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Overcoming various health challenges through research

Research is the art of the soluble. It proffers solutions to myriads of health problems leading to generalizable knowledge. This issue of the journal disseminates information from sixteen research studies that cover the areas of toxicology, non-communicable diseases, infections, dental care and ethnobotany. Starting with avoidable circumstances, Michael and colleagues reported on methanol contamination of locally brewed drink that resulted in high case fatality in Ode-Irele Community of Ondo State. Extremely high levels of methanol and low levels of zinc were found in the sera of the victims. Individuals drinking locally brewed alcoholic drinks should be aware of such risks and, even, possible death. The other manuscript on toxic exposure by Obilor showed evidence of possible endocrine disrupting capacity of bisphenol-A exposure in plastic factory workers. Animal and in-vivo experiments provide foundational evidence for subsequent studies and product development. Four articles report on i) potential damage by trivalent chromium on the rat stomach and possibly the brain; ii) the anticarcinogenic effects of the leaf extract of *Momordica charantia; iii) Thonningia sanguinea* as a source of plant antioxidant and iv) the known anti-inflammatory effect of diclofenac emulsion prepared in *Vitellaria paradoxa* (Shea butter).

Early presentation of patients for medical attention was re-echoed by the authors of two articles on non-communicable diseases. The first by Iyare and colleagues who carried out a 10-year review of chemoradiation in head and neck tumors and reported better outcome when the affected individuals presented and commenced therapy early. The concurrent use of chemoradiation was encouraged. The same applied to individuals with acute kidney injury according to findings from a multi-site study by Adelaja *et al*. They also advocated early identification of cases for intensive unit care management. A key component for informed health choices is education which appeared to influence preference for either orthodox or informal maternal facility for ante-natal care and delivery according to Ogedegbe *et al*. Adequate education and counselling are reported to bring about safer and healthier reproductive behaviours by individuals living with the human immunodeficiency virus (Oladoyin and Sekoni). Ifeorah *et al*. reported co-infection of Hepatitis B virus with Hepatitis C (5.2%) or delta virus (5.7%) and advocated blood screening to avoid the terrible consequences of such co-infections. Lastly, Abdus-salam and colleagues reported that nosocomial infections affected the healing of repaired urinary fistula. The dexterity of the surgeon is as important as the steps taken to avoid infections.

Ibiyemi and Bankole related enamel defect in primary teeth of 4-year-old children to fluoride contents in tooth paste and in the drinking water while Dosumu et al in 2 separate articles, focused on communication with patients in simple understandable language to ensure adherence to dental care. Combination of verbal and written instructions is even better. Lastly, dermatoglyphic traits have relevance in ethnic identification and have a place in forensic service. Igbigbi excellently show-cased the sub-Saharan African pattern.

These articles should arouse the curiosity of our readers and inspire others to replicate the findings. We encourage our readers to send comments in the form of Letters to the Editor towards initiating interesting intellectual discourse, an essential ingredient for growth of science.

A. Ogunniyi
Editor-in-Chief

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Free radical scavenging activity, total phenolic and flavonoid constituents of medicinal plants used in Nigerian ethnobotany

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Abstract
Introduction: Plant polyphenols have the ability to protect biomolecules and cell membrane from oxidative stress thereby offering protection against the development of wide range of diseases. This study evaluated the antioxidant property of the methanol extract of twelve medicinal plants, identified and selected from a previous ethnobotanical studies conducted in South-western region of Nigeria.
Methods: 2, 2-Diphenyl 1-picryl hydrazyl (DPPH) free radical scavenging and nitric oxide inhibitory assays were used to evaluate the antioxidant activities. Total phenolic content (expressed as garlic acid equivalent), total flavonoid (expressed in terms of quercetin equivalent) and total antioxidant capacity were also determined by standard methods. Garlic and ascorbic acid were included in the study as reference standards. Results were expressed as the mean ± SEM. Differences between means were tested for statistical signiûcance using Student t- test ($p \geq 0.05$).
Results: The extracts exhibited scavenging activities against the DPPH radical. *Uvaria chamae* (stem bark) and *Thonningia Sanguinea* (whole plant) had IC_{50} values of 4.30 and 6.12 respectively, though not comparable to standard drug (ascorbic acid) with IC_{50} value of 1.4 µg/mL However, *T. sanguinea* extracts exhibited the highest nitric oxide inhibitory activity with IC_{50} values of 1.3±0.1 µg/mL significantly comparable to garlic acid with an IC_{50} of 1.1 ±0.1 µg /mL ($p \geq 0.05$). Methanol extract of *T. sanguinea* and *K. senegalensis* (stem bark) had the highest phenolic contents 343 ± 1.79 and 346 ± 0.02 mg/g respectively. *Thonningia sanguinea* had the highest total antioxidant capacity (TAC) of 374.46 ± 8.41 AAE (mg/g). *Uvaria chamae,* had the highest flavonoid content of 84.84 ± 6.26 QE mg/g of quercetin equivalent.
Conclusion: This study suggests that the screened plants, especially *Thonningia sanguinea* can serve as a valuable source of plant antioxidants.

Key words: *DPPH and Nitric Oxide Assays, fFlavonoid Compounds, Folin-Ciocalteu method, Thonningia sanguinea and Total Phenol,*

Résumé
Introduction : Les polyphénols végétaux ont la capacité de protéger les biomolécules et la membrane cellulaire du stress oxydatif, offrant ainsi une protection contre le développement d'un large éventail de maladies. Cette étude a évalué la propriété antioxydante de l'extrait de méthanol de douze plantes médicinales, identifié et sélectionné parmi des études ethnobotaniques antérieures menées dans la région du sud-ouest du Nigéria.
Méthodes : 2, 1-picryle 2-diphényl hydrazyl (DPPH) piégeage des radicaux libres et des dosages d'inhibition d'oxyde nitrique ont été utilisés pour évaluer les activités antioxydantes. La teneur en composé phénolique totale (exprimée en équivalent d'acide d'ail), en flavonoïde totale (exprimée en termes de quercétine équivalent) et la capacité antioxydante totale ont également été déterminées par des méthodes standard. L'ail et l'acide ascorbique ont été inclus dans l'étude en tant que référence standard. Les résultats ont été exprimés sous forme de moyenne ± ESM. Les différences entre les moyennes ont été testées pour la signification statistique à l'aide du test t d'Elève (p Ã 0,05).
*Résultats***:** Les extraits ont montré des activités de balayage contre le radical DPPH. *Uvaria chamae* (écorce de tige) et *Thonningia Sanguinea* (plante entière) avaient des valeurs de CI_{50} de 4,30 et 6,12 respectivement, comparables à celles de l'acide ascorbique avec une valeur de CI_{50} de 1,4 µg / mL. *Les* extraits de *T. sanguinea* présentaient l'activité inhibitrice d'oxyde nitrique la plus élevée, avec des valeurs de CI_{50} de 1,3 ± 0,1 µg / mL comparables à celles de l'acide d'ail avec une CI_{50} de 1,1 ± 0,1 µg / mL. Les extraits au méthanol de *T. sanguinea* et *K. senegalensis* (écorce de tige)

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avaient les teneurs en phénol les plus élevées, 343 ± 1,79 et 346 ± 0,02 mg/g. *Thonningia sanguinea* avait la capacité antioxydante totale (CAT) la plus élevée, de 374,46 ± 8,41 AAE (mg / g). *U. chamea,* avait la plus forte teneur en flavonoïdes de 84,84 ± 6,26 QE mg / g de quercétine équivalent.
Conclusion: Cette étude suggère que les plantes criblées, en particulier *Thonningia sanguinea,* peuvent constituer une source précieuse d'antioxydants.

Mots-clés : *tests de DPPH et d'oxyde nitrique, composés flavonoïdes, méthode de Folin-Ciocalteu, Thonningia sanguinea et Phénol total,*

Introduction

Nature has continued to be a source of medicinal agents all through human history. A remarkable number of contemporary drugs have been isolated from natural resources which also serve as a reservoir of rare medicinal agents [1]. Plant produces various anti oxidative metabolites to counteract reactive oxygen species (ROS) in order to survive. Natural antioxidants either as crude raw herbs or their chemical constituents are very effective in preventing the destructive processes caused by oxidative stress [2, 3]. Free radical reactions have been known to be involved in many acute and chronic disorders in human beings, including diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration [4]. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells [5].

Studies on medicinal plants have indicated the presence of phenolics with their concomitant bioactivities which explains the presence of such phenolic constituents. Plant phenolics are commonly found in both edible and non-edible plants, which play important roles in plant development in addition to eliciting multiple biological properties. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [6]. The importance of natural phenolic compounds has been on the rise among scientists, food manufacturers, and consumers [7].

The present study was designed to characterize the radical scavenging capacity, nitric oxide inhibitory activity, the total phenolic and flavonoid contents as well as total antioxidant capacity of methanol extracts of twelve selected medicinal plants. The studied plants are: *Thonningia sanguinea* Vahl (fruiting body), *Khaya senegalensis* (Ders.) A. Juss (bark & leaf), *Parquetina nigrescence* (Afzel) Bullock (root bark), *Spondia mombin* Linn (bark and leaf), *Tetrapluera tetraptera* (Schum&Thonn.) Taub. (Fruit) *Secamone afzelii* (Schult.) K. Schum, *Abutilon mauritianum* (Jacq) Medic (leaf), *Senna siamea* Linn (bark), *Chrysophyllum albidum* G. Don(seed), *Zephyranthes candida Lindl* (whole plant), *Uvaria chamae* P. Beauv (stem bark), *Lippia multiflora* Poir (leaf). They were identified and selected from the Nigeria ethno medicine, where they have been indicated for the treatment of various diseases especially inflammation, malaria and viral infections [8, 9]

Materials and methods

Drugs and chemicals

Griess reagent (Sulfanilamide, napthylethylene diamine hydrochloride), Sodium nitroprusside, Sodium nitrite, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhdrazyl (DPPH), Sodium bicarbonate, Gallic acid, Quercetin, Ascorbic acid, Sodium phosphate ammonium molybdate, sodium acetate and aluminium chloride were obtained from Sigma Chemicals, USA. All other chemicals and reagents were of analytical grade and were used without further purification.

Plant collection and authentication

Plant materials were obtained at different times of the year. Specimens were collected from the botanical garden, University of Ibadan and seeds were purchased from Bode market, Ibadan, Oyo State, Nigeria. Plant materials were authenticated and voucher specimens were deposited at the forest herbarium, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria under the following FHI numbers; *Khaya senegalensis* (leaf) 108856*, Khaya senegalensis* (bark) 108856*, Chrysophyllum albidum* (seed) 108851*, Zephyranthes candida* (whole plant) 110045*,Abutillon mauritianum* (leaf) 109985*, Senna siamea* (bark) 110047*, Tetrapluera tetraptera* (seed)108850*, Parquetina nigrescence (*root*)*108852*, Secamone afzelii* (leaf)109987*,Thonnigia sanguinea* (whole plant) 108854*, Uvaria chamae* (bark) 109900*, Spondias mombin* (leaf) 109901*, Spondias mombin* (bark) 109901*, Lippia multiflora* (leaf) 108858

Extract preparation

Plant materials were air-dried for two weeks and pulverized into coarse powder with the aid of a milling machine. Pulverized plant materials (400 g each) were extracted by maceration in redistilled methanol for 72h at room temperature with occasional stirring. Methanol was used in this study because it is an amphiphilic solvent, a lot of non-polar components are also dissolved in methanol along with polar bio-active components of the plants

and also because its extracts can be concentrated easily due to its low boiling point in comparison with water and ethanol. Extracts were filtered and concentrated using a rotary evaporator at 40 ºC. The dried extracts were stored in the refrigerator at 4 ºC until needed for analyses.

Antioxidant assays

Each extract was dissolved in methanol to prepare a stock concentration of 1 mg/mL; serial dilutions were then prepared for various antioxidant assays. All assays were performed in triplicates. Reference chemicals were used for comparison in all assays.

DPPH free radical scavenging activity

The *in vitro* free radical scavenging activity of the fractions was assessed using 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay according to a previously described method [10]. A fresh working solution of DPPH at 0.04 mg/mL was prepared by diluting DPPH solution with methanol. The DPPH solution (150 µL) was added to 100 µL of the graded concentration of the sample (6.25 – 400 µg/mL). The reaction mixture was well-shaken and incubated in the dark for 15 min at RT and the absorbance was taken at 517 nm using a spectrometer (SPECTRA max PLUX, Analytik and Biotechnologie, Germany). The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\% \text{ Inhibition} = \left[1 - \left(\frac{A1}{A0}\right) * 100\right]$$

A_0 =absorbance of the control
A_1 =absorbance of the extracts

Concentration of sample required to scavenge 50% of free radicals (IC_{50} values) were determined using non-linear regression. Assays were performed in duplicates and repeated twice.

Nitric oxide inhibitory assay

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described by Janetia et al [11]. Sodium nitroprusside (10 mM) in phosphate-buffered saline (pH 7.4) was mixed with different concentrations of the extract dissolved in phosphate-buffered saline in a test tube and incubated at 25 °C for 180 minin the dark. Samples from the above (100 µL) was transferred into a 96 well plate and reacted with 100 µL Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamme was read at 550 nm and referred to the absorbance of standard solutions of sodium nitrite treated in the same way with Griess reagent.

Inhibition activities of nitrite formation by the plant extracts and the standard antioxidant garlic acid were calculated relative to the control. The percentage inhibition was linearized against the concentration of each extract and Gallic. The IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation was determined.

Estimation Total Flavonoid Contents (TFC)

The total flavonoids content was determined in accordance with the methods of Milauskas et al [12]. The reference drug used for the assay was quercetin. Extract (150 µL) was introduced into the wells of microtiter plate (in triplicates), 50 µL of aluminium chloride and 100 µL of sodium acetate buffer respectively were added to each of the wells. Absorbance was measured at the wavelength of 412 nm. A calibration curve was constructed and used in the estimation of the total flavonoids content.

Phosphomolybdate assay/Total Antioxidant Capacity Assay (TAC)

The total antioxidant capacity was measured according to the method reported by Prietto et al [13] with a slight modification. Extract (100 µL) and 100 µL of ascorbic acid (reference standard) were taken, placed separately in a test tube with 1mL of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. After incubation in a thermal block at 95°C for 1 hour 30 min, samples were cooled at RT and the absorbance was measured at 695 nm against a blank which was incubated at 1 mg/mL concentration under the same conditions. The experiment was conducted in triplicate and values were expressed as mg ascorbic acid equivalent per gram of extract.

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using spectrophotometric method [14]. Methanolic solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.0 mL of 7.5% $NaHCO_3$. Blank was concomitantly

prepared, containing 0.5 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.0 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean values of absorbance were obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of garlic acid equivalent (GAE mg/g of extract).

Table 1: DPPH Free Radical Scavenging Activity of Investigated Plant Extracts

Plant Extracts	% Yield of extract	IC_{50} (µg/mL)
Khaya senegalensis(L)	5.20	12.16 ± 0.06
Khaya segalensis (B)	14.92	10.85 ± 0.21
Chrysophyllum albidum(S)	14.00	25.34 ± 0.01
Zephyranthes candida (W)	1.84	47.43 ± 0.11
Abutillon mauritianum(L)	3.90	7.76 ± 0.68
Senna siamea (B)	4.04	15.60 ± 0.43
Tetrapluera tetraptera (S)	21.70	44.12 ± 0.44
Parquetina nigrescence(R)	5.30	71.02 ± 078
Secamone afzelii(L)	5.30	97.60 ± 0.19
Thonnigia sanguinea (W)	2.50	6.12 ± 0.11
Uvaria chamae (B)	6.96	4.30 ±0.82
*Spondias mombim (*L)	6.19	14.92 ± 0.03
*Spondias mombim (*B)	0.53	20.04 ± 0.45
Lippia multiflora	1.98	17.10 ± 0.75
Ascorbic Acid	-	1.40± 0.22

Key: L=Leaf, B=Bark, W=Whole plant, F=Fruit R=Root

Statistical analysis

Data obtained were expressed as the mean ± SEM. Means of results (data) obtained from various assays were tested for statistical signiûcance using student t- test of a commercially available GraphPad® package version 5.0 (San Diego, USA). Mean differences were considered significant at $P < 0.05$.

Result

The extracts exhibited scavenging activity against the DPPH radical and had IC_{50} values, ranging from 4.30 to 97.60 µg/mL (Table 1). *Uvaria chamae*, *T. sanguinea* and *K. senegalensis* (sb) had IC_{50} values of 4.30, 6.12 and 10.85 µg/mL respectively. Ascorbic acid used as a reference in this study, had IC_{50} value of 1.4 µg/mL *Secamone afzelii and Parquetina nigrescence* (r) and had the lowest scavenging activities against the DPPH radical among the test extracts with IC_{50} value of 71.02 and 97.60 µg/mL, respectively, Other results are displayed in Table 1.

Furthermore, the results of NO scavenging activity of the selected plant extracts are shown in Figure 1. The IC_{50} values for NO inhibition ranged from 1.3 ± 0.7 to 44 ±1.7 µg/mL (Fig. 1). *Thonnigii sanguinea*, and *K. senegalensis* (l) had the highest inhibitory activity with IC_{50} values of 1.31 ± 0.7 and 3.1 ± 0.1 µg/mL respectively. The total phenolic content of the plants extract ranged from 14.71 ± 0.05 to 346 ± 0.02 (Fig. 2). *Khaya senegalensis* (b), *T. sanguinea* and *S. mombin* (b) methanolic extracts gave high phenolic contents of 346.77 ± 0.02, 343.47 ± 1.79 and 357.4 ± 0.02 mg/g, respectively. On the other hand, *Abutilon mauritianum* had the lowest phenolic contents of 14.7 ± 0.05 mg/g as shown in figure 2

In addition, the total antioxidant capacity (TAC) of the extracts expressed as ascorbic acid Equivalent (AAE) ranged from 11.17 ± 1.37 to 374.66 ± 8.41 AAE mg/g (Fig. 3). *Thonnigii sanguinea* had the highest TAC value of 374.46 ± 8.41 AAE mg/g followed by *S. mombin* (b) with TAC value of 359 ±4.47 AAE mg/g (Fig.3). For the total flavonoid contents, *Uvaria chamea* had the highest contents of 84.84 ± 6.26 mg/g, *P. nigresence* (r), *Z. candida* and *C. albidum* had little or no flavonoid content (Fig 3)

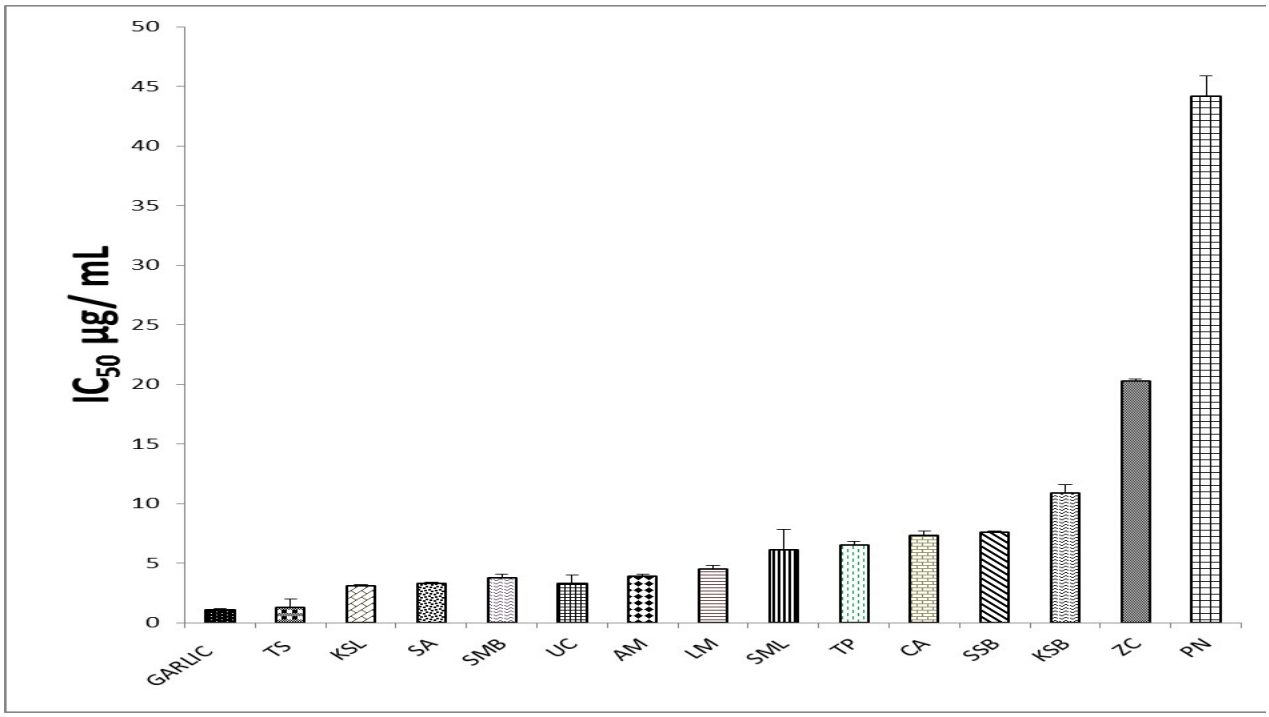

Fig. 1: Nitric oxide inhibitory activity of investigated plant extracts
Key: AM- *Abutilon mauritianum, TT- Tetrapluera tetraptera*(F) , **CA**- *Chrysophyllum albidum*(S), **PN**- *Parquetina nigresence* (R), **ZC**- *Zephyranthes candida* (W), ***SS***- *Senna siamea* (B),**SM-** *Spondias mombin*(B&W),**SA**- *Secamone afzelii,* **TS**- *Thonnigii sanguinea* (W*),* **KS** –*Khaya senegalensis*(B&L), **LM** - *Lippia multiflora* and **UV**- *Uvaria chamae*(B)

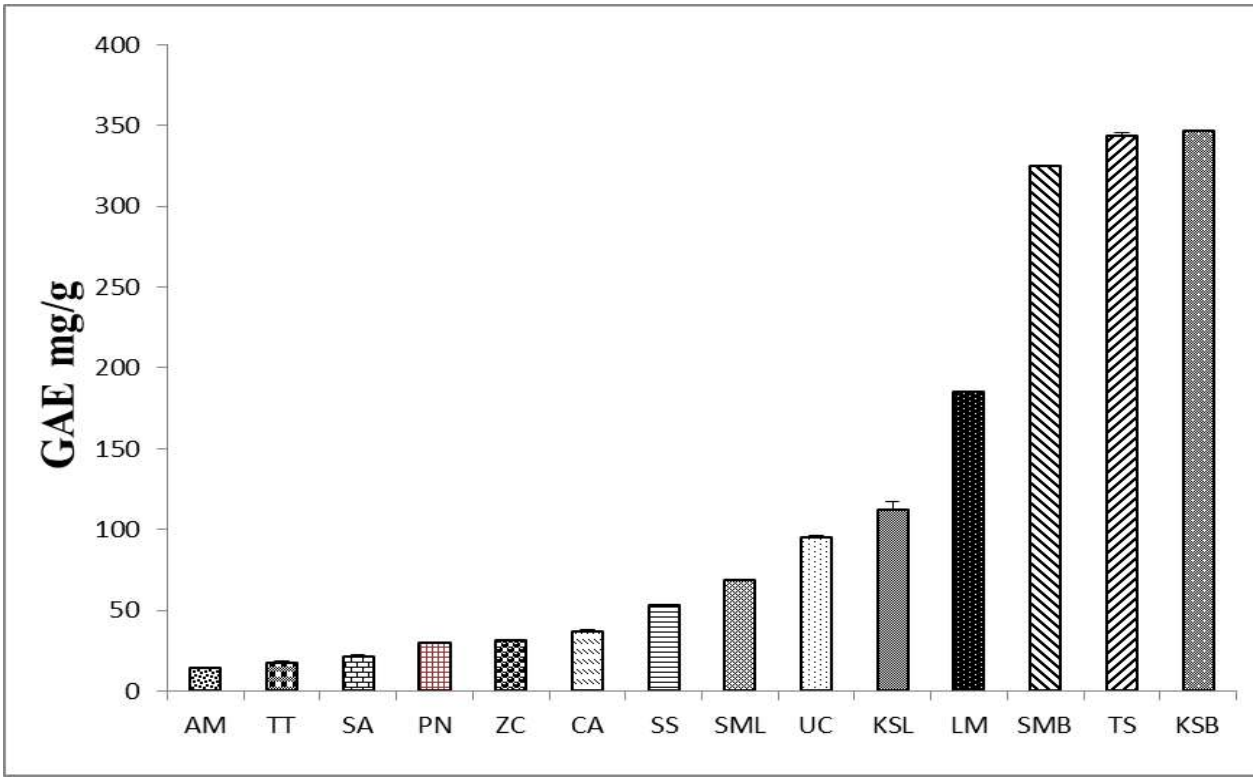

Fig. 2: Total phenolic contents expressed as Gallic Acid Equivalent (GAE mg /g)
AM- *Abutilon mauritianum,* **TT**- *Tetrapluera tetraptera*(F) , **CA**- *Chrysophyllum albidum*(S), **PN**- *Parquetina nigrescence* (R), **ZC**- *Zephyranthes candida* (W), ***SS***- *Senna siamea* (B),**SM-***Spondias mombin*(B&W),**SA**- *Secamone afzelii,* **TS**- *Thonnigii sanguinea* (W*),* **KS** –*Khaya senegalensis* (B&L), **LM** - *Lippia multiflora* and **UV**- *Uvaria chamae*(B)

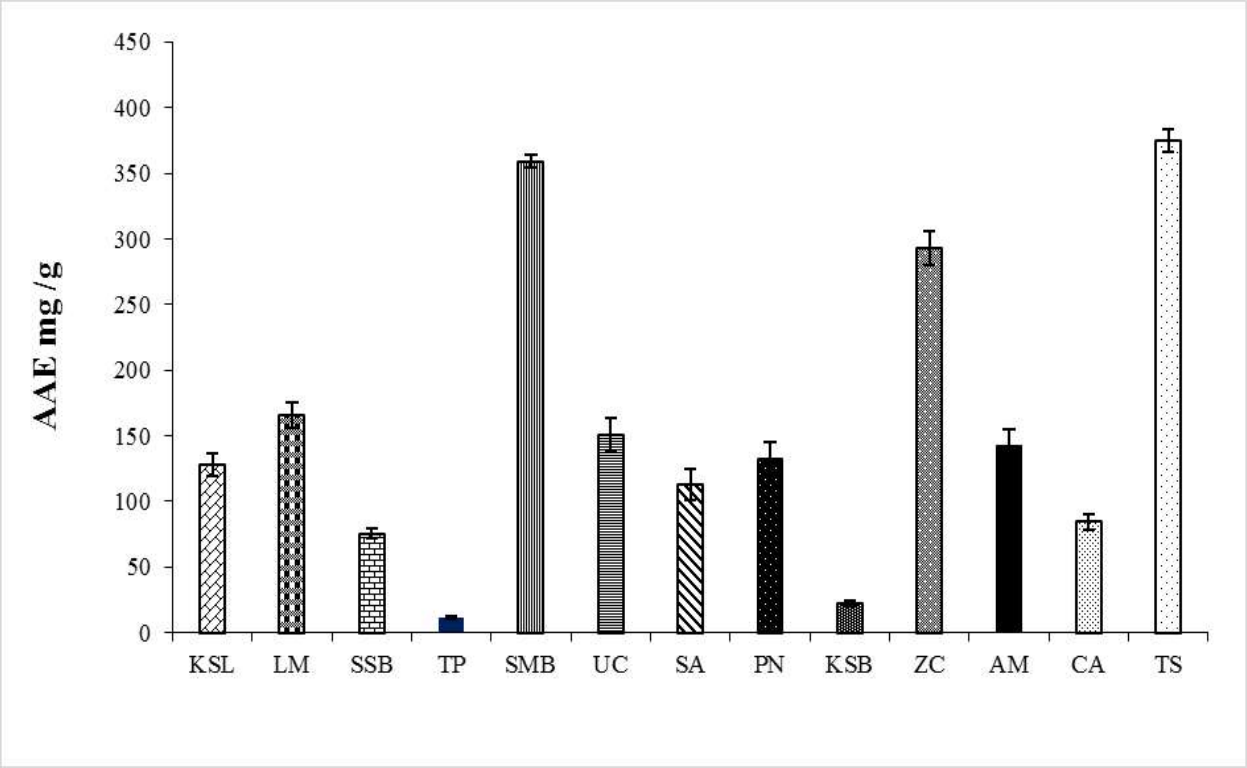

Fig. 3: Total antioxidant property of extracts expressed as Ascorbic Acid Equivalent AAE mg/g
AM- *Abutilon mauritianum,* **TT**- *Tetrapluera tetraptera* (F) , **CA**- *Chrysophyllum albidum*(S), **PN**- *Parquetina nigrescence* (R), **ZC**- *Zephyranthes candida* (W), ***SS***- *Senna siamea* (B), **SM-***Spondias mombin* (B&W),**SA**- *Secamone afzelii,* **TS**- *Thonnigiia sanguinea* (W*),* **KS** –*Khaya senegalensis* (B&L), **LM** - *Lippia multiflora* and **UV**- *Uvaria chamae*(B)

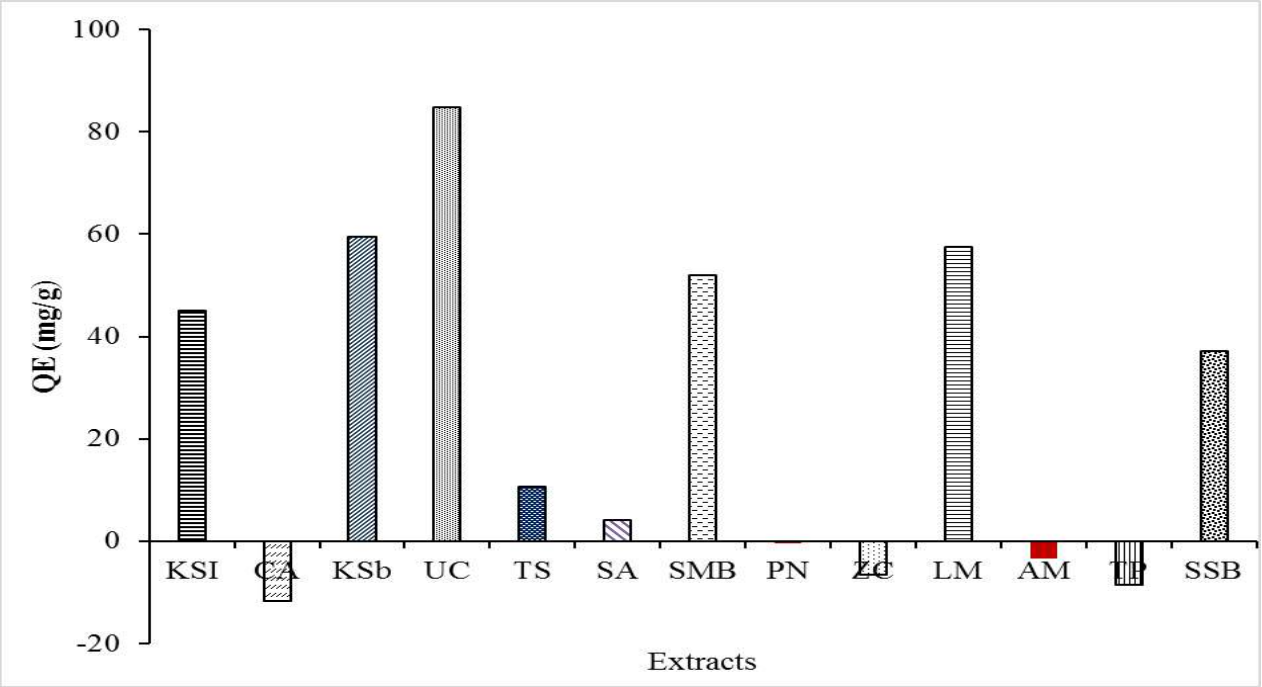

Fig. 4: Total flavonoid contents of the Extracts expressed as Quecertin Equivalent QE (mg/g)
AM- *Abutilon mauritianum,* **TT**- *Tetrapluera tetraptera* (F) , **CA**- *Chrysophyllum albidum* (S), **PN**- *Parquetina nigrescence* (R), **ZC**- *Zephyranthes candida* (W), ***SS***- *Senna siamea* (B),**SM-** *Spondias mombin* (B&W),**SA**- *Secamone afzelii,* **TS**- *Thonnigiia sanguinea* (W*),* **KS** –*Khaya senegalensis* (B&L), **LM** - *Lippia multiflora* and **UV**- *Uvaria chamae* (B)

Discussion

Excessive production of free radicals causes decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to cell death [15]. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, inflammation, rheumatoid arthritis, epilepsy and degradation of essential fatty acids [16]. Therefore, substances that can scavenge the excess free radicals are important agents that can protect the body from the deleterious effect of free radicals.

In search of new antioxidant agents from plants, the antioxidant activity of 12 medicinal plants was evaluated using the DPPH and Nitric oxide based assays. The Diphenyl-2-picryl-hydrazyl (DPPH) contains an odd number of electrons, which gives a purple colour to the compound. As an antioxidant agent donates an electron, the DPPH gets paired with the hydrogen from the antioxidant and becomes decolorized [17]. Thus, interaction of an antioxidant agent with DPPH results in a colour change that can be estimated in a colorimetric assay [18]. In this study, most of the extracts evaluated exhibited various scavenging activities against the DPPH radical. The radical scavenging activity of the five most active extracts followed this order *Uvaria chamae* (sb) > *Thonnigia sanguinea* (w) > *Abutillon mauritianum* (l) > *Khaya senegalensis* (sb)> *Khaya segalensis* (l). DPPH radical scavenging activity of different plant part of *Uvaria chamae* had been previously reported; the seed extract [19], root extracts [20,21] the leaf, the result from the stem bark in this assay established the fact that radical scavenging agent is well distributed in the various parts of the plant although the DPPH scavenging activity is good but it is does not significantly comparable to ascorbic acid in this study ($p= 0.032$) . Similarly, there has been a previous report of the DPPH scavenging activity of *T. sanguinea* from Ghana [22]. Gyamfi and co-workers also reported the isolation of two antioxidant ellagitannins; thonningianins A and B from *T. sanguinea* [23, 24]. *Thonnigiia sanguinea* had the highest Total antioxidant capacity (TAC) in this study.

Nitrite ions were detected in this study using the Griess reagent. Nitrite ions react with Griess reagent to form a purple azo and in the presence of nitric oxide scavengers the amount of nitrites decreased [25]. Interestingly seven of the plant extracts scavenged nitric oxide in the following order of increasing activities *Thonningia sanguinea* >*Uvaria chamae* > *Khaya senegalensis* leaf > *Secamone afzelii* > *Spondia mombin* bark >*Abutilion mauritanium* > *Lippia multiflora*. The NO scavenging activity of *T. sanguinea* is comparable to gallic acid, a standard antioxidant agent ($p = 0.79$). *In vivo,* nitric oxide interacts with superoxide anion, another free radical to produce peroxynirite. Generation of peroxynitrite, can result in subtle modulation of cell signalling to overwhelming oxidative injury resulting in cells necrosis or apoptosis [26]. The ability of these extracts to scavenge NO will result in pharmacological inhibition of peroxynitrite induced damages thereby preventing ischaemia-perfusion injury, circulatory shock, inflammation, pain vascular and neurogenerative diseases associated peroxynitrite induced oxidative stress [27-29]

It has been reported that phenols and polyphenolic compounds, such as flavonoids possess antioxidant activity and including them in human nutrition might results in positive effect which can be of vital importance to general well-being. It has been postulated that the mechanism of action of phenolics such as flavonoids may be through scavenging or chelating process [30, 31]. Phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby upsetting chain oxidation reactions in cellular components and it has been said that their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals [32]. This finding suggests that the observed antioxidant activities of *T. sanguinea* correlate with its phenolic content; *T. sanguinea* had one of the highest phenolic content in this study. Also it had the highest total antioxidant content and its NO scavenging activity is significantly greater than other extracts (pd" 0.005). *K. senegalensis* stem back had the highest TPC, but there is no significant difference between its TPC and that of *T. sanguinea* ($p > 0.05$). In addition, *K. senegalensis* had good radical scavenging activity in both DPPH and NO scavenging assays. *Uvaria chamae* with the highest total flavonoid content also had the highest DPPH radical scavenging activity. Previous studies showed that antioxidant activity directly correlates with phenolic content in medicinal plants [33-37] and this corroborates the results of our studies.

One of the major findings in this study was that *T. sanguinea* demonstrated much stronger antioxidant activity and contained significantly higher phenolic content than common vegetables and fruits earlier studied by our group [38] and are considered as good natural sources of dietary antioxidants. Thus the significance of the various activity of these medicinal plants cannot be overlooked since they have been indicated in the

traditional treatment of various illness including; antiviral, anti-inflammatory and antimalarial studies. Their ability to greatly scavenge free radicals might be one of the ways through which they militate against various disease states.

Conclusion

The results obtained in this study showed that some of the extracts especially *T. sanguinea,* and *U. chamae* exhibited potent free radical scavenging activity; they also have high total phenolic, flavonoid and antioxidant content. This suggests that the plants have potentials as sources of natural antioxidants and justified their use in the Nigerian ethno-botany as therapeutic agents in preventing or slowing the progress of oxidative stress-related degenerative diseases.

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Palmar and digital dermatoglyphic traits of sub-Saharan African subjects

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Abstract
Introduction: Dermatoglyphic traits in conjunction with other morphological, molecular and biochemical markers are extremely important in biological anthropology to explore affinities and differences between human population groups. Towards this end, not much work has been documented for sub-Saharan African subjects.
Materials and methods: Bilateral finger and palmar prints were obtained by the standard technique of Cummins and Midlo on samples of Nigerians of different tribes namely Ibos, Yorubas, Hausas and Urhobos, Kenyans and Tanzanians, Malawians and Zimbabweans, who are apparently physically healthy subjects and whose parents and grandparents were from the countries indicated above. The palmar prints obtained were classified into arches, loops and whorls. Ridge count, atd angles and pattern intensity indices were calculated according to standard methods.
Results: Ulnar loops were the most predominant palmar digital ridge patterns observed in all the studied subjects. However, the percentage frequencies of these loops vary between the regions with the East Africans having a higher percentage than Nigerians. Among South Africans, Zimbabweans had higher percentage of ulnar loops than Malawians especially the female subjects. Furthermore, the Southern Africans had the highest percentages of arches when compared with West and East Africans. The mean TFRC was highest in Southern Africans, high in East Africans but least in Nigerians while the mean atd angle and a-b ridge counts were highest in East Africans high in Southern Africans and again least in Nigerians except for Malawian females. PII values were, however, highest in Nigerians than East and Southern Africans whose values were less than 10.
Conclusion: This study has demonstrated significant regional dermatoglyphic variability between Nigerians, East and Southern Africans in atd angles a-b ridge counts, TFRC and PII an indication of the usefulness of dermatoglyphic traits in the study of population dynamics. However, inspite of these differences, sub-Saharan African populations fit within the well-established clinal distribution of traits, showing a comparatively high whorl frequency among northernly located population groups.

Keywords: *Palmar, digital, dermatoglyphics, sub – Saharan, African subjects.*

Résumé
Introduction : Les traits dermatoglyphes en conjonction avec d'autres marqueurs morphologiques, moléculaires et biochimiques, sont extrêmement importants en anthropologie biologique pour explorer les affinités et les différences entre les groupes de population humaine. À cette fin, peu de travaux ont été documentés sur des sujets d'Afrique subsaharienne.
Matériels et méthodes : Des empreintes digitales et palmaires bilatérales ont été obtenues selon la technique standard de Cummins et Midlo sur des échantillons de différentes tribus Nigérianes notamment Ibos, Yorubas, Haoussas et Urhobos, Kenyans et Tanzaniens, Malawiens et Zimbabwéens, sujets apparemment en bonne santé physique dont les parents et grands-parents étaient originaires des pays indiqués ci-dessus. Les empreintes ulnaires obtenues ont été classées en arches, boucles et tours. Le dénombrement de la crête, les angles atd et les indices d'intensité de motif ont été calculés selon les méthodes standard.
Résultats : Les boucles ulnaires étaient les motifs numériques de crêtes palmaires observés les plus prédominants chez tous les sujets étudiés. Cependant, les fréquences en pourcentage de ces boucles varient d'une région à l'autre, les pourcentages étant plus élevés chez les Africains de l'Est que chez les Nigérians. Parmi les Africains du Sud, les Zimbabwéens présentaient un pourcentage plus élevé de boucles ulnaires que les Malawiens, en particulier les sujets féminins. En outre, les pourcentages d'arcades étaient les plus élevés parmi les Africains du Sud, par rapport aux Africains de l'Ouest et de l'Est. La TFRC moyenne était plus élevée parmi les Africains du Sud, élevée parmi les Africains de l'Est, mais moins chez les Nigérians, tandis que les dénombrements moyens entre angles

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et arêtes étaient plus élevés chez les Africains de l'Est et élevés parmi les Africains du Sud, mais encore moins chez les Nigérians, à l'exception des Malawiennes. Les valeurs PII étaient toutefois plus élevées chez les Nigérians que chez les Africains de l'Est et du Sud dont les valeurs étaient inférieures à 10.

Conclusion : Cette étude a démontré une importante variabilité des dermatoglyphes régionales entre les Nigérians, les Africains de l'Est et du Sud - dans le nombre des angles atd de crêtes ab, TFRC et PII une indication de l'utilité des traits dermatoglyphes dans l'étude de la dynamique des populations. Cependant, malgré ces différences, les populations d'Afrique subsaharienne s'inscrivent dans la distribution clinal bien établie des traits, la distribution, montrant une fréquence de spores relativement élevée parmi les groupes de population situés au nord.

Mots - clés *:* *Palmaire, numérique, dermatoglyphes, subsaharienne, sujets africains.*

Introduction

The principle of dermatoglyphics focuses on the ridges formed in the raised apertures of sweat glands that have unique detailed formations, are much less fortuitous in origin and anatomical regularities [1]. Dermatoglyphic traits, along with other morphological, molecular and biochemical markers have traditionally been used in biological anthropology to explore affinities and differences among human groups [2]. There are also normal variation in these traits, which represent hereditary differences between members of separate populations and members of the same population or family. Thus, dermatoglyphic traits are most useful in studying population dynamics [3].

The use of dermatoglyphic traits as racial indicators has demonstrated that human races did not differ in the expressions of any of these traits, but they differed only in the relative frequencies of the traits [4]. This initial view was, however, later altered slightly when Jantz and Parham [5] showed that ridge breadth was found to differ among populations. However, a few instances have shown that dermatoglyphic traits could substantiate racial history interpretations as shown in the confirmation of blood group evidence[6] and separate lines of population history for African and Asian (Oceanic) pygmy populations [7]. The value of dermatoglyphic traits in tracing population history was further enhanced by Birdsell [8] when he postulated that complex genetic traits (those under polygenic control) are more useful than simple genetic traits in terracing widely separated or distantly related populations. Rife [9] and Newman[10]confirmed the above assertion when they concluded that the polygenic nature of dermatoglyphic traits make them less subject to random genetic drift, and in addition they are free of assortative mating effect hence less subject to environmental influences and gene flow. This makes dermatoglyphic traits most suitable for "classifying older and more basic relationships between populations".

Studies on Caucasians [11-13] have demonstrated that dermatoglyphic traits could he used in tracing the history and relationships of population. In this connection, Wilder [14] showed that racial differences existed in the palm and finger configuration of Germans. European—Americans, Chinese and Japanese. The distribution of fingerprint characteristics in Whites and Negroes living in Brazil have also been documented and the most marked difference observed between Whites and the total Negroid group was in the prevalence of radial loops [15].

Studies in sub-Saharan Africans have shown total finger ridge count (TFRC) variability among various African population groups [16-19]. Ogunye and Sagay [20], for example, showed that sexual dimorphism was exhibited by the atd angle of the Zulus of South Africa, who also demonstrated a lower 'TFRC' when compared with Southern Nigerians. On the other hand, Boroffice [21], Jantz and Brehme [22] and Igbigbi *et al* [23] studied the digital and palmar dermatoglyphics of the Yoruba and Ibo tribes of Nigeria, showing dermatoglyphic differences between both tribes. . In the same vein, Igbigbi and Msamati [24,25] have reported on the digital and palmar dermatoglyphics same parameters in Malawian and Zimbabwean subjects. Other studies on Southern African populations have documented the dermatoglyphics of South African Negro [26] and the Pandamatenga Bush-Bantu hybrid of Botswana [27]. These studies have clearly demonstrated that dermatoglyphic traits differ among the various African groups, hence can be used to differentiate them.

Despite the importance of dermatoglyphic traits enumerated above, not much work has been documented for African subjects especially those in sub-Saharan African region. This study aimed at filling this gap in knowledge and also in presenting the palmar and digital dermatoglyphic trait profiles of sub-Saharan Africans using subjects from West, East and Southern Africa.

Materials and methods

Nigerians

The sample consisted of 390 Ibo (250 males, 140 females), 383 Yoruba (250 males, 133females), 625 Hausa (320 males, 305 females) and 612 Urhobo (342 males, 270 females). The subjects were apparently healthy volunteers aged 13-25 years whose parents and grandparents were of the Nigerian tribes indicated above. Furthermore, the subjects were asked individually if there was any non-member of the tribes above that contributed to their ancestry

for as far back as they know, and anyone who gave a positive answer was excluded. In the case of related individuals in the sample, only the print of one of them was included in the analysis as is conceivable that statistics indicating the occurrences of particular features might be distorted by the introduction of several members of a family in a relatively small collection [28].

Malawians and Zimbabweans

The sample consisted of 231 Malawians (142 females and 89 males) aged between 11-39 years form Chichiri Secondary School and from members of staff of the College of Medicine all in Blantyre. The Zimbabweans sample consisted of 135 males and 135 females aged between 11-47 years from Mufakose high density township in the cosmopolitan cities of Harare and Gweru cities in Zimbabwe's midlands. This gave a good mixture of social backgrounds to allow for the inclusion of quantitative palmar variables [29]. The subjects were apparently physically healthy with Malawian and Zimbabwean parents and grandparents, respectively. The subjects were also asked individually if there were any non-Malawian or Zimbabwean ancestry for as far back as they know, and anyone who gave a positive answer was excluded.

Bilateral finger and palmar prints were obtained by the standard technique of Cummins and Midlo [28]and only clear prints were classified into arches, loops and whorls (Fig 1) and ridge counts were performed according to the method described by Arrieta et al.,[30].Inter-observe variations were eliminated as one person examined all the prints. The following features were examined;

The total finger ridge counts (TFRC) are the sum of the ridge-counts (largest count only when there is more than one) on all ten fingers for each gender.

Pattern intensity index (PII) was the mean number of triradii on digits per individual subject. This reflects the complexity of the finger patterns in an

Table 1a: The Percentage frequency of digital patterns of Yorubas and Ibos of Nigeria.

Pattern Types	Yorubas						Ibos					
	Male			Female			Male			Female		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
Arch	10.20	10.10	10.10	11.60	10.20	10.90	14.40	11.40	12.90	12.90	11.40	12.40
Radial Loop	10.90	2.70	2.30	2.30	1.10	1.70	2.30	2.60	2.50	3.10	2.10	2.60
Ulnar Loop	61.20	58.70	60.00	60.50	62.40	62.00	62.00	61.80	61.90	55.70	58.30	57.00
Whorl	26.70	28.50	25.70	23.30	23.30	24.50	22.30	24.20	22.30	28.30	28.10	28.20

Kenyans and Tanzanians

The sample consisted of 304 Kenyans (164 males, 140 females) aged 12-14 years from Nairobi Primary School and 300 Tanzanians (180 males, 120 females) aged 19-24 years who are students of Muhimbili University College of Health Sciences. The subjects were apparently healthy volunteers whose parents and grandparents were either Kenyans or Tanzanians respectively.

They were selected from the cosmopolitan cities of Nairobi and Dar-es-salaam respectively, giving a good mixture of social backgrounds to allow for the inclusion of quantitative palmar variables [29]. Furthermore, the subjects were asked individually if there were any non-Kenyan or Tanzanian contribution to their ancestry for as far back as they knew, and anyone who gave a positive answer was excluded. In the case of related individuals in the sample, only the print of one of them was included in the analysis as is conceivable that statistics indicating the occurrences of particular features might be distorted by the introduction of several members of a family in a relatively small collection [28].

Table 1b: The total finger ride count (TFRC) and pattern intensity indices of Yoruba and Ibos of Nigeria

	Yorubas		Ibos	
	Male	Female	Male	Female
TFRC Mean	101.60	121.60	113.80	111.40
SD	37.90	39.20	44.30	40.20
*PII Mean	11.74	11.36	11.03	11.60

**PII was calculated from pattern intensity type frequency totals; hence standard deviations were not obtained .n.s= not significant.*

Table 1c: The atd angle and a-b ridge counts of Yorubas and Ibos of Nigeria

	Yorubas			Ibos		
	Male	Female		Male	Female	P
atd angles Mean	76.30	77.90	n.s	77.10	76.60	
SD	4.70	11.80		4.10	7.80	
Mean a-b ridge	72.80	74.40	P<0.05	74.20	73.40	n.s
SD	8.50	5.90		8.50	8.90	

n.s = not significant

Table 1d: Comparison of dermatoglyphic variables between Yorubas and Ibos of Nigeria

	Yorubas		bos	
	Male	Female	Male	Female
TFRC Mean	101.60	121.60	113.60	11.40
SD	37.90	39.20	44.30	40.20
N	250	133	250	140
Atd angles Mean	76.30	77.90	77.10	76.60
SD	4.70	11.80	4.10	7.80
a-b ridge count Mean	72.80	74.40	74.20	73.40
SD	8.50	5.90	8.50	8.90
PII Mean	11.74	11.36	11.03	11.60

individual or population. In this system, arches having no triradii are scored 0; loops representing pattern formation with one triradii are scored 1; and whorls or composite patterns with two (or more) triradii are scored 2. The pattern frequency of a population is therefore summarized by the finger pattern intensity index representing the mean number of digital triradii per person.

The *a-b ridge* **count** represents the number of ridges observed between triradii a and b.atd angle: This was the angle between two straight lines joining the radial (a) and ulnar (d) triradii to the hypothenar triradius (Fig 1).

The results of the ridge patterns, TFRC, PII, atd angle and a-b ridge counts were analyzed statistically using the X^2 test with 2 degrees of freedom for pattern type intensity, and t tests for matched or unmatched pairs as appropriate for TFRC, atd angles and a-b bridge counts. The data were then compared between all the studied subjects in the respective countries.

Table 2a: The Percentage frequency of digital patterns of Hausas and Urhobos of Nigeria.

Pattern Types	Hausas						Urhobos					
	Male			Females			Males			Females		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
Arch	9.49	7.05	8.27	16.07	13.71	14.89	14.56	11.80	13.18	14.10	11.80	12.9
Radial Loop	1.28	2.38	1.83	2.10	1.72	1.91	2.74	3.70	3.22	2.00	1.90	1.95
Ulnar Loop	54.93	53.79	54.36	51.87	53.07	52.47	53.00	49.40	52.20	51.30	54.70	53.0
Whorl	34.30	36.78	35.54	29.96	31.50	30.73	29.70	35.11	32.40	32.60	31.60	32.1

Results

Palmar ridge patterns

Palmar ridge patterns did not exhibit gender dimorphism in all our sampled subjects. This notwithstanding, differences were shown in the various ridge patterns within and between the groups but again these differences were not statistically significant (P>0.05)

Digital ridge patterns

Table la shows the digital pattern type distributions for both gender in the Ibo and Yoruba subjects. With regards to gender dimorphism, only the Ibos showed

Table 2b: The total finger ride count (TFRC) and pattern intensity indices of Hausas and Urhobos of Nigeria

	Hausas			Urhobos		
	Males	Females		Males	Females	P
TFRC	130.11	127.74	P<0.05	115.46	110.48	P<0.05
SD	9.13	8.20		16.83	15.47	
*PII Mean	12.64	11.59		11.20	11.40	

Table 2c: The atd angle and a-b ridge counts of Hausas and Urhobos of Nigeria

	Hausas			Urhobos		
	Males	Females		Males	Females	P
atd angles Mean	78.04	79.72	n.s	59.90	76.60	P<0.05
SD	7.91	6.26		8.70	7.80	
a-b ridge count Mean	72.95	78.66		74.40	73.40	n.s
SD	10.38	9.35	P<0.05	8.94	8.90	

n.s = not significant

Table 2d: Comparison of dermatoglyphic variables between Hausas and Urhobos of Nigeria

	Hausas		Urhobos	
	Males	Females	Males	Females
TFRC Mean	130.11	124.74	113.60	11.40
SD	9.13	8.20	44.30	40.20
N	320	305	342	270
atd angles Mean	78.04	79.72	59.90	68.10
SD	7.91	6.62	8.70	15.47
a-b ridge counts Mean	72.95	78.66	74.40	74.40
SD	10.38	9.35	6.10	8.94
P II Mean	12.64	11.59	11.20	11.40

Table 3a: The Percentage frequency of digital patterns of Kenyan and Tanzanian subjects

Pattern Types	Kenyans						Tanzanians					
	Males			Females			Males			Females		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
Arch	5.71	4.28	4.99	3.16	2.63	2.89	5.11	4.67	4.89	3.33	3.33	3.33
Radial Loop	6.25	6.42	6.34	6.32	6.32	6.71	6.67	7.04	6.86	7.50	7.50	7.50
Ulnar Loop	69.05	76.19	172.62	69.12	70.18	69.65	65.66	68.89	69.22	79.17	70.83	75.00
Whorl	18.99	13.11	16.05	21.40	20.08	20.75	22.66	19.40	21.03	10.00	18.34	14.44

a significant difference in the rather unusual direction of males having fewer whorls and more arches than females (P<O.01). In terms of ethnic group comparisons, the gender of both groups were found to be significantly different from each other (P<0.0l). There was, however, a striking contrast between the Yoruba and Ibo females who had more whorls and fewer arches than their counterpart. For both groups, the most predominant digital ridge pattern was the ulnar loop.

Tables lb and 1c show quantitative dermatoglyphic variables of TFRC, PII, atd angles

Table 3b: The total finger ride count (TFRC) and pattern intensity indices of Kenyan and Tanzanian subjects

	Kenyans			Tanzanians		
	Males	Females		Males	Females	P
TFRC Mean	125.60	116.26		115.05	114.9	
SD	39.0	32.16	<0.01	32.14	32.50	n.s >0.5
*PII Mean	7.94	8.91		8.23	7.29	

Table 3c: The atd angle and a-b ridge counts of Kenyan and Tanzanian subjects

	Kenyan			Tanzanian		
	Males	Females		Males	Females	P
atd angle Mean	85.20	86.78	<0.	72.981	78.00	<0.05
SD	10.24	11.50		8.80	7.22	
Mean	89.60	87.00	0.001	83.42	83.42	<0.01
SD	15.36	17.34		19.80	18.90	

n.s = not significant

and a-b ridge counts. For TFRC, significances were observed within and between the Ibo and Yoruba subjects (P<0.01). The mean TFRC in Yoruba female was significantly higher than the mean for Yoruba males while the reverse was the case for the Ibo. Similarly the mean a-b ridge count of Yoruba females was significantly higher than that of their males counterpart (P< 0.05). The mean PII showed remarkably little variation between the gender and across the two groups. However, the other variables of atd angles and a-b ridge counts showed little inter-gender and inter group variations (Table 1d).

Table 3d: Comparison of dermatoglyphic variables between Kenyan and Tanzanian subjects

	Kenyan		Tanzanian	
	Male	Female	Male	Female
TFRC Mean	125.60	116.26	115.05	114.90
SD	39.0	32.16	32.14	32.50
N	164	140	180	120
Atd angle Mean	85.20	86.78	77.98	78.00
SD	10.24	11.50	8.86	7.22
a-b ridge count Mean	89.60	87.00	85.42	83.42
SD	15.34	17.34	19.80	18.90
P II Mean	7.94	8.91	8.23	7.09

Table 4a: The percentage frequency of digital patterns Malawian and Zimbabwean subjects

Pattern types	Malawians						Zimbabweans					
	Males			Females			Males			Females		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
Arch	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Radial Loop	8.67	6.67	7.67	5.00	5.45	5.21	5.55	5.55	5.55	6.67	6.67	6.67
Ulnar Loop	73.33	46.67	60.00	41.67	37.50	39.59	77.77	66.66	72.22	78.88	77.77	78.33
Whorl	8.00	36.66	22.33	43.33	47..08	45.20	66.86	17.79	4.45	10.00	4.56	5.00

Digital patterns

gender (P>0.05). While there was a significant difference in the mean atd angle for Urhobo females

Table4b: The total finger ride count (TFRC) and pattern intensity indices of Malawian and Zimbabwean subjects

	Malawians			Zimbabweans		
	Males	Females		Males	Females	P
TFRC Mean	123.72	140.15		126.65	123.71	
SD	39.82	39.82	<0.001	27.38	27.51	0.5
*PII Mean	7.65	6.66		10.83	10.18	

Table 4c: The atd angle and a-b ridge counts of Malawian and Zimbabwean subjects

	Malawians			Zimbabweans		
	Males	Females		Males	Females	P
atd angle Mean	72.06	80.66		72.70	82.40	P<0.01
SD	8.30	8.50	P <0.001	5.98	10.08	
a-b ridge count Mean	79.74	64.66		79.34	79.40	<0.5
SD	16.88	13.22	<0.001	9.58	8.18	

n.s = not significant

Ulnar loops were the most prevalent finger ridge patterns and arches were the least type in the sampled populations. Significant gender differences were exhibited in arches, ulnar loops and whorls in the sampled populations (P<0.05, Table 1a).

Table 2a shows that Hausa females had more arches than their male counterpart but slightly less ulnar loops and whorls. The Urhobo females on the other hand had slightly more ulnar loops but slightly less arches, radial loops and whorls when compared with their male counterpart. Significant gender differences where observed in TFRC in Hausa and Urhobo subjects (P<0.05; Table 2b). Hausas showed little or no difference in mean atd angle between the and males (P<0.05; Table 2c). The mean a-b ridge count was, however, significantly higher in Hausa females than in males (P<0.05; Table 2d). Like the Ibo and Yoruba subjects, the Urhobo and Hausa subjects showed that ulnar loops were the most predominant ridge pattern.

Ulnar loops were the most prevalent finger ridge patterns and arches were the least type in Kenyan and Tanzanian subjects. Significant gender differences were exhibited in arches, ulnar loops and whorls in both groups (Table 3a).

Table 3b shows the mean total finger ridge count (TFRC), mean atd angles, mean ab ridge counts and pattern intensity index (PII) by gender. There

Table 4d: Comparison of dermatoglyphic variables between Malawian and Zimbabwean subjects

Variables	Malawians		Zimbabweans	
|	Males	Females	Males	Females
TFRC Mean	123.72	140.15	126.65	123.71
SD	39.82	39.82	27.38	27.51
N	89	142	135	135
atd angle Mean	72.74	80.66	79.70	82.40
SD	8.30	8.50	5.98	10.08
a-b ridge count Mean	79.74	64.66	79.34	79.40
SD	16.88	13.22	9.58	8.18
P II				
Mean	7.65	6.66	10.83	10.18

was significant gender difference in TFRC in Kenyans (P<0.01) but none in Tanzanian subjects (P<0.05). In both groups men showed significantly higher mean a-b ridge counts than women (P<0001, Kenyans; P<0.01, Tanzanians). Women had higher mean atd angles than men in both groups but the difference were not statistically significant (P>0.5) table 3c). Women had also higher PII than Kenyan men and the reverse was the case in Tanzanians.

Similar significant differences also existed in all the above parameters between Malawians and both Kenyans and Tanzanians (P<0.001). However, the level of significance between Malawian and Tanzanian men in TFRC, and between Tanzanian men and women in atd angle differed (P<0.01). The TFRC in females was higher in Malawians, high in Kenyans and least in Tanzanians; for men it was higher in Kenyans, high in Malawians and least in Tanzanians. A similar trend emerged with the a-b ridge counts. The PII of Kenyans was closer to the indices of Tanzanians than those of Malawians (Table 3d).

Tables 5: Comparism of dermatoglyphic variables of Nigerian, Kenyan, Tanzanian, Malawian and Zimbabwean Subjects

Varibles	Nigerians		Kenyans		Tanzanians		Malawins		Zimbabweans	
	males	females	males	females	males	females	males	females	males	females
TFRC Mean	115.24	117.80	125.60	116.26	115.05	114.90	123.72	140.15	126.65	123.71
SD	27.04	25.77	39.00	32.16	32.14	32.50	39.82	39.82	27.38	27.51
N	1162	848	164	140	180	120	89	142	135	135
atd angle mean	72.84	77.71	85.20	86.78	77.98	78.00	72.06	80.66	72.70	82.40
SD	6.35	8.50	10.24	11.50	8.86	7.22	8.30	8.50	5.98	10.08
a-b ridge count	73.59	74.97	89.60	87.00	85.42	83.42	79.74	64.66	79.34	79.40
mean	9.08	8.26	15.34	17.34	19.80	18.90	16.88	13.22	9.58	8.18
SD										
PII Mean	11.65	11.49	7.94	8.91	8.23	7.09	7.65	6.66	10.83	10.18

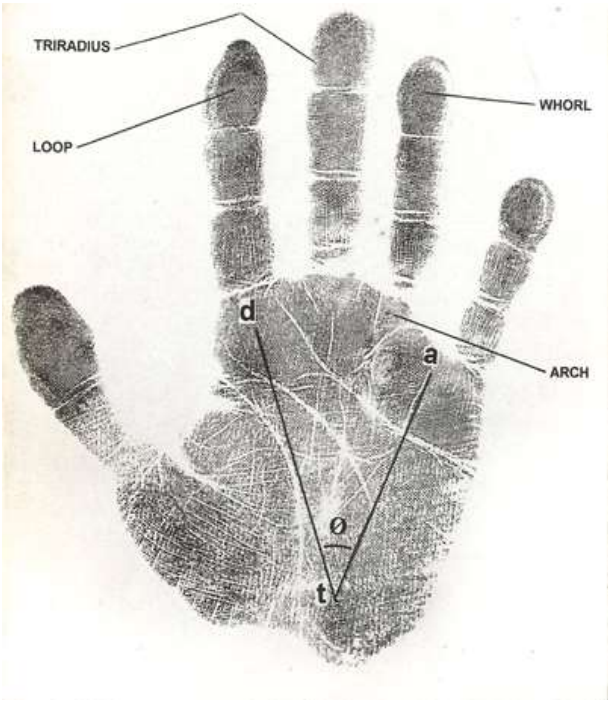

Fig. 1: *Palmar and digital print showing arches, loops, whorls, triradius and atd angles*

The overall distribution of the palmar patterns was not significantly different between hands or between gender in Zimbabwean subjects (P>0.05). Ulnar loops were the most predominant pattern type in both gender, followed by whorls in males and arches in females; however, the gender differences between these digital pattern types were not statistically significant (P >0.05, Table 4a).

Table 4b shows the total finger ridge count (TFRC), atd angle, a-b ridge count and pattern intensity index (PII) by gender. Females had significantly wider atd angles than males (P <0.01); males, however, had slightly higher PII values than females, but the difference was not statistically significant (Chi P >0.5). There were no significant differences between the gender in TFRC and a-b ridge counts (P> 0.5) (Table 4c)

Similarly significant difference also existed between Zimbabwean and Malawian females and between Zimbabwean and Nigerian men in TFRC and a-b ridge counts (P <0.001). Significances were also found between Zimbabwean and Malawian males; male Zimbabweans and Nigerians. Male Zulus and Yorubas also showed significant differences in atd angles (P <0.001).

Table 5 shows the comparison of the dermatoglyphic variables of Nigerian, Kenyan, Tanzanian, Malawian and Zimbabwean subjects. The mean TFRC of South Africans represented by

Malawian and Zimbabwean subjects was significantly higher than those of East Africans (Kenyans and Tanzanians) and was followed by Nigerians representing West Africa. Similarly significant differences were also recorded in a-b ridge counts between Nigerians and East Africans ($P<0.01$). However, the mean atd angle was significantly highest in East Africans, followed by South Africans and then Nigerians ($P<0.01$). In the same vein, the mean a-b ridge count was highest in East Africans, followed by South Africans and then Nigerians except Malawian female subjects. However, PII was highest in Nigerians, followed by Zimbabweans, then East Africans, while Malawians were the least.

Discussion

Palmar ridge patterns

This study has demonstrated that palmar ridge patterns do not show gender dimorphism and therefore are not useful in gender differentiation in a given population. Previous studies in African subjects [16,21,26,32], Caucasians [30-32], Chorote Indians [34] Japanese and Chinese [14] support the above finding. Wilder [14] had also shown that there was a greater frequency of occurrence of the hypothenar patterns in the 'White' over that seen in the 'yellow' races hut no gender differences in the pattern peculiarities were shown. Despite this lack of gender dimorphism palmar ridge patterns tend to show close relationship between members of the same group. This was demonstrated in this study as exemplified by the similarities of palmar ridge patterns amongst Nigerians. Kenyans and Tanzanians, and then Malawians and Zimbabweans. Similarly, individuals from the same countries tended to have similar palmar ridge patterns than their close neighbours.

The present study has also shown the prevalence of digital ulnar loops followed by whorls as, indeed, was the case with other African populations earlier studied[17,26,27,35]. However, Kenyan men had more ulnar loops than women, as was the case with the Zulus of South Africa [26]. This is an interesting finding for a number of reasons. It emphasized that prints of the finger palmar surface provide a distinct technical record in physical anthropology [36] because they have greater phylogenetic stability [37]. However, in Caucasians, high frequencies of digital arches and radial loops in both gender have been reported [30]. These findings underline the usefulness of digital patterns in differentiating population groups. In this connection, De sa Benevides and Salzano [15] had also shown that the most marked difference between whites and the total Negroid group was the prevalence of radial loops in the latter than the former. In another study on the Hehe tribe of Tanzania it was shown that the finger pattern types of women showed rather greater than usual elevation in the total frequency of loops and a diminution in whorls [32], which was also shown in this study. As far as we know the only other female sample with high loop frequency was in Mozambique [32]. The present study has also demonstrated a higher frequency of loops in both gender of both groups, suggesting dermatoglyphic similarity between Kenyans and Tanzanians.

In this light, therefore, Nigerians of West Africa could be differentiated from the East Africans and also from Southern Africans. Although these differences, which existed in the palmar surfaces, were statistically not significant, they may provide a bird's eye view of the differences between population groups.

Digital ridge patterns

The study has further demonstrated that population groups can be differentiated using digital ridge patterns. Among Nigerians, the predominant digital ridge pattern was ulnar loops followed by whorls. In Malawians and Zimbabweans, ulnar loops were the most predominant pattern type on both gender followed by whorls in males and arches in females while in Kenyans and Tanzanians it was also loop with arches the least. For Caucasians, however, high frequencies of arches and radial loops in both gender have been reported [30]. Holt [2] showed that certain patterns tend to occur more frequently on some digits than on others and these seem to be constant for any population group. Holt's observation supported the findings of this study. It appeared that digital ridge patterns were more specific than palmar ridge patterns and they also exhibited gender dimorphism. The distribution of characteristic digital ridge patterns also differentiated members of the same country, as indeed was the case with tribal groups in Nigeria.

Within the East African countries of Kenya and Tanzania, the most predominant digital ridge pattern variation between them could be used to differentiate them. This was also the case with Malawians and Zimbabweans.

Furthermore, as previous study on the Hehe tribe of Tanzania had shown that among women, there were a usual elevation in the total frequency of loops and a diminution in whorls. Boroffice [21] had also shown this phenomenon that among sub-

Saharan Africans; there was a well-established clinal distribution of a comparatively high whorl frequency among northernly located population groups.

The mean TFRC was significantly higher in females than males (P<0.001) in Malawians[24] contrary to the findings of Arrieta et al.,[30] in Caucasians where males had higher mean TFRC than females, even though the differences were not statistically significant between the limbs. This finding suggests racial differences between Malawian and Caucasian samples. The mean TFRC did not show gender dimorphism in Zimbabweans and was significantly higher in males than females [25]. A report on Nigerian subjects also showed gender dimorphism in mean TFRC [23]. Among Kenyans the mean TFRC showed gender dimorphism while in Tanzanians none was exhibited. However, in East Africans the mean TFRC was higher in men than women [38]. These findings suggest that mean TFRC can differentiate population groups among sub-Saharan Africans. A Comparison of the dermatoglyphic traits of Kenyans, Tanzanians and of Malawians previously reported[24], revealed significant differences between Kenyans and Tanzanians with respect to TFRC, atd angle and a-b ridge counts (P<0.001). Indeed it had been shown that racial differences do exist between Africans and Caucasians in mean TFRC [30]. Blecher [27] also showed that mean TFRC was lower in African groups than in British samples with the difference most marked in males. Basu and Namboodiri [39] also demonstrated that males generally had higher values of mean TFRC than females. They also showed the importance of TFRC as a strong inherited variable and a useful parameter to describe, compare and contrast various populations. The importance of TFRC shown in this study between Malawians and Zimbabweans and Kenyans and Tanzanians supports the observation of Ho1t [40]. The mean TFRC was least in Nigerians of West Africa, higher in subjects of Southern Africa and high in East Africans.

The study has demonstrated gender dimorphism in TFRC in Kenyans but none in Tanzanians. However, in both groups the mean TFRC was higher in men than women. This is the opposite of what was reported in Malawians [24] but it was in agreement with what was reported among valley Basques when compared with other Spaniards [30]. Previous study had indeed shown that racial differences do exist between Africans and Caucasians in mean TFRC [30]. Other authors have also demonstrated that the mean TFRC was lower in African than in British samples but the difference was most marked in men [27]. Our findings tend to suggest that mean TFRC can also differentiate African population groups as was demonstrated in the comparison of the dermatoglyphic traits of the Zulus of South Africa from a previously documented study with the Yorubas of Western Nigeria, which showed significant differences in TFRC and a-b ridge counts (P<0.001). Difference in total finger ridge count frequencies between different populations may also be expected, since the frequencies of arches, loops and whorls vary between races [27].

The mean PII was higher in Nigerians, high in Zimbabweans and East Africans and it was least in Malawians. Similarly the mean PII in both gender was higher among Zimbabwean subjects than Malawians, and in both gender are comparable with those of Pandamatenga hybrids of Botswana [27]. The mean PII was higher in Kenyan women than men but the reverse was the case in Tanzanian and Malawian subjects. There was no clear-cut male/female divide in mean PII in Nigerians and there may be a closer dermatoglyphic link between Malawians and Tanzanians exhibited by the mean P11. Some researchers [41-43] had shown that mean TFRC was a more qualified and specific dermatoglyphic variable than P11. This study has also demonstrated this fact. PII could not differentiate the studied populations into west, east, and southern dermatoglyphic groups hut the mean TFRC had done so.

Moreover, it has been shown that the PII was higher in Kenyan women than men but the reserve was the case in Tanzanian and Malawian subjects (Tables 3b, 4b). This may indicate a closer dermatoglyphic link between Malawians and Tanzanians.

The mean atd angle was also found to be higher in women than men in this study but the differences were not statistically significant, thus confirming the reports in other populations [24,27,35]. The mean atd angle was also found to be higher in women than men in all our studied subjects. This has been reported in other human populations [24,27]. However, the mean atd angle was higher in East Africans, high in Southern Africans and least in Nigerians. In addition there was a gradual increase of atd angle form northernly located population down south, and a West-to-East increase. A previous study by Penrose [1] had highlighted the important role of atd angle in differentiating population group because of its genetic determination but its only drawback was the fact that it was influenced by age and early foetal environment.

The a-b ridge count of a person is the total of the a-b counts of the two hands. This ranges from below 70 to over 100. Counts of 78 or below are classified as "low", those of over 78 are "high"[44]. The genetic factors, which determined variation in the a-b ridge counts, had been analyzed and consisted of a main gene, whose expression was affected by multiple modifying factors. The allele that determines a high count (79) ridges and over was dominant over the allele determining a low count. Modifying factors affect the place in the high or low category the individual occupied [44]. The study showed Nigerians had least counts, followed by East Africans and high for Southern Africans. There was an increase in the counts from West to East with Malawians having the lowest counts probably reported for Africans for the first time. However, all subjects had low ridge counts based on pons classification. Pons [45] showed that racial differences existed in the human palm with regards to a-b ridge counts. He showed that the a-b ridge counts for white populations vary between 80 and 86 ridges, black populations between 74 and 80, Indian subcontinent 68 and 80, while American Indians have between 80 and 90. This study has also shown that a-b ridge count can be used to differentiate the different population groups in sub-Saharan subjects. The higher values reported by Pons are applicable to West Africans as values in Nigerians have shown.

Furthermore, this study has demonstrated that using all dermatoglyphic variables, significant differences existed with Nigerians having the least, Southern Africans being high and the East Africans showed the highest mean atd angles and a-b ridge counts. Similarly Nigerians also had the least TFRC, high for East Africans and higher for Southern Africans ($P < 0.01$). The mean PII was also highest for Nigerians; these regional findings could be explained on linguistic grouping disparities advanced by Jantz and his colleagues [17] when they showed that linguistically all black Africans were represented in two groups namely, Niger-Congo and Benue-Congo. Another explanation may be the linguistic grouping proximity and by extension common ancestry [17]. The dermatoglyphic distance and the admixture of other language groups like Arabic, Chinese and Portuguese as was the ease with Kenyans and Tanzanians may indeed explain some of these dermatoglyphic differences between countries from the same linguistic grouping of Jantz [17].

Conclusion

This study has, however, shown the usefulness of dermatoglyphic traits in the study of population dynamics, which was demonstrated by Chai [46]. However, in spite of the differences, sub-Saharan African populations fit within the well-established clinal distribution, showing a comparatively high whorl frequency among northernly located population groups. But for the first time these West-to-East increasing trends is described within palmar and digital ridge patterns.

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Factors of sexual function in males occupationally exposed to bisphenol-A in a plastic industry in Ibadan

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Abstract

Introduction: Bisphenol-A, a constituent of plastic and an endocrine disrupting chemical has been implicated in the aetiology of sexual dysfunction.This study was aimed at evaluating sexual function in males occupationally exposed to Bisphenol-A in a plastic industry in Ibadan, Nigeria.

*Methodology***:** Eighty apparently healthy males aged 18-62 years with normal renal function were enrolled into this case-control study. They were forty male employees of a plastic industry (PIW) age matched with forty males who were non-employees of any plastic industry (Controls). Sexual history, blood pressure, socio-demographic and anthropometric indices were obtained by standard methods. Venous blood (10mL) was obtained from participants for sex hormones analyses by enzyme linked immunosorbent assay while nitric oxide and superoxide dismutase activities were estimated spectrophotometrically. Bisphenol-A was estimated in spot urine samples using high performance liquid chromatography-tandem mass spectrometry. Data analysed were statistically significant at $p<0.05$.

Result: Bisphenol-A was detected in both groups but was significantly raised in controls compared with PIW ($p<0.003$). The controls also showed significantly raised diastolic blood pressure and adiposity indices but lower nitric oxide compared with PIW ($p<0.05$). In controls, bisphenol-A had a direct relationship with systolic BP and waist circumference but indirect relationship with diastolic blood pressure and waist height ratio ($p<0.05$). Although bisphenol-A was not associated with physical sexual function indices, it had a direct relationship with oestradiol in PIW ($p<0.010$).

Conclusion: Bisphenol-A was present in both exposed and unexposed groups but was not associated with sexual function. However, its endocrine disrupting capacity especially in the exposed group is suggested.

Keywords: *Bisphenol-A, Endocrine disrupting chemicals, Hormones, Industrialisation, Sexual dysfunction.*

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Résumé

Introduction : Bisphénol-A, un constituant du plastique et un agent chimique perturbant le système endocrinien, a été impliqué dans l'étiologie de la dysfonction sexuelle. Cette étude visait à évaluer la fonction sexuelle chez les hommes exposés professionnellement au bisphénol- A dans une industrie du plastique à Ibadan, au Nigéria.

Méthodologie **:** Quatre-vingts hommes apparemment en bonne santé âgés de 18 -62 ans avec la fonction rénale normale étaient enrôlés dans cette étude cas-témoins. Ils y'avaient quarante employés masculins d'une industrie plastique (PIW) appariés pour l'âge avec quarante hommes qui étaient non-employés de toute l'industrie plastique (Contrôle). Les antécédents sexuels, la pression artérielle, les indices socio-démographiques et anthropométriques ont été obtenus par des méthodes standard. Le sang veineux (10 ml) a été prélevé chez les participants pour une analyse des hormones sexuelles par dosage d'immunosorbant lié à une enzyme, tandis que les activités d'oxyde nitrique et de superoxyde dismutase étaient estimées par spectrophotométrie. Le bisphénol-A a été estimé dans des échantillons ponctuels d'urine en utilisant une spectrométrie de masse à haute performance par chromatographie en phase liquide et tandem. Les données analysées étaient statistiquement significatives à $p <0,05$.

Résultat : Le bisphénol-A a été détecté dans les deux groupes, mais il était significativement élevé chez les témoins par rapport au PIW ($p <0,003$). Les témoins ont également montré une augmentation significative de la pression artérielle diastolique et des indices d'adiposité, mais une réduction de l'oxyde nitrique par rapport à l'IPW ($p <0,05$). Chez les témoins, le bisphénol-A avait une relation directe avec la pression artérielle systolique et le tour de taille, mais une relation indirecte avec la pression artérielle diastolique et le rapport hauteur / taille ($p <0,05$). Bien que le bisphénol-A n'ait pas été associé à des indices de fonction sexuelle physique, il existait une relation directe avec l'œstradiol dans IPW ($p <0,010$).

Conclusion : Le bisphénol- A était présent dans les groupes exposés et non exposés, mais n'était pas associé à la fonction sexuelle. Cependant, sa capacité de perturbation endocrinienne, en particulier dans le groupe exposé, est suggérée.

Mots - clés : *Bisphénol-A, produits chimiques perturbateurs du système endocrinien, hormones, industrialisation, dysfonctionnement sexuel.*

Introduction

Bisphenol-A is a multipurpose compound that is widely used in the modern industrial world [1]. It is a key monomer of plastic materials that are widely used in daily life; there is thus widespread human exposure [2]. It is utilized in the production of phenol resins, polycarbonates, polyacrilates, polyesters and lacquer coatings on food cans [3, 4].

Occupational exposure appears to be a potential source of endogenous bisphenol-A in the human system [5]. Essentially, all absorbed bisphenol-A is largely excreted unaltered in human urine and faeces as glucuronide and sulphur conjugates. Analysis of total urinary bisphenol-A has generally been used as a biomarker of exposure to Bisphenol-A in bio-monitoring studies [6].

An intact hypothalamo-pituitary-testicular axis is necessary in the initiation and maintenance of sexual function [7]. Bisphenol-A has been shown to disturb endogenous hormone signaling pathways causing various effects among which is hypogonadism [8]. The structure and function of Bisphenol-A and oestradiol are similar. Bisphenol A binds and activates the same oestrogen receptor as the natural hormone [9]. Oestrogen receptors are consistently present in abundance in efferent ducts of male reproductive tract but less so in other accessory organs. It is well established that efferent ducts reabsorb luminal fluids, ions and proteins as well as transport sperm [10]. Studies have shown that workers occupationally exposed to Bisphenol-A are more likely to have ejaculation difficulties, reduced sexual functions and sexual difficulties within one year of their employment [11, 12].

The effect of Bisphenol-A is through epigenetic modification, cytokine release and oxidative stress (OS) [13]. Oxidative stress occurs when there is an imbalance between pro-oxidants and ability of the antioxidants to scavenge excess ROS [14]. The role of OS in the pathophysiological mechanism of erectile dysfunction (ED) has been of concern [15]. Reactive oxygen species are cytotoxic agents causing oxidative damage by attacking cell membrane and deoxyribonucleic acid (DNA) [16]. Bisphenol-A has been reported to cause sperm DNA damage in exposed rodents. Chronic exposure to low-dose BPA induces DNA breaks and produces reactive oxygen species (ROS) in mice and rats [17, 18]. Bisphenol-A has been shown to generate ROS such as superoxide radical ($O_2^{\cdot-}$), which is injurious to the testis, epididymal sperm and other organs in rodents [19]. It has been demonstrated that two doses (25mg/kg and 10mg/kg) of Bisphenol-A given to adult male rats resulted in a significant decrease in nitric oxide (NO) levels after 6 and 10 weeks respectively [20].

The production of NO plays a central physiological role in erection. The endothelium is the primary source of NO [21]. The interaction between NO and superoxide radical ($O_2^{\cdot-}$) is one of the important mechanisms implicated in the pathophysiological process of ED [22]. Superoxide radical ($O_2^{\cdot-}$) interacts with NO reducing the bioavailability of NO and resulting in pyroxinitrite ($ONOO^-$) formation [23]. Blockade of nitric oxide synthetase and/or inactivation of superoxide dismutase (SOD) and increased basal superoxide radical production in penile arteries have been demonstrated [24, 23]. This suggests that the release of superoxide radical ($O_2^{\cdot-}$) is modulated by its interaction with endothelial-derived NO, probably by producing peroxynitrite that reduces the bioavailability of both radicals [25]. Plastics are ubiquitous in Ibadan and are used for water and food storage. Moreover, they are littered in the environment, thus contributing to pollution, which has poor regulations in Nigeria. Studies that demonstrate the association of Bisphenol–A with sexual function especially in those that are occupationally exposed in Ibadan are sparse. This study is aimed at evaluating sexual function in males occupationally exposed to Bisphenol-A in a plastic industry in Ibadan, Nigeria.

Materials and methods

Study design

This is a case-control study, conducted in a plastic industry in Ibadan, Nigeria. The study protocol was approved by the University College Hospital Ethical Review Committee. Written informed consent was obtained from the participants before enrolment.

Study population

A total of 80 apparently healthy males aged 18-62 years were enrolled into this study. The test group comprised of forty male employees of a plastic industry occupationally exposed to bisphenol-A (PIW). These were age matched with forty apparently healthy males with no occupational exposure to bisphenol-A (Controls). Both PIW and Controls had normal plasma levels of creatinine (0.12±0.0 mmol/L, 0.11±0.0 mmol/L) and urea (4.50±1.2 mmol/L, 3.84±1.0 mmol/L) respectively indicative of normal renal function [normal reference intervals: Creatinine=0.04-0.13 mmol/L, Urea=3.3-7.7mmol/L).

Sample collection

Table 1. Demographic characteristics and social habits of workers occupationally exposed to bisphenol A and the controls

Indices	PIW (n=40)	Controls (n=40)	t	X^2	p
+Age	37.1±11.2	33.6±9.4	1.466		0.147
+Parity	3.6±1.5	2.8±1.3	1.620		0.117
Marital status					
Single	14(35.0)	20(54.1)		2.83	0.073
Married	26(65.0)	16(45.9)			
Educationalstatus					
None	4(10.0)	1(2.9)		21.38	<0.001*
Primary	9(22.5)	1(2.9)			
Secondary	15(37.5)	4(11.4)			
Tertiary	12(30.0)	29(82.9)			
Social habits					
Smoking (Cigarette)					
No	34(85.0)	34(97.2)		4.149	0.126
Occationally	2(5.0)	1(2.8)			
Frequently	4(10.0)	0(0.0)			
Alcohol intake					
No	26(65.0)	21(56.8)		4.348	0.114
Ocasionally	11(27.5)	16(43.2)			
Frequently	3(7.5)	0(0.0)			

*values in mean (%), +=values in mean±SD, *=significant at p<0.05, X2= Chi Square, t= t-test, PIW= plastic industry workers, Controls= non-plastic industry workers. 36 participants of Controls responded to the questions as indicated above.*

Ten millilitres of venous blood was obtained aseptically by venepuncture from each participant. The tourniquet was applied 3-4 inches above the collection site. The puncture site (mainly the medial cubital vein in the antecubital fossa) was cleaned with 70% alcohol pad. The blood was obtained with a vacutainer plain tube. Serum was obtained by centrifugation at 500g for 5 minutes and separated into storage tubes in aliquots. 10 mLs of spot urine were collected into clean glass universal bottles, which were previously rinsed with acetonitrile (bisphenol-A free bottles). Both serum and urine samples were stored at -20°C until analysis.

Demography, social, reproductive indices and duration of exposure

Demography (age, educational status, occupation and marital status), social habits (smoking and alcohol consumption), reproductive history (parity, libido, normal erection during sex, early morning erection and frequency of erection) and duration of exposure to bisphenol A at work place were obtained from each participant using a semi-structured pretest questionnaire. *Anthropometric, blood pressure,* body weight, height, body mass index (BMI) waist circumference (WC), hip circumference (HC), waist hip ratio (WHR), waist height ratio (WHtR) and blood pressure (BP) [systolic (SBP) and diastolic (DBP)] were obtained from the participants by standard methods as described elsewhere [26].

Biochemical indices

Hormones (oestradiol and testosterone), bisphenol-A, SOD and NO were analyzed in PIW and Controls. Hormones were determined using enzyme linked immunosorbent assay (Bio-Inteco, UK) [27]. Bisphenol-A was determined by high performance liquid chromatography-tandem mass spectrometry (Altus HPLC, USA) [28]. SOD activity was determined by modified epinephrine assay method [29] while NO was determined using Griess reagent [30].

Functional indicator

Physical indicators of sexual function were sustained erection during sex, early morning erection, sexual satiety and libido. Hormones (oestradiol and testosterone) and OS indicators (SOD and NO) were biochemical factors of sexual function while bisphenol-A and duration of employment in the plastic industry were indicators of occupational exposure to bisphenol-A.

Statistical analysis

The statistical package for the social science (SPSS version 22.0) was used for statistical analysis. Individual parameters were expressed as mean ± SD.

Student's t-test, analysis of variance and Post hoc test were used for comparison of quantitative variables while Chi Square test was used for analysis of qualitative variables. Mann Whitney U test was used for comparing variables that were not normally distributed. Kruskal Wallis test was also used for the comparison of groups with small number of participants. Multiple regressions were used to find relationships. Data obtained were considered significant at $p<0.05$.

Results

Table 1 compares demographic characteristics and social habits of PIW with Controls. Only the association in educational qualification showed that PIW were less educated than the Controls ($p<0.05$).

Table 2 shows comparisons of anthropometric measures, blood pressure, biochemical indicators of sexual function and occupational exposure indicators in PIW and Controls. DBP, anthropometry (height, body weight, BMI, WC and HC) and Bisphenol-A were significantly higher in the controls compared with PIW ($p<0.05$). These measures in both groups were within the normal reference intervals.

Table 3 shows associations of physical indicators of sexual function in both PIW and controls. All physical indicators of sexual function (sustained erection during sex, early morning erection, sexual satiety and libido) in PIW showed no significant associations with controls ($p>0.05$). Similar observations were made in sex history (urinary tract infection, dysuria, inguinal hernia, and scrotal pain) in the association of PIW with Controls (Table 4).

Table 5 shows comparison of age, blood pressure, anthropometric measures, biochemical indicators and occupational exposure indicators in PIW based on their different operational areas. Significant differences were observed in age, duration of occupational exposure and NO across the groups in PIW ($p<0.05$). The drivers were the oldest among the groups. Workers in production department and Machine Operators had significantly greater NO levels than Store keepers, Engineers and

Table 2. Blood pressure, anthropometric measures, biochemical indicators of sexual function and occupational exposure indicators of workers occupationally exposed to bisphenol-A and the controls

Indices	PIW(n=40)	Controls(n=40)	t	p
Blood pressure				
SBP (mmHg)	115.3±15.4	114.3±11.4	0.298	0.766
DBP (mmHg)	74.3±7.8	78.7±7.9	-2.460	0.016*
Height (m^2)	1.7±0.1	1.7±0.1	-1.755	0.083
Body Weight (kg)	61.8±9.8	68.2±9.9	-2.835	0.006*
BMI (kg/m^2)	21.6±3.1	23.2±3.6	-2.046	0.044*
WC (cm)	77.1±8.0	81.2±9.7	-2.077	0.041*
HC (cm)	90.1±6.1	96.1±7.5	-3.850	<0.001*
WHR	0.9±0.1	0.9±0.1	0.597	0.552
WHtR	0.5±0.0	0.5±0.1	-1.374	0.174
Biochemical indicators of sf				
Hormones				
Oestradiol (nmol/L)	8.2±3.7	7.4±4.7	0.696	0.489
Testosterone(nmol/L)	23.1±8.0	23.7±7.6	-0.335	0.739
Oestradiol/Testosterone	3.5(2.6-4.6)	3.2(1.4-4.3)	-1.614^{+}	0.106
OS Indicators				
SOD (u/mL)	1.0±0.5	1.1±0.4	-1.355	0.179
NO (µmol/mL)	12.4(6.9-23.1)	9.5(6.9-14.3)	-1.737^{+}	0.082
OE indicators				
Duration in years	9.6±7.3			
BPA (ng/mL) 53.0(44.0-58.5)	56.5(52.3-62.5)	-2.163^{+}	0.031*	

values in mean±SD except those of Oestradiol/Testosterone, NO and BPA(which are in median with range in parentheses), *=significant at $p<0.05$, Z= Z value using mann whitney u test, DBP=diastolic blood pressure, SBP= systolic blood pressure, BMI=body mass index, WC=waist circumference, HC= hip circumference, WHR= waist hip ratio, WHtR=waist height ratio,SF=sexual function, OS Indicators= oxidative stress indicators, SOD= superoxide dismutase, NO= nitric oxide, BPA= bisphenol-A, PIW= plastic industry workers, Controls= non-plastic industry workers, OE= occupational exposure

Table 3. Physical Indicators of Sexual Function of Workers Occupationally Exposed to Bisphenol-A and the Controls

Indices	PIW (n= 40)	Controls (n= 40)	X^2	p
Sustained erection during sex				
Yes	35(89.7)	31(91.2)	0.43	0.578
No	4(10.3)	3(8.8)		
Early Morning Erection				
Yes	36(90.0)	35(100.0)	3.70	0.075
No	4(10.0)	0(0.0)		
Sexual Satiety				
Yes	36(92.3)	31(91.2)	0.31	0.595
No	3(7.7)	3(8.8)		
Libido				
High	31(77.5)	29(80.6)	0.11	0.483
Low	9(22.5)	7(19.4)		

Values in mean (%), X2=Chi Square, PIW= plastic industry workers, Controls= non-plastic industry workers.

Table 4. Sex history of workers occupationally exposed to bisphenol-A and the controls

Indices	PIW(n= 40)	Controls(n= 40)	X^2	p
UTI				
Yes	2(5.0)	1(2.8)	0.247	0.540
No	38(95.0)	35(97.2)		
Dysuria				
Yes	1(2.5)	0(0.0)	0.091	0.526
No	39(97.5)	36(100.0)		
Inguinal Hernia				
Yes	2(5.0)	0(0.0)	1.850	0.274
No	39(97.5)	36(100.0)		
Scrotal Pain				
Yes	1(2.5)	0(0.0)	0.912	0.526
No	39(97.5)	36(100.0)		

*Values in mean (%), *=significant at p<0.05, X2=Chi Square, UTI=urinary tract infection, PIW= plastic industry workers, Controls= non-plastic industry workers.*

Drivers. However, the Printers spent longer time than other workers in the plastic industry ($p<0.05$).

Table 6 shows comparison of age, blood pressure, anthropometric measures, biochemical indicators and bisphenol-A of Controls based on their occupation. Bisphenol-A levels and WHR showed significant differences across the groups ($p<0.05$). Civil Servants, Artisans and Laboratory Scientists had significantly increased WHR compared with Students and Doctors. However, Civil Servants and Doctors had significantly higher levels of BPA than Artisans, Students and Laboratory Scientists ($p<0.05$).

Table 7 shows multiple regression of BPA with blood pressure, anthropometric measures and biochemical indicators in workers occupationally exposed to BPA. Oestradiol had a positive and significant relationship with BPA in PIW only ($p<0.010$). Although all other parameters measured in PIW had no significant relationships with BPA, the overall relationship was significant (R^2 adj. =28%, F= 8.4, p= 0.010).

Table 8 shows multiple regression of BPA with blood pressure, anthropometric measures and biochemical indicators in the Controls. Systolic blood pressure and WC had a positive and significant relationship with BPA while DBP and WHtR had negative but significant relationship with BPA. Their overall relationship was not significant (R^2 adj. 21%, F= 1.5, p= 0.256). Sex hormones: testosterone and oestradiol as well as oestradiol-testosterone ratio did

Table 5. Age, Blood Pressure, Anthropometric Measures, Biochemical Indicators of Sexual Function and Duration of Occupational Exposure in Workers in Various Areas of Operations of the Plastic Industry

Indices	Printers (n= 9)	S. Keepers (n= 6)	Engineers (n= 6)	Drivers (n=2)	Producers (n=10)	M. Oper (n= 7)	p
Age	27.9±8.2[a]	31.7±7.5[a]	47.0±4.4[b,c]	52.5±10.6[c]	39.9±9.8[a]	40.1±12.0[b]	0.010*
Blood Pressure							
SBP(mmHg)	112.2±8.3[a]	115.0±10.5[a]	113.3±5.8[a]	145.0±21.2[b]	116.0±17.8[a]	110.0±20.8[a]	0.304
DBP(mmHg)	74.4±7.3[a]	73.3±5.2[a]	76.7±5.8[a]	85.0±7.1[a]	76.0±9.7[a]	68.6±6.9[a]	0.177
Anthropometric Meas.							
Height(m^2)	1.7±0.1[a]	1.8±0.0[a]	1.7±0.1[a]	1.7±0.0[a]	1.7±0.1[a]	1.6±0.1[a]	0.238
Body Weight(kg)	59.0±8.4[a]	59.3±8.9[a]	67.3±6.4[b]	65.5±27.6[a]	65.3±8.9[a]	55.9±7.7[a]	0.266
BMI(kg/m^2)	20.7±2.2[a]	19.3±2.4[a]	23.3±2.5[a]	23.0±8.6[a]	22.6±2.4[a]	20.9±2.2[a]	0.140
WC(cm)	72.9±3.7[a]	77.5±6.1[a]	81.3±8.1[a]	90.5±23.3[b]	78.6±6.8[a]	72.9±6.1[a]	0.086
HC(cm)	87.9±4.6[a]	89.7±4.6[a]	96.3±3.8[b]	91.5±13.4[a]	90.8±6.5[a]	88.4±5.3[a]	0.406
WHR	0.8±0.0[a]	0.9±0.0[a]	0.8±0.1[a]	1.0±0.1[a]	0.9±0.1[a]	0.8±0.1[a]	0.144
WHtR	0.4±0.0[a]	0.4±0.0[a]	0.5±0.0[a]	0.5±0.1[a]	0.5±0.0[a]	0.5±0.0[a]	0.319
Biochemical Indicators							
Hormones							
Oestradiol (nmol/l)	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]	0.1±0.1[a]	0.358
Testosterone (nmol/l)	26.1±7.6[b]	23.9±8.9[a]	19.3±5.4[a]	16.2±5.1[a]	20.8±6.7[a]	27.5±10.2[b]	0.840
Oestradiol/ Testosterone	0.004±0.0[a]	0.003±0.0[a]	0.004±0.0[a]	0.005±0.0[a]	0.004±0.0[a]	0.003±0.0[a]	0.159
OS Indicators							
SOD	1.0±0.4[a]	1.0±0.5[a]	0.6±0.36[a]	0.7±0.7[a]	1.2±0.4[a]	0.9±0.6[a]	0.525
NO(µmol/L)	16.6±6.1[b]	12.6±1.7[a]	11.4±1.8[a]	13.8±2.7[a]	17.3±2.8[b]	16.1±4.7[b]	0.025*
OE Indicators							
Number of Hrs/day	9.0±0.0[c]	8.7±0.5[b]	8.7±0.6[b]	8.5±0.7[b]	8.0±0.0[a]	8.0±0.0[a]	<0.001*
Number of Years	8.9±5.8[a]	6.8±5.9[a]	6.5±4.8[a]	15.0±7.2[b]	13.3±6.3[b]	9.5±3.5[a]	0.325
BPA(ng/mL)	52.8±14.8[b]	49.7±17.6[a]	34.7±21.3[a]	39.5±31.8[a]	49.7±20.5[a]	43.0±20.5[a]	0.395

*Values in mean±standard deviation, n= number of participants, P=probability value, *=significant at p<0.05, M.Oper= Machines operators, S. Keepers= store keepers, OE Indicators= occupational exposure indicators, Number of hrs/day- number of hours per day, OS Indicators= oxidative stress indicators, SOD= superoxide dismutase, NO= nitric oxide, BPA= bisphenol-A, SBP= systolic blood pressure, DBP= diastolic blood pressure, Meas.= measures, WC= waist circumference, HC= height circumference, BMI= body mass index, WHR= weight hip ratio, WHtR= waist height ratio. Means with same letter are not significant. Kruskal Wallis test was used for comparisons.*

not show significant relationship with BPA in controls ($p < 0.05$)

Discussion

Industrialization has led to massive introduction of chemicals and their products into the environment. Among such chemical compounds are endocrine disrupting chemicals such as bisphenol-A. There is widespread human exposure to bisphenol-A because it is a multipurpose compound in the modern industrial world [1, 2]. Its abundance in various house hold products has raised a serious public health concern [31]. This is corroborated in this study as detectable levels of bisphenol-A were observed in both PIW and Controls. Occupational exposure to BPA containing substances has previously been reported in Enugu state, Nigeria [5].

Surprisingly, higher levels of bisphenol-A were observed in the controls than the occupationally exposed group (PIW). Ninety eight percent of men from the general population had been shown with detectable urinary levels of BPA [32].

Although the number of participants was small, comparison of individuals in various occupational groups among the Controls showed doctors and civil servants with the highest levels of BPA (75.3 and 65.5 ng/mL respectively). The reason

Table 6. Age, blood pressure, anthropometric measures, biochemical indicators of sexual function and bisphenol-A of control according to their occupation

Indices	C.Servants (n = 8)	Artisans (n = 7)	Students (n = 11)	Lab.Scientists (n = 9)	Doctors (N = 5)	p
Age	31.0±2.7[a]	43.4±17.6[b]	31.00±4.3[a]	32.9±4.9[a]	29.3±1.5[a]	0.134
Blood pressure						
SBP(mmHg)	118.6±12.2[a]	115.7±16.2[a]	115.0±10.9[a]	107.1±7.6[a]	115.0±5.8[a]	0.263
DBP(mmHg)	81.4±9.0[a]	80.0±11.5[a]	78.3±7.2[a]	77.1±4.9[a]	75.0±5.8[a]	0.583
Anthrop.measures						
Height(m^2)	1.7±0.0[a]	1.8±0.1[a]	1.7±0.1[a]	1.7±0.0[a]	1.7±0.0[a]	0.105
Body Weight(kg)	68.4±13.6[a]	64.1±4.4[a]	66.3±11.5[a]	70.9±7.3[a]	75.8±4.5[a]	0.176
BMI(kg/m^2)	23.2±4.9[a]	20.6±2.5[a]	22.9±3.3[a]	24.9±3.2[a]	25.4±1.5[a]	0.070
WC(cm)	82.9±10.6[a]	84.1±8.9[a]	76.4±7.2[a]	88.1±9.8[a]	75.8±9.6[a]	0.086
HC(cm)	94.4±9.4[a]	95.9±5.2[a]	96.6±8.7[a]	97.9±7.1[a]	94.5±6.2[a]	0.844
WHR	0.9±0.1[b]	0.9±0.1[b]	0.8±0.1[a]	0.9±0.1[b]	0.8±0.1[a]	0.005*
WHtR	0.5±0.1[a]	0.5±0.1[a]	0.5±0.0[a]	0.5±0.1[a]	0.4±0.1[a]	0.149
Biochem. indicators						
Hormones						
Oestradiol (nmol/l)	0.07±0.0[a]	0.11±0.1[b]	0.07±0.1[a]	0.08±0.0[a]	0.03±0.0[a]	0.858
Testosterone (nmol/l)	19.9±6.0[a]	25.6±8.3[b]	23.3±9.5[a]	25.3±7.3[b]	22.4±5.4[a]	0.180
Oestradiol/Testosterone	0.004±0.0[b]	0.004±0.0[b]	0.003±0.0[b]	0.003±0.0[b]	0.001±0.0[a]	0.167
Oxidative stress indicators						
SOD (u/mL)	0.8±0.4[a]	1.1±0.2[a]	1.2±0.4[a]	1.3±0.4[a]	1.2±0.5[a]	0.314
NO(µmol/mL)	11.9±10.2[b]	8.5±4.7[b]	12.4±6.2[b,c]	9.4±6.7[b]	5.8±3.6[a]	0.468
O. Exposure indicators						
BPA(ng/mL)	65.5±8.1[b]	54.6±10.5[a]	53.3±3.4[a]	54.0±7.2[a]	75.3±24.0[c]	0.006*

*Values in mean±standard deviation, *=significant at p<0.05, n= number of participants, P= probability value, SBP= systolic blood pressure, DBP= diastolic blood pressure, Anthrop. Measures= anthropometric measures, WC= waist circumference, HC= hip circumference, WHR= waist hip ratio, WHtR= waist height ratio, Biochem.Indicators= biochemical indicators, SOD= superoxide dismutase, C.Sercants=Civil servants, NO= nitric oxide, O.Exposure Indicators= occupational exposure indicator, BPA= bisphenol-A. Means with same letter are not significant. Kruskal Wallis test was used for comparisons..*

for this is not clear. However, significantly raised BPA levels have been attributed to the free consumption of water packed in light polythene bags and stored under high tropical heat for long period as well as canned and micro waved food [5]. Food products are the major sources of BPA exposure, which in an order of magnitude are more than other routes [33]. Heating cans during sterilization or food preparation can cause leaching of BPA into the can content from epoxy coating of the can wall or from plastic packs of food thereby increasing the potentials of dietary BPA exposure [34]. It has been observed that BPA released into the environment led to increased pollution and contamination of the soil and ground water [35].

Bisphenol-A is a lipophylic compound, thus, it accumulates in the lipid-rich components of the body such as membranes [36]. Adiposity measures (weight, BMI, WC and HC) are consistent with sedentary life style [37]. These were significantly higher in the Controls compared with PIW. It is possible that the increased bisphenol-A levels in the controls are related to the increased adiposity observed as WC and SBP showed positive association with bisphenol-A in this group. Diastolic blood pressure was also significantly increased in controls compared with PIW. The relationship between increased adiposity and increased blood pressure is known [26]. However, WHtR (also an adiposity measure) and DBP showed negative association with bisphenol-A in Controls.

Although this observation may relate to metabolic alteration resulting from regional fat deposition [38], the association of bisphenol-A with different measures of abdominal fat-WC and WHtR is not clear. Waist-to-height and WC are proxy measures of abdominal fat and were increased in obese and hypertensive traders compared with normal weight and normotensive traders respectively in our previous studies. Moreover, WHtR, which is a simple and practical index for assessing central fat distribution and metabolic risk in men and women, is an improved index over

Table 7. Multiple regression of bispenol-A with blood pressure, anthropometric measures and biochemical indicators in of workers occupationally exposed to bisphenol-A

Dependent Variable	Predictor	Beta	t	p
BPA	*Blood Pressure*			
R^2Adj.=28%	SBP (mmHg)	-0.071	-0.346	0.734
F=8.4	DBP (mmHg)	-0.300	-1.475	0.159
P=0.010	*Anthropometric Measures*			
	Height	-0.264	-1.334	0.200
	Weight (kg)	-0.007	-0.036	0.972
	BMI (kg/m^2)	0.568	0.140	0.999
	HC(cm)	0.115	0.582	0.568
	WC (cm)	0.117	0.588	0.564
	WHR	0.083	0.408	0.688
	WHtR	0.179	0.905	0.378
	Biochemical Indicators			
	Hormones			
	Oestradiol (nmol/l)	0.225	0.922	0.010*
	Testosterone (nmol/l)	2.770	0.127	0.669
	Oestradiol/Testosterone	0.081	-7.260	0.580
	Oxidative Stress Indicators			
	SOD (u/mL)	0.077	0.355	0.727
	NO (µmol/mL)	0.227	1.173	0.257

**= Significant at p < 0.05, Beta= regression coefficient, SBP= systolic blood pressure, DBP= diastolic blood pressure, BPA= bisphenol-A, BMI= body mass index, WC= waist circumference, HC= hip circumference, WHR= waist hip ratio, WHtR= waist height ratio, SOD= superoxide dismutase, NO= nitric oxide, number of participants=40*

Table 8. Multiple regression of sispenol-A with blood pressure, anthropometric measures and biochemical indicators in the controls

Dependent Variable	Predictor	Beta	T	p
BPA	*Blood pressure*			
R^2 Adj.=21%	SBP (mmHg)	0.722	2.255	0.044*
F=1.5	DBP (mmHg)	-0.948	-2.427	0.032*
P=0.256	*Anthropometric measures*			
	Height(m^2)	-2.036	-0.971	0.351
	Weight (kg)	-9.149	-1.551	0.147
	BMI (kg/m^2)	10.807	1.619	0.131
	HC (cm)	-2.113	-0.907	0.382
	WC (cm)	26.833	2.214	0.047*
	WHR	-1.960	-0.730	0.479
	WHtR	-25.58	-2.245	0.044*
	Biochemical indicators			
	Hormones			
	Oestradiol (nmol/l)	-0.168	-0.167	0.609
	Testosterone (nmol/l)	0.566	1.053	0.746
	Oestradiol/Testosterone	0.297	0.159	0.736
	Oxidative Stress Indicators			
	SOD (u/mL)	0.067	0.178	0.862
	NO (µmol/mL)	-0.010	-0.450	0.965

**= Significant at p < 0.05, Beta= regression coefficient, SBP= systolic blood pressure, DBP= diastolic blood pressure, BPA= bisphenol-A, BMI= body mass index, WC= waist circumference, HC=hip circumference, WHR= waist hip ratio, WHtR= waist height ratio, SOD= superoxide dismutase, NO= nitric oxide.*

WC [26, 38]. It is possible that both measures assess different regions of body fat.

Obesity is associated with elevated oestrogen in men. It is postulated that the enzyme, aromatase located in adipose tissue, converts androgens to oestrogens. Though currently controversial, oestrogen has long been associated with cardioprotection. Oestrogen is thought to prevent the development of atherosclerosis through favourable effects on an intact endothelium and direct protective effect against ischemia/reperfusion [38]. Thus Bisphenol A, which is similar in structure to oestrogen, may mimic its function, which probably explains the inverse correlation between Bisphenol-A and DPB observed in this study.

A higher bisphenol-A level in tissue samples from placenta, which has a modestly higher fat content than in serum samples from the same women has been reported [39]. Another report demonstrated that perinatal and postnatal exposure to bisphenol-A increased body tissue mass and serum total cholesterol and triglyceride levels [40]. Additionally, bisphenol-A at environmentally minimal doses inhibits the release of adiponectin that protects humans from the metabolic syndrome [41]. Bisphenol-A, however, showed no relationship with hormones in the Controls. Reports had shown no correlation between BPA exposure and testosterone levels in two cross-sectional studies of 167 and 302 fertile men [42]. Contrarily, in this present study, the PIW group with lower levels of bisphenol-A, oestradiol had a positive correlation with bisphenol-A suggesting the endocrine disrupting ability of bisphenol-A in this group. This is in tandem with the previous reports that demonstrated the association of urinary bisphenol-A concentration with serum oestradiol levels [32, 43].

Different pathophysiologic mechanisms in this respect have been postulated, firstly, bisphenol-A and oestrogens have similar structure [44]. In vitro studies have shown that bisphenol-A can interact with oestrogen receptors, ERα and ERβ, as a partial agonist and, in the presence of oestradiol, exhibit competitive inhibition by blocking the binding of the more potent natural oestrogen to the ER [32]. This action disrupts the normal activity of oestrogens [45]. Secondly, the mechanism of the fertility disrupting potential of bisphenol-A in men seems to be due to its oestrogenic activity in the hypothalamus, which in turn disrupts the proper function of the gonadotropin releasing hormone (GnRH) pulse generator. Oestrogen sends a signal to the pituitary to reduce the production of gonadotropins (negative feedback mechanism) [46]. Thirdly, aromatase (CYP19) catalyzes conversion of androgens (testosterone) to oestrogens [47, 48] and bisphenol-A exposure increases its expression as reported in animal studies [49, 50]. Additionally, bisphenol-A is a xenoestrogen that binds to sex hormone binding globulin (SHBG), with a reversible and competitive binding activity for both testosterone and oestradiol. This action produces a dose-dependent increase in concentrations of human SHBG-unbound testosterone and/or oestradiol. Bisphenol-A may thus displace endogenous sex steroid hormones from human SHBG binding sites and disrupt the androgen-to-oestrogen balance that is required for normal spermatogenesis [51, 47]. However, no relationship was observed between duration of occupational exposure and bisphenol-A levels/ hormones.

Acetylcholine is a neural transmitter that motor neurons of the nervous system releases in order to activate muscles. It plays important role in arousal [52]. It is released from carvanosal nerve endings and stimulates the neuronal NOS enzyme which leads to release of NO from the endothelium. NO plays important role in male sexual function. Nitric oxide in this study was not significantly increased in PIW compared with the Controls. However, no significant differences were observed in the physical indicators of sexual function between the PIW and Controls. These observations do not support the demonstration of an inverse relationship between bisphenol-A and NO in rats [53]. Moreover, no significant association was observed between bisphenol-A and SOD as well as reproductive history in and between PIW and Controls. These findings also contrast the hypothesis that the interaction between NO and superoxide radical is one of the important mechanisms implicated in the pathophysiological process of ED especially in males exposed to BPA [22]. These postulates suggested that the release of superoxide radical ($O2^{\cdot}>$) is modulated by its interaction with endothelial-derived NO, probably by producing peroxynitrite, which reduces the bioavailability of both radicals in the sustenance of penile erection [25].

Conclusion

The general population may be exposed to Bisphenol-A. Although bisphenol-A had a positive

relationship with oestradiol probably indicative of its endocrine disrupting activity, it had no relationship with the physical indicators of sexual function or duration of occupational exposure in the exposed group.

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Effect of trivalent chromium (Cr_2O_3) on stomach morphometry and some vital organs in male wistar rats

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Abstract

Background: Trivalent chromium (Cr_2O_3) is required in trace amount and has health benefits. Its deficiency is linked to symptoms associated with endocrine and cardiovascular diseases. Its essentiality and presumed functions in body system is poorly understood. This study evaluated the effects of Cr_2O_3 on gross morphology of the stomach, liver, kidneys and brain of rats.

Methodology: Eighteen male Wistar rats (91.1 ± 4.2 g, 7 weeks old) were equally assigned to three groups: group 1 (control) received drinking water while groups 2 and 3 received 10 and 100 ppm Cr_2O_3 respectively for 12 weeks through drinking water. Animals were weighed weekly, sacrificed after 12 weeks and blood chromium concentration was determined and full blood count estimated. The stomach, colon, liver, kidney and brain were excised and weighed. Stomach was assessed for gross, histology and histomorphometry alterations. Liver, kidney and brain histology were also evaluated using standard methods.

Results: Blood chromium level was significantly higher in the group treated with 10 ppm Cr_2O_3 (0.17 ± 0.01ppm); 100 ppm (0.19 ± 0.01ppm) compared with control (0.11 ± 0.02 ppm). Platelet count was significantly lower in control (72.3 ± 3.1 x 10^3/µL) compared to 10 ppm (107.7 ± 3.7 10^3/µL) and 100 ppm (101.3 ± 4.4 x 10^3/µL). The stomach mucosa width was significantly high in group treated with 10 ppm (7097±130 µm) and 100 ppm (7306 ± 632 µm) compared with control (4623 ± 247 µm). Brain histology revealed few deranged cells in the chromium treated groups.

Conclusion: This study underscores possible stomach and few derangements in the brain cells from trivalent chromium treatment.

Keywords: *Trivalent chromium, Stomach, Brain, Histomorphometry, Rats*

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Résumé

Contexte : Le chrome trivalent (Cr_2O_3) est requis en quantité minime et présente des avantages pour la santé. Sa carence est liée aux symptômes associés aux maladies endocriniennes et cardiovasculaires. Son caractère essentiel et ses fonctions présumées dans le système corporel sont mal compris. Cette étude évalue les effets du Cr_2O_3 sur la morphologie globale de l'estomac, du foie, des reins et du cerveau des rats.

Méthodologie : Dix-huit rats Wistar mâles (91,1 ± 4,2 g, âgés de 7 semaines) ont été également répartis dans trois groupes : le groupe 1 (témoin) a reçu de l'eau de boisson, tandis que les groupes 2 et 3 ont reçu respectivement par l'eau de boisson 10 et 100 ppm de Cr_2O_3 pendant 12 semaines. Les animaux ont été pesés chaque semaine, sacrifiés après 12 semaines et la concentration de chrome dans le sang a été déterminée et la formule sanguine complète estimée. L'estomac, le côlon, le foie, les reins et le cerveau ont été excisés et pesés. L'estomac a été évalué pour les altérations macroscopiques, histologiques et histomorphométriques. Les histologies du foie, des reins et du cerveau ont également été évaluées à l'aide de méthodes standard.

Résultats : Le taux de chrome sanguin était significativement plus élevé dans le groupe traité avec 10 ppm de Cr_2O_3 (0,17 ± 0,01 ppm). 100 ppm (0,19 ± 0,01 ppm) par rapport au contrôle (0,11 ± 0,02 ppm). La numération plaquettaire était significativement plus faible chez les témoins (72,3 ± 3,1 x 10^3 / µL) par rapport aux groupes à 10 ppm (107,7 ± 3,7 x 10^3 / µL) et à 100 ppm (101,3 ± 4,4 x 10^3/ µL). La largeur de la muqueuse gastrique était significativement élevée dans le groupe traité avec 10 ppm (7097 ± 130 µm) et 100 ppm (7306 ± 632 µm) par rapport au groupe contrôle (4623 ± 247 µm). L'histologie cérébrale a révélé peu de cellules perturbées dans les groupes traités au chrome .

Conclusion : Cette étude met en évidence un possible dérangement de l'estomac et peu de dérangements dans les cellules du cerveau à la suite d'un traitement au chrome trivalent.

Mots - clés : *Chrome trivalent, Estomac, Cerveau, Histomorphométrie , Rats*

Introduction

A number of trace elements and heavy metals have direct effect on gastrointestinal tract when ingested

and /or indirect impact on other body organs, thereby presenting health challenge and health in general. The heavy metals which are majorly metalloids could induce toxicity at minimal exposure dose [1]. Human exposure to these metals is on exponential increase particularly due to increasing environmental pollution from industrial, domestic and technological activities [2, 3], especially in developing world.

`Chromium, a trace element and heavy metal (transitional metal) occurs naturally in two main forms either as trivalent or hexavalent chromium [4]. The trivalent form is the focus of this study and it is available food substances such as meat and vegetables among others [5]. Trivalent chromium is an essential nutrient in both animals and human, and it plays important role in glucose, fat and protein metabolism by potentiating the action of insulin [6] whereas; hexavalent chromium exposure in humans is reportedly toxic to the exposed tissues [7, 8]. Over 300,000 workers are estimated to be exposed annually to hexavalent chromium especially in places of work [6]. Non-occupational exposure occurs through food and drinking water whereas, occupational exposure occurs majorly through inhalation [9].

The increase in consumption of trivalent chromium supplement [10] has been reported with dearth of information on its effects on the stomach majorly. The inadequate information has created vital gap in the knowledge base for possible interaction between the stomach and chromium. In this study, we evaluated the effect of chromium consumption on gross morphology of the stomach, liver, kidneys and brain of rats.

Materials and methods

Chemicals and drugs

Trivalent chromium ($Cr_2O_{3)}$ (analytical grade) was obtained from Koshin Chemicals, Japan.

Animals and treatment protocol.

Eighteen male Wistar rats (91.1 ± 4.2 g, 7 weeks old) were purchased from the Preclinical Animal House, College of Medicine, University of Ibadan and were used for the experiments. Rats were acclimatized under standard laboratory conditions, fed with Ladokun® feeds with free access to water. They were grouped into 3: group 1, control (n=6) were allowed free access to clean drinking water and groups 2 and 3, were administered oral 10 ppm, (n=6) and 100 ppm (n=6) Cr_2O_3 respectively through their drinking water for 12 weeks. The study was carried out in conformation to the Guidelines of the National Institute of Health - *Guide for the Care and Use of Laboratory Animals [11].*

Termination of the experiment and harvest of organs

The stomach and metabolic organs; liver, colon and kidneys were removed and weighed following laparotomy under cocktail of both xylazine (0.0005 ml/g b.wt) and ketamine (0.015 ml/g b.wt) anaesthesia.

Determination of blood chromium

After 12 weeks of oral exposure to Cr_2O_3, 2 ml of blood was collected from retro-orbital sinus of rats and was quickly transferred into a test tube and subsequently digested with 2 ml of nitric acid (HNO_3) overnight. The digested blood was placed in water-bath and heated for 30 min at 100ºC and allowed to cool to room temperature. The sample was made up to 15 ml with addition of 12 ml distilled water and was filtered. The chromium concentration of the filtrate was now read at 530 nm using flame atomic absorption spectrophotometer (FAAS).

Haematological Analysis

Blood samples were obtained from all animals through retro-orbital sinus for full blood count [12].

Histological and Histomorphometrical Evaluation

Ketamine (100 mg/kg) was injected intraperitoneally and cervical dislocation done to sacrifice the animals, followed by harvesting of the tissues. Sections of stomach, liver, and kidney were fixed in 10% buffered neutral formalin. The tissues were processed and embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (H&E) according to the method described by Bancroft and Gamble [13]. Accuscope TS view, China was used to capture images and to evaluate morphological changes and stomach histomorphometry. From each section of the stomach, ten fields per mucosa were randomly selected and measured using image J in each slide per field and the average of the fields was determined[14]. Slides from the rat brain were examined under Leica DM 500 digital light microscope (Germany) and images captured with Leica ICC50 E digital camera (Germany) using an objective lens (x 40) and an ocular lens (x 10).

Statistical analysis

Results were expressed as Mean ± SEM and one-way ANOVA with Newman-Keuls comparison *post hoc* test was adopted using GraphPad Prism version 5.0 for Windows (GraphPad software Inc., San Diego, CA), $p<0.05$ was considered significant.

Results

Effect of trivalent chromium on body weight, relative organ weights and blood chromium level.

Figures 1, 2 and 3 describe the findings on percentage body weight, relative organ weight and blood chromium concentration after period of exposure to chromium respectively. There was no significant difference in the percentage body weight of chromium treated groups compared with the control. The relative organ weights of the two chromium groups also were significantly higher compared with control. However, the blood chromium level increased significantly ($p<0.05$) in the chromium treated groups, 10 ppm (0.17 ± 0.01ppm); 100 ppm (0.19 ± 0.01ppm) compared with control (0.11 ±0.02 ppm).

Effect of trivalent chromium on haematological variables.

The result was significant in all the variables measured except for the platelet counts that was significantly lower, $p<0.05$ in control (72.3 ± 3.1 x $10^3/\mu L$) compared to 10 ppm (107.7 ± 3.7 $10^3/\mu L$) and 100 ppm (101.3 ± 4.4 x $10^3/\mu L$) (Table 1) respectively.

Effects of trivalent chromium on stomach gross morphology and histology.

There were no disruptions in the stomach mucosa integrity in the chromium treated rats compared with control at both gross and histology levels (Tables 2). However, there was significant increase ($p<0.05$)

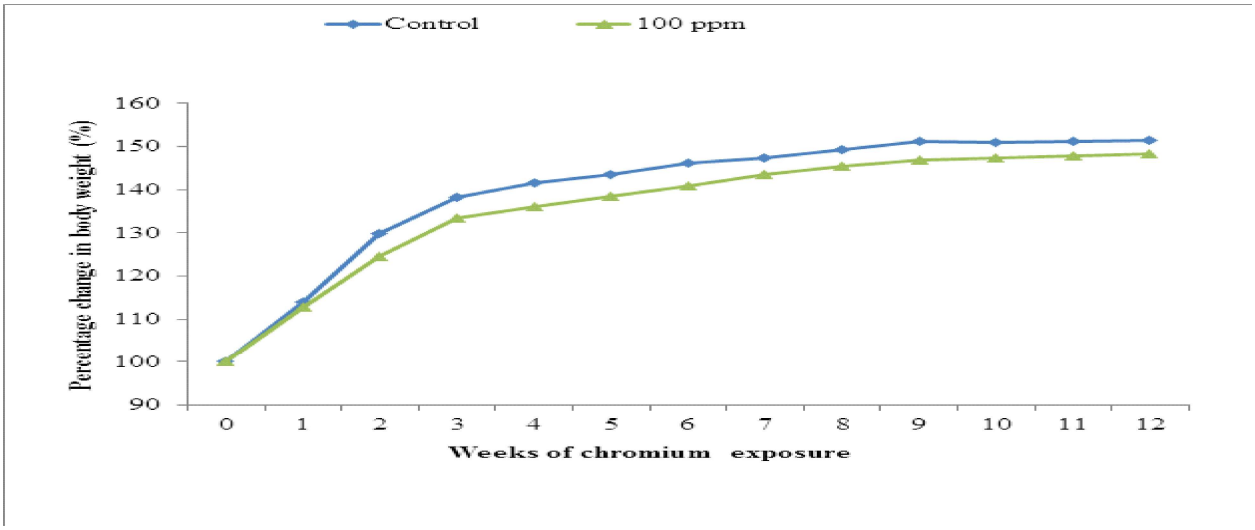


Fig. 1: Percentage body weight change following 12 weeks of chromium exposure.
No significant difference in chromium treated groups compared with control percentage body weight differences.

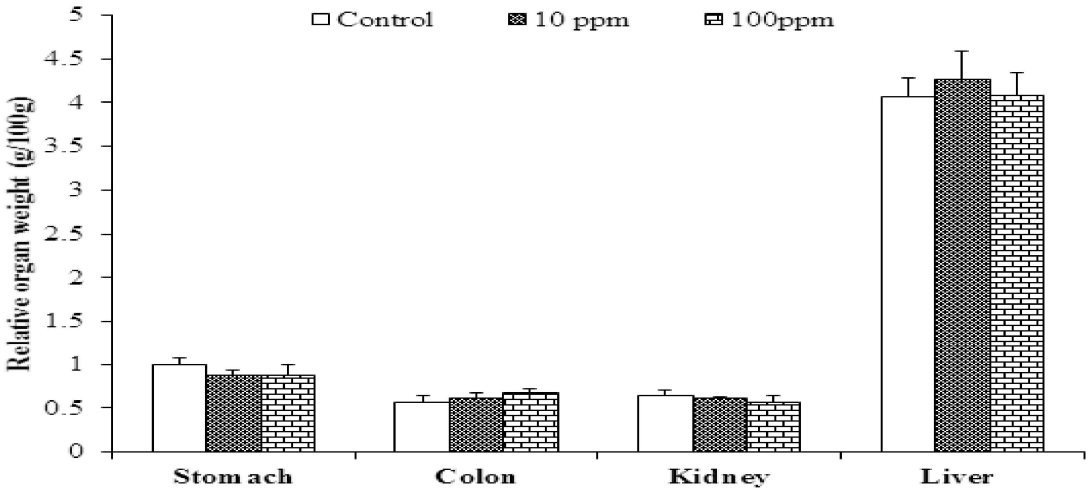


Fig. 2: Mean relative organ weights following 12 weeks of chromium exposure. No significant difference in test groups compared with control in organ weights

Table 1: Effects of chromium exposure on some haematological variables

	Control	10 ppm	100 ppm
Packed Cell Volume (%)	41.1 ±1.2	41.7±1.3	41.9±0.7
Haemoglobin Conc. (g/dL)	13.5 ±1.3	12.3±0.9	13.1±0.7
Red Blood Cell count(x 10^6/µL)	7.1 ± 0.3	7.4±0.3	7.0±0.1
White Blood Cell count(x 10^3/µL)	2.2 ± 0.3	2.5±0.2	2.6±0.3
Platelets Count (x 10^3/µL)	72.3 ± 3.1	107.7±3.7+	101.3±4.4+
Lymphocyte Count (%)	64.2 ± 1.3	62.3±9.7	63.0±2.2
Neutrophil count (%)	30.8 ± 2.1	32.33±5.3	33.3±3.1
Monocyte (%)	0.3 ± 0.1	2.1±1.17+	2.0±0.7+
Eosinophil (%)	2.0 ± 0.3	2.0±1.0	2.0±0.3

+ Significantly different at $p<0.05$ compared with control

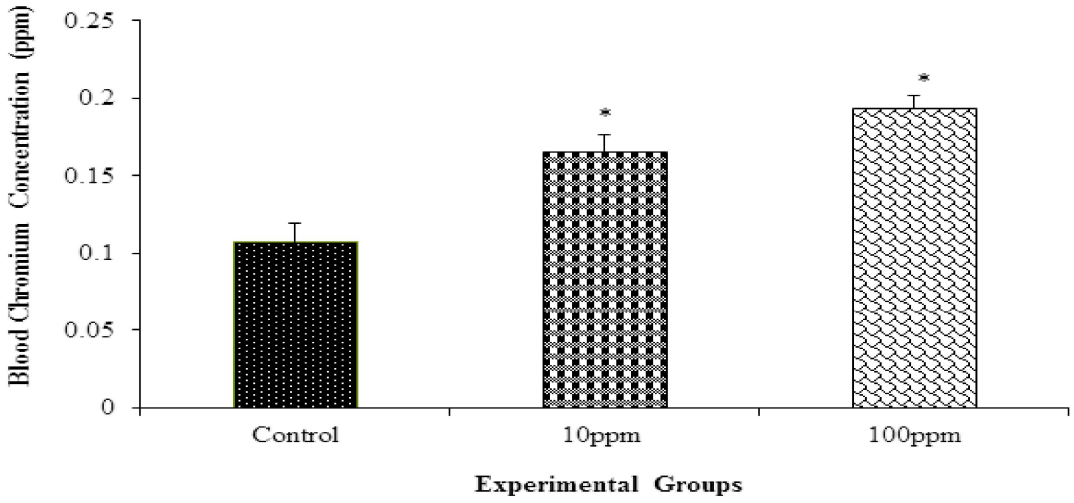


Fig. 3: Mean blood chromium concentration following 12 weeks of chromium exposure.
* Significant at $P<0.05$ compared with the control

in the stomach mucosa width of animals exposed to 10 ppm (7097 ± 130 µm) and 100 ppm (7306 ± 632 µm) chromium compared with control (4623 ± 247 µm).

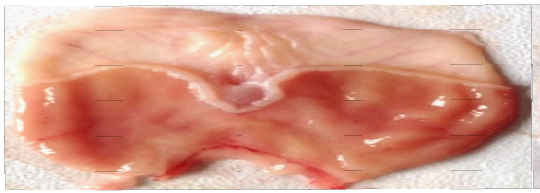
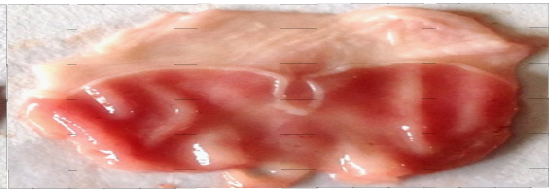
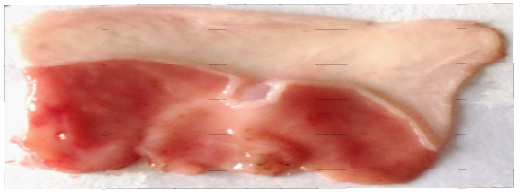
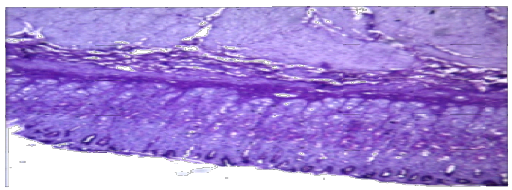
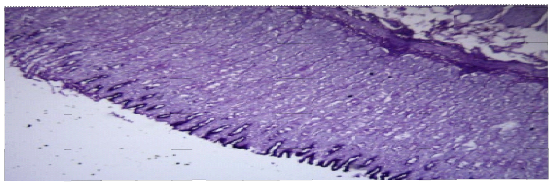
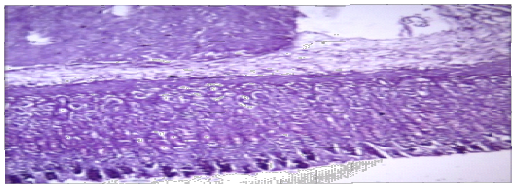
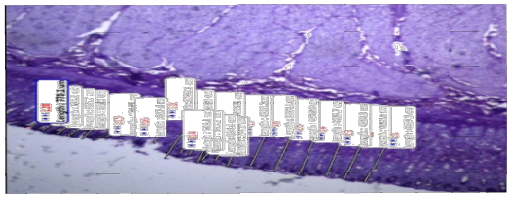
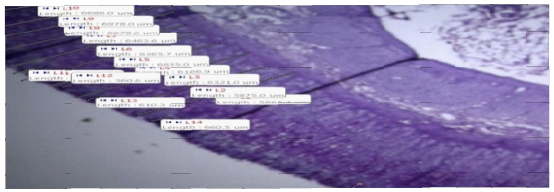
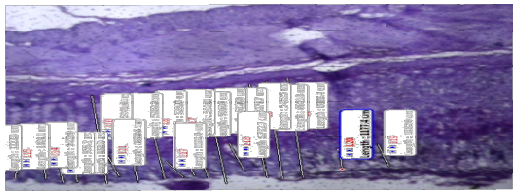
Effects of trivalent chromium on the histology of the liver, kidney and brain.

Liver (plate 1) and kidney (plate 2) histology shows no significant distortion in the chromium exposed groups compared with control. Plates 3, 4 and 5 display sections of cornu ammonis, dentate gyrus and cerebral cortex of the rats' brain respectively.. The three layers of the CA3 (stratum oriens, stratum pyramidalis, stratum radiatum) and the pyramidal neurons of the cornu ammonis 3 (CA3) of the hippocampal retained their normal architecture in all the groups. However, the granule neurons of the dentate gyrus were distorted in the chromium groups compared with the control. There were few pyknotic (dead) cells in the cerebral cortex region more in the chromium treated. Other brain areas examined were essentially normal as compared with control.

Discussion

Heavy metals and trace elements play significant roles in health and disease conditions of man [15]. The burden of these metals on cellular metabolism is enormous and could be harmful, while in some instances it might be vital for sustaining physiologic metabolic activities of cells and tissues. Excess or deficiencies of some heavy-trace metals have been implicated in some cancer burdens [16, 17]. However, trivalent chromium has no reported risk in the development or progression of gastric diseases from ingestion. This study was conducted to assess the impact of trivalent chromium on the structure of

Table 2: Effects of oral trivalent chromium on the stomach gross, histology and histomorphometry in rats.

	Control	10 ppm	100 ppm
Macroscopic			
Microscopic			
Histomorphometry			
Gross mucosa lesion scores(mm^2)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Histology scores	0.24 ± 0.11	0.28 ± 0.11	0.20 ± 0.09
Mucosa width (µm)	4623 ± 247.5	7097 ± 130**	7306 ±632**
Pit depth (µm)	893.9 ± 25.8	922.6 ± 29.2	036.1 ± 66.9

**Significant difference at $p<0.01$ compared with control (Mag. X 100). H & E stain

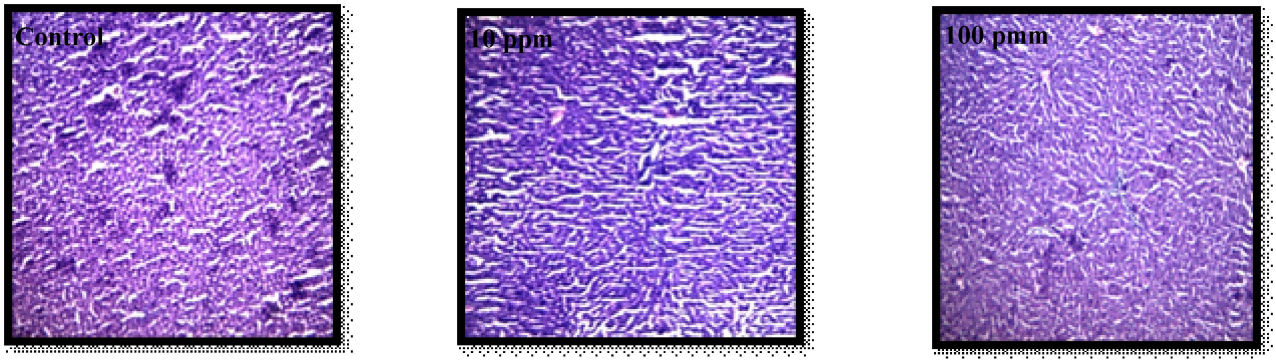


Plate 1: *Representative micrographs of transverse section of rats' liver (Mag. X100), after exposure to chromium. Tissue stained with H&E. No visible lesion is seen in the liver parenchyma in all groups.*

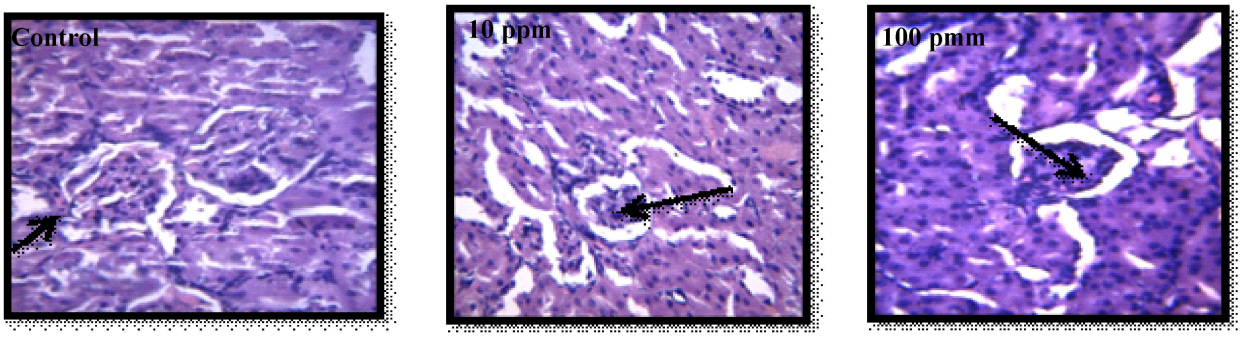


Plate 2: *Representative micrographs of the transverse section of kidney after trivalent chromium exposure (Mag. X 100). Tissue stained with H & E. There is no visible lesion seen in all groups, the glomerulus apparatus (black arrows) appeared normal in all groups.*

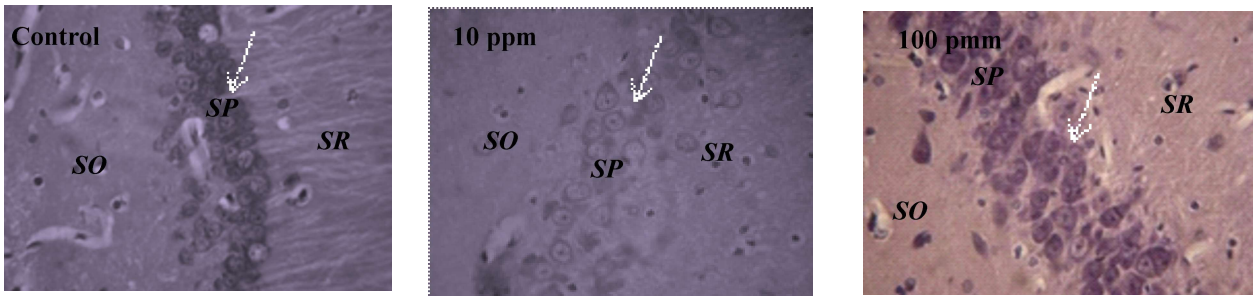


Plate 3: *Representative stained sections of the Cornu Ammonis 3 of hippocampus after oral chromium treatment for 12 weeks. The neurons appear normal in all groups (white arrows) – (SO- stratum oriens, SP- stratum pyramidalis, SR- stratum radiatum) (Mag. X 400).*

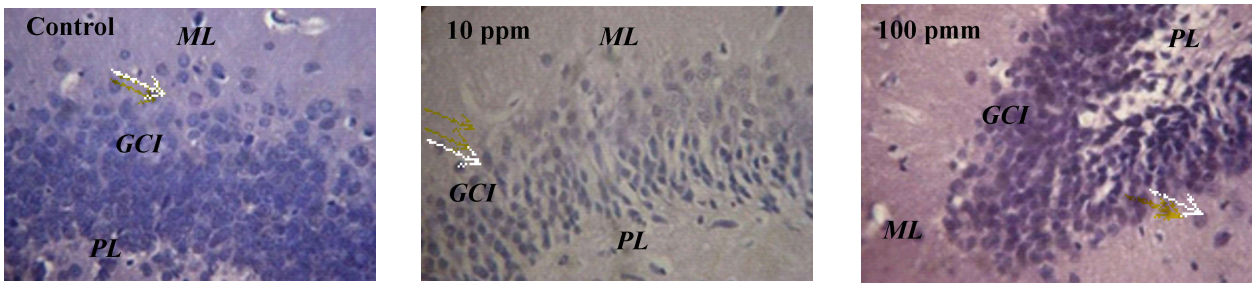


Plate 4: *Representative stained sections of the dentate gyrus of hippocampal formation of rats after oral chromium treatment for 12 weeks. The control and chromium treated hila cells appear normal with normal Purkinje cell staining. The granule cell layer (GCL) appears mildly distorted in the chromium groups. ML and PL represent the molecular layer and polymorphic layer (Mag. X 400).*

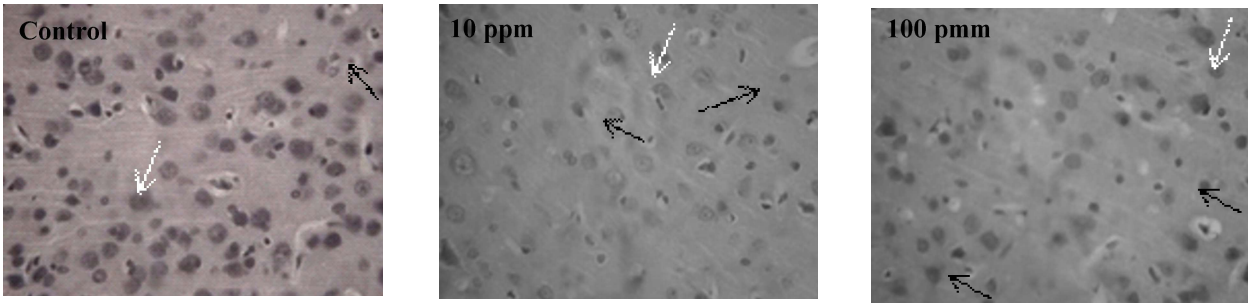


Plate 5: Representative stained sections of the cerebral cortex of rats after oral chromium treatment for 12 weeks. The neuronal cells in control and chromium groups appear essentially normal (white arrows), except for a few pyknotic cells across the groups but appear more in the test groups (black arrow) (Mag. X 400)

the stomach, brain, colon, liver and kidney, in view of the recent increase in its consumption rate [10].

Findings from the present study revealed no significant changes in body weight throughout the period of trivalent chromium administration. The result is in agreement with previous findings from Bunting *et al.,* [18] as well as Mathison and Engstrom [19] and Swanson *et al*., [20] who found no positive effect of chromium on body weight. However, Chang and Mowat [21], Moonsie-Shageer and Mowat [22] and Kegley *et al*., [23] reported a gain in weight. Pittler *et al.,* [24] from their studies agreed to loss of body weight following chromium supplementation.

Earlier reports where studies were conducted for periods beyond twelve months of chromium administration, reported no change in the relative organ weights following period of chromium treatments when compared with control [19, 20]. Chromium supplements have been claimed to reduce body fat and increase lean (muscle) mass [25]. A review of 24 studies that examined the effects of 200 to 1,000 mcg/day of chromium (in the form of chromium picolinate) on body composition reported no significant benefits on body weight [25]. In another clinical trial, findings show insignificant change in body weight after chromium supplementation [24].

The blood chromium level is similar with the findings of Anderson and Kozlovsky [26] who reported a significant increase in blood chromium after supplementation for three months. The haematological indices did not change significantly except for the increased platelet count in chromium exposed groups. Increased platelets protect the mucosa against anti-platelets agents. Platelets contain many agents that help in promoting tissue growth and neovascularization including vascular endothelial growth factor (VEGF), transforming growth factor-b and platelet factor-4 [27, 28]. The increased platelet count reported could be an important factor constituting protection to the stomach mucosa of rats in chromium groups.

Normally, gastric mucosa and other mucosae are constantly regenerated with continuous mucosal cell proliferation, and this also depends on the degree of assaults involved. Mucosa of the GI tract is maintained by ongoing cell renewal and any deviation from the replicative processes might compromise both the structural and functional integrity [29]. The observed increase in mucosa width of the chromium group could be as a result of reaction to the chromium water over time and might be a protective factor for the stomach mucosa. This may be a coping mechanism which could permit the gastric mucosa to withstand frequent exposure to damaging factors or agents.

The brain tissues evaluated appeared essentially normal in all the groups but for few necrotic neurons which was expressed more in the chromium groups. Krikorian *et al*., [30] reported a beneficial effect on cognitive functions of laboratory rats following supplementation with trivalent chromium. It was also reported that chromium supplementation attenuated post-stroke infarction in rats [31].

This present study and findings underscore possible stomach tissue toxicities from trivalent chromium administration to rats. The increase in mucosa architecture in the stomach tissue might suggest cyto-protection to the mucosa integrity when exposed to oral trivalent chromium. It could also side line possible toxicity to other tissues examined. In the case of the brain tissue, it is certainly not clear what the effect might be on cognition, memory and behavior if evaluated. A prolonged period of chromium administration could be more revealing.

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Outbreak of methanol poisoning at a rural community in Southwest Nigeria: Results of laboratory analysis

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Abstract

Background: In the month of April 2015, an epidemic initially attributed to mysterious causes broke out in a rural community (Ode-Irele, Ondo State) of Nigeria. Twenty-five adults were affected, with a mortality of 18/25 (a rate of 72%). This is a report of laboratory analysis conducted at the College of Medicine, University of Ibadan, and the University College Hospital, Ibadan, Nigeria.

Methods: Samples of blood and urine (both coded A, B, and C) of the three victims of the disease, and three plastic containers of locally brewed alcoholic beverages (coded (D, E, F) reported to have been consumed by the victims were made available. All the samples were tested for copper, lead, zinc, and cadmium toxicity. The blood samples were tested for cholinesterase inhibition as an indirect test of probable pesticide poisoning. Methanol content of the samples was determined by gas chromatography.

Results: The urine and blood samples showed concentrations of lead, copper, zinc, and cadmium that were not uncommonly elevated. Zinc levels in the blood of two of the victims were low. There was a significant inhibition of cholinesterase activity in the three blood samples as follows; sample A 96.2%, sample B 31.8%, and sample C 2.5%. Methanol content of the alcoholic beverages ranged from 48 g/L (4.8%) to 625 g/L (62.5%), far above allowable limits of 0.005 g/L (0.0005%) in Nigeria.

Conclusions: Methanol poisoning was concluded to be the cause of the unknown disease. This finding guided measures that rapidly controlled and eventually ended the epidemic.

Keywords: *Methanol poisoning, Heavy metals, Nigeria*

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Résumé

Contexte : Dans le mois d'avril 2015, une épidémie initialement attribuée à des causes mystérieuses s'est déclarée dans une communauté rurale (Ode-Irele, État d'Ondo) du Nigéria. Vingt-cinq adultes ont été touchés, avec une mortalité de 18/25 (un taux de 72%). Ceci s'agit d'un rapport d'analyse laboratoire réalisée au Collège de Médecine de l'Université d'Ibadan et au Collège Hospitalier Universitaire d'Ibadan au Nigéria.

Méthodes : Des échantillons de sang et d'urine (tous deux codés A, B et C) des trois victimes de la maladie et trois récipients en plastique de boissons alcoolisées brassées localement (codés (D, E, F) qui auraient été consommés par les victimes ont été soumis à des tests de toxicité sur le cuivre, le plomb, le zinc et le cadmium, ainsi que sur l'inhibition de la cholinestérase dans les échantillons de sang, en tant que test indirect d'intoxication probable par un pesticide. La teneur en méthanol des échantillons a été déterminée par chromatographie en phase gazeuse.

Résultats : Les échantillons d'urine et de sang ont montré des concentrations de plomb, de cuivre, de zinc et de cadmium qui n'étaient pas inhabituellement élevées. Les concentrations de zinc dans le sang de deux des victimes étaient faibles. Il y avait une inhibition significative de l'activité de la cholinestérase dans les trois échantillons de sang comme suit; échantillon A 96,2%, échantillon B 31,8% et échantillon C 2,5%. La teneur en méthanol des boissons alcoolisées variait de 48 g / L (4,8%) à 625 g / L (62,5%), bien au-dessus des limites autorisées de 0,005 g / L (0,0005%) au Nigéria.

Conclusions: il a été conclu que l'intoxication au méthanol était la cause de la maladie inconnue. Cette découverte a guidé les mesures qui ont rapidement maîtrisé et éventuellement mis fin à l'épidémie.

Mots clés: *intoxication au méthanol, métaux lourds, Nigéria*

Introduction

Methanol (methyl alcohol) is structurally similar to ethanol and both alcohols share many chemical characteristics and metabolic pathways. When

ingested, methanol is rapidly absorbed and distributed in body tissues in proportion to water content. The lethal dose of methanol has been reported to be approximately 1g/kg, with a wide range of variations [1]. This dose is dependent on individual metabolism, and other factors that include age, liver enzyme activity, and renal function [1, 2]. In Nigeria, the National Agency for Food Drug Administration and Control (NAFDAC) in 2005 put the allowable limits of methanol in alcoholic beverages at 0.0005% (5mg/L) [3]. Epidemic methanol poisoning is rare and when it occurs is often unrecognized and generates public concern.

In the month of April 2015, a series of deaths attributed to unknown and mysterious causes occurred at Ode-Irele, a remote community of Ondo State, Southwestern Nigeria (Figure 1). At the first wave of the epidemic, 25 adults developed sudden onset of vomiting, abdominal pain, and blurred vision that resulted in blindness; the victims rapidly became unconscious within an hour of onset of symptoms. While approximately 18 of the 25 victims of the mysterious disease died, a few remained unconscious for several weeks under close medical care. One factor that was common to all the victims was the consumption of a locally brewed alcohol drink. Initial analysis of blood samples sent to laboratories at Lagos, Nigeria's commercial center, and the Zonal office of the World Health Organization were done on the basis of the possibility of Insecticide and heavy metal poisoning [4]. The mysterious deaths caused community anxiety as the deaths occurred at about the same period of Ebola Virus Disease (EVD) epidemic in Nigeria.

On April 18, 2015, a pathologist with the Ondo State Ministry of Health contacted the Chief Medical Director and sought the assistance of the University College Hospital Ibadan, Nigeria, in the management of five patients who were suffering from clinical effects of the Ode-Irele mysterious disease but were blind and unconscious. The victims of the mysterious epidemic were transferred to the medical ward of the University College Hospital, Ibadan for care and further investigation where they were managed by a team of neurologists, nephrologists, and ophthalmologists. Blood and urine samples that were obtained from the victims were delivered to the laboratory for analysis.

Of the five victims, fresh urine and blood samples were obtained from three; Mr. S.O, a 25 year old man, Mr. G.A a 70 year old man, and Mr. O.J., a 35 year old man, with similar histories of acute onset of vomiting, severe abdominal pain, visual disturbances, and ultimately unconsciousness. They were also reported to have ingested locally brewed alcoholic beverages, samples of which were brought to the hospital. The hospital management assembled a team of experts comprising laboratory scientists, Clinical Pharmacologists, toxicologists, and a Chemical Pathologist to identify the cause of the disease. Blood and urine samples obtained from the three patients were systematically analyzed to identify the toxicants responsible for the clinical state of the patients.

In a 2016 publication, Adeyanju and co-authors described the case identification, epidemiological investigation, and public health response of the methanol intoxication outbreak that we investigated in the laboratory [5]. The authors briefly stated the results of the laboratory analysis carried out at UCH. In this manuscript we are providing the details of the methods used in the laboratory analysis and the results. This will be a ready resource for investigation of similar outbreaks should they occur in the future.

Methods

A systematic approach, as described below, was used in the analysis of samples of the alcoholic beverage reportedly ingested by the victims as well as blood, and urine samples obtained from the three admitted victims of the unknown disease. The samples were analyzed for the presence of heavy metals, pesticides, and methanol in that order. Universal precautions guiding the handling of potentially infectious samples were strictly adhered to throughout the analysis.

Coding of samples

About 7 mL of blood and 40 mL of urine samples were obtained from the patients. Along with these were about 400 mL of three alcoholic beverage said to have been ingested by the victims. Triplicate blood samples were transported in plain, sodium EDTA, and heparinized plastic bottles. Urine samples were transported in 50 mL plain plastic bottles. The blood and urine samples were transported in ice packs. The beverages were transported in large plastic kegs in which they were sold. The samples were obtained at the general hospital at Ondo State, and sent immediately to the University College Hospital Ibadan, before the victims were transferred to Ibadan. On arrival at the laboratory the samples were coded in the following order, S.O. (sample A), G.A. (sample B), and O.J. (sample C). The alcoholic beverages reportedly ingested by the patients were also coded as beverage D, beverage E, and beverage F; it was not certain which alcoholic beverage was taken by

which victim. Samples were kept in a 4^0C refrigerator; analyses were done within 48 hours of receiving the samples. It is however noteworthy that the samples were obtained from the victims three days after the acute onset of symptoms.

Analysis for zinc, copper, lead, and cadmium
This was done by flame Atomic Absorption Spectrophotometry (AAS). The analyses were carried out at the Multidisciplinary Central Research Laboratory (MCRL) of the University of Ibadan, Nigeria. Double distilled deionized water (ddH_2O) was used as negative control and a calibration curve was prepared using certified laboratory reference standards of the heavy metals analyzed. All methods complied with those reported in the manual by Perkin Elmer [6], except for the analysis of blood lead levels in which nitric acid digestion was done. Only analytic grade reagents were used. All the samples (beverage, blood, and urine) were analyzed for Pb, Zn, Cu, and Cd. Two repeat measurements were done for every sample. The alcoholic beverages and urine samples were centrifuged at 4000g for 7 minutes and aliquots (4mL) taken from the supernatants. The aliquots of beverages and urine were diluted with ddH_2O to a dilution ratio of 1:5 to a final volume of 20 mL before injection into the flame AAS. Final contents of the metals were determined after due considerations of the dilution factors.

Analysis of blood for zinc, copper, lead, and cadmium
Clotted blood samples were centrifuged at 4000g for 7 minutes and serum was aspirated into plain plastic bottles. Aliquots of the serum were taken and diluted with ddH_2O to a dilution of 1:5 before injection into the flame AAS for determination of Zn, Cu, and Cadmium. Whole blood was used for analysis of lead levels. Lead extraction was done using the nitric oxide digestion method before analysis with AAS. Briefly, 3.8 mL of 0.1 N nitric acid was added to 0.2 mL of whole blood and allowed to stand for > 6 hours (overnight) at 4 ^{o}C (to liberate bound lead). The solution was then centrifuged at 4000g for 10 minutes. The clear supernatant was diluted with ddH_2O to a ratio of 1:5 before aspiration into the flame AAS.

Evaluation for pesticide intoxication
The possibility of pesticide intoxication was assessed indirectly by determination of cholinesterase activity. The test was performed using heparinized blood obtained from the three victims. Whole blood acetylcholinesterase enzyme activity was determined according to the modified Ellman's method as published by Worek *et al*. in 1999 [7]. Briefly, whole blood (control and samples from the victims) was diluted a hundred times in 0.1M sodium phosphate buffer (pH 7.4) containing 0.03% Triton-X-100. Acetylcholine (28.4 mM) and DTNB (10mM) were added to the mixture and incubated at 37^0 C for 10 minutes. Absorbance was read at 436 nm in a UV-VIS Spectrophotometer (BIO-RAD, SMART-SPEC PLUS, USA). An inhibition of 30% is considered clinically significant.

Analysis for methanol and ethanol levels
Determination of methanol and ethanol concentrations in the beverages, blood, and urine samples was done by gas chromatography using the method described by Helena Pontes *et al* [8]. Reagents, calibration procedures, sample preparation, and instrument setting were as described in the publication. The beverages were centrifuged at 4000g for 7 minutes, supernatants taken and diluted with ddH_2O to 1:5 dilution; 0.5 µL of the supernatants were injected sequentially into the chromatographic system. Urine samples were similarly treated and diluted before injection into the chromatographic system. Plasma was then prepared as described by Helena Pontes *et al* [8]. Prepared plasma was then diluted to 1:5 using ddH_2O before injection into the chromatographic system. All the chemicals used were of analytical grade.

Results

Heavy metal contents in samples of blood, urine, and beverage from the victims Zinc, copper, lead and cadmium
The urine and blood samples showed concentrations of Pb, Cu, Zn, and Cd that were not out of acceptable limits. Zinc levels in the blood samples of patients A and B were low. Analysis of the alcoholic beverages showed concentrations of analyzed metals within recommended allowable limits. Table 1 shows a summary of the results of metals analysis. It should be noted that alcoholic beverage coded D, E, and F do not correspond to patients A, B, and C. as it was not certain the alcoholic beverage each of the patients ingested.

Analysis for possible pesticide toxicity
Cholinesterase activity: There was a significant inhibition of the activity of the enzyme in the three blood samples as follows; average values of inhibition were sample A 96.2%, sample B 31.8%, and sample C 2.5%.

Table1: Heavy metal contents (Mean mg/L ± SD) in samples of blood, urine, and beverage from the victims

Sample	Heavy metal	Level of metals in individual patient's samples		
		A	B	C
Blood	Lead	0.00 ± 0.03	0.00 ± 0.03	0.00 ± 0.07
	Cupper	0.06 ± 0.00	0.28 ± 0.05	0.28 ± 0.09
	Zinc	0.19 ± 0.01	0.41 ± 0.02	1.21 ± 0.01
	Cadmium	0.00 ± 0.01	0.00 ± 0.01	0.01 ± 0.01
Urine	Lead	0.06 ± 0.03	0.09 ± 0.05	0.00 ± 0.07
	Cupper	0.00 ± 0.02	0.00 ± 0.01	0.05 ± 0.01
	Zinc	0.19 ± 0.02	0.11 ± 0.01	0.05 ± 0.01
	Cadmium	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Beverage	*Heavy metal*	*D*	*E*	*F*
	Lead	0.04 ± 0.05	0.00 ± 0.04	0.00 ± 0.01
	Cupper	0.19 ± 0.02	0.00 ± 0.01	0.01 ± 0.01
	Zinc	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.02
	Cadmium	0.00 ± 0.01	0.00 ± 0.01	0.01 ± 0.01

SD = Standard Deviation

Table 2: Average Methanol and Ethanol levels in the blood, urine, and beverages (g/L and percentage alcohol)

Sample		Methanol content - g/L	Ethanol content – g/L
Blood	A	1.40 (0.14%)	0.695 (0.07%)
	B	1.05 (0.11%)	1.095 (0.12%)
	C	0.25 (0,03%)	0.235 (0.02%
Urine	A	0 (0%)	0.04 (0.004%)
	B	0.18 (0.02%)	0.015 (0.001%)
	C	Inadequate sample	Inadequate sample
Beverage	D	625 (62.5%)	127.5 (12.75%)
	E	48 (4.8%)	428.5 (42.85%)
	F	325 (32.5%)	205 (20.5%)

beverages

Methanol content: Methanol was detected in the blood in concentrations above tolerable limits. Methanol content of the alcoholic beverages was also above allowable limits. Table 2 shows a summary of findings of tests for methanol and ethanol contents.

Discussion

Methyl alcohol (Methanol) is a widely available solvent with industrial and domestic applications. Methanol has a relatively low intrinsic toxicity; however, it is metabolized to highly toxic compounds, such as formic acid which can cause

Fig 1: Map of Nigeria showing Ondo State

Methanol and Ethanol levels in the blood, urine, and

blindness, coma, and metabolic disturbances and that

can be life-threatening [9]. Victims of methanol poisoning often seek medical care after significant delay, mainly because there is a lag period between ingestion and toxic effects and due to protean nature of signs and symptoms [10].

Methanol levels were very high in the alcoholic beverages consumed by the victims of the epidemic reported in this paper. However, the source of methanol in the alcoholic beverages remains to be determined. Possible sources of the contamination could have been from illegal addition of methanol by vendors or contamination during the fermentation process [3]. Epidemic methanol poisoning of this scale had not been previously reported from Nigeria to the best of our knowledge and this may have led to a delay in recognizing the cause of the symptoms and deaths that occurred. Levels of methanol detected in the blood and urine of the victims tested supported the diagnosis of methanol poisoning. It is also of note that the blood‘ and urine levels detected may have been reduced by the time the samples were collected. The level of inhibition of acetylcholinesterase detected in the analysis could be attributed to the effects of methanol, bearing in mind that this could also be due to other causes that may include exposure to organophosphates.

Low levels of zinc were also detected in the victims tested. Zinc is a micronutrient required for the activities of over 300 enzymes, including alcohol dehydrogenase (ADH), may have been reduced where there is high demand. There is evidence that severe infections and other forms of stress, particularly when encountered in hospitalized adults or accompanied by fever or other indicators of an acute phase response, produce a drop-in plasma zinc concentration [11]. Probably, zinc levels were low in the blood samples of the victims on account of increased demand for Zn due to increased metabolism of methanol during the illness. This may have contributed to the progression of the observed signs of methanol poisoning in the victims including the loss of vision. There is a need to conduct studies aimed at determining the relationship between methanol toxicity and plasma concentration of zinc.

Once ingested, methanol is metabolized by dehydrogenation to formaldehyde and then to formic acid leading to profound acidosis. These two metabolites are highly reactive, binding readily to tissue proteins with resultant inhibition of metabolic enzymes and cytochrome oxidase systems [2, 12]. The toxic effects and ocular effects of methanol are attributable to toxicity of formic acid [13, 14]. The same enzymes responsible for ethanol metabolism are responsible for the metabolism of methanol with higher Km for methanol. Presence of ethanol will inhibit the metabolism of methanol. Clinical features of toxicity have been shown to be dose-dependent, and can be ameliorated by concomitant administration of ethanol diverting the pathway to that of intermediates less toxic than formic acid.

Accidental ingestion of methanol-containing solvents in homes is an alternative source of toxicity. Methanol-containing products may be found in homes, as constituents of a large variety of solvents, chemicals, and pesticides. However, epidemics of methanol poisoning are more likely the result of ingestion of alcoholic products containing high concentrations of methanol. In 1951, an epidemic of methanol poisoning was reported to have occurred in Atlanta [15]. The epidemic was caused by city-wide distribution of methanol containing illicit whisky. Over a five-day period, a total of 323 cases, including 41 deaths, occurred during the epidemic [15]. An outbreak of acute methyl alcohol intoxication was also reported to have occurred in Port Moresby, Papua New Guinea, in March 1977. Twenty-eight young men attended a drinking party and drank methyl alcohol. All 28 became ill within 8 to 36 hours after drinking and were hospitalized. The most commonly observed clinical syndromes among the Port Moresby patients were: acute metabolic acidosis, severe visual impairment and acute pancreatitis. Four died within 72 hours after admission to the hospital [1]. Another report of acute methanol poisoning from Chaoyang County [17]. The Chaoyang episode was due to poisoned wine which caused acute vertigo, headache, weakness, vomiting, night sweat, dyspnea and blurring of vision within 6 to 120 hours of ingestion. Twenty-nine people were reported to have drunk the wine, fourteen of them died, two of them became blind. On further analysis, high content of methanol was detected in the spirit; the victims’ blood and urine also had methyl alcohol. Laboratory analysis showed that the content of methyl alcohol was between 16.6 and 40.69 g/100 mL [17]. The Ode-Irele methanol poisoning epidemic is consistent with previously reported occurrences.

Methanol toxicity occurs uncommonly worldwide, it is usually not recognized early and causes a lot of confusion in the society. The diagnosis is often elusive and requires a high index of suspicion. The Nigeria epidemic reported in this case occurred in an area that is within the belt stretching from the Midwestern to the South-South regions of the country where locally brewed alcohol is widely produced and consumed.

Victims of the epidemic developed acute episodes of severe abdominal pain, vomiting seizure, visual disturbances and loss of consciousness within

five days of ingestion of local alcohol. Initially, the community attributed the deaths to spiritual 'attacks' and the people blamed the 'gods' for the disease. The laboratory investigation conducted at the University College Hospital, Ibadan, provided the first evidence that implicated methanol as the cause of the mysterious disease. An important observation was that after collation of all laboratory data, a remarkable congruence or correlation was strikingly evident with clinical data; the patient with the lowest blood concentration of methanol recovered faster than the other victims whose samples were analyzed. These results guided the government's measures that rapidly ended the epidemic. Confirmations of the findings by other laboratories added to the evidence provided by the research team at Ibadan.

In developing nations, rare epidemics (when they occur) pose many challenges to community healthcare institutions due to unpreparedness. The delay in recognition of the etiology causes panic and unnecessary deaths. In addition, due to the epidemic being uncommon, index of suspicion is usually low. In the case reported here the affected community within the local alcohol belt described above should have raised index of suspicion. An additional challenge was the lack of readiness of the laboratory in conducting the required analyses. Most of the reagents used in the analysis were not available and had to be rapidly procured during the epidemic. The methods used were run for the first time and analysis for formic acid content could not be done. Such are some challenges faced in resource-scarce countries, however, with the close collaboration of several disciplines (clinical and laboratory) and full support of the hospital management, the team was able to identify the cause of the widely reported 'mysterious disease'. The success reported here showed that even with little resources, with the right leadership and human resources, a lot can be achieved locally. This calls for need to have well equipped toxicology laboratories to avoid unnecessary morbidity & mortality. In addition, it is recommended that index of suspicion should be kept high especially when episodes occur within the belt described above. Laboratory and human capacity should be strengthened for rapid response in similar circumstances. In the present era of infectious disease epidemics, healthcare workers and stakeholders should remain vigilant for epidemic or sporadic cases of methanol poisoning or other uncommon conditions that may occur at any time. The government should put in place mechanisms of routinely sampling locally made alcohol for methanol content to prevent future outbreaks of methanol poisoning while the dangers of illicitly brewed alcohol should be made known to communities.

Conclusions

The clinical and laboratory findings in affected victims during the widely reported epidemic of a mysterious disease in the month of April 2015 in Southwest Nigeria was due to consumption of locally brewed alcohol contaminated with high concentrations of methanol. The findings of associated inhibition of acetylcholinesterase, low levels of zinc in tested victims, and the high degree of correlation between laboratory and clinical presentation support the diagnosis. It is recommended that rapid analysis of beverages, blood, and urine be carried out should there be fresh cases of the disease, with prompt and appropriate management of victims at tertiary hospitals. The public should be informed of the dangers of locally brewed alcohol; in the communities affected by the disease. People should be admonished to stop drinking improperly brewed alcohol and to be aware of the possibility of contamination with methanol. Finally, one key lesson from this report is that with increasing urbanization and industrialization the risk of chemically- induced disease should be given high index of suspicion and priority in Nigeria and many other developing countries.

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Pattern and prognostic factors of acute kidney injury in an intensive care unit in Nigeria

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Abstract

Introduction; Acute kidney injury (AKI) is a clinical entity with significantly high morbidity and mortality rates especially in the intensive care setting. Few previous studies in this area have employed less sensitive criteria with limited results. Thus, application of newer criteria and prognostic scores will give a true picture of the magnitude of the problem in this particular setting.

Methodology; This study was carried out among 100 consecutive patients admitted to the intensive care unit (ICU) over an eighteen-month period to determine the occurrence, frequency, and outcomes, and to also seek a relationship between the diagnostic criteria of AKI such as the Risk, Injury, Failure, Loss and End stage (RIFLE) and the Acute Kidney Injury Network (AKIN) criteria respectively and the Acute Physiological and Chronic Health Evaluation, (APACHE) IV. The outcome measures in these patients were as follows; need for haemodialysis, survival without haemodialysis or death.

Results; The patients studied were aged between 18 and 70 years (Mean± SD ; 41.5±16.3) and the male to female ratio of 1.4:1. The incidence of AKI in patients admitted into the ICU was 54 (54%) . Using the RIFLE criteria, 37% were in the Injury stage while 46.3% were in stage 2 using the AKIN criteria. Surgical cases such as head injuries and advanced carcinomas constituted the major primary aetiology (72.2%). The presence of other organ system failure (apart from the kidney) was largely predictive of outcome among ICU patients ($p<0.001$). 47 patients had other organ system failure. Out of these, only 7 (14.9%) of them survived. There was a higher APACHE IV scores(61.1± 24.3) in patients that developed AKI compared to those that did not develop AKI (55.2 ± 19.9).

Conclusion; Acute kidney injury is a huge burden in the intensive care setting, early identification using newer diagnostic parameters and risk stratification with more sensitive diagnostic scores could help in identifying patients at risk

Keywords, *Acute, kidney, injury, intensive, care*

Résumé

Introduction : L'injurie rénale aiguë (IRA) est une entité clinique présentant des taux de morbidité et de mortalité significativement élevés, en particulier dans le contexte des soins intensifs. Peu d'études antérieures dans ce domaine ont utilisé des critères moins sensibles avec des résultats limités. Ainsi, l'application de critères et de scores pronostiques plus récents donnera une image fidèle de l'ampleur du problème dans ce contexte particulier.

Méthodologie : Cette étude a été réalisée sur 100 patients consécutifs admis en unité de soins intensifs (USI) sur une période de dix-huit mois afin de déterminer l'occurrence, la fréquence et les résultats ainsi que de rechercher une relation entre les critères de diagnostic de l'IRA tels que les critères de risque , blessures, échec, perte et étape ultime (RIFLE) et critères du réseau de lésions rénales aiguës (RIRA) respectivement et évaluation de l'état de santé aiguë physiologique et chronique (APACHE) IV. Les mesures de résultats chez ces patients étaient les suivantes : besoin d'hémodialyse, de survie sans hémodialyse ou mort.

Les résultats ; Les patients étudiés étaient âgés de 18 à 70 ans (moyenne ± ET ; 41,5 ± 16,3) et le ratio hommes / femmes de 1,4 : 1. L'incidence d'IRA chez les patients admis en USI était de 54 (54%). En utilisant les critères RIFLE, 37% se trouvaient à l'étape lésion alors que 46,3% étaient à l'étape 2 en utilisant les critères RIRA. Les cas chirurgicaux tels que les blessures à la tête et les carcinomes avancés constituaient la principale étiologie primaire (72,2%). La présence d'autres défaillances du système organique (hormis le rein) était largement prédictive du résultat chez

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les patients en USI (p <0,001). 47 patients ont eu une autre défaillance du système d'organe. Parmi eux, seulement 7 (14,9%) ont survécu. Les scores APACHE IV étaient plus élevés (61,1 ± 24,3) chez les patients ayant développé une IRA par rapport à ceux qui ne présentaient pas d'IRA (55,2 ± 19,9).

Conclusion : Les lésions rénales aiguës représentent un fardeau énorme en soins intensifs. Une identification précoce à l'aide de nouveaux paramètres de diagnostic et une stratification du risque avec des scores de diagnostic plus sensibles pourraient aider à identifier les patients à risque.

Mots-clés : *Aiguë, rein, blessure, intensive, soins*

Introduction

Acute kidney injury (AKI) refers to an abrupt but often reversible decline in the glomerular filtration rate (GFR) occurring over a period of minutes to days with retention of blood urea nitrogen and serum creatinine. [1-2]. The kidney Disease Improving Global Outcomes (KDIGO) work group further defined AKI as any of the following (i) an increase in serum creatinine by $\geq$ 0.3mg/dl ($\geq$ 26.5µmol/L) within 48hours, (ii) an increase in serum creatinine to $\geq$ 1.5 baseline, or (iii) urine volume < 0.5ml/kg/hr for 6 hours [3].

It occurs in different settings ranging from community-acquired to others seen in the general hospital wards and in the intensive care units (ICU) with very high mortality rates in the latter. [4-5]. Monitoring and support of threatened or failing vital functions in critically ill patients is done in this setting [6]. These categories of patients managed invariably have a higher incidence of AKI and a poorer outcome when compared to the general population [7].

There are several scoring systems used in the ICU to estimate the severity of illness and to predict outcomes, these includes, amongst others, the Mortality prediction model (MPM)[8], Multiple Organ dysfunction system (MODS)[9], LIANO scores [10] , and the APACHE scores [11] . The latter are used to assess the severity of illness estimation and estimates risk based on data on the first 24 hours of ICU stay using vital signs, co-morbid conditions, physiological and neurological variables.

The APACHE IV, which is a third generation of ICU scoring system, is based on the study of an advanced standard of care of a more recent patient population. Additional variables include mechanical ventilation, disease specific coefficient, rescaled Glasgow coma scale, need for thrombolysis, partial arterial oxygen and fraction of inspired oxygen [12]. Results from various studies have shown that APACHE IV is a better prognostic scoring system and it predicts mortality rate better than APACHE II scoring system as it provides the basis for the calculation of both the estimated mortality ratio or risk of death and the estimated length of stay [13]. It has 142 variables in 3 domains of vital signs/ laboratory data, chronic health conditions and admission information and diagnosis [10].

About a decade ago, studies on AKI in ICU from our environment, used generally older and less sensitive tools such as the APACHE II, and Liano severity scoring index, to assess the aetiological factors and outcomes. [5]

Furthermore, more specific criteria for defining AKI have been developed over the last fifteen years , these include the RIFLE (Risk, Injury, Failure, Loss of renal function and End stage renal disease) criteria which shows that increase in serum creatinine levels over 7 days correlates with disease severity in correlation with the urinary output and the glomerular filtration rates [14]. Also, the Acute kidney Injury network (AKIN) further proposed a modified version of RIFLE criteria i.e. an increase in serum creatinine over 48hour rather than 7days, using three different stages for acute kidney injury [15].

Outcome studies in the intensive care units using the above two definitions in other environments have not yielded any superior advantage of either in prognosticating [16].

The incidence and mortality of AKI in ICU patients were much lower when compared to what obtains in the developed world from a previous study in our environment and this may give a false sense of security [15]. This might have been due to the use of less sensitive and non-specific defining criteria.

We felt that a realistic and true picture of the magnitude of AKI in the ICU setting would be obtained using newer and more specific criteria in detecting the actual incidence and the mortality pattern in the patients. This will help to inform a strategy in the provision of acute renal care by the medical personnel in the ICU to prevent, recognize early, and assist in the management of AKI in these patients.

We therefore set out to study the occurrence, prognostic indicators and outcome of acute kidney injury in the intensive care unit by using the RIFLE and AKIN criteria, to assess the global illness severity in the ICU using the APACHE IV scoring systems and to seek a relationship between these AKI criteria and APACHE IV.

Methods

This study was carried out at the intensive care unit of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria over an 18-month period. The institution provides specialist health services to over one million people in Osun State, South West Nigeria.

One hundred consecutive patients between the ages of 18 and 70 years, who fulfilled the criteria for ICU admission, were recruited for the study over the same period. The criteria included critically ill patients in a medically unstable state who require an intensive level of care for monitoring and treatment. Patients with hypertension, diabetes mellitus, clinical features of obstructive uropathy and those with previous history suggestive of chronic kidney disease were excluded from the study.

Presence of AKI in the ICU patients was determined using both the RIFLE and AKIN criteria which have been shown to be equally effective in defining AKI in this particular setting[16]. Within 24 hours of admission of these patients, their respective death risk and severity index score were determined using the APACHE IV scoring system. The APACHE foundation software generated a list of scores, which represented the actual versus the predicted hospital mortality, and ICU length of stay for the 100 patients recruited from the ICU. A standardized mortality ratio (SMR) was also generated for all the patients. This represented the ratio between the actual hospital mortality value and the patient's actual ICU length of stay compared to the predicted mortality value and predicted ICU length of stay. A SMR ratio of 1.0 indicated a match between actual and predicted values. Ratio above 1.0 represented actual mortality rates above predicted, and ratio below 1.0 represented rate below predicted. A ratio of 1.0 indicates a precise match between actual and predicted values.

The actual length of stay of patients in ICU and their outcome along each stages of RIFLE and AKIN criteria was noted and compared to other ICU patients who did not developed AKI. Major Burns was defined as thermal injury involving complete full thickness of the skin characterized by eschar formation and complete loss of sensation [19]

The presence of one or more organ failure (apart from kidneys) was determined using some clinicopathological parameters as defined by Knaus and Wagner [20]. Sepsis was defined as a microbiologically proven focus of infection (such as urine, blood, catheter, wound site and endotracheal tubes and others) and deterioration of the clinical state evidenced by at least one of the following: temperature $>39^0$C on 2 or more occasions, leucocytes $>10 \times 10^9$/L, or positive blood culture[20].

Patients in RIFLE-F or Acute Kidney Network Stage 3 with indications requiring renal replacement support as per the following criteria were offered heamodialysis viz: symptomatic ureamia, severe hyperkaleamia (serum potassium>6.5 mmol/L) Ureamic pericarditis, acute pulmonary oedema especially in the setting of anuria or oliguria, intractable acidosis especially with serum bicarbonate <12mmol/L ; azotemia with serum creatinine > 600µ mol/L and serum urea > 25mmol/L.

Heamodialysis was done through femoral vein cannulation using a single lumen femoral catheter with indwelling life span of not more than 48hours. Such patients were treated as emergency cases and received some sessions of heamodialysis with one or two days interval. The conventional intermittent heamodialysis with low blood flow rate of 150ml/min, with heparin anticoagulation was used. Great attention was paid to their blood pressure during heamodialysis sessions and the use of low ultrafiltration and vasopressor support (low dose dopamine 2µg/kg/min) was administered when necessary

Conservative management was based on our unit protocol of practice which included attempts at reversing the underlying cause of the disease and corresponding fluid and electrolytes abnormalities. The fluid intake was restricted to 500ml to 1 litre in oliguric patients to match measurable plus insensible losses. The protein intake was restricted to 0.6g/kg/day of high biological value and calories of at least 35cal/kg/day. Energy supplementation for patients with severe vomiting included the administration of 50% glucose boluses. Mechanical ventilation was given by the ICU specialists to critically ill patients who required some form of assisted respiration.

Clinical outcomes of all patients were determined by the following: (i) mortality {death from ureamia, death not due to ureamia or other condition (but from the primary condition)}, (ii) need for commencement on RRT, (iii) patients survival (daily reduction of serum creatinine by 100µmol/lit/day), and other electrolytes to normal or near normal levels.

Data obtained was analyzed using the Statistical Package for Social Sciences (SPSS) for windows version 16 computing software. Variables were summarized in percentages, ratios, frequencies, proportions, means and standard deviation.

Chi-square statistics was used for defining associations between categorical variables such as age, sex and duration of stay. While the Kaplan-Meier survival test was used to determine the outcome and the length of hospital stay across the different stages of AKI. . The correlation between quantitative variables was tested using the Pearson correlation analysis. Severity and prognosis of illness was determined by using APACHE IV scoring system while the Standardized Mortality Ratio (SMR) and the ICU/LOS ratio was determined using APACHE foundation software.

Results

One hundred critically ill patients admitted into the ICU constituted the study population. They were aged between 18 to 70 years with a mean age of 41.5±16.3 years (Mean±SD). Males constituted 59% of the total population with a male to female ratio of 1.4:1. The incidence of AKI in ICU patients is 54 (54%) by both RIFLE and AKIN criteria. The mean age of the patients with AKI was 42.7±15.4 years (Mean±SD), while that of the 46 non AKI patients was 40.0±17.4 years (Mean±SD). (Table 1)

Among all the ICU patients seen, surgical cases (78%) were the leading cause of admission. This was followed by medical (13%) and obstetrics (9%). Head injury was complicated by AKI in 24.1% (13) of cases. AKI was also found in 18.5% (10) cases of advanced carcinoma (carcinoma of breasts, stomach, pancreas and colon), 14.8% (8) cases of sepsis, 9.3% (5) cases of cardiothoracic surgery and obstetrics respectively (Table 1).

The pattern of outcome showed that patients with head injury had worst outcome with 28.2% deaths and 13.3% survival. This was followed by advanced carcinoma with 20.5% deaths and 13.3% survival. However, patients with vasculitidies and specifically, post maxillofacial surgery all survived. (Table 2)

Using the RIFLE criteria, 17 (31.5%) of AKI cases were at the Risk level, 13 (24.1%) at Injury, 20 (37.0%) at Failure and 4 (7.4%) cases were at the loss of renal function levels respectively. No patient was found with End Stage Renal Disease. At the Risk level ,6 (35.3%) survived while 11 (64.7%) died and at the injury level, 5 (38.5%) survived while 8 (61.5%) died. Furthermore, none of our AKI patients at Loss of renal function level survived. It was observed that as the severity of AKI progressed the outcome across each level of AKI according to RIFLE criteria became poorer ($p<0.339$).(Table 3).

Using the AKIN criteria, 17 (31.5%) of AKI cases were at stage l, 25 (46.3%) at stage 2 and 12

Table 1: Socio-demographics and clinical characteristics among patients with AKI/NON-AKI in the ICU

Age Range (years)	AKI Frequency (n) n=55(%)	Non AKI Frequency n=46(%)
Mean Age	*42.7±15.4*	40.0±17.4
≤19	-	2(4.3)
20-39	24(44.4)	25(54.3)
40-59	20(37.1)	9(18.5)
≥60	10(18.5)	10(21.7
Gender		
Male	30(55.6)	29(63)
Female	24(44.4)	17(37)
Aetiology (by specialty)		
Surgical	39(72.2)	39(84.8)
Medical	9(16.7)	4(8.7)
Obstetrics and Gynaecology	6(11.1)	3(6.5)
Primary Diagnosis		
Sepsis	8(14.8)	4(8.7)
Nephrotoxins	4(7.4)	1(2.2)
Advanced Carcinomas	10(18.5)	9(19.6)
Head Injury	13(24.1)	12(26.1)
Obstetrics	5(9.3)	5(10.9)
Vasculitidies	1(1.9)	1(2.2)
Multiple fractures	2(3.7)	6(13.0)
Haematological Malignancy	1(1.9)	0
Major burns	2(3.7)	1(2.2)
Post Major Surgeries	8(22.9)	7(17.4)

Table 2: Relationship between presence of AKI and outcome in ICU patients

Aetiology	Survived Presence of AKI n (%)			Dead Presence of AKI n (%)		
Sepsis	4(100)	0(0)		5(50)	4(50)	
Nephrotoxins	1(50)	1(50)		3(100)	0(0)	
Advanced Carcinoma	2(22.2)	7(77.8)	*LRx²*	8(80)	2(20)	*LRx²*
Head Injury	2(28.6)	5(71.4)		11(61.1)	7(38.9)	*=15.259*
Obstetrics	2(40)	3(60)	*=19.967*	3(60)	2(40)	
Vasculitidies	1(100)	0(0)		0(0)	1(100)	*(P<0.171*
Multiple Fracture	0(0)	5(100)	*(P<0.030)*	2(66.7)	1(33.3)	
Major burns	0(0)	1(100)		2(100)	0(0)	
Haem malignancy	1(100)	0(0)		0(0)	0(0)	
Major Surgeries* *(Abd/Cardiothoracic)	2(50)	2(50)		6(60)	4(5\40)	

* Significant

LRX^2 = Likelihood ratio chi-square test

Table 3: AKI staging and outcome in the icu using the rifle and the akin criteria.

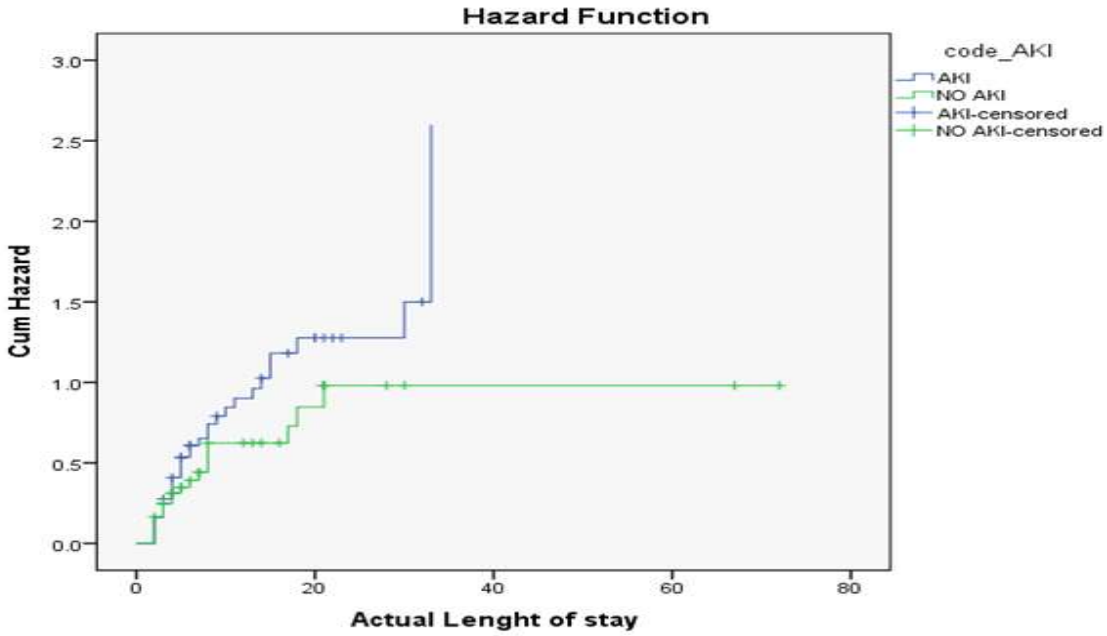
ICU: RIFLE	n = 54 n(%)	Outcome n (%) Survived n(%)	Death n(%)	Total	Test Statistics	Df	p-value
R	17 (31.5)	6 (35.3)	11(64.7)	17			
I	13 (24.1)	5 (38.5)	8 (61.5)	13	**LRX²** =3.360	4	0.339
F	20 (37.0)	4 (20)	16 (80)	20			
L	4(7.4)	0 (0)	4 (100)	4			
E	0 (0)	0 (0)	0 (0)	0			
AKIN							
Stage 1	17 (31.5)	7(41.2)	10 (58.8)	20			
Stage 2	25 (46.3)	7(28.0)	18(72.0)	17	LR $\chi2$=3.783	0.151 2	
Stage 3	12 (22.2)	1(8.3)	11(91.7)	12			

LR χ2*= Likelihood ratio Chi-square*
RIFLE (Risk, injury, loss, end stage renal failure)
AKIN (A*cute Kidney* ***I****njury* ***N****etwork) criteria and outcome of patients in the ICU.*

(22.2%) at stage 3. In stage 1, 7 (41.2%) survived while 10 (58.8%) died and in stage 2, 7 (28.0%) survived while 18 (72.0%) died. Furthermore, only 1 (8.3%) survived in stage 3 while 11 (91.7%) died. As the severity of AKI progressed from one stage to another the outcome became poorer, (p<0.151) (Table 3). The mean actual length of stay among AKI patient was shorter (10.2 ± 9.4, Mean±SD) than those without AKI (11.1 ± 10.0, Mean±SD). (p = 0.721). (Figure 1)

The odds of an AKI patient in ICU dying increases with 3 units in the presence of organ system failure (p<0.001), while the odds of a non AKI patient dying in the presence of organ system failure increases by 1.6 (P<0.023). These findings were statistically significant. (Table 4)

Among the 23 (100%) AKI patients in ICU that developed organ system failure, 11 (47.8%) of them survived while 12 (52.2%) died. Furthermore,



Cumulative hazard rate difference for non AKI versus AKI tested by log rank test.

Table 4: Relationship (including regression analysis) between organ system failure, AKI and outcome in ICU

Variables	Outcome			Chi Square	df	P-Value
	Survival n= 15	Dead n=39	Total n=54	X^2		
Organ System failure						
Present	11(47.8)	12(52.2)	23(100)	8.027	1	<0.005*
Absent	4(12.9)	27(85.1)	31(100)			
Variable	Outcome					
	B	S.E		CI		
OSF	2.130	0.056		3.118-22.696		<0.001*
AKI Status	-0.882	0.476		0.163-1.051		0.064

B= Regression coefficient, S.E= standard error, CI= confidence interval, OSF = organ system failure, AKI= acute kidney injury, df = degree of freedom.

** Significant*

among the 31 (100%) AKI patients who did not develop organ system failure, only 4 (12.9%) survived while 27 (87.1%) died. (Table 4)

The mean APACHE IV score of the 100 patients recruited for this study was 58.4±20.0. The range of their APACHE IV score was 14 to 119. The mean APACHE IV score for those patients that died was 66.0±23.2 (Mean±SD), with a median of 57.5 while that of those that survived was 48.2±16.9 , median of 55. This higher APACHE IV score in those patients that died was statistically significant with a p<0.0001, when compared to those patients that survived. There was no statistical difference in the APACHE IV score of patients with AKI, 61.1±24.3 (Mean±SD) when compared to that of those patients who did not develop AKI (55.2±19.9) during their admission in ICU.

The APACHE IV illness severity scores for those that died were high in both groups, i.e. 63.46±24.25 for AKI patients and 54.87±24.0 (Mean±SD) for non AKI patients (Table 5). This difference was not statistically significant (p<0.248 and p<0.765 respectively). This may imply that APACHE IV scoring system could not estimate and differentiate between AKI severity from that of the primary illness of the patients that necessitated their admission into ICU. Also the relationship that exist between APACHE IV illness severity score and AKI

diagnostic criteria (RIFLE and AKIN) for patients across different stages of AKI was not statistically significant. The correlation result for RIFLE was r 0.054, p<0.698 and AKIN was r 0.116, p<0.0404.

Discussion

The magnitude of AKI worldwide is poorly defined due to under reporting, regional disparities, differences in definition, aetiological and environmental factors [21]. In this study, the incidence of 54% was higher than what was reported in this environment previously which was 19.6% [5]. This may be attributed to the use of more sensitive criteria in this study. It is also higher when compared to those from the developed world which ranged from 20- 35% [22]. In our setting, apart from an increased acuity as well as increasing recognition of AKI, most cases needing further close monitoring and interventions are generally managed in the ICU with little triaging, while the comparatively lower incidence in developed countries might be due to active triaging, the use of early AKI diagnostic methods and prompt advance interventions.

The presence of AKI in critically ill patients also conferred on them a poor outcome, with a significant mortality rate of 65% compared to non-AKI patients with a mortality rate of 35 %. This mortality rate was similar to what has been reported from other local studies but lower than what obtains in the developed world which ranged from 75-80% [22]. This might be explained by the relatively large number of patients studied in such works and also the diverse number of cases managed in such ICUs.

The leading cause of admission among the AKI patients in the ICU was majorly surgical in this study as also reported elsewhere. Cases of head injury were the leading primary diagnosis in a quarter of patients and this portends a dismal prognosis especially in the setting of AKI. There have been some clinical and experimental evidence that the traumatized brain especially the cerebral frontal lobe can specifically affect the kidney causing renal cortical ischemia [5]. Jennet et al interpreted some clinical findings in a way that there exists a relationship between AKI and brain injury [5][24]. Furthermore, patients whose cases of head injury are complicated by AKI tends to have higher occurrence of organ system failure and death when compared to those with head injury without AKI in ICU.

The presence of AKI in critically ill patients confers 3 times higher risk of developing other organ system failure apart from kidney failure (OR 3.047, p<0.001) , acute injury often seen in the setting of multiple organ dysfunction is precipitated by a pro-inflammatory mechanism that involves neutrophil cell migration, cytokine expression and increased oxidative stress[25]. Survival after AKI is thus influenced by the severity of the underlying etiological factors and organ system failure in the ICU setting..

Using the RIFLE and AKIN criteria in this study, there were more patients in the early stage of AKI. However, as the severity of AKI progresses, the number of patients reduced, but the outcome in terms of survival and death worsened. This may be due to the severity of their illnesses and higher occurrence of multiple organ system failure in them. Abosaif et al reported that the patients in the failure stage showed the worst parameters with regards to the APACHE criteria as mortality was often increased in them[26].

The shorter duration of stay in ICU by patients with AKI compared with those that did not develop AKI may be due to their earlier exit from ICU as a result of death. This observation was also confirmed along the different stages of AKI. AKI patients in the early stages of AKI either by RIFLE or AKIN criteria had a longer duration of ICU stay compared to their counterparts with AKI at more severe stages of AKI .This might be due that the duration or length of stay of patients in ICU being shortened as as the severity of AKI progressed. A

Table 5: The mean APACHE IV general scores and outcome for AKI and Non AKI patients in the ICU

Variables	AKI (n =54)	Non AKI (n = 46)	Test statistics	df	p-value
Apache IV Score					
x ± SD	61.1 ± 24.3	55.2 ± 19.9	t = 1.315	98	0.192
Alive	54.87 ± 24.0	53.36 ± 20.44	t = 1.169	2	0.248
Dead	63.46±24.25	56.14±19.61	t= 0.300		0.765

df = degree of freedom

similar observation was reported by Hoste et al in which patients with RIFLE class F incur significantly increased length of stay and increased risk of in hospital mortality compared with those in early stages even after adjusting for baseline severity of illness, gender and age [27].

From this study, we could not determine the significance of dialysis therapy because most of our patients could not have adequate dialysis due to a number of factors ranging from delays in the referral to the nephrologist, the patient's heamodynamic status and financial constraints in accessing haemodialytic support. This observation was also reported a decade earlier in a similar work done in the same centre.

Furthermore, the mean APACHE IV of those AKI patients that died was higher than those non AKI patients that died though this difference did not reach a statistically significant level. This is probably because patients with AKI had high APACHE IV scores ab initio due to the severity of their illness.

The standard mortality ratio (SMR) for our patients in the ICU was greater than 1. This was because the actual mortality (60%) that was recorded in this study was higher than the predicted mortality of 40.12%. Furthermore the predicted mean length of stay of our patients in the ICU was shorter compared to the actual length of stay of our patients with SMR ratio greater than 1. This reflects a lower survival rate of our patients than what is expected of them. Similar finding was observed by Dahhan et al [28].

Preventive strategies in ensuring reduction in the high level of morbidity and mortality in critically ill patients generally must therefore be holistic and anticipatory. Those in developing countries must include the need to avoid nephrotoxic precipitants particularly in high risk patients, and aggressive fluid resuscitation of the traumatized patients.

This is one of the few studies that had specifically compared the APACHE IV scores with the newer diagnostic criteria for AKI. Preliminary results have suggested that the scores which measure the severity of illness in the critically ill patients is an important general predictor of outcome in our study population and might thus be routinely employed in the ICU. More studies with a larger number of patients are advocated for a more robust conclusion.

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Critical appraisal of post-repair nosocomial infection: a trigger for failed repair of urinary fistula

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Abstract

Background: Failed fistula repair is an emotive outcome for surgeons and patients. It is usually characterized by anger, frustration and depression. Postoperative urinary tract infection could cause failed repair. Serial urine samples for microscopy, culture and sensitivity with prompt treatment of infection if it exists will usually prevent this unwanted outcome.

Objectives: To describe the pattern of nosocomial infection post-urinary fistula repair among women with failed repair.

Methods: This was a retrospective review of medical records of women with failed urinary fistula from January to December 2012. Of the 25 patients repaired during the period, patient who had failed repair due to proven microbiological specimen urinary infection were adjudged nosocomial infection. The routine practice is to ensure preoperative sterile urine. The same surgeon performed all the surgeries.

Results: Five patients out of the 25 patients operated during the period had evidence of nosocomial infections. The entire urine samples microscopy and culture tests yielded same organism- Klebsiella species; and the sensitivity as well as resistance patterns to antibiotics were the same. We also observed that all women that developed nosocomial infections were nursed on the same ward at the time.

Conclusion: Nosocomial infections could negatively influence the outcome of fistula repair. We recommend that attention should be focused beyond the dexterity of the surgeon but also on drivers of post repair nosocomial infections to reduce the occurrence of failed repair. Training of support staff such as nurses in this highly specialized management is imperative including infection control.

Key words*: Urinary fistula, nosocomial infection, urinary tract infection, urinary infection fistula*

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Résumé

Contexte : L'échec de la réparation de la fistule est un résultat sensible pour les chirurgiens et les patients. Il se caractérise généralement par la colère, la frustration et la dépression. Une infection des voies urinaires postopératoire peut entraîner une défaillance de la réparation. Des échantillons d'urine en série destinés à la microscopie, à la culture et à la sensibilité, avec un traitement rapide de l'infection, si elle existe, préviendront généralement ce résultat indésirable .

Objectifs **:** Pour décrire le schéma d'infection nosocomiale après la réparation de la fistule urinaire chez les femmes dont la réparation a échoué.

Méthodes **:** Il s'agissait d'un examen rétrospectif des dossiers médicaux de femmes atteintes d'une fistule urinaire défaillante de janvier à décembre 2012. Sur les 25 patientes réparées au cours de la période, les patientes dont la réparation avait été manquée en raison d'un échantillon microbiologique prouvé ont été considérées comme une infection nosocomiale. La pratique courante consiste à s'assurer de l'urine stérile préopératoire. Le même chirurgien a effectué toutes les chirurgies.

*Résultats***:** Cinq patients sur les 25 opérés au cours de cette période présentaient des signes d'infections nosocomiales. La microscopie et les tests de culture des échantillons d'urine complets ont révélé le même organisme: l' espèce Klebsiella ; et la sensibilité ainsi que les profils de résistance aux antibiotiques étaient les mêmes. Nous avons également observé que toutes les femmes développant des infections nosocomiales étaient soignées dans le même service à l'époque.

Conclusion: **les** infections nosocomiales pourraient influer négativement sur l'issue de la réparation de la fistule. Nous recommandons que l'attention soit portée au-delà de la dextérité du chirurgien mais également aux conducteurs d'infections nosocomiales post-réparation afin de réduire le risque de défaillance de la réparation. La formation du personnel de soutien tel que les infirmières à cette gestion hautement spécialisée est impérative, y compris la prévention et control des infections.

Mots-clés: *fistule urinaire, infection nosocomiale, infection des voies urinaires, fistule d'infection urinaire*

Introduction

Genital tract fistula is a major reproductive and public health concern that is largely preventable [1]. Of all the causes, obstetric fistula is the commonest and it has been described as a true reflection of the quality of maternity services [1]. In addition, it is also characterized by being young, illiterate, married at an early age, poor, rural and associated with poor access to antenatal care.[2] Nigeria accounts for 40% of the global fistula prevalence and about 12,000 new cases occurring each year.[2,3] According to the 2008 DHS report, the national average of obstetric fistula was 0.4% with a geographic variation of 0.5% in the North and 0.3% in the South. [4] Urinary fistula may also occur as a result of iatrogenic urethro-vaginal fistula following surgical repair of genital tract abnormalities such as transverse vaginal septum [5], or pelvic surgery like hysterectomy.

Surgical intervention as a treatment modality is in itself associated with anxiety for many patients. The patient and relations are also apprehensive of the outcome of surgical repair. A failed fistula repair with leakage of urine is characterized by emotional blow, anger, expression of frustration, and sometimes frank manifestation of mental health problems by the patient [6,7]. The failed repair sometimes sets clients against their health care providers leading to a tensed relationship. The feeling of disappointment by both the woman and health-care team is due to the cost implication of a subsequent attempt, poor outcome of repeat surgery and fear of subsequent surgeries amongst others.

Evidence from previous studies showed that successful fistula repair is usually dependent on factors such as site, size and number of the fistula; extent of vaginal tissue scarring, number of previous repair attempt, route and technique of repair, skill of the surgeon; infection and postoperative nursing and medical care [8-10] In general, wound healing is affected by age, tissue blood supply, tissue oxygenation; infection, viability of tissue, steroid therapy, vitamins and micronutrients, diabetes mellitus and smoking.[11,12]

Nosocomial infections are hospital-associated or hospital-acquired infections [13] and the risk of acquiring any of these infections increases with hospitalization. Other risk factors are surgical wound infection, prosthesis, implants, foreign body and use of other hospital devices [13]. The site of a nosocomial infection depends on the risk factor and type of surgical procedure performed. For example, urinary tract infection could occur in catheterized patients, bacteraemia in intravascular catheter use and pneumonia in ventilated and intubated patients [13]. Urinary tract infection is the second most common nosocomial infection. This usually results from ascending infection following catheterization or following contamination of catheter and urine collection bag [13, 14]. It may be due to ascending infection through the urinary tract or via the urinary drainage system.

Postoperative infection could cause failed repair and serial sampling of the urine for microscopy, culture and sensitivity will lead to early detection and prompt treatment [15] thus preventing failure of a successful repair. Nosocomial infection as a cause of failed repair is sparingly considered and reported. Our initial review of literature revealed that there has not been any proactive intention to analyze all post-repair infection in this direction. This gap is crucial because a failed repair attributable to nosocomial infection is easily avoidable and preventable. This will ultimately improve the quality of care. Furthermore, it could also be an eye opener to ensuring that infection control is taken seriously within the health facility. This case series was undertaken to showcase the significance of nosocomial infection in a tertiary obstetric fistula unit and its implication on outcome of care.

Methods

This was a retrospective study involving a one year review of all fistula repairs performed in 2012 at the University College Hospital Ibadan. The hospital has been offering fistula repair since her inception in 1957 as part of routine gynaecological consultation. In 2008, the genitourinary medicine and urogynaecology unit (GUU) was fully established with the mandate to offer sub-specialized services including Obstetric Fistula Care with four consultant staffs. Two trained nurses in fistula care also joined the unit to compliment the unit activities.

The unit protocol for obstetric fistula management is as follows. Each patient is seen at the weekly outpatient clinic for initial clinical evaluation (history and examination) and thereafter, she is scheduled for a dye test under conscious sedation at the outpatient theatre. On the week of the surgery, routine investigations – haematocrit, urinalysis, group and cross matching of blood and pipette specimen urine for microscopy, culture and sensitivity are performed. Surgery is mostly

performed under regional or general anaesthesia where indicated; and post-operative care administered for 10 to 14 days. In our unit, failed repair is defined as leakage of urine before or after discharge post operatively.

Table 1: Socio-demographic characteristics of the participants

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	35	39	23	35	24
Parity	4	2	0	1	1
No of living Children	3	1	0	0	0
Occupation	Trader	Trader	Student	Trader	Patent medicine attendant
Marital Status	Married/ Supportive	Married/ Supportive	Single	Single	Separated

Table 2: The clinical features of the participants

Type of fistula	Patient 1 Vesicocervical fistula	Patient 2 VVF + Uretero-VF	Patient 3 Urethero-VF + VVF	Patient 4 VVF +RVF	Patient 5 VVF
Antecedent history	Prolonged obstructed labour/ CS	Ruptured uterus/ Hysterectomy	Iatrogenic VVF due to failed vaginoplasty for a Congenital transverse vaginal septum	Prolonged obstructed labour	Prolonged obstructed labour/ CS
Outcome of Pregnancy	Live baby/ Alive & well	Stillborn	-	Stillborn	Live baby/ Early neo-natal death
Duration of fistula (years)	3.5	6	5/12	17	10
No. Previous attempt	1	0	2	3	0
Size of fistula (cm)	1 X 2	4 X 6	3	2X2 VVF 2X2 RVF	6 X5
Location of fistula	Vesicocervical	Vaginal vault	Anterior Vaginal wall	Mid-Vaginal	Mid-Vaginal
Duration of Surgery(min)	220	270	120	180	60
UrinalysisLeucocyte;	Negative	Positive	Negative	Positive	Negative
Nitrites.	Negative	Positive	Negative	Positive	Negative
Onset of postoperative leakage(day)	8th days	2nd day	10th day	3rd day	7th day
Perioperative	Ceftriaxone antibiotic	Amoxycillin + clavulanate	Nitrofurantoin	Nitrofurantoin	Amoxycillin + clavulanate

VVF- Vesico-vaginal fistula
UVF-Uretero-vaginal fistula
RVF-Recto-vaginal fistula
CS-Caesarean section

In 2012, the GUU ward underwent renovation and the unit services were temporarily offered in another ward within the hospital by another set of support staff (nurses and ward assistants). During this period, we reviewed all cases of failed repair out of the 25 women that underwent surgery. Five patients had failed repair that were associated with urinary tract infection (UTI). The medical records of these five patients were further evaluated and the data collected included socio-demographic characteristic, clinical features (type of fistula, size, location; number of previous repair attempt, duration of urine leakage, extent of vaginal scarring, and duration of surgery), and urinalysis with microscopy, culture and sensitivity. In this analysis, UTI was defined as a microbiologically proven urinary tract infection detected from catheter specimen urine sample taken from the third postoperative day or from when urine leakage was detected after repair within the first 10 days of surgery. All the five patients reviewed had sterile preoperative urine microscopy and culture.

Results

Twenty-five women with urinary fistulae were repaired during the period. Five patients had failed fistula repair secondary to post repair urinary tract infections. They were similar in socio-demographic characteristics. The ages of the patients ranged between 23 – 39 years. All the patients were artisans; two of them were still married and had social support from their spouses. (Table 1)

The type and size of fistula, number of previous attempts, and duration of surgery varied among the patients. The duration of surgery varied from 60 to 270 minutes. Two patients had vesicovaginal fistula with either urethral or ureteric involvement and a third patient had combined fistula. Prolonged obstructed labour was the commonest cause with iatrogenic fistula due to caesarean hysterectomy and failed vaginoplasty were seen in two patients. Two of the five patients delivered live babies following prolonged obstructed labour. The onset of urinary leak ranged from the second to the tenth post-operative day. Two patients with urine analysis positive for leucocytes and nitrites had early onset leakage of urine despite a sterile pre-operative urine microscopy. All the patients had prophylactic peri-operative antibiotic therapy. (Table 2)

All patients had preoperative urine microscopy which was sterile and postoperative urine microscopy done between day 3 and day 10 post-operation yielded growth of Klebsiella Species as the major organism in addition to Escherichia coli in two patients. The microbiologic studies among the patients showed the same antibiotic sensitivity and resistance pattern. (Table 3)

Discussion

This study reviewed a common factor among five patients with failed fistula repair who were admitted into the same gynaecologic ward at the same time. We observed that all the women that had failed repair after remaining dry for variable number of days had microbiological evidence of urinary tract infection. Prior to surgery, these five patients had sterile urine samples, suggesting that there was no evidence of either asymptomatic or symptomatic UTI. Furthermore, the urine samples from the five patients grew the same organism, had similar sensitivity and resistance pattern and the culture suggested nosocomial as a possible source. Klebsiella infection is one of the commonest causes of hospital acquired bacterial infection [16].

Although previous studies had focused on other recognized causes of failed fistula repair such as surgical expertise, judgement and appropriateness of technique [17], fistula characteristics (large fistula size, bladder neck lesions, degree of vaginal scarring, reduced vaginal capacity, circumferential fistulae, urethral involvement, previous fistula repair, juxta-urethral fistulae) to determine outcome of care [5,18-21], it may be more important to now focus on other avoidable practices that could potentially mar surgical repair outcome. The presence of urinary tract infection after surgical repair of fistula should be aggressively investigated and promptly treated to avoid the catastrophe of failed repair.

Patients with prolonged catheterization are prone to urinary tract infection and this is why observing universal best practices such as good hydration, antibiotics and observing universal precaution at patient bedside is paramount.[13,22-24] The unit routinely follows strict preoperative, intraoperative and post-operative guidelines to manage patients. We had never experienced such accident and the only suspicion was that the infection coincided with the temporary transfer of post-operative patients to a new ward while the GUU ward was undergoing renovation. The support staffs (nurses and assistants) in this ward had neither recently managed post repair obstetrics fistula patients nor undergone any special training like the trained fistula nurses in the primary ward. However, this mishap led to a multidisciplinary meeting and subsequent crash training for the affected support staffs as an interim measure. Thereafter, none of the

Table 3: Microbiological pattern on Urine Microscopy/Culture/Sensitivity

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Preoperative urine m/c/s:	Sterile	Sterile	Sterile	Sterile	Sterile
Postoperative urine m/c/s: day3	WBC: numerous RBC: 1-2 No growth	WBC:18-20 RBC: 1-2 No growth	WBC: 28-30 RBC: 6-8 No growth	WBC: numerous RBC: numerous No growth	WBC: numerous RBC: numerous No growth
Postoperative urine m/c/s: day 5	WBC: numerous RBC: numerous Klebsiella Spp	WBC: 3-4 RBC: 2-3 Klebsiella Spp	WBC: numerous RBC: numerous Klebsiella Spp	WBC: numerous RBC: numerous No growth	WBC: numerous RBC: numerous No growth
Postoperative urine m/c/s: day 10	–	–	–	WBC: 8-10 RBC: 25-30 No growth	WBC:2-4 RBC: numerous No growth
Postoperative urine m/c/s: > 10 days	–	–	–	Catheter tip Gram negative Klebsiella Spp/ E. coli	Catheter tip Gram negative WBC: ++ Klebsiella Spp / E. coli
Sensitivity pattern	Levofloxacin Nitrofurantoin	Levofloxacin	Levofloxacin Nitrofurantoin	Levofloxacin Nitrofurantoin	Levofloxacine, Ofloxacine, Nalixidic acid, Pefloxacin
Resistance pattern	Amikacin,genticin, cefuroxime, Ceftazidime, Ciprofloxacin,	Amikacin, genticin Ceftazidime, ofloxacine, ciprofloxacin, ceftriaxone, amoxycillin clavulanate	Amikacin,Genticin Ceftazidime,ciprof loxacin,ceftriaxone, ofloxacine, amoxycillin/ clavulanate/	Amikacin, Genticine, Ofloxacine,	Nitrofurantoin, Genticin, Amoxycillin + clavulanate

E. coli- Eschericia coli.

patient operated before relocating to the GUU ward had any noticeable infection. The principles of post-operative care of urinary fistula patients include high fluid intake, adequate urine output and maintain dry beddings. These components of postoperative care require the commitment of nurses trained in fistula care and support staff.

Leakage of urine began in all patients reviewed after the third day except one after 48hours suggesting that closure of fistulous defect in the operating room was successful. The leakage was therefore more likely to have occurred following postoperative infection probably from the new location. In addition, we demonstrated evidence of infection with microscopy culture and sensitivity from the laboratory since clinical features would have been masked by routine antibiotic use. Although, it can be argued that prolonged catheterization (usually after 72 hours) is a potential nidus for urinary tract infection. However, we have always used broad-spectrum antibiotics based on our experience and local evidence of sensitivity pattern without any complication. The risk of infection is higher with transurethral than suprapubic catheter due to the risk of ascending infection. [25]

An unpublished data from the laboratory surveillance annual report of the hospital infection control unit [26] for the period January to December 2012 showed an overall infection rate of 2.9% with a total of seventeen different organisms. The predominant organisms isolated then were Klebsiella spp 31.0%, Staphylococcus aureus 26.8%, Pseudomonas aeruginosa 13.4% and Escherichia coli 12.2% among others. Of all infections caused by Klebsiella spp, majority were surgical wounds followed by urinary tract infections. The most common infection caused by Escherichia coli in the surveillance was urinary tract infection.[26] Escherichia coli was reported in two of the patients with failed repair.

In conclusion, patients on admission or those undergoing surgical procedures in the hospital are at risk of nosocomial infections. The findings from this study suggest that post-operative nosocomial infections could negatively influence the outcome a fistula repair. Intraoperative closure of fistula defect does not in its entirety guarantee successful outcome, a post-operative nursing and supporting care are equally important to achieve the desired surgical outcome. The unfortunate events and experience presented in this review is to raise the awareness of nosocomial infection as an avoidable cause of failed fistula repair among health care providers. In order to prevent a failed obstetric fistula repair, the fistula health providers should consider the drivers of post-repair nosocomial infections repair in their health facility.

We advocate regular training of nurses and support staff in highly specialized fistula management including the use of simple but effective strategies such as universal precaution and infection control. Routine hospital infection surveillance and control should be practiced to prevent avoidable disasters after treatment.

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Formulation and *in vivo* anti-inflammatory properties of diclofenac multiple emulsions prepared using *Vitellaria paradoxa* fat (Shea Butter)

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Abstract
Background: In the present study, diclofenac multiple emulsion was formulated using shea butter (*Vitellaria paradoxa*) as the oil phase and the anti-inflammatory properties of the multiple emulsion assessed in Wistar rats.
*Methods***:** The multiple emulsions were prepared using the double emulsification technique and the properties (mean globule size, viscosity and creaming index) of the emulsions were assessed. The *in-vivo* anti-inflammatory activity of the multiple emulsion was assessed after topical application using two models of inflammation induction, namely formalin-induced paw lick and egg albumin-induced paw oedema.
Results: Stable diclofenac multiple emulsion was obtained with shea butter as the oil phase and surfactant mixtures, Tween 80: Span 80 ratio of 1:1 with water content of 20 %; and Tween 80: Span 80 ratio of 1:1.5 with water content of 25 %. The ranking of inhibition of inflammation after topical application of the formulation was shea butter emulsion < 2 % diclofenac emulsion < standard diclofenac gel < 1 % diclofenac emulsion < shea butter < < No treatment. This indicates that the formulation of shea butter as multiple emulsion significantly ($p < 0.05$) increased its anti-inflammatory properties while diclofenac multiple emulsion gave a dose dependent activity. In addition, the serum myeloperoxidase activity was significantly lower in treated animals compared to untreated animals.
Conclusion: Stable diclofenac multiple emulsions possessing anti-inflammatory activity was successfully developed using shea butter as carrier.

Keywords: *Shea butter, Vitellaria paradoxa, diclofenac, multiple emulsion, anti-inflammatory properties.*

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Résumé
Contexte: Dans la présente étude, des émulsions multiples de diclofénac ont été formulées en utilisant du beurre de karité (*Vitellaria paradoxa*) comme phase huileuse et les propriétés anti-inflammatoires de l'émulsion multiple évaluée chez le rat Wistar .
*Méthodes***:** Les émulsions multiples ont été préparées en utilisant la technique de la double émulsification et les propriétés (taille moyenne des globules, viscosité et indice de crémage) des émulsions ont été évaluées. L'activité anti-inflammatoire *in vivo* de l'émulsion multiple a été évaluée après application topique à l'aide de deux modèles d'induction d'inflammation, à savoir le léchage de la patte induit par le formol et la patte d'œdème induit par l'albumine d'œuf.
Résultats **:** Une émulsion multiple stable de diclofénac a été obtenue avec du beurre de karité en tant que phase huileuse et mélanges tensioactifs, rapport Tween 80 : Span 80 de 1:1 avec une teneur en eau de 20%; et rapport Tween 80 : Span 80 de 1:1,5 avec une teneur en eau de 25%. Le classement de l'inhibition de l'inflammation après l'application topique de la formulation était émulsion de beurre de karité < 2% émulsion diclofénac < gel diclofénac de norme <1% émulsion diclofénac < beurre de karité << Pas de traitement. Cela indique que la formulation de beurre de karité en émulsion multiple a significativement augmenté ($p < 0,05$) ses propriétés anti-inflammatoires, tandis que l' émulsion multiple de diclofénac a donné une activité dépendante de la dose. De plus, l'activité de la myéloperoxydase dans le sérum était significativement plus faible chez les animaux traités que chez les animaux non traités.
Conclusion: Des émulsions multiples stables au diclofénac possédant une activité anti-inflammatoire ont été développées avec succès en utilisant du beurre de karité comme support.

Mots clés : *beurre de karité, Vitellaria paradoxa, diclofénac , émulsion multiple, propriétés anti-inflammatoires*

Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) have been a main-stay in the management of pain occasioned by inflammatory arthritis.

Diclofenac, belonging to the phenylacetic acid class of NSAIDs, has been widely used for the treatment of arthritis but its gastrointestinal (GI) side effects had limited its potential for long term therapy [1]. Diclofenac is also poorly water-soluble especially in acidic medium (about 15 µg/ml) and unstable in aqueous solutions resulting in its poor oral bioavailability [2, 3]. The shortcomings of diclofenac when used orally have necessitated the need for alternative routes of administration with a view to improve its usefulness in the treatment of arthritis for long term therapy.

Topical preparation has proved to be a formulation of choice for both the prescribers and patients. Prescribers can give long term effective drug management of arthritis without the concern of creating other complications and the patients are able to comply with the dosage regimen due to the ease of use, non-invasiveness and minimal side effects, thus optimising therapy. A randomized controlled clinical trial has demonstrated that adverse drug reactions (ADRs) from topical NSAIDs were lower compared to the orally ingested dosage forms [4].

Multiple emulsions are a class of emulsions in which both oil-in-water and water-in-oil emulsions exist simultaneously within a single system and are stabilised by hydrophilic and lipophilic surfactants, respectively [5]. In contrast to macroemulsions, multiple emulsions consist of oil (O) dispersed in water (W) and the emulsion formed is further dispersed in oil making O/W/O or water in W/O/W. This creates the internal, middle and external phases which present an improved opportunity to enclose the active drug. Thus, the active drug contained in the innermost phase is partitioned between several phases (oil, water and emulsifier) which provide an additional reservoir for drug partitioning that would act as depot for gradual release of drugs over a given period [6 - 8] and enhance dermal absorption [9]. The embedded drug in multiple emulsions is released to elicit its therapeutic activity by different mechanisms. The drug moves from the internal phase to the external through the middle layer by diffusion, carrier mediated transport, micelle transport, thinning of oil membrane, rupture of oil phase or solubilisation of internal phase in oil membrane. Diffusion is the most common of all the mechanisms where unionized drug moieties which are hydrophobic in nature diffuse through the semi-permeable liquid membrane which is the oil layer [10]. The drug release rate and effectiveness of such agent are affected by factors such as droplet size, pH, phase volume ratios, viscosity and the nature of entrapped material. Multiple emulsions are promising drug delivery system due to their thermodynamic stability, macroscopic homogeneity, ease of preparation and small droplet size [6].

Shea butter obtained from the kernels of the African Shea tree – *Vitellaria paradoxa* C.F Gaertn (family Sapotaceae) formerly known as *Butyrospermum paradoxum* C.F Gaertn and *Butyrospermum parkii* G. Don [11], which is indigenous to the Savannah belt of Africa; extending from Nigeria and Mali in the West, to Ethiopia and Uganda in the East. Shea butter is an off-white or ivory-coloured fat, which is solid at room temperature but readily softens at body temperature when applied to the skin [12]. Shea butter has a remarkable composition of unsaponifiable fats in comparison with other oils and this portion is responsible for keeping the skin young by stimulating the tissue and helping the skin make its own collagen, making shea butter invaluable in cosmetic industries [13]. Shea butter, due to its high yield and fatty content, has been used traditionally in West Africa as cooking oil. It is used for medicinal purposes such as rheumatism, nasal inflammation, nasal congestion, cough, leprosy and in minor bone dislocation. Shea butter is also used for soothing and accelerating healing after circumcisions and prevention of stretch marks in pregnant women [14]. Studies have shown that Shea butter possesses anti-inflammatory [16], moisturising and skin healing properties [14] and is useful as a vehicle in the delivery of sulphur [15]. Hence, shea butter has the unique potential of being both an active ingredient and excipient. Thus, in the present study, diclofenac multiple emulsions have been formulated for topical application using shea butter as a carrier and the *in vivo* anti-inflammatory activity of the formulations evaluated in Wistar rats in comparison to a marketed brand of diclofenac sodium gel.

Materials and methods

The materials used were diclofenac sodium powder (Caesar and Loretz GmBH, Hilden, Germany), Tween 80 and Span 80 (Sinopharm Chemical Reagent Company Limited, China) and 1 % diclofenac sodium gel (Olfen® gel, Merckle, Ulm, Germany). Shea butter was obtained from local shea butter producer (Alheri Women Co-operative/ Global Shea Alliance, Bosso LGA, Niger state, Nigeria). All other reagents used were of analytical grade.

Preliminary formulation studies

Multiple emulsions were prepared using the double emulsification technique described by Florence and

Whitehill [17]. Preliminary formulation studies were carried out to optimise the composition of the multiple emulsion, temperature, stirring speed, stirring time and phase-volume ratio. The composition of the multiple emulsions is presented in Table 1.

using a heavy-duty laboratory mixer (Model L2R, Silverson Machines Limited, Chesham Bucks, England) for five minutes. The primary emulsion obtained was then re-emulsified in melted shea butter containing a low HLB surfactant, Span 80, also maintained at 50-60 °C, making up the remaining 50

Table 1: Composition and stability of Shea butter multiple emulsion

Code	Tween 80 conc ($\%^{v}/_{v}$)	Span 80 conc ($\%^{v}/_{v}$)	Water content ($\%^{v}/_{v}$)	Shea butter content ($\%^{v}/_{v}$)	Stirring speed (rpm)	Type of instability	Instability observed (days)
D1	2.5	0.5	22.5	74.5	600	Phase separation solidification	3
D2	2.5	1.0	22.5	74.0	600	Phase separation and solidification	3
D3	2.5	2.5	22.5	72.5	600	Solidification	6
D4	2.5	5.0	22.5	70.0	600	Solidification	6
D5	2.5	7.5	22.5	67.5	600	Solidification	6
D6	5.0	2.5	20	72.5	600	Solidification	6
D7	5.0	5.0	20	70.0	600	Stable	-
D8	5.0	7.5	20	67.5	600	Stable	-
D9	7.5	0.5	17.5	74.5	600	Solidification	6
D10	7.5	2.5	17.5	72.5	600	Phase separation	0
D11	5.0	5.0	25	65.0	600	Solidification	20
D12	5.0	5.0	30	60.0	600	Phase separation	2
D13	5.0	5.0	40	50.0	600	Phase separation	0
D14	5.0	7.5	25	62.5	600	Stable	-
D15	5.0	7.5	30	57.5	600	Phase separation	1
D16	5.0	7.5	40	47.5	600	Phase separation	0
D17	5.0	2.0	20.5	72.5	600	Stable	-
D18	5.0	7.5	25	72.5	800	Stable	-
D19	5.0	7.5	25	72.5	1000	Stable	-

Preparation of Vitellaria paradoxa multiple emulsion

Fifty percent of the total emulsion volume was first prepared as the oil-in-water primary emulsion by emulsifying equal volume of melted shea butter with distilled water containing a high HLB surfactant, Tween 80. The oil, water and surfactant were maintained at 60 °C using an electric hot water bath (OC-4743-E, Gallenkamp, England). The emulsification was done under high stirring intensity

% of the multiple emulsion. This was done at low shear rate using a magnetic stirrer (SHC-1 Maple Scientific Instruments, Staffordshire, England) maintained at 600 rpm for five minutes, to avoid rupturing of the primary emulsion globules.

Diclofenac (1 and 2 $\%^{w}/_{v}$) was incorporated into the multiple emulsion by mixing half of the required quantity of diclofenac into the oily phase of the primary emulsion and the other half into the

Table 2: Composition and stability of diclofenac multiple emulsion

Code	Conc. of diclofenac sodium ($\%^{v}/_{v}$)	Tween 80 conc. ($\%^{v}/_{v}$)	Span 80 conc. ($\%^{v}/_{v}$)	Water content ($\%^{v}/_{v}$)	Shea butter content ($\%^{v}/_{v}$)	Type of instability	Instability observed (days)
D20	1	5	5	20	69	Stable	-
D22	2	5	5	20	68	Solidification	4
D23	1	5	7.5	25	61.5	Stable	-
D24	2	5	7.5	25	60.5	Solidification	4

oily phase of the secondary emulsion to obtain an even distribution of the drug within the emulsion system. The composition of diclofenac multiple emulsions are presented in Table 2.

Viscosity measurements

The viscosity of the emulsion was measured using a Brookfield viscometer (DV-2+ Pro, Brookfield Engineering Laboratories Inc., Middleboro, USA) at 100 rpm using spindle size 5. The viscosity of the emulsion was conducted at different time intervals (0, 1, 7, 14 and 30 days).

Phase separation/ solidification

The emulsion was observed visually for signs of phase separation and solidification at different time intervals and the observations were recorded.

Determination of globule size

The globule sizes of the multiple emulsions were determined using a light microscope (Barska Monocular Compound Microscope AY11240, Barska Technology, Pomona CA, USA). A quantity of the multiple emulsion was stained with crystal violet and mounted on the slide to view under the microscope. The diameter for 100 globules was determined at different time intervals and the mean globule size was calculated.

Determination of creaming index

The creaming index was determined using the method described by Odeku *et al* [18]. Briefly, a one in ten dilution of the multiple emulsion was made by diluting 5 ml of the emulsion to 50 ml with distilled water to facilitate discernible differences in the rate and extent of creaming of the emulsions. The diluted emulsions were kept for observation in 50 ml plain bottles and the height of creaming was determined. The creaming index was then calculated from equation 1[19]:

$$Creaming\ index\ (\%) = \frac{Height\ of\ cream\ layer\ (cm)}{Total\ heigh\ t\ of\ emulsion\ (cm)} \times 100 \quad (1)$$

In-vivo *anti-inflammatory studies*

The *in vivo* anti-inflammatory activity of the prepared formulations was carried out on 60 healthy albino Wistar rats of both sexes (99 ± 12 g). The animals were procured from the Faculty of Veterinary Medicine Experimental animal house at the University of Ibadan, Ibadan, Nigeria. The animals were allowed free access to food and water and allowed to acclimatize for 7 days before commencement of the experiment.

The animal experiments were conducted in compliance with the guidelines stated in Principle of Laboratory animal care [20]. The protocols were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/ App/2015/052). The animals were randomized into six different groups as follows:

Group A - negative control (no treatment)
Group B - positive control (brand of 1 %w/w diclofenac gel)
Group C - shea butter alone
Group D - shea butter multiple emulsion
Group E - 1 %w/w diclofenac multiple emulsion
Group F - 2 %w/w diclofenac multiple emulsion

Formalin-induced paw-lick test

The analgesic property of the formulations was determined using the formalin-induced paw-licking test [21]. Briefly, the formulation was gently rubbed into the plantar surface of the left fore-paw for about 30 seconds and 50 µl of 2 % formalin was injected into the sub-plantar surface after 30 minutes. The total paw-lick time by the rats immediately after formalin injection was recorded for both the early phase (0-5 minutes) and late phase (15-30 minutes) inflammation; the early phase shows the initial acute neurogenic nociceptive response while the late phase shows the chronic inflammatory response [21].

Egg albumin-induced paw oedema

The anti-inflammatory activity of the formulations was determined using the egg albumin induced paw oedema method [22]. The rats were pre-treated by gently rubbing the microemulsion into the plantar surface of the right fore-paw for about 30 seconds. Thirty minutes after pre-treatment, the rats were injected with 0.2 ml of undiluted fresh egg albumin into the sub-plantar surface of the rat paw. The paw size (cm) was determined by measuring the circumference of the oedematous paw with a thread wrapped around the paw, which is then placed on a metre rule to determine the diameter. The measurement, which represented the inflamed paw size of the rats [22], was done immediately before egg albumin injection, immediately after injection and at 30 minutes intervals post inflammation induction for a period of 120 minutes. The percentage inhibition was calculated using the equation [23]:

$$\% Inhibition = \frac{[(Ct-Co)\ control-(Ct-Co)\ treated]}{(Ct-Co)control} \times 100$$

(2)

where C_t is the mean paw size at time t and C_o is the initial mean paw size.

Evaluation of serum myeloperoxidase activity

The myeloperoxidase (MPO) activity was determined using the method of Xia and Zweier [24]. Blood collected from the rats were analysed biochemically for the neutrophil infiltration marker-myeloperoxidase. The blood was collected into plain sample bottles and then centrifuged to separate the serum using a centrifuge (Model 80-2, GB Medical Ltd, Hampshire, England,) at 4000 rpm for 30 minutes. The serum was carefully decanted using a Pasteur pipette into labelled plain tubes capped and stored in a freezer for the assay. The reagent (O-dianisidine mixture) for the myeloperoxidase assay was prepared with 16.7 mg O-dianisidine, 100 ml of 0.05 M potassium phosphate buffer and 50 µL of diluted hydrogen peroxide. Two millilitres of O-dianisidine mixture was placed into a cuvette and into it was added 70 µL of the serum sample. The cuvette was then immediately placed into its compartment in the spectrophotometer (Gumpton Medical and Scientific England, Model S23A) and the absorbance read at 450 nm at 30 and 60 seconds. One unit of MPO activity was defined as that degrading one micromole of peroxide per minute at 25 p C and was calculated using equation:

$$MPO\ activity\ \left(\mu \frac{mol}{L}\right) = \frac{Absorbance}{1.13} \times 100$$

(3)

Statistical analysis

Data are presented as mean ± standard error of mean (SEM) except for data of MPO activity, which were expressed as mean ± standard deviation (SD). The differences between groups were analysed using one-way analysis of variance (ANOVA) test while the data for formalin-induced paw-lick test was analysed using t-test. The level of significance was taken as $p \leq 0.05$.

Results and discussion

Preliminary formulation studies

Instability is a common occurrence in emulsion systems because of its heterogeneous nature. This makes optimisation of formulation parameters an important aspect to obtain products that are sufficiently stable over time. The preliminary formulation studies to optimize the surfactant concentration, phase-volume ratio, and secondary emulsification speed, period that the formulations retained its semi-solid consistency and physical form after storage at room temperature (27±2 °C) are shown in Table 1. Shea butter was successfully used in the formulation of multiple emulsions, which were generally creamy-white to white in colour, free flowing, easily spreadable and smooth in texture. Stable diclofenac multiple emulsions were obtained with Tween 80 to Span 80 ratios of 1:1 (5:5 %) and 1:1.5 (5:7.5 %). Increasing the water content from 20 to 25 % reduced the stability of the formulation with surfactant ratio of 1:1 (formulations D7 and D11) but did not affect the stability of formulation containing surfactant ratio 1:1.5 (D8 and D14). Increasing the secondary emulsification speed from 600 to 1000 rpm had no significant effect on physical form of the emulsions (formulations D17 to D19). Thus, two multiple emulsion formulations consisting of Tween 80 to Span 80 ratio of 1:1, water content of 20 % (D7) and Tween 80 to Span 80 ratio of 1: 1.5, water content of 25 % (D14), which exhibited better stability were selected for the incorporation of 1 % (D20 and D23) and 2 % diclofenac (D22 and D24) as shown in Table 2.

Properties of diclofenac multiple emulsion

The properties of diclofenac multiple emulsion shown in Table 2 indicate that incorporation of drugs generally led to a decrease in the viscosity of the multiple emulsion although the viscosity increased with time. Formulations containing 1 %w/v diclofenac exhibited good consistency over the period of study whereas formulations containing 2 %w/v diclofenac showed a significant ($p<0.005$) increase in viscosity to a semi-solid consistency after 4 days. This indicates that increasing the drug content in the emulsion system led to an increase in the viscosity of the formulations. This is because the more solid materials are added, the more viscous the product. Viscosity has been described as the ability of a material to produce internal resistance to friction when one layer of molecules is involved in motion relative to the next, due to attractions between such molecules [25]. Low viscosity formulations have been shown to release the drug faster probably due to faster diffusion of drug through the vehicle with lower viscosity as a result of less resistance to the movement of the molecules of active ingredient [26]. The viscosity of the multiple emulsions presented

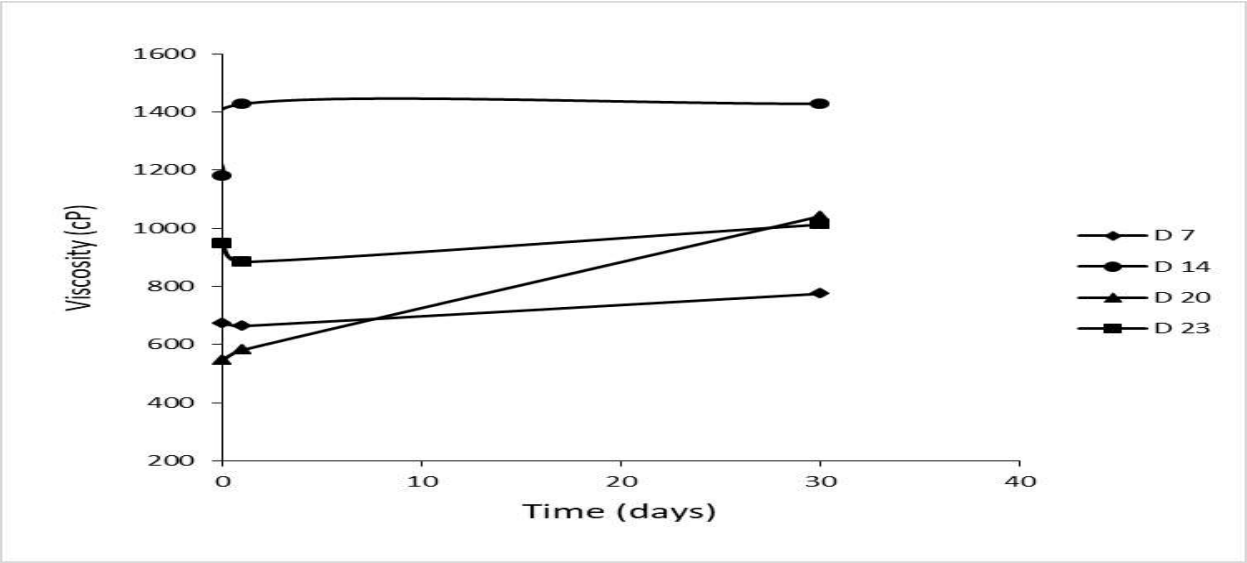

Fig.1: Viscosity changes of unmedicated (D 7 and D 14) and medicated (D 20 and D 23) shea butter multiple emulsion containing different oil:surfactant:water ratio over time.

in Figure 1 indicates that the viscosities of the formulations generally increased with storage.

Formulation with surfactant concentration of 7.5 % and water content of 25 % (D14) showed significantly ($p<0.05$) higher viscosity probably due to a higher concentration of surfactant and water available to facilitate emulsification of the oil. Formulations for topical application should possess adequate viscosity to prevent run-off from the skin surface after application. A major advantage of high viscosity formulations is the ability of such preparations to offer prolonged activity at the site of action because of adherence to the skin [27], which is also desirable since the frequency of application is reduced. Thus, the multiple emulsions possess adequate viscosity to facilitate the adherence of the formulation to the skin.

Increase in globule size due to coalescence has been shown to be a sign of instability and deterioration in disperse systems like multiple emulsions. The results of the mean globule size of the multiple emulsions shown in Figure 2 indicate that the mean globule size increased with time although the increase was generally not significant ($p > 0.05$). Generally, the addition of diclofenac did not appear to have significant ($p > 0.05$) effect on

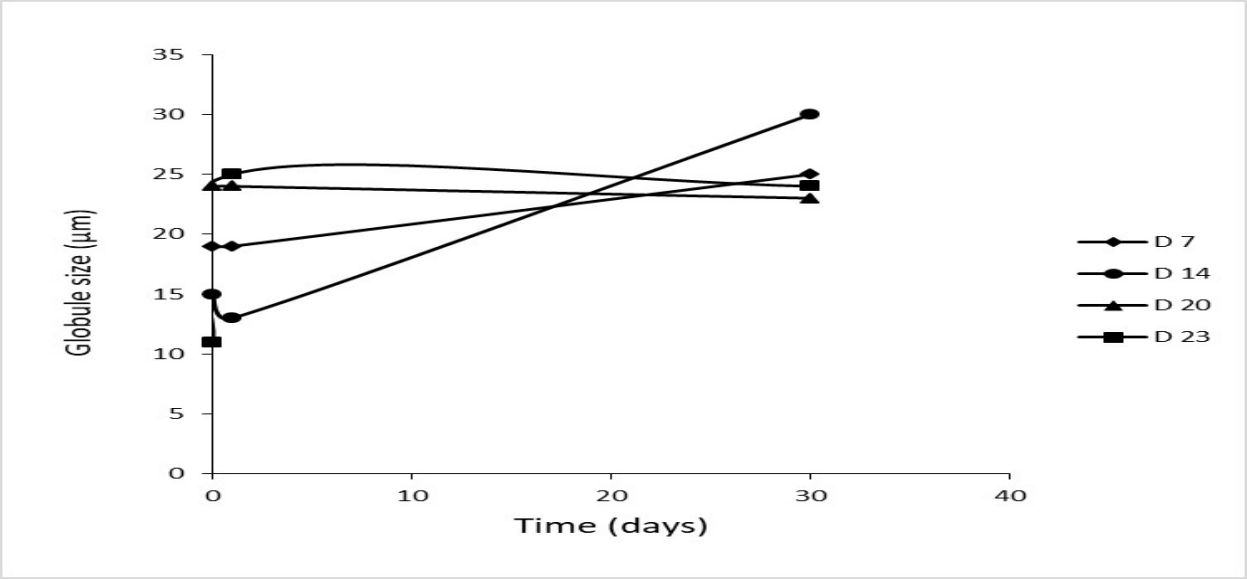

Fig. 2: Globule size of unmedicated (D 7 and D 14) and medicated (D 20 and D 23) shea butter multiple emulsion containing different oil:surfactant:water ratio over time

the globule size of the multiple emulsions. Thus, the emulsion can be said to exhibit reasonable stability.

Creaming is a form of instability that is common in emulsion systems, although a creamed emulsion is not necessarily bad provided it can be re-dispersed with moderate agitation; it is preferable for the emulsion to exhibit low degree of creaming. The result of the percentage creaming in Figure 3 showed that the creaming index of the formulations ranged between 7 to 24 %. The cream was easily redispersible with moderate agitation.

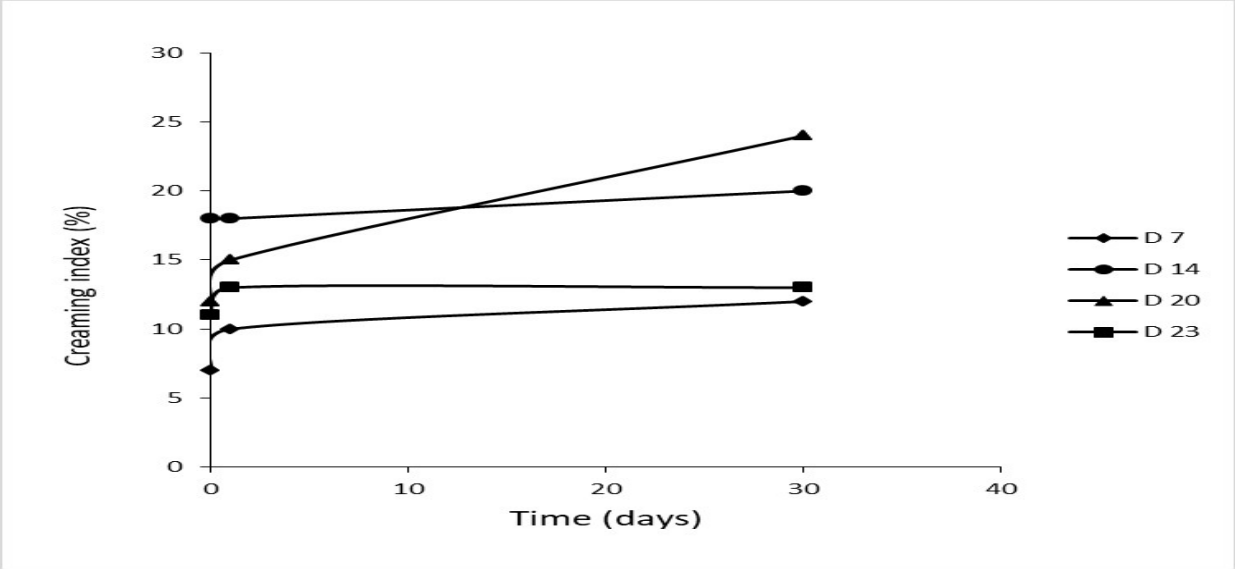

Fig. 3: Creaming index of unmedicated (D 7 and D 14) and medicated (D 20 and D 23) shea butter emulsion containing different oil:surfactant:water ratio over time

Stability studies over a period of twelve months indicated that there were no significant changes in the viscosity, mean globule size and creaming index of the emulsion (data not shown). It appeared that the changes that occurred in the multiple emulsions, i.e. either phase separation or solidification occurred within the first one week after preparation (Table 1). This indicates the relative stability of the multiple emulsions once the appropriate ratio of the oil: surfactant: water is used in the formulation.

In vivo *anti-inflammatory properties of diclofenac multiple emulsion*

The formalin induced paw-link test in mice has been used as a valid and reliable model of inflammation and nociception. Dilute formalin injected into the dorsal surface of the right hindpaw of rats, serves as a harmful stimulus causing an immediate and intense increase in the spontaneous activity of C-fiber afferent and evoke a distinctive and measurable behaviour indicative of pain confirmed by the amount of time the animals spend licking the injected paw [28-30]. Formalin test is a highly specific method which involves a biphasic response identified as: the initial phase (first 5 minutes) as a result of direct stimulation of the paw which gives a neurogenic nociceptive response that is centrally mediated; and the second phase otherwise known as inflammatory response (15 to 30 min after formalin injection) as a result of the peripheral release of pro-inflammatory mediators such as bradykinnin, histamine, serotonin and prostaglandins [21, 31, 32]. Centrally acting analgesics such as morphine, codeine, nefopam and orphenadrine can inhibit both phases; while peripherally acting drugs, such as NSAIDs such as indomethacin and naproxen and the corticosteroids, inhibits only the late phase [33].,

The *in vivo* anti-inflammatory property of the diclofenac multiple emulsions was compared with a known brand of diclofenac sodium topical gel containing 1 % diclofenac sodium and the results of the formalin-induced paw lick test are shown in Table 3. The result showed that the number of paw licking was higher at the late than the early phase. The ranking of the paw licking at the early phase of inflammation was 1 % diclofenac emulsion < standard diclofenac gel < shea butter emulsion < 2 % diclofenac emulsion = No treatment < shea butter. This shows that the 1 % diclofenac multiple emulsion gave higher activity than the standard diclofenac gel, while shea butter emulsion was more active than unformulated shea butter. However, during the late phase, the ranking was shea butter emulsion < 2 %

Table 3: Effect of diclofenac-shea multiple emulsion on formalin-induced paw lick test

Group	Treatment	Number of licking	
		Early phase (0 – 5 min)	Late phase (15 – 30 min)
A	No treatment	72.2 ± 10.0	287.2 ± 29.0
B	Standard (1 % diclofenac gel)	54. 6 ± 14.0	159.4 ± 18.0
C	Shea butter	79.6 ± 13.0	187.2 ± 32.0
D	Shea butter multiple emulsion	65.0 ± 18.0	93.2 ± 18.0
E	1 % diclofenac multiple emulsion	49.4 ± 14.0	172.0 ± 39.0
F	2 % diclofenac multiple emulsion	72.2 ± 7.0	123.0 ± 28.0

Table 4: Effect of formulation on the percentage inhibition of inflammation in rats

Group	Treatment	Inhibition (%)	
		0-5 min	15-30 min
A	No treatment	0	0
B	Standard 1 % diclofenac sodium gel	24	45
C	Shea butter	10	35
D	Shea multiple emulsion	10	68
E	1 % diclofenac multiple emulsion	32	40
F	2 % diclofenac multiple emulsion	0	57

diclofenac emulsion < standard diclofenac gel < 1 % diclofenac emulsion < shea butter < < No treatment. The anti-inflammatory activity of diclofenac multiple emulsion was also found to be dose dependent and was comparable to that of diclofenac sodium gel. The higher concentration of diclofenac in the 2 % formulation was able to increase its anti-inflammatory activity in the late phase compared to the 1 % formulation suggesting prolonged action which could be as a result of the higher viscosity and long contact time.

significantly ($p< 0.05$) higher percent inhibition of inflammation and paw licking than plain shea butter indicating that formulation of shea butter as multiple emulsion significantly increased its anti-inflammatory properties. This is because Shea butter served as the oil phase which has been emulsified using a blend of hydrophilic (tween 80) and hydrophobic (span 80) emulgents. The emulsification produced small droplet sizes of the oil phase distributed within the emulsion system. These small globules offered improved spreadability and penetration into the skin to elicit its activity [9].

Table 5: Effect of formulations on egg albumin-induced paw oedema at various time intervals (mean ± SEM, n=5)

Groups	Treatment	Paw size (cm)			
		30 min	60 min	90 min	120 min
A	No treatment	0.64 ± 0.11	0.72 ± 0.11	0.50 ± 0.15	0.36 ± 0.09
B	Standard diclofenac sodium gel	0.64 ± 0.18	0.46 ± 0.10	0.32 ± 0.09	0.18 ± 0.06
C	Shea butter	0.40 ± 0.08	0.38 ± 0.04	0.36 ± 0.06	0.12 ± 0.08
D	Shea multiple emulsion	0.56 ± 0.04	0.52 ± 0.04	0.60 ± 0.05	0.24 ± 0.09
E	1 % diclofenac sodium multiple emulsion	0.66 ± 0.00	0.48 ± 0.08	0.44 ± 0.07	0.34 ± 0.07
F	2 % diclofenac sodium multiple emulsion	0.66 ± 0.11	0.52 ± 0.10	0.32 ± 0.09	0.18 ± 0.06

The results of the percent inhibition of inflammation for the formulations presented in Table 4 showed that shea butter multiple emulsions gave

Shea butter exhibited remarkable anti-inflammatory action in the late inflammatory phase thus supporting previous claim of its anti-inflammatory activity [12].

In addition, shea butter, its multiple emulsions and diclofenac multiple emulsion formulations produced inhibition at both early and late phases like diclofenac gel. This indicates that the formulations acted both centrally and peripherally, and enhanced effects were observed at the late phase. While the early phase is basically nociceptive (analgesic), the late phase establishes the anti-inflammatory properties of the formulations. Furthermore, NSAIDs such as diclofenac exerts its slower onset of action with the 2 % formulation exhibiting increased anti-inflammatory activity in the later phase of the experiment to give a similar inflammation inhibition comparable with the standard diclofenac gel. This adds credence to the effectiveness of diclofenac sodium multiple emulsion in inhibiting inflammation.

Myeloperoxidase (MPO) is used as a marker in systemic inflammation since it is released into the extracellular fluid in the inflammatory process on

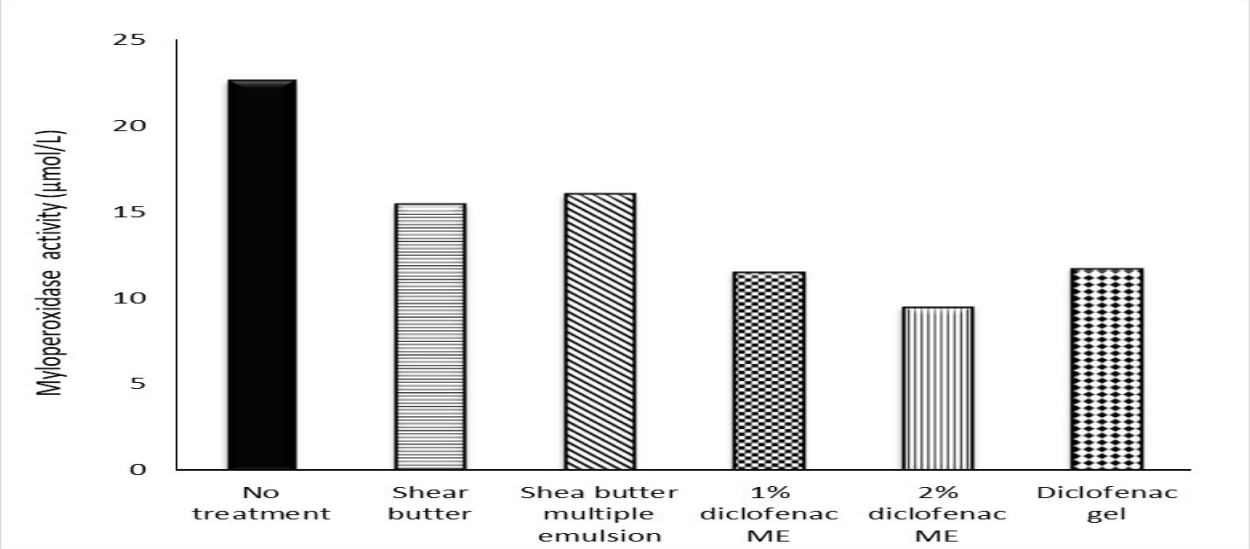

Fig. 4: Myeloperoxidase activity for formalin-induced paw lick test

action in a multimodal and novel mechanism of action indicating analgesic, antipyretic and anti-inflammatory properties [34]. Thus, shea butter elicits it anti-inflammatory effect in similar manner to diclofenac [35]. In addition, shea butter, which serves as the oil phase for encapsulation of the active drug also elicit anti-inflammatory activity leading to increased pharmacological activity.

Egg albumin-induced paw oedema is caused by the release of histamine and 5-hydroxytryptamine, which are mediators of inflammatory response [36]. The effect of formulations on egg albumin-induced paw oedema at varying times is presented in Table 5. The results showed that there was general reduction in paw size of the rats with time. There was a significant difference ($p < 0.05$) between paw size of all the treatment groups compared with the untreated group suggesting the effectiveness of all the formulations in inhibiting inflammation. Similar to the formalin-induced model, shea butter multiple emulsion facilitated higher reduction in the paw size than shea butter. The diclofenac multiple emulsion gave a activation of neutrophils and macrophages. This is achieved by MPO catalysing the conversion of chloride and hydrogen peroxide to hypochlorite which is secreted in inflammatory conditions [37]. Myeloperoxidase is characterised by powerful pro-oxidative and pro-inflammatory properties [38, 39] thereby making it a useful tool in assessing anti-inflammatory processes. The results (Figures 4 and 5) showed significant reduction in activity of the systemic inflammation marker, MPO, in treated groups compared to the untreated group for both models of inflammation induction, suggesting the effectiveness of the formulations in reducing systemic inflammation when applied topically.

Conclusion

Diclofenac sodium multiple emulsions were successfully formulated using shea butter as the oil phase. Stable emulsions were obtained with surfactant ratio of Tween 80: Span 80 of 1:1 and 1:1.5 and water content of 20 and 25 %, respectively. *In vivo* anti-inflammatory studies showed that shea butter and diclofenac multiple emulsions showed

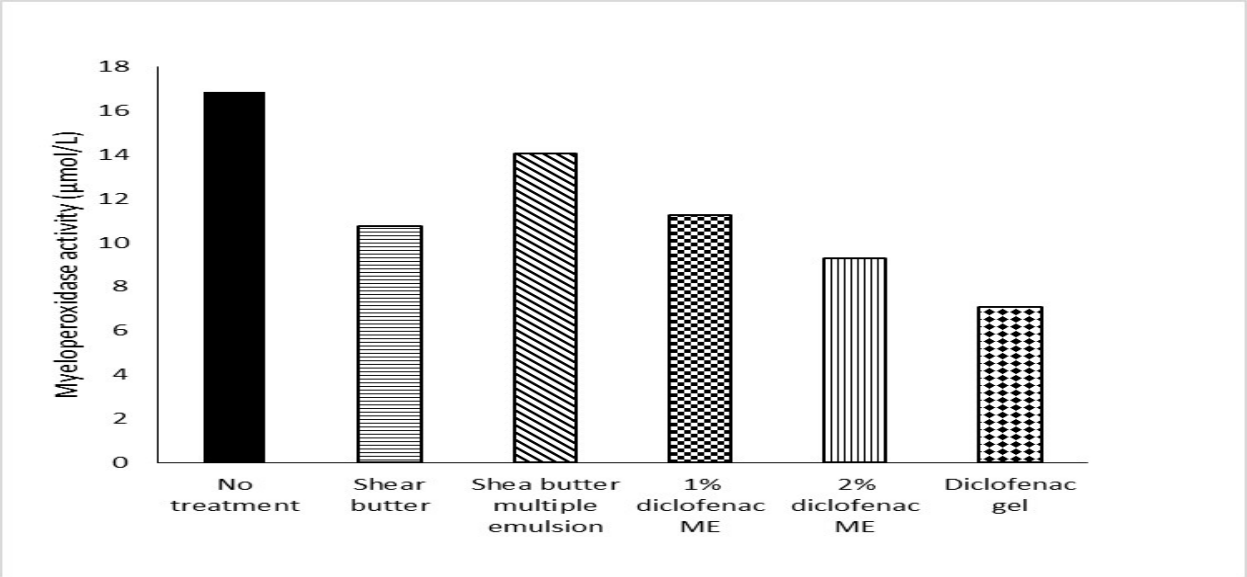

Fig. 5: Myeloperoxidase activity for egg albumin-induced paw oedema

topical anti-inflammatory properties comparable with diclofenac gel. The type and quantity of surfactant employed in the formulation of diclofenac multiple emulsions need to be carefully chosen to enable fast onset of action, high penetration and sustained anti-inflammatory properties.

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Hepatitis C and delta viruses among HBV positive cohort in Abuja Nigeria

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Abstract
Introduction: Hepatitis B virus (HBV) is a transfusion transmissible viral pathogen known to cause chronic liver diseases associated with cirrhosis and hepatocellular carcinoma. The disease becomes more aggressive and severe in Hepatitis B, C and D co or tri-infected population which further complicates treatment options. Therefore, this study aimed to evaluate the burden of HCV and HDV infections among prospective donors tested positive for Hepatitis B surface Antigen (HBsAg) in selected health facilities in Abuja, Nigeria.
Methodology: This cross-sectional study was carried out among 193 (M=99; F=94) consenting HBV infected prospective blood donors, between age range of 18 to 60 years with mean age 31.6 (SD=12.4) years, initially intended to donate blood but were disqualified due to their HBV status in four health facilities in Abuja Nigeria. The demographic and other relevant information were captured using a structured questionnaire anti-HCV, anti-HDV and HBsAg were detected by commercial qualitative ELISA kits according to the manufacturer's instructions.
Results: Overall rates of 5.2% and 5.7% were detected for anti-HCV and anti-HDV among HBV infected cohort respectively. The rates were similar in male (7.1%) for HCV and HDV but higher in male (7.1%) than in female (3.4% and 4.3%) counterparts respectively. Furthermore, the males have 1.5 times higher risk of HCV/HDV with significant association (p=0.0065) than in females (OR=1.47, 95%CI 1.03-2.21) for both infections. The rate (7.7%) for HCV peaked at age group ≤20 years while anti-HDV rate (10.3%) was highest within the age groups 41-50 years. However, HCV/HDV/HBV tri-infection rate (3.6%) was only found in male age ranged 21-30 years. Among other predisposing risk factors for HCV/HDV/HBV co and tri-infections multiple sexual partnership was significantly associated (p<0.0423; OR=1.19, 95%CI 0.93-1.60) However, no significant association (p=0.059; OR=1.06, 95%CI 1.23-2.11) was found between study participants with HCV/HDV/HBV tri-infection and age/sex.
Conclusion: The study identified that the rate of HCV and HDV co-infection was high while tri-infection was rare among the study population. Therefore, blood screening for HCV and HDV is recommended among individuals with chronic HBV infection.

Keywords: *HCV, HDV/HBV co-infection, Tri-infection, Blood donors, ELISA, Abuja, Nigeria*

Résumé
Introduction : Le virus de l'hépatite B (VHB) est un agent pathogène viral transmissible par transfusion connu pour provoquer des maladies hépatiques chroniques associées à la cirrhose et au carcinome hépatocellulaire. La maladie devient plus agressive et plus grave chez les co-patients atteints d'hépatite B, C et D ou les trois infections, ce qui complique encore les options de traitement. Par conséquent, cette étude visait à évaluer le fardeau des infections à VHC et à VHD chez les donneurs potentiels testés positifs à l'antigène de surface de l'hépatite B (AgHBs) dans des établissements de santé sélectionnés à Abuja, au Nigéria.
Méthodologie : Cette étude transversale a été menée auprès de 193 (M = 99; F = 94) donneurs éventuels de sang infectés par le VHB, âgés de 18 à 60 ans et âgés en moyenne de 31,6 ans (SD = 12,4 ans), initialement destinés à ont donné du sang mais ont été disqualifiés en raison de leur statut au VHB dans quatre centres de santé à Abuja au Nigéria. Les informations démographiques et autres informations pertinentes ont été saisies à l'aide d'un questionnaire structuré anti-VHC, anti-HDV et AgHBs ont été détectées par des kits qualitatifs commercial ELISA, conformément aux instructions du fabricant.
Résultats : Des taux globaux de 5,2% et 5,7% ont été détectés pour les anticorps anti-VHC et anti-VHD dans la cohorte infectée par le VHB, respectivement. Les taux étaient similaires chez les hommes (7,1%) pour le VHC et le VHD, mais plus élevés chez les hommes (7,1%) que chez les femmes (3,4% et 4,3%) respectivement. De plus, les hommes

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présentent un risque 1,5 fois plus élevé de VHC / VHD avec association significative (p = 0,0065) que les femmes (OR = 1,47, IC 95% 1,03-2,21) pour les deux infections. Le taux (7,7%) pour le VHC a atteint un sommet au groupe d'âge ≤ 20 ans alors que le taux d'anti-VHD (10,3%) était le plus élevé au sein des groupes d'âge 41-50 ans. Cependant, le taux de tri-infection par le VHC / VHD / VHB (3,6%) n'a été observé que chez les hommes âgés de 21 à 30 ans. Parmi les autres facteurs de risque prédisposant de co et de tri-infections VHC / VHD / VHB, le partenariat sexuel multiple était associé de manière significative (p <0,0423 ; OR = 1,19 ; IC à 95% 0,93-1,60). Cependant, aucune association significative (p = 0,059; OR = 1,06 ; IC 95% 1,23-2,11) a été trouvé entre les participants à l'étude atteints de tri-infection VHC / VHD / VHB et leur âge / sexe.

Conclusion **:** L'étude a révélé que le taux de co-infection par le VHC et le VHD était élevé, alors que l'infection par trois était rare dans la population étudiée. Par conséquent, le dépistage sanguin du VHC et du VHD est recommandé chez les personnes présentant une infection chronique au VHB.

Mots-clés : VHC, co-infection VHD / VHB, tri-infection, donneurs de sang, ELISA, Abuja, Nigeria

Introduction

Hepatitis B virus (HBV), Hepatitis Delta virus (HDV) and Hepatitis C virus (HCV) share some significant similarities such as modes of transmission, considerable global spread, ability to infect the liver and the capacity to induce a chronic infection which may result to liver cirrhosis and hepatocellular carcinoma (HCC) [1]. In 2015, the number of individuals living with chronic HBV and HCV infection globally was estimated to be about 257 and 71 million respectively, of which African region was most affected [2]. Additionally, HBV, HCV and HDV co-existence is common especially in regions with high endemicity for the viruses [3]. Specifically about 5-20% of people with chronic HBV infection are also co-infected with HCV [4,5,6] and an estimated 5% rate of HBV/HDV co and /or super-infection have been reported [7].

According to a WHO report about 1.34 million deaths was caused by viral hepatitis in 2015 while this was similar to the number of deaths from tuberculosis, it was higher than the number of deaths from HIV [2]. Of this number, while HCV was responsible for 30%, HBV and /or HDV accounted for 66% while the remaining 4% was due to other viral hepatitis agents [2]. Reports have suggested an increase in mortality caused by liver diseases in the last two decades making liver cancer the second foremost cause of cancer deaths globally, after lung cancer [2,8]. Specifically, increased and rapid progression rate to complications associated with viral hepatitis has been reported in tri and/ or co-infection than in mono-infection [9].

Although unsafe blood may no longer be a health concern in the developed countries, the situation may be different in low and middle income countries, where the prevalence of transfusion transmissible infections is high [10,11]. Consequently due to the rising health risks associated viral hepatitis with co and/or tri-infections [3], we therefore aimed to assess the rates of HDV and HCV co and/or tri infections in HBsAg positive prospective blood donors in Abuja, Nigeria.

Materials and methods

Study location

A cross sectional sampling method was carried out among consenting prospective unremunerated blood donors who tested positive for HBsAg in a cohort from blood banks of four selected healthcare facilities in Abuja, the Federal Capital Territory of Nigeria. The four locations within Abuja community in which the sampling was done included the following: General Hospital Wuse, General Hospital Nyanya, General Hospital Kuje, and General Hospital Asokoro. The consenting participants were asymptomatic dwellers located in densely populated part of the city who had come to donate blood in the blood bank of the selected health facilities. They claimed not to be intravenous drug users.

Enrolment of the participants

The enrolment of the consenting participants was done between April and October 2016. During this period, a total of 193 HBV-positive cohort including 99 male and 94 female participants, age ranged 18 to 60 years, were enrolled (mean age = 31.6; SD = 12.4). They were unremunerated potential blood donors who were disqualified from donation solely due to their HBV status. A well-structured questionnaire was used to capture demographic and other relevant information from each participant while ethical approval for the study was obtained from the Federal Capital Territory Health and Research Ethics Committee (FHREC/2016/01/24/ 06–04-16).

Sample collection

Blood volume of about 5ml collected by venipuncture from each participant after obtaining their consent and dispensed into an appropriately

labeled EDTA sterile container. These samples were subsequently conveyed to the laboratory in an ice-filled Jablow box. They were centrifuged at a low speed of 500 g for 5 minutes, the plasma was then separated and two aliquots were made into a well-labeled cryovials for each sample using a sterile disposable pipette. Each aliquot was stored at "80°C

performed according to manufacturer's instructions. The optical density was read using the Emax endpoint ELISA micro-plate reader (Molecular Devices, Sunnyvale, CA, USA), and the results were interpreted according to the manufacturer's instructions.

Table 1: Overall seroprevalence of anti-HCV, anti-HDV among HBV positive cohort in Abuja

Age Range (years)	No Tested	No (%) HCV Positive	No (%) HDV-Ab Positive
≤ 20	26	2(7.7)	1(3.8)
21-30	68	2(2.9)	5(7.4)
31-40	54	4(7.4)	2(3.7)
41-50	29	2(6.9)	3(10.3)
≥ 51	16	0(0.0)	0(0.0)
Total	193	10(5.2)	11(5.7)

at the Defense Research Laboratory repository. At the end of the whole sample collection, the stored cryovials were moved using an ice-filled chest container to the Institute for Advance Medical Research and Training, College of Medicine, University of Ibadan, where they were stored at

Statistical analysis

Statistical analyses were performed using SPSS software, version 21. Chi square ($\chi 2$) test was used to determine the association of HCV, HDV, and HBV markers with age and gender. Results were considered statistically significant at $p < 0.05$.

Table 2: Gender distribution of HCV among HBV positive population in Abuja (n=193)

Age range (years)	Male		Female		Total	Total
	No Tested	No (%) Positive	No Tested	No (%) Positive	No Tested	No (%) Positive
≤ 20	10	2(20.0)	16	0(0.0)	26	2(7.7)
21-30	28	2(7.1)	38	0(0.0)	64	2(3.1)
31-40	29	2(6.9)	27	2(7.4)	58	4(6.9)
41-50	20	1(5.0)	9	1(11.1)	29	2(6.9)
≥ 51	12	0(0.0)	4	0(0.0)	16	0(0.0)
Total	99	7(7.1)	94	3(3.4)	193	10(5.2)

p=0.735
(OR=1.49, 95%CI 1.03-2.21)

"80°C until tested using one of the vials while the other set was used for further studies.

ELISA screening for HBsAg, anti-HCV and anti-HDV

All the 193 blood samples were retested for HBsAg to ascertain their true status and subsequently tested for HCV and HDV antibodies using enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic Automation/Cortez Diagnostic, Woodland Hills, California, USA). Both sensitivity and specificity of these test kits are 100%. The assays were

Results

This study reported overall rates of 5.2% and 5.7% for anti-HCV and anti-HDV among HBV infected population respectively (Table 1). The same rate (7.1%) was found in male for HCV and HDV while in female counterparts, the rate detected for HCV (3.4%) was lower than 4.3% found for HDV (Tables 2 and 3). Also by gender, the males have 1.5 times higher risk of HCV/HDV with significant association (p=0.0065) than in females (OR=1.47, 95%CI 1.03-2.21) for both infections respectively (Tables 3). The infection rate (7.7%) for HCV peaked within age

Table 3: Gender distribution rate of anti-HDV among HBV positive population

Age range (years)	Male		Female		Total	Total
	No Tested	No (%) Positive	No Tested	No (%) Positive	No Tested	No (%) Positive
≤ 20	10	0(0.0)	16	1(0.0)	26	1(3.8)
21-30	28	2(7.1)	38	3(5.3)	64	5(7.8)
31-40	29	2(6.9)	27	0(0.0)	58	2(3.4)
41-50	20	3(15.0)	9	0(0.0)	29	3(10.3)
≥ 51	12	0(0.0)	4	0(0.0)	16	0(0.0)
Total	99	7(7.1)	94	4(4.3)	193	11(5.7)

p=0.0065
(OR=0.99, 95%CI 0.63-1.57)

group ≤20 years while that for anti-HDV (10.3%) detected among participants at age groups 41-50 years (Tables 2 and 3). However, HBV/HCV/HDV tri-infection rate (1.6%) was only found in male participants among age group 21-30 years. There were no established significant associations (p=0.059; OR=1.06, 95%CI 1.23-2.11) between study participants with HCV/HDV/HBV tri-infection and age/sex (table 4).

had only primary education, 26(13.5%) did not have any formal education while the rest 66(34.2% and 18(9.2%) had secondary and tertiary education respectively. The subjects also consisted of 74(38.3%) individuals with multiple sexual partners and 119(61.7%) people with one or no sexual partner while 63(32.6%) had history of blood/ and blood products transfusion against 130(67.4%) who did not. Of these predisposing risk factors, multiple

Table 4: Co-infection of HCV/HDV by gender among HBV positive population (Tri-infection)

Age range (years)	Male		Female		Total	Total
	No Tested	No (%) Positive	No Tested	No (%) Positive	No Tested	No (%) Positive
≤ 20	10	0(0.0)	16	0(0.0)	26	0(0.0)
21-30	28	1(3.6)	38	0(0.0)	64	1(1.6)
31-40	29	0(0.0)	27	0(0.0)	58	0(0.0)
41-50	20	0(0.0)	9	0(0.0)	29	0(0.0)
≥ 51	12	0(0.0)	4	0(0.0)	16	0(0.0)
Total	99	1(1.0)	94	0(0.0)	193	1(0.5)

p=0.059
(OR=1.06, 95%CI 1.23-2.11)

Predisposing/risk factors for HDV in HBV and HCV infections

Association of sociodemgraphic profiles of the study participants with HDV in HBV and HCV infections showed that only 54 (28.0%) participants had prior knowledge while 139(72.0%) were ignorant. Fifty(25.6%) participants claimed to have been vaccinated against the virus against 143(74.4%) who were not vaccinated against HBV. Only 60(31.1) of them had no incisions on their bodies while 133 (68.9%) were incised at one point or the other in their lives. A total of 83(43.0%) of the participants sexual partnership was significantly associated (p<0.0423; OR=1.19, 95%CI 0.93-1.60) (Table 5).

Discussion

Dual and triple infections with hepatotropic viruses (HCV, HBV, HDV) are often associated with acute or chronic hepatitis with potential rapid progression to cirrhosis and Hepatocellular Carcinoma (HCC) [3,9]. This study has found overall rates of 5.2% and 5.7% for HCV and HDV antibody respectively among HBsAg positive cohort (Table 1). The rate for HCV/HBV co-infection detected in this study is

Table 5: Association of socio-demographic profiles with predisposing factors for HDV in HBV and HCV infections among the study population

Characteristic	No Tested (N=193)	No (%) Positive	p-value	OR (95% CI)
Age range (years)				
<20	26(13.5)	1(3.8)	0.609	1.22(159-1.91)
21-30	64(33.5)	5(7.8)		
31-40	58(30.1)	2(3.4)		
41-50	29(15.0)	3(10.3)		
≥ 51	16(8.9)	0(0.0)		
Marital status				
Single	62(32.1)	2(3.2)	0.087	1.13((0.78-1.90)
Married	94(48.7)	5(5.3)		
Separated	25(12.6)	3(12.0)		
Widowed	12(6.2)	1(0.0)		
Sex				
Male	99(51.3)	7(7.1)	0.0065*	0.99(0.63-1.57)
Female	94(48.7)	4(4.3)		
Level of Education				
Primary	83(43.0)	4(4.8)	0.215	0.8((0.58-1.88
Secondary	66(34.2)	2(5.0)		
Tertiary	18(9.3)	1(5.8)		
None	26(13.5)	4(16.4)		
Knowledge about hepatitis B/D/C viral infections				
Yes	54(28.0)	2(3.7)	0.370	
No	139(72.0)	9(5.7)		
Vaccination against HBV				
Yes	50(25.6)	3(6.0)	0.152	
No	143(74.4)	8(6.6)		
Having multiple sexual partners				
Yes	74(38.3)	6(8.2)	0.0423*	1.19(0.93-1.6)
No	119(61.7)	5(4.2)		
Have tattoo, incision/tribal in any part of the body				
Yes	133(68.9)	4(3.6)	0,216	
No	60(31.1)	7(11.7)		
History of blood/blood products transfusion?				
Yes	63(32.6)	4(6.3)	0,0721	
No	130(67.4)	7(5.4)		

**p<0.05 was considered statistically significant (using Chi square test)*

5.2% which connotes the fact that both virus share similar route of transmission. This rate while higher than 0.4% reported in a presumed low risk group in Jos [12] falls within the rate of 5.2% reported by Strickland, [13]. Our rate however is lower than the rates of 8.6%-14.5% found in other regions of the country [14,15,16]. Although variation in population may account for this difference, however, improved healthcare delivery which included but not limited to improved screening of blood units and safe needle practices in various health centers over the years may also account for the decline in rate of co-infection [10,11].

Large variations in anti-HDV prevalence across and within countries have been reported [17]. Our study detected, the prevalence of 5.6% for anti-HDV which falls within the range of 2.1% and 12.5% reported in different population groups from different parts of Nigeria [18,19,20]. Although there is low public awareness, medical interest, and research support for HDV co-infection probably due to the gains in HBV control [21]. There is need for renewed

interest in understanding the epidemiology as reports has shown sudden uprising in the prevalence of HDV infection even in regions previously known to be in HBV low endemicity region [22,23]. Specifically improved surveillance for HDV co/superinfection is of a major clinical importance since HDV in HBV causes the most aggressive form of viral hepatitis, with rapid progression to cirrhosis and hepatic decompensation in comparison to other viral hepatitis counterparts [23].

In this study, the rate of HCV and HDV co-infection by gender shows that the males have higher rate of infection (7.1%) than their female (3.4% and 4.3%) counterparts (p=0.0065) for both infections respectively and this is however not significant (Tables 2 and 3). This finding is in concordance with other reports which showed higher prevalence of hepatotropic virus co-infections in male than their female counterparts [5,19,20]. Furthermore differences in life styles of males associated with risky behaviours compared with their female counterparts who are known to eliminate the virus more frequently (24) may explain the reason for this.

Age-wise analysis in the present study found HCV to be higher (7.7%) among individuals belonging to the ≤20years (Tables 2 and 3). While this is in agreement with (16) which reported a lower co-infection rate among younger age group than with older age group for HCV/HBV, this however contrasted with the findings of Ifeorah *et al.* [20] which showed a higher infection rate for HDV in HBV among younger population who are more involved in high risky practices including multiple sexual partnerships (p<0.0423) than their older counterparts (Table 5). Nevertheless, a rate of 10.3% for anti-HDV in found among participants within the 41-50 years age groups agrees with the findings of Opaleye *et al.* [19], indicating that chronicity of HDV infection is proportional with advancement in age. A contrary finding was reported for heterotropic viruses investigated in another population group and this is not in consonance with our findings [1,16]. The reason for this difference seemed unclear but may be attributed to the variations in population involved in these studies among other reasons.

The present study also found HCV/HDV/HBV tri-infection rate of 0.5% (Table 4). This rate is lower but comparable with the findings of 0.7% and 0.8% respectively reported in similar other studies [20,25] among other population groups. The lower rates for triple infection could be associated with various mechanisms which bring about inhibition processes and repression of hepatotropic organisms in different scenario. Specifically while active HCV replication has been suggested to be involved in suppression of replication of other hepatotropic viruses and may dominate in triple infection [26], other reports provide evidence for suppression of HCV replication by HDV and HBV [27,28]. Now that the scenario in which the problems of tri-infection is becoming more obvious thereby making management of the victims more complex, it is therefore necessary for a renewed interest in areas of Hepatitis tri/co infection due to underlining health consequences.

Various confounders have been associated with predisposing/risk factors associated with hepatotropic viral infections including knowledge about HDV/HBV/HCV mode of transmission, vaccination history of HBV, level of education, multiple sexual partnerships, having tattoo, body incisions/tribal marks and history of blood/body fluid transfusions among others. As clearly demonstrated in this study, practice of multiple sexual partnerships was significantly and independently associated with HDV/HBV/HCV transmission (p<0.0423; OR=1.19, 95%CI 0.93-1.60) (Table 5). In line with this norm, Oliveira *et al.* (24) reported a significant association of practice of having multiple sexual partners predisposes such individuals to HDVHBV/HCV infections and in agreement with our study. However, lack of knowledge of the mode of transmission of hepatotropic viruses such as HDV in HBV and HCV helps to maintain the transmission (5,7) particularly among those with risky lifestyle such as injection drug users and others with tattoos, body incisions/tribal marks and blood transfusion. Hence, in places where appropriate laboratory investigations are lacking, many improperly screened blood/blood products may be certified pathogen free and this scenario of public health importance (19).

Conclusion

This study has reported high rates of HCV and HDV co infection in hepatitis B surface antigen-positive cohort and these findings are comparable with high risk population groups from previous studies. The tri-infection rate though low does not under rate the health implication especially with increased incidence of death due to liver cancer as reported by WHO in 2015. We therefore recommend further strengthening of the health care system with regards to viral hepatitis control especially among people already living with one form of viral hepatitis.

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Evaluation of patients's post denture delivery instructions recall ability in a Nigerian population

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Abstract
Aim: To evaluate the recall ability of patients to post-denture delivery instructions and eventual compliance to the instructions in a Nigerian population.
Method: Three hundred and fifty eight consecutive adult patients who consented to participate in the study were recruited from Prosthetic unit of the Dental Centre, University College Hospital, Ibadan, Nigeria over a period of five years. Institutional ethical approval was obtained. The patients were divided into two groups each; verbal (''V'') only and both verbal & written (''V and W'') instructions. The instructions were post-denture delivery (DD) and both the verbal and written instructions were identical. Inclusion into any particular group was done by simple random sampling. Twenty-two stem self and interviewer administered questionnaire was used to evaluate patients' demography, postoperative clinical assessment and assessment of recall abilities of the two groups. Data was analyzed using the SPSS version 20.0 and descriptive statistics was used to summarize the variables. The independent sample student t-test and Chi square were used to test association involving descriptive data and level of significance was set at $P<0.05$.
Result: Recall ability of three elements of the instructions were statistically significantly ($P\leq0.05$) while ten elements were not ($P>0.05$) between the two groups.
Conclusion: For adequate recall ability and eventual compliance to postoperative instructions, the instructions should be simple and in a language that the patient understands clearly with both types (verbal and written) given. A good level of patient-professional interaction should also be established.

Keywords: *Recall, ability, post-denture, delivery, instructions.*

Résumé
Objectif **:** Pour évaluer la capacité de rappel des patients à suivre les instructions relatives à la livraison post-dentier et leur éventuelle conformité aux instructions dans une population nigériane.
Méthode **:** Trois cent cinquante huit patients adultes consécutifs ayant consenti à participer à l'étude ont été recrutés au sein de l'unité de prothèse du Centre Dentaire, Collège Hospitalier, Universitaire d'Ibadan, au Nigéria, pour une période de cinq ans. L'approbation éthique institutionnelle a été obtenue. Les patients ont été divisés en deux groupes chaque : verbal seulement (‘’ V ‘’) et les deux instructions verbales et écrites (‘’ V & W ‘’). Les instructions étaient la livraison post-dentier (DD) et les instructions verbales et écrites étaient identiques. L'inclusion dans un groupe particulier a été réalisée par un simple échantillonnage aléatoire. Vingt-deux branches de questionnaires auto-administrés et par intervieweur ont été utilisés pour évaluer la démographie des patients, l'évaluation clinique postopératoire et l'évaluation des capacités de rappel des deux groupes. Les données ont été analysées à l'aide de SPSS version 20.0 et des statistiques descriptives ont été utilisées pour résumer les variables. L'association impliquant des données descriptives a été réalisée à l'aide du test t d'élève et du chi carré de l'échantillon indépendant, et le niveau de signification a été fixé à $p <0,05$.
Résultat **:** La capacité de rappel de trois éléments des instructions était statistiquement significative ($P\leq0,05$) alors que dix éléments n'étaient pas ($P> 0,05$) entre les deux groupes.
Conclusion **:** Pour une capacité de rappel adéquate et le respect éventuel des instructions postopératoires, les instructions doivent être simples et dans un langage que le patient comprend clairement avec les deux types (verbal et écrit) donnés. Un bon niveau d'interaction patient-professionnel devrait également être établi.

Mots-clés : *Rappel, capacité, post-dentier, livraison, instructions*patient-professionnel devrait également être établi.

Introduction

Inflammation of the mucosa of edentulous areas adjacent to the pontics of a fixed partial denture most probably occurs in response to the accumulation and retention of dental plaque [1]. No statistical relationship was found between denture stomatitis and frequency of teeth brushing and denture cleaning methods but there was a statistically significant

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relationship between denture stomatitis, yeasts' presence and denture cleanliness [2]. Denture plaque containing Candida could cause candidiasis in the form of oral thrush or denture-induced stomatitis and also caries and periodontitis of abutment teeth [3]. Trauma from denture wearing or plaque accumulation on the dentures can induce inflammation of the mucosa [3].

Post operative period is influenced by the understanding of the patient and the subsequent implementation of the guidelines presented by the professional in order to minimize morbidity complications and to improve the quality of life of the patient [4]. Some researchers reported that the main elements that could interfere with the understanding of post operative care instructions are how they are presented by the professional (verbally and /or written) and the socio-cultural level of the patient [5-7]. Compliance or non-compliance to post operative instructions may be influenced by adherence to these instructions and the level of the patients' pre operative anxiety [4]. Factors that may interfere with the extent and quality of information are reported to be how the information is presented, the need to provide additional information, level of preoperative anxiety sociocultural level and age [4].

Atchinson *et al* [8] suggested that there is room for improvement in post operative instructions because of the gaps in patient understanding of postoperative care. More attention is required in the preparation of written educational materials for dental patients to ensure better understanding [9]. Kessel [10] reported that memory for medical information is often poor and inaccurate especially when the patient is old or anxious. He [10] stated that patients tend to focus on diagnosis-related informations but fail to register postoperative instructions and treatment to prevent any possible complication. He [10] further stated that simple and specific instructions are better recalled than general statements and therefore suggested that verbal instructions should be supported with written and or visual materials.

The aim of this study therefore is to evaluate the details of understanding and recall ability of patients to post-denture delivery instructions and eventual compliance to the instructions in a Nigerian population.

Materials and methods

Three hundred and fifty-eight consecutive adult patients aged 18yaers and above, who consented to participate in the study were recruited from the Prosthetic unit of the Dental Centre, University College Hospital, Ibadan, Nigeria over a period of five years. Institutional ethical approval was obtained (UI/EC/10/0035). This was a cohort study and patients were blinded on the group they will belong to by picking ''V'' or ''V & W'' from a ballot box and the patients were subsequently divided into corresponding group that they picked; verbal (''V'') instructions only and both verbal & written (''V &W'') instructions. The instructions were post-denture delivery and both the verbal and written instructions were identical given in English and Yoruba languages being the most common languages spoken where this study was carried out. Inclusion criteria were: patients who consented to participate after full explanation of the study; in good health, not on any routine medication, coherent and first time denture wearers. The exclusion criteria were:; patients with language limitations (English or Yoruba), with underlying systemic conditions that may affect the oral tissues such as Diabetes Mellitus, might have worn dentures before. Twenty-two stem self and interviewer administered questionnaires were used to evaluate patients' demography, postoperative clinical assessment and assessment of recall abilities of the two groups of patients to post-operative instructions. The postoperative clinical parameters were assessed and data collected on the dental chair in the clinic with adequate illumination and dental mirror on the first day of recruitment of the participants into the study. The clinical parameters assessed were gingival inflammation using the PMA (Papillary Marginal Attached) Index (Schour & Massler); mucosal ulceration and hyperplasia, denture cleanliness and fracture in which zero (0) score is when inflammation ulceration or hyperplasia is absent while one (1) is when inflammation, ulceration or hyperplasia is present. Both groups were given the postoperative instructions verbally and explained in the language that they understood while the ''V and W' group was additionally given same instructions in written format. Patients's recall ability was reassessed by their ability recall the instructions correctly when interviewed with direct questions related to the instructions previously given. An examiner was calibrated and trained in the post-operative clinical assessments of the patients and giving the verbal instruction. All the patients were reassessed one month post- denture delivery both clinically and verbally from the instructions in the questionnaire. The clinical reassessment after one month included PMA Index score, presence or absence of mucosal ulceration and hyperplasia, denture cleanliness and whether denture was fractured or not in order to correlate with the instructions while the verbal

reassessment was done by verbal interview using direct questions related to the postoperative instructions previously given.

Data was analyzed using the Statistical Package for Social Sciences (SPSS) Version 20.0 and descriptive statistics was used to summarize the variables. The independent sample student t-test and Chi square were used to test association involving descriptive data and level of significance was set at $P<0.05$.

and written instructions group. One hundred and eighty-eight (52.5%) were males, 162 (45.3%) were females while we had no response from 8 (2.2%). Two hundred and ninety-five (82.4%) had formal education (primary, secondary or tertiary), 47 (13.1%) had no formal education while there was no response from 16 (4.4%). Two hundred and sixty-three (73.5%) are employed, 93 (26%) were housewives or students while we had no response to this from 2 (0.6%). Two hundred and fifty-nine

Table 1: Post-denture delivery clinical findings of participants.

	Verbal only		Verbal and written	
	Present	Absent	Present	Absent
PMA Index(Schour & Massler) before	93(54.7)	77(45.3)	54(28.7)	134(71.3)
PMA Index(Schour & Massler) after	170(100.0)	0 (0.0)	178(94.7)	10(5.3)
Ulceration site	0 (0.0)	170(100.0)	0 (0.0)	188(100.0)
Mucosa hyperplasia	1(0.6)	169(99.4)	0 (0.0)	188(0.0)
Denture condition(accumulation of debris)	Clean	Dirty	Clean	Dirty

Table 2 A and B : Denture and mucosal condition one month post denture delivery

2A

Denture condition	Grp verbal only	verbal & written only	Total	Chi square	P-value
Clean	84	96	180		
	46.7%	53.3%	100.0%		
Dirty	86	91	177		
	48.6%	51.4%	100.0%		
Fractured	0	1	1		
	0.0%	100.0%	100.0%		
Total	170	188	358		
	47.5%	52.5%	100.0%	1.039	=0.595

2B

Mucosal hyperplasia	Group		Total	Chi-square	p-value
	Verbal only	Verbal and written			
None	169	188	357	1.109	0.292
	47.3%	52.7%	100%		
Present	1	0	1		
	100%	0%	100%		
Total	170	188	358		
	47.5%	52.5%	100%		

Results

Three hundred and fifty-eight participants were assessed. One hundred and seventy (47.5%) were in the verbal instruction group and one hundred and eighty-eight (52.5%) were in the combined verbal

(72.4%) were of Yoruba ethnic group where the center of this study is located, 68 (19%) were of Igbo ethnic group while 24 (6.7%) were of Hausa ethnic group.

Tables 3A-D: Ability to Recall Post Denture Delivery Instructions by Participants

Grp What were you instructed to do before eating your food	verbal only	verbal and written only	Total	Chi square	P-value
Feel the food temperature	56	93	149		
	37.6%	62.4%	100.0%		
Unable to recall	49	55	104		
	47.1%	52.9%	100.0%		
Nothing	61	38	99		
	61.6%	38.4%	100.0%	13.786	=0.001
Total	166	186	352		
	47.2%	52.8%	100.0%		

3B

When were u instructed to use your denture	Grp verbal only	verbal and written only	Total	Chi square	P-value
Eat and talk	161	185	346	3.435	=0.064
	46.5%	53.5%	100.0%		
When going out	7	2	9		
	77.8%	22.2%	100.0%		
Total	168	187	355		
	47.3%	52.7%	100.0%		

3C

How often were you instructed to clean your denture	Grp verbal only	verbal and written only	Total	Chi square	P-value
Once daily	101	127	228		
	44.3%	55.7%	100.0%		
Twice daily and rinse	30	31	61		
	49.2%	50.8%	100.0%		
Can't recall	16	11	27		
	59.3%	40.7%	100.0%		
Whenever it is dirty	23	18	41		
	56.1%	43.9%	100.0%		
No response	0	1	1		
	0.0%	100.0%	100.0%		
Total	170	188	358	4.624	=0.328
	47.5%	52.5%	100.0%		

Out of the thirteen post-operative instruction elements that were tested for levels of significance between the two groups of participants, three were found to be statistically significant while ten were not statistically significant, Tables 3A-3D. The recalled instructions that were found to be statistically significant between the two groups were those related to the question '' what were you instructed to do before eating your food?'' with options of ''feel the food temperature''; ''can't remember'' and ''nothing''. The post-operative clinical findings between the two groups were also not statistically significant as shown in Tables 2A and 2B.

Discussion

In this cohort study, out of the total number of 358 participants, 96% (343) had formal education (primary, secondary or tertiary). This shows a high level of percentage of education of the participants in this study. Only three out of the thirteen post-denture delivery instruction elements recall ability

3D

How were you told to prevent your denture from breaking?	Grp verbal only	verbal and written only	Total	Chi Square	P-value
Washing over a bowl of water	169	185	354		
	47.7%	52.3%	100.0%		
Handle carefully	0	1	1		
	0.0%	100.0%	100.0%		
Eating soft diet	0	2	2	2.72	0.257
	0.0%	100.0%	100.0%		
Total	169	188	357		
	47.3%	52.7%	100.0%		

of the participants were statistically significant between the two goups of participants while ten are not statistically significant. In this study, out of the total number of 358 participants, 96% (343) had one formal education (primary, secondary or tertiary). This shows a high level of percentage of education of the participants in this study. This may therefore support the findings of Weiner and Lonitt [10] that education is a poor parameter of the patients' ability to understand medical instructions. Some studies also emphasize that the reading ability or the comprehension of spoken language affects the post operative compliance to the instructions and not the level of education [11,12] Comprehension of written material by patients is often a few grades lower than their level of education [13].

This statistical significance between the two groups that we found may also be due to the fact that the post-denture delivery instructions given were voluminous and the patients may not understand or remember all of them. This may be supported by the reports of Correa *et al* [14] that non compliance to post operative instructions by patients could be because the instructions given may be difficult to remember or comprehend. It is also reported that the ability of the patients to remember instruction is decreased if verbal advice is not reinforced by written instructions. [15]. Inability to remember or understand instructions may result in non compliance and compliance can therefore be improved by ensuring that patients comprehend the instructions [5].

Lack of reinforcement of post operative instructions could also be a factor for non compliance. Compliance with post operative instructions can be improved by ensuring patients comprehension of advice and patient education on the dangers of non compliance [14].

The main elements that could interfere with the understanding of post operative care instructions are how they are presented by the professional (verbally and/or written) and the patients socio-cultural level [5,6]. We did not find any statistical significance in the clinical parameters probably because of the short period of reassessment (one month) which might not have shown enough differences.

We had good compliance to instructions by the participants probably because majoriy (72.4%) of the participants had good understanding of the languages (English and Yoruba) that the instructions were given by the instructors which might have established a good level of patient-professional interaction. This also supports the report that patient –professional interaction and degree of literacy and ignorance of the language are crucial elements in understanding and compliance of the instructions [4-6]. We did not find any statistical significance in the clinical parameters probably because of the short period of reassessment (one month) which might not have shown enough differences

Conclusion.

Within the limits of this study which majorly was the short period of reassessment (one month), it may be concluded that in order to have adequate recall ability and eventual compliance to postoperative instructions, the instructions should be simple and in a language that the patient understands clearly with both types (verbal and written) given. A good level of patient-professional interaction should be established and further research with a longer period of reassessment is suggested

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Comparative evaluation of the effectiveness of verbal and combined verbal and written post-operative instructions following some dental procedures

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Abstract

Aim: To determine recall ability of patients of post-operative instructions and to evaluate differences in compliance to verbal instructions and combined verbal and written instructions.

*Method***:** Eight hundred and fifteen consecutive adult patients who consented to participate in the study were recruited from two units (Oral and maxillofacial surgery (407) and Periodontology (408) of the dental centre, University College Hospital, Ibadan, Nigeria over a period of three years. Institutional ethical approval was obtained. The patients were divided into two groups each by random sampling; verbal (''V'') only and both verbal and written (''V and W'') post-extraction and post- professional oral prophylaxis instructions. Questionnaires (self and interviewer administered) were used to evaluate patients' demography, postoperative clinical assessment and assessment of recall abilities. Data was analyzed using SPSS Version 20.0 and descriptive statistics was used to summarize the variables. Independent sample student t-test and Chi square were used to test association involving descriptive data and level of significance was set at P<0.05.

Result: Recall ability of ten elements of the post-extraction instructions were statistically significant between the ''V and W'' group and the ''V'' group (p values were 0.001, 0.014 and 0.001) while recall ability of the post-oral prophylaxis instruction of two elements between the two groups were not statistically significant (p values were 0.807 and 0.992) while one was statistically significant (p value was 0.036), p value was set at p< 0.05.

Conclusion: The ''V'' and ''W'' group was found to be more effective because it gave more significant differences in the recall abilities between the two groups especially in the post-extraction instructions in this study.

Keywords: *Verbal, written, instructions, dental procedures.*

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Résumé

But: Déterminer la capacité de rappel des patients des instructions postopératoires et évaluer les différences de conformité aux instructions verbales et aux instructions combinées verbales et écrites.

Méthode: Huit cent quinze patients adultes consécutifs ayant accepté de participer à l'étude ont été recrutés dans deux unités (chirurgie buccale et maxillo-faciale (407) et parodontologie (408)) du centre dentaire de l' University College Hospital à Ibadan, au Nigéria, pendant une période donnée. de trois ans. L'approbation éthique institutionnelle a été obtenue. Les patients ont été divisés en deux groupes chacun par échantillonnage aléatoire; instructions verbales (‘’ V ‘’) et verbales et écrites (‘’ V & W ‘’) post-extraction et post-professionnelles de prophylaxie orale. Des questionnaires (auto-administrés et interrogés) ont été utilisés pour évaluer la démographie des patients, l'évaluation clinique postopératoire et l'évaluation des capacités de rappel. Les données ont été analysées à l'aide de SPSS version 20.0 et des statistiques descriptives ont été utilisées pour résumer les variables. Un test t d'élève et un chi carré indépendants ont été utilisés pour tester l'association impliquant des données descriptives et le niveau de signification a été fixé à p <0,05.

Résultat: La capacité de rappel de dix éléments des instructions post-extraction était statistiquement significative entre le groupe «V & W» et le groupe «V» (les valeurs p étaient de 0,001, 0,014 et 0,001), tandis que la capacité de rappel des Les instructions de prophylaxie de deux éléments entre les deux groupes n'étaient pas statistiquement significatives (les valeurs p étaient de 0,807 et 0,992), tandis que l'un était statistiquement significatif (la valeur p était de 0,036), la valeur p était fixée à p <0,05.

Conclusion: le groupe ‘’ V ‘’ et ‘’ W ‘’ s'est avéré plus efficace car il donnait des différences plus significatives dans les capacités de rappel entre les deux groupes, en particulier dans les instructions post-extraction de cette étude.

Mots - clés : *Verbale, écrite, instructions, procédures dentaires*

Pre-operative instructions can generally be enforced easily because non- compliance will lead to delay in the operation which the patient will want to avoid unlike post operative instructions which is very difficult to enforce because the patient has already had the operation [1]. The understanding and adherence to post operative care instructions are factors that aid the recuperation process after any surgical procedure [2]. Post operative period is influenced by patients understanding and subsequent implementation of the post operative instructions in order to minimize morbidity, complications and improve the quality of life [2]. Post operative care instructions reduce post operative morbidity and improve the quality of life during the recovery period [3-6]. Pre operative anxiety is also reported to represent an obstacle for the patients which limits their attention and compliance of any post operative instructions. [3,7-11].

Poor oral hygiene is associated with bacteremia and the clinical implication of the improvement in oral hygiene may reduce the risk of developing infective endocarditis from bacteremia [12[. The incidence of bacteremia following dental procedures such as tooth extraction, endodontic treatment, periodontal surgery and root scaling have been documented [13]. The emphasis in the prevention of infective endocarditis has now shifted from the use of antibiotics prior to dental procedures to the maintenance of good oral hygiene in patients at risk of developing infective endocarditis [14,15]. There are several mechanisms by which dental plaque bacteria may initiate or worsen atherosclerotic process such as activation of innate immunity, bacteria related to dental treatment and direct involvement of mediators activated by dental plaque and involvement of cytokines and heat shock proteins from dental plaque bacteria [16].

Post operative treatment with medication such as analgesics and antibiotics is documented in the literatures while less attention has been given to patient-surgeon relationships, the mode of transmission of post operative instructions from the surgeon to the patient and how the patient understands the instructions and applies them correctly [17]. Non compliance to post operative instructions by patients could be because the instructions given may be difficult to remember or comprehend [1].

The ability of the patients to remember instruction is decreased if verbal advice is not reinforced by written instructions [1]. Inability to remember or understand instructions may result in non compliance and compliance can therefore be improved by ensuring that patients comprehend the instructions [1]. Factors that may interfere with the extent and quality of information are reported to be how the information is presented, the need to provide additional information, level of preoperative anxiety, sociocultural level and age [2]. Compliance or non compliance to post operative instructions may be influenced by adherence to these instructions and the level of the patients pre operative anxiety [2]. The improper use of antibiotic medication by the patients is reported to be primarily due to popular beliefs and ignorance about the prescribed medication [18]. It is reported that patients prefer both verbal and written information about the medication that was prescribed to them [19]. Alvira Gonzalez and Gary –Escoda [2] reported no statistical differences between adherence to post operative guidelines or methods by which information was presented to the patient. Kasse [7] reported that patients forget 40% to 80% of the information given to them by professionals almost immediately depending on the socio-cultural level and age as influencing factors in comprehension and implementation of post operative instructions.

The aim of this study therefore was to assess the level of significance of recall abilities between participants that received verbal (V) instructions only and those that received both verbal and written (V and W) instructions and compliance of the participants to the postoperative instructions because these have been reported to aid the recuperation process after any surgical procedure [2]. .

Materials and methods

Eight hundred and fifteen (815) consecutive adult patients aged 18yers and above, who consented to participate in the study were recruited from two units (Oral and Maxillofacial surgery (407) and Periodontology (408) of the Dental Centre, University College Hospital, Ibadan, Nigeria over a period of three years. Institutional ethical approval was obtained. The participants were divided into two groups by simple random sampling each; participants were blinded on the group they will belong to by picking ''V'' or ''V & W'' from a ballot box and they were subsequently divided into corresponding group that they picked. An examiner from each of the units were calibrated and trained in the post-operative clinical assessments, administration of the questionnaire and assessment of the call abilities of the participants. The instructions were standard instructions that are usually given to patients after these procedures (extraction and oral prophylaxis); both the verbal and written instructions were

administered in English and Yoruba languages been the most common languages spoken in the location of this study. In order to be sure that the participants understood the instructions, they were asked questions that are related to the instructions given immediately the instructions were given. The interviewed questions to assess participants recall abilities post-oral prophylaxis were; ''How soon were you instructed to start the WSMW (warm saline mouth wash)?''; ''How often were you instructed to use the WSMW?'' and ''what else did you use in addition to the WSMW?''. The interviewed questions to assess the participants recall abilities post-extraction were; ''How did you prepare the WSMW?''; When did you start the WSMW?'' and How often did you do the WSMW?. Inclusion criteria were: patient consenting to participate; no underlying systemic conditions that may influence the postoperative clinical outcome and coherent; patient wouldn't have done scaling and polishing in the past one year and patient wouldn't have had tooth extraction done in the last one year.

The exclusion criteria were: patients with language limitations; patient must have had scaling and polishing or tooth extraction done in the past one year. Self and interviewer administered questionnaires (17 stem for post- extraction & 15 stem for post-professional oral prophylaxis) were used to evaluate patients' demography, postoperative clinical assessment and assessment of recall abilities of the two groups of patients to post-operative instruction differently for the two procedures. The number of items on the questionnaire for the post-extraction instructions were more than the post-oral prophylaxis because the extraction procedure is more invasive and deeper structures like the alveolar bone might be tampered which will affect the extraction socket healing. All the extraction patients were reassessed one week post-extraction clinically for socket cleanliness, infection, dryness, pain, bleeding, inflammation, necrosis and healing status and both the extraction while oral prophylaxis patients were also clinically reassessed post-operation for level of oral hygiene and gingival inflammation. Both the extraction and oral prophylaxis patients were assessed at this one week post-operative period for their recall abilities of the respective instructions through interview by the trained and calibrated examiners.

Data was analyzed using the Statistical Package for Social Sciences (SPSS) Version 20.0 and descriptive statistics was used to summarize the variables. The independent sample student t-test and Chi square were used to test association involving descriptive data and level of significance was set at $P<0.05$.

Results

Post professional oral prophylaxis instructions

Four hundred and eight participants were assessed. Two hundred and one (49.3%) were in the verbal instructions only while 207 (50.7%) were in the verbal and written instructions group. One hundred and sixty six (40.7%) were males while 242 (59.3%) were females. Three hundred and seventy-three (91.4%) had one level of education or the other, 23 (5.6%) had no education while we had no response on this question from 12 (2.9%). Three hundred and five (74.8%) are employed while 103 (25.3%) were housewives or students.

No statistical significant difference was found in the recall abilities of the ten options in the interviewed questions between the two groups (V; V and W); Tables 1A-C.

Post-extraction instructions

Table 1 A-C : Recall Abilities to Some Post- Oral Prophylaxis Instructions by the Participants.

Table 1A How soon were you instructed to start the WSMW	Group Verbal and Written	Verbal only	Total	Chi square	P-valve
As soon I get home	190	195	385		
	49.4%	50.6%	100.0%		
24hrs after	5	5	10		
	50.0%	50.0%	100.0%		
One week after	0	1	1		
	0.0%	100.0%	100.0%	0.977	0.807
Unable to Recall	6	6	12		
	50.0%	50.0%	100.0%		
Total	201	207	408		
	49.3%	50.7%	100.0%		

Four hundred and seven participants were assessed. Two hundred and twenty-one (54.3%) were in the verbal instruction group and 186 (45.7%) were in the combined verbal and written instructions group. One hundred and eighty-seven (46.6%) were males, 218 (53.6%) were females while we had no response from 2 (0.5%). Three hundred and fifty- three (86.7%) had one level of education or the other, 50(12.3%) had no formal education while there was no response from 4 (1%). Two hundred and sixty-three (64.6%) are employed, 93 (22.9%) were housewives or students while we had no response to this from 2 (0.5%).

Table 1B

What else did you use in addition to the WSMW?	Group verbal and written	Verbal only	Total	Chi square	P-value
Hydrogen peroxide	1	2	3		
	33.3%	66.7%	100.0%		
Others	0	5	5		
	0.0%	100.0%	100.0%	6.647	0.036
None	217	175	392		
	55.4%	44.6%	100.0%		
Total	218	182	400		
	54.5%	45.5%	100.0%		

Table 1C

How often were you instructed to use WSMW	Group Verbal and written	Verbal only	Total	Chi square	P-value
After each meal	86	89	175		
	49.1%	50.9%	100.0%		
Once daily	31	31	62		
	50.0%	50.0%	100.0%		
Twice daily	84	87	171		
	49.1%	50.9%	100.0%	0.016	=0.992
Total	201	207	408		
	49.3%	50.7%	100.0%		

Tables 2 A-C Recall Abilities to Some Post-Extraction Instructions by Participants.

Table 2A

How did you prepare your WSMW	Group verbal only	verbal and written	Total	Chi square	P-value
Half tea spoonfull of	87	104	191		
salt & warm water	45.5%	54.5%	100.0%		
Half tea spoonfull of	22	5	27		
salt & hot water	81.5%	18.5%	100.0%		
Half tea spoonfull of	0	4	4	22.208	<0.001
salt & warm tea	0.0%	100.0%	100.0%		
One tea spoonfull of	112	71	183		
salt & warm water	61.2%	38.8%	100.0%		
Total	221	184	405		
	54.6%	45.4%	100.0%		

The one week clinical assessment of extraction sockets healing status of ‘’V & W” was satisfactory in 68.3% and unsatisfactory in 31.7% of the participants (Fig 1), 70.6% of the sockets were clean, 22.2% dirty and none of the sockets were dry nor bleeding (Fig 2).

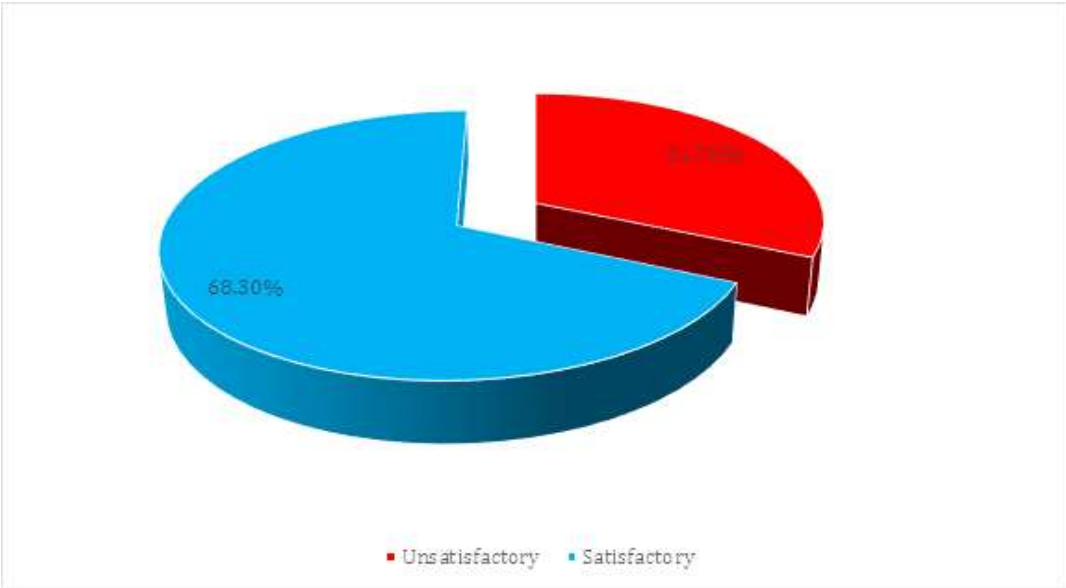

Fig. 1: Extraction Socket Healing Assessment In The ''V and W'' Group

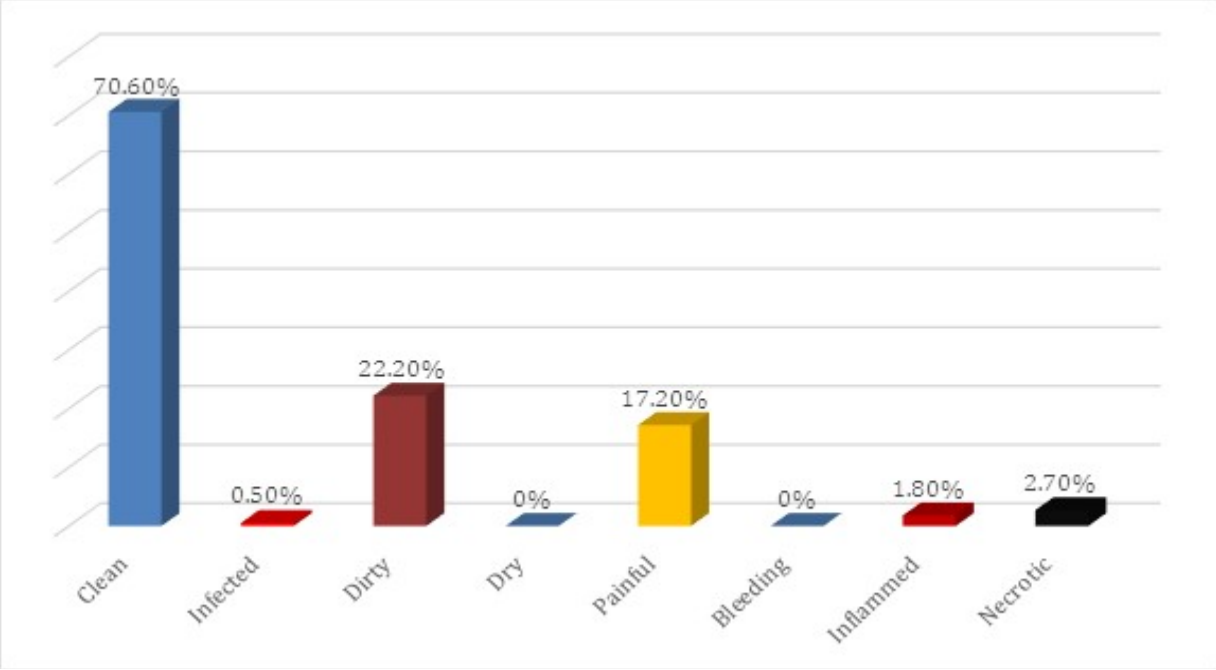

Fig. 2: Extraction Socket Clinical Features In ''V and W'' group

The recall abilities of the participants of all the ten options related to the interviewed post-extraction instructions between the two groups (V; V and W) were statistically significant, tables 2A-C. In post-extraction instruction, 78.7% of the ''V and W'' were able to correctly recall the instructions compared to 73.3% of the ''V'' group, while 89.2% of the ''V and W'' complied with the post-extraction instructions, 83.4% in the ''V'' group. The actual proportions that recalled the instructions rightly were 52.3% and 52.4% in the ''V and W'' group compared to 47.8% and 47.6% in the ''V'' group for both post-extraction and post-oral prophylaxis instructions respectively.

Discussion

We found a significant difference between the recall abilities of the post-extraction instruction participants that received both the verbal and written (''V and W'') and those that received the verbal

Table 2B

When did you start WSMW?	Group Verbal and written	Verbal only	Total	Chi square	P-value
As soon as I got home	10	20	30		
	33.3%	66.7%	100.0%		
24hrs later	210	162	372		
	56.5%	43.5%	100.0%	5.988	0.014
Total	220	182	402		
	54.7%	45.3%	100.0%		

Table 2C

How often did you do the WSMW?	Group verbal and written	Verbal only	Total	Chi Square	P-value
Once daily	21	0	21		
	100.0%	0.0%	100.0%		
3times daily	1	7	8		
	12.5%	87.5%	100.0%		
Before & after meals	196	160	356	33.682	<0.001
	55.1%	44.9%	100.0%		
Unable to Recall	3	15	18		
	16.7%	83.3%	100.0%		
Total	221	182	403		
	54.8%	45.2%	100.0%		

(''V'') instructions alone. This finding may be supported with the report of Blunder *et al* [17] in their study that recommended verbal and written post operative instructions and that verbal explanation alone are insufficient while written instruction is mandatory.

In this study, we also found that a higher percentage of the ''V and W'' (78.7%) participants were able to correctly recall the post-extraction instructions than 73.3% of the ''V'' participants and the corresponding compliance to these instructions by these groups were 89.2% and 83.4% respectively. This may agree with the findings of Adebayo and Dairo [20] who reported that provision of both written and verbal postoperative instructions to patients after minor oral surgery enhances compliance. Vallerrand *et al* [3] reported that providing post operative instructions both verbal and written improved post operative instruction compliance after third molar surgery. Recovery from the pre operative state, from a patients perspective signifies a return to normal function and they feel that they have recovered therefore they do not have to comply with the post operative instructions [3]. Lack of reinforcement of post operative instructions with written instruction could also be a factor for non compliance [17].

Compliance with post operative instructions can be improved by ensuring patients comprehension of advice and patient education on the dangers of non compliance [13]. While some authors [8,9] reported that verbal instructions alone were ineffective, we found both forms to be effective because of the high percentages of the correctness in the recall abilities and the level of compliance of both groups except that the ''V&W'' group showed a more significant result than the ''V'' group. Houts *et al* [21] also reported that patients in their study remembered only 14% of the information when given orally, this is however not the case in this study in which the correct recall abilities of the participants in both groups was between 73.3% and 78.7% for the ''V'' and ''V and W'' respectively which may be due to a better patient-professional relationship established during the interview administration of the questionnaires. The increased patient compliance in this study may also be as a result of possible reduction in their level of anxiety due to proper explanation of importance of complying with the post-operative instructions. This supports

Alexander's [22] suggestion that adapting the instructions to the needs of each patient or case (especially regarding limitations of understanding certain terms and language of ignorance) reduces patient anxiety and encourages adherence to the instructions.

Akpata *et al* [23] also reported significant association of non-compliance to post extraction regimen and incidence of localized alveolar osteitis. They emphasized the need to properly educate patients on the effect of compliance to various combination of post extraction regimen in reducing the incidence of localized alveolar osteitis. In this study 86.7% and 91.4% of the post-extraction instructions and post-professional oral prophylaxis participants respectively had one level of education or the other which could also be responsible for their comprehension of the instructions and afterward correctness in the recall ability and compliance to the instructions. This may corroborate Atchinson *et al* [8] suggestion that there is room for improvement in post operative instructions because of the gaps in the patient understanding of post operative care. We did not find any statistically significant differences in the correctness in recall ability and compliance levels between the two groups (''V and W'' and ''V'') in the post-professional oral prophylaxis instructions participants in this study. This is probably because the procedure is less invasive, the instructions are less voluminous and this procedure is frequently done by people. The participants also might have received less apprehensive post-operative reports from previous patients which might have led to less anxiety and subsequent compliance by participants in this study.

Conclusion

Both types of postoperative instructions (''V'' and ''W'') gave significant difference of recall abilities than the verbal (''V'') instructions alone and the ''V and W'' group had appreciable satisfactory post-extraction clinical features in this study. Recommendation: In order to have adequate recall ability and eventual compliance to postoperative instructions especially for more invasive procedures such as teeth extractions, a good level of patient-professional interaction should be established and both types of instructions (''V'' and ''W'') should be given.

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Enamel defects in primary teeth: A study among 4-year-old Nigerian children

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Abstract

Objective: To assess the prevalence, extent and risk factors of enamel defects in the primary dentition of 4-year-old Nigerian children.

Methodology: Three hundred and two healthy 4-year-olds were divided into 4 groups according to fluoride concentration in ground water supplies. Clinical dental examinations were undertaken under field conditions using dmft, modified Developmental Defects of Enamel (DDE) and Thystrup and Fejerskov indices. Samples of toothpaste, drinking and cooking water were analysed for fluoride concentration. Parents/legal guardians provided information on infant/childhood diseases, infant feeding and tooth cleaning practices. Descriptive analyses were undertaken and association between categorical variables were performed using chi-square. Differences between means (> 2 groups) were performed using ANOVA. Correlation and binary regression were also used as appropriate.

*Results***:** The overall mouth prevalence of DDE was 77.8%, which was high. The mean (SD) number of teeth affected was 4.0 (3.33) teeth. Dental fluorosis mouth prevalence was 5.6% with a mean (SD) of 0.24 (1.26) teeth affected. Prevalence and extent of DDE and dental fluorosis were greater in higher water F area than lower water F area ($p<0.001$). A weak positive correlation was seen between extent of dental fluorosis and drinking water F concentration ($\rho = 0.12$). The amount of toothpaste used per brushing and fluoride toothpaste exposure were statistically significant predictors of dental fluorosis with Odd Ratio of 9.66 (CI = 1.28 – 73.16) and 0.03 (CI = 0.02 – 0.70) respectively.

Conclusion: The overall mouth prevalence of DDE was 77.8%, which was high. The mean (SD) number of teeth affected was 4.0 (3.33) teeth. The corresponding figures for dental fluorosis and dental caries were 5.6% and 0.24 (1.24) and 10.6% and 0.29 (1.07) respectively. There was an association between prevalence of DDE and dental fluorosis and fluoride concentration in water and a weak \

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relationship between extent of dental fluorosis and fluoride exposure in drinking water. Other environmental factors such as a history of infant/ childhood disease and fluoride toothpaste use were positive predictors of DDE and dental fluorosis respectively.

Keywords: *Developmental enamel defects, dental fluorosis, dental caries, primary teeth, children, Nigeria*

0.02 – 0.70) respectively.

Conclusion: The overall mouth prevalence of DDE was 77.8%, which was high. The mean (SD) number of teeth affected was 4.0 (3.33) teeth. The corresponding figures for dental fluorosis and dental caries was 5.6% and 0.24 (1.24) and 10.6% and 0.29 (1.07) respectively. There was an association between prevalence of DDE and dental fluorosis and fluoride concentration in water and a weak relationship between extent of dental fluorosis and fluoride exposure in drinking water. Other environmental factors such as a history of infant/childhood disease and fluoride toothpaste use were positive predictors of DDE and dental fluorosis respectively.

Keywords: *Developmental enamel defects, dental fluorosis, dental caries, primary teeth, children, Nigeria*

Résumé

Objectif **:** Pour évaluer la prévalence, l'étendue et les facteurs de risque de défauts d'émail dans la dentition primaire des enfants nigérians âgés de 4 ans.

Méthodologie: Trois cent deux enfants de 4 ans en bonne santé ont été divisés en 4 groupes en fonction de la concentration de fluorure dans les eaux souterraines. Des examens dentaires cliniques ont été entrepris sur le terrain en utilisant les indices dmft, de défauts du développement de l'émail (DDE) et de Thystrup et Fejerskov. Des échantillons de dentifrice, d'eau de boisson et de cuisson ont été analysés pour déterminer leur concentration en fluorure. Les parents / tuteurs légaux ont fourni des informations sur les maladies infantiles, l'alimentation de l'enfant et les pratiques de

nettoyage des dents. Des analyses descriptives ont été entreprises et l'association entre les variables qualitatives a été réalisée à l'aide du chi-carré. Les différences entre les moyennes (> 2 groupes) ont été réalisées à l'aide d'une ANOVA. La corrélation et la régression binaire ont également été utilisées, le cas échéant.

*Résultats***:** La prévalence globale du DDE dans la bouche était de 77,8% , ce qui était élevé. Le nombre moyen (ET) de dents touchées était de 4,0 (3,33) dents. La prévalence buccale de fluorose dentaire était de 5,6% avec une moyenne (ET) de 0,24 (1,26) dents affectées. La prévalence et l'étendue de la DDE et de la fluorose dentaire étaient plus élevées dans les zones F à fort débit d'eau que dans les zones F à faible débit d'eau ($p<0,001$). Une faible corrélation positive a été observée entre l'étendue de la fluorose dentaire et la concentration de F dans l'eau de boisson ($\rho = 0,12$). La quantité de dentifrice utilisée par brossage et l'exposition au dentifrice au fluorure étaient des facteurs prédictifs statistiquement significatifs de la fluorose dentaire avec un rapport impair de 9,66 (IC = 1,28 - 73,16) et 0,03 (IC = 0,02 - 0,70) respectivement.

Conclusion: La prévalence globale du DDE dans la bouche était de 77,8% , ce qui était élevé. Le nombre moyen (ET) de dents touchées était de 4,0 (3,33) dents. Les chiffres correspondants pour la fluorose dentaire et les caries dentaires étaient de 5,6% et 0,24 (1,24) et 10,6% et 0,29 (1,07) respectivement. Il existait un lien entre la prévalence du DDE et la fluorose dentaire et la concentration de fluorure dans l'eau et une faible relation entre l'ampleur de la fluorose dentaire et l'exposition au fluorure dans l'eau de boisson. D'autres facteurs environnementaux tels que des antécédents de maladie infantile / infantile et l'utilisation de dentifrice au fluorure étaient des prédicteurs positifs de la DDE et de la fluorose dentaire, respectivement.

Mots-clés : *Défauts développemental de l'émail, fluorose dentaire, carie dentaire, dents primaires, enfants, Nigeria*

Introduction

Enamel defect, developmental or acquired is a major public health problem worldwide. In developed countries, dental caries, an acquired enamel defect is known to be a public health problem especially among children where it affects 60 – 90% of school children [1] however, the prevalence is said to be declining due to increase use of fluoride in its various forms [2]. On the contrary, in certain developing countries, dental caries is reported as a major public health problem due to a shift from traditional to a more westernized life-style of increased consumption of refined sugars as well as inadequate exposure to fluoride [2]. The optimal and judicious use of fluoride offers maximum caries protection while excessive intake of fluoride may lead to chronic fluoride toxicity which insults the enamel organ during enamel formation resulting in dental fluorosis [3]. Other forms of developmental enamel defects (DED) such as enamel opacities and hypoplasias can also occur when enamel organ is insulted [4].

The prevalence and extent of dental caries and developmental enamel defects including dental fluorosis in primary teeth have been reported by several studies in different parts of the world. Studies considering the prevalence and covariates of dental caries and DDE vary with respect to characteristics of the populations investigated, measurement aspects and study design utilized [5]. This variability should be considered when comparing results of various research reports. Limited works in Nigeria have shown a range in prevalence of dental caries between 10.5% reported for 6 months to 5-year-olds [6] and 17.4% reported for 4 to 6-year-olds [7] residing in naturally fluoridated areas while there is dearth of information on the prevalence of developmental enamel defects in primary teeth in Nigeria. Developmental enamel defects present major clinical significance since they affect aesthetics, cause early childhood caries [8,9], severe tooth sensitivity and dentofacial anomalies usually when there is loss of tooth structure as in hypoplasia and severe dental fluorosis. Dental caries and DDE can be adequately managed with the limited resources available in Nigeria if the prevalence and extent of these conditions is determined. Furthermore, information about the main risk factors for the development of dental caries and developmental defects of enamel are needed to help prevent them or at least mitigate their effect where possible. Compared to the permanent dentition, risk factors for development of DED in the primary dentition has not been well reported.

Therefore, the aim of this study is to first determine the prevalence and extent of enamel defects in primary dentition of 4-year-old Nigerian children and second to identify risk factors of the occurrence of developmental enamel defects.

Materials and methods

Two Local Government Areas (LGAs) – one rural (Ibarapa Central with a population of 102,979) and one urban (Ibadan North with a population of 306,795) were randomly selected from the total of 33 LGAs in Oyo State (population 5.6 million), South-western Nigeria [10]. Opinion leaders who

had resided locally since birth and who knew common community groundwater supplies used by the public identified 124 groundwater sources (boreholes and wells) across rural and urban sites in these two LGAs. Water samples taken from these sources were analysed for fluoride concentration (in mg/L or ppmF) in Oral Pathology Laboratory at the University of Ibadan, Nigeria using a Fluoride Ion-Selective-Electrode (Model 9409 Thermo orion, Boston, MA USA) directly after adding TISAB III [11].

Four water fluoride areas within the two LGAs were identified as rural higher (2.13 ppm F), urban higher (0.85 ppm F), rural lower (0.09 ppm F) and urban lower (0.07 ppm F) based on the F analyses of these common community ground water supplies. The study was subsequently undertaken in 25 randomly selected nursery schools across these 4 areas (9 rural and 12 urban). A sample size of 302 was determined based on a power of 95% at an alpha level of 5% to determine a mouth difference of DED or dental caries of 3% between areas and with an expected non-completion rate of 30%. A cluster sample of healthy 4-year-old children of both genders in nursery school 2 was undertaken in the randomly selected nursery schools in these 4 study locations. A clinical dental examination was undertaken by one dentist who had been trained and calibrated in the diagnosis of DDE, dental fluorosis and dental caries with the support of appropriate reference and calibrated materials [12, 13]. This examination was undertaken using a wooden spatula, dry gauze and a disposable mouth mirror (DenLite Illuminated Dental Mirror, Miltex Inc., York, PA, USA). Presence of developmental defects of enamel, dental fluorosis and dental caries were recorded using modified DDE [14], TF [12] and dmft [13] indices. Intra-examiner reproducibility was determined by re-examining a random sample of 36 (11.9%) participants for all dental indices.

Each participant's toothbrushing behavior, infant and early childhood feeding habits as well as infant/childhood diseases were collected from parents or legal guardians via an interviewer-administered semi-structured questionnaire developed from a standard questionnaire [15] and translated into Yoruba, the local language of inhabitants of the study locations and back translated to English Language prior to data management. The questionnaire also contained pictorial scale of the amount of toothpaste routinely used by the participants. The developed questionnaire was pre-tested among nursing mothers with similar socio-demographic characteristics as the mothers of study participants. To ensure that the questionnaire retained its reliability and validity, the local language wording was modified after the pre-test. Drinking and cooking water samples consumed by the participants were also collected from parents or legal guardians. Parents or guardians of study participants mentioned the type of toothpaste used by the study participants. The toothpaste mentioned was then purchased from local shops. Fluoride concentration in waters and toothpaste was assayed using fluoride ion-selective-electrode (F-ISE) by measuring directly unbounded free ionic fluoride in water and toothpaste [11, 16].

Table 1: Mean (SD) fluoride concentration (mg F/L) in water samples for 4-year-old participants (n=302) by area

Water samples	Fluoride concentration (mg F/L) Rural higher water F	Urban higher water F	Rural lower water F	Urban lower water F	All areas	P-value	Tukey's Post hoc following ANOVA
Community water supply							
Mean (SD)	2.13[a] (0.64)	0.85[a,b] (0.19)	0.09[a,b] (0.02)	0.07[a,b] (0.02)	0.84 (0.99)	<0.001	[a,b] P < 0.001
Drinking water							
Mean (SD)	1.10[a,b] (1.04)	0.35[a] (0.31)	0.25[b] (0.15)	0.53 (0.74)	0.76 (0.90)	< 0.001	[a]P = 0.01; [b]P<0.001
Cooking water							
Mean (SD)	1.10[a,b,c] (0.99)	0.35[a] (0.23)	0.29[b ,c] (0.16)	0.31[b] (0.54)	0.69 (0.82)	< 0.001	[a]P = 0.001; [b]P=0.01; cP<0.001

Notes a,b,c,d – Tukey Post Hoc Test values with the same superscript letters were statistically significant different at P=0.01; P=0.001; P < 0.001

Table 2: Mouth prevalence (No. of children (%) and extent (Mean (SD) No. of teeth affected) for developmental defects of enamel (DDE score 1-8), dental fluorosis (TFI > 0) and caries experience (dmft > 0) in primary teeth of 4-year old participants by area.

Enamel defects	Rural higher water F (n = 78) No. (%)	Urban higher water F (n = 78) No. (%)	Rural lower water F No. (%)	Urban lower water F (n = 76) No. (%)	All areas (n= 302) No. (%)	P-value	Tukery's Post Hoc test following ANOVA
Developmental defects of enamel							
Mouth prevalence (DDE score 1 – 8) No. (%)	66 (84.6)	73 (93.6)	36 (51.4)	60 (78.9)	235 (77.8)	< 0.001^{+}	
Demarcated opacities	28 (35.9)	10 (12.8)	1 (1.4)	25 (32.9)	64 (21.2)		
Diffuse opacities	38 (48.7)	73 (93.6)	20 (28.6)	32 (42.1)	163 (54.0)		
Hypoplasia	3 (3.8)	6 (7.7)	5 (7.1)	9 (11.8)	23 (7.6)		
Other defects	32 (41.0)	0 (0)	12 (17.1)	30 (39.5)	74 (24.5)		
Demarcated and diffuse opacities	1 (1.3)	0 (0)	0 (0)	2 (2.6)	3 (1.0)		
Demarcated opacities and hypoplasia	1 (1.3)	1 (1.3)	0 (0)	1 (1.3)	3 (1.0)		
Diffuse opacities and hypoplasia	5 (6.4)	14 (17.9)	5 (7.1)	8 (10.5)	32 (10.6)		
Demarcated and diffuse opacities and hypoplasia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Extent (No. of teeth affected) Mean (SD)	4.64^{a,b} (3.25)	6.14^{a,c,d} (3.25)	1.39^{b,d,e} (1.85)	3.55^{c,e} (2.85)	4.00 (3.33)	<0.001$^{\#}$	^{a,b,c,d,e}P<0.001
Dental fluorosis							
Mouth prevalence (Deans index) No. (%)	9 (11.5)	12 (15.4)	0 (0)	0 (0)	21 (7.0)		
Mouth prevalence (TFI > 0) No. (%)	8 (10.3)	9 (11.5)	0 (0)	0 (0)	17 (5.6)	<0.001^{+}	
Extent (No. of teeth affected) Mean (SD)	0.54^{a,b} (2.00)	0.40 (1.40)	0^{b} (0)	0^{a} (0)	0.24 (1.26)	0.01$^{\#}$	^{a,b,c}P=0.01
Dental caries experience							
Mouth prevalence (dmft > 0) No. (%)	3 (3.8)	14 (17.9)	5 (7.1)	10 (13.2)	32 (10.6)	0.02^{+}	
Extent (No. of teeth affected) Mean (SD) dmft	0.06^{a} (0.34)	0.54^{a} (1.43)	0.23 (1.09)	0.34 (1.08)	0.29 (1.07)	0.04$^{\#}$	^{a,b}P=0.04

Notes: $^{+}$ Chi-square; $^{\#}$ One way ANOVA; a,b,c,d – Tukey Post Hoc Test values with the same superscript letters were statistically significant different at P=0.01; P=0.001; P < 0.001

The mean proportion of toothpaste ingested per brushing by the participants was estimated by multiplying the pictorial recorded amount of toothpaste used per brushing (mg) by its fluoride concentration (mg/kg) and recorded frequency of daily use. The mean proportion of toothpaste ingested per brushing by the participants was then multiplied by 41%, the mean percent of toothpaste ingested per toothbrushing session reported for Iranian 4-year-olds [17] and UK 6-year-olds [18].

The intra-examiner reproducibility was determined using kappa statistics. Descriptive statistics was undertaken using SPSS version 21 (SPSS, Chicago, IL, USA) to derive proportions and mean (SD) for each group. One way ANOVA and Tukey Post hoc Tests were used to examine differences in the mean fluoride concentration in drinking and cooking water of more than 2 groups at $p < 0.05$. The water fluoride concentration of drinking and cooking waters consumed by participants was classified [19] into 3 groups: lower (<0.7 ppm F), moderate (0.7 – 1.2 ppm F) and higher (> 1.2 ppm F) and their correlation with the extent of dental fluorosis was explored. Binary logistic regression analysis was undertaken to determine the associations between the dichotomous dependent variables (DDE 1-8: yes/no and TFI > 0 yes/no) and explanatory independent variables at $p < 0.05$.

The study protocol was approved by the Ethics Committee, Newcastle University, UK and the University of Ibadan Ethical Review Board and was performed in accordance with the ethical standards as laid down in 1964 Declaration of Helsinki and its later amendments or comparable ethical standards [20]. The study was undertaken between February, end of dry season and July, mid-rainy season in 2013.

Results

Males and females comprised 51.3% and 48.7% of the sample respectively across the four areas ($p>0.05$).

Table 1 showed the mean (SD) fluoride concentration ranged from 0.07 (0.02) mg/L in urban lower water fluoride area to 2.13 (0.64) mg/L in rural higher water fluoride area ($p < 0.001$). However, the mean (SD) fluoride of actually consumed drinking water ranged from 0.25 (0.15) mg/L in rural lower water fluoride area to 1.10 (1.04) mg/L in rural higher water fluoride area ($P < 0.001$). The range of fluoride concentrations was similar for the actually consumed cooking water, from 0.29 (0.16) mg/L in rural lower water fluoride area to 1.10 (0.16) mg/L in rural higher water fluoride ($P < 0.001$).

Table 3: Correlation between F concentration (mg/L) in drinking and cooking water and the extent of dental fluorosis (no. of teeth affected) in primary teeth of 4-year-old participants (n=302[1]).

Water F (mg/L)	n	Mouth prevalence of dental fluorosis No. (%)	No. of teeth affected Mean (SD)	*P*	P
<0.7					
Drinking water	254	15(5.91%)	0.82 (1.1)	0.169	0.01
Cooking water	253	14(5.53%)	0.71 (1.3)	0.107	0.09
0.7 – 1.2					
Drinking water	23	1(4.35%)	0.14 (1.4)	-0.125	0.57
Cooking water	25	2(8.0%)	0.24 (1.2)	- 0.066	0.76
>1.2					
Drinking water	16	1(6.25%)	0.62 (1.3)	- 0.174	0.52
Cooking water	15	1(6.67%)	0.58 (1.5)	- 0.161	0.57
All areas					
Drinking water	293[1]	17(5.80%)	0.8 (1.4)	0.115	0.04
Cooking water	293[1]	17(5.80%)	0.7 (1.5)	0.092	0.12

Notes: ρ = Spearman correlation coefficient. [1] Of the 302 four-year-olds dentally examined, 293 provided drinking and cooking water samples

The kappa score for intra-examiner agreement in the recording of presence or absence of DDE using the modified DDE index for 37 (10.5%) of the 4-year-old participants who were re-examined was 0.963 ($p < 0.001$) showing excellent

Table 4: Binary logistic regr ession analysis model for DDE (Yes/No) in primary teeth of 4 year olds (n=302).

Predictors	Developmental dental defects (Yes/No) (R^2=0.075[a]; % Predicted =77.2%)				
	B	Sig (p)	OR[c] (Exp B)	95% CI Lower	Upper
Age (Years)	0.45	0.47	1.57	0.47	5.28
Gender (Male/Female)	-0.23	0.43	0.79	0.45	1.41
F Concentration Drinking Water (mg/L)	0.20	0.69	1.22	0.46	3.21
F Concentration Cooking Water (mg/L)	0.50	0.37	1.64	0.55	4.89
Exclusive Breast Feeding (No/Yes)	20.35	1.00	6.32b	0.00	[a]
Age of stopping Breast Feeding (Months)	20.35	1.00	6.81b	0.00	[a]
Infant/childhood disease (No/Yes)	0.45	0.02	1.57	0.88	2.81
Age of tooth brushing (Months)	0.11	0.72	1.12	0.61	2.07
Frequency of tooth brushing (1x, 2x & >2x)	0.33	0.56	1.39	0.46	4.26
Amount of toothpaste used per brushing (g)	0.61	0.23	1.85	0.68	5.00
Fluoride toothpaste exposure (µg/g)	-1.03	0.16	0.36	0.09	1.50
Normal birth (No/Yes)	0.78	0.32	1.18	0.48	10.0
Family history - tooth discolouration (No/Yes)	0.31	0.62	1.36	0.42	4.44

Note: [a] – Nagelkerle R^2; [a] – Not reported because it is very negligible, b - x10-[8]; [c] Odds Ratio

Table 5: Binary logistic regression analysis model for dental fluorosis (Yes/No) in primary teeth of 4 year olds (n=302).

Predictors	Dental fluorosis (Yes/No) (R^2=0.090[a]; % Predicted =94.1%)				
	B	Sig (p)	OR[c] (Exp B)	95% CI Lower	Upper
Age (Years)	0.10	0.93	1.11	0.12	9.93
Gender (Male/Female)	0.15	0.79	1.16	0.41	3.27
F Concentration Drinking Water (mg/L)	-0.27	0.71	0.76	0.18	3.19
F Concentration Cooking Water (mg/L)	0.35	0.63	1.42	0.34	5.90
Exclusive Breast Feeding (No/Yes)	-18.20	1.00	0.00	0.00	[a]
Age of stopping Breast Feeding (Months)	-17.97	1.00	0.00	0.00	[a]
Infant/childhood disease (No/Yes)	-0.50	0.37	0.61	0.21	1.81
Age of tooth brushing (Months)	-0.19	0.74	0.83	0.28	2.49
Frequency of tooth brushing (1x, 2x & >2x)	0.97	0.29	2.64	0.44	15.91
Amount of toothpaste used per brushing (g)	2.27	0.03[b]	9.66	1.28	73.16
Fluoride Toothpaste exposure (µ/g)	3.39	0.03[b]	0.03	0.02	0.70
Normal birth (No/Yes)	-0.63	0.59	0.53	0.05	5.32
Family history - tooth discolouration (No/Yes)	0.61	0.48	1.83	0.35	9.73

Note: [a] – Nagelkerle R^2; [b] – Statistically significant at P<0.05; [a] – Not reported because it is very negligible b - x10-[8]; [c] Odds Ratio

agreement while for the TF index, the kappa value was 0.828 (P < 0.001) also demonstrating excellent agreement. As shown in Table 2, overall mouth prevalence of DDE was 77.8%, ranging from 51.4% in rural lower water fluoride area to 93.6% in rural higher water fluoride area (P < 0.001). The mean (SD) number of affected teeth in the primary dentition was 1.39 (1.85) in rural lower water fluoride area compared to 6.14 (3.25) in rural higher water fluoride area (P < 0.001). Overall, the most commonly observed DDE across all the 4 areas was diffuse opacities, with a mouth prevalence of 7.6%, 21.2% and 54.0% reported for hypoplasia only, demarcated opacities only and diffuse opacities only in the primary dentition respectively (Table 2). Table 2 also shows that overall mouth prevalence of dental

fluorosis was 5.6%, with no participant in the lower water fluoride areas having dental fluorosis while 11.5% and 10.3% of the participants had fluorosis in urban and rural higher water fluoride areas respectively ($P < 0.001$). The overall mouth prevalence of dental caries was 10.6% with a range from 3.8% in rural higher water fluoride area to 17.9% in urban higher water fluoride area ($P = 0.02$). In terms of the extent of caries experience, the mean (SD) dmft ranged from 0.1 (0.34) in the rural higher water fluoride area to 0.54 (1.43) in the urban higher water fluoride area ($P = 0.04$).

Table 3 shows the association between extent of dental fluorosis in primary teeth and F concentration of the actual drinking and cooking waters consumed by these 4-year-olds. Overall, the correlation for all areas was low and the relationship between the fluoride concentration of drinking water and extent of dental fluorosis in primary teeth was weak and positive (Spearman's correlation coefficient = 0.115).

As shown in Table 4, infant/childhood disease was the only explanatory variable associated with DDE. The presence of infant/childhood disease was associated with higher risk of DDE occurrence (B coefficient was 0.45, odds ratio was 1.57 (95% CI – 0.88, 2.81). Amount of toothpaste used per brushing and fluoride toothpaste exposure were statistically significant predictors of dental fluorosis in the primary teeth of these 4-year-olds when the association between dental fluorosis and the independent variables was modelled (Table 5). Increase in the amount of fluoride toothpaste used per brushing was associated with a higher risk of dental fluorosis (B coefficient was 2.27, odds ratio was 9.66, (95% CI – 1.28, 73.16) and increase in the amount of fluoride toothpaste exposure was associated with a higher risk of dental fluorosis (B coefficient was 3.39, odds ratio was 0.03, (95% CI – 0.02, 0.70). The binary regression model was able to make a correct prediction for 77.2% and 94.1% of children having DDE or dental fluorosis or not. From the binary regression model, the Nagelkerke R^2 value which is similar to the R^2 used in linear regression and provides a statistical measure of how well the independent variable(s) account for the dependent variable was 0.08 and 0.09 for DDE and dental fluorosis respectively, indicating that 1% of the variability in the occurrence of DDE and dental fluorosis were accounted for by the independent variables.

Discussion

There is dearth of population based studies on developmental enamel defects in primary dentition in developing countries. To the best of our knowledge this is the first study that determined the prevalence and extent of enamel defects in primary dentition of 4-year-old Nigerian children as well as identified risk factors of the occurrence of developmental enamel defects. Fluoride analysis of actual drinking and cooking water samples showed that the F concentrations were similar indicating that different water sources but with the same F concentration or same water source might have been used for both drinking and cooking. The mean fluoride concentration of the community ground water supplies which was used to select the study location into high and low water fluoride areas ranged from 0.07 to 2.13 mg F/L varied widely from 0.25 to 1.10 mg F/L and 0.29 to 1.10 mg F/L, the mean fluoride concentration of actual drinking and cooking waters respectively. This difference in fluoride concentrations suggests that drinking and cooking water samples provided might not have been from the community water supply, but rather they might have been from other sources within the community. In addition, some of this variability might be due to different aquifers outside of study location, collection of water from shallow wells and seasonal differences since waters were sampled between end of dry season and middle rainy season. A previous study from Iran [17] also reported high variability in the F concentration in water obtained from shallow wells and another study [21] reported that water collected from shallow wells during rainy season tend to be lower in F than those collected during dry season. The mean F concentration of drinking and cooking water was 0.76 mg/L and 0.69 mg/L respectively. However, in the rural higher water F, it was 1.10 mg/L which is high when compared to the recommended F concentration for tropical countries like Nigeria [22]. As a result, chronic excessive consumption of water during tooth development in this area could increase the risk of development of developmental enamel defects.

In this present study, the mouth prevalence of DDE was between 51.4% in rural lower water F area and 93.5% in rural higher F area. The corresponding values for diffuse opacities in these two areas was 1.4% and 34.9%. The higher prevalence of DDE among those who lived in high water F area might be the reason for the higher prevalence of diffuse opacities thereby demonstrating the association between fluoride

induced diffuse opacities and fluoride concentration in community water supplies. These findings are in agreement with previous studies [23, 24] where diffuse opacities were more prevalent in areas with fluoridated water. The overall prevalence of DDE 77.8% falls within the range of 3.9% to 81.3% reported for 4 and 9-year-old American [25] and 1 to 4-year-old Brazilian [26] children respectively. However, this prevalence when compared with data from Nigeria was higher than 11.7% and 42.5% reported for 10 to 19-year-old [27] and 12 to 15-year-old [28] Nigerians respectively living in naturally fluoridated areas. The differences observed might be due to differences in age group studied and examination conditions. Caution should be observed when making comparisons of prevalence since age groups studied and diagnostic criteria used may vary across studies.

Dental fluorosis in primary teeth is considered to be relatively rare [29] due to the protective action of the placental barrier which prevents the transfer of fluoride from mother's blood to the fetus [30]. Fewer studies have assessed the prevalence of dental fluorosis in primary teeth in sub-saharan Africa. In this present study, the presence of dental fluorosis was assessed using both the Deans and Thylstrup and Fejerskov indices. These indices are very popular and widely used in various population surveys of dental fluorosis. Using the Deans index, the prevalence of dental fluorosis was 5.6% which falls within 0% and 100% reported for Swedish children living in less than 0.2ppm [31] and 10 ppm [32] water fluoride areas respectively. This prevalence was lower than 18%, 76.5% and 96.6% reported for 6 to 8-year-old Kenya children living in non-fluoridated areas [33], 5 to 6-year-old Iranian children living in water fluoridated areas [34] and 7-8-year-old Chinese children living in 7.6 ppm fluoride water areas [35]. The prevalence of dental fluorosis in primary teeth of 4 year olds was similar to 5.8% reported for 4.5 to 5-year-old American children who lived in 0.1 ppm water fluoride areas [29]. The differences in the frequency distribution of dental fluorosis in the primary teeth might be due to differences in fluoride exposure and investigative methods used. Fluorosis in primary teeth should be given the required attention because a previous study [36] on enamel defects in primary teeth reported that children with such kind of enamel defect in their primary teeth are also likely to have these defects in their permanent teeth. Therefore, the primary teeth may act as a biomarker of fluoride exposure and thus give an indication of what to expect in permanent dentition.

In this study, the mouth prevalence of dental fluorosis was slightly higher when Dean's index was used to assess the defects than when TFI was used which was at variance with result of a previous study [37] where the scoring systems produced identical prevalence of fluorosis. The ease with which Dean's index can be employed in epidemiological studies could be the reason why a higher prevalence of dental fluorosis was observed in this present study. The prevalence and extent of dental fluorosis was higher in areas of higher water F in both rural and urban areas than their counterparts when both the Deans and Thylstrup and Fejerskov indices were used. In addition, there was a statistically significant correlation (ρ=0.115; p=0.04) between fluoride concentration in drinking water and the extent of dental fluorosis though the correlation was weak. These findings were in agreement with previous studies [23, 38, 39]. The reason for the higher prevalence of fluorosis in higher water fluoride areas might be due to higher ingestion of fluoride water used for drinking and cooking. Further research is needed to better characterize the link between total fluoride intake and dental fluorosis in primary dentition.

In Nigeria, the prevalence and extent of dental caries varies because studies were conducted in different age groups using different methods and in diverse populations. In this present study, the prevalence of dental caries was 10.6% while the mean dmft was 0.29, indicating that the prevalence and extent of caries were low and falls within the range reported for Nigeria [40]. This prevalence and extent of dental caries were in agreement with previous studies [40, 41] and highest in urban compared to rural areas probably due to greater exposure to cariogenic diets in urban environments.

Although parents or legal guardians of study participants were not asked about previous history of malarial infection, an endemic tropical disease, the occurrence of infectious diseases which sometimes present like malaria or occur together with malaria during infancy or childhood among study participants was reported by some parents or legal guardians. Infectious diseases caused by bacteria and viruses such as chicken pox, rubella, measles, mumps and influenza have been associated with DDE in primary teeth [5, 42]. Consistently with other studies [43, 44], diseases during infancy or childhood were associated with the occurrence of DDE in this present study. This might be due to fever and derangement of acid-base balance from infections and damage to

ameloblasts as well as developing enamel prisms formed during mineralization [5]. In accordance with findings from other studies [45-47], increased toothpaste used per brushing and fluoride toothpaste exposure (amount of toothpaste used and frequency of brushing) were statistically significant predictors of dental fluorosis in primary teeth. In this present study, for a 1 unit increase in the amount of fluoride toothpaste used per brushing, the risk of having dental fluorosis was 9 times more while the odds of having fluorosis from 1 unit increase in fluoride toothpaste exposure was low. Though it was surprising to observe these findings because calcification of primary tooth buds of incisors would be largely complete before 6 months when 4-year-olds start using toothpaste but effect of toothpaste on primary canine and molars especially when large amount of it is swallowed will be noticeable since they complete their calcification between 9 to 12 months. Conversely, in other studies [48, 49], the association between toothpaste use and dental fluorosis was not statistically significant in primary teeth. Further studies are required to explore the relationship between toothpaste use and other environmental factors on the occurrence of DDE and dental fluorosis.

Conclusions

For this group of 302 four-year-old Nigerian children, the mouth prevalence of DDE was 77.8% with a mean (SD) of 4.0 (3.33) teeth affected. The mouth prevalence of dental fluorosis and dental caries 5.6% and 10.6% respectively. The mean (SD) number of teeth affected by dental fluorosis and dental caries was 0.24 (1.24) and 0.29 (1.07) respectively.

There was an association between prevalence of DDE and dental fluorosis and fluoride concentration in water and a weak relationship between extent of dental fluorosis and fluoride exposure in drinking water. Other environmental factors such as a history of infant/childhood disease and fluoride toothpaste use were positive predictors of DDE and dental fluorosis respectively.

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Chemoradiation in head and neck tumours in patients receiving treatment tertiary hospital in Nigeria: A ten-year review

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Abstract

Background: Head and neck tumours are diverse, heterogeneous, and are relatively difficult to diagnose/stage. The use of chemoradiation for these cancers has been associated with better outcomes but has not been fully studied in this environment. Thus, this study aims at describing the pattern of presentation and mode of management of patients with head and neck cancers while exploring the impact of chemoradiation on treatment outcomes and survival in these patients.

*Methods***:** Using a retrospective study design, clinical data was obtained for 406 patients who were treated for head and neck cancers between January 2001 and December 2011.

Results: The mean age was 49±17.1 years with a 2:1 male to female ratio. More tumours were located in the nasopharynx (18.0%) than anywhere else, and the bulk of patients presented with stage 4 diseases (48.5%). Most patients (37.5%) presented with a neck mass, then nasal blockage (23.4%). Squamous cell carcinoma was more prevalent (57.4%) and 73.6% had biopsy before and during surgery, while 26.4% had definitive surgical procedures done. Radiotherapy was the sole treatment in 41.4% and 29.1% had chemoradiation. Post-treatment, 63.1% experienced complete response; 28.3% partial response, and 14% recurrence. Most patients (39.8%) survived for six months post-treatment, 30.7% for 7-12 months, and 17.5% for 13-24 months and patients who received chemoradiation had longer survival.

Conclusion: Early presentation for diagnosis and treatment will definitely improve treatment outcome and survival duration. In addition, concurrent use of chemoradiation improves the treatment outcomes.

Keywords: *Chemoradiation, cancer, Nigeria, head and neck, radiotherapy*

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Résumé

Contexte: Les tumeurs de la tête et du cou sont diverses, hétérogènes et relativement difficiles à diagnostiquer / classer. L'utilisation de la chimioradiothérapie pour ces cancers a été associée à de meilleurs résultats, mais n'a pas été entièrement étudiée dans cet environnement. Cette étude vise donc à décrire le schéma de présentation et le mode de gestion des patients atteints de cancers de la tête et du cou, tout en explorant l'impact de la chimioradiothérapie sur les résultats du traitement et la survie de ces patients.

*Méthodes***:** En utilisant une structure d'étude rétrospective, les données cliniques ont été obtenues pour 406 patients traités pour un cancer de la tête et du cou entre janvier 2001 et décembre 2011.

Résultats: L'âge moyen était de 49 ± 17,1 ans avec un ratio hommes / femmes de 2:1. Plus de tumeurs étaient localisées dans le nasopharynx (18,0%) que partout ailleurs et la majorité des patients présentaient des maladies de l'étape 4 (48,5%). La plupart des patients (37,5%) ont présenté une masse au cou, puis un blocage nasal (23,4%). Le carcinome épidermoïde était plus prévalent (57,4%) et 73,6% avaient subi une biopsie avant et pendant la chirurgie, tandis que 26,4% avaient subi une intervention chirurgicale définitive. La radiothérapie était le seul traitement chez 41,4% des patients et 29,1% avaient une chimioradiothérapie . Après le traitement, 63,1% ont eu une réponse complète; 28,3% de réponse partielle et 14% de récurrence. La plupart des patients (39,8%) ont survécu six mois après le traitement, 30,7% de 7 à 12 mois et 17,5% de 13 à 24 mois, et les patients ayant reçu une chimioradiothérapie ont eu une survie plus longue.

Conclusion: Une présentation précoce pour le diagnostic et le traitement améliorera définitivement le résultat du traitement et la durée de survie. En outre, l'utilisation simultanée de la chimioradiothérapie améliore les résultats du traitement.

Mots-clés: *Chimioradiothérapie, cancer, Nigéria, tête et cou, radiothérapie*

Introduction

Tumours of the head and neck are the sixth most common malignancy globally with annual incidence of 533,100 cases.[1] They are a diverse and heterogeneous group of diseases arising in all structures cephalad to the clavicles except for the brain, spinal cord, base of skull and usually skin. Each tumour has its own distinct epidemiologic, anatomic and pathologic feature. About 90-95% of head and neck cancers are squamous cell carcinomas. [2-13] The staging of head and neck cancer is complex and depends on the anatomic location of the tumour and there are three clinical stages: early, loco-regionally advanced, and metastatic or recurrent.[6,7,14]

One of the earliest references to head and neck cancers in Nigeria was made by Elmes and Baldwin [15] who reported cases of nasopharyngeal cancer but noted it was rare. Later, cases of oesophageal carcinoma and salivary tumours were reported, although they were also found to be rare in Nigeria [16,17]. However, it has been established that cancer of the nasopharynx is not uncommon in Nigeria as previously reported [18]. Presently, the prevalence of head and neck tumour is on the increase in Lagos, Nigeria; and late presentation is a major problem [18-24]. Similar to foreign studies, there is a male preponderance of head and neck cancers in Nigeria [10-12].

The risks of developing head and neck cancers have been associated with cigarette smoking (by 25-fold) and alcohol use (by 2-6-fold) [13,14,25]. Other risk factors include infections with Epstein Barr virus and Human Papilloma virus especially in younger age groups [13,14]. The common sites of occurrence are in the oral cavity, pharynx, nasopharynx, larynx, and hypopharynx while paranasal sinuses and salivary gland tumours are less common. Also, sarcomas, lymphomas and melanomas of the head and neck region are less common.[3,-5,12,19] Most patients with tumours of the head and neck present with advanced local disease with lymph node metastasis already present in many cases. Salivary gland and nasopharyngeal tumours occur in younger age groups at about the age of 40 years [26] and approximately one-third of these patients are females.[27] As the age increases, the incidence of head and neck cancers increases.[14,28]

Presenting features depend on the primary site involved. Usually, the early symptoms do not produce functional limitations or cosmetic problems because they are mostly vague and non-specific, and are therefore often ignored or not suspected. This makes many patients in this environment present at advanced stage of the disease.[18-20] Ignorance, poverty and late referral additionally contribute to the dismal state of late presentation. In Nigeria, patients consulting traditional healers and spiritual faith healers also tend to contribute to delay in presentation.

The diagnosis of head and neck cancers requires a good knowledge of disease pattern and a high index of suspicion. Also, treatment depends on the initial localization of tumour on patients, occurrence of co-morbidities, and/or the potential side effects of treatment. Surgical resection, radiotherapy, chemoradiation radio-biotherapy with anti-epidermal growth factor receptor (EGFR) such as cetuximab, and other anti-EGFR are the therapeutic methods used in locally advanced cases.

Chemotherapy can be used singly, depending on stage or in combination to achieve a better outcome. It is also used as an adjunct and can be given as induction chemotherapy or adjuvant to surgery or radiotherapy or given concurrently with radiotherapy which is called "chemoradiation" and this has been observed with better outcome in patients and improves the rate of curability.[29]

There is some paucity in knowledge with regards to head and neck cancers in the local Nigerian population. This study focuses on documenting the pattern of presentation and mode of management of patients with head and neck cancers; and to compare treatment outcome and side effects of different treatment modalities: chemotherapy alone, chemotherapy with radiotherapy given either as induction chemotherapy or adjuvant to radiotherapy in comparison with chemoradiation (concurrent chemotherapy and radiotherapy CCRT).

Methods

This ten-year (1st January 2001 – 31st December 2011) retrospective study reviewed the case files of four hundred and six (406) patients with head and neck cancers in the hospital.

Data extracted for analysis include: Age and sex of the patients, Clinical staging of the cancer, histopathologic diagnosis, tumor site, diagnosis and investigations, mode of treatment given and outcomes.

Selection Criteria.

All patients with head and neck cancers who had biopsy and histological confirmation and receiving any form of treatment i.e. chemotherapy, radiotherapy, or concurrent chemoradiation (CCRT) at the radiotherapy clinic in LUTH were included in this study. The exclusion criteria include: (i) absence of histological confirmation, (ii) non-commencement of treatment for histologically confirmed disease, (iii) defaulting or non-completion of treatment.

Each patient was assessed and staged based on information on clinical examinations and radiological investigations such as head and neck CT scan, abdominal ultrasound, and chest x-rays. Patients were treated on the linear accelerator machine (6MV) where radiotherapy field and techniques depended on the anatomical location of the tumour but encompassed the primary disease and all regional lymph nodes. The given treatment dose was 60-70 Gray as 2-Gray daily fractions over 6-7 weeks. For concurrent chemoradiation: radiotherapy was given with concurrent weekly intravenous cisplatin 40mg/m[2] with weekly reaction review. For other patients who received chemotherapy alone or as adjuvant therapy, a variety of cytotoxic drug combinations were used for them or in adjuvant therapy.

Results

Sample characteristics.

The records of four hundred and six (406) patients with head and neck carcinomas seen at the radiotherapy clinic of Lagos University Teaching Hospital (LUTH) between 2001 and 2011 were reviewed. The patients' ages ranged from 2 to 90 years (Fig. 1). The mean age was 49 years (SD 17.1), while the median age was 50 years. There were two hundred and fifty six (256) males (63%) and one hundred and fifty (150) females (37%), with a male to female ratio of about 2:1.

The locations of the tumours are shown in Table 1. The majority of tumours were located in the nasopharynx (18.0%), followed by maxillary antrum (11.3%), and larynx (10.3%). Other major sites include the parotid (8.9%), orbit (8.6%), and mandible (7.4%).

At the time of presentation, majority of patients (48.5%) presented with stage 4 disease. Stage 3 disease followed closely with 37% of the patients presenting at this stage. Only 1% and 14% of the patients presented with stages 1 and 2 respectively. The most common pattern of presentation (Table 2) was neck mass in 37.5% of the respondents, and followed by nasal blockage in 23.4%.The histopathological types of the different cancers seen are shown in Table 3. Squamous cell carcinoma (57.4%) was the most common subtypes of this group.

Treatment modalities

A significant proportion of the patients (73.6%) had biopsy before and during surgery, while 26.4% had definitive surgical procedures done. Radiotherapy was the sole treatment in 41.4%. The results in table 4 show that most of the patients had either radiotherapy alone or radiotherapy in combination with chemotherapy. Just 8.1% had chemotherapy alone.

After the required course of treatment, the nature of the patients' response to treatment (two months after completion of treatment) is reflected in Table 5. Complete response is defined as total disappearance of all clinical disease while partial response represent a greater than 50% reduction in size of some or all lesions. No response is defined as a reduction in size of less than 50% of some or all lesion or progressing disease. For those with recurrence it is defined as the reappearance of cancer in the same location or distant after patient has been disease free for a time interval1 year after having complete response to treatment. A significant proportion of the patients (n=256, 63.1%) had complete response following treatment; while 28.3% had partial response and required more treatment. In addition, 35 patients (8.6%) had progression of disease despite the treatment, and 57 (14%) had recurrence.

Table 1. Anatomical locations of the tumours

Site	No	%
Nasopharynx	73	18.0
Maxillary antrum	46	11.3
Larynx	42	10.3
Parotid	36	8.9
Orbit	35	8.6
Mandible	30	7.4
Neck	19	4.7
Nasal	18	4.4
Oral cavity	18	4.4
Tongue	17	4.2
Palate	16	3.9
Cancer of Unknown Primary	16	3.9
Submandibular	14	3.4
Salivary Gland	7	1.7
Ear	7	1.7
Oesophagus	6	1.5
Lip	5	1.2
Parietal	1	0.2
Total	406	100

It is important to note that 14% of the patients experienced recurrence after treatment. The disease recurrence in these patients in relation to the treatment modalities were as follows: radiotherapy alone (n=34, 59.6%), chemotherapy alone (21, 36.8%), and chemoradiation (n=2, 3.5%). The outcome of patients following treatment also varied. Up to 240 patients (59.1%) were lost to follow up or

Table 2. Pattern of presentation

Presentation	Frequency	%
Neck mass	93	37.5
Nasal blockage	58	1.6
Epistaxis	50	20.2
Hoarseness	20	8.1
Metastasis	20	8.1
Dysphagia	7	2.8
Total	268	100

Table :3. Histopathological types of head and neck cancers

Type	Freq	%
Squamous cell carcinoma		
SCC Moderately differentiated	82	20.2
SCC Well differentiated	70	17.2
SCC Poorly differentiated	65	16
Adenoid cystic	40	9.9
Mucoepidermoid	21	5.2
Undifferentiated	15	3.7
Adenocarcinoma	15	3.7
Non Hodgkins	12	3.0
Rhabdomyosarcoma	10	2.5
Hodgkins lymphoma	9	2.2
Lymphoepithelioma	8	2.0
Pleiomorphic adenoma	7	1.7
Anaplastic	6	1.4
Others	46	11.3
	406	100

Table: 4. surgical procedures and types of treatment modalities

	Frequency	%
Surgical Procedures		
Definitive surgery	107	26.4
Biopsy	299	73.6
Types of Treatment Modalities		
Radiotherapy alone	168	41.4
Chemoradiation	118	29.1
Radiotherapy before chemotherapy	46	11.3
Chemotherapy before radiotherapy	41	10.1
Chemotherapy alone	33	8.1
Total	406	100

Table: 5. Nature of response, recurrence and treatment outcomes

	Frequency	%
Response		
Complete response	256	63.1
Partial response	115	28.3
No response	35	8.6
Recurrence after . . .		
Radiotherapy alone	34	20.2 (34/168)
Chemotherapy alone	21	63.6 (21/33)
Chemo radiation	2	1.7 (2/118)
Treatment Outcomes		
On follow-up	136	33.5
Lost to follow-up	240	59.1
Dead	30	7.4

Table 6. Overall survival following individual treatment modalities

Periods			Radiotherapy	Chemoradiation	Chemotherapy
1 - 6 months	66	39.8%	23	38	5
7 - 12 months	51	30.7%	19	30	2
13 - 24 months	29	17.5%	10	18	1
25 - 36 months	11	6.6%	3	8	0
37 - 48 months	5	3%	2	3	0
49 - 60 months	4	2.4	2	2	0
	166	100%	59 (35.6%)	99 (59.6%)	8 (4.8%)

defaulted, 136 (33.5%) were on regular follow up at the clinic while 30 patients (7.4%) died.

The survival period following treatment in this study ranged from two weeks to five years. 66 (39.8%) survived for six months after treatment, 51 (30.7%) survived for seven to twelve months after treatment, 29 (17.5%) survived for thirteen to twenty-four months, 11 (6.6%) survived for twenty-five to thirty-six months, 5 (3%) survived for thirty-seven to forty-eight months while 4 (2.4%) survived for forty-nine to sixty months (Table 6).

Discussion

This study showed that most of the patients were within the age range of 40-49 and 50-59 years, which represents the 4th and 5th decades of life. This suggests that head and neck cancer is most common around the age of 50, a statistic that has been highlighted in similar studies from Lagos and Ilorin, Nigeria [3,7]. However, studies conducted in northern Nigeria showed that the peak incidence was in the 3rd and 4th decade [5,9,30] while in Ibadan

the peak incidence is in the 6th decade of life [4,9]. This variation in age may be due to geographical and environmental differences. The predominance of males noticed in this study is similar to the sex ratios seen in previously reported studies, locally and international [3-6,8,10-12,15,24,30,31].

The nasopharynx was found to be the commonest site of head and neck cancers. This is similar to reports in many studies [3-5,19,31]. About 4% of the patients presented with metastatic spread to a cervical lymph node, for which the primary site or origin remained undetermined. There has been similar report which described unknown metastatic neck nodes without an obvious primary source [3,4,6,7,19,32,33]. Also, carcinoma of the lip was observed to be a hundred percent in females. This is not in agreement with reports in the literature where lip carcinomas were 8 times commoner in males. In this study, cancer of the ear was also observed to be few which is similar to previous studies in literature [5,8,19,31].

The pattern of histologic types of head and neck cancers found in this study was not different from those previously reported. The most common histological type was squamous cell carcinoma which accounted for 61.3% of the total cases in this study. Other studies have reported a 66.7% incidence for squamous cell carcinoma. [3,5-7,9,19,31]. Adenoid cystic carcinoma (9.9%) was the second most frequent cell type, with sarcoma being the third most frequent type (7.3%). This finding was at variance with the studies of Nwawolo *et al* [3] , Lilly-Tariah [25], Okoye *et al* [8] and Ajayi *et al* [28] who all reported sarcomas as the second most common occurring histological type. Reports from other health institutions within the country showed that lymphoma was the second most frequent cell type [5,6,16,19,30]. This was not so with the review study where lymphomas accounted for 5.2% representing the 4th most common histologic type.

The presenting features depended on the primary site of the disease. At presentation, enlarged cervical lymphadenopathy was the predominant symptom accounting for 54%. This is similar to existing presentation patterns. Similarly, late presentation in this environment may have contributed to bulky diseases and extensive nodal involvement at presentation. This late presentation has also been identified in similar studies as most patients usually present in the hospital after failure of traditional and spiritual treatments. By the time they finally present in the hospital for care, the disease would have often progressed significantly to an advanced stage and sometimes with distant metastasis which makes such tumours become almost unresectable. The stage of disease at the time of presentation varied among the patients. The majority presented with stage IV (48.5%) [18,19,30].

The manner of treatment of these patients with head and neck cancers varied, as the major influencing factors were the stage and location of the tumour; which is similar to what has been previously reported by Bernier *et al* [34] and other researchers [35,36]. Many tumours of the head and neck are not surgically accessible and, in some cases, where surgery may play a role, late presentation makes it unsuitable or cosmetic results are taken into consideration.

As a result, surgical treatment will be limited to biopsy alone. Radiotherapy is the main stay of treatment in most head and neck tumours. Chemotherapy can be used to down stage the disease in those with locally advanced disease and this can be used concurrently with radiotherapy. Combinations consisting of cisplatin, taxane and 5FU have given satisfactory results in previous studies. it has also been observed that chemoradiation has a better outcome and improves the rate of curability and survival of patients also depends on stage and treatment modalities and outcome [29,37,34] In this study, patients with head and neck cancer who receive chemoradiation had the least number of recurrences and also had better overall survival (Table 5 and 6) .

One major challenge of this study was follow up as many patients abandoned hospital treatment and follow up once they experienced slight improvement in symptoms. Other patients who were referred from far centres or location are lost when they decide to go to their referral centres due to proximity, thereby abandoning follow up.

Conclusion

From the findings of this this study, head and neck cancers are commoner in males, and the commonest symptom is neck mass. The commonest type found was nasopharyngeal carcinoma with squamous cell carcinoma being the commonest histopathological type seen. Most patients presented late with advanced and metastatic disease. The advent of concurrent chemotherapy and radiotherapy has improved rate of curability and survival outcome. Despite the treatment options available, factors like, the grade of disease, histological type, age, presence of metastasis and the type of treatment and other co-morbid factors, should be considered when treating patients.

This study has shown the pattern of presentation and treatment modalities employed in managing head and neck cancers in LUTH. Concurrent use of chemotherapy and radiotherapy in the management of head and neck cancers improves the treatment outcomes. For developing nations like Nigeria, more radiotherapy centres are needed to improve accessibility to treatment since most patients in this study had to travel far distances to get treatment. Also, there is need for more awareness of the importance or necessity of presenting to an orthodox medical practitioner immediately whenever any persistent mass or symptom is noticed in the head and neck region.

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Determinants of choice of orthodox and informal maternity facilities among women in an urban community in Ibadan, Southwest Nigeria.

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Abstract
*Background***:** Utilization of Antenatal Care (ANC) and skilled assistance during delivery are required to reduce maternal mortality and morbidity. Lack of skilled care during pregnancy and delivery is associated with poor pregnancy and delivery outcomes. The facility chosen for antenatal care and delivery determines whether women receive care from skilled or unskilled personnel. More information is needed on facility preferences of Nigerian women for ANC. This study was therefore conducted to assess antenatal care utilization, choice of facility for antenatal care and delivery among women in an urban community in Ibadan, Nigeria.
Methods: This was a cross-sectional study. A two-stage cluster sampling technique was used to select 351 women in Yemetu area. A pretested semi-structured questionnaire was used to obtain information on socio-demographic characteristics, antenatal care component, facility chosen for antenatal care and delivery, as well as the reasons for utilizing these facilities. Private and government-owned health facilities were classified as orthodox facilities while mission homes and facilities run by traditional birth attendants were classified as informal facilities.
Results: The mean age of respondents was 28.8 ± 5.6 years. 81.5% had at least secondary education. Sixty-two (17.7%) of respondents chose informal facilities for antenatal care and 76 (21.7%) delivered in informal facilities. Good component of antenatal care was received by 93.8% of women in orthodox facilities compared to 74.2% in informal facilities (p= 0.001). Respondents' educational attainment was the single predictor of choice of informal facilities for antenatal care (OR=2.6; 95%CI= 1.4 - 4.9). The predictors of the choice of informal facilities for delivery were respondents who did not have antenatal care with skilled personnel at least once (OR=252.4; 95%CI= 78.2 – 817.9), and those who did not have someone to take them to the hospital during labour (OR= 4.38; 95%CI= 1.6 – 12.3).
*Conclusion***:** There is a need to promote utilization of orthodox facilities for antenatal care especially among women of lower educational status.

Keywords: *Antenatal care utilization, Antenatal care components, Informal health facilities*

Résumé
Contexte: L'utilisation des soins prénatals et une assistance qualifiée lors de l'accouchement sont nécessaires pour réduire la mortalité et la morbidité maternelles. Le manque de soins qualifiés pendant la grossesse et l'accouchement est associé à de pièttres résultats en termes de grossesse et d'accouchement. L'établissement choisi pour les soins prénatals et l'accouchement détermine si les femmes reçoivent les soins d'un personnel qualifié ou non qualifié. Des informations supplémentaires sont nécessaires sur les préférences des femmes nigérianes en matière d'établissement pour les soins prénatals. Cette étude a donc été menée pour évaluer l' utilisation des soins prénatals , le choix de l'établissement pour les soins prénatals et l'accouchement chez les femmes d'une communauté urbaine d'Ibadan, au Nigéria.
Méthodes: Il s'agissait d'une étude transversale. Une technique d'échantillonnage en grappes en deux étapes a été utilisée pour sélectionner 351 foyers de femmes dans la région de Yemetu. Un questionnaire semi-structuré prétesté a été utilisé pour obtenir des informations sur les caractéristiques sociodémographiques, la composante de soins prénatals, l' établissement choisi pour les soins prénatals et l'accouchement, ainsi que les raisons de l'utilisation de ces établissements. Les établissements de santé privés et appartenant à l'État ont été classés entant qu'établissements orthodoxes, tandis que les maisons de mission et les établissements gérés par des accoucheuses traditionnelles ont été classés entant qu'établissements informels.
Résultats: L'âge moyen des répondants était de 28,8 ± 5,6 ans. 81,5% avaient au moins une éducation secondaire. Soixante-deux (17,7%) des répondants ont choisi des établissements informels pour les soins prénatals et 76 (21,7%) ont accouché dans des établissements informels. Une bonne composante de soins prénatals a été reçu par 93,8% des femmes fréquentant les structures orthodoxes, par rapport à 74,2% dans les structures informelles (p = 0,001). Le niveau de scolarité du répondant était l'unique facteur prédictif du choix d' un établissement informel pour les soins prénatals (OR

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= 2,6; IC à 95% = 1,4 à 4,9). Les prédicteurs du choix des structures informelles pour l'accouchement étaient les répondants qui n'avaient pas eu de soins prénatals avec du personnel qualifié au moins une fois (OR = 252,4 ; IC 95% = 78,2 - 817,9), et ceux qui n'avaient personne pour les emmener à l'hôpital au moment de labeur (OR = 4,38 ; IC 95% = 1,6 - 12,3). *Conclusion:* Il est nécessaire de promouvoir l'utilisation des services orthodoxes pour les soins prénatals, en particulier chez les femmes moins scolarisées.

Mots-clés: *Utilisation des soins prénatals, Composants des soins prénatals, Établissements de santé informels*

Introduction

Nigeria accounts for 14% of global maternal deaths, with a maternal mortality ratio of 814 deaths per 100,000 live births in 2015 [1,2]. Utilization of skilled antenatal, delivery and postnatal care services is a major strategy for the reduction of maternal mortality and the improvement of maternal health [3]. Antenatal care is the totality of health services rendered to a pregnant woman by a doctor or a health worker in a medical facility or at home, with the aim of achieving good maternal and foetal outcomes. It is an aspect of health care that deals with pre-symptomatic diagnosis of general medical disorders, nutrition, immunology, health education and social medicine in addition to prevention and early detection of pregnancy disorders [4]. In order to achieve the maximum impact, antenatal care services must be provided in accordance with the stipulated recommendations.

Globally, 86% of pregnant women access antenatal care with a skilled health personnel at least once, and 62% of pregnant women had at least four antenatal visits. In sub-Saharan Africa 52% of women had at least four antenatal visits [5]. The proportion of women who had at least four antenatal visits have been reported in various countries in Africa; Gambia, 78% [6]; Tanzania, 62% [7]; Uganda, 59.9% [8] and Kenya 58% [9]. In Nigeria 51% had at least 4 antenatal care visits during pregnancy.

The WHO guidelines for focused antenatal care are specific as regards the timing and content of antenatal care visits according to gestational age [10]. Each ANC visit consists of a well-defined set of activities related to three important general areas:

- Screening for conditions likely to increase the risk of developing adverse pregnancy/delivery outcomes – screening tests/examinations carried out include, measurement of blood pressure, testing of urine for bacteriuria and proteinuria, and blood tests to detect syphilis and severe anaemia. Routine weight and height measurement at each visit is considered optional in this model [3].
- Providing therapeutic interventions that have been proven to be beneficial. These include; presumptive treatment and case management of malaria in pregnancy, iron and folic acid supplementation, HIV counseling and testing, antiretroviral therapy if required, deworming, tetanus toxoid vaccination and management of preexisting conditions such as diabetes mellitus and sickle cell anaemia.
- Health promotion and education concerning proper nutrition, hygiene and infection prevention, early recognition of danger signs, health seeking behaviour, infant care and feeding and postpartum family planning.

Having antenatal care with skilled personnel is one of the strategies for the reduction of maternal mortality and morbidity. According to the World Health Organization (WHO), a skilled health worker is "an accredited health professional such as: a midwife, doctor, or nurse who has been educated and trained to proficiency in the skills needed to manage normal (uncomplicated) pregnancies, childbirth and the immediate post-partum period, and in the identification, management, and referral of complications in women and newborns"[11]. Receiving antenatal care in formal or orthodox health facilities is essential for the promotion of maternal and child health, since skilled personnel will most likely be found in such health facilities. Although a large body of knowledge exists on antenatal care utilization and timing of first antenatal care visits, literature is sparse on the type of facilities women choose to go for antenatal care. In addition to the skills of personnel assisting women with delivery, place of childbirth is a factor that influences birth outcome and the health of both the mother and infant [12]. This is because most skilled care is provided in formal or orthodox health facilities. Increasing the percentage of births delivered in health facilities is an important factor in reducing deaths arising from the complications of pregnancy. This is because if complication arises during delivery, a skilled health worker can manage it or refer the mother to the next level of care [12]. Some studies have reported the place of delivery of women. Utz *et al.,* (2013) reported that 18% of women in Bangladesh, 19% in Nepal, 39% in Pakistan, and 47% in India were attended to by skilled personnel in 2011[13]. In Africa, proportion of women who delivered in health facilities and assisted by skilled personnel have been reported; Gambia, 57% (6); Kenya, 61% [9], Zambia, 64% [14] and Uganda, 78.6% [8]. In Nigeria 61%

of women delivered in orthodox facilities while 37% of delivery took place at home (informal facilities) and 38% of deliveries were assisted by skilled personnel [12].

Research shows that women fail to utilize health facilities during childbirth for several reasons. Blondel *et al*. reported that 7% of women delivered at home because maternity facility was 30km away from their homes. Women that delivered at home had higher parity and lower educational attainments than those that delivered in orthodox facilities [15]. A study conducted in Bangladesh revealed that, distance, cost of transportation and user fees in health facilities were the major barriers to accessing skilled care during delivery [16].

A qualitative research conducted in a rural community in Zambia (in sub-Saharan Africa) had similar findings: women did not deliver in hospitals because of unaffordable user fees, poor quality of care received in available hospitals, long distance to health facilities and lack of transportation, including lack of adequate knowledge on the importance of delivering in health facilities [17]. Waiswa *et al*. (2008) in a study conducted in rural Uganda also had similar findings; women were willing to have their deliveries in health units/facilities but were unable to do so due to several barriers. These included, health workers' rudeness, corrupt tendencies and unavailability; inability to afford health facility-based care or maama kits (the standard clean delivery kits required for delivery in health facilities); commencement of labour at night in the absence of transportation and also because health facilities were usually closed at night. Women therefore preferred to deliver with the help of TBAs who were more readily available and willing to provide services on credit [18].

In South-west Nigeria, eighty-seven percent (87%) of pregnant women received antenatal care from a skilled provider and approximately 75% delivered in orthodox facilities [12]. It is necessary to understand the pattern of utilization of antenatal care and delivery services to identify suboptimal utilization patterns and reasons for choice of women's preferred facilities for delivery. Factors associated with delivery in informal facilities need to be determined. The aim of this study was to assess antenatal care utilization, choice of facility for antenatal care and delivery and reasons for facility preferences among women in an urban community in Ibadan, Nigeria.. This would create evidence to inform and guide interventions to accelerate the progress being made in the improvement of maternal health in the country.

Methods

Study Area

The study was conducted in Yemetu, an urban community with a population of 30, 861 people of which about 6,790 of them are women of reproductive age and are located in Ibadan North Local Government Area (LGA), in south western Nigeria. A number of health facilities are within reach of community members. These are a primary health care facility (Kola Daisi Foundation Community Health Centre), a government owned secondary health facility (Adeoyo Maternity Hospital), a tertiary hospital (University College Hospital) and several private health care facilities.

Study population

The study was conducted among women of reproductive age 15-49 residing in Yemetu community. Women residing in Yemetu Community who had a delivery in the year preceding the study and gave their written informed consent were included in the study. Women who did not have antenatal care during their last pregnancies and those who received antenatal care from more than one site were excluded from the study. This is because "antenatal care" is a key outcome variable for the study, and multiple booking would introduce confounding.

Study design and sampling

A cross sectional design was used. Sample size was estimated using the Leslie Kish (1965) formula for cross-sectional studies [19] using a 'p' of 87.1% which was percentage of ANC utilization rate among women receiving antenatal care from a skilled provider in South western Nigeria according to 2008 NDHS. The minimum sample size was estimated to be 288. A cluster sampling technique was used to select study participants. Four out of ten settlements were selected by simple random sampling using the ballot method. All the women in the 4 settlements who delivered in the year preceding the study, had antenatal care in only one site throughout their immediate past pregnancy and gave consent to participate in the study were interviewed.

Data collection method

Data was collected using a semi-structured interviewer administered questionnaire which comprised questions on the socio-demographic characteristics of respondents, their obstetrics and gynaecology history, antenatal care utilization pattern (number of visits and timing of booking), the type of facility chosen for antenatal care, the content of care they received during antenatal care

visits, the place of delivery and the outcome of their pregnancy. The questionnaire was pretested among 25 women outside the study area and revised to remove ambiguity. Questionnaires were administered in English and Yoruba (the local dialect) by three trained female interviewers.

Ethical considerations

Ethical approval for this study was obtained from the Oyo State Ministry of Health Ethical Review Committee. Participation in the study was completely voluntary and informed consent forms were signed or thumb printed by consenting respondents. Questionnaires were filled anonymously. Information collected was safe guarded and made available only to those who were directly involved in the research.

Data analysis

Data entry and analysis were performed using Statistical Package for the Social Sciences (SPSS) version 15. Univariate analysis was done using frequency tables, means, standard deviations and graphs. Bivariate analysis to compare proportions of discrete variables was done using Chi-square tests or Fisher's exact tests. Bivariate analysis was done to determine the association of certain variables with choice of facility for antenatal care and for delivery. Multivariate analysis to adjust for the effect of potential confounders was done using binary logistic regression analysis. Only the factors which were significantly associated with choice of facility for antenatal care and for delivery were included in the logistic regression. The level of significance for the two-sided tests was 5%.

Government owned (public) health facilities and private hospitals were classified as orthodox facilities while traditional birth attendants and mission homes were classified as informal/ unorthodox facilities.

Independent variables

There were three main independent variables in this study: type of facility chosen for antenatal care, content of antenatal care received during antenatal care and type of facility chosen for delivery. Content of ANC: Respondents were asked if they received 30 items recommended by the World Health Organization to be part of the services provided to pregnant women during antenatal care visits. The adequacy of ANC content received by each respondent was determined using an ANC content assessment index (Trinh *et al*., 2006). Adequacy of ANC content was determined in terms of the total number of items provided and classified as;

Good if respondents had $\geq$23 items ($\geq$75% of items); Fair if they had 15-22 items (50-<75% of items); Poor if they had 0-14 items (<50% of items)

Dependent variables

Key dependent variables for this study were: Socio-economic status of respondents, Respondents' level of educational attainment, parity and having skilled antenatal care at least once during their last pregnancy. The socio-economic classification was determined using a wealth index. The wealth index was created using nine equally scored items with respect to the ownership of household items that are in good working condition, the type of toilet, floor and wall in respondents' homes. Respondents were then categorized into three socioeconomic groups based on their total scores, maximum score was 9; low socioeconomic group - score of $\leq$3; middle socioeconomic group - score of 4-6; high socioeconomic group - score of $\geq$7

Results

Socio-demographic characteristics

Table 1 shows the socio-demographic characteristics of respondents. Three hundred and fifty-one (351) women participated in this study. The mean age of respondents was 28.8 ± 5.6 years with a range of 17 to 49 years. Majority of the respondents (91%) were Yoruba and 81.5% had secondary or higher education. Thirty one percent (31%) of respondents were in the low socioeconomic group, 28.2% in the middle socioeconomic group and 41.3% in the high socioeconomic group.

Choice and type of facility for antenatal care

Table 2 shows the type of facilities where women had antenatal care during their last pregnancy, the choice of facility, and the main reasons for choosing informal facilities for antenatal care.

Sixty-two (17.7%) women chose informal facilities for antenatal care of which 56 (90.3%) had antenatal care in mission homes and 6 (9.7%) with traditional birth attendants.

Multiple responses

The main reasons why women chose to have antenatal care in informal facilities included: perception of the quality of care 55 (88.7%), religious reasons 27 (43.5%) and affordability of cost of health care services provided in informal facilities 10 (16.1%).

Table 1: Socio-demographic characteristics of respondents N=351

Characteristics	N	%
Age of respondents (n=351)		
<19	9	2.6
20-29	178	50.6
30-39	149	42.5
>40	15	4.3
Mean age: 28.8 (5.6) years		
Age range: 17-49 years		
Marital status (n=351)		
Single	4	1.1
Married/Cohabiting	347	98.9
Type of marriage (n=347)		
Monogamous	303	87.3
Polygamous	44	12.7
Highest level of educational attainment (n=351)		
No formal	5	1.4
Primary	60	17.1
Secondary	239	68.1
Post-secondary	47	13.4
Religion (n=351)		
Christianity	184	52.4
Islam	167	47.6
Ethnicity (n=351)		
Yoruba	319	90.9
Ibo	5	1.4
Hausa	22	6.3
Others (Delta, Edo, Egun, Ghanian, Kogi)	5	1.4
Socioeconomic group (n=351)		
Low	107	30.5
Middle	99	28.2
High	145	41.3
Parity (n=351)		
Primiparous	87	24.8
Multiparous	264	75.2

Factors associated with choice of facility for antenatal care

Table 3 shows bivariate analysis of socio demographic variables and choice of facility for antenatal care. A higher proportion of respondents who had at least primary education (32.3%) chose informal facilities for antenatal care compared to those who had secondary education and above (14.3%). A higher proportion of respondents in the low socio-economic group (27.2%) chose informal facilities for antenatal care compared to those in the middle and high socio-economic groups (13.2% and 11.3% respectively). These results are statistically significant ($p<0.05$).

Table 2: Type of facility chosen for antenatal care N=351

Type of facility chosen for antenatal care	N	%
Type of facility (n=351)		
Orthodox facilities	289	82.3
Informal facilities	62	17.7
Type of orthodox facility (n=289)		
Government-owned (public)	179	61.9
Private	110	38.1
Type of informal facility (n=62)		
Mission home	56	90.3
TBA	6	9.7
Main reasons for choosing informal facilities for antenatal care *		
Perception of quality of care	55	88.7
Religious reasons (mainly for prayer)	27	43.5
Affordable	10	16.1
Proximity	6	9.7
Husband's preference	5	8.1

Adequacy of antenatal care components received

Table 4 shows the ratings of the components of antenatal care received by the respondents per health facilities. A total of 317 women (90.3%) had good antenatal care content, 31 women (8.8%) had fair content and 3 (0.9%) had poor antenatal care content. A higher proportion of women who received antenatal care in orthodox facilities (93.8%) reported that the components of antenatal care was good compared to 74.2% of women who received antenatal care in informal facilities ($p< 0.05$, $p= 0.001$).

Type of facility chosen for delivery

Table 5 shows the type of facilities chosen by women for delivery during their last pregnancy and the main reasons for delivering in informal facilities. Two hundred and seventy-five women (78.3%) delivered in orthodox facilities. Seventy-six (21.7%) of the women delivered in informal facilities, of which 57(75%) delivered in mission homes, 10(13.2%) at home, 7(9.2%) by traditional birth attendants, one woman (1.3%) delivered by herself in a vehicle while on the way to the hospital, and another woman (1.3%), who had twins, delivered one of the babies in a mission home and the second in a private hospital.

Reasons for delivering in informal facilities were: perception of quality of care 57 (89.1%), religious reasons (mainly to be prayed for) 20 (31.3%), the closeness of the informal facility to the respondents 12 (18.8%) and affordability 10 (16.1%) among others.

Table 3: Socio-demographic characteristics and choice of facility for antenatal care
Socio-demographic

Socio-dermographic characteristics	Type of facility chosen for antenatal care Orthodox n=289 n(%)	Informal n=62 n(%)	Total	N= 351 p value
Age				
Below 35	242(82.0)	53(18.0)	295	0.73
35 and above	47(83.9)	9(16.1)	56	
Respondent's educational attainment				
Primary or no formal	44(67.7)	21(32.3)	65	0.001*
Secondary or post-secondary	245(85.7)	41(14.3)	286	
Husband's educational attainment				
Primary or no formal	19(86.4)	3(13.6)	22	0.78
Secondary or post-Secondary	266(82.45)	57(17.6)	323	
Socio-economic groups				
Low	91(72.8)	34(27.2)	125	0.002*
Middle	112(86.8)	17(13.2)	129	
High	86(88.7)	11(11.3)	97	
Parity				
Monoparous	69(79.3)	18(20.7)	87	0.39
Multiparous	220(83.3)	44(16.7)	264	
History of obstetric Complication				
Yes	176(85.0)	31(15.0)	207	0.11
No	113(78.5)	31(21.5)	144	

*$p < 0.05$

Table 4. Adequacy of overall antenatal care components received by respondents N=351

Type of facility	Good	Fair	Poor	Total
Orthodox facilities	271 (93.8%)	17(5.9%)	1(0.3%)	289(100%)
Informal facilities	46 (74.2%)	14(22.6%)	2(3.2%)	62(100%)
All respondents	317(90.3%)	31(8.8%)	3(0.9%)	351(100%)

X^2=23.24 p value-0.00

Socio-demographic characteristics and choice of facility for delivery

Table 6 shows bivariate analysis of factors associated with choice of facility for delivery. A higher proportion of respondents who had primary school education (35.4%) delivered in informal facilities compared to those who had secondary education and above (18.5%).

A higher proportion of respondents in the low socio-economic group (32%) delivered in informal facilities compared to those in middle and high socio-economic groups (16.3% and 15.5% respectively).

A higher proportion of those who did not have skilled attendant at antenatal care (93.5%) delivered in informal facilities compared to those who did

Table 5: Type of facilities chosen for delivery

Place of delivery (for all respondents)	N	%
Choice of facility for delivery (n= 351)		
Orthodox facility	275	78.3
Informal facility	76	21.7
Type of orthodox facility (n=275)		
Government-owned hospital	163	59.3
Private hospital	112	40.7
Type of informal facilities (n=76)		
Mission home	57	75
TBA	10	13.2
Others		
At home	7	9.2
In a vehicle, on the way to the hospital	1	1.3
Gave birth to twins – one in a mission house and the second in a private hospital, when the delivery became complicated		
Main reasons for delivering in informal facilities[*]		
Perception of quality of care	57	89.1
Religious reasons (mainly for prayer)	20	31.3
Affordable	10	16.1
Proximity	12	18.8
Husband's preference	4	6.3
Healthcare providers' attitudes	2	3.1
Government-owned hospitals were on strike	2	3.1

[*] *Multiple response*

(6.2%). A higher proportion of those who did not have support from family members (especially husband) during labour (37%) delivered in informal facilities compared to those who did (19.4%).

Predictors of utilization of informal facilities for antenatal care and choice of informal facilities for delivery

Table 7 shows multivariate analysis of predictors of choice of informal facilities for antenatal care and delivery. Respondents who had no formal or primary education were three times more likely to choose informal facilities for antenatal care than those who had secondary education (OR = 2.6; 95% CI= 1.4-4.9; p= 0.002).

Respondents who did not have skilled attendant at least once in the last pregnancy were about two hundred and fifty times more likely to deliver in informal facilities (OR= 252.8. 95% CI= 78.2- 817.9). Respondents who did not have someone to take them to the hospital during labour were four times more likely to deliver in informal facilities (OR= 4.4; 95% CI= 1.6- 12.3).

Discussion

Choice of facility for antenatal care

Majority of respondents had antenatal care in orthodox facilities and more than half of the women had antenatal care in government owned (public) health facilities. Majority of pregnant women in the study population made good choices with regards to the type of facilities where they received antenatal care. One third of the respondents received antenatal care in private hospitals. This shows that the private sector plays a key role in the delivery of antenatal care services. The World Bank report shows that the private sector accounts for approximately 50% of health service delivery in Africa [21]. Health industry stakeholders need to create an enabling environment for optimal private sector involvement in the delivery of health services to ensure increased coverage of the population.

NDHS 2013 reported that 87.3% of women received antenatal care from orthodox (skilled birth attendants) [12], 1.9% from traditional birth attendants, and 10.6% did not receive antenatal care at all. A study conducted in Sagamu, Southwest

Table 6: Socio-demographic characteristics and choice of facility for delivery

Socio-demographic characteristics	Type of facility chosen for delivery			N= 351
	Orthodox (275) n(%)	Informal (76) n(%)	Total	p value
Age of respondents				
Below 35	229(77.6)	66(22.4)	295	0.45
35 and above	46(82.1)	10(17.9)	56	
Respondent's educational Attainment				
Primary or no formal	42(64.6)	23(35.4)	65	0.00*
Secondary or post-secondary	233(81.5)	53(18.5)	286	
Socio-economic groups				
Lowest	85(68.0)	40(32.0)	125	0.002*
Middle	108(83.7)	21(16.3)	129	
Highest	82(84.5)	15(15.5)	97	
Had ANC with a skilled Attendant at leastonce				
Yes	271(93.8)	18(6.2)	289	0.00*
No	4(6.5)	58(93.5)	62	
Time of the day when labour started				
Day	143(80.8)	34(19,2)	177	0.24
Night	130(75.6)	42(24.4)	172	
*Availability of someone To take respondents to The hospital during labou*r				
Yes	245(80.6)	59(19.4)	304	0.007*
No	29(63.0)	17(37.0)	46	

**P<0.05*

Nigeria reports that 84.2% women had antenatal care in orthodox facilities [22]. The proportion of women that had antenatal care with a traditional birth attendant in this study is however slightly lower, 9.2% than those of the other studies. This may be due to the availability of a good number of health facilities in this community, minimizing access-related barriers. The tertiary and secondary hospitals and several private clinics are within easy reach of members of this community.

Reasons for choice of facility for antenatal care

Reasons that influenced women's preferences with regards to choice of facility for antenatal care were respondents' perception of quality of care provided in different centres, preference of respondents' husband/partner, proximity, cost and religious reasons. These findings were similar to reports by Iyaniwura and Yussuf (2009), in which the main reasons for the choice of facility for antenatal care were perception of quality of care, distance/ proximity and husband's preference [22]. In this study, affordable cost of healthcare and desire to be prayed for during antenatal care (by 1/6th of the women) were major reasons why respondents chose informal facilities especially mission homes for antenatal care. It is likely that women preferred traditional birth homes because of cultural beliefs passed down from their mothers or mothers in-law because they themselves utilized 'traditional birth attendant care' when they were pregnant. This is similar to reports by Idowu *et al*. (2005) [23].

Educational attainment of respondents was found to be a predictor of choice of facility for antenatal care. Women who had no formal or primary education were three times more likely to choose informal facilities for antenatal care than those who

Table 7: Predictors of choice of informal facilities for antenatal care and delivery

	OR	95% CI Lower	Upper	p value
Predictors of choice of informal Facilities for ANC				
Respondent's educational attainment				
Primary or no formal	2.6	1.4	4.9	0.002*
Secondary or post-secondary			1	
Socioeconomic group				
Low socioeconomic group	1.7	0.8	3.4	0.152
High socioeconomic class	1			
Predictors of choice of informal Facilities for delivery				
Respondent's educational attainment				
Primary or no formal	0.99	0.32	3.08	0.99
Secondary or post-secondary	1			
Socio economic group				
Low socioeconomic group	1.04	0.38	2.92	0.93
High socioeconomic group	1			
Had ANC with a skilled attendant at least once				
No	252.4	78.2	817.9	0.000*
Yes	1			
Availability of someone to take respondent to the hospital during labour				
No	4.38	1.6	12.3	0.005*
Yes	1			

*$p < 0.05$

had at least secondary education. This is comparable to findings from NDHS 2013 where 20.5% of women who had no formal education and had no antenatal care of which 6.3% chose informal facilities for antenatal care and 50.7% of women who had primary education, had no antenatal care and 4.8% chose informal facilities for antenatal care [12]. This finding underscores the importance of maternal education in the promotion of good health seeking behaviours and highlights the need for the government and other relevant stakeholders to invest in the improvement of the educational status of Nigerian women.

Choice of facility for delivery

Majority of respondents 78% delivered in orthodox facilities and 22% delivered in informal facilities, mainly in mission homes. The proportion of women that delivered in orthodox facility in this study is higher than the national figure of 36% but comparable to 74.7% of women who delivered in orthodox facility in Oyo state [12]. The proportion of women who delivered in orthodox facilities was also higher than that obtained in Thailand (12%), Lebanon (32.4%), and Kenya (61%) [9,24,25].

In this study, one-fifth of women delivered in informal facilities. This is much lower than the national figure of 63% but comparable to 24.7% of women who delivered at home in Oyo state according to NDHS (2013) (12). The proportion of women delivered in informal facilities is lower than that reported in Kenya (38%), Lebanon (52.5%) and Thailand (88%) [9,24,25].

In our study, women who had no antenatal care with a skilled provider at least once were significantly more likely to deliver in an informal facility compared to those who had antenatal care with skilled attendant. This is similar to findings from a health facility-based study conducted in Sokoto (in Northern Nigeria) in which 68.5% of women that had 4 antenatal care visits in a health facility delivered there [26]. Research has shown that majority of women who had the recommended four or more antenatal care visits while pregnant usually delivered in health facilities, with access to skilled providers [3]. The high rate of utilization of orthodox health facilities for delivery

in this study is encouraging and is evident that good health seeking behaviour and its attendant benefits are achievable in low resource settings in urban areas.

A point of concern however is that in spite of several orthodox facilities around them, one-sixth of all the women interviewed in our study delivered in mission homes, where the skills/ qualifications of the personnel that attend deliveries are doubtful. Women delivered in informal facilities because of their perception of the quality of care they would receive, availability of opportunity to be prayed for, affordability of cost of health care and how close they were to the hospital. Qualitative studies need to be carried out to understand what women considered as quality care. Other reasons given by women who delivered in informal facilities include husband/partner's preference, poor attitude of health workers, industrial action by health workers in public facilities and cost of hospital-based care. These results are similar to findings from studies conducted in Zambia [17], Rural Uganda [18] and Northern Nigeria [26]. Women in Zambia delivered in informal facilities because of long distances, lack of transport, user fees, inadequate health education given during antenatal care attendances, poorly staffed and ill-equipped orthodox health facilities with poorly skilled personnel [17]. Waiswa *et al* (2008) reported that rural Ugandan women delivered in informal facilities because of the high cost of drugs and supplies in orthodox facilities [18]. In Northern Nigeria, women in Sokoto delivered in informal facilities because of privacy and lack of transportation to orthodox facilities [17].

These reasons provide a basis for measures to be put in place to improve utilisation of orthodox facilities for delivery such as improved accessibility to orthodox facilities.

Factors associated with delivering in informal facilities

In this study, women who did not have antenatal care in health facilities (at least once) in their last pregnancy were significantly more likely to deliver in informal facilities. Also, women who did not have someone around to take them to the hospital when labour started were four times as likely to deliver in informal facilities as those who had help. Although one-third of the respondents are of low socio-economic status, their socioeconomic status was not a significant predictor of delivering in informal facilities, perhaps because the study area is a low-income community where health facilities are within a walking distance for all the community members.

These findings are major contributions to the existing body of knowledge on antenatal care and delivery practices among urban women and highlights the determinants and reasons for utilization of informal facilities.

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HIV treatment optimism and fertility intention of HIV-infected persons in Oyo State, Nigeria

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Abstract

Background: Fertility intention of People Living with HIV (PLHIV) creates a potential for increased sexual transmission of HIV and other STIs during natural pregnancy conception attempts. There is however paucity of data on the association between attitude and belief of PLHIV about HIV treatment and their fertility intention. This study aimed to determine the association between HIV treatment optimism and fertility intention among PLHIV and the role of other predictors in mediating this association.

Methods: A cross-sectional survey of heterosexual adults living with HIV was conducted using a questionnaire survey. HIV treatment optimism scores ranged from 5 to 20. Scores ≤14 were considered as realistic and >14 as optimistic. Data were analysed using descriptive and inferential statistics at 5% significance level.

Results: Mean age of the respondents was 35.8 ± 7.1years and 82.4% were females. HIV treatment optimism was associated with fertility intention (p=0.037). Predictors of fertility intention were being HIV treatment optimistic [OR 1.76 (1.19-2.61)], being a female [OR 1.67(1.02-2.74)], non-disclosure of HIV status to partner [OR 2.28(1.13-4.62)], partner being HIV negative [OR 7.88(1.59-39.12)] or positive [OR 15.72(2.65-93.42)], unknown partner HAART status [OR 10.31(2.18-48.80)], being in a short relationship [OR 0.99(0.99-1.00)] and having ≤ 2 living lifetime children [OR 10.02(1.79-55.99)].

Conclusion: Attitudes and beliefs of PLHIV about HIV treatment influences their fertility intentions. To attain the goal of zero new HIV infection, adequate education and counselling that will bring about safer and healthier reproductive attitudes and behaviours would be of value particularly among individuals with characteristics influencing the association.

Keywords: *HIV treatment optimism, Fertility intention, Confounders, Heterosexual HIV adults, People Living with HIV, South-western Nigeria*

Résumé

Contexte: L'intention de fécondité des personnes vivant avec le VIH (PVVIH) crée un potentiel d'augmentation de la transmission sexuelle du VIH et d'autres IST lors des tentatives de conception d'une grossesse naturelle. Il existe cependant peu de données sur le lien entre l'attitude et la conviction des PVVIH concernant le traitement du VIH et leur intention de fécondité. Cette étude visait à déterminer le lien entre l'optimisme lié au traitement du VIH et l'intention de fécondité chez les PVVIH et le rôle des autres prédicteurs dans la médiation de cette association.

Méthodes: Une enquête transversale sur des adultes hétérosexuels vivant avec le VIH a été réalisée à l'aide d'un questionnaire. Les scores d'optimisme pour le traitement du VIH allaient de 5 à 20. Les scores ≤ 14 étaient considérés comme réalistes et > 14 comme optimistes. Les données ont été analysées à l'aide de statistiques descriptives et déductives inférentielles à un niveau de signification de 5%.

Résultats: L'âge moyen des répondants était de 35,8 ± 7,1 ans et 82,4% étaient des femmes. L'optimisme du traitement du VIH était associé à l'intention de fécondité (p = 0,037). Les prédicteurs de l'intention de fécondité étaient d'être optimistes quant au traitement du VIH [OR 1,76 (1,19-2,61)], en tant que femme [OR 1,67 (1,02-2,74)], non divulgation du statut VIH au partenaire [OR 2,28 (1,13-4,62)], partenaire séronégatif [OR 7.88 (1.59-39.12)] ou positif [OR 15.72 (2.65-93.42)], inconnu statut HAART du partenaire [OR 10.31 (2.18-48.80)], étant dans une courte relation [OR 0.99 (0.99- 1,00)] et ayant ≤ 2 enfants dans la vie entière [OR 10.02 (1.79-55.99)].

Conclusion: Les attitudes et croyances des PVVIH concernant le traitement du VIH influencent leurs intentions en matière de fécondité. Pour atteindre l'objectif de zéro nouvelle infection par le VIH, une éducation et des conseils adéquats permettant de créer des attitudes et des comportements en matière de procréation plus sûrs et plus sains seraient particulièrement utiles, notamment chez les personnes dont les caractéristiques influent sur l'association.

Mots-clés: *optimisme face au traitement du VIH, intention de fécondité, facteurs de confusion, adultes hétérosexuels vivant avec le VIH, personnes vivant avec le VIH, sud-ouest du Nigéria*

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Introduction

Infection with the human immunodeficiency virus (HIV) affects the sexual and reproductive health and well-being of People Living with Human Immunodeficiency Virus (PLHIV) [1]. Usually, all the dimensions of their sexual and reproductive health including fertility intention are potentially affected [1]. However with the major strides made in expanding access to Antiretroviral Therapy (ART) and comprehensive care for HIV infected men and women, a significant return to normal sexual activity and varying reproductive health decisions among HIV infected individuals has been enabled [2-6]. Antiretroviral therapy has the potential to influence the fertility desire and intention of PLHIV through improvement in their health, quality of life, survival and HIV treatment optimism (or antiretroviral optimism) as depicted by the conceptual framework for the potential impact of antiretroviral therapy on fertility in sub-Saharan Africa [7].

In the context of HIV and Highly Active Antiretroviral Therapy (HAART), optimism represent some shifts in attitudes and beliefs (realistic or optimistic) about the sexual and reproductive risk related with HIV/AIDS due to the availability of HAART [8]. HIV treatment optimism represents the potential negative consequences of having an optimistic view of HIV as a less severe and less dangerous disease [8]. It reflects individuals' optimism about the use and efficacy of HAART and the corresponding attitude and beliefs concerning sexual and reproductive behaviours [8, 9]. Given that over 80% of PLHIV are in their reproductive years and many of them continue to desire and intend to achieve pregnancy especially during this era of antiretroviral therapy scale up [2, 4, 10-12], this perception arguably creates a potential for increased sexual transmission of HIV and other sexually transmitted infections during natural conception attempts by married and co-habiting couples in Nigeria for various reasons. First, quite a number of the new HIV infections occur in persons who are not engaging in high risk sex, a sub-population that includes cohabiting or married sexual partners, according to the mode of transmission studies conducted in Nigeria [13]. Secondly, resources for assisted reproductive technology, treatment as prevention (TasP) and pre-exposure prophylaxis (PrEP) are in short supply in Nigeria [14].

To date most research on HIV treatment optimism has tended to focus more on its influence on sexual risk behaviours in developed countries [15-20]. Far too little attention has been placed on the role of HIV treatment optimism on reproductive behaviours and fertility intention which can also fuel the HIV epidemics in resource poor settings like Nigeria where donor agencies are gradually withdrawing [21]. HIV treatment optimism has been shown to influence fertility intentions with evidence of such being documented in the United States of America [22], Australia [23] and Uganda [5]. However, the peculiarities and the cultural context of Nigeria could not have been captured by these studies and local decisions cannot be based on global findings. Focusing on the role of HIV treatment optimism on fertility intention of PLHIV in Nigeria where HIV prevalence is high [13, 24], resources and programs for antiretroviral (ARV) drugs and assisted reproductive technology are in short supply [14] and fertility is particularly of value [25] is therefore very important for the designing of programmes for safer sexual and reproductive health for PLHIV in resource poor settings like Nigeria.

Also, beyond the observed association between HIV treatment optimism and fertility intention that has been documented in previous research [5], there is a need to assess the role of other potential predictors in mediating the association between HIV treatment optimism and fertility intentions.

Therefore this study, conducted among PLHIV attending two comprehensive ART sites in South-Western Nigeria, aimed to determine the association between HIV treatment optimism and fertility intention as well as the role of other potential predictors in mediating this association. Findings from this study will help guide efforts to support PLHIV in resource constrained countries who are considering their reproductive options while reducing HIV transmission and sexually transmitted infections.

Methods

This cross-sectional study was conducted in two comprehensive ART sites within two government-owned secondary level health facilities (Adeoyo Maternity Hospital and Saki State Hospital) in Oyo state, Nigeria using a questionnaire survey. The two comprehensive ART sites were purposively selected based on their high patient load.

Oyo state, located in the Southwest region of Nigeria, is predominantly a Yoruba speaking state where fertility is particularly of value [25]. According to the 2006 census in Nigeria, the total number of males aged 15-64 years and females aged 15-49 years was 1,609,850 and 1,465,628 respectively [26]. At the time this study was conducted in 2015, this population was projected to be 2,095,412 and

1,907,689 for males aged 15-64 years and females aged 15-49 years respectively assuming an annual growth rate of 3.35% [27]. The HIV prevalence for the state in 2012 was 5.6% [24]. Located within Oyo state are various non-governmental organizations (NGOs) involved in the provision of prevention, care and treatment for PLHIV. These NGOs have clinics located within government-owned and private health facilities in the state. Adeoyo Maternity Hospital is located in Ibadan North Local Government Area (LGA) which is an urban LGA within Oyo state while Saki State Hospital is located in Saki West LGA which is a semi-urban LGA within Oyo state. They are both government-owned secondary level health facilities which serve as referral centres for many primary health centres and private clinics within the LGAs and its environs. They both have comprehensive ART sites within them.

The study population consisted of adult women (18-49 years) [24] and men (18-64 years) [24] attending the adult antiretroviral clinic in the selected health facilities who were screened using ELISA and/or Western blot and were found to be HIV sero-positive. Included in the study were those who had been in the HIV programme for at least six months. Also include were those who had current and steady partners (18-64 years old male partners for the female respondents and 18-49 years old female partners for the male respondents) with whom they were sexually active.

For the purpose of this study, being sexually active was defined as self-reported sexual activity in the six months preceding the study [28, 29]. Excluded were women and men living with HIV who were too ill to grant an interview; women 18-49 years old (or male respondents with female partners 18-49 years old) who had attained menopause (natural or artificial) and respondents who were pregnant (or whose partners were pregnant). Eight hundred and fourteen women and men living with HIV were interviewed in the selected comprehensive ART sites. However, only eight hundred and eight questionnaires had sufficient information to be used in analysis. A systematic random sampling technique was employed to obtain the unit of enquiry using a sampling interval of 3 and 2 for Adeoyo Maternity Hospital and Saki State Hospital respectively. The sampling fraction was calculated by dividing the proposed sample size per day by the average number of patient seen per day in each location (Table 1).

The proposed sample size per day for each comprehensive ART site was determined by spreading the minimum sample size of four hundred and seven for each location over a period of twelve clinic days. The first respondent for each site was selected by balloting. If a patient was not eligible, the next patient was selected. Respondents were approached before the start of full clinic activities and peradventure any respondent was called before the interview ended, he or she was implored to come back for completion of the interview when he or she was through.

Data collection involved the use of a semi-structured, interviewer, administered questionnaire. The section on HAART optimism was adapted from the women's HAART optimism monitoring and evaluation scale version 1 (WHOMEN'S scale) developed by Kaida in Uganda [5]. The questionnaire was used to obtain data on socio-demographics, HIV history, partner grid, reproductive decision-making, health provider interaction on fertility option and HIV treatment optimism. The questionnaire was in English language but was translated to Yoruba language and back translated to English language to ensure that its original meaning was retained. To assure data quality, the questionnaire was pretested on a similar population outside the study sites after research assistants were trained to use the instrument over a period of two days.

The questionnaires were checked daily for consistency and completeness and were coded before computer entry. Data analysis was done using SPSS version 22. The categorical variables of interest were summarized using frequencies and proportions while the continuous quantitative variables were summarised using means, medians, standard deviations and interquartile ranges.

The dependent variable was fertility intention. It was assessed based on a question that had been used to determine fertility intention in previous surveys [30, 31] which was: ''How many children do you expect to give birth to in the future?'' The variable was dichotomized into ''no intention'' if respondent indicated ''0''and ''intends pregnancy'' if respondent indicated ''1 or greater''. If the respondent did not answer this question but responded ''Never'' to the question regarding when in the future the respondent (or partner) planned to be pregnant, the respondent was assumed to have no intention of pregnancy. Respondents who did not answer that question but provided a time frame for future pregnancies or responded that ''that they did not know'' were assumed to have intention of pregnancy in the future.

The main independent variable was HIV treatment optimism. It was assessed using the women's HAART optimism monitoring and evaluation scale (WHOMEN'S scale) developed by

Kaida in Uganda [5]. The original scale was an eight item scale which incorporated concerns about becoming pregnant and risks of vertical transmission of HIV in addition to those related to HIV transmission risk to sexual partners and severity of HIV disease. Only five items which generated a high cronbach's alpha after the pretest were however used for analysis in this study. Responses to each statement were scored from 1 (Strongly Disagree/ Highly realistic) to 4 (Strongly Agree/Highly optimistic). The minimum obtainable score (indicating high realism) was 5 and the maximum obtainable score (indicating high optimism) was 20. Using the median score of 14 as cut off, HIV treatment optimism was dichotomized into realistic and optimistic. Respondents with median score and below were considered as realistic while scores above the median score were considered as optimistic.

Bivariate analysis (Chi-square test and Mann-Whitney U test) was carried out to determine the associations between the outcome variable and the independent variables of interest at 5% level of significance. Thereafter, all variables that were significant at the 10% level of significance on bivariate analysis were selected and fit into the logistic regression model to identify the predictors of fertility intention at 5% level of significance.

Ethical consideration

Ethical approval for the study was obtained from the Oyo State Ministry of Health Ethical Review Board. Permission to conduct the study was also obtained from the Chief Hospital Consultants of the selected health facilities. In addition, the purpose of the study was explained to the respondents and written informed consent obtained before commencement of data collection. Confidentiality of the data collected was assured and the study caused no harm to the participants.

Table 1: Calculation of sampling interval

Location	Average number of patients seen per day (N)	Proposed sample size per day (n)	Sampling fraction (1/K=n/N)	Sampling interval (K = N/n)
Adeoyo Maternity Hospital	100	34	1/3	3
Saki State Hospital	70	34	1/2	2

Results

Participants' and their partners' characteristics

Of the 814 questionnaires administered, 808 (99.3%) had sufficient information to be used in analysis, 403 (49.9%) in the urban LGA and 405 (50.1%) in the semi-urban LGA. The mean age of the respondents was 35.8 ± 7.1 years. Majority (82.4%) were females, about half (51.5%) had below secondary education and 87.1% were Yoruba (Table 2).

The median time since respondents have been diagnosed to have HIV was 13.0 (IQR: 16.0 - 56.8) months. Most (97.3%) were on HAART and of these, the median time since commencement of HAART was 28.0 (IQR: 14.0 – 52.0) months. Majority (87.6%) had disclosed their HIV status to their partners (Table 3).

Most (93.7%) of the participants in this study were in a marital relationship and the median duration of the respondents' relationship with their partners was 120.0 (IQR: 60.0 – 180.0) months. About two-fifth of the respondents' partners were HIV seronegative (62.7%) and HAART naïve (64.7%). Approximately 79% of the current partners of the respondents were not their first partners (Table 3).

HIV treatment optimism

The median HIV treatment optimism score in this study was 14.0 (IQR: 12.0 – 16.0). When the HIV treatment optimism score was dichotomized, more than half (55.9%) of the respondents were realistic about HIV treatment (Table 4).

Obstetric history and fertility intention

The obstetrics history and fertility intention of the respondents is summarized in Table 5. Many (63.2%) of the respondents had had 3 or more childbirths during their lifetime. Four hundred and fifty-nine (58.4%) of the respondents with children in their lifetime had 3 or more of such children alive. Most (93.8%) of the respondents had a child for their current partner, and 98.3% of the children were alive. About three-fifth (62.0%) of the respondents had future pregnancy intentions. One hundred and twelve (22.4%) of those who intended to have a pregnancy in the future expected to have 3 or more children in the future.

Table 2: Socio-demographics of respondents

Variables	Frequency	Percentage (%)
Location of health facility		
Urban	403	49.9
Semi-urban	405	50.1
Age (in years)		
≤30	213	26.4
> 30	595	73.6
Age: mean ± SD[a]	35.8 ± 7.1	
Sex		
Male	142	17.6
Female	666	82.4
Highest level of education completed		
None	119	4.7
Primary	171	21.2
Junior Secondary	126	15.6
Senior secondary	283	35.0
Tertiary	109	13.5
Religion		
Christianity	370	45.8
Islam	431	53.3
Traditional	7	0.9
Tribe		
Yoruba	704	87.1
Hausa	25	3.1
Igbo	41	5.1
Non-nationals[b]	14	1.7
Others[c]	24	3.0
Occupation		
Unskilled/Unemployed	31	3.8
Skilled manual	65	8.0
Skilled non-manual	628	77.7
Professional/Managerial	84	10.4
Monthly income (in naira)(n = 798)		
< 18,000	265	33.2
≥ 18,000	533	66.8
Monthly income in naira: median (IQR)[d]	28,000 (12,000 – 80,000)	

[a]*Standard deviation* [b]*Ghana, Sierre-Leone, Togo* [c]*Baruba, Edo, Igala, Igbira, Ijaw, Taraba* [d]*Interquartile range*

Association between HIV treatment optimism and fertility intention

In the bivariate analysis, a significant association between HIV treatment optimism and fertility intention was seen. More of the respondents who were optimistic about HIV treatment intended pregnancy compared to those who were realistic about HIV treatment (p = 0.037).

Further analysis to assess the role of other predictors in mediating the association between HIV treatment optimism and fertility intentions using a multivariate analysis revealed that being optimistic about HIV treatment significantly increased the likelihood of intending pregnancy among the respondents [OR 1.76 (CI: 1.19 – 2.61)]. Covariates that predicted fertility intention in this study were location of the health facility, sex, disclosure of HIV status to partner, duration of relationship with partner, partner's HIV status, partner's HAART status and number of lifetime children who are alive. (Table 6).

Discussion

This study, which aimed to identify the association between HIV treatment optimism and fertility intention as well as the role of other predictors in mediating this association, revealed that although there is an association between HIV treatment

Table 3: HIV characteristics of respondents and their partners

Variables	Frequency	Percentage (%)
Time since HIV diagnosis in months: median (IQR)[a]	30.0 (16.0 – 56.8)	
HAART status		
HAART naive	22	2.7
On HAART	786	97.3
Time since on HAART in months: median (IQR)[a](n=786)	28.0 (14.0 – 52.0)	
Ever disclosed HIV status		
No	67	8.3
Yes	741	91.7
Disclosure of HIV status to Partner (n = 741)		
No	92	12.4
Yes	649	87.6
Relationship with partner		
Married	757	93.7
Cohabiting	51	6.3
Duration of relationship in months: median (IQR)[a]	120.0 (60.0 -180.0)	
Partner's HIV status		
Negative	507	62.7
Positive	227	28.1
Unknown	74	9.2
Partner's HAART status		
On HAART	182	22.5
HAART naive	523	64.7
Unknown	103	12.7
Is partner respondent's first partner		
No	168	20.8
Yes	640	79.2

[a]*Interquartile range*

Table 4. HIV treatment optimism

Variables	Frequency	Percentage (%)
HIV treatment optimism score: median (IQR)[a]	14.0 (12.0 – 16.0)	
HIV treatment optimism		
Realistic	452	55.9
Optimistic	356	44.1

optimism and fertility intention, the effect of other factor should be considered in interpreting this association.

Concerning HIV treatment optimism, this study showed that more respondents were realistic about HIV treatment even in this era of widespread HAART. This result is in agreement with other published works which also reported that more PLHIV were realistic about HIV treatment [5, 8]. This finding suggests that although HAART is widely available, PLHIV still exhibited some caution. Worthy of mention however is that although more of the respondents in this study were realistic about HIV treatment, the fact that some (44.1%) were still optimistic is disturbing as optimism about HIV treatment has been documented to be associated with some sexual and reproductive behaviours which can fuel HIV transmission [18, 32, 33]. The implication of this is that as coverage for HIV treatment improves, there is also a need to monitor the attitudes and beliefs of PLHIV towards treatment.

Regarding fertility intention, respondents in this study, like in other Nigerian [3, 4, 34] and non-Nigerian [2, 35-38] studies, intend to have (more) children in the future. The personal desires to experience biological parenthood, which is influenced by the cultural value placed on

Table 5. Obstetrics history and fertility intention of respondents

Variables	Frequency	Percentage (%)
Lifetime childbirth		
0	22	2.7
1	90	11.1
2	185	22.9
≥ 3	511	63.2
Number of lifetime children who are alive(n = 786)		
0	6	0.8
1	107	13.6
2	214	27.2
≥ 3	459	58.4
Number of childbirth for current partner		
0	50	6.2
1	127	15.7
2	198	24.5
≥ 3	433	53.6
Number of living children for current partner (n = 758)		
0	13	1.7
1	133	17.5
2	217	28.6
≥ 3	395	52.1
Fertility intention		
No intention	307	38.0
Intends pregnancy	501	62.0
Number of children expected in the future (n = 501)		
1	219	43.7
2	170	33.9
≥ 3	112	22.4

childbearing is a possible explanation for this finding [3, 39].

Before adjusting for other factors, HIV treatment optimism was found to be associated with fertility intention in this study. Kaida and colleagues [5] also documented an association between HIV treatment optimism and fertility intention in their study. Antiretroviral therapy brings about a return to near normal life as previously documented by published literature [40, 41]. So expectedly, this will affect the attitude and beliefs of PLHIV about various life choices including fertility intention as noticed among respondents in this study and that of Kaida and colleagues [5].This association is however quite disturbing given the fact that the natural process of conception remains a dilemma especially for those in sero-discordant relationships as stated in published literature [33], and hence decreasing heterosexual transmission of HIV may not be in view in this part of the world. This discussion is corroborated by the limitation of the HIV Prevention Trials Network (HPTN 052) study conducted in 9 countries across America, Asia and Africa to evaluate the effect of combination antiretroviral therapy on the prevention of HIV-1 transmission to uninfected partners and on clinical events in infected persons [42]. Although the HPTN052 study documented a large decrease in sexual transmission of HIV among HIV serodiscordant couples through early use of HAART, the authors sounded that it should be interpreted with caution. The authors sounded that the ongoing couples counselling and the condom provision might have contributed to the decreased transmission which might not be the scenario in real life situations.

After adjusting for other factors, this study showed that being HIV treatment optimistic predicts fertility intention. When interpreting this association however, the effect of other predictors should be taken into account as revealed by our study. The other predictors of fertility intention from this study were being a female, non-disclosure of HIV status to partner, partner being HIV negative or positive, unknown partner HAART status, being in a short relationship, having ≤ 2 living lifetime children and attendance of an antiretroviral clinic located in an urban area. All these should be factored in when

Table 6. Bivariate and multivariate analysis for the association between HIV treatment optimism and fertility intention

Characteristics	Fertility intention		P-value[a]	Adjusted OR (CI)[b]	P-value[c]
	No intention	Intends pregnancy			
HIV treatment optimism					
Realistic	186(41.2)	266(58.8)	0.037	1	
Optimistic	121(34.0)	235(66.0)		1.76(1.19-2.61)	0.004
Location of health facility					
Urban	130(32.3)	273(67.7)	0.001	2.03(1.32-3.11)	0.001
Semi-urban	177(43.7)	228(56.3)		1	
Age (in years)					
≤ 30	53(24.9)	160(75.1)	<0.001	1.02(0.63-1.66)	0.928
> 30	254(42.7)	341(57.3)	1		
Sex					
Male	80(56.3)	62(43.7)	<0.001	1	
Female	227(34.1)	439(65.9)		1.67(1.02-2.74)	0.043
Highest level of education completed					
<Senior Secondary	173(41.6)	243(58.4)	0.030	1	
≥ Senior secondary	134(34.2)	258(65.8)		1.12(0.75-1.65)	0.586
Religion					
Islam	162(37.6)	269(62.4)	0.798		
Others	145(38.5)	232(61.5)			
Tribe					
Yoruba	275(39.1)	429(60.9)	0.104		
Others	32(30.8)	72(69.2)			
Occupation					
Unskilled/Unemployed	8(25.8)	23(74.2)	0.407		
Skilled manual	28(43.1)	37(56.9)			
Skilled non-manual	237(37.7)	391(62.3)			
Professional/Managerial	34(40.5)	50(59.5)			
Monthly income (in naira) (N = 798)					
< 18,000	92(34.7)	173(65.3)	0.151		
≥ 18,000	213(40.0)	320(60.0)			

[a]P-value in bivariate analysis [b]Adjusted odds ratio (confidence interval) [c]P-value in logistic regression [d]Interquartile range

considering the association between HIV treatment optimism and fertility intention. No published work was identified to have assessed the role of other predictors in mediating the association between HIV treatment optimism and fertility intentions.

The limitations of this study must be acknowledged. First, this study recruited PLHIV who were attending adult ARV clinics in two government health facilities in Oyo state such that findings may not be generalizable to all PLHIV in the country. Second, the measures used were all self-reported, so a degree of biased reporting was possible. Lastly, the wide confidence interval observed for some of the adjusted odds ratio indicates low precision and that more information may be needed on the associations with wide confidence interval to guide decision-making. Despite this limitation however, this study provides insight into the attitudes and beliefs about HAART in the light of reproductive decision-making.

Conclusions

In addition to determining the association between HIV treatment optimism and fertility intention among PLHIV, this study was also able to utilise multivariate analysis to assess the role of other

Table 6 (continued). Bivariate and multivariate analysis for the association between HIV treatment optimism and fertility intention

Characteristics	Fertility intention		P-value[a]	Adjusted OR (CI)[b]	P-value[c]
	No intention	Intends pregnancy			
Time since HIV diagnosis in months: median (IQR)[d]	31.0(12.0 -58.0)	29.0(18.0 -54.5)	0.738		
HAART status					
HAART naive	8(36.4)	14(63.6)	0.873		
On HAART	299(38.0)	487(62.0)			
Time since on HAART in months: median (IQR)[d]	31.0(12.0 -57.0)	27.0(15.0 -50.0)	0.387		
Disclosure of HIV status to Partner					
No	25(27.2)	67(72.8)	0.011	2.28(1.13-4.62)1	0.022
Yes	266(41.0)	383(59.0)			
Relationship with partner					
Married	288(38.0)	469(62.0)	0.910		
Cohabiting	19(37.3)	32(62.7)			
Duration of relationship in months: median (IQR)[d]	156.0(96.0 -228.0)	96.0(48.0 -144.0)	<0.001	0.99(0.99-1.00)	<0.001
Partner's HIV status					
Negative	194(38.3)	313(61.7)	0.026	7.88(1.59-39.12)	0.012
Positive	95(41.9)	132(58.1)		15.72(2.65-93.42)	0.002
Unknown	18(24.3)	56(75.7)		1	
Lifetime childbirth					
≤ 2	36(12.1)	261(87.9)	<0.001	0.7(0.12-4.16)	0.703
>2	271(53.0)	240(47.0)		1	
Number of lifetime children who are alive					
≤2	44(13.5)	283(86.5)	<0.001	10.02(1.79-55.99)	0.009
>2	263(57.3)	196(42.7)		1	
Number of childbirth for current partner					
≤2	62(16.5)	313(83.5)	<0.001	1.20(0.20-7.24)	0.841
>2	245(56.6)	188(43.4)		1	
Number of living children for current partner					
≤2	63(17.4)	300(82.6)	<0.001	1.00(0.17-5.91	0.997
>2	238(60.3)	157(39.7)		1	

[a]*P-value in bivariate analysis* [b]*Adjusted odds ratio (confidence interval)* [c]*P-value in logistic regression* [d]*Interquartile range*

predictors in mediating the association between HIV treatment optimism and fertility intentions.

The findings from this study underscore the importance of giving equal attention, or more, to prevention messages as is given to HIV treatment during this period of upscale in HIV treatment. This is very important so that the overstated benefits of HIV treatment do not lead to a reduction in other prevention measures which may ultimately lead to a rise in the incidence of HIV because of the high risk attitudes and behaviours PLHIV are likely to adopt. Prevention messages should pay special attention to PLHIV who are HIV treatment optimistic and possess characteristics that have been identified to mediate the relationship between HIV treatment optimism and fertility intentions.

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Inductive effects of fractions of crude water-soluble extract of *Momordica charantia on* rat liver mitochondrial membrane permeability transition pore

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Abstract

Background: The mitochondrial membrane permeability transition (MMPT) pore opening has been implicated as a final cell death pathway in numerous diseases and therefore understanding conditions dictating this opening is crucial for developing targeted therapies. Cells committed to suicide, signal the release of cytochrome c from the inner mitochondrial membrane; a point of no return in the intrinsic/mitochondrial apoptotic pathway. The efficacy of *Momordica charantia* (MC) against certain cancers has been linked to its ability to induce apoptosis; however, the underlying mechanism of the induction is still unknown. This study was designed to evaluate the effect of leaf extract of MC on the opening of MMPT pore in normal Wistar rat liver mitochondria.

*Methodology***:** The leaves of MC were obtain from the Botanical Garden, OAU, Ile-Ife Campus and was cold-extracted in distilled water to obtain the Crude Water-Soluble Extract (CWSE). N-hexane, dichloromethane, ethylacetate, butanol and aqueous fractions were obtained from the CWSE via solvent partitioning. The *in vitro* effects of these fractions on rat liver MMPT pore and ATPase activity at various concentrations (75, 100 and 125 µg/ml) were spectrophotometrically assayed.

Result: At all concentrations, all fractions of CWSE of MC show significant induction of the MMPT pore but the highest induction was observed at 125µg/ml of butanol fraction with a 23.56-fold increase when compared with the control group. In the same vein, the ATPase activities were also significantly enhanced by in vitro treatment with all but the ehtylacetate fraction; peaking at 14.13 mMPi/mg protein/min for the butanol fraction at 125µg/ml in comparison with the control group.

Conclusion: We thus conclude that the fractions of interest derived from the CWSE of MC are both potent inducers of and enhancers of the MMPT pore and mitochondrial ATPase activity respectively, the butanol fraction being the most potent.

Keywords: *Momordica charantia, Crude water soluble extract, ATP, Mitochondrial membrane permeability transition pore, Mitochondrial ATPase activity, Mitochondrial swelling*.

Résumé - 4010

Contexte: L'ouverture des pores de la transition de perméabilité membranaire mitochondriale (MMPT) a été impliquée en tant que voie de mort cellulaire finale dans de nombreuses maladies. Par conséquent, la compréhension des conditions dictant cette ouverture est cruciale pour le développement de thérapies ciblées. Les cellules suicidaires signalent la libération du cytochrome c de la membrane mitochondriale interne; un point de non-retour dans la voie apoptotique intrinsèque / mitochondriale. L'efficacité de *Momordica charantia* (MC) contre certains cancers a été liée à sa capacité à induire l'apoptose; néanmoins, le mécanisme sous-jacent de l'induction est encore inconnu. Cette étude visait à évaluer l'effet de l'extrait de feuille de MC sur l'ouverture des pores de MMPT dans les mitochondries de foie de rat Witsar normal.

Méthodologie: Les feuilles de MC ont été obtenues du jardin botanique, UOA, du campus Ile-Ife et ont été extraites à froid dans de l'eau distillée pour obtenir l'extrait brut soluble dans l'eau (CWSE). Le N-hexane, le dichlorométhane, l'acétate d'éthyle, le butanol et des fractions aqueuses ont été obtenus à partir de CWSE via un partage par solvant. Les effets in vitro de ces fractions sur l'activité des pores et de l'ATPase de la MMPT dans le foie de rat à diverses concentrations (75, 100 et 125 µg / ml) ont été analysés par spectrophotométrie.

Résultat: A toutes les concentrations, toutes les fractions de CWSE de MC présentent une induction

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significative du pore de MMPT, mais l'induction la plus élevée a été observée à 125 µg / ml de fraction de butanol avec une augmentation de 23,56 fois par rapport au groupe témoin. Dans la même veine, les activités ATPase ont également été significativement augmentées par un traitement in vitro avec toutes les fractions sauf la fraction acétate d'éthyle; culminant à 14,13 mMPi / mg de protéine / minute pour la fraction butanol à 125 µg / ml par rapport au groupe témoin.
Conclusion: Nous concluons donc que les fractions d'intérêt dérivées de la CWSE de MC sont à la fois des inducteurs puissants et des amplificateurs de l'activité du pore de MMPT et de l'ATPase mitochondriale, la fraction butanol étant la plus puissante.

Mots-clés: *Momordica charantia, extrait brut soluble dans l'eau, ATP, pore de transition de la perméabilité de la membrane mitochondriale, activité ATPase mitochondriale, gonflement mitochondrial.*

Introduction

The mitochondrial permeability transition pore is a presumed proteinaceous entity in the inner mitochondria membrane. MMPT pore opening has generally been attributed to a structural change in a protein embedded within the membrane, which in certain conditions seems to usually perform a physiological role [1, 2]. Mitochondrial permeability transition has been found to be involved in the regulation of apoptosis, as the mitochondrial pro-apoptotic factors such as cyt. C., AIF and Smac/Diablo, which are normally confined to the mitochondrial matrix are released through it into the cytosol. Once released, Cyt. C binds with Apaf-1 which prompts the activation of caspases in the presence of ATP/dATP [3]. Kerr and colleagues in 1972 raised the possibility that a large percentage of cell loss from tumors was due to apoptosis and this hypothesis has been confirmed by subsequent studies which revealed a high frequency of apoptosis in spontaneously regressing tumors and in tumors treated with cytotoxic anticancer agents [4].

These observations therefore suggest that apoptosis contributed to a high rate of cell loss in malignant tumors and could promote tumor regression [5]. It is now well established that anticancer agents induce apoptosis, and that disruption of apoptotic programs can reduce treatment sensitivity [6]. Sun *et al*. (2004), identified representatives from various classes of chemopreventive agents from *in vitro* studies with sufficient evidence to provide a detailed account of their apoptotic mechanisms. Most of these compounds can activate caspases through intrinsic effector mechanisms that are regulated by $Bcl-_2$ family members (e.g inhibition of $Bcl-_2$ expression or induction of Bax expression) or the mitochondrial permeability transition (e.g dissipation of mitochondrial inner trans membrane potential) [7].

The popularity of *Momordica charantia* in various systems of traditional medicine for several ailments suggests that the plant contains bioactive agents that could be potentially useful in drug development. Several studies using modern techniques have authenticated its use in diabetes and its complications. Most importantly, some of these studies have shown its efficacy in various cancers including breast cancer, skin tumor, prostatic cancer, and Hodgkin's disease [8].

The aim of this study was therefore to assess the inductive effect of fractions obtained from CWSE of MC on MMPT pore opening; a vital pre-requisite for the intrinsic apoptotic pathway. The effects of the fractions were also assessed on rat liver mitochondrial ATPase activity.

Materials and methods

Plant

Fresh leaves of *Momordica charantia* were obtained from medicinal plant garden and other locations within Obafemi Awolowo University Ile-Ife Campus, Osun State. The plant was authenticated by Mr. I. I. Ogunlowo, at the Faculty of Pharmacognosy Herbarium, of the same institution. The plant herbarium no is FPL-1783.

Preparation of extracts

The leaves of MC were weighed, rinsed with clean water and air-dried after which it was blended to macerate. One kilogram of the collected macerate was soaked in 1litre of distilled water and left for 12 hours at room temperature and then sieved using a muslin bag to obtain both the filtrates and the residues. The filtrate obtained was subjected to filtration and then concentrated using Rotatory evaporator at 65°C. The moist extract obtained after concentration was freeze dried using a Freeze drier. The powdery extract was partitioned to obtain n-hexane, dichloromethane, ethylacetate, butanol and aqueous fractions. All fractions were stored at 4°C.

Experimental animal

Twenty five Wistar strain albino male rats (4 months old; 180-200g) were obtained and kept at the Faculty of Basic Medical Sciences' Animal House, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, under light-controlled conditions (12h"light/12h"dark cycle) and in well-ventilated

plastic cages. The animals received feeds and water *ad libitum*, were allowed to acclimatize over a period of two weeks and cared for in accordance with good laboratory animal care practice prescribed by the Faculty of Basic Medical Sciences' Animal Care and Use Committee.

Chemicals and reagents

Sodium Carbonate (Na_2CO_3), Sodium Hydroxide (NaOH), Sodium-Potassium Tartarate (Na-K-C_4O_6), Hydrated Copper Sulphate ($CuSO_4.5H_2O$), Calcium Chloride ($CaCl_2$), Potassium Hydroxide (KOH), Methanol were Products of BDH Poole, UK Ltd. and Co., while Folin Ciocalteau Reagent, BSA, Mannitol, Sucrose, HEPES [4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid], EGTA, Cyclosporine, Rotenone, and Sodium Succinate hexahydrate were Products of Sigma-Aldrich Co, USA. All Chemicals were of analytical grade.

Mitochondrial Fraction Isolation

Overnight-fasted animals were sacrificed by cervical dislocation and liver mitochondrial fraction were isolated essentially according to the method of Olorunsogo *et al.,* (1984) and as reported by Lapidus and Sokolove (1993) [9,10]. Livers were rapidly excised, trimmed to remove excess tissues and washed in a buffer containing 210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES, 1 M KOH, and 1 mM EGTA, pH 7.4. Thereafter the livers were weighed, chopped and suspended in the same buffer to make a 10% homogenate.

The suspension was immediately homogenized on ice using a Porter glass homogenizer. The homogenate was centrifuged in an SM-18B High Speed Refrigerated Centrifuge twice at 2500 rpm for 5 min to remove the nuclear fraction and cellular debris. Supernatants obtained were centrifuged at 13000 rpm for 10 min and the mitochondrial fractions obtained were washed three times at 12000 rpm for 10 min with a washing buffer which contained 210mM Mannitol, 70mM Sucrose, 5mM HEPES-KOH and 0.5% BSA, pH 7.4. The mitochondrial pellets were suspended in swelling buffer (210 mMMannitol, 70 mM Sucrose, and 5 mM HEPES-KOH, pH 7.4) and immediately dispensed in 1 ml Eppendorf tubes.

Mitochondrial swelling assay

Mitochondrial permeability transition opening was determined according to the method of Lapidus and Sokolove [10]. This was monitored by measuring the changes in absorbance of mitochondria at 540 nm in the presence and absence of calcium ion (triggering agent) in a Spectrumlab 752s UV/Visible spectrophotometer. Mitochondria (0.4 mg protein/ml) were pre-incubated in the presence of 8μM rotenone in a medium containing 210mM mannitol, 70mM sucrose, 5mM HEPES-KOH (pH 7.4) for 3 minutes at 30°C prior to the addition of 300μM $CaCl_2$, while 50μM sodium succinate was added 30 seconds later and MMPT pore opening was measured at 540 nm for 12 minutess at 30 seconds interval. The inhibitory effect of cyclosporine on the induction of pore opening was carried out prior to the addition of $CaCl_2$. The inductive effects of fractions were monitored when the fractions were replaced with $CaCl_2$.

Determination of ATPase activity

Mitochondrial ATPase assay was done by modifying the method of Lardy and Wellman (1953) [11]. Each test medium contained 65 mM Tris-HCL (pH 7.4), 0.5 mg protein (mitochondria), 0.5 mM KCl, 1 mM ATP and 25 mM sucrose. The final assay volume was 2 mL. Changing concentrations of the MC portions (n-hexane, dichloromethane, ethylacetate, butanol and aqueous fractions) were included as needs be. The reaction was begun by the addition of the ATP and permitted to continue for 30 minutes with consistent shaking at 37°C. One milliliter of 10% sodium dodecyl sulfate was added to the mixture in each test tube to convey the reaction to a stop. After which four milliliter of distilled water was added to each test tube and then one milliliter of the resulting solution removed into fresh test tubes where one milliliter of 1.25% Ammonium molybdate in 6.5% Sulphuric acid was added. One milliliter of 9% ascorbic acid was further added for colour development which was estimated at 660 nm. All analysis was completed in triplicate.

Estimation of Inorganic Phosphate Released

The concentration of inorganic phosphate released following the hydrolysis of ATP was determined according to the method described by Bassir and as modified by Olorunsogo and Malomo [12] using Disodium Hydrogen phosphate (Na2HPO4) as standard. Ammonium molybdate (1.25%) and freshly prepared 9% ascorbic acid were added to 1mL of the reaction mixture and allowed to stand for 30 minutes. The intensity of the blue colour was read at 660 nm in a spectrophotometer.

Statistical Analysis

The data were statistically evaluated using one way analysis of variance (ANOVA) and student's T-test. All the results were expressed as Mean ± Standard Deviation (SD). The $p < 0.05$ were considered to be statistically significant.

Results

Figure 1 shows no significant changes in the volume of intact mitochondria respiring on succinate in the absence of calcium, while calcium ion induced significant opening of mitochondrial permeability was reversed by cyclosporine, the standard inhibitor of the pore by about 62%. As shown in figure 2, butanol fraction triggers the *in vitro* opening of the MMPT pore in rat liver mitochondria in a concentration-dependent manner such that (75<100<125µg/mL). a 23.5-fold increase (Δ540nm= -0.636) in permeability transition was observed at the highest concentration of 125µg/mL when compared to the control. Δ540nm of -0.065 and -0.481 which translates to 2.41 and 17.81 fold increases respectively were observed at 75µg/mL and 100µg/mL.

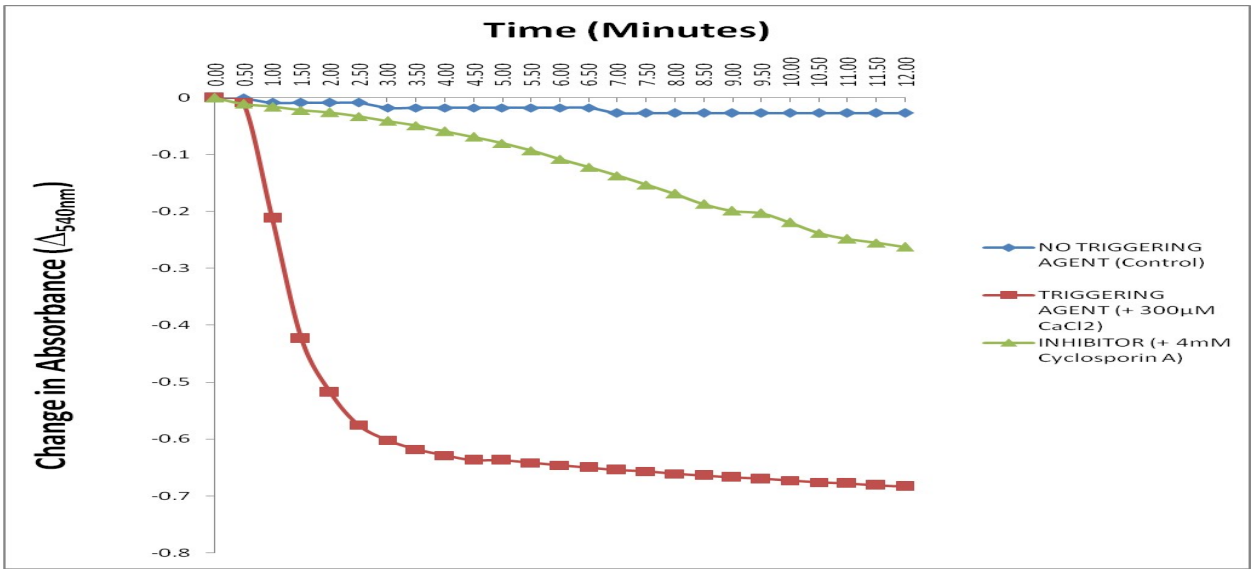

Fig. 1: In vitro induction of the opening of MMPT pore by Ca^{2+} and inhibition by cyclosporine in a male Wistar rat strain.

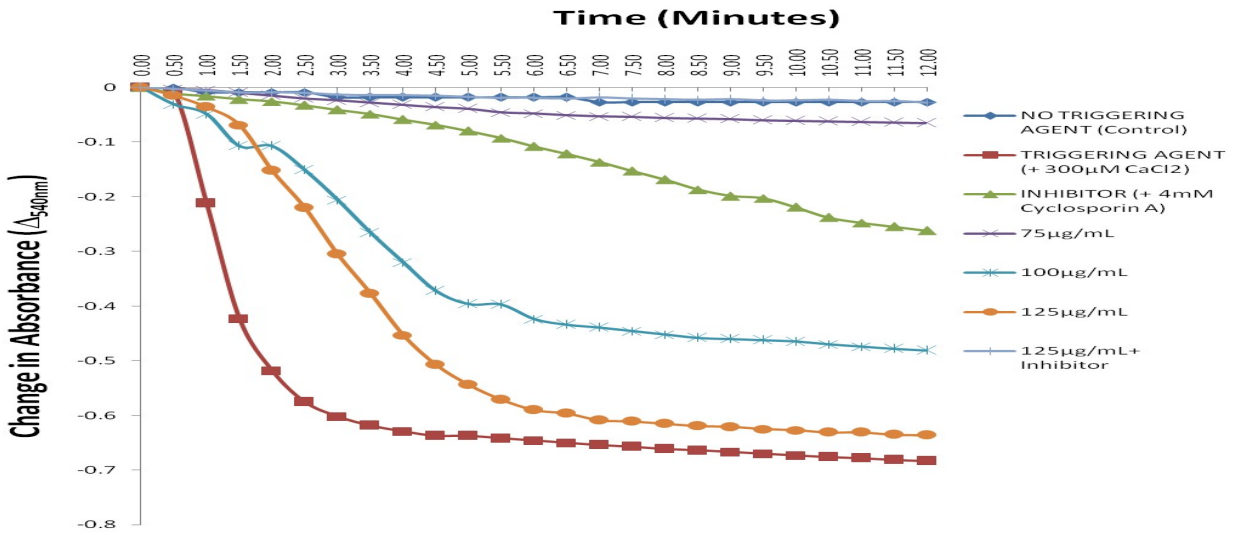

Fig. 2: The effect of butanol fraction of CWSE of *Momordica charantia* on MMPT pore at varying concentration.

transition pore up to about 10 folds in the presence of succinate and rotenone. This observed induction All inductions being significant at (P<0.05). figure 3 also shows a maximal induction of 22.11-fold

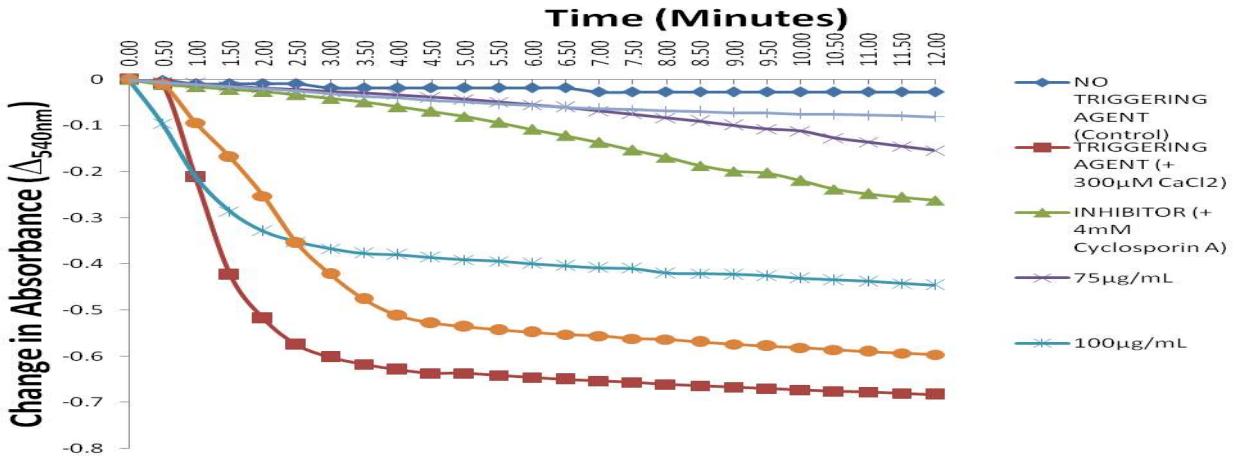

Fig. 3: The effect of ethylacetate fraction of CWSE of *Momordica charantia* on MMPT pore at varying concentration**.**

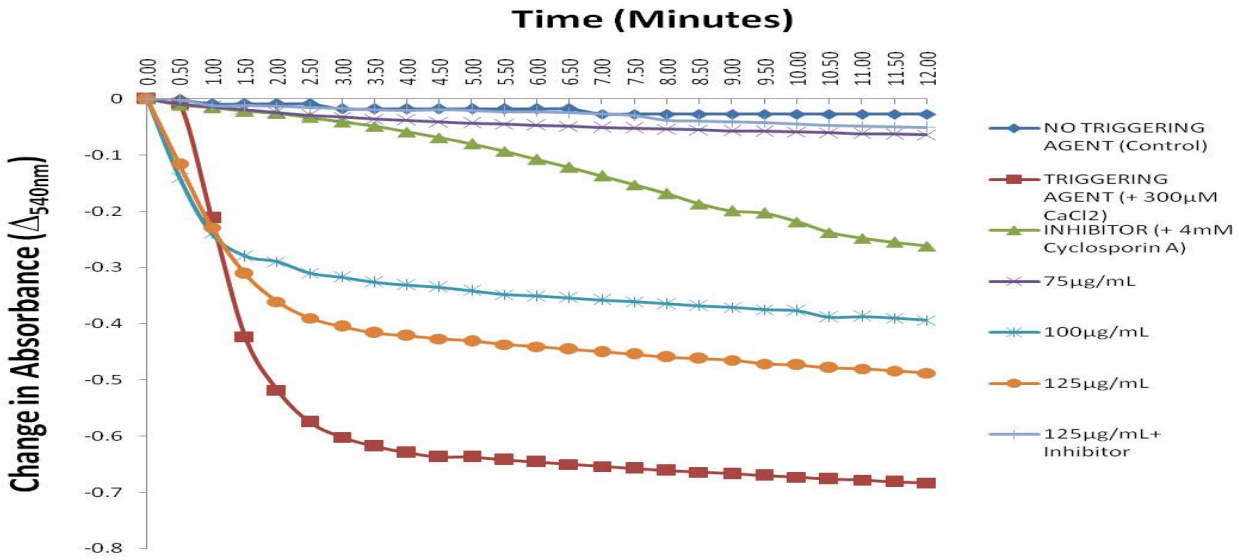

Fig. 4: Effect of the n-hexane fraction of CWSE on MMPT pore opening at varied concentrations.

increase (Δ540nm = -0.597) at highest concentration (125µg/mL) as compared with control while at 75µg/mL and 100µg/mL, Δ540nm of -0.154 and -0.446 which translates to 5.7 and 16.52 fold increases were observed respectively. At all concentrations, ethylacetate fraction of CWSE of MC triggered significant (P<0.05) openings of MMPT pore. As shown in figure 4, all concentration of n-hexane fraction of CWSE of MC trigger significant (P<0.05) induction such that a 2.37-fold increase was observed at 75µg/ml with a Δ_{540nm} of -0.064, at 100µg/ml, Δ_{540nm} = -0.394 (14.59-fold increase), while at 125µg/ml a Δ_{540nm} of -0.488 which translates to 18.07 fold increase was observed.

As shown in figure 5, all concentrations of dichloromethane fraction of CWSE of MC show significant (P < 0.05) induction. Such that a Δ_{540nm} of –0.059 (2.19-fold increase) was observed at 75µg/ml. At 100µg/ml, Δ_{540nm} = –0.299 (11.07-fold increase). The highest induction was observed at 125µg/ml, with Δ_{540nm} of –0.343 which translates to 12.70-fold increase. According to figure 6, The aqueous fraction of CWSE of MC has a concentration-dependent inductive effect on MMPT pore in an increasing order such that at 75µg/ml, (Δ_{540nm} of –0.052a"1.95-fold increase); at 100µg/ml, Δ_{540nm} of –0.536 a"19.85-fold increase) and at 125µg/

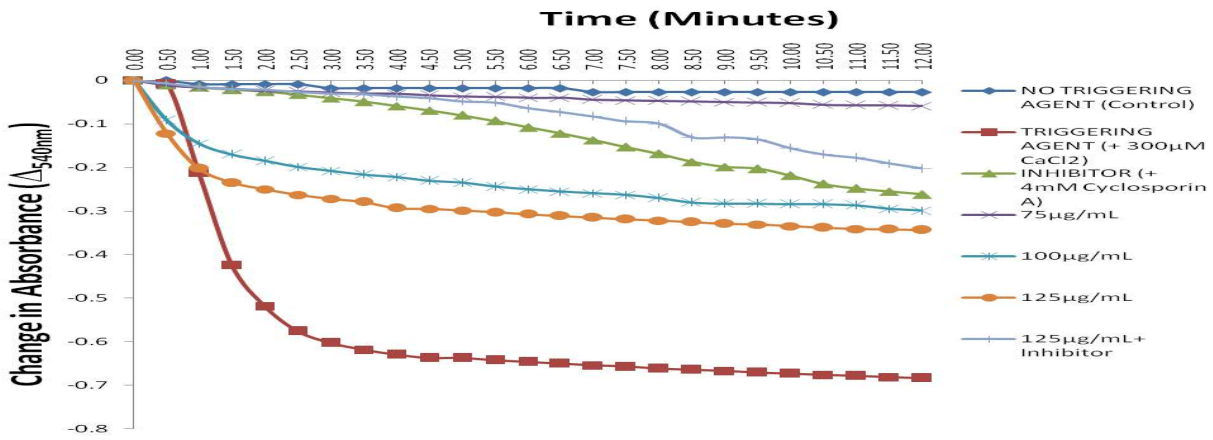

Fig. 5: Effect of the dichloromethane fraction of CWSE on MMPT pore opening at varied concentrations.

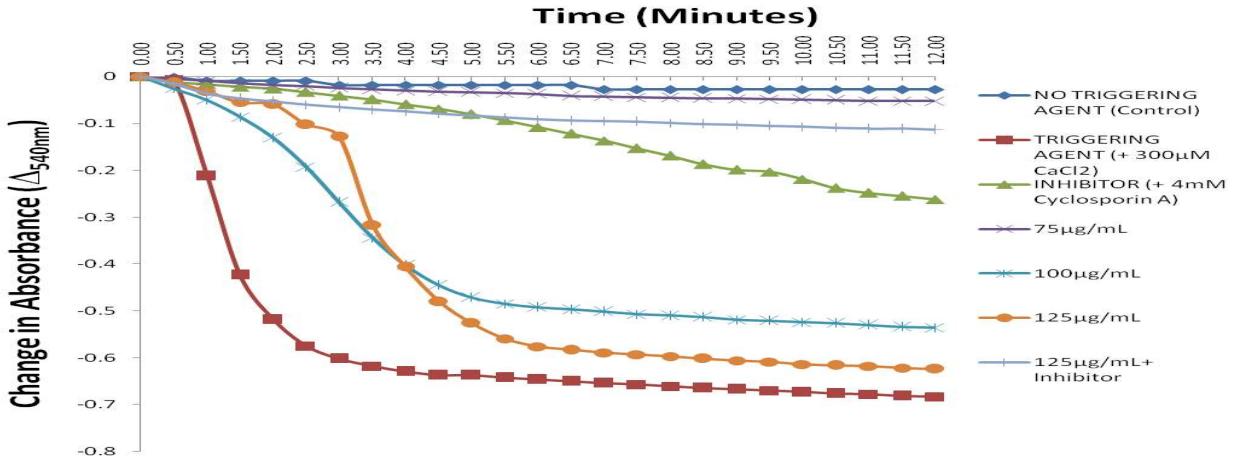

Fig. 6: Effect of the aqueous fraction of CWSE on MMPT pore opening at varied concentrations.

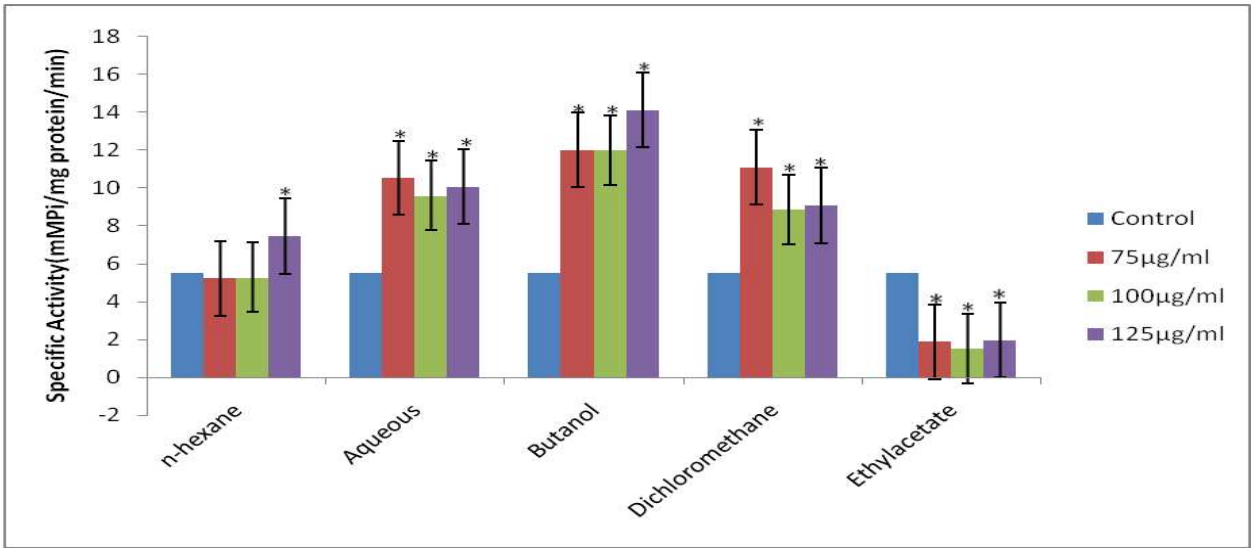

Fig. 7: The comparative effects of different fractions of CWSE of *MC on Mitochondrial* ATPase activity

ml, a Δ_{540nm} of –0.624=23.11-fold increase). All MMPT pore openings were significant at ($P<0.05$).

Mitochondrial ATPase activities were significantly enhanced by the aqueous, butanol and dichloromethane fractions at all concentrations, however, the n-hexane fraction shows significantly ($P<0.05$) increased ATPase activity only at 125µg/ml while the ethylacetate fraction show significant ($P<0.05$) decreases of ATPase activities at the various tested concentrations (figure 7).

Discussion

Cancer is a genetic disorder characterized by dysregulation of various cellular pathways that orchestrate cell proliferation, differentiation and death. Cancers are caused by carcinogens and mutagens [13]. A cell becomes cancerous when a series of oncogenes and/or onco-suppressors become dysfunctional hence inducing a neoplastic phenotype which causes high proliferation kinetics and loss of cell-cell contact inhibition in the cells [14]. Mitochondria have emerged as an intriguing target for anti-cancer drugs, inherent to vast majority if not all types of tumors. Drugs that concentrate on mitochondria to exert anti-cancer activity has become the center of attention of recent analysis due to their effective clinical potential (which has not been maximized thus far). The exceptional potential of mitochondria as a target for anti-cancer agents has been reinforced by the discouraging finding that even tumors of the same type from individual patients differ in a number of mutations. This is consistent with the idea of personalized therapy, an elusive goal at this stage which is in line with the notion that tumors are unlikely to be treated by agents that focus on only a single gene or a single pathway. This endows the mitochondrion an invariant target present in all tumors, with an exceptional momentum [15].

A critical stage of apoptosis is the opening of the mitochondrial membrane permeability transition pore because the release of cytochrome C into the cytosol finally commits the cell to self destruction [16-18]. Mitochondria are the cells' powerhouse, but also their suicidal weapon store. Many lethal signal transduction pathways converge on mitochondria to cause the permeabilization of the mitochondrial outer membrane, prompting the cytosolic release of pro-apoptotic proteins to the hindrance of the bioenergetic elements of mitochondria. The mitochondrial metabolism in malignant growth cells is deregulated inferable from the utilization of glycolytic intermediates, which are regularly bound for oxidative phosphorylation, in anabolic reactions. Initiation of the cell death mechanism in cancerous cells by repressing tumor-explicit modifications of the mitochondrial digestion or by invigorating mitochondrial membrane permeabilization could consequently be promising restorative methodologies.

Previous observations have shown that bioactive agents that alter mitochondrial membrane function and/or dissipate the mitochondrial potential can induce apoptosis. For example, epigallocatechin galate (EGCG) in green tea, depolarizes mitochondria in numerous human cell lines including prostate and lung cells, leading to apoptosis [19]. The vanilloid curcumin, found in tumeric, and capsaicin, found in chili peppers, can open the MMPT pore and collapse mitochondrial potential, leading to induction of apoptosis [19]. Curcumin, a polyphenol, induces mitochondrial swelling and collapses the MMPT, resulting in apoptosis in numerous cell types [20, 21]. Beta carotene, a carotenoid found in carrot, can induce release of cytochrome c from mitochondria and alter mitochondrial membrane potential in different tumor cell lines derived from leukemia, colon adenocarcinoma, and melanoma cells [22]. Interestingly, prior studies in our laboratory have also confirmed the MMPT pore opening potentials of *Momordica charantia,* first as a decoction and also in different solvent extracts [23, 24].

We found out that of the tested extracts, the crude water soluble extract was the most potent; hence the present study was conceived. The results showed concentration-dependent large amplitude mitochondrial swelling in all fraction-treated groups, suggesting the undisputable essence of MC as an MMPT pore inducer. All fractions obtained had their maximal inductive effect at highest concentration (125µg/mL) such that fold-increases of 23.56, 22.11, 18.07, 12.70 and 23.11 were observed for groups treated with butanol, ethylacetate, n-hexane, dichloromethane and aqueous fractions respectively compared with the control group. These observed large amplitude swellings caused by the fractions of the CWSE of MC suggest a possible role for the medicinal plant in the treatment of ailments arising from apoptosis deregulation.

Opening of the MPTP allows free entry into the mitochondria of any small molecule (<1500 Daltons) including protons. [25]. An important consequence of opening of the MPTP is uncoupling of oxidative phosphorylation [26]. Loss of membrane potential interferes with the production of ATP, the cells main source of energy, because mitochondria must have an electrochemical gradient to provide

the driving force for ATP production. In cell damage resulting from conditions such as neurodegenerative diseases and head injury, opening of mitochondrial permeability transition pore can greatly reduce ATP production, and can cause ATP Synthase (through its reversal) to begin hydrolyzing, rather than producing ATP [27]. Opening of the MPTP leads to permeability transition (PT), a sudden increase of inner mitochondrial permeability to solutes with molecular mass up to 1.5 kDa which is implicated in apoptosis or necrosis as an important event in the control of cell death or survival [28]. This opening generates a colloidal osmotic pressure across the inner mitochondrial membrane which drives water into the matrix and causes swelling. The inner membrane being extensively folded into cristae can expand to compensate but the outer membrane cannot and this ruptures, releasing intermembrane proteins. It is the release of these proteins such as Cytochrome C that enables the mitochondria play a role in apoptosis i.e the release of cytochrome C causes cells to go through apoptosis by activating pro-apoptotic factors [29-31].

In consonance with the induction of the MMPT pore observed in this study, fractions obtained from CWSE of MC except for ethylacetate fraction enhanced ATPase activities, in a non concentration-dependent manner. For example, ATPase activities at the three tested concentrations for butanol fraction were all significantly enhanced with respect to the control group while for the n-Hexane fraction, significant increase in the activity of ATPase was only observed at 125µg/ml. The highest ATPase activity observed across all fractions was at 125µg/ml for butanol fraction.

The observed increases in the activity of the mitochondrial ATPase must have been due to the release of inorganic phosphate (Pi), an indication of the uncoupling of phosphorylation in the mitochondrion, a process which is synonymous with MPT pore opening and mitochondrial swelling. Also, we observed that the CWSE fractions at all concentrations induced opening of the MMPT in a concentration-dependent manner, that is, the inductive effect increased as concentration increased. This may have to do with the assumption that the active components of the plant may be interacting with specific components of the pore such as adenine nucleotide translocase (ANT). Although it is yet to be determined which of the active components exerts the observed effect, there is incontrovertible evidence that exposure to *MC will* possibly elicit opening of the pore and subsequently the release of cytochrome C and activation of the execution caspases. A process which will be useful in the development of drugs which rely on the permeabilization of the mitochondrial membrane in the treatment of diseases caused by dysregulated apoptosis.

Conclusion

Conclusively, fractions obtained from the CWSE of *Momordica charantia* leaves significantly induced large amplitude mitochondrial swelling consequent to the opening of the MMPT pore, and mostly enhanced mitochondrial ATPase activity. An indication that the plant's phytochemicals are potent agents with possible usefulness in the treatment of diseases arising from the down-regulation of apoptosis and responsive to the up-regulation of the same via opening of the MMPT pore.

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