pour A-L + formulation antiacide étaient 5,92 µg / ml (IQR: 2,08-14,44) et 4,42 µg / ml (IQR: 3,84-14,30) respectivement. Les intervalles QTc moyens obtenus avant administration de la dose, 6, 72 et 504 heures après administration étaient de 390,08 ± 19,84 ; 406,23±19,04 ; 394,60±19,91 et 396,33±23,94 ms respectivement. L'allongement de l'intervalle QTc à 6 heures post-dose par rapport à zéro (0) h était statistiquement significative ($P0<05$).

Conclusion: Dans cette étude préliminaire, les antiacides ne semblent pas modifier la biodisponibilité erratique de la luméfantrine chez les humains. De plus, l'augmentation limitée de l'intervalle QTc causée par la luméfantrine n'était pas cliniquement significative.

Mots - clés: *artéméther-luméfantrine, intervalle QTc, antiacides, antipaludéen, interaction médicamenteuse*

Introduction

Malaria disease burden continues to increase as the countries in which it is endemic face the risk of widespread resistance of the parasite to conventional anti-malarial drugs [1]. It is an important cause of death and illness in children and adults, especially in tropical countries. World Health Organization (WHO) recommended the use of artemisinin-based combinations (ACTs) in 2001 [2]. Artemether-lumefantrine (A-L) is the most widely used ACT today and is recommended as the first-line treatment in many tropical countries including Nigeria.

The combined effect of the drugs in the battle against malaria parasites is additional benefit of combination therapy. In the case of artemether-lumefantrine as stated by Lefevre *et al*, artemether has a short half-life (2-3 hrs in healthy individuals), and a fast onset of action, while lumefantrine with longer half-life has slow onset of action and hence clears residual parasite and prevents recrudescence [3]. A-L has a wide therapeutic index with high variability in lumefantrine plasma levels, mostly influenced by food intake. Lumefantrine plasma level has also shown to be influenced by other drugs such as mefloquine when the two drugs are co-administered [3].

This suggests the possibility of drug-drug interaction between A-L and other drugs. Although, A-L has a wide therapeutic window and is very effective against multi-drug resistant *Plasmodium falciparum* malaria, significant interaction may alter plasma concentration to such a degree that the clinical efficacy of the drug may be affected. *In-vitro* study showed that antacids *markedly* adsorbed lumefantrine [4], suggesting that antacids may decrease the bioavailability of lumefantrine and diminish its anti-malarial activity. Peptic ulcer may co-exist with malaria, hence such ulcer patients may be taking A-L with antacids.

Generally, drug-drug interaction is a common phenomenon in polypharmacy. It is therefore essential to investigate and confirm this *in vitro* result with *in-vivo* studies to predict the clinical implications. In addition, an antimalarial drug, halofantrine (an aryl amino alcohol), an antimalarial with similar structure to lumefantrine has been reported to have significant pharmacokinetic interaction with antacid in Nigerians [5].

Hence, it is important to also investigate *in-vivo* for possible interaction between lumefantrine and antacids, as information on this is lacking. Also, cardiotoxic potential of aryl amino class of antimalarial agents including halofantrine [6-8] and quinidine [9] has been reported. Although the reports so far indicated that lumefantrine has no potential cardiotoxic effect [10-12], QTc interval was monitored in this study to evaluate the effect of concomitant administration of antacids with lumefantrine on the QTc interval of lumefantrine. This study therefore evaluated the effects of antacids on the bioavailability of lumefantrine, it also monitored the QTc interval changes in healthy subjects.

Methods

Subjects

Thirteen (13) healthy subjects (10 males and 3 females) aged between 19 and 39 years and weighing between 51 and 88.5 Kg participated in the study. However, complete data for pharmacokinetic determination were obtained for six subjects (4 males and 2 females) aged between 19 and 35 years and weighing 19 to 70 Kg. Their vital signs (blood pressure, temperature) were checked by a physician prior to commencement of the study to ensure subject's eligibility to participate in the study. Subjects that are pregnant were excluded from the study. This study was approved by the joint University of Ibadan/University College Hospital (UI/UCH) Ethical Review Committee, University of Ibadan, Nigeria. The subjects were recruited after giving their informed consent.

Study design

The study was a randomized two-way, open label, crossover design in which subjects were randomized into two groups (Group 1 and Group 2). On the first day (first arm) of the study, after an overnight fast, 80/480mg Coartem® tablet was administered to the subjects under group 1 while subjects in group 2 were given 30ml of antacid (Moko® Mist.Mag Trisilicate) formulation commercially available, followed by 80/480mg Coartem® 10mins later. The subjects remained in a fasted state for up to 3 hrs post dose.

The antacid formulation used contains magnesium trisilicate, light magnesium carbonate and sodium bicarbonate. The formulation containing mixture of antacids was used in this study since in ideal clinical situation, antacids are usually prescribed as formulation of different antacids. After a washout period of three weeks, the drugs were interchanged between the two groups (Group 2 now took only 80/480mg Coartem® while group 1 took 80/480mg Coartem® and 30ml antacid). Drug administration was carried out by a Pharmacist. The subjects remained in the study site for 12 hours during each treatment period. Only twelve subjects returned for the second arm of the study. One subject

did not report. The same type of foods were given to the subjects during the study periods (During the first 12 hours of the two study arms) and none of the subjects took any other antimalarial drug for at least two weeks before commencement of the study. This study was carried out in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan. No other drug, alcohol or caffeine-containing beverage was allowed during the study periods. Participants were interviewed from time to time for possible adverse drug reaction.

Electrocardiographic evaluation

Electrocardiographic screening was performed on volunteers recruited for the study prior to drug intake (0), at 6, 72 and 504 hours post dose. This was performed by a consultant cardiologist at cardiology unit of the University College Hospital (UCH), Ibadan. The computer ECG readings were confirmed manually by the cardiologist. The mean QTc interval at 0 hour was compared with the mean QTc interval at 6, 72, and 504 hours post dose.

Sample collection

Venous blood (4ml each) was collected at pre-dose (0) and at, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 96 hours post dose from each volunteer in the first arm of the study, while in the second arm, venous blood (4ml each) were collected at pre-dose (0) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, 144, 192 and 240 hours post dose from each volunteer. The blood samples were collected by venipuncture by Phlebotomists and transferred into lithium heparin tubes. The samples were centrifuged immediately at 4000 rpm for 5 minutes and the plasma was transferred into cryo vials and stored at -20^{o}C until analyzed.

Lumefantrine analysis

The plasma samples were analyzed for lumefantrine using High Performance Liquid Chromatography (HPLC) method.

Chemicals and reagents

Lumefantrine reference standard was obtained from United State Pharmacopoeia (USP), USA through the Centre for Drug Discovery, Development and Production (CDDDP), University of Ibadan. All chemicals and solvents used in this study were of analytical grade. HPLC grade acetonitrile and methanol were purchased from suppliers. The brands used were; Sigma Aldrich (Germany) for acetonitrile and methanol, and ortho-phosporic acid; JT Baker (USA). Tetrahydrofuran (THF) obtained was also Sigma Aldrich (Germany) and potassium dihydrogen phosphate was SCP (England).

Chromatographic condition

Chromatography was performed with a HPLC system (Agilent Technologies 1200 series) consisting of a quaternary pump, a syringe loading sample injector with a 20 µl sample loop coupled to a variable wavelength detector (VWD) which was operated at 265nm. Chromatographic separations were performed on a C_8 reversed phase Zorbax Eclipse XDB 150 x 4.6mm, 5 µm particle size at ambient temperature. The mobile phase consisted of acetonitrile: 25 mM KH_2PO_4 buffer (70:30 v/v) adjusted to pH of 4.0 with orthophosphoric acid and pumped at a flow rate of 1ml/min. Halofantrine was used as an internal standard.

Extraction procedure

For plasma drug extraction, 12 µl of 500 µg/ml halofantrine internal standard was added to 0.4 ml of plasma in a 5ml extraction tube. An amount (0.788 ml) of chilled acetonitrile was added (for protein precipitation) to the measured plasma containing the internal standard to obtain a final volume of 1.2 ml. Thereafter, it was vortex mixed for 1 minute and centrifuged at 4000 rpm for 5 minutes. The supernatant was injected into the HPLC. The concentration of halofantrine in the final solution was 5µg/ml.

Preparation of lumefantrine and halofantrine stock solutions

Stock solutions containing 1 mg/ml lumefantrine was prepared by first dissolving lumefantrine in THF and making up to the required volume with 50% tetrahydrofuran in acetonitrile. Series of standard solutions were made from the stock solution using the same solvent. Stock solution containing 1 mg/ml halofantrine was also prepared in methanol and 500 µg/ml halofantrine was made from the stock. The standard solutions of lumefantrine and halofantrine prepared were used to spike the drug free plasma to make a calibration curve. The percent coefficient of variation (% CV) for intra day precision was lower than 4% with a range of 1.31-3.96%, while % CV for the interday ranged from 4.0-19.34% with 19.34 % obtained for the lowest concentration. The percentage deviation of the mean value for the three concentrations determined from the true value (a measure of the accuracy) ranged from 0.7-4.2%

Pharmacokinetics and statistical analysis

Pharmacokinetic parameters were determined using WinNonlin version 5.3. The Mann-Whitney U test

was used to compare pairs of data between treatments for Area Under the Curves (AUCs) and peak plasma concentration (Cmax). The student t-test was used to compare difference in the mean QTc intervals at 0 hour compared to 6, 72 and 504 hours. A *P*-value of 0.05 was considered significant.

Results

Pharmacokinetic parameters of lumefantrine

No adverse reaction was reported by any of the volunteers hence artemeter-lumefantrine was well tolerated. Lumefantrine was also well resolved from halofantrine (internal standard) with retention times of 2.9 and 4.1 minutes for halofantrine and lumefantrine respectively.

The median lumefantrine AUC_{0-96} when the drug was taken alone and in combination with antacids were 173 (IQR: 72.11- 688.51) and 221.96 µg.hr/ml (IQR: 64.21- 465.47) respectively, while the median peak plasma concentrations of lumefantrine were 5.92 µg/ml (2.08- 14.44) and 4.42 µg/ml (3.84- 14.30) following administration of the drug alone and after co-administration with antacids respectively. Table 1 shows the result of the Mann-Whitney U test for the comparison of pairs of data for AUC and C_{max} while Table 2 shows other pharmacokinetic paramaters obtained for lumefantrine from each of the six volunteers after administration of 80/480 mg artemether-lumefantrine (coartem®) and when coartem® was co-administered with 30 mls of antacid formulation.

Table 1: Comparison of AUC and C_{max} between treatments

Outcome	Drug Lumenfantrine *Median (IQR)*	Lumenfantrine + Antacid *Median (IQR)*	U test	P
AUC_{0-96} (µg.hr/ml)	173 (72.11, 688.51)	221.96 (64.21, 465.47)	17.00	0.94
C_{max} (µg/ml)	5.92 (2.08, 14.44)	4.42 (3.84, 14.30)	14.00	0.59

IQR- Interquartile range

Electrocardiographic evaluation

Of the thirteen volunteers with electrocardiograms recorded, 76.9% were males and 23.1 % were females. Table 3 compares mean QTc interval values at pre-dose with mean QTc interval at 6, 72 and 504 hours post- dose for the thirteen volunteers evaluated. However, only ten and twelve volunteers were evaluated at 72 and 504 hours respectively. The other subjects did not report at that time. For the group that took A-L with antacid (with lower mean AUC), there was 2.95% lengthening of the mean QTc interval from zero (0) to 6 hours, while for the group that took A-L alone, there was about 5.5% lengthening of the mean QTc interval from 0 to 6 hours.

Table 3: Comparison of mean QTc interval at 0 hr for the thirteen volunteers with mean QTc intervals at 6, 72 and 504 hr.

	No of volunteers (N)	Mean QTc ± SD (ms)	p-value
QTc 0 hr and QTc 6 hr	13 13	390.08 ± 19.84 406.23 ± 19.04	0.01[a]
QTc 0 hr and QTc 72 hr	10[b] 10	389.40 ± 16.66 394.60 ± 19.91	0.30
QTc 0 hr and QTc 504 hr	12[c] 12	390.08 ± 19.20.73 396.33 ± 23.94	0.13

a *Statistically significant*
b 10 volunteers reported at 72 hr, their mean QTc value at 72 hr was compared to the mean QTc value for ***only*** *the 10 volunteers at 0 hr.*
c 12 volunteers reported at 504 hr

Discussion

A-L is the most widely used ACT today and is recommended as the first-line treatment in many tropical countries. It is essential that the safety and pharmacokinetics of this treatment be well characterized when A-L is co-administered with antacids since patients can have medical conditions warranting that. Based on the evidence that antacid affected the pharmacokinetics of halofantrine and on the fact that no information exists on the *in-vivo*interaction between antacids and lumefantrine, we hypothesized that antacids may affect the plasma levels of lumefantrine. To test this hypothesis, we carried out an *in-vivo* study in humans and plasma samples were analysed for lumefantrine.

Table 2: Pharmacokinetic parameters of lumefantrine (LF) obtained following single oral dose of 80/480 artemether-lumefantrine alone and when co-administered with antacids to six volunteers.

Sub.	Weight (kg)[a]	C_{max} (µg/ml)[b]		t_{max}(hr)[a]		AUC_{0-96} (µg.hr/ml)[b]		$t_{1/2}$ (hr)[a]		Cl/f (L/hr/kg)[a]		Vd/f (L/kg)[a]	
		A-L	A-L+	A-L	A-L+	A-L	A-L+	A-L	A-L+	A-L	A-L+	A-L	A-L+
Ant.	Alone	Ant.	Alone	Ant.	alone	Ant.	alone	Ant.	alone	Ant.			Alone
002	54	8.60	4.74	4	12	197.056	227.39	66.22 (2.75 days)	46.0 (1.92 day)	0.03	0.03	2.78	2.12
005	58	2.39	3.47	4	3	73.42	60.24	14.79	57.59 (2.4 days)	0.11	0.08	2.30	6.66
006	59.5	1.16	4.09	96	1	68.19	65.53	-	59.74 (2.49 days)	-	0.08	-	6.93
014	70	3.24	20.91	96	10	149.56	395.18	130 (5.42 days)	44.32 (1.85 days)	0.02	0.01	2.91	0.80
015	61.5	11.99	12.10	72	48	603.38	676.33	268.38 (11.18 days)	53.35 (2.22 days)	0.008	0.007	0.70	0.51
016	51	21.77	3.96	48	72	943.88	216.52	92.04 (3.84 days)	-	0.009	-	1.21	-
±	±	5.92 ± 14.44)	8.21 (72.11-7.01	53.33 (64.21-42.16	24.3 28.95	173 ± 688.51)	221.96 ± 465.47)	114.29 ± 95.78	52.2 ± 6.85	0.035 ± 0.043	0.041 0.036	1.98 0.98	3.40 3.16	(2.08-

a = mean ± SD, b = median (IQR), Sub. = subjects, A-L = artemether-lumefantrine (Coartem®), Ant. = Antacid

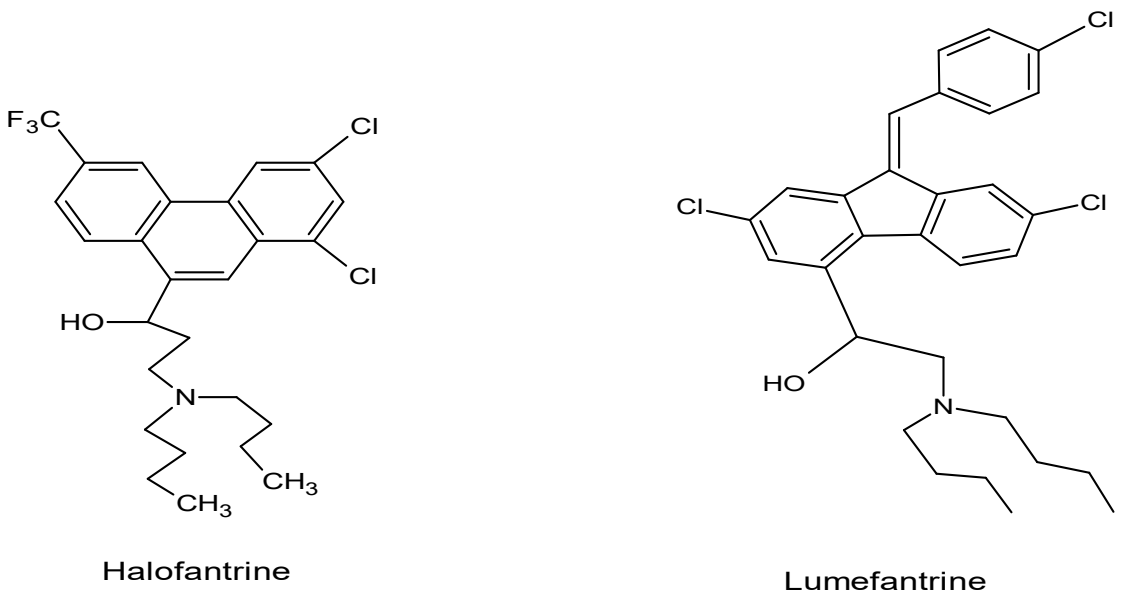

Fig 1: Structures of halofantrine and lumefantrtine

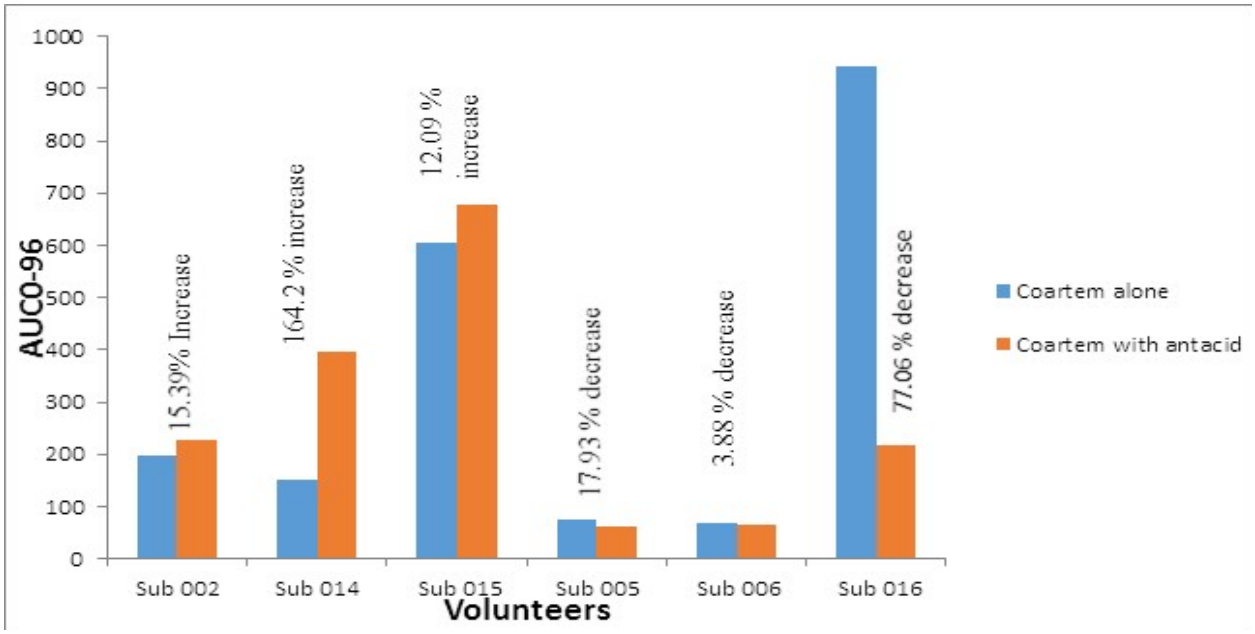

Fig 2: Comparison of AUC_{0-96} of lumefantrine when artemether-lumefantrine was taken alone and when taken with antacids.

A wide variability in AUC existed within and between subjects. This is comparable to the reports that lumefantrine has a low and variable bioavailability, with very erratic absorption. The absorption of lumefantrine is dependent on food especially fatty foods hence, a 16-fold increase in bioavailability of lumefantrine when taken with fatty food has been reported [13, 14]. Levefre *et al.* reported mean AUC_{0-264} value of 195±119 µg.hr/ml for lumefantrine [15]. In another study, they reported mean AUC_{0-816} value of 2290±1450 µg.hr/ml and a median time of peak plasma concentration (t_{max}) of 64 hour (54-70) for lumfantrine [3], in healthy Caucasian subjects. This result is comparable to the large variations in AUCs observed in the present study. However, there was no significant difference between the median AUCs obtained in this study for both treatments ($P > 0.05$).

The volunteers were in a fasted state during drug administration and for up to 3 hours post dose. The fasting state may contribute to the low and variable AUCs observed. In addition, lumefantrine is a substrate of permeability glycoprotein (p-gp) [16] and active efflux by P-gp across the intestine could partly contribute to the low/variable bioavailability of lumefantrine. Also, p-gp is polymorphic [17] and therefore may be expressed differently in different individuals thereby leading to variation in lumefantrine AUC in volunteers. Furthermore, lumefantrine is predominantly metabolized by CYP3A4 [18] and this enzyme was shown to be polymorphic [19].

In the presence of polymorphism, high C_{max} and AUC may be observed due to poor metabolism of the drug. The AUCs reported from previous studies [3, 15] were in healthy Caucasian volunteers, this study is unique in that it was conducted on Africans. The difference in values obtained compared to the reported AUCs in Caucasians may be as a result of genetic polymorphism of CYP3A4. It has also been reported that lumefantrine concentration and AUC values measured in two Malaysian volunteers were much higher than the values obtained with Chinese volunteers [20].

The median C_{max} obtained after administration of A-L alone was higher than the value obtained when the A-L was given in combination with antacids. However, the difference in the median C_{max} was not statistically significant ($P > 0.05$). This is in contrast with significant reduction in C_{max} and AUC reported when halofantrine, a similar drug, was co-administered with magnesium carbonate [5]. Figure. 2 which compares the AUCs obtained when A-L was taken alone and when combined with antacids, revealed that 50% of the volunteers had their AUCs increased when A-L was co-administered with antacids. Weakly acidic or weakly basic drugs are normally absorbed in their unionized form and lumefantrine is a weakly basic drug with pKa of 9 [21].

In the intestine (where most drug absorption occur) with pH of about 6.8, lumefantrine exists more in ionized compared to unionized form. Antacids increase the pH of a medium and consequently, can cause rise in gastric pH [22]. Hence, taking antacid formulation with A-L, may raise the pH of the gastrointestinal tract thereby causing more of the lumefantrine to be in unionized absorbable form. The rise in pH could increase the percentage of the drug absorbed, hence increase in the AUCs observed in some subjects. On the other hand, the other half of the volunteers had their AUCs decreased when A-L was given together with antacids. This decrease is similar to the *in-vitro* result where antacids were found to directly adsorb lumefantrine significantly [4]. The observed decrease in AUCs in some subjects could be as a result of adsorption of the drug by the antacids which in turn, reduced the percentage of the drug absorbed. The reason why one half of the participants showed decrease and the other half increase in AUC is unclear.

These differences in C_{max} and AUC confirmed the erratic nature of lumefantrine bioavailability between individuals and races. Genetic polymorphism in CYP3A4 may be a contributing factor to these variations. However, conclusion can only be made if genotype studies are carried out in different races and individuals to determine the expression of CYP3A4 and the effect of the genotypes on lumefantrine concentration. The small sample size in our study is a limitation and may have biased the results obtained. Another limitation of this study was the 17 months delay in analysis of plasma samples after sample collection, also the stability of lumefantrine was not determined.

However, the samples were constantly monitored to ensure they remained in frozen states during storage. Again, blood sample collection was truncated at 96 hour in the first arm which did not allow for evaluation of the terminal phase of lumefantrine pharmacokinetic parameters because of its long half-life. Nevertheless, the sampling time was extended to 240 hour to allow evaluation of the terminal phase pharmacokinetic parameters in the second arm but the effect of antacids on the C_{max} and AUC was evaluated from zero time to 96 hours.

Furthermore, abnormal QTc prolongation on the electrocardiogram is an independent risk factor for sudden cardiac death [23]. From the results of electrocardiographic recording carried out in this study as shown in Table 3, it could be seen that at 6 hour, which was about the t_{max} for some volunteers, there was significant ($P < 0.05$) prolongation of QTc interval. Again at 72 and 504 hour, the mean QTc interval became lower than what was obtained at 6 hour. This is an indication that, QTc interval prolongation depended on the plasma concentration of lumefantrine. Similar report was given for halofantrine (a similar drug); the QT interval lengthening of halofantrine was dependent on the dose [6]. In addition, the 2.95% lengthening (from zero to 6 hours post dose) of the mean QTc interval for the group that took A-L with antacid (with lower median C_{max}) compared to 5.5% lengthening for the group that took A-L alone, may not be significant, however, lower percentage lengthening of the QTc interval observed for the group that took A-L with

antacids (with lower median C_{max}) further suggests the dependence of QTc interval prolongation on the plasma concentration of lumefantrine.

Although, there was statistically significant prolongation of mean QTc interval at 6 hours post dose (406.23±19.04 ms) when compared to the mean value at zero hour (390.08±19.84 ms), the prolongation was still within normal limits and hence may not lead to cardiotoxicity since abnormally prolonged QTc interval in men should be >450 and >470 ms in women [23]. This result showed that lumefantrine is well tolerated which is in agreement with previous reports which suggested that lumefantrine has no cardiotoxic potential [10-12, 24, 25].

In conclusion, this preliminary study showed that antacids did not significantly influence the bioavailability of lumefantrine in human. Lumefantrine's erratic bioavailability was also observed in co-administration of antacid with lumefantrine. Also the significant prolongation of the QTc interval by lumefantrine at 6 hours post-dose showed no evidence of potential cardiotoxic effect. Hence the treatment was well tolerated. However, further studies with larger sample size as well as in fed state is recommended. This may be necessary to confirm this finding and the clinical implications since QTc interval prolongation seem to be dependent on lumefantrine concentration and food is also known to cause significant increase in the lumefantrine area under the curve (AUC).

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The International HIV Dementia Scale, a valuable screening instrument for HIV-Associated Neurocognitive Disorder (HAND) in HIV-Infected adults in North Central Nigeria

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Abstract

*Background***:** The study sought to determine the usefulness of the International HIV Dementia Scale (IHDS) as a screening tool for HIV-Associated Neurocognitive Disorder (HAND) in HIV-positive adults in Jos, North Central Nigeria.

Design: The frequency of HAND is largely unknown in resource-limited settings. In Nigeria, there is paucity of data on the prevalence of HAND because very little research has previously being carried out in this area. We therefore studied HIV-positive adults as cases and HIV-negative individuals as controls to determine the usefulness of the IHDS as a screening instrument.

Methods: HIV-positive adults in an HIV outpatient clinic were matched to HIV-negative subjects for age, sex and education. Screening for HAND was carried out using IHDS. The study participants were further subjected to full neuropsychological assessment to confirm or exclude HAND

Results: Overall, 87 HIV-positive individuals and 87 HIV-negative subjects were screened. The HIV-positive subjects had a significantly lower IHDS mean total score of 8.4±0.2 compared with the HIV-negative subjects with a mean score of 11.1±0.7 ($p < 0.001$). Abnormal scores (≤ 10) on the IHDS were found in 67.8% of the HIV-positive subjects and in 0% of the HIV-negative subjects ($p < 0.001$).

Conclusions: The results suggest that the frequency of HAND may be higher than the previous estimates in North Central Nigeria and demonstrates that the IHDS can be used as a screening tool for HAND in Nigeria. We therefore advocate that all studies on HAND in Nigeria should strategically start with the IHDS as a screening tool.

Keywords: *HIV/AIDS, neurocognitive impairment, IHDS, HAND.*

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Résumé

Contexte **:** L'étude visait à déterminer l'utilité de l'Echelle Internationale de Démence VIH (IHDS) en tant qu'outil de dépistage du Trouble Neurocognitif Associé au VIH (HAND) chez les adultes séropositifs à Jos, Nord Central du Nigéria.

Conception: La fréquence de HAND est largement inconnue dans les environnements à ressources limitées. Au Nigéria, il y a peu de données sur la prévalence de l'HAND car très peu de recherches ont déjà été menées dans ce domaine. Nous avons donc étudié les adultes séropositifs en tant que cas et les personnes séronégatives comme témoins afin de déterminer l'utilité de l'IHDS en tant qu'instrument de dépistage.

Méthodes: Des adultes séropositifs dans une clinique VIH pour patients externes ont été appariés à des sujets séronégatifs pour l'âge, le sexe et l'éducation. Le dépistage de l'HAND a été effectué à l'aide de l'IHDS. Les participants à l'étude ont été soumis à une évaluation neuropsychologique complète pour confirmer ou exclure l'HAND.

Résultats: Dans l'ensemble, 87 personnes séropositives et 87 sujets séronégatifs ont été dépistés. Les sujets séropositifs avaient un score total moyen inférieur de 8,4 ± 0,2 à l'IHDS par rapport aux sujets séronégatifs avec un score moyen de 11,1 ± 0,7 ($p < 0,001$). Des scores anormaux (≤ 10) sur l'IHDS ont été trouvés chez 67,8% des sujets séropositifs et chez 0% des sujets séronégatifs ($p < 0,001$).

Conclusions: Les résultats suggèrent que la fréquence de l'HAND peut être plus élevée que les estimations précédentes dans le centre nord du Nigeria et démontre que l'IHDS peut être utilisé comme un outil de dépistage pour l'HAND au Nigeria. Nous préconisons donc que toutes les études sur l'HAND au Nigeria devraient commencer stratégiquement avec l'IHDS en tant qu'outil de dépistage.

Mots-clés: *VIH / SIDA, déficience neurocognitive, IHDS, HAND.*

Introduction

HIV-associated neurocognitive disorder (HAND) is an important spectrum of neurological complication of HIV infection. The frequency of this disorder is

largely underreported in resource-limited countries although preliminary surveys in Uganda [1] and India [2] suggest a relatively high frequency of cognitive dysfunction. A study done in Cameroun found that 6.6% of 108 subjects with HIV had HIV-Associated Dementia (ADC) [3], this suggested that HIV Associated Neurocognitive Dementia (HAND) was frequent in sub-Saharan Africa in the pre-HAART era. With improved access to HAART in our setting, this picture is expected to have changed remarkably for the better. However, because of the paucity of data in Nigeria on HAND, this study was carried out using a simple screening tool: the International HIV Dementia Scale (IHDS) to assess the current trend of HAND in the post HAART era.

With the advent of HAART, the incidence of HIV-Associated Dementia (HAD), the most severe form of HAND has been decreasing in the United States [4] but with continued survival, the prevalence of this disorder has actually increased [5]. Given the increased prevalence of HAD and its negative impact on quality of life [6], the morbidity of HAD is potentially significant especially in developing countries like Nigeria where large numbers of people are infected with HIV. HAD is associated with an increased morbidity and mortality [7,8].

HAND is treatable with highly active antiretroviral therapy [9,10]. It can affect patients' ability to work, adhere to medication instructions and carry out instrumental and basic activities of daily living [11]. However, improvements in function and prognosis have been achieved in such patients by the use of HAART [12]. The presence of HAND can thus be used as a clinical indicator to facilitate the commencement of antiretroviral therapy hence the need to screen these patients for this condition.

The diagnosis of HAND requires subjecting suspected persons to a time consuming battery of neuropsychological tests that usually requires experts. Therefore, it is important to have a simple screening tool that can be used to identify subjects who are at risk and would need to undergo a battery of neuropsychological testing for confirmation so that appropriate management can be instituted.

Objectives

The specific objectives of this study were (1) to validate IHDS as a screening tool for HAND in HIV patients, (2) to determine the prevalence of HAND and assess the value of the IHDS as a screening tool in our context, and (3) to propose a strategy for future studies on HAND in Nigeria.

Methods

Study setting

The site of this study was the AIDS Prevention Initiative in Nigeria (APIN) supported HIV clinic at the Jos University Teaching Hospital (JUTH), Jos. This clinic provides comprehensive HIV care services for the city of Jos, which is located in the Jos North Local Government Area (LGA) of Plateau State. The clinic serves as a referral centre for both health facilities in the other LGAs of the state and some neighboring states in the country. Plateau State has a population of about 3,206,531 with the state capital having a population of approximately 900,000 [13]. Plateau state has an HIV seroprevalence of 2.3% [14]. On regional basis, the North Central zone has a seroprevalence rate of 3.4% [14] in the country.

Study design

We used a case–control study design involving HIV positive adults as cases and an equal number of HIV-negative individuals as controls to determine the usefulness of the IHDS as a screening tool, having complied with the standard requirements of the ethics committee of the Jos University Teaching Hospital, Jos.

Patients and data collection

This study was conducted using a questionnaire developed by the AIDS Clinical Trial Group (ACTG A5199 team) which was translated into Hausa, a language generally spoken in Jos, North Central Nigeria to assess demographic parameters, medical history, depression history and neurological symptoms. Assessment of functional impairment was done with the Karnofsky Performance Scale. All recruited patients and controls were screened for HAND using the International HIV dementia scale (IHDS) and a 5-test neuropsychological battery comprising; Grooved Pegboard, Finger Tapping test, Timed Gait, Semantic Verbal Fluency and Digit Span. A detailed general, systemic and neurological examination was performed on each subject. All the HIV-positive subjects had their full blood count, serum biochemistry, CD4 cell counts, viral loads and serological tests for hepatitis B surface antigen and anti-hepatitis C antibody performed.

The IHDS and adaptation

The IHDS is an adjustment to that proposed by Power *et al* (1995)[15] which was later adapted by Sacktor *et al* [16] (2005). The IHDS if validated could be used to screen and identify patients at risk of HAND without the need for the laborious and expensive neuropsychological tests that are not readily available in Nigeria.

The IHDS consists of 3 subsets: Motor speed, assessed by timed finger tapping; timed alternating hand sequence for psychomotor speed and recall of 4 items to assess registration and recall. The subtests above were rated on a scale of 4 each. Memory was assessed with the 4-word recall at 2 minutes, which assesses memory, registration and recall. This was done by reciting 4 words to the subject (rat, chair, orange, and blue) saying each of these words one per second. The subject was then asked to repeat the words. If the subject failed to repeat all the words immediately, the examiner repeated all the words until the subject could repeat all the 4 words correctly. The subject was then asked to recall the 4 words after performing the other 2 subtests. For words not recalled, the subject was prompted with a semantic clue as follows: animal (rat), furniture (chair), fruit (orange), color (blue).

One point was given for each word recalled spontaneously and 0.5 points for each word recalled with prompting. For the assessment of motor speed, the number of finger-taps of the first 2 fingers of the non-dominant hand was measured by instructing the participant to open and close the fingers as widely and as quickly as possible over a 5-second period. Points were assigned as follows: 4 = $\geq$15 taps/5s; 3 = 11–14 taps/5s; 2 = 7–10 taps/5s; 1 = 3–6 taps/5s; and 0 =0–2 taps/5s. In the alternating hand sequence for assessing the psychomotor speed, the subject was asked to perform the following movement in succession with the non-dominant hand as quickly as possible over a 10-second period: (1) clench the hand in a fist on a flat surface, (2) put the hand flat on the surface with the palm down, and (3) put the hand perpendicular to the flat surface on the side of the fifth digit. The 3 hand positions were demonstrated to the participant by the examiner, and the participant was then asked to perform the sequence correctly twice for practice before the 10-second subtest was performed.

The task was scored as follows: 4 = 4 sequences in 10 seconds; 3 = 3 sequences in 10 seconds; 2 = 2 sequences in 10 seconds; 1 = 1 sequence in 10 seconds; and 0 = 0 sequence in 10 seconds. Timing was done using a Professional Quartz Timer. The total score out of 12 was calculated for each participant, with each of the 3 subtests contributing 4 points maximum to the total score. For our study, an IHDS score of $\leq$10 was considered abnormal. The sensitivity and specificity for the detection of HAND for a score of $\leq$10 have been shown to be 80% and 55%, respectively in a Ugandan cohort and 80% and 57% in a US cohort [16]

Data analysis

The mean scores of the neuropsychological parameters of the control subjects provided the normative data against which the neuropsychological test scores of each HIV+ve subjects were compared and classified as either neurocognitively impaired or unimpaired in each cognitive test based on standard definitions ; HIV+ve subjects that scored one standard deviation below mean for age and education appropriate norm in at least 2 of the 5 tested domains was diagnostic of Asymptomatic cognitive impairment (ANI), those that had Mild neurocognitive disorder (MND) met the criteria for ANI but also had impairment of activities of daily living and HIV-associated dementia (HAD) scored 2-SD below the normative mean in at least 2 cognitive domains with marked impairment in activities of daily living.

The data was entered and analyzed using Epi-info 3.5.4, Atlanta, Georgia, USA. HAND was the dependent variable and all other variables were independent variables. Standardized- z- scores of the neuropsychological test scores were calculated for the HIV negatives group. The HIV positive subjects then had their neuropsychological scores converted to z scores using the demographically adjusted means of the normative sample.

Neurocognitive impairment was stratified based on IHDS as normal or abnormal. Group comparison was made on demographic, medical history, neurologic symptoms and current functional and immune status. Categorical variables were compared using the chi square test and continuous variables compared using ANOVA. The sensitivity and positive predictive values of the neuropsychological assessment were also determined. In all tests of associations, critical p value of < 0.05 was regarded as statistically significant. A logistic regression model was developed to determine significant predictors and their strength of association with HAND. Subjects gave a written informed consent to participate in the study and this research was approved by the Ethics committee of the Jos University Teaching Hospital, Jos, Nigeria.

Results

Eighty seven HIV-positive subjects were matched for age, gender and educational attainment with 87 HIV-negative controls. The sociodemographic characteristics of the HIV+ve subjects are shown in Table 1. There were no statistically significant differences with respect to age, sex and educational status between the HIV-positive and HIV-negative

Table 1. Sociodemographic and Clinical Characteristics of the study subjects

Variable	HIV Positive	HIV Negative	Total	P-Value
Sex				
Male	28(32.2%)	28(32.2%)	56(32.2%)	-
Female	59(67.8%)	59(67.8%)	118(67.8%)	
Mean Age in Years	35.9±8.2	35.6±7.9	35.6±8.1	0.77
Male	42.0±6.8	41.9±5.8	42.0±6.3	0.95
Female	33.1±7.2	32.6±7.0	32.8±7.1	0.72
Mean Education(Year)	12.1±4.7	12.8±4.5	12.4±0.4	0.37
Mean CD4 Count	338.5±183.9	NA		NA

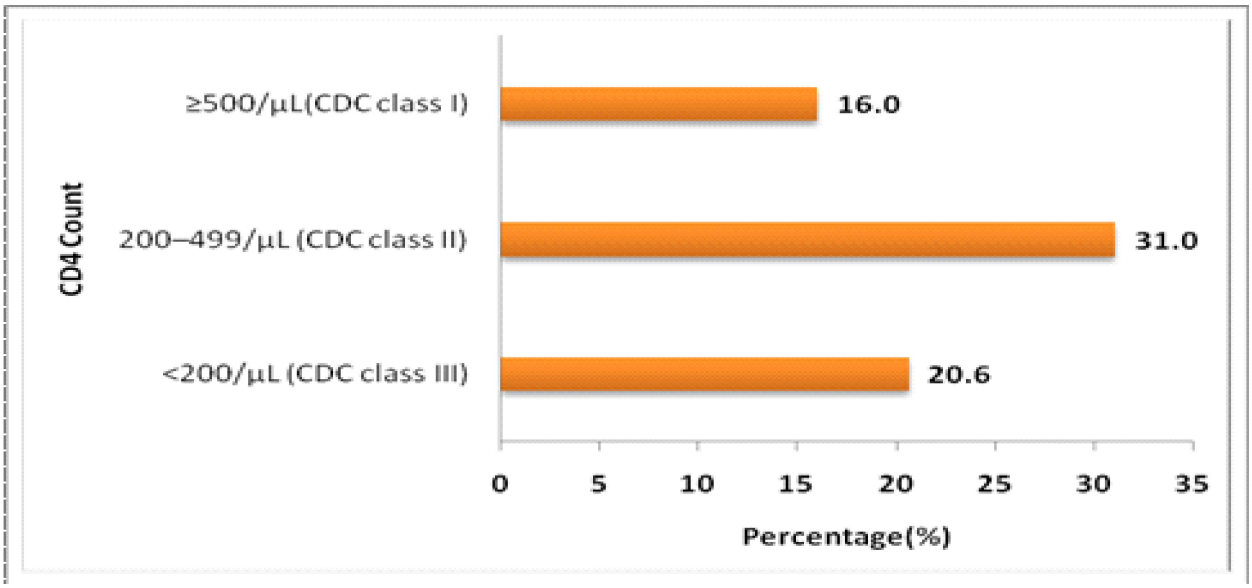

Fig 1: CD4 Levels and CDC Class among HIV-Positive Subjects

Table 2: Comparison of the IHDS scores between the HIV-positive and HIV-negative subjects

Variable	HIV Positive Controls (n = 87)	HIV Negative Cases (n = 87)	Total	P-Value
IHDS total score	8.4±1.8	11.1±0.7	9.7±1.9	<0.001
Finger-tapping Test (Non Dominant)	34.3±9.2	42.5±5.9	40.0±9.6	<0.001
Finger-tapping Test (Dominant)	34.6±9.5	45.5±5.8	38.4±8.8	<0.001
Time Gait	13.3±3.2	10.7±1.2	12.0±2.7	<0.001
Semantic Verbal Fluency	9.3±3.3	10.6±3.3	10.0±3.4	0.01
Digit Span Forward	5.0±1.1	5.8±0.9	5.4±1.1	<0.001
Digit Span Backward	3.1±1.7	4.7±0.8	3.9±1.5	<0.001
IHDS 4-word recall	2.1±0.9	2.7±0.5	2.4±0.8	<0.001

Values are given as mean values (± SD).

subjects. The mean ages of the HIV-positive and HIV-negative subjects are as shown in table 1, likewise their sex distribution, mean education years as well as the mean CD4 cell count of the HIV-positive subjects.

The HIV-positive subjects had a mean of IHDS score of 8.4±1.8 while the HIV-negative subjects scored 11.1±0.7 ($p < 0.001$). Fifty nine (67.8%) of HIV-positive subjects had IHDS score ≤10 (abnormal) and thus had possible

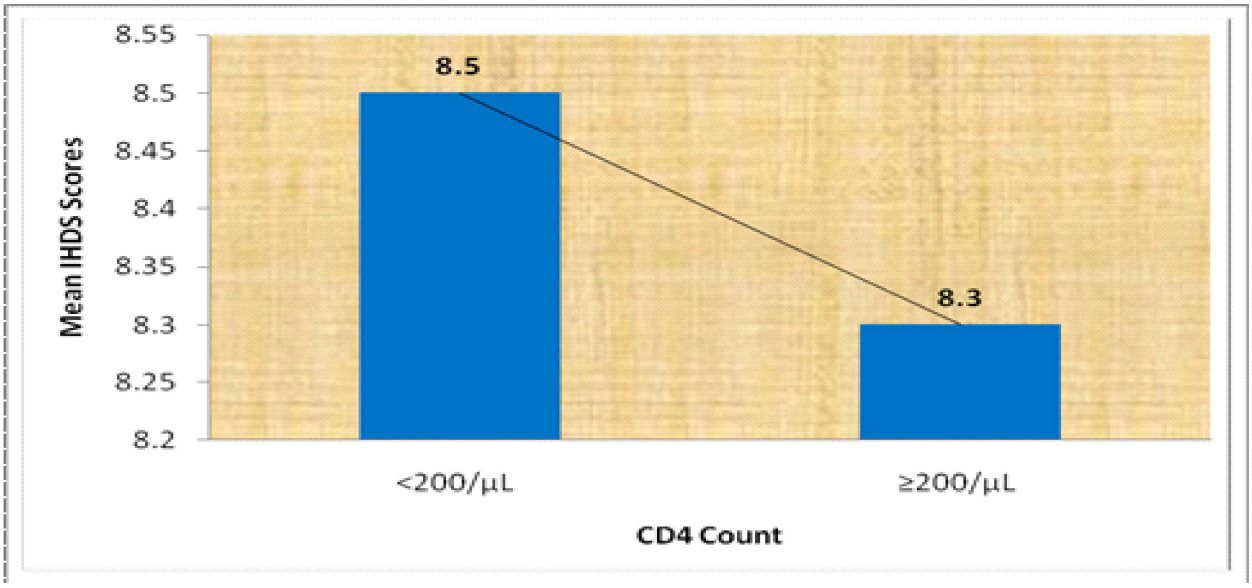

Fig. 2: Mean IHDS scores by CD4 count cut-offs

Table 3: Comparing HIV years and ARV weeks by IHDS score of HIV+ subjects

Variables	Normal(>10)	Abnormal(10 and Below)	t	95% Confidence Intervals	P-Value
HIV Years (Mean±SD)	2.7±1.4	3.5±1.7	2.2	(-1.6, -0.08)	0.03
ARV Weeks (Mean±SD)	151.3±71.0	170.95	0.8	(-71.8, 32.5)	0.5

Table 4: Comparing mean IHDS score across ARV regimen category for HIV+ subjects

ARV Regimen Category	Mean±SD	95% Confidence Interval for Mean	
		Lower Bound	Upper Bound
None	8.7±2.0	7.9	9.5
1st Line	8.3±1.5	8.0	8.8
2nd Line	6.3±3.4	0.8	11.7
Total	8.4±1.8	8.0	8.7

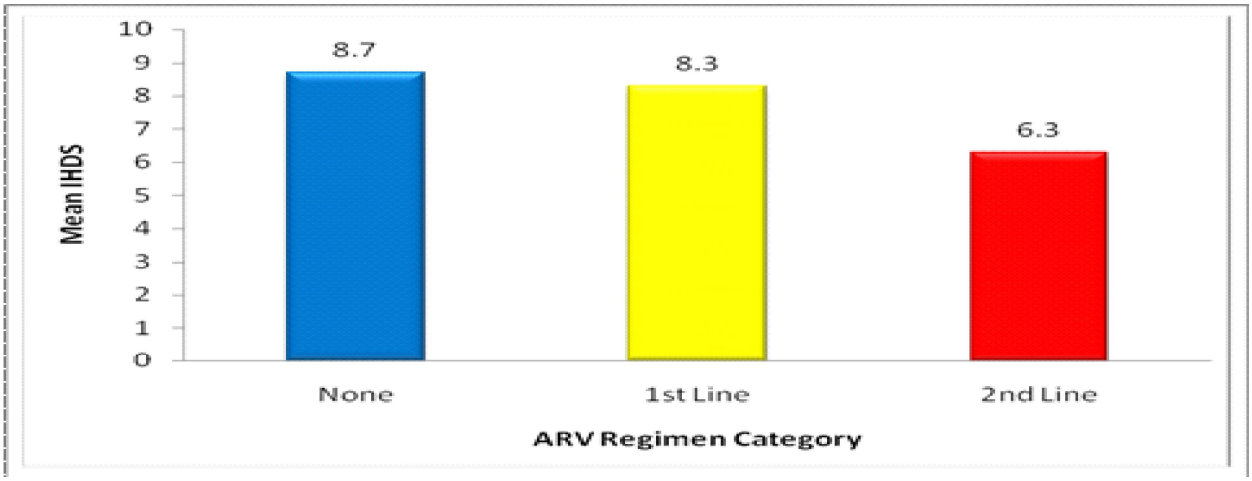

Fig. 3: Comparing Mean IHDS Score across ARV Regimen Category for HIV+ Subjects

neurocognitive impairment whereas there were no HIV-negative subject (0.0%) who had an IHDS score of ≤10 (95% confidence interval: 56.9% to 77.4%) p <0.001.

However, after neuropsychological assessment, the proportion of HIV-positive subjects that had HAND was 33 (37.9%). Using the neuropsychological assessment test as gold standard, the sensitivity and specificity of the IHDS screening tool were found to be 72.7% and 75.2% respectively. The overall accuracy of the test was 74.7%. The mean total IHDS score of HIV-positive subjects with abnormal scores was 7.6 ±1.6, and that of the HIV-negative subjects was 11.1±0.7 (p <0.001). Table 2 shows the comparison between the 2 groups with respect to the mean IHDS scores on the 8 components of the neuropsychological test. In the Finger-tapping test (Non Dominant), the HIV-positive subjects scored an average of 34.3±9.2 as compared to the HIV-negative group which recorded a mean of 42.5±5.9 (p <0.001). The Finger-tapping test (Dominant) recorded a mean of 34.6±9 among the HIV- positive subjects whereas the HIV-negative had a mean of 45.5±5.8 (p <0.001). The Time Gait test (TG), revealed that the HIV-positive subjects recorded a mean of 13.3±3.2sec whereas the HIV-negative group had a mean of 10.7±1.2sec to cover a distance of 60 meters with marked significant differences (p <0.001). The Semantic Verbal Fluency (SVF) test scores revealed that the HIV-positive subjects recorded an average of 9.3±3.3 while their HIV-negative counterparts had a mean of 10.6±3.3 (p <0.001). Digit span forward test showed that the HIV-positive subjects scored a mean of 5.0±1.1 when compared with the HIV-negative group that had a mean of 5.8±0.9 (p <0.001). Observation with the Digit Span Backward revealed same trend where the HIV-positive recorded an average of 3.1±1.7 as compared to the HIV-negative arm that had a mean of 4.7±0.8 (p <0.001)

In the HIV-positive group, 18 of the 87 subjects (20.6%) with CD4 counts <200/μL (CDC class III) had an IHDS score ≤10, while 27 of the 87 subjects (31.0%) with CD4 counts 200–499/μL (CDC class II) had a score <10, and only14 of the 87 subjects (16.0%) with CD4 counts ≥500/μL (CDC class I) had an abnormal score (p < 0.001). The mean IHDS score of subjects with CD4 ≥200/μL was 8.5±1.5 while that of patients with CD4 <200/μL was 8.3±1.9 (p = 0.67) as shown in figure 2. The proportion of cases with abnormal IHDS for subjects with CD4 ≥200/uL was 69.5.0% (49/59), confidence limits: 24.2%–70.5% while that of patients with CD4 <200/μL was 5/28 (17.9%) with confidence limits of 15.5%–40.3%.

Discussion

This study reveals the usefulness of the IHDS in evaluating patients with HIV/AIDS. The study established that IHDS can be successfully used for screening of cases of HAND. It was identified that the mean total IHDS score of the HIV-positive subjects was significantly lower than that of HIV-negative controls (p <0.001). This marked differences in the IHDS score between both groups (Cases and Control) is not very different from the figure obtained in a similar study in Yaoundé, Cameroun [17]. In this study, the HIV-positive subjects had a mean of IHDS score of 8.4±1.8, as compared with the HIV-negative subject's score of 11.1±0.7 (p <0.001), which is much lower than 10.87± 0.91 for cases and 11.28 ± 0.56 for controls obtained from the Cameroonian study and 9.9±1.6 for cases and 11.0±1 for controls in a study done in Uganda. These differences may be due to differences in disease stage of subjects as well as the age differences between the study populations in the three studies. Another possibility is the age difference between the HIV positive subjects and the HIV-controls in the case of the Ugandan study as well as differences in methodologies.

The sub-tests of the IHDS which include Finger-tapping Test (Non Dominant), Finger-tapping Test (Dominant), Time Gait, Semantic Verbal Fluency, Digit Span Forward and Digit Span Backward revealed statistically significant differences between the HIV-positive and HIV negative subjects. For instance the Finger-tapping Test (Non Dominant) scores of HIV positive subjects differed significantly from that of the HIV negative subjects.(P<0.001). Similar differences were observed in the scores of Finger-tapping Test (Dominant), Time Gait, Semantic Verbal Fluency, Digit Span Forward and Digit Span Backward. These research findings are very different from the studies conducted in Yaoundé, United states, Uganda and India where differences in performance were found only in memory recall and psychomotor speed and it was thought that these subtests were the surrogate markers for picking up the early changes associated with HAD. Furthermore, in contrast to our finding, there was no difference in the finger tapping subtest between the HIV- positive and HIV-negative controls in the Yaoundé and Indian studies. However, our finding was similar to the finding in the Ugandan study where there was a statistically significant difference in the finger tapping subtest between the HIV-positive and HIV-negative controls, although this was attributed to the fact that the HIV-positive cohort was significantly older than the HIV-

negative cohort and age is associated with motor performance decline, especially in the fifth and sixth decades of life. In our study cases and controls were matched for age so the influence of the age factor was excluded.

This study showed that HIV-positive subjects were as high as 67.8% at risk of HAND using IHDS tool. However, upon subjecting these subjects to extensive neuropsychological tests battery, only 37.9% of them had HAND demonstrating that using a cut off of IHDS $\leq$ 10 is associated with false positive result. Interestingly, there were no subjects among the HIV-negative subjects that were found to have an IHDS score $\leq$ 10. This observation differs from reports from other studies where up to 2.5% and 15% of HIV negative controls had abnormal IHDS scores [17,18].

In our study as in the Ugandan study a cut off value of IHDS of $\leq$ 10 was used because of its sensitivity of 80% at this point. In the Cameroonian and Indian studies a cut off of >10 was used, the differences in this cut off may be responsible the differences in the prevalence of HAND in the different studies and the high false positive rate noted in our study using the IHDS. In contrast to the Cameroonian study, full neuropsychological screening was done on the HIV-positive subjects and HIV-negative control making it possible to identify false positive subjects.

We identified the population of HIV-positive subjects at risk for potential HAND; they were subsequently subjected to full neuropsychological assessment for confirmation. We do recognize that the IHDS does not replace the neuropsychological tests for diagnosing HAND, but it is useful in directing limited resources for the diagnosis of HAND to those at risk of developing this complication and therefore seems to be quite suitable for resource-limited countries like Nigeria. We therefore suggest that future studies investigating HAND in Nigeria and other limited settings in Africa should utilize this tool for screening before employing subsequent neuropsychological assessment for those at risk for good surveillance and HIV management and control.

Conclusion

In this study we have successfully screened for the risk of HAND using the IHDS and subsequently confirmed HAND using a 5-test neuropsychological battery in Jos, North Central Nigeria. The performance scales was found to be a good method for identifying HIV patients at risk of HAND and normal healthy subjects. It is therefore suggested that neurological clinical care in Nigeria adopts this method of screening for the identification of those as risk of HAND in clinical care and practice. HAND seems to be an important complication of HIV infection in North Central Nigeria with a potential risk prevalence of 67.8% with IHDS screening tool, however neuropsychological assessment is necessary to confirm the diagnosis.

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Saliva based analysis of biochemical factors in patients with chronic kidney disease undergoing hemodialysis

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Abstract

Background: Use of saliva as alternative to blood in monitoring systemic diseases is still subject to continued research. Hence, this study evaluated changes in biochemical composition of saliva and plasma before and after hemodialysis and also determined the correlation between these factors in saliva and plasma of patients with chronic kidney disease (CKD).

Methods: A cross sectional study that included 50 patients with CKD undergoing hemodialysis. Whole saliva and blood samples were collected from the participants before and after dialysis. Samples were analyzed for urea, creatinine, total protein, sodium, potassium, calcium, chloride, and bicarbonate. Data were compared using Related Samples Wilcoxon Signed Rank test. Correlation between plasma and salivary parameters was determined using Spearman's correlation test.

Results: Levels of salivary urea and creatinine were reduced in the post dialysis state in consistence with reduced plasma levels. Salivary and plasma bicarbonate levels were elevated in the post dialysis state compared to pre-dialysis while both salivary and plasma levels of total protein, sodium, potassium, calcium and chloride did not show significant change. There were positive correlations between salivary and plasma creatinine and potassium in the post dialysis state as well as calcium in both pre and post dialysis states.

*Conclusion***:** Findings of this study suggest that saliva reflects plasma levels of biochemical factors in patients with CKD in the pre and post dialysis states. Hence, saliva may be an alternative to blood in monitoring patients with CKD undergoing dialysis.

Keywords: ***S****aliva; blood; chronic kidney disease; dialysis*

Résumé

Contexte: L'utilisation de la salive comme alternative au sang dans la surveillance des maladies systémiques fait toujours l'objet de recherches continuées. Par conséquent, cette étude a évalué les changements dans la composition biochimique de la salive et du plasma avant et après l'hémodialyse et a également déterminé la corrélation entre ces facteurs dans la salive et le plasma des patients atteints d'insuffisance rénale chronique (IRC).

Méthodes : Une étude transversale qui a inclus 50 patients atteints d'IRC sous hémodialyse. Des échantillons entiers de salive et de sang ont été prélevés chez les participants avant et après la dialyse. Les échantillons ont été analysés pour l'urée, la créatinine, les protéines totales, le sodium, le potassium, le calcium, le chlorure et le bicarbonate. Les données ont été comparées à l'aide du test du Rang Signé Wilcoxon des échantillons reliés. La corrélation entre les paramètres plasmatiques et salivaires a été déterminée en utilisant le test de corrélation de Spearman.

Résultats : Les niveaux d'urée salivaire et de créatinine ont été réduits dans l'état post dialyse en accord avec des taux plasmatiques réduits. Les taux salivaires et bicarbonatés plasmatiques étaient élevés dans l'état post dialyse par rapport à la pré-dialyse, tandis que les taux salivaires et plasmatiques de protéines totales, de sodium, de potassium, de calcium et de chlorure n'ont pas montré de changement significatif. Il y avait des corrélations positives entre la créatinine salivaire et plasmatique et le potassium dans l'état post dialyse ainsi que le calcium dans les états de dialyse pré et post.

Conclusion **:** Les résultats de cette étude suggèrent que la salive reflète les taux plasmatiques de facteurs biochimiques chez les patients atteints d'IRC avant et après la dialyse. Par conséquent, la salive peut être une alternative au sang dans la surveillance des patients atteints d'IRC subissant une dialyse.

Mots-clés: *Salive; sang; maladie rénale chronique; dialyse*

Introduction

Chronic kidney disease (CKD) is one of human diseases that have global impact for which diagnosis and monitoring require supplementing clinical evaluation with laboratory testing [1]. The increasing global burden of CKD and resultant end-stage renal disease (ESRD) continues to present serious challenges for the entire world [1, 2]. Management of CKD includes renal replacement therapy (RRT) and the available options are dialysis or kidney

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transplantation. Hemodialysis as a form of RRT requires frequent blood collection for adequate monitoring [3]. The frequent blood sampling associated with hemodialysis may result in blood loss [4]. In addition, the individuals involved in the management of patients with CKD are at more risk of blood borne diseases because of their frequent contact with blood. Hence, a non-invasive diagnostic test with minimal risk as well as ability to provide a dependable evaluation of disease condition and adequacy of hemodialysis would be invaluable to both the healthcare professionals and the patients.

It had been reported in several studies that changes occur in salivary secretions in some systemic diseases for which saliva has been suggested to be an alternative to blood in monitoring these diseases [5-7]. Also, the diagnostic potential of salivary urea and creatinine analyses in patients with CKD had been reported [8-11]. Saliva has great potential as a diagnostic fluid and offers many advantages over blood and other biological fluids because of its economic and noninvasive method of collection which promotes its use for monitoring systemic diseases [12, 13]. In addition, salivary analysis holds great promise as an effective tool for the diagnosis, prognosis and monitoring adequacy and effectiveness of dialysis in patients with CKD [11]. Other advantages of saliva as a clinical tool over blood and other body fluids include use of smaller aliquot samples, good cooperation from patients, cost effectiveness, easy storage and transportation, good sensitivity and correlation with levels of some factors in blood [14, 15]. Saliva is indeed a very useful diagnostic fluid. However, its potential for clinical medicine is not used as much as it should. Hence, its use as alternative to blood in monitoring of systemic diseases as well as efficacy of dialysis therapy is still subject to continued research. This study was therefore designed to evaluate changes in salivary biochemical factors before and after hemodialysis and to determine correlation between plasma and salivary biochemical factors before and after hemodialysis.

Methods

Study design

This was a prospective study of patients with CKD who were being dialyzed in a tertiary hospital. Following ethical approval from the institution Research Ethics Committee, the study was carried out among consecutive patients with CKD that required hemodialysis. Chronic kidney disease was defined as estimated glomerular filtration rate eGFR< 60mls/min/1.73m^2. Inclusion criteria were age >18 years, diagnosis of CKD and presence of indication for hemodialysis. Exclusion criteria were age < 18 years, diagnosis of acute kidney injury, patient who did not require hemodialysis, history suggestive of upper gastrointestinal bleeding, unconsciousness and inability to produce saliva.

This study included 50 patients with CKD composed of 35 males and 15 females with a mean age of 49.5 ± 16.07 years. The patients were in stages 3, 4 or 5 and there clinical data are as shown in table 1.

Table 1: Demographic and clinical data of participants

Variables	Mean ± SD / Value (%) N = 50
Age (years)	49.5 ± 16.07, Range: 19 to 77
Gender	
Male	35 (70%)
Female	15 (30%)
CKD grading	
Stage 3	6 (12%)
Stage 4	24 (48%)
Stage 5	20 (40%)

Note. CKD: chronic kidney disease

Each participant completed a self-administered questionnaire to obtain demographics, stage of CKD and dialysis history. Whole saliva samples were collected by asking patients to spit into a graduated universal bottle until about 3ml of saliva was produced just before and after dialysis when the patient was stable. Samples were transferred aseptically to sterile tubes and stored at -20^{o}C until the time for laboratory analysis. Simultaneously, venous blood samples (5ml each) were collected in sample tubes with lithium heparin. Whole saliva and plasma (separated from the blood samples) were used for the biochemical analysis.

Following manufacturer's instructions (RANDOX Creatinine Reagent, UK), estimation of creatinine was done using modified Jaffe's method [16] while, estimation of urea was done using the method of Marsh *et al*., [17] (RANDOX Urea Reagent, UK). Estimation of total protein was done using Biuret method [18]. Sodium and potassium levels were determined using flame emission spectrophotometry, while estimation of calcium was done using indirect colorimetric method [19]. Concentrations of chloride and bicarbonate were determined by Schales method using mercuric nitrate [20].

Statistical analysis

All statistical analyses were performed using IBM - Statistical analysis Software Package (SPSS Statistics) Version 22. 0. Armonk, New York. The main quantitative variables were salivary and plasma levels of urea, creatinine, total protein, sodium, potassium, calcium, bicarbonate and chloride before and after dialysis. Data are presented using descriptive statistics such as mean, median and interquartile range. Data were tested for normality using Kolmogorov-Smirnov test. Serum and salivary levels of most analytes were not normally distributed; hence the Related Samples Wilcoxon Signed Rank test was used to compare median values of the factors before and after dialysis. Correlation between plasma and salivary biochemical parameters was determined using Spearman's correlation test. All analyses were done at p-value < 0.05.

Results

Salivary levels of urea and creatinine were significantly reduced in the post dialysis samples compared to the pre dialysis samples ($p < 0.001$ and $p < 0.001$ respectively). These findings were similar to what was observed in plasma samples (Figs. 1 and 2). Levels of bicarbonate were significantly elevated in saliva and plasma in post dialysis samples compared to the pre dialysis samples ($p = 0.04$ and 0.02 respectively). Salivary levels of total protein, sodium, potassium, calcium, chloride and bicarbonate before and after dialysis are shown in table 2.

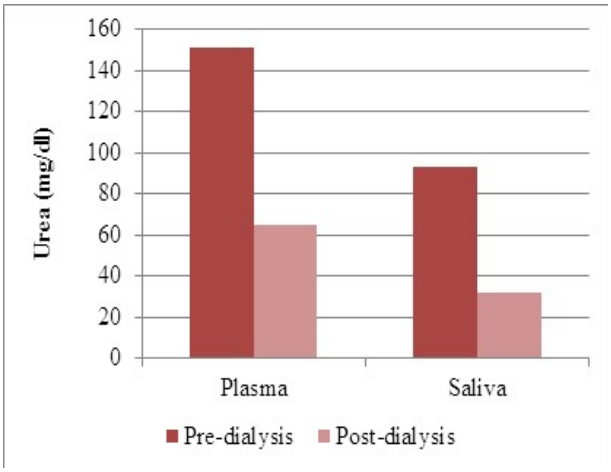
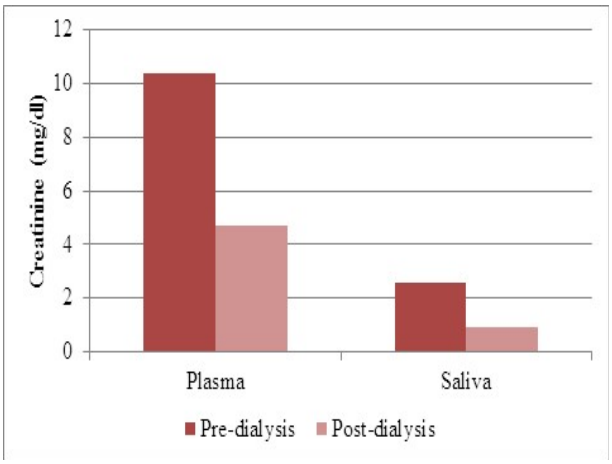


Fig. 1: Levels of salivary/plasma urea before and after dialysis

Plasma levels of total protein, sodium, potassium, calcium, chloride and bicarbonate before and after dialysis are shown in table 3. As shown in figures 1 and 2, plasma levels of urea and creatinine were significantly reduced in the post dialysis



Data are presented as median values

Fig. 2: Levels of salivary/plasma creatinine before and after dialysis

samples ($p < 0.001$ and $p < 0.001$ respectively). The mean percentage reductions of urea and creatinine in plasma were 57% and 55% respectively while the mean percentage reductions of urea and creatinine in saliva were 66% and 65% respectively.

Table 2: Salivary biochemical factors before and after dialysis

	Before dialysis	After dialysis	p-value
Sodium (mEq/L)	4.3(3.9)	5.4(4.6)	0.29
Potassium (mEq/L)	18.7(13.4)	19.2(8.5)	0.40
Chloride (mEq/L)	12(12)	14.1(11.8)	0.13
Bicarbonate (mg/dl)	14(16)	18(14)	0.04*
Total Protein (mg/dl)	17(21)	21(14)	0.32
Calcium (mg/dl)	7.5(3.4)	7.2(5.8)	0.26
Urea (mg/dl)	93(141)	32(58)	< 0.001**
Creatinine (mg/dl)	2.6(2.5)	0.9(1.4)	< 0.001**

Data are presented as median (Interquartile range)

Table 3: Plasma biochemical factors before and after dialysis

	Before dialysis	After dialysis	p-value
Sodium (mEq/L)	125(12)	129 (6)	0.06
Potassium (mEq/L)	5.3(2.2)	5.1 (3.6)	0.08
Chloride (mEq/L)	97(10)	99 (9)	0.24
Bicarbonate (mg/dl)	17(4)	18 (3)	0.02*
Total Protein (mg/dl)	60(10)	64 (14)	0.11
Calcium (mg/dl)	8.1(3.3)	8.7 (3.8)	0.37
Urea (mg/dl)	151(126)	65 (36)	< 0.001**
Creatinine (mg/dl)	10.4(7.18)	4.7 (3.6)	< 0.001**

Data are presented as median (Interquartile range)

There were positive correlations between salivary and plasma creatinine and potassium in the post dialysis state as well as calcium in both pre and post dialysis states (table 4).

Table 4: Correlation between salivary and plasma factors before and after dialysis

	Before dialysis	After dialysis
Sodium	-0.26 (0.08)	0.26 (0.09)
Potassium	-0.05 (0.75)	0.30 (0.04*)
Chloride	-0.27 (0.08)	0.17 (0.28)
Bicarbonate	-0.17 (0.27)	0.15 (0.34)
Total Protein	0.25 (0.08)	0.16 (0.28)
Calcium	0.39 (0.01*)	0.46 (< 0.01*)
Urea	-0.16 (0.26)	0.08 (0.59)
Creatinine	0.07 (0.63)	0.34 (0.02*)

Data are presented as the correlation coefficient (p value)

Discussion

The main findings from this study were reduced levels of salivary urea and creatinine and elevated levels of salivary bicarbonate after dialysis consistent with their levels in plasma. In addition, similar to what was observed in plasma, that there was no significant difference in the levels of salivary sodium, potassium, chloride, phosphate and total protein post dialysis compared to their pre dialysis levels.

In agreement with previous studies, our findings showed elevated concentration of salivary urea and creatinine in the patients with CKD before dialysis [21-23]. . In the present study, the salivary concentrations of urea and creatinine decreased substantially (66% and 65%, respectively) after haemodialysis probably because saliva reflected adequate changes in concentrations of urea and creatinine in the blood. Furthermore, the presence of urea and creatinine in the saliva indicates their passive diffusion from plasma to saliva through the salivary glands [24]. These findings also suggest that the salivary concentrations of urea and creatinine could be useful in monitoring the efficacy of dialysis. In addition, a good correlation was observed between the salivary and plasma levels of creatinine after dialysis. The usefulness of salivary creatinine in monitoring dialysis in patients with CKD is also corroborated by the positive correlation between the salivary and plasma levels after dialysis.

The post dialysis concentration of salivary bicarbonate was higher compared to what was observed in plasma. This finding may be explained by the contribution of the bicarbonate dialysate commonly used to correct the metabolic acidosis which is often associated with advanced CKD [25]. However, there was no appreciable difference in the concentrations of other salivary electrolytes (sodium, potassium and chloride), calcium and total protein. This finding is similar to what was observed in plasma. The lack of change in the levels of the salivary sodium after dialysis is similar to the report by previous studies [23, 26]. These observations in the concentrations of salivary electrolytes before and after dialysis could be explained in many ways. This could be because majority of the participants undergoing hemodialysis had more of uremia rather than electrolyte derangements and thus the pre and post dialysis levels of these parameters were similar. Also, the lack of change in the salivary concentrations of the electrolytes (sodium, potassium, calcium, chloride and phosphate) may be explained by the concurrent lack of change in their plasma levels although, the salivary concentrations of these ions do not depend exclusively on their plasma concentrations [27]. Sodium and potassium ions undergo active transport along the salivary duct via Na-K-Cl symporters apart from passive diffusion [28]. Contrary to our finding, Shasha *et al*. observed that salivary concentration of sodium and potassium level in hemodialysed patients fell until it reached values close to those of the controls in the post-dialysis state [29].

There were positive correlations between salivary and plasma levels of calcium in the pre and post dialysis states as well as concentrations of potassium in the post dialysis state. Contrary to our finding, a previous study reported no correlation between plasma and salivary levels of calcium in patients with CKD [30]. The differences in the findings could be explained by the variations in the study population as well as laboratory methods used.

In conclusion, saliva based biochemical analysis in patients with CKD showed reduced salivary concentrations of urea and creatinine while bicarbonate concentration was elevated after dialysis similar to what was observed in plasma. In addition, there was positive correlation between salivary calcium in the pre and post dialysis states as well as creatinine and potassium in the post dialysis state. However, there was no significant change in the salivary as well as plasma concentrations of sodium, potassium, calcium and total protein after dialysis. Also there was no correlation between saliva and plasma concentrations of sodium, potassium, and total protein. Findings of this study indicate that saliva reflects levels of the assessed biochemical factors in the plasma of patients with CKD in the pre and post dialysis states, with some of the saliva

factors showing correlation with the plasma counterparts. Hence, saliva may be an alternative to blood in monitoring chronic kidney disease especially in patients undergoing dialysis.

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Hepatotoxicity and clastogenicity of dichlorvos at high doses in male Wistar rats

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Abstract

Introduction: Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate (DDVP) is widely used in Nigeria for the preservation of stored grains, especially dry beans. Residues of this organophosphate pesticide may therefore inadvertently be present in such products. The present study investigates the hepatotoxic and clastogenic effects of high doses of DDVP in rats as a mimic to its indiscriminate use on store products.

Methods: Male rats (100-150 g) were randomly divided into six groups of five each. Treatments (p.o) were as follows: Group 1-distilled water; Group 2 (control)-corn oil; Groups (3-5)-varying doses of DDVP (5-20 mg/kg b.w) and Group 6-(2.5 mg/kg) sodium arsenite. After 28 days, indices of hepatotoxicity [serum activities of gamma-glutamyl transferase (γGT), alkaline phosphatase (ALP), alanine and aspartate aminotransferases (ALT and AST)] and clastogenicity [relative number of micronucleated polychromatic erythrocytes (mPCEs)] were determined.

Results: DDVP at all the tested doses induced significant ($p < 0.05$) increase in activities of γGT, ALP, ALT and AST. It also significantly ($p < 0.05$) induced mPCEs formed in the bone marrow as compared with the control. The level of induction was dose dependent in both cases. In addition, there was significantly ($p < 0.05$) higher number of hepatic cells in the cell/mm^2 assay for the group treated with DDVP. Histopathological analysis of liver samples from the treated groups revealed lesions corroborating the biochemical indices above.

Conclusion: Findings from this study suggest that DDVP has clastogenic and hepatotoxic effects in rats. There is therefore a need for strict regulatory control and monitoring of the use and residues of DDVP in stored products.

Keywords: *Aminotransferases, Micronucleated polychromatic erythrocytes (mPCEs), DDVP.*

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Résumé

Introduction : Le dichlorvos (2, 2-dichlorovinyl diméthyle phosphate) (DDVP) est largement utilisé au Nigéria pour la conservation des grains entreposés, en particulier des haricots secs, et des résidus de ce pesticide organophosphoré peuvent donc être présents par inadvertance dans ces produits. Cette étude présente enquête sur les effets hépatotoxiques et de clastogènes à fortes doses de DDVP chez les rats, comme une imitation de son utilisation sans discernement sur les produits en réserve.

Méthodes : Des rats mâles (100-150 g) ont été divisés au hasard en six groupes de cinq chacun. Les traitements (p.o) étaient les suivants: Groupe 1 - eau distillée; Groupe 2 (témoin) - huile de céréale; Groupes (3-5) - doses variables de DDVP (5-20 mg / kg de poids corporel) et du groupe 6- arsénite de sodium (2,5 mg / kg). Après 28 jours, indices d'hépato-toxicité [activités sériques du gamma-glutamyl transférase (γGT), la phosphatase alcaline (ALP), l'alanine et l'aspartame aminotransférase (ALT et AST)] et la clastogénicité [nombre relatif d'érythrocytes polychromatiques micro-nucléés (mPCEs)] ont été déterminées.

Résultats : Le DDVP à toutes les doses testées a induit une augmentation significative ($p <0,05$) des activités de γGT, ALP, ALT et AST. Il a également induit significativement ($p <0,05$) des mPCEs formés dans la moelle osseuse par rapport au témoin. Le niveau d'induction dépend de la dose dans les deux cas. En outre, il y avait un nombre significatif ($p <0,05$) plus élevé de cellules hépatiques dans le test cellule/mm ^{2} pour le groupe traité avec DDVP. L'analyse de l'histopathologie des échantillons hépatiques des groupes traités a révélé des lésions corroborant aux indices biochimiques ci-dessus.

Conclusion : Les résultats de cette étude suggèrent que le DDVP a des effets clastogènes et hépatotoxiques chez les rats. Il existe donc un besoin de contrôle réglementaire strict et de surveillance de l'utilisation et des résidus de DDVP dans les produits stockés.

Mots - clés: *taminotransférase, micro-nucléés érythrocytes polychromes (mPCEs), DDVP*

Introduction

Significant progress has been made in understanding the molecular basis of cancer and other diseases. This often involves defects in diverse number of genes [1]. Such defects can be initiated by environmental, biological, physical and chemical agents. Some of the agents are deliberately taken as with excessive ethanol in drinks while others, are inadvertently introduced into the biological system. Pesticides that are used in crop processing and production belong to the latter category. Increasing use of pesticides in agriculture has been attributed as a factor behind recent all year food availability and increase in agricultural productivity [2].

One such pesticide is dichlorvos (2, 2-dichlorovinyl dimethyl phosphate, DDVP), an organophosphate pesticide that is effective against aphids, spider, mites, caterpillars, thrips, and white flies [3, 4]. It is used for space treatment during food processing, handling, and in storage plants, feedlots, stockyards, corrals, holding pens, animal buildings, poultry houses, as well as commercial and institutional buildings. In Nigeria, it is widely used in the preservation of stored grains especially dry beans. European Union recently placed a ban on importation of Nigerian dry beans due to presence of high concentration of DDVP beyond permissible limit of 0.01 mg/g [5, 6].

Although there are concerns regarding the environmental and ecological impact of DDVP, only little is known about its toxicity in the mammalian system [7, 8]. There is a need for increased understanding of the toxic effects of this class of chemicals used indiscriminately in food preservation in order to define the health risk associated with exposure to them. This study was designed to assess the hepatotoxic and clastogenic effects of DDVP when administered at high doses in experimental male Wistar rats.

Materials and methods

Test substances

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) was purchased from Amenss Agrochemicals, Mokola Ibadan. The stock solution was prepared by making up 0.5 ml of dichlorvos (1 g/ml) to 50 ml using corn oil to give a concentration of 10 mg/ml.

Kits and Chemicals

Aspartate aminotransferase (AST), alanine aminotransferase and γ-glutamyl transferase kits were purchased from Randox Laboratories, United Kingdom. May Grünwald stain, DPX mountant, Giemsa stain, and colchicine were from Sigma Chemical Co. USA.

Experimental animals and treatments

Thirty male albino rats weighing between 100-150 g were obtained from Olufarms, Ibadan; and housed in the experimental animal facility of our department. The animals were kept under the condition of 12 hours light/ dark cycle and temperature of 28 ± 2 °C with free access to feed pellets (Vita Feeds, Mokola, Ibadan) and water *ad libitum* throughout the duration of the experiments.

The rats were divided into six groups of five rats each and treated as follows:

Group 1: Given distilled water

Group 2: Given corn oil only.

Group 3: Administered DDVP at 5 mg/kg body weight

Group 4: Administered DDVP at 10 mg/kg body weight

Group 5: Administered DDVP at 20 mg/kg body weight

Group 6: Given sodium arsenite at 2.5 mg/kg body weight (this is $1/10^{th}$ LD_{50} in rats [9].

Sodium arsenite was used as a standard genotoxin and hepatotoxin [10, 11]. The test substances were administered every other day for a period of four weeks by oral gavage. Experimental animals were treated and sacrificed following standard rules laid down by the University Ethics Committee on the treatment of experimental animals.

Serum samples preparation

The rats were sacrificed by cervical dislocation. Through cardiac puncture, blood was collected from each of the sacrificed animals into plain tubes and allowed to clot at room temperature for about 2 hours. The clotted blood samples were centrifuged at 3000 g for 10 minutes at 4 °C using Beckman L5-50B ultracentrifuge (Ramsey, MN, USA). The supernatant (the serum) was separated and used immediately or stored at -20 °C until required.

Biochemical assays

Micronuclei assay

The method of Schmid [12] was adopted in the preparation of bone marrow smears. The femurs were removed from the sacrificed rat and stripped clean of muscle. A pair of scissors was used to make an opening in the iliac region of the femur. A small pin was then introduced into the marrow canal at the epiphyseal end. As the pin was pushed inside the canal, the marrow exuded through the hole at the iliac end. The marrow was placed into a slide and a drop of fetal calf serum was added to the smear using a Pasteur pipette. The marrow and fetal calf were mixed to homogeneity using a clean edge of another

slide. The homogeneous mixture was then spread on the slide as a smear and allowed to dry. The cells on the slides were viewed under light microscope to detect the presence of micronucleated polychromatic erythrocytes (mPCEs). Micronuclei and mPCEs stain blue while mature erythrocytes stain red. Tally counter was used to aid the scoring of mPCEs.

Protein determination

The protein concentrations of the various samples were determined by Biuret method as described by Gornal and his research group [13]. Potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide. In some cases the serum or supernatants from liver homogenate were diluted 100 times with distilled water. Then, 1 ml of the original or diluted sample was taken and added to 3 ml of Biuret reagent. The determination was done in triplicates. The mixtures were incubated at room temperature for 30 minutes after which the absorbance was read at 540 nm using distilled water as blank. The protein concentration for each of the samples was obtained by extrapolated from the standard curve.

Enzyme assay

Gamma glutamyl transferase activity (γGT)

Serum γGT was assayed using the reconstituted γGT diagnostic reagent following the method of Szasz [14].

Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities

Serum ALT and AST activities were assayed according to Reitman and Frankel [15] by monitoring an intensely coloured hydrazone read at 546 nm using a Spectronic-20 spectrophotometer (Thermo Scientific, Surrey, UK).

Determination of lipid peroxidation

Lipid peroxidation level was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. An aliquot of 0.4 ml of the sample was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30 % TCA was added. 0.5 ml of 0.75 % TBA was added and placed in a water bath for 45 minutes at 80 °C. This was then cooled on ice and centrifuged at 3000 g for 10 minutes. Clear supernatant was collected and absorbance measured against reference blank of distilled water at 532 nm. The MDA (malondialdehyde) level was calculated according to an established method [16].

Histopathological analysis

Liver tissues from the animals were immersed in 10 % buffered formal-saline. These were left for 24 hours for fixation of the organs after which cross-sections of the organs were cut at 3 mm thickness and placed in a processor overnight. In the processor, the tissues were placed first in 70 % alcohol for 2 hours, followed by 90 % alcohol for another 2 hours, xylol for 4 hours, and finally, in wax for 5 hours. The tissues were removed, embedded in molten fibro wax and allowed to solidify under a running tap. The tissues, mounted on wooden blocks, were then chilled on ice. Sections of the tissue were cut at a thickness between 3 and 5 mm using the rotary microtome and then allowed to float in 20 % alcohol, followed by water at 58°c (in an incubator). It was then placed on albumized glass slides and dried on a hot plate at 60 °C. The slides so prepared were initially placed in xylol and washed with decreasing concentration of absolute alcohol, 90 % alcohol, 80 % alcohol and finally, 70 % alcohol. They were washed in water stained with Cole's haematoxylin, washed again with water, followed by 1 % hydrochloric acid, running tap water and rinsed in saturated lithium carbonate. These glass slides were transferred to 1 % aqueous solution of eosin for 2 minutes, and washed in a running tap. They were cleaned, mounted on Depex after treatment in absolute alcohol. The slides were finally allowed to dry on the bench at room temperature and then viewed under the microscope.

Cells per mm^2 analysis

The numbers of hepatocytes per mm^2 on stained slices, prepared from the liver were counted under a Nikon light microscope at x400 with the aid of a grid and tally counter.

Haematological analysis

White blood cell (WBC) (total and differential), total red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), and platelets were determined from blood samples collected in EDTA bottles using standard techniques.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation and analysed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) to test for significant differences among the groups of rats using Statistical Package for Social Sciences (SPSS) program. P values less than 0.05 were considered statistically significant.

Results

Administration of sodium arsenite (2.5 mg/kg body weight), used as standard toxicant in this study, resulted in significant ($p<0.05$) reduction in percentage change in body weight compared with the group given distilled water only (Group 1). Also, administration of DDVP in corn oil, at all doses, caused significant ($p < 0.05$) reduction in the percentage change in body weight when compared to the control Group 2 treated with corn oil (Fig. 1).

In addition, findings from the micronucleus assay showed that DDVP at all doses tested, induced formation of micronuclei at levels similar to sodium arsenite. The number of mPCEs scored per 1000 PCEs in the bone marrow cells of rats in each of the treated groups (Groups 3 -6) were significantly higher than observations made in the respective control (Groups 1 and 2) (Fig. 2). Furthermore, administration of sodium arsenite at 2.5 mg/kg body weight or DDVP (at 10 mg and 20 mg/kg body weight)

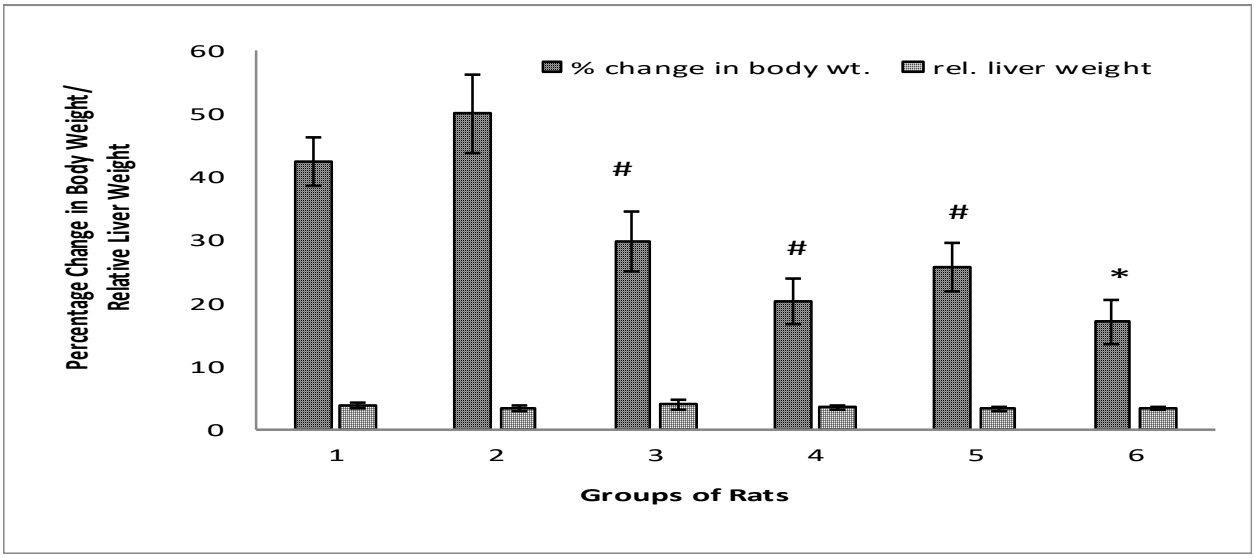

Fig. 1: Effects of DDVP on percentage change in body weight and relative liver weights of rats. Values are mean + SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only (corn oil was used as vehicle for DDVP); group 3 rats were treated with DDVP at 5 mg/kg; group 4 rats were treated with DDVP at 10 mg/kg; group 5 rats were given DDVP at 20 mg/kg; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite dissolved in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

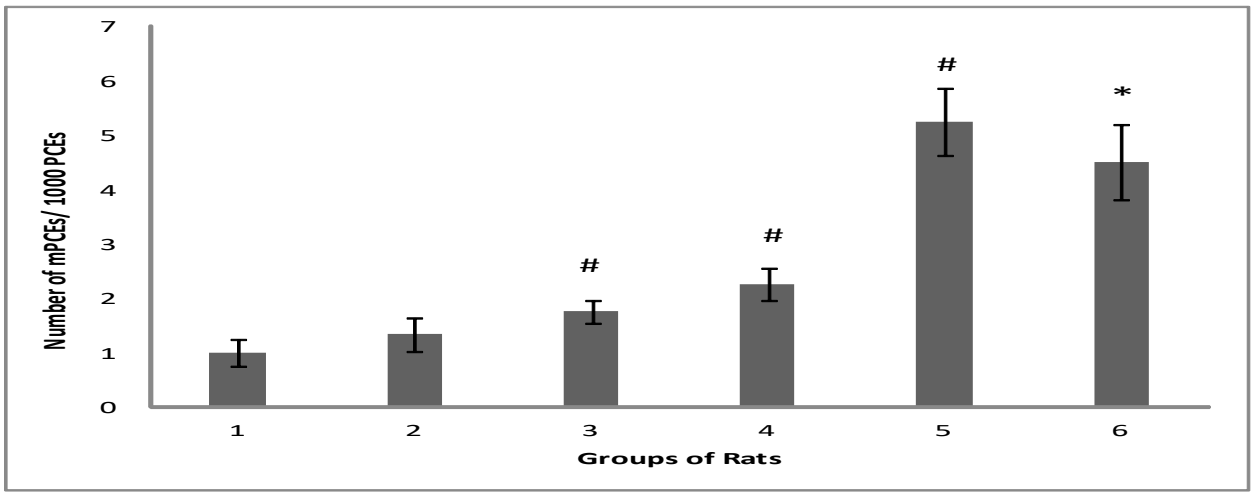

Fig.2: Induction of micronuclei formation in the bone marrow cells by DDVP. Values are means $\pm$ SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with DDVP at 5 mg/kg; group 4 rats were treated with DDVP at 10 mg/kg; group 5 rats were given DDVP at 20 mg/kg; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite dissolved in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

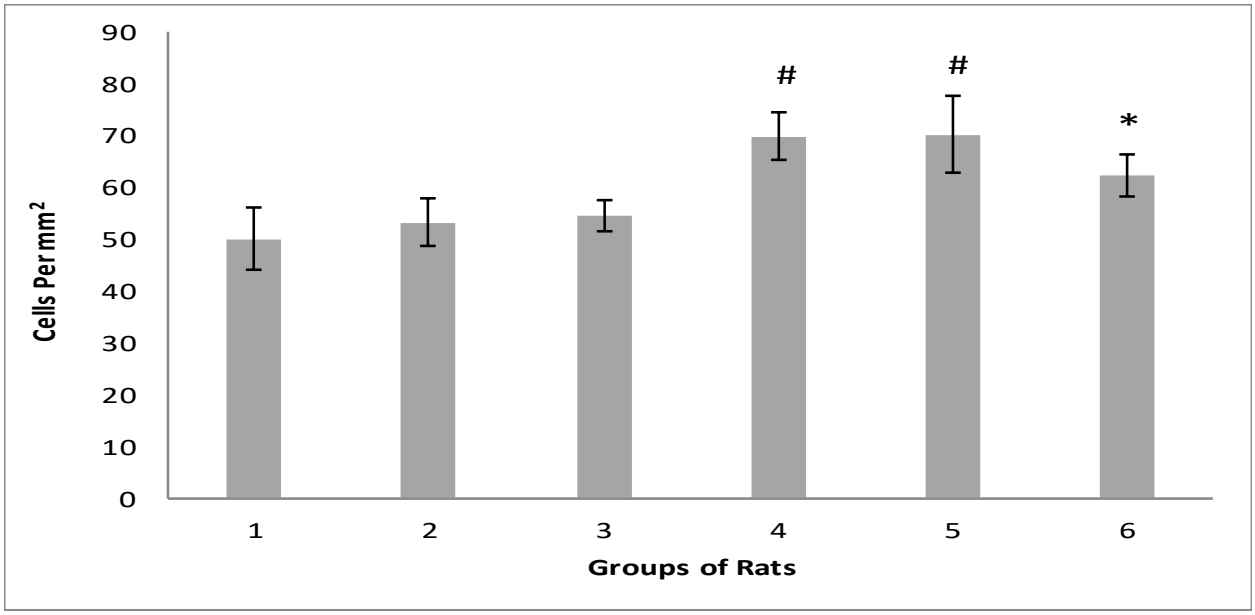

Fig. 3: High doses of DDVP induced hepatic cells proliferation in rats. Values are means ± SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with 5 mg/kg DDVP; group 4 rats were treated with 10 mg/kg DDVP; group 5 rats were given 20 mg/kg DDVP; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

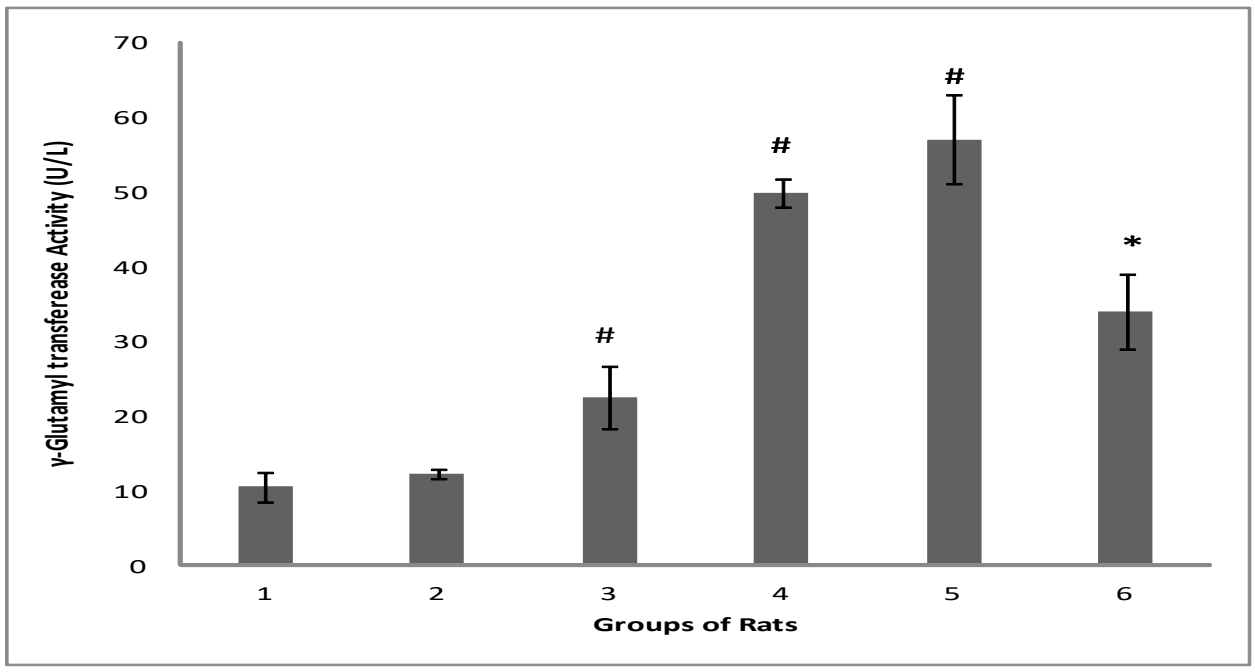

Fig. 4: Effects of DDVP on serum γ-glutamyl transferase activity in rats. Values are means ± SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with 5 mg/kg DDVP; group 4 rats were treated with 10 mg/kg DDVP; group 5 rats were given 20 mg/kg DDVP; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

triggered significant increase in the liver cell per mm^2 compared with the control group (Fig. 3).

Treatment of rats with sodium arsenite or DDVP (at all doses) produced significant increase in mean serum γGT activities compared with the control groups treated with distilled water or corn oil alone. The increase in the enzyme activity in groups treated with DDVP is dose dependent (Fig. 4). Observation made with AST and ALT activities (Fig.5), and ALP activity (Figure 6) in the treated

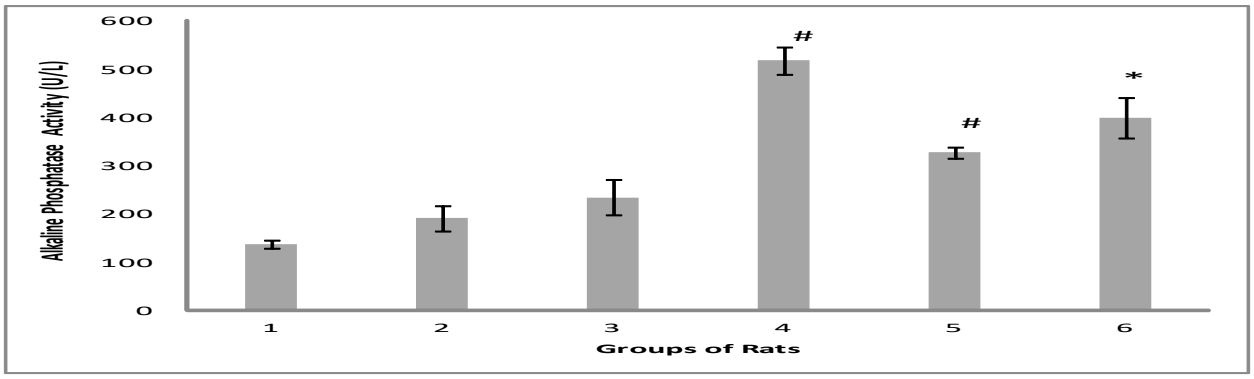

Fig. 5: Effects of DDVP on serum alkaline phosphatase activity in rats. Values are means ± SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with 5 mg/kg DDVP; group 4 rats were treated with 10 mg/kg DDVP; group 5 rats were given 20 mg/kg DDVP; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

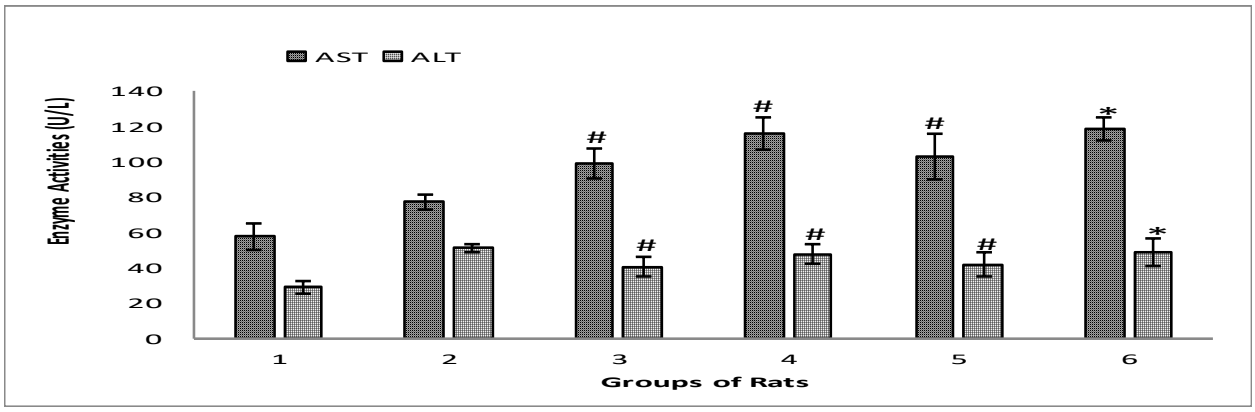

Fig.6: Administration of DDVP produced raised serum alanine and aspartate aminotransferases (ALT and AST) activities in rats. Values are means ± SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with 5 mg/kg DDVP; group 4 rats were treated with 10 mg/kg DDVP; group 5 rats were given 20 mg/kg DDVP; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

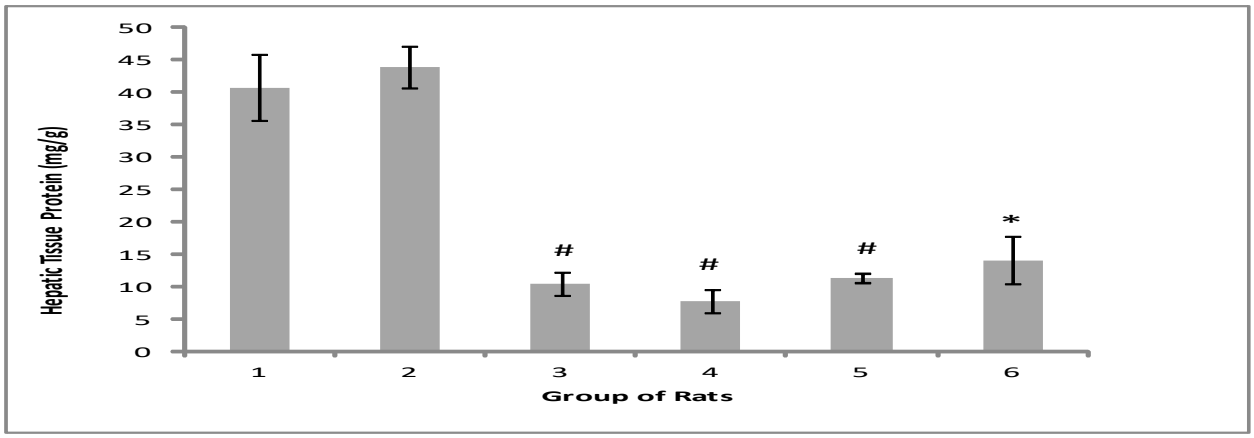

Figure 7: Effect of DDVP on tissue hepatic protein in rats. Values are means ± SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with 5 mg/kg DDVP; group 4 rats were treated with 10 mg/kg DDVP; group 5 rats were given 20 mg/kg DDVP; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

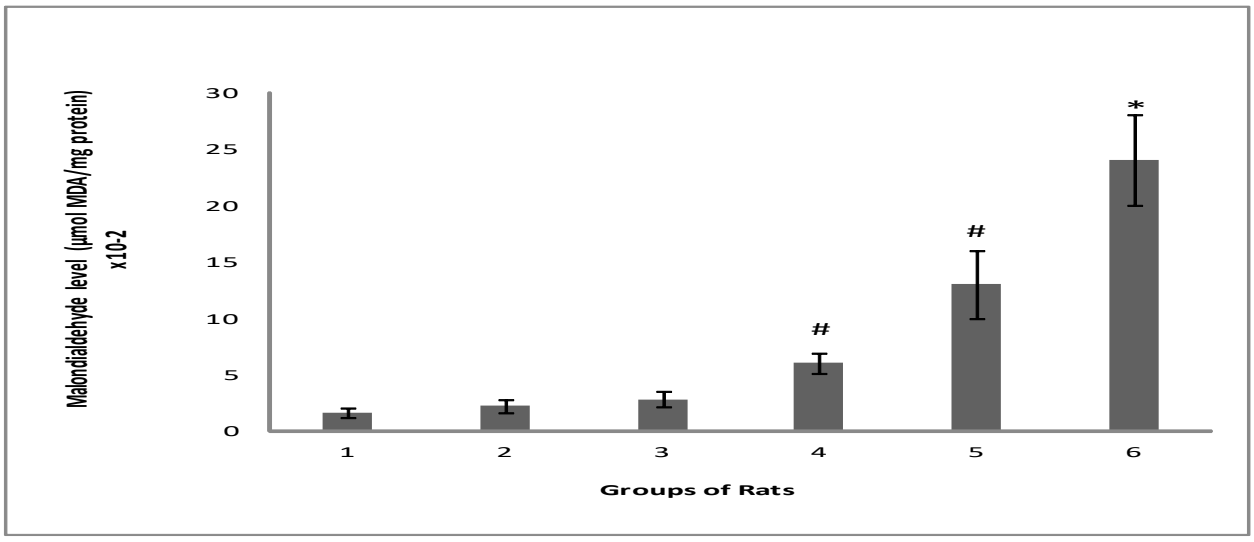

Fig. 8: Lipid peroxidation index (MDA) in rats treated with DDVP. Values are means ± SD (n =5); group 1 represents negative control given distilled water; group 2 rats were given corn oil only; group 3 rats were treated with 5 mg/kg DDVP; group 4 rats were treated with 10 mg/kg DDVP; group 5 rats were given 20 mg/kg DDVP; group 6 served as positive control group treated with 2.5 mg/kg sodium arsenite in distilled water. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1.

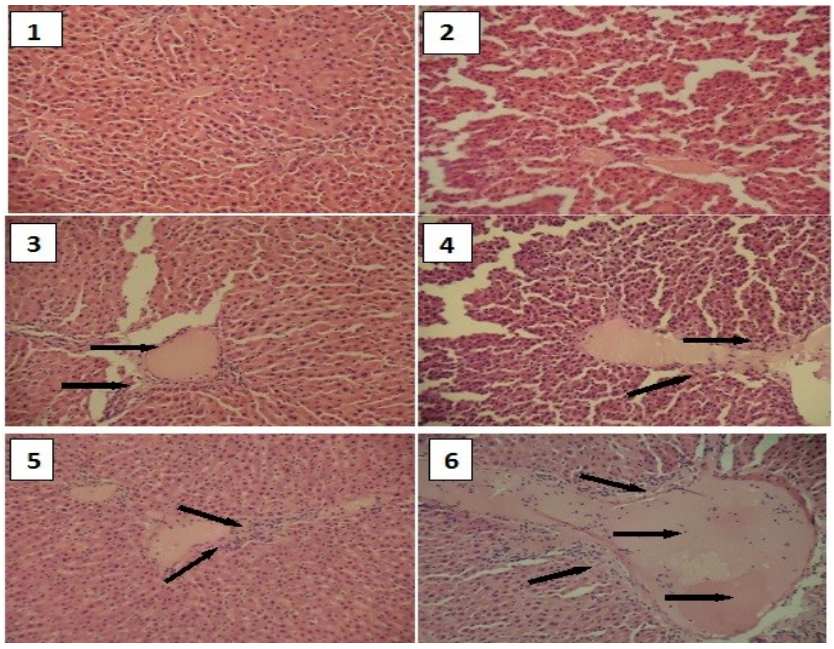

Fig. 9: Selected photomicrograph of the liver sections (x40) rats treated and untreated with DDVP. Negative control group (1) distilled water: no visible lesions seen. Negative control group (2) rats given corn oil only: no visible lesions seen. Rats treated 5 mg/kg DDVP (3): There is moderate portal fibroplasia and cellular infiltration by mononuclear cells. Rats treated with 10 mg/kg DDVP (4): There is mild to moderate periportal infiltration by mononuclear cells. Rats given 20 mg/kg DDVP (5): There is extensive and sinusoidal congestion with periportal and some extent of diffuse cellular infiltration. Positive control group (6) (Rats treated with 2.5 mg/kg sodium arsenite): There is very severe portal congestion, cellular infiltration and fibroplasia.

and control groups followed pattern similar to the findings for γGT activity. Moreover, significant reduction ($p<0.05$) in total hepatic protein was observed in all the groups treated with sodium arsenite or DDVP compared with the negative control (Fig.7). On the other hand, lipid peroxidation, measured as µmol MDA/mg protein, increased significantly ($p<0.05$) in groups treated with sodium arsenite (group 6) and DDVP (at 10 mg or 20 mg/kg body weight) (groups 4 and 5) compared with the control. There was no significant difference ($p>0.05$) between groups treated with DDVP at 5 mg/kg and control (Fig.8).

The histological assessment for liver integrity showed that while groups 1 and 2 given distilled water and corn oil alone respectively had no visible lesion, rats treated with 5 mg/kg DDVP (group 3) showed a moderate portal fibroplasia and cellular infiltration by mononuclear cells. Rats treated with 10 mg/kg DDVP (group 4) showed a mild to moderate periportal infiltration by mononuclear cells. In rats given 20 mg/kg DDVP (group 5), there is an extensive and sinusoidal congestion with periportal and some diffuse cellular infiltration. In the positive control (group 6), treated with 2.5 mg/kg sodium arsenite, there is very severe portal congestion, cellular infiltration and fibroplasias (Figure 9).

The effect of DDVP treatment on rats' haematological parameters is shown on Table 1. Overall, administration of DDVP did not produce significant effects ($p>0.05$) on RBC counts, PCV and Hb values compare with the control given corn oil only. However, at all doses of DDVP, WBC increased significantly ($p<0.05$) compared with control (Table 1).

pesticide for the duration of exposure has no effect on the overall liver mass turnover.

It was observed that treatment with DDVP (at 10 and 20 mg/kg body weight) produced increased liver cell counts compared with the control, suggesting that the insecticide is able to promote hepatocytes proliferation. The induction of increased liver cell populations in the exposed animals may be an adaptation by the rats to get rid of the potentially toxic insecticides. Benford and others [17] have also demonstrated an enforced cell proliferative property of dichlorvos in B6C3F1 strain of mice. However, another research group [18] has reported DDVP induction of cell death *in vitro*. In addition, we observed that the administration of (DDVP) or sodium arsenite promotes significant ($p<0.05$) formation of micronuclei in the polychromatic erythrocytes (PCEs) of the rats bone marrow cells suggesting that DDVP caused chromosome aberrations and nuclear anomalies during mitosis. This is consistent with earlier observations made with sodium arsenite [11,19] and carbofuran [20]. Similar findings were also reported

Table 1: Effects of Dichlorvos on Haematological parameters of Wistar male rats

Groups	Treatment	RBC	WBC	PCV	Hb
1	Distilled water	7.30±0.14	7383.3±275.4	44.3±1.20	59.63±4.52
2	Corn oil	7.51±0.05	5533.33±464.6	45.33±1.15	15.6±0.1
3	5 mg/kg DDVP	7.75±0.13	10466.67±202.1#	44±1.73	13.93±0.95#
4	10 mg/kg DDVP	7.76±0.30	8566.67±208.2#	47±1.00#	15.43±0.15
5	20 mg/kg DDVP	8.41±0.27	7150±132.3#	49.67±2.89	17.1±1.15
6	2.5 mg/kg S.A.	8.11±0.31*	5850±475.2*	48.67±1.53	16.23±0.25*

*DDVP= 2, 2-dichlorovinyl dimethyl phosphate; S.A. = sodium arsenite. # = significantly different ($p < 0.05$) from control group 2. * = significantly different ($p < 0.05$) from control group 1. Values are means ± SD (n =5).*

Discussion

This study was designed to investigate hepatotoxicity and clastogenicity of high doses of DDVP in Wistar rats using sodium arsenite as standard toxicant [10,11]. Our findings showed a significant decrease in percentage body weight change in the groups treated with sodium arsenite and DDVP compared with the control groups. This suggests that DDVP or sodium arsenite, within the duration of the study, exert detrimental effects on the systemic activities in the body of experimental rats. Moreover, a loss of appetite was observed in the rats administered the chemicals. However, there was no significant difference in the relative liver weights between the groups of rats treated with sodium arsenite or DDVP compared with the control groups indicating that the in the culture of human peripheral blood lymphocytes with DDVP [21].

Serum enzymes, γGT, ALP, AST and ALT, activities are used in the diagnosis of hepatic injuries and diseases, and elevation in the levels of these enzymes is an indication of a liver lesion [22-24]. The finding in the present study, showing that DDVP induces activities of the transaminases in treated groups of rats compared with the observation made with the control group, suggests that orally administered DDVP is hepatotoxic in the animals and causing damage to the liver cells resulting in the release of these enzymes into the blood. Moreover, histopathological analyses of the liver sections indicated that DDVP (and sodium arsenite) induced degeneration in the liver cells. Observations

range from a mild to severe periportal infiltration of mononuclear cells, an extensive and severe sinusoidal congestion, diffuse cellular infiltration, severe central venous and portal congestion and cellular infiltration and fibroplasia. Hepatotoxicity of DDVP in the aquatic organism, *Misgurnus anguillicaudatus*, has also been reported recently [25].

The observed total hepatic protein in the treated groups was found to be much lower than the control group. This finding is similar to earlier observation [26] which was reported as a significant decrease in protein level in *Channa gachua* exposed to DDVP. In addition, DDVP along with other insecticides have been reported to alter metabolism of proteins, glucose and fats [27]. Moreover, treatment with DDVP or sodium arsenite resulted in a significant increase in the level of lipid peroxidation products (TBARS) compared to the control groups. Several studies have also associated the toxicity of DDVP to its induction of oxidative stress [28-31].

Administration of DDVP did not produce significant effect on RBC count, PCV and Hb in the experimental rats ($p > 0.05$) compare with control. Significant decrease in mean corpuscular volume after five weeks of exposure to DDVP has been reported [32]. The disparities between the findings in this report and that of Edem and co-workers [32] may be due to differences in route of exposure and duration of exposure to DDVP. On the other hand, we recorded that DDVP at all doses enhanced the WBC counts ($p < 0.05$) compared with control group treated with corn oil only. This suggests that DDVP is recognised by the body defense as foreign or pathogenic leading to proliferation of WBC. This finding supports the carcinogenic potential of long term intoxication of DDVP [33].

In conclusion, the findings from this study suggest that DDVP has clastogenic and hepatotoxic effects in rats and may be carcinogenic long term. Considering the profile of toxicities of DDVP in experimental rats, it could constitute a big risk to human health. There is therefore an urgent need for strict regulatory control for the use of DDVP for food preservation, and effective monitoring system of its residues in stored products before they gain access to the consumers. This regulation will not only provide health safety measures but will enhance the international consumer confidence and provide a much needed thriving source of foreign exchange for developing countries.

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Sickle cell disease management in Nigeria: Understanding the challenges from the physicians' perspectives

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Abstract

Introduction: Nigeria has the greatest burden of sickle cell disease (SCD) in sub-Saharan Africa. The disorder is usually associated with a huge psychological and financial toll on families and individuals in developing countries. However, SCD diagnosis and management are still largely rudimentary especially in Africa. This study therefore aims to explore the views of physicians about challenges facing SCD management in Nigeria as well as the health system responses to these challenges.

Methods: This qualitative cross-sectional study was conducted in 2015. A total of ten in-depth interviews (IDIs) were conducted among purposively selected resident doctors at the Hematology Department of the University College Hospital (UCH), Ibadan, Southwest Nigeria. Data from the IDIs were transcribed and analyzed with the aid of the NVIVO (version 10) software using the thematic framework approach to qualitative data analysis.

Results: In the opinion of the study participants, many of the SCD patients, cannot access up-to-date medical care because of poor health financing and poverty, inadequate health infrastructures and medical facilities including obsolete blood transfusion services and medical equipment. There were myriads of medical challenges facing individuals with SCD in Nigeria: frequent illnesses and crises which may comprise bone pains, recurrent anemia, malaria, chronic leg ulcer and even risk of HIV and Hepatitis B from frequent blood transfusion. Similarly, SCD patients may experience psychological challenges, according to the respondents, due to the frequent illnesses, discrimination suffered as well as stigmatization. Some respondents submitted that there is still significant ignorance about the disease and its pathophysiology among the patients themselves, their caregivers and the general population thereby feeding a lot of superstitious beliefs. Some health systems challenges highlighted in SCD management include inadequate financial support as well as poor infrastructures for diagnosis and treatment.

Conclusion: The knowledge gap in the community about sickle cell disease should be bridged through constant health education in order to alleviate stigma. There is also a need for an effective policy to protect persons living with SCD from discrimination in the labor market as well as the workplace. Better funding for research as well as the strengthening of the social health insurance will go a long way to promote the management of SCD and reduce catastrophic expenditure and poverty among SCD patients and their families.

Keywords: *Sickle cell disease, Health systems management, Physicians' perspective, Out-of-pocket health expenditure*

Résumé

Introduction: Le Nigéria a le plus grand fardeau de drépanocytose en Afrique subsaharienne. Le trouble est généralement associé à une lourde charge psychologique et financière sur les familles et les individus, en particulier dans les pays en voie de développement. Cependant, le diagnostic et la prise en charge de la drépanocytose sont encore largement rudimentaires surtout en Afrique. Cette étude vise donc à explorer les points de vue des médecins sur les défis de la prise en charge de la drépanocytose au Nigeria ainsi que les réponses du système de santé à ces défis.

Méthodes : Cette étude transversale qualitative a été menée en 2015. Au total, neuf entretiens approfondis ont été menés parmi des médecins résidents sélectionnés au sein du département d'hématologie du Collège Hospitalier Universitaire (UCH) à Ibadan, Sud - Ouest du Nigeria. Les données des entretiens approfondis ont été transcrites et analysées à l'aide du logiciel NVIVO (version 10) en utilisant l'approche du cadre thématique pour l'analyse qualitative des données.

Résultats: De l'avis des participants à l'étude, beaucoup de patients drépanocytaires ne peuvent pas accéder à des soins médicaux à jour en raison du mauvais financement de la santé et de la pauvreté, des infrastructures sanitaires et des installations médicales inadéquates, notamment des services de transfusion sanguine et du matériel médical

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obsolètes. Il y avait une myriade de défis médicaux auxquels sont confrontés les personnes atteintes de la drépanocytose au Nigeria: maladies fréquentes et crises qui peuvent comprendre des douleurs osseuses, anémie récurrente, paludisme, ulcère de jambe chronique et même risque de VIH et d'hépatite B provenant de transfusions sanguines fréquentes. De même, les patients drépanocytaires peuvent rencontrer des difficultés psychologiques, selon les répondants, en raison des maladies fréquentes, de la discrimination subie ainsi que de la stigmatisation. Certains répondants ont fait valoir que la maladie et sa physiopathologie demeurent ignorantes chez les patients eux-mêmes, chez leurs soignants et dans la population en général, alimentant ainsi de nombreuses croyances superstitieuses. Certains défis liés aux systèmes de santé mis en évidence dans la gestion de la drépanocytose comprennent un soutien financier inadéquat ainsi que des infrastructures médiocres pour le diagnostic et le traitement.

Conclusion: Le manque de connaissances sur la drépanocytose dans la communauté devrait être comblé par une éducation sanitaire constante afin de réduire la stigmatisation. Il est également nécessaire de mettre en place une politique efficace pour protéger les personnes vivant avec la drépanocytose contre la discrimination sur le marché du travail et sur le lieu de travail. Un meilleur financement pour la recherche ainsi que le renforcement de l'assurance de santé sociale contribueront grandement à promouvoir la prise en charge de la drépanocytose et à réduire les dépenses catastrophiques et la pauvreté parmi les patients atteints de drépanocytose et leurs familles.

Mots-clés: *Drépanocytose, Gestion des systèmes de santé, Point de vue des médecins, Dépenses de santé directes*

Introduction

Nigeria bears the greatest burden of sickle cell disease (SCD) in sub-Saharan Africa. [4]About 25% of Nigerians have the sickle cell trait.[6, 7]The disorder is usually associated with a huge psychological and financial burden on families and individuals especially in developing countries with limited social security.[1, 5]

Furthermore, despite being one of the most common monogenic disorders globally, SCD diagnosis and management are still largely rudimentary especially in Africa where the clinical course of the disease is more aggressive and there exists a wide knowledge gap with lack of novel therapies. [3, 12]Many studies have examined the pathophysiology and molecular nature of the disease but studies exploring the views of physicians, who are primary care givers, about the disease, the management modalities and health systems response are sparse. It is thus imperative to understand the views and perspectives of the doctors primarily involved in their management. This information will promote our understanding of the sickle cell disease process and how the health system may better respond to these challenges.

The findings of this study will have implications for patient education and policy formulation in healthcare delivery. This study therefore aims to explore the views of physicians at the University College Hospital about challenges facing SCD management in Nigeria as well as the health system responses to these challenges.

Methods

This cross-sectional study was conducted in 2015 and it is part of a larger study on management of sickle cell disease in Nigeria. A total of ten in-depth interviews (IDIs) were conducted at the University College Hospital (UCH), Ibadan, Southwest Nigeria. The University College Hospital Ibadan is a flagship referral center for Nigeria and many parts of the West African sub-region. The hospital has a department of haematology that provides clinical care for patients with sickle cell disease.

The study population consisted of resident doctors who had worked in the Department of Hematology for at least a year. A total of 10 resident doctors in Hematology were interviewed. All were men with ages ranging from 25 to 40 years(Median=30.5 years). However, nationality, gender or religions were not prerequisites for the selection of participants for the IDIs. The authors facilitated some of the interviews while the others were facilitated by research assistants who were also doctors in community medicine/health management. Each interview was recorded, with the participant's consent, using a digital voice recorder. The interviewer also took note of non-verbal expressions of participants. All the interviews were conducted in English.

Participants in this study were recruited through a purposive sampling method based on their ability to provide relevant information on the subject of interest and availability. An in-depth interview guide was used to facilitate the interviews. Issues explored in the interviews included views and opinions of participants presentation of SCD patients in this environment, causes of crises in SCD, challenges faced by people living with SCD in Nigeria as well as the challenges with the management of SCD in Nigeria. [14]

Data from the IDIs were transcribed and analyzed with the aid of the NVIVO (version 10)

software using the thematic framework approach to qualitative data analysis [15].This was an iterative process of analysis which started right after the first interview and continued throughout the research. A thematic framework was developed from emerging themes in the interviews. As themes emerged, these were indexed and compared with themes from subsequent interviews until a sense of attainment of saturation was achieved [15].

Ethical considerations

Ethical approval was obtained from the University of Ibadan/University College Hospital Ethical Review Board. Written informed consent was also gotten from participants before the interviews.

Results

The results of the qualitative enquiry into the subject of the perception of physicians about the challenges of individuals living with SCD in Nigeria as well as the physicians' view of SCD management in Nigeria:

Challenges faced by SCD patients in Nigeria

The interviewed physicians thought that SCD patients in Nigeria are confronted by a number of challenges. Some participants described some general/background challenges which include ignorance and superstitious beliefs on the part of parents/caregivers of SCD and the patients themselves on what causes the disease, the symptoms and management options. The ignorance and superstition may affect their compliance with medical management. As stated by some of the respondents:

"...it starts from the ignorance of the parents. Let us start from a child being born, if the parents do not have idea what sickle cell is, that is a challenge on its own. Now when the child begins to have problem ... the quality of medical care from the childhood is substandard" (IDI 10).

"...other challenge we find in this environment is poor education, some people still `believe some myths that sickle cell disease is a punishment from some gods whatever... There is this dangerous belief people have, that when they attain a particular age they are immune from sickle cell crisis - that is another challenge. You will see people will not come for follow up in a long time" (IDI 6).

These challenges were described as "…lifelong …and enormous" (IDI 3). According to the respondents these challenges include medical, psychological, social and financial challenges. These challenges are highlighted below and summarized in Figure 1.

Financial challenges

The management of SCD is said to be financially tasking. Many of the SCD patients, in the opinion of this study participants, cannot access good medical care or keep up with prescribed line of management because of inadequate finances and poverty. In the word of one of the physicians, "…the greatest challenge is finance. It is a chronic disease that takes away the little earnings that they have and recurrent crises and recurrent need for medical treatment and their drugs. It tells more on them and most of them are mainly from low socioeconomic class" (IDI 6). While alluding to the importance of lack of finances among all other challenges facing SCD patients in Nigeria, a participant said: "…If I would arrange in descending order, I would first of all consider the financial challenge…" (IDI 9). Furthermore, another respondent corroborated: "…it requires a lot of resources in managing the crises so I think by and large the limitation to resources available to the patient is one of the most serious challenges that they face" (IDI 4).

Physicians' perception of medical challenges faced by patients

According to the participants, there are myriads of medical challenges facing individuals with SCD in Nigeria. These medical challenges include frequent illnesses and crises and slow growth. The illness may comprise bone pains, recurrent anemia, malaria, chronic leg ulcer and even risk of HIV and Hepatitis B from frequent blood transfusion. Also, the chronic nature of SCD and the need for constant medications sometimes affect patients' compliance with prescriptions. These issues, some participants opined, are challenging both for the patients and the primary caregivers. Some verbatim quotes from respondents are presented below:

"…most of them (SCD patients) when they come in, you think it is just bone pain; you cannot estimate when the patient is likely to be discharged…their disease has unpredictable pattern. I have seen a case that came in with pain and ended up with severe haemolytic crisis thereafter went into anemic heart failure, sequestration crises. She spent like almost a month before we could discharge her" (IDI 5).

"…when they are exposed to some conditions you and I … are exposed to everyday because of their condition, they find it very difficult to manage it, for example: malaria" (IDI 3).

"…We have some patients who do not believe they have to be on medications (hematinics) perpetually…. That in itself is a challenge because it then becomes difficult for you to convince a patient

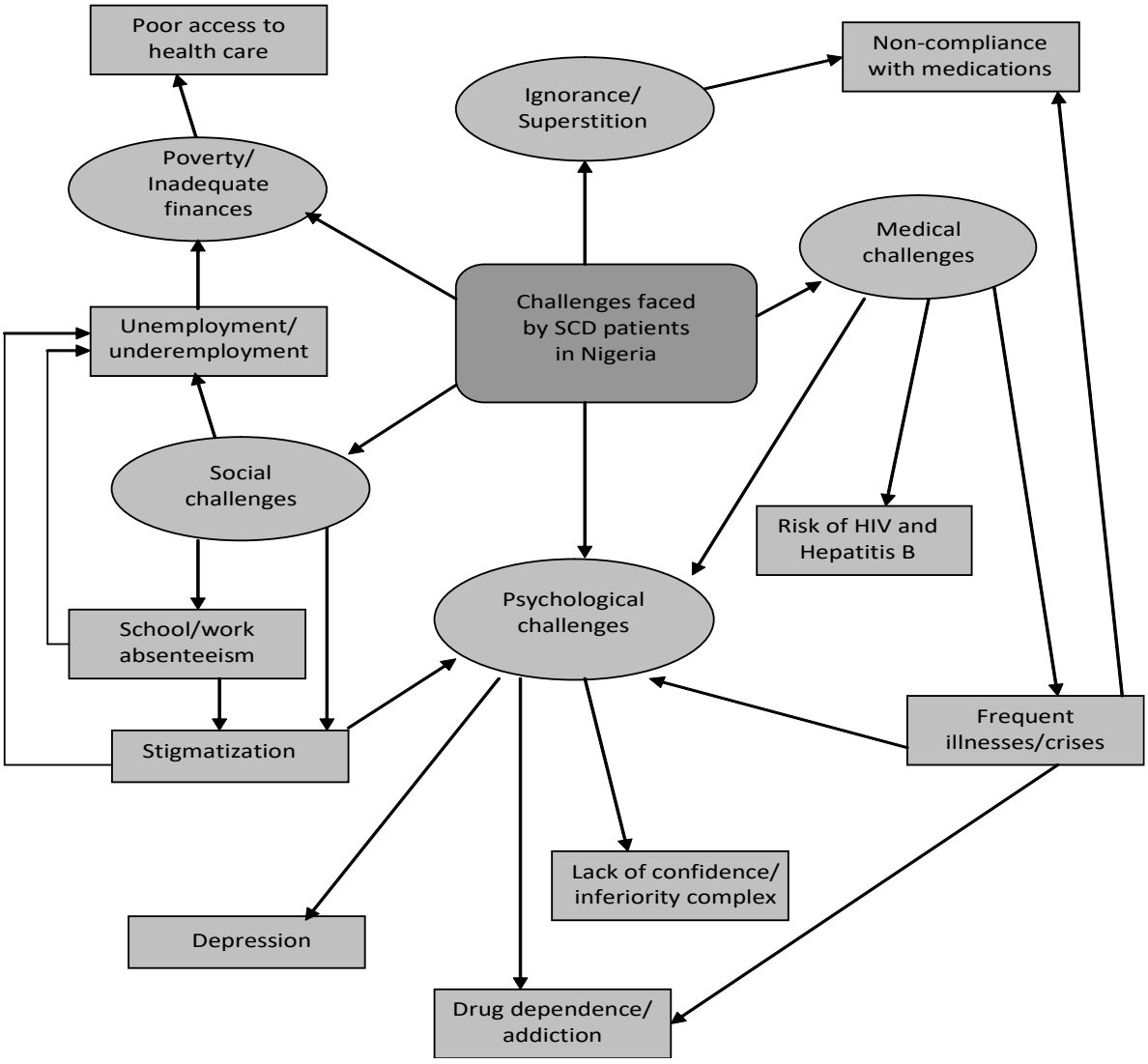

Fig. 1: Respondents' view on challenges facing sickle cell disease patients in Nigeria

to be adherent/compliant to the management when stable or there is no apparent sickness" (IDI 3).

""As a result of repeated transfusion, some SCD clients also come down with infections (like) HIV, Hepatitis B which could also lead to liver diseases and a host of others." (IDI 7)

Psychological challenges

There are a number of psychological issues SCD patients confront. Sometimes, these issues are intertwined with the medical challenges. Some respondents suggest that it is not only persons living with SCD that struggle with these psychological challenges but also the parents/ caregivers and families of SCD patients. Some of the issues that may bring psychological stress include the physique (which may be smaller than that of persons of the same age and gender), frequent illness and the attendant suffering.

"…*in those days people just get married to each other without apparently checking their hemoglobin composites and eventually give birth to children with sickle cell disease and because of the chronic nature of the disorder and attendant manifestation, many a times you see family separated because the father will blame the mother for bringing the problem into the family." (IDI 4)*

"…if you look at their physique, in fact it is enough to make them psychologically affected, because often times with their physique you can actually describe the classical sickle cell habitus even before patients are subjected to hemoglobin electrophoresis. (IDI 8)

Also some respondents opined that SCD patients often suffer from lack of confidence, doubtful marital prospects, stigmatization and drug dependency and addiction. One participant said: *"…when one is subjected to severe stress, there could be some form of depression. A few might come down*

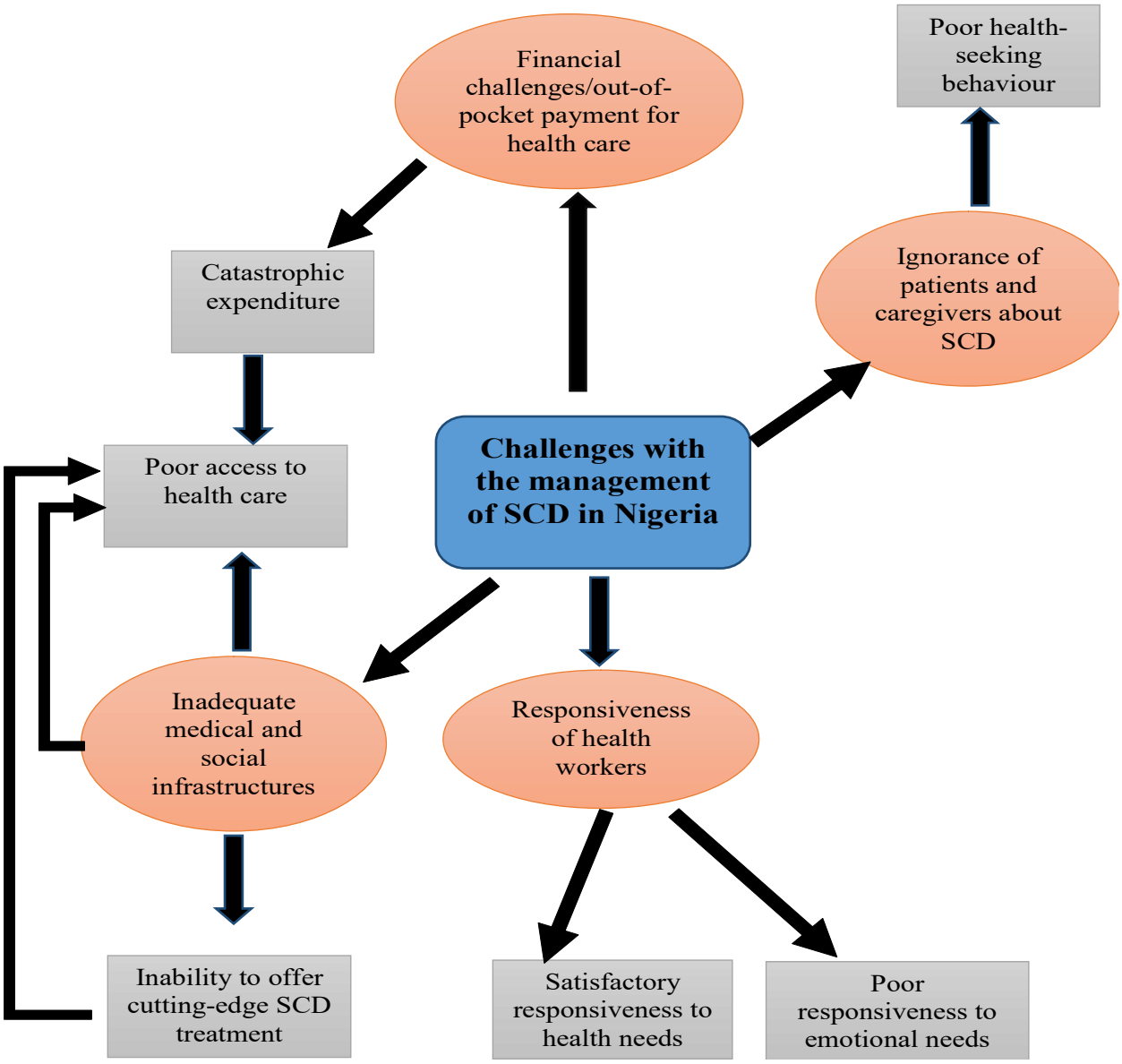

Fig.2: Respondents' views on challenges with the Management of SCD in Nigeria

with depression. In fact, some of them get addicted to things like morphine. In a bid to control the pain they develop that dependence."

According to another respondent, SCD patients tend "…to be less confident and they need a lot of support to improve their confidence" (IDI 10). In the opinion of another respondent, parent may stigmatise their children with SCD unknowingly: "Also a form of stigmatization - parents do not want other people to be aware of the child's condition…" (IDI 2). In addition, there could be regrets from the SCD patient (for being born with such an ailment) or the parent/caregiver (for entering the union that produced such suffering in an individual). To buttress this, a participant said: "…some of them have these feelings of wanting to blame their parents for not choosing rightly and having children with such an abnormality. So their parents have these … feeling of regret to have married in such a way as to predispose them to producing an offspring with Sickle cell disease " (IDI 5).

Also there could be fear – of death and getting sick, feeling of guilt and inferiority complex and this could make the SCD patient withdraw from other people. A few quotes from participants to support this assertion include:

"You talk about … fear of death. He/she may not be socially inclined, few friends, probably stays indoor most of the time, then fear of getting sick" (IDI 10)

"They feel guilty at times for not performing to expectation; they don't like the fact that people look at them as being "subnormal", sort of. Then even when they are present at work, those (SCD patients)

always want to perform in a way that people won't see them as being subnormal…" (IDI 5)

"…they withdraw from others - colleagues and activities" (IDI 5)

"…So the one (SCD patient) who goes in and out of the hospital, with time may begin to feel inferior and want to ask questions: what is really wrong with me? Am I normal?"(IDI 3)

Social challenges

Respondents alluded to the fact that there are numerous social challenges facing persons with SCD ranging from school or work absenteeism due to frequent illnesses, employability and hence income and ability to pay for healthcare, challenges with relationships, difficult pregnancy and delivery for women. "…It affects the child education; you could have poor attendance; when the child is sick, he/she misses exams. Now how is the child going to end up? How employable is this person? This now contributes to the person inability to pay for health care?" (IDI 10)

As par relationships, it becomes a little challenging as they seem to have a very narrow pool to choose from with regards to genotype compatibility. According to one respondent, "Some of the social issues they may see would include choice of marriage partner, because they have to be sure of the genotype of the person they likely to marry. They cannot just marry anybody." (IDI 8)

A respondent also reported that the stigma of being called a 'sickler' or being treated like an invalid is also a real challenge in the Nigerian context: "…people say 'you are a sickler', that is a stigma... She is a sickler; she can die. That stigma is there and then in the workplace, how fit is the person for the kind of work? (IDI 10). A more terrible kind of stigma however is to refer to children with SCD as *Ogbanje* – a mythical demonized child who would not stay on earth but would die after a while. A participant narrated his experience:

"...I remember as a child we used to hear what they call Ogbanje i.e. some little kids who come from the spirit world; they don't want to stay and they keep falling sick until they end up dying and that is the belief which hopefully education and enlightenment have come to clear but I can see that some people still believe their existence. There is that concept of bewitched children."

Challenges with the management of SCD in Nigeria

There are a number of challenges with the effective treatment/management of SCD patients in Nigeria. In the opinion of respondents, some of these challenges have to do with how much patients and their caregivers know about the condition whereas some are as a result of health systems challenges in the country. The health systems challenges include out-of-pocket payment for healthcare, poor hospital infrastructures, inadequate blood transfusion facilities. These challenges are examined in greater depth below with verbatim quotes from participants. Figure 2 below also summarizes these challenges.

Limited knowledge of patients and caregivers about SCD

Many SCD patients as well as their parents/ caregivers are still said to be ill-informed about the disease and how to take care of themselves or provide support to a patient before coming to the hospital. According to a participant, "…some patients don't even know what is happening to them; most of them don't know how to take care of themselves; they don't know what to do and what not to do, things to avoid and things not to avoid. So having a good understanding of their own condition is also a problem for many of the patients." (IDI 8). About caregiver ignorance, a respondent remarked thus: "…it starts from the ignorance of the parents. … if the parents do not have idea what sickle cell is, that is a challenge on its own." (IDI 10).

Financial challenges/out-of-pocket payment for health care

Due to the chronic nature of the disease and the need for relatively frequent consultations and hospitalization, with the predominant out-of-pocket-payment for healthcare, many SCD patients and their caregivers experience financial stress and catastrophic expenditure. In the words of a respondent, "…the fact that they (SCD patients) have to be hospitalized; pay for consultation; do investigations, so most of them have financial challenges" (IDI 5).The participant added that "When patient are asked to do test they can't afford; when they buy this drug, they may resort to begging and sourcing for funds… We have some of them that are homeless, most of them are jobless; owing to the fact that some have been made handicapped from the disease". In the opinion of another participant, "…the greatest challenge is finance. It is a chronic disease that takes away the little earnings that they have and recurrent crises and recurrent need for medical treatment and their drugs. It tells more of them and most of them are mainly from low socioeconomic class" (IDI 6).

Although the National Health Insurance Scheme (NHIS) is operational in Nigeria, many SCD

patients are not covered. One of the participants suggested: "…we still need more financial support in terms of NHIS, I mean Health Insurance Scheme to be available to almost all patients. Maybe we can have NGO, bodies that can really support them" (IDI 5).

Inadequate medical and social infrastructures

In the opinion of the interviewed doctors, challenges of SCD management in Nigeria span from diagnosis to actual treatment of patients. According to a respondent, "We have so many challenges in diagnosis. Though by and large, we are able to diagnose accurately with electrophoresis most of the time but there is this pocket of patients that have non typical usual hemoglobin variant" (IDI 4).In addition, a participant added: "I believe that with the recent advancement in management of this SCD, we should be able to offer them more than we do now. Precisely …bone marrow transplant if they can afford it…I will not say we have got everything to take care of them" (IDI 6).

Furthermore, participants were of the opinion that many SCD patients cannot access good healthcare when they need it as a result of inadequate health infrastructures and medical facilities including blood transfusion services and medical equipment. According to a respondent, "Availability of blood for transfusion… is an issue" (IDI 10). The respondent added: "For instance we need a pulse oximeter which is very important, especially for our triage centerwhich we don't have…I think the problem is more about facilities and can be improved upon" (IDI 10). Some respondents believed that it is difficult for SCD patients to access good healthcare in the Nigerian context of weak health system. In the words of one of the respondents, "Health facilities in Nigeria generally, I will not say it is adequate, but we are just trying to offer the best we can offer with the little that is available to us" (IDI 5).In the opinion of another respondent, "I think in this center we have a comprehensive care… except for the sophisticated… bone marrow transplantation which is the gold standard therapy for people who are eligible at that age" (IDI 4).

However, a respondent proposed a special unit to take care of SCD patients in order to take care of their special needs; "I think special people like these should actually have special form of care…I would suggest … a special unit in the hospital, maybe a special ward that will take of care of sickle cell patient..."(IDI 3).

Responsiveness of health workers

Generally, participants agree that health workers are very responsive to taking care of the health needs of SCD patients including their emotional needs. A respondent said: "…most of the time the management of SCD is an empathic kind of a relationship. In our own setting here, we have dedicated workers who empathize with people who have this disease … to assist them in overcoming their distressing circumstance …" (IDI 4). One of the participants buttressed the need for emotional responsiveness from the health workers with a personal story:

"…there is a medical student who was always coming down with pain. I just decided to be a friend to the guy and I think for some time now he has been doing well. I am just trying to say some of them need support. Some of them will just come to the hospital with crises and after their parents show them some level of love they get better and they go home" (IDI 6).

However some respondents believe that health workers' responsiveness to SCD patients' emotional needs is poorer probably due to stress. As a participant puts it: "…the stress of work sometimes gets to some of us and we do not have empathy. I think many of us do try" (IDI 6). Furthermore, one of the participants interviewed believed that it takes training to be able to handle emotional needs of these patients better. "Yes it comes with the training, yes as a doctor you don't only look at the physical part but you consider the emotional part" (IDI 3).

Respondents' satisfaction with treatment received by SCD patients

Many of the respondents believe the care given to SCD patients in a tertiary hospital like theirs is much better than what obtains in other centers. Most respondents rated the SCD management in their center – the University College Hospital as fair although there is a consensus that the management can be better. According to a respondent, "…from what I have seen, I think our patients prefer our care because it is better than what they get from outside facilities" (IDI 5).

However, the respondent added that there are patients "…complaining about the kind of care they were given at referral centers that are not really into special care of sickle cell disease". Nonetheless, another doctor interviewed believed that patients are usually treated well especially because management has provision for indigent patients. His words: "…based on my experience, they (SCD patients) are well attended to…regardless of the financial aspect of the patient, you go to the management, talk with them and you are in for the treatment" (IDI 9).

Discussion

We present in this study physicians' views and opinions about the challenges with the management of sickle cell disease in Nigeria as well as the challenges facing individuals living with the disease in the country. As expected, participants have a good understanding about the etiology, distribution and presentation of the disease in line with what has been described in literature [1-3,5-7]. However, while making a case for better physician education on SCD, Adewoyin [4] submitted that Nigerian doctors need to know more on SCD phenotypes and comprehensive management of the disease in order for the quality of lives of persons living with the disease to improve.

Also, our study suggests that ignorance, poor education are still issues among persons living with SCD, their parents or caregivers in Ibadan. In a British study [16], researchers reported that ignorance about the natural course of SCD and the poor information about the disease's epidemiology hampered the effective management of the disease in Britain. Similarly, Burnes and colleagues [17] demonstrated through their Canadian study that the issue of SCD stigma is not limited to developing countries and that it has implications for health system responsiveness. Their study also showed that stigma can lead to social isolation for SCD patients and their families as well as reluctance to join a support group.

Also, our study highlighted other challenges facing SCD patients and their families including reported high out-of-pocket expenditure in paying for healthcare leading to poverty, stigma which leads to isolation in the immediate communities and in the labor market. Just like this study revealed, Abuosi and colleagues [18]in a study done in Ghana posited that there is a huge financial burden on families treating children with non-communicable diseases [NCDs] like SCD as a result of the ineffectiveness of the national health insurance system which offered no protection for children with NCDs from poor families and rural areas. This picture of high financial burden and poor insurance coverage among SCD patients and households was also demonstrated in Nigeria. [19]

In addition, studies have shown a vicious cycle between illnesses from SCD, poverty from low productivity as well as social stigma against SCD patients, their families [19,20]. Mubyazi and Njunwa [20] suggested that more resources need to be invested in research, public enlightenment and education in order to reduce the social stigma as well as in engaging policy makers in order to get these issues on the policy agenda.

Furthermore, apart from stigma, SCD patients and their families may also experience family disharmony, poor self-confidence and guilt. Good family support, reduced daily stress and conflicts have been suggested as essential for good psychological adjustment among SCD patients [21,22].

Physicians who participated in this study were of the opinion that the infrastructures for managing SCD were lacking or at best poor in Nigeria despite great advances in the management of the disease including hydroxyurea therapy, chronic blood transfusion and haemopoietic stem cell transplantation [4]. However, research has shown that SCD management requires a holistic approach which is mindful of the psychological, physical and financial needs of the affected individuals in a milieu of a responsive health system and good social support [23].

Conclusion

Physicians interviewed in this study were of the opinion that sickle cell disease patients and their families face myriads of challenges in Nigeria which range from medical, psychological, social and even that of poor health systems to address their needs. It is imperative that the knowledge gap in the community about the disease be bridged through constant health education and enlightenment in order in order to alleviate stigma about the disease. There is also a need for an effective policy to protect persons living with SCD from discrimination in the labor market as well as the workplace.

Furthermore, the government as well as the private sector need to invest more in research that promote better management of SCD including improved blood transfusion services and bone marrow transplantation. Special protection should also be given to SCD patients in the National Health Insurance Scheme in order to ameliorate catastrophic health expenditure and poverty among SCD patients and their families. A possible way of doing this is to ensure greater coverage of the people in the informal sector [where a greater proportion of the poor falls] through a greater spread of the community based health insurance. [24]

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Metabolic alterations in Africans with prostate cancer before bilateral orchidectomy

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Abstract

Introduction: Prostate cancer (PCa) is the commonest male malignancy worldwide with African descent and age greater than 50 years as important risk factors. Men (75%) with PCa undergo bilateral orchidectomy (BO) as preferred mode of treatment due to late presentation. Reports show poor management outcome with rapid progression to key features of metabolic syndrome (MS) and increased mortality rate of 64% within 2 years. This study was aimed at identifying pre-existing metabolic changes in blacks with PCa before BO.

Methods: 100 participants were recruited by convenience sampling. Demographic, clinical history and life style measures were obtained from pre-test questionnaire. Anthropometric indices and blood pressure readings were obtained by standard methods. Serum testosterone and prostate specific antigen (PSA) were estimated by enzyme immunoassay. Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides and high density lipoprotein (HDLC) were determined by enzymatic methods while low density lipoprotein was calculated. pÂ0.05 was considered significant.

Results: The mean PSA level in PCa group was higher than BPH and control groups. On the other hand, the mean serum testosterone levels in PCa and BPH were significantly lower than the corresponding value in the control group. 25.8%, 30.6%, 30.0% of PCa, BPH and control groups respectively had MS. Elevated BP, FPG and reduced HDLC were the most prevalent MS components while elevated WC and elevated triglyceride were the least MS components in all groups.

Conclusion: Testosterone is reduced in prostatic diseases. Preexisting MS in males with PCa before BO may inform the high mortality observed after BO.

Keywords: *Metabolic syndrome, testosterone, benign prostatic hyperplasia, bilateral orchidectomy, prostate specific antigen, prostate cancer*.

Résumé

Introduction: Le cancer de la prostate (CaP) est la malignité masculine la plus fréquente dans le monde avec une descendance africaine et l'âge de plus de 50 ans comme facteurs de risque importants. Les hommes (75%) atteints de CaP subissent une orchidectomie bilatérale (OB) en tant que mode de traitement préféré en raison d'une présentation tardive. Les rapports montrent des résultats de gestion médiocres avec une progression rapide vers les principales caractéristiques du syndrome métabolique (SM) et une augmentation du taux de mortalité de 64% dans 2 ans. Cette étude visait à identifier les changements métaboliques préexistants chez les Noirs avec CaP avant OB.

Méthodes: 100 participants ont été recrutés par échantillonnage de commodité. Des données démographiques, d'histoire clinique et de style de vie ont été obtenues à partir d'un questionnaire pré-test. Les indices anthropométriques et les lectures de pression sanguine ont été obtenus par des méthodes standard. La testostérone sérique et l'antigène prostatique spécifique (APS) ont été estimés par essai-immunitaire enzymatique. La glycémie à jeun (FPG), le cholestérol total (TC), les triglycérides et les lipoprotéines de haute densité (HDLC) ont été déterminés par des méthodes enzymatiques tandis que les lipoprotéines de basse densité ont été calculées. pÂ0,05 était considéré comme significatif.

Résultats : Le taux moyen d'APS dans le groupe CaP était supérieur à celui du BPH et du groupe témoin. D'autre part, les taux moyens de testostérone sérique dans CaP et BPH étaient significativement plus bas que la valeur correspondante dans le groupe témoin. 25,8% ; 30,6% ; 30,0% des groupes CaP, BPH et témoin avaient respectivement SM. La PA élevée, la glycémie à jeun et les HDLC réduites étaient les composantes les plus fréquentes du SM, tandis que les valeurs élevées de WC et les triglycérides élevés étaient les composantes les moins importantes du SM dans tous les groupes.

Conclusion : La testostérone est réduite dans les maladies prostatiques. Un SM préexistant chez les mâles atteints de CaP avant l'OB peut renseigner sur la mortalité élevée observée après OB.

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Introduction

Prostate cancer (PCa) is the commonest male malignancy worldwide, rising with an aging population [1,2]. African descent is an important risk factor [3,4]. In Nigeria, PCa accounts for 16.5% of all cancers [5]. North and South regional reports emphasize late presentation with metastatic disease in two-thirds of PCa patients [6].

Elevated level of prostate specific antigen (PSA) of >4ng/mL is the non-invasive and laboratory index of diagnosis of PCa [7]. PSA is a glycoprotein that is expressed by both normal and neoplastic prostate tissue. Early prostate cancer usually causes no symptoms and is found by a PSA test and/or digital rectal examination. When symptomatic, PCa can cause urological problems such as inability to urinate or difficulty starting or stopping the urine flow, urinary urgency, nocturia, the need to urinate more frequently, weak or interrupted urine flow, pain or burning during urination [8]. These symptoms are also present in men with benign prostatic hyperplasia (BPH) and are more likely to be caused by BPH than cancer [7,8].

Haematuria, haematospermia and erectile dysfunction are signs of advanced PCa. A small percentage of men present with non-specific symptoms due to metastatic disease such as bone pain or, rarely, spinal cord compression [8]. Unfortunately, elevated PSA levels in males may be due to age or result from PCa and BPH [7].

The most common explanation for an elevated serum PSA in BPH is the very high prevalence of this condition in men over the age of 50 as BPH produces more PSA per gram than normal prostate tissue. Malignant prostate tissue generates more PSA than normal or hyperplastic tissue, probably because of increased cellularity and disruption of the prostate-blood barrier [8].

Elevated serum testosterone is thought to be a feature of PCa. Thus, androgen deprivation therapy (ADT) is targeted specifically to cause tumour regression by reducing or preventing androgens from reaching prostate cancer cells [9]. This is the choice treatment for advanced disease, which is usually achieved by bilateral orchidectomy (BO) in 75% of patients in sub-Saharan Africa, with only a few treated with either anti-androgens (flutamide / casodex) or LHRH agonist [10,11].

Evidence in men with no preexisting metabolic dysfunction, shows poor outcome of this management with rapid progression to key features of the metabolic syndrome (MS) leading to advanced disease and increased cancer mortality [12]. Langlois and Blaton [8] reported that PCa often grows so slowly and most men die of other causes before the disease becomes clinically advanced. Abdominal obesity, hypertension, dyslipoproteinemia, hypertriglyceridemia and hyperglycemia characterize MS culminating in cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM) and castrate resistant PCa [13,14]. Our previous studies showed 12.4% prevalence of MS in apparently healthy Nigerian males and increase of MS with age has been reported [15].

The antioxidant and cardio protective roles of testosterone have been suggested [15,16]. Reduced serum testosterone levels have been associated with MS [14]. Hypotestosteronaemia due to increased conversion of testosterone to oestrogen in increased adipose tissue has been observed in males with MS and T2DM [14,17]. Increased mortality rate of 64% within 2 years after ADT has long been reported [18]. It is uncertain if preexisting MS in males with PCa before BO informed the high mortality observed after BO. Studies addressing this perspective are scarce in Nigeria. This study was aimed at identifying MS, its components and other associated metabolic alterations in black Africans with PCa and BPH.

Methods

Study design

Convenience sampling method was used for this prospective case control study. Ethical approval (UI/UCH EC Registration Number: NHREC/05/01/2008a) was obtained from the Health Research Ethics Committee of University of Ibadan (UI)/University College Hospital (UCH), Ibadan.

Participants

A total of 100 participants aged 51-90 years consisting of 49 and 31 newly diagnosed males with PCa and BPH, respectively age matched with 20 apparently healthy asymptomatic males (control) enrolled into the study. The males with PCa and BPH were clinically examined by Urologists at the Urology Clinic. Both groups had PSA levels of $\geq$4µg/L and digital rectal examination. Prostate biopsies were obtained from the PCa group for confirmation of diagnosis. The males in the control group were confirmed healthy by the Physicians in the Geriatric Clinic of the UCH, Ibadan. They were asymptomatic of prostate diseases, had no previous surgery, no urinary tract malignancy or infection and

were not on medications. They attended the clinic for routine medical check, eye check and medically certified travel document.

Diagnosis of metabolic syndrome

The Joint Interim Criteria [19] was used for MS diagnosis and include any 3 of the following 5 risk factors (components): central obesity (waist circumference (WC)): ≥ 94 cm), raised triglycerides:1.7 mmol/L, reduced HDL cholesterol (HDLC):<1.03 mmol/L, raised blood pressure (BP): systolic BP (SBP) ≥130 or diastolic BP (DBP) ≥85 mm Hg and raised fasting plasma glucose (FPG): ≥5.6 mmol/L).

Demography, life style, sexual and prostate cancer history

Demographic Indices (education and occupation), lifestyle measures (cigarette smoking, alcohol consumption, exercise and dietary history); sexual and prostate cancer history were obtained from semi structured pretest questionnaires administered to participants.

Anthropometry and blood pressure

Anthropometric indices (body weight, height, BMI, WC and hip circumference (HC)) and BP (SBP and DBP) were determined by standard methods described elsewhere [17,20].

Sample collection

Venous blood (10mL) was aseptically obtained by veni puncture from each participant after an overnight fast (10-14h). Three mL was dispensed into potassium ethylene diamine tetra acetic acid (K_3EDTA) tube for the determination of lipids; 2mL was dispensed into fluoride oxalate tubes for glucose estimation while 5mL was dispensed into plain tubes kept for 1-2hours to clot to obtain serum for the determination of total PSA and testosterone. The samples were centrifuged at 500g to obtain plasma/serum, which were stored in aliquots at -20^{0}C until analyses.

Biochemical indices

Serum testosterone and PSA were estimated by enzyme immunoassay. Glucose (FPG), total cholesterol (TC), high density lipoprotein cholesterol (HDLC) and triglyceride were estimated in plasma by enzymatic methods while low density lipoprotein cholesterol (LDLC) was calculated using the Friedwald's formula described elsewhere [21]. All analytical kits were obtained from Dialab Produktion, Austria.

Statistical analyses

Data were analyzed using the Statistical Package for Social Science (SPSS) software 20.0 version. Analysis of variance (ANOVA) followed by Post Hoc tests were used for comparison of quantitative variables. Multiple regression model was used to assess the relationship between variables while Chi-square test was used to investigate the associations between qualitative values. Data obtained were significant at $p<0.05$.

Results

Health and diet history

The health and diet history of study participants in each of the three groups is as follows: 11 (28.2%), 10 (35.7%) and 1 (5%) of the BPH, PCa and control groups respectively had previously been treated for BPH (p=0.048). Forty-five (95.7%), 20 (66.7%) and 16 (80.0%) of BPH, PCa and control groups respectively did not use sexual performance enhancing drugs (p=0.004). Seventeen (85.0%) of control group consumed vegetables daily, while 22 (44.9%) and 17 (54.8%) of BPH and PCa groups consumed vegetables daily (p=0.020). The percentage intake of red meat and pork by BPH and PCa groups were 19.6% and 19.4% respectively, while the control group was 55.0% (p=0.018).

Age, physical and biochemical parameters

Intra-group comparisons (Table 1), shows statistically significant differences only in the mean HC, PSA and testosterone values (p=0.022,<0.001,<0.001) respectively. In BPH, the mean HC (97.3±1.2cm) was significantly higher (p=0.006) while the serum testosterone (25.0±1.0nmol/L) was significantly lower (p=<0.001) than the respective values in the control group (91.6±1.6cm, 41.6±3.5nmol/L). The mean PSA concentrations in PCa (53.3±6.7µg/L) were significantly elevated when compared with the corresponding values in BPH (14.8±2.8µg/L, p=<0.001) and control groups (8.7±2.9µg/L, p=<0.001) while the slight difference between BPH and control value did not reach level of statistical significance (p=0.433). The mean serum testosterone concentrations in both PCa and BPH (25.6±2.4nmol/L, 25.0±1.0nmol/L) were significantly lower than the control value (41.6±3.5nmol/L) (p=<0.001, p=<0.001) respectively while the slight difference between the values in PCa and BPH was not statistically significant (p= 0.782).

Obesity, metabolic syndrome and its components

Table 1: Age, Physical and Biochemical Parameters

Variables	PCa n=31	BPH n=49	Control n=20	P	p_1	p_2	p_3
Age (years)	71.9±1.6	65.8±1.8	66.6±2.7	0.055	0.019	0.101	0.792
SBP (mmHg)	138.5±4.1	143.6±3.6	129.2±4.7	0.068	0.346	0.166	0.021
DBP (mmHg)	82.1±2.6	86.4±3.2	75.6±2.3	0.069	0.295	0.199	0.022
Height (m)	1.7±0.03	1.7±0.01	1.7±0.02	0.424	0.324	0.824	0.256
Weight(kg)	67.0±1.9	69.5±2.0	64.4±2.4	0.273	0.394	0.477	0.117
BMI (kg/m^2)	23.8±0.8	24.3±0.6	23.6±0.9	0.795	0.627	0.889	0.548
WC (cm)	89.1±1.8	91.2±1.7	87.5±2.0	0.367	0.413	0.609	0.175
HC (cm)	95.5±1.6	97.3±1.2	91.6±1.6	0.022*	0.358	0.095	0.006*
FPG(mmol/L)	5.8±0.3	6.0±0.2	6.0±0.4	0.889	0.665	0.687	0.950
TC (mmol/L)	5.4±0.3	5.2±0.2	4.9±0.3	0.572	0.634	0.297	0.460
HDLC(mmol/L)	1.0±0.1	1.0±0.1	1.2±0.1	0.062	0.746	0.086	0.021
LDLC(mmol/L)	3.8±0.3	3.7±0.2	3.2±0.3	0.407	0.761	0.226	0.250
TG (mmol/L)	1.0 ±0.1	1.1±0.1	0.9±0.1	0.356	0.403	0.657	0.171
PSA (µg/L)	53.3±6.7	14.8±2.8	8.7±2.9	0.000*	0.000*	0.000*	0.433
Testosterone (nmol/L)	25.6±2.4	25.0±1.0	41.6±3.5	0.000*	0.782	0.000*	0.000*

values are in means ± SEM,=significant at pÂ0.05,PCa=Prostate cancer group, BPH=benign prostatic hyperplasia, controls=apparently healthy controls, p= probability, p_1= probability value of PCa and BPH, P_2 = Probability value of PCa and control, p_3 = probability value of BPH and control, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, WC=waist circumference, HC=hip circumference, FPG=fasting plasma glucose, TC=total cholesterol, TG=triglyceride, HDLC=high density lipoprotein, LDLC=low density lipoprotein, PSA=prostate specific antigen, n=number of participants.*

Table 2: Metabolic Syndrome and Obesity

Participants	Zero Component of MS		MS		MS+ BMI $\geq$ 30 (Kg/m^2)	
	n	%	n	%	n	%
PCa	4	12.9	8	25.8	0	0
BPH	0	0	15	30.6	3	6.0
Control	4	20.0	6	30.0	1	5.0

n= frequency of participants, %= percentage of frequency, MS= metabolic syndrome, BMI= body mass index, PCa= prostate cancer group, BPH= benign prostatic hyperplasia group, control=apparently healthy group

Table 2 shows the frequency of zero MS component, MS and obesity with MS in PCa, BPH and control groups while Table 3 shows the frequency of 0 - 5 components of MS in the three groups. All BPH had one or more components of MS, while 12.9% PCa and 20.0% control had no component of MS. The distribution of the types of MS components showed that 8 (25.8%), 15 (30.6%), 6 (30.0%) of PCa, BPH and control groups respectively had MS at diagnosis (e" 3MSC). Twenty one (67.7%) of males with PCa had elevated blood pressure (BP) as a component (most prevalent), 13 (41.9%) had reduced HDLC, 11 (35.5%) had elevated FPG, 10 (32.3%) had increased WC while 0 (%) had elevated triglycerides (least component).None of the males in the PCa group was metabolically obese (MS and BMIe"30 Kg/m^2).

Similarly, 30 (61.2%), 29 (59.2%) and 28 (57.1%) of males in the BPH group had elevated BP, FPG and reduced HDLC (most prevalent MS components) respectively, while 16 (32.7%) and 3 (6.1%) had elevated WC and triglyceride (least MS component), 3 (6.0%) of them were metabolically obese. In the control group, 9 (45.0%) males had elevated BP, 9 (45%) males had elevated FPG and 6 (30.0%) males had reduced HDLC (most prevalent MS components) while 5 (25%) males had increased WC and 2 (10.0%) males had elevated triglycerides (least components). 1 (5%) male in the control group was metabolically obese.

Table 3: 0-5 Components of Metabolic Syndrome

nMSC	n (%)	WC(%)	BP(%)	TG(%)	HDLC(%)	FPG(%)
PCa						
0	4 (12.9)	0	0	0	0	0
1	11 (35.5)	1	7	0	2	2
2	8 (25.8)	2	6	0	5	3
3	5 (16.1)	4	5	0	3	3
4	3 (9.7)	3	3	0	3	3
5	0 (0)	0	0	0	0	0
Total	31 (100)	10 (32.3)	21 (67.7)	0 (0)	13 (41.9)	11 (35.5)
BPH						
0	0 (0)	0	0	0	0	0
1	14(28.6)	0	6	0	5	3
2	20(40.8)	7	11	0	9	13
3	9 (18.4)	4	7	1	8	7
4	5 (10.2)	4	5	1	5	5
5	1 (2)	1	1	1	1	1
Total	49(100)	16(32.7)	30(61.2)	3 (6.1)	28(57.1)	29(59.2)
Control						
0	4(20)	0	0	0	0	0
1	8(40)	1	3	0	2	2
2	2(10)	0	1	0	1	2
3	5(25)	3	4	2	2	4
4	1(5)	1	1	0	1	1
5	0(0)	0	0	0	0	0
Total	20(100)	5(25)	9(45)	2(10)	6(30)	9(45)

Values in frequency, %=percentage is in parentheses, nMSC=number of metabolic syndrome components, MS=metabolic syndrome, PCa=prostate cancer group, BPH=benign prostatic hyperplasia group, control= apparently healthy group, elevated WC=waist circumference, BP=elevated blood pressure; TG=high triglyceride, reduced HDLC=high density lipoprotein cholesterol, elevated FPG=fasting plasma glucose, n=number of participants

Metabolic risk factors

Table 4 shows the pair-wise comparisons in mean values of serum PSA, testosterone and metabolic risk factors within each group (PCa, BPH and control). In PCa, only the mean WC and FBG in those with MS were significantly elevated when compared with the respective values in those without MS. In the BPH, only the mean WC and HC in those with MS were significantly elevated when compared with the corresponding values in those without MS. On the other hand, in the control group with MS, the mean body weight, BMI, WC, HC, FPG and TG were significantly elevated when compared with the corresponding values in those without MS.

Risk factors in one metabolic syndrome component

The comparisons of anthropometric and clinical indices of MS among PCa, BPH and control groups with one component of MS are as follows: The intra group comparison showed statistically significant differences in the mean height, HC and PSA values. In PCa, the mean height and PSA were significantly elevated when compared with both BPH and control groups while the HC was significantly higher than the control group. In BPH, the mean height was significantly higher than the respective value in the control group.

Risk factors in two metabolic syndrome components

The comparisons of anthropometric and clinical indices of MS among PCa, BPH and control groups with two components of MS are also as follows: The mean PSA and testosterone values were statistically significant at p=0.002 and p=0.004 respectively. The mean PSA was significantly higher in PCa compared with BPH and control, while the mean testosterone values were lower in PCa and BPH when compared with the corresponding value in the control group. No significant differences were observed in the other parameters.

Risk factors in $\geq$3metabolic syndrome components

The group comparisons of physical and biochemical parameters in participants with MS in the three different groups are as follows: The data showed intra group variations in serum PSA and testosterone

Table 4. Metabolic risk factors between metabolic and non-metabolic syndrome in prostate cancer, benign prostate hyperplasia and control groups

Variables	PCa			BPH			Control		
	MS n=8	Non-MS n=23	P	MS n=15	Non-MS n=34	P	MS n=6	Non-MS n=14	P
Age (years)	72.6±2.4	71.7±2.0	0.802	69.7±2.4	64.0±2.3	0.136	67.5±2.7	66.14±3.8	0.826
SBP (mmHg)	148.5±8.7	134.6±4.4	0.131	150.3±7.0	140.3±4.1	0.197	138.3±6.0	125.2±6.0	0.209
DBP (mmHg)	88.4±4.7	79.7±3.1	0.143	94.3±8.4	82.5±2.0	0.076	82.3±4.9	72.7±2.2	0.053
Height (m)	1.6±0.1	1.7±0.03	0.306	1.7±0.02	1.7±0.01	0.307	1.7±0.03	1.6±0.02	0.670
Weight(kg)	71.8±2.8	64.9±2.4	0.094	73.4±3.4	67.8±2.4	0.198	75.7±3.4	59.6±2.1	0.001*
BMI (kg/m^2)	25.3±0.6	23.2±1.1	0.241	25.4±1.1	23.8±0.7	0.214	27.4±1.1	22.1±0.9	0.002*
WC (cm)	97.0±2.4	85.6±1.7	0.001*	96.1±3.0	88.7±1.9	0.039*	96.5±2.6	83.6±1.8	0.001*
HC (cm)	100.3±2.0	93.6±1.8	0.053	100.9±2.4	95.3±1.1	0.020*	99.5±2.3	88.2±1.2	0.000*
FPG(mmol/L)	6.9±0.5	5.5±0.3	0.047*	6.2±0.2	5.9±0.3	0.515	7.4±1.2	5.4±0.1	0.024*
TC (mmol/L)	4.9±0.4	5.6±0.4	0.196	5.5±0.4	5.1±0.3	0.379	4.9±0.5	4.8±0.4	0.654
HDLC(mmol/L)	0.8±0.1	1.1±0.1	0.108	0.9±0.1	1.0±0.1	0.214	1.1±0.1	1.3±0.1	0.160
LDLC(mmol/L)	3.6±0.3	4.0±0.5	0.544	4.1±0.3	3.5±0.3	0.287	3.4±0.5	3.2±0.4	0.733
TG (mmol/L)	1.0±0.2	0.9±0.1	0.909	1.2±0.1	1.0±0.1	0.088	1.3±0.2	0.7±0.1	0.007*
PSA (µg/L)	57.3±14.0	52.1±7.8	0.753	12.3±2.9	16.0±3.9	0.551	11.5±7.9	7.3±2.5	0.523
Testosterone (nmol/L)	21.8±5.2	27.0±2.8	0.390	25.7±1.0	24.6±1.4	0.673	44.7±3.1	39.9±5.2	0.540

*values are in means ± SEM, *Significant difference at pÂ0.05, MS=metabolic syndrome group, non-MS=participants with 0-2 MS components, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, WC=waist circumference, HC=hip circumference, FPG=fasting plasma glucose, TC=Total Cholesterol, TG=Triglyceride, HDLC=high density lipoprotein cholesterol, LDLC=low density lipoprotein cholesterol, PSA=prostate specific antigen, n=number of participants, PCa=prostate cancer group, BPH=benign prostatic hyperplasia group, control= apparently healthy group*

Table 5: Relationships between metabolic risk factors in participants with prostate cancer, benign prostate hyperplasia, controls

PCa			BPH			Controls		
Indices	β	p	Indices	β	p	Indices	β	p
Age/HDLC	- 0.335	0.002*	Age/PSA	0.153	0.029*	Age/PSA	0.602	< 0.001*
PSA/FPG	0.439	0.017*	Height/weight	0.002	<0.001*	T/Height	0.010	0.023*
BMI/Height	-13.653	0.007*	WC/Weight	0.606	0.012*	HC/FPG	0.089	0.044*
Height/BMI	- 0.024	0.001*	WC/BMI	0.177	0.009*	WC/BMI	0.373	0.007*
FPG/HC	0.274	<0.001*						

PSA=prostate specific antigen, HDLC=high density lipoprotein cholesterol, FPG=fasting plasma glucose, BMI=body mass index, HC=hip circumference, WC=waist circumference, T=testosterone, PCa=Prostate cancer group, BPH=Benign prostatic hyperplasia, Controls= apparently healthy group

concentrations. The mean PSA in PCa was significantly higher than the respective values in BPH and control with no significant difference between the value in BPH and control groups. On the other hand, there were significant reductions in testosterone in both PCa and BPH when compared with the corresponding control value.

Relationships between metabolic risk factors

Table 5 presents a summary of the multiple regressions of variables in PCa, BPH and control groups. HDLC negatively related with age in PCa group (β=-0.335, p=0.002) while PSA related positively with age in both BPH (β =0.153, p=0.029) and control (β= 0.602, p<0.001) groups. PSA related directly with FPG in PCa group only (β=0.434, p=0.017).BMI related indirectly with height (β=-0.024, p=0.001) and vice versa (β=-13.653, p=0.007) in PCa, body weight related positively with height in BPH (β=0.002, p<0.001) while testosterone related directly with height in control (β=0.010, p=0.023) groups. FPG related directly with HC in PCa (β=0.274, p<0.001) and control (β=0.089, p=0.04) groups. WC related directly with weight

(β=0.606, p=0.012) and BMI (β=0.177, p=0.009) in BPH as well as BMI (0.373, p=0.007) in control groups.

Discussion

The health history showed that among the males newly diagnosed with PCa in this study, as high as 35.7% had been previously treated for benign prostate disease. An earlier study suggested that African-Americans previously treated for BPH were at a higher risk of developing PCa as compared to their Caucasian counterparts [4,22]. This may indicate its importance in the aetiology of PCa in populations of African descent. Use of performance enhancing drug was low among the study population. This may be beneficial as adverse health consequences including cardiovascular risk have been attributed to their use [23]. However, daily consumption of vegetable and red meat/pork was higher in controls than in PCa and BPH. Lassed *et al*. [24] demonstrated the reduction of prostate cancer risk through increased intake of vegetable. However, our results negate previous studies that showed positive association between high consumption of red meat and prostate cancer [25,26].

Metabolic syndrome describes a cluster of co-morbidities including abdominal obesity, elevated blood glucose, high cholesterol and hypertension which increase the risk of developing diabetes and cardiovascular disease [27]. The prevalence rates of MS among PCa was comparable to the respective values in BPH and control groups, probably suggesting that MS is common in elderly Nigerian males irrespective of their prostate status. The prevalence of MS were 25.8%, 30.6% and 30.0% in PCa, BPH and controls respectively. Although the prevalence of MS was 12.4% in apparently healthy Nigerian males in the general population in Ibadan, increase in MS with age was observed [15,28].

The mean WC value in this study was significantly raised in MS across the groups (PCa, BPH and control) (pÂ0.039). WC reflects abdominal fat, which contains higher amount of visceral fat. Visceral fat is made by liver, turned into cholesterol and released into the blood stream where it forms plaque in the artery walls, resulting in high blood pressure and cardiovascular disease [29]. Elevated blood pressure was the most prevalent MS component while triglyceride was the least prevalent MS component in all the groups. Gender-specific differences in MS have been demonstrated as metabolic syndrome appears to be more common in females with obesity whereas hypertension appears to be more common in males [30]. However, our earlier study in the general population (in young and elderly men) showed reduced HDLC as the most prevalent MS component while triglyceride was the least component [15]. It is also noteworthy that as high as 41.9% of newly diagnosed PCa had low HDLC as a MS component. From our findings, it appears that unfavourable lipid profile and other components of MS that may inform the high mortality observed after orchidectomy may already be present at diagnosis before surgical intervention in Nigerians with PCa.

In the present study, the reduced testosterone concentrations in PCa and BPH with or without MS when compared with the corresponding values in the control group is striking. The mean testosterone in the control group was higher than the respective values in both PCa and BPH groups. This is not in agreement with some earlier reports [9] indicating that testosterone was elevated in prostate cancer thus the need for androgen deprivation therapy (ADT). Prostate cancer has been known for decades as an androgen dependent disease and androgen-deprivation therapy (ADT) has been the first-line treatment and fundamental management for men with advanced PCa to suppress functions of androgen/androgen receptor (AR) signaling [31].

On the other hand, some other studies suggested that low testosterone levels might predict more aggressive disease and poorer prognosis in men with advanced PCa [32,33]. Some theorize that PCa cells can inhibit testosterone production, and this hypothesis would suggest that low testosterone levels could play a role as a marker for high grade prostate cancer [34]. Another study suggested that testosterone levels are altered by the presence of PCa, supporting the possibility that PCa may inhibit serum testosterone levels by negative feedback in the hypothalamic- pituitary axis [35], while Chodak *et al*. [36] demonstrated that the higher the serum testosterone prior to treating men with metastatic PCa with a gonadotropin-releasing hormone analogue, the better the survival [36].

Androgen deprivation therapy (ADT) reduces the levels of androgens in the body, or prevents them from reaching prostate cancer cells, resulting in tumor regression [9]. In Africa, most males with prostate cancer present with advanced disease, thus hormone ablation (ADT) remains the only treatment of these stages of the disease, this is usually achieved with bilateral orchidectomy (75%), with only a few patients being treated with either anti-androgens (flutamide/casodex) or LHRH agonist as most treatment strategies are unavailable or unaffordable [10]. The implications of our

observations for the management of PCa patients in this community need further evaluation.

Elevated level of prostatic specific antigen (PSA) of >4ng/mL is the laboratory index of diagnosis of PCa, elevated PSA levels in Nigerian males may result from PCa, BPH or BPH with prostatitis (prostate diseases presenting with similar symptoms) [7]. Irrespective of MS status, this study shows higher level of PSA in males with PCa than BPH and asymptomatic controls. Interestingly, the mean levels of PSA in PCa, BPH and control groups were >4ng/mL, with PSA increasing with age in BPH group and controls but not in PCa group. Increased PSA level related directly with fasting plasma glucose in males with PCa only. HDLC was inversely related with age in males with PCa.

Conclusion

PSA may be an unreliable laboratory measure of prostatic diseases in older Nigerian men. Elevated BP, reduced HDLC and elevated FPG appear to be the most prevalent MS components in all groups. Testosterone is reduced in prostatic diseases and may be important in specific regional fat redistribution. Elevated FPG and reduced HDLC were found in this study and need confirmation in other settings in this population. The prevalence of MS in older males is high irrespective of their prostate status and may predispose them to CVD and/or T2DM. Patients with diagnosis of PCa, being considered for bilateral orchidectomy should have metabolic screening before surgery to pre-empt a possible worsening of MS after procedure.

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Healthcare-associated infections in a Newborn Unit in Nigeria

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Abstract

Introduction: Due to immaturity of neonatal immune function, prolonged hospitalisation, invasive procedures and inadequate infection control measures, healthcare-associated infections (HAI) remain a perennial problem in neonatal units globally and impact negatively on outcome. It is essential to quantify the burden and assess the risk factors in order to address this problem.

Objectives: To determine the prevalence, bacterial aetiology of and risk factors for HAI in the neonatal unit of the University College Hospital, Ibadan, Nigeria.

*Materials and methods***:** Fifty-six out of the 435 neonates admitted who developed symptoms or signs of neonatal sepsis after 48 hours of admission or symptom free interval had their clinical history and physical examination recorded. Blood, urine and where indicated, cerebrospinal fluid (CSF) samples were taken for culture and sensitivity before antibiotic therapy.

Results: The incidence of HAI was 4.1% (18 babies) of neonatal admissions, 4.4/1000 patient days (72.2% of infections was septicaemia). There were 7 Gram positive isolates [all Methicillin Resistant *Staphylococcus aureus* (MRSA)] and 11 Gram negative isolates. The blood culture isolates were MRSA (6), *Klebsiella pneumoniae* (3), *Hafnia alvei* (2) and one each of *Stenotrophomas maltophilia* and *Escherichia coli*. The two cerebrospinal fluid isolates were *Chryseobacterium meningosepticum* and *Klebsiella ozaenae,* other isolates *were Escherichia coli* - abscess aspirate, MRSA and *Escherichia coli* urethral catheter tips. MRSA demonstrated 33.3% and 50.0% susceptibility to gentamycin and vancomycin respectively. The Gram negatives demonstrated 81.8%, 45.6% and 90.1% susceptibility to ceftazidime, ciprofloxacin and meropenem respectively. The case fatality rate was 38.9% (7 babies).

Conclusion: MRSA and *Klebsiella pneumoniae* are the common causes of HAI in the neonatal unit and are associated with high mortality.

Keywords: *Healthcare Associated Infections; Neonatal Septicaemia; Bacterial pathogens*

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Résumé

Introduction: En raison de l'immaturité de la fonction immunitaire néonatale, d'une hospitalisation prolongée, de procédures invasives et de mesures inadéquates de contrôle des infections, les infections nosocomiales demeurent un problème chronique dans les unités néonatales et ont un impact négatif sur les résultats. Il est essentiel de quantifier la charge et d'évaluer les facteurs de risque afin de résoudre ce problème.

Objectifs: Déterminer la prévalence, l'étiologie bactérienne et les facteurs de risque d'infection nosocomiale dans l'unité néonatale du Collège Hospitalier l'Universitaire, Ibadan, Nigeria.

Matériel et méthodes **:** Cinquante-six des 435 nouveau-nés admis qui ont développé des symptômes ou des signes de septicémie néonatale après 48 heures d'admission ou un intervalle sans symptômes ont eu leurs antécédents cliniques et un examen physique recordé. Du sang, l'urine et dans le cas échéant, des échantillons de liquide céphalo-rachidien (LCR) ont été prélevés pour la culture et la sensibilité avant l'antibiothérapie.

Résultats: L'incidence des infections nosocomiales était de 4,1% (18 bébés) d'admissions néonatales, 4,4 / 1000 jours-patients (72,2% des infections étaient des septicémies). Il y avait 7 isolats Gram positifs [tous *Staphylococcus aureus* résistants *à la méthicilline* (SARM)] et 11 isolats Gram négatifs. Les isolats de culture de sang étaient SARM (6), *Klebsiella pneumoniae* (3), *Hafnia alvei* (2) et un chacun des *sténotrophomes maltophilie* et *Escherichia coli*. Les deux isolats de liquide céphalorachidien étaient *Chryseobacterium meningosepticum* et *Klebsiella ozaenae*, autres isolats *Escherichia coli* - aspiration d'abcès, SARM et extrémités de cathéter urétrale *Escherichia coli*. SARM a démontré une sensibilité de 33,3% et 50,0% à la gentamycine et à la vancomycine respectivement. Les Gram négatifs ont montré une sensibilité de 81,8% ; 45,6% et 90,1% à la ceftazidime, à la ciprofloxacine et au méropénème respectivement. Le taux de létalité était de 38,9% (7 bébés).

Conclusion: SARM et *Klebsiella pneumoniae* sont les causes les plus fréquentes des infections nosocomiales dans l'unité néonatale et sont associées à une mortalité élevée.

Mots-clés: *Infections nosocomiales; Septicémie néonatale ; Pathogènes bactériens*

Introduction

Nosocomial infections more appropriately referred to as healthcare-associated infections (HAI) are infections acquired in the hospital that were neither present nor incubating at the time of admission [1]. They are common in paediatric and neonatal intensive care units (NICU) due to relative immunodeficiency especially among babies who are preterm and low birth weight, have prolonged hospital stay and undergo invasive procedures. Health-care associated infections may be local or systemic, evidenced by positive culture from a usually sterile site, acquired after 48 hours of admission [2].

The incidence of HAI in neonatal units varies widely depending on the definitions used [3] as well as practices within the unit. The prevalence ranges between 6 and 25% of NICU admission or 8.9 to 62 infections per thousand patient days [4]. It is more prevalent in developing countries where the neonatal units are often overcrowded, understaffed and poorly equipped and lack written infection control policies and antibiotic protocols [5-8].

Healthcare-associated infections are associated with high cost of care, longer hospital stay and higher morbidity and mortality especially in developing countries [5-8]. They also contribute to the incidence and severity of impaired long term outcomes among survivors [9, 10]. Neonatal infection rates are high in developing countries with rates as high as 6.5 to 38 per thousand live hospital born babies and up to 68 per thousand live births among the low birth weight in Nigeria, but the burden due to HAI has not been specifically quantified [4, 7].

The study was carried out to determine the prevalence, bacterial aetiology and risk factors for HAI at the neonatal wards of the University College Hospital (UCH), Ibadan, Nigeria.

Materials and methods

The study was conducted in the neonatal unit of the University College Hospital, Ibadan comprising two neonatal wards, one ward admits babies born within the hospital (inborn) and those born outside the hospital (outborn) but presenting within 48 hours of age. The second ward admits all other neonates. It was a cross sectional study of all 435 neonates admitted into the unit over a four month period out of which 56 developed symptoms or signs of neonatal sepsis after 48 hours of admission or symptom free interval [11].

Maternal demographic data, details of pregnancy, delivery and perinatal history including antibiotic use were documented.

Each neonate that developed symptoms and signs of illness had blood, suprapubic or catheter urine and cerebrospinal fluid (CSF) cultures done as indicated prior to institution of new antibiotics. In cases where there were abscesses, aspirates were also taken for culture. Urethral catheter tips were also sent for cultures in cases of catheterized patients. Between 1.0ml and 1.5ml of blood was taken for cultures, sent to the Microbiology laboratory immediately and incubated in the Bactec 9050 machine at 37^0 Celsius for four days.

Lumbar puncture for cerebrospinal fluid analysis was performed in 43 neonates who had indications for the procedure.

Descriptive statistics are presented using tables and means (± Standard Deviation) as appropriate. Association between socio-demographic characters, perinatal, maternal and dependent variables were analysed using the chi squared test. Statistical significance was set at a p value of <0.05. Binary logistic regression analysis was conducted on variables that had a p value of <0.20 on bivariate analysis in order to determine the clinical predictors of HAI.

Approval for the study was obtained from the Joint University of Ibadan/ University College Hospital, Ibadan Ethics Committee and informed written consent from parent or caregiver was obtained before recruitment

Table 1: Distribution of birth weight and gestational age of neonates screened for HAI

Characteristics	Subjects screened for HAI n (%)	Proportion of total admissions (%)
Birth weight (unit)		
<1.5	24(42.9)	28.5
1.5 - <2.5	12(21.4)	33.8
>2.5	20(35.7)	37.7
Gestational age(weeks)		
< 28	7(12.5)	3
28 – 32	23(41.1)	19.5
33 – 36	18(32.1)	25.4
>37	8(14.3)	52.1

Results

Of the 56 neonates screened, 26(46.4%) were delivered in UCH (inborn) while 30(53.6%) were delivered in facilities other than UCH (out-born). There were 29(51.8%) males and 27 (48.2%) females. The distribution of birth weight and gestational age of the neonates are as shown in Table 1. All episodes occurred within the first 28 days of

admission. The duration of hospital stay prior to episode ranged from 3 to 26 days (mean 7.7±3.8 days). Of the 56 neonates, 44 had prior antibiotic exposure for a mean duration of 4.2±3.3 days prior to episode. Half (28) of the subjects had previous negative blood cultures on admission while one had a previous positive blood culture and had been completely treated with normalized C-reactive protein (CRP) before the episode. The repeat episode was 72 hours after normalization of CRP and completion of initial treatment. The remaining 27 did not have indications for an initial sepsis screening on admission.

The nurse to patient ratio in the unit during the study period was one nurse to 5 – 7 babies.

Table 2: Presenting clinical features necessitating HAI screening

Clinical features	Frequency n (%)
Respiratory distress	41(73.2)
Fever	30(53.6)
Abdominal distension	28(50.0)
Lethargy/reduced activity	22(39.3)
Apnoea	20(35.7)
Poor feeding	20(35.7)
Bleeding diathesis	20(35.7)
Tachypnoea	17(30.4)
Temperature <36°C	17(30.4)
Hepatomegaly	15(26.8)
Mottled skin	13(23.2)
Regurgitation/vomiting	12(21.4)
Jaundice	9(16.1)
Seizures	8(14.3)
Sclerema	6(10.7)
Cyanosis	4(7.1)
Palor	4(7.1)
Significant pre gavage aspirate	1(1.8)
Periumbilical redness	1(1.8)
Prolonged capillary refill	1(1.8)
Coma	1(1.8)

Clinical features

Respiratory distress was the most common presenting feature; fever 30(53.6%), abdominal distension 28 (50.0%), and lethargy 22(39.3%) were the other common features (Table 2).

Culture results

There were 18 culture-proven HAI corresponding to an incidence of 4.1% of all admissions and 4.4 episodes of infection per thousand patient days**:** 13 (72.2%) of the infections were blood stream infections, 2 (11.1%) were meningitis, 2 (11.1%) were catheter tip isolates and the last from an abscess. The prevalence rate of meningitis was therefore 3.6% of cases of HAI. The Gram negative organisms predominated, 11(61.1% of all isolates), while the only Gram positive organism was Methicillin Resistant *Staphylococcus aureus* (MRSA) 7(38.9%). The Gram negative organisms were *Klebsiella* spp, 4(22.2%), *Escherichia coli* 3(16.7%) and the other Gram negatives 4(22.2%) [*Hafnia alvei* 2(15.4%), *Chryseobacterium meningosepticum* 1(7.7%) and *Stenotrophomas* maltophilia 1(7.7%)].

Of the 13 blood culture isolates, 6 (46%) were MRSA, 3 (23.1%) *Klebsiella pneumoniae*, 2 (15.4%) *Hafnia alvei* and 1 (7.7%) each were *Stenotrophomas maltophilia* and *Escherichia coli.* One patient who previously had been treated for *Klebsiella Pneumoniae* septicaemia but had to be rescreened 72 hours after completion of initial treatment had blood culture positive for *Hafnia alvei*.

The two positive CSF cultures yielded *Chryseobacterium meningosepticum* and *Klebsiella ozaenae,* the two positive urethral catheter tips yielded MRSA and *Escherichia coli*. *Escherichia coli* was isolated from the aspirate of an abscess.

One of the *Hafnia alvei* was isolated from a preterm neonate after an initial culture which yielded *Klebsiella pneumoniae* had been completely treated. Both subjects with positive CSF cultures had negative blood cultures at that time of the positive CSF culture. Both babies were inborn babies with late onset sepsis (LOS) who previously had negative early onset sepsis (EOS) screening.

There were no isolates obtained from the urine of the subjects screened. The neonates with positive urethral catheter tips were previously catheterised for more than three days for urine output monitoring and later developed signs of sepsis.

Antimicrobial susceptibility pattern

The MRSA demonstrated 50%, 0%, 33.3% and 40% susceptibility to Amikacin, cefotaxime, gentamycin and vancomycin respectively. Generally, the Gram negatives demonstrated 81.8%, 45.6% and 90.1% susceptibility to ceftazidime, ciprofloxacin and meropenem respectively. Escherichia coli demonstrated 100% susceptibility to Amikacin, Gentamycin, ceftazidime and meropenem while Klebsiella demonstrated 80%, 66.7%, 60% and 100%

respectively to Amikacin, Gentamycin, ceftazidime and meropenem. The other Gram negative isolates demonstrated 75%, 50% and 75% susceptibility to ceftazidime, ciprofloxacin and meropenem respectively as shown in Table 3.

Table 3: Percentage sensitivity of HAI isolates to antibiotics

Organisms Antibiotics	MRSA	Kleb.	E.Coli	Other Gram negatives
Ampicillin	0	0	50	0
Ampicillin sulbactam	0	66.7	-	0
Amoxicillin	0	25	0	0
Ampiclox	0	0	-	-
Amikacin	50.0	80	100	100
Gentamicin	33.3	66.7	100	75
Vancomycin	40.0	0	-	-
Ciprofloxacin	66.7	66.7	0	50
Ceftazidime	0	60	100	75
Cefotaxime	0	60	0	-
Cefuroxime	0	60	0	100
Cefepime	0	100	50	0
Meropenem	14.2	100	100	75
Chloramphenicol	50	66.7	0	0

Risk factors for HAI

Very low birth weight and gestational age less than 32 weeks were associated with four and threefold increased risk respectively of HAI as shown in Table 4.

Table 4: Risk factors for HAI

Postnatal risk factor	OR	CI	p value
Male sex	1.250	0.405-3.856	0.698
Birth weight <1.5kg	4.710	1.424-15.576	0.006
GA<32weeks	3.034	1.141-8.065	0.020
Exchange blood transfusion	1.486	1.236-1.787	0.546
Umbilical venous catheterization	2.675	0.949-7.542	0.054
Endo tracheal Intubation	2.431	0.277-21.365	0.408
Urethral catheterization	1.998	0.676-5.909	0.203
Prior antibiotic use	1.238	0.180-8.504	0.828

Outcome

More than a third, 7 (38.9%), of neonates with culture proven HAI died compared to 70(16.9%) of those who were culture negative (OR = 3.123, CI**:** 1.146-8.512, p = 0.020).

The mean length of hospital stay was 20.8±9.4 among neonates with culture proven HAI and 9.3±7.00 among those without (p = 0.000).

Discussion

Healthcare-associated infections in neonates continue to be a major challenge to improved outcomes despite the technological advances in the area of respiratory and nutritional support particularly for the extreme preterms. The rate of culture proven HAI in this study was 4.4 per 1000 patient days and 4.1% of total neonatal admissions. This figure includes all babies who had either a septicaemia or positive culture in sites like the CSF and urine which should normally be sterile. These rates are much lower than 13.8 per 1000 patient days reported from Australia and 19.2% and 21.4% of total admissions reported in Saudi-Arabia, and Egypt respectively [6, 12].

This might be due to the fact that the unit does not use or indeed, have facilities for invasive neonatal intensive care such as mechanical ventilation, total parenteral nutrition or even central lines which are known to predispose to HAI [13, 14].

The HAI rate in this study was also slightly lower than the 8.9% reported by Osinupebi *et al* [15], the reason for this lower figure is not clear as the special care baby unit at Sagamu is in the same geopolitical zone of the country, with similar facilities.

Majority of the positive cultures were from the blood. The blood stream is known to be the most common site for isolation of hospital acquired organisms [16]. This is not surprising in neonates who are known to have limited ability to localise infections hence infections from any site readily disseminate into the blood stream.

The 3.6% prevalence of positive CSF culture in this study was similar to the 5% documented in a review of HAI in neonatal intensive care units by Carey [17] though this 5% included non-bacterial causes.

There were isolates from the urethral catheter tips of two babies who developed features of illness after urethral catheterisation. Though the

Centre for Disease Control (CDC) definition of HAI does not include cultures from catheter tips, the positive cultures from the catheter tips in this study were regarded as significant because the babies developed signs of illness necessitating the screening.

As shown in this study, all the infections occurred within 26 days of admission with a mean of 7.7±3.8 days, which is consistent with previous reports that HAIs occur in the early days in 85% of cases [18]. It is therefore necessary to intensify efforts at prevention of HAI during this critical period when they are likely to be undergoing the most intensive care for their primary conditions.

The most prevalent organisms found in this study were methicillin resistant *Staphylococcus aureus* (MRSA), Klebsiella spp and other Enterobacteriaceae which are known to thrive in solutions, containers and other hospital equipment if not adequately sterilised. This is consistent with what is reported in developing countries [5] though Klebsiella predominated in this review. *Staphylococcus aureus* continues to be an important HAI pathogen in neonatal units in developing countries as seen in this study and its spread is mainly attributed to the hands of healthcare workers [5]. On the contrary coagulase negative staphylococcus (CONS) and *Staphylococcus aureus* are more commonly reported in developed countries [14].

Other pathogens like Candida and viruses have also been implicated in the aetiology of HAI in the neonatal intensive care units in developed countries [19]. No case of Candida was found in the present study probably because there was a policy of prophylactic fluconazole for high risk babies in place at the time of the study [6, 14, 18, 20]. Other organisms known to be associated with HAI in neonates include respiratory syncytial virus and rotavirus in cases of respiratory and gastrointestinal infections but none of those were sought in this study.

The organisms found in this study are known to be more virulent than coagulase negative staphylococcus reported in developed countries, which is associated with less morbidity and mortality [21]. The challenge of HAI in this set up is therefore of a higher magnitude in terms of illness severity and risk of death. These organisms especially the MRSA are often associated with outbreaks in NICUs and with prompt institution of necessary infection control strategies and specific measures like isolation, cohorting, use of antiseptic agents such as chlorhexidine and mupirocin and microbiological surveillance**,** they are brought under control [22].

MRSA infections are largely preceded by colonization. Colonized babies then serve as a reservoir for the cross-transmission among babies through the hands of handlers who may themselves be carriers. MRSA outbreaks are known to be difficult to contain and may go on to become endemic if not promptly controlled [23, 24]. It is not known if the finding of 46% of the HAI in this study being due to MRSA was actually endemic in the unit or an outbreak because no previous data was available.

In some other developing countries, *Staphylococcus aureus* is also the most commonly isolated organism in LOS in the hospital setting, with a few studies from Nigeria also reporting CONS [25, 26]. The finding of *Klebsiella pneumoniae* as a common cause of HAI in our study is also consistent with previous reports from our centre and other parts of Nigeria [27-30]. This was a common cause of HAI in the developed world in the 1960s but remains a major burden in Nigeria till date [19]. Klebsiella and the other Enterobacteriaceae isolated in this study are known to be prevalent where there is unrestricted use of broad spectrum antibiotics such as third generation cephalosporins [21]. Unrestricted antibiotic use is prevalent in centres in resource limited settings such as ours where financial and logistic challenges with laboratory support for routine patient care exist. Many of this Gram-negative Enterobacteriaceae are able to express extended spectrum beta lactamases and even transfer resistance across other organisms.

Chryseobacterium meningosepticum was found as a cause of neonatal meningitis in one of the neonates who had no septicaemia at that time. This organism is a recognised cause of nosocomial meningitis especially in immunosuppressed patients and it has been cultured from swabs of incubators in the neonatal intensive care unit [31].

Healthcare-associated infections are frequently due to resistant organisms be they Gram positive or negative hence the difficulty in treating them and the associated poor outcomes. The MRSA found in this study demonstrated significant resistance to vancomycin as is now being increasingly reported from other parts of the world [32]. This particularly portends danger in developing country settings with limited availability of antibiotics. This is of special significance in developing countries in view of the other challenges with staffing, overcrowding, laboratory support and antibiotic availability and cost in the area of hospital care of newborn babies. The Gram negative organisms demonstrated reasonable susceptibility to ceftazidime and meropenem expectedly. It is

therefore crucial that these drugs be protected from overuse at this time to prevent development of resistance. Having written evidence-based antibiotic guidelines and regular microbial surveillance is essential to achieve this.

Very low birth weight and gestational age below 32 weeks were associated with the highest risk of HAI. This observation conforms to what obtains globally as this group of babies have compromised immunity and often require the most invasive procedures.

Prolonged use of empiric antibiotics in the face of negative cultures will alter the normal flora of the neonate and predispose to colonisation and ultimately infection especially by resistant organisms [33]. Prior use of empiric antibiotic, was not significantly associated with an increased risk of HAI in this study as reported in previous studies [21].

The mortality rate among babies with culture proven HAI in this study was higher than those who were negative as previously documented. The mean length of hospital stay was also significantly longer among those with proven HAI as documented in the literature. This adds to the challenge of health care costs in this group of babies. Apart from costs to parents, it also adds to the burden of care on the already overstretched human and material resources.

The high HAI rate, virulent type of organisms, low susceptibility to "reserve" antibiotics and poor outcomes of the babies underscore the need emphasize appropriate prevention and control measures like scrupulous hand hygiene and proper antibiotic stewardship.

Conclusion

The prevalence of HAI at the UCH is high and the most common organisms responsible for HAI are MRSA and Klebsiella pneumoniae. Very low birth weight neonates and neonates with gestational age below 32 weeks were found to be at a higher risk of HAI. The high mortality rate among neonates with culture proven HAI underscores the need for intensifying infection control measures in order to improve neonatal outcomes especially in developing countries.

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Notes for Contributors

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This Journal has agreed to accept manuscripts prepared in accordance with the Vancouver style and the Editor will consider only papers conforming to this style.

References should be numbered in the order in which they are cited in the text. At the end of the article, the references should be listed as numbered in the text. Each reference should give the names and initials of all authors (unless there are more than six, when only the first three should be given, followed by *et al.*). The authors' names should be followed by the title of the article, the journal title (abbreviated according to the style of *Index Medicus)*, the year of publication, the volume number and the first and last page numbers. Titles of books should be followed by the publisher, place of publication, and year. Examples of format for references are as follows:

Edington GM, Osunkoya BO and Smith JA.
Immunopathology of Burkitt's lymphoma. West Afr Med J 1986; 85: 76 – 87.

Brown A. Primary Health Care and the Medical Curriculum Edinburgh: Universities Press. 1977.

Lewis A. Primary liver cell carcinoma. In: Ajose A. Odeku EL, Eds. Priorities in Health Planning. Ibadan: University Press, 1983; 110 - 117

Reference to tables should be in Arabic numerals, e.g. Table 3, and tables should include titles, which make therein without reference to text. Tables should be typed separately from the text. Referencing within the body of the article should be in block form, i.e [1,2].

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