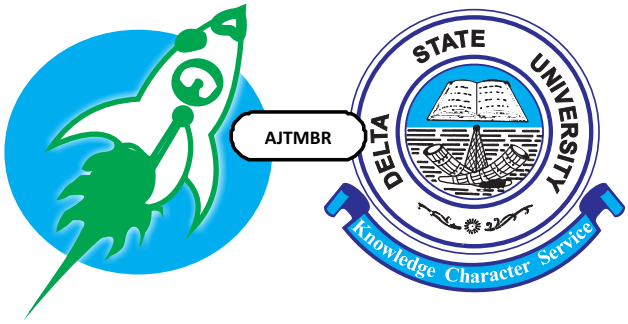
African Journal of Tropical Medicine and Biomedical Research (AJTMBR)



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The *African Journal of Tropical Medicine and Biomedical Research* is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (anatomy, biochemistry, physiology, pharmacology, psychology, nursing etc) and clinical sciences (internal medicine, surgery, obstetrics and gynaecology, dental surgery, child health, laboratory sciences, radiology, community medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Table of Contents

Editorial

The Challenges of Diagnosis and Management of Fetal Anomalies in Low Resource Settings 7
Enabudoso E & Ikubor J

Original Articles

Screening for Diabetes Mellitus and Its Risk Factors Among Senior Staff of Delta State University, Abraka. 13
Adje DU, Arute J, Aghoja CO & James M

Antibacterial Activity Against Clinical Isolates from a Tertiary Hospital in Nigeria Exhibited by Methanol and Aqueous Extracts of Root and Stem Bark of *Persea Americana* (Laureaceae) 22
Ching FP, Joseph CG & Nwaeze N

Family Size Preferences Among Married Men in A Semi-urban Community in Delta State, South-south, Nigeria 33
Oyibo PG, Onohwakpor EA, Abedi HO

An Estimation of the Some Antioxidant Vitamins in the Serum of Diabetes Mellitus Patients 42
Mordi JC

Relationship between Obesity indicators and Reduced Renal Function in a Nigerian Rural Population 47
Okoye O, Oviasu E & Ojogwu L

Assessment of Needle Stick Injuries and Post Exposure Prophylaxis Utilization among Doctors in Benin City, Edo State 55
Obi AI & Ofili AN

Lower Extremity Amputation Surgeries in a Sub-urban Teaching Hospital in Nigeria 65
Odatuwa-Omagbemi DO

Pattern of Tooth Loss in a Niger Delta Region of Nigeria 73
Etetafia MO Okoro SI

Review Articles

HIV Associated Neurocognitive Disorders Among Adult Nigerians in the Era of Highly Active Anti-retroviral Therapy 81
Obiabo YO

Molecular Techniques: Its role in Microbial Diseases and Antibiotic Resistance Detection 96
Adomi P O, Owhe-Ureghe UB & Nwafor OB

Case Reports

Vitamin K Deficiency Bleeding Masquerading as a Malignant Disease 109
Osarogiagbon WO

Erratum 116

Editorial

The Challenges of Diagnosis and Management of Fetal Anomalies in Low Resource Settings

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Keywords: Fetal anomalies, low resource, fetal Medicine, Nigeria, obstetric ultrasound

Congenital anomalies are structural or functional anomalies present at birth. They are significant causes of perinatal morbidity and mortality. In areas where the perinatal mortality rate is low as in high income countries, their contribution to perinatal morbidity and mortality is more obvious. However, in low resource settings, where perinatal mortality is high, the major aetiologic factors include neonatal sepsis and birth asphyxia. These conditions usually obviate the contribution from congenital malformations[1-3]. However, fetal malformations remain a source of significant intrauterine and newborn deaths as well as significant childhood morbidity exerting its toll on an already overstretched health system[1-3]

Fetal diagnosis is an essential aspect of fetal care. It mainly involves fetal biometry, fetal anatomical survey and fetal biophysical assessment. Advances in medical ultrasound has revolutionalised the imaging of the fetus thus giving views for the various assessments. Concurrent advance in human genetics, embryology, mechanisms of fetal dysmorphology and endoscopy has culminated in practice that manages the fetus as a distinct entity as against the previous practice of the fetus being an appendage of the mother. One area in which this has proven useful is in the detection and management of fetal anomalies.

Fetal anomaly ultrasound (often also called fetal anatomical survey or detailed ultrasound scan) can detect majority of these abnormalities. In addition, ultrasound scan can be used as screening for certain chromosomal anomalies such as Trisomy 21 (Down syndrome) and also to guide invasive procedures like amniocentesis, chorionic villus sampling and cordocentesis. These functionalities have made ultrasound an indispensable tool in the evaluation of the fetus for congenital anomalies.

Routine fetal anomaly scan is practiced in many high income countries. This is usually

done at the 18 to 22 week gestation. In some high risk women, an earlier 11 to 13 week scan may be done for early detection of major anomalies [5,6,7]. This is usually in a bid to facilitate first trimester termination of affected pregnancies which is considered safer than later terminations. High risk indications for this includes women with previous congenitally malformed babies, those who had exposure to teratogens in the index pregnancy, presence of chronic medical diseases, family history of congenital malformations, amongst others[8].

The practice of routine fetal anomaly ultrasound in pregnancy has been proposed to confer numerous advantages. These include the provision of information for earlier termination of pregnancy for lethal anomalies, planning of place, mode and timing of delivery, provision of therapy either medical or surgical, etc[5,9]. Anomalies like holoprosencephaly, anencephaly and severe ventriculomegaly (hydrocephalus) are considered lethal and termination is usually offered. Medical induction of fetal lung maturity using Dexamethasone is offered in situations of anticipated delivery before 34 weeks gestation. Laser ablation of vessels is offered for twin-twin transfusion syndrome, fetoscopic closure of open spina bifida and balloon tracheal occlusion for congenital diaphragmatic hernia are examples of beneficial fetal surgical procedures. Presence of large teratomas may be indications for caesarean section to prevent rupture of the tumor or obstructed labour from the mass, just as they may indicate frequent monitoring of the fetus for early detection of fetal hydrops.

Furthermore, early detection of congenital disorders has multiple effects on the person of the baby, the mother, family and the community. On the baby, early detection of the defect may lead to planned delivery which may prevent some long term complications or offer a chance at prenatal therapy as in intrauterine surgery for spinal bifida. On the mother, early detection of some conditions may prevent some maternal complications as may occur in Mirror's syndrome as a complication of fetal hydrops or severe abdominal pains from polyhydramnios resulting from anencephaly. It may also obviate the need for certain interventions like caesarean section for fetal distress in a severely malformed fetus. Some fetal defects exert a high toll on the family and the community at large. It is also known that optimizing fetal care could prevent perinatal mortality hence preventing the 'replacement syndrome' occasioned by the loss of a fetus or baby[4].

Despite these perceived advantages, there have been opponents of routine ultrasound screening especially for fetal anomalies. Many studies have failed to prove its beneficial effect over indicated scans in reducing major perinatal outcome measures like mortality and cerebral palsy[10,11] despite being more expensive and requiring on the average, more scans to be performed. Landmark studies including the RADIUS study and its various reviews have however opined that for there to be significant reduction in perinatal mortality following a system of routine ultrasound screening including detection of congenital anomalies, the detection rate for anomalies has to be high (unfortunately, this hardly obtains on a large scale even in high income countries) and there has to be a high uptake of pregnancy termination following detection[12]. This regime in reality substitutes a perinatal

death with an abortion. This essentially means that a fetus that should have been counted as a perinatal death if it gets to viability, is aborted before viability and does not end up in the perinatal records hence statistically is not counted as a perinatal death.
Whilst these lofty ideals rave in high resource countries, the same cannot be said of low resource countries. High quality obstetric ultrasound services are hardly available, early pregnancy screening services are nonexistent neither is there facility for ultrasound guided investigative procedures. The implications of these for detection of congenital anomalies is obvious – most anomalies are only detected when there is fetal demise or following delivery or if they manifest with other obvious clinical complications. In a few instances where they are detected, it is by chance and not a product of a systematic fetal anomaly scan. Many factors are responsible for this abysmal state.

Lack of access to fetal diagnostic services is one strong factor that drives this sorry state of affairs. In many low resource countries, medical service including obstetric diagnostic service is on fee for service basis. This exerts enormous cost on an already impoverished population. A regime of routine fetal anomaly scan is beyond the reach of many mothers. Furthermore, majority of mothers book late. In a recent study, it was found that the median gestational age at booking was between 18 and 20 weeks with a large percentage booking after 24 weeks[13]. These would have missed the window for optimum performance of the 18 to 22 week detailed scan.

Non availability of fetal anomaly scans is another factor militating against the detection of congenital anomalies. This service requires good quality ultrasound machines, highly motivated and trained sonologists and the availability of ample time to perform each evaluation. Ultrasound machines are expensive and the top range ones are well beyond the reach of the average health facility. Furthermore, there is dire lack of ultrasound reporting softwares that assist in visual diagnosis of features of some conditions following biometric measurements obtained at evaluation. The need for training of sonographers and sonologists for fetal anomaly scan and other high level scans has been reported by many researchers [14,15]. Some have even advocated the need for training in some of the more standard centres abroad[16]. However, this has been hampered by the lack of the necessary funding or sponsorship. Even when there are trained hands, the enormous demands on the few sonologists make them have insufficient time to carry out the time consuming fetal anomaly scans hence giving room for errors and inadequate diagnosis.

The non availability of growth charts customized for the local populations is another militating factor. Most growth charts in use in low resource regions of the world are mainly Caucasian. Rigorous scientific work to show compatibility of these charts with the local population is lacking. Therefore, any deviation noticed may not be predictive of abnormality as it may just be constitutional. Though a few studies have reported customized growth charts for some local populations[17], they have not gained wide applicability as they are not incorporated in the commonly available ultrasound systems.
Detection and management of congenital diseases requires a multidisciplinary approach. For an effective system, there is the

need for specialists in Fetal Medicine and morbid anatomists with interest in fetal dysmorphology, embryologists, human geneticists, chemical pathologists, neonatologists, paediatric surgeons and other essential sub-specialists. There is paucity of laboratories to perform karyotypic assessments and even for chemical analysis on maternal blood specimen for screening for fetal anomalies.

The lack of accurate records of incidence, prevalence and type of fetal anomalies prevalent in some of these countries also creates factors that prevent the design of programmes geared towards prevention or management. Training programs, sub-specialty needs and equipment should be tailored to the needs of the particular countries. These needs can only be known through proper and diligent research. The considerations for ultrasound in countries differ from one another hence the desire for a needs assessment. Furthermore, cultural acceptability of these emerging technologies differ. It cannot be taken for granted that women will like to know about anomalies in the fetus and even if present, that they will be willing to commit funds to therapy or to accept termination [18,19].

The prevailing laws in the countries also affect fetal diagnosis. Diagnosed severe fetal disorders may be lethal hence termination of pregnancy is often offered on medical grounds. However, many countries have abortion laws that restrict such pregnancy terminations [20,21]. In such instances, it becomes even more controversial whether a programme of routine anomaly scans should be done and if done, if whether the reports should be made known to the parents.

Having explored the myriads of factors that hamper the diagnosis and management of fetal anomalies in low resource settings, there is need for concerted efforts to improve standards and practices to reduce the gap in terms of fetal care between the regions of the world. It is essential to look at issues concerning research, training and implementation. These are imperative to institutionalizing an enduring programme.

Research is necessary to address the prevalence and type of fetal anomalies in specific locales and the possible associations and local connotations. It should also assess the acceptability of interventions for such conditions by the would-be beneficiaries. The cost implication and the effect on the overall health budget vis-à-vis other health issues in the society also needs to be explored.
Addressing training needs is imperative to success. While it is important to have foreign training in established centres abroad, paramount to a successful and sustainable service is the provision of locally provided training programme. Previous reports have established severe shortage of health workers including sonographers in the most underserved areas of the world mainly sub-saharan Africa and South Asia[22,23]. Fetal anomaly detection depends crucially on the knowledge and experience of the sonographer. Carrera reported recently in a survey that only about 15% of sonographers in Africa have attended a formal practical training and less than 5% in a hospital environment[23]. This therefore casts doubt on the quality of fetal scans in these environments as obstetric scans constitute the vast majority of scans done by sonologists[24,25,26].

While there are training institutions in many of the countries, there is need for upgrading of programmes and standardization through improved training for the tutors. Training also of the allied subspecialties especially medical genetics[12], counseling, biochemists and provision of laboratories for karyotypic and other assessments is essential to a useful programme. While there have been some local based training in many countries including Nigeria, the recent International training programme in Fetal Medicine[27] that focused on practical ultrasound skills facilitated by the lead author of this commentary and other seasoned international experts is highly commendable. There are plans to upgrade this particular training programme to cover the West African subregion and even sub-Saharan Africa. This we believe is a step in the right direction.

Training needs can also be addressed by establishing regional centres of excellence locally. These can act as training and service rendering facilities where experience can be rapidly built up. The experience of the Western Cape in South Africa is worth emulating[28]. Using trained sonographers who screened patients in the periphery and referred suspicious cases to the tertiary centres, Geerts et al reported a marked increase in the detection of fetal anomalies. These centres of excellence obviously will need capacity in treatment of remediable conditions and this they will acquire as the years roll by.

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Original Articles

Screening for Diabetes Mellitus and Its Risk Factors Among Senior Staff of Delta State University, Abraka.

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ABSTRACT

Introduction: The prevalence of undiagnosed diabetes mellitus is on the rise worldwide. Long term life threatening complications can be avoided by early detection and prompt intervention through health screening. The aim of the study therefore is to determine the prevalence rate of undiagnosed diabetes mellitus (DM) among senior staff of Delta State University, Abraka, explore the presence of known risk factors among subjects screened and to determine the percentage of the screened population referred to the physician.

Materials and Method: A prospective cross sectional study of 312 senior staff of Delta State University, Abraka. selected by systematic random sample was done. Fasting blood glucose, body mass index (BMI), waist circumference and blood pressure were measured and entered into a data collection form. A health check card was given to each participant. Data analysis was done using SPSS version 20. P values less than 0.05 were considered significant.

Results: There were more males 177(57%) than females 135(43%). Mean age was 45.95±6.89 years. About three quarters (74%) were either overweight or obese (BMI >25). There was a significant association between waist circumference, hypertension and diabetes (P<0.05) . The prevalence rate of undiagnosed DM (FBG) was 9.3%. The percentage of screened subjects referred to physicians for expert management was 12.8.

Conclusion: Prevalence rate for undiagnosed diabetes among senior staff of Delta State university was 9.3%. About 13 percent of screened subjects were referred to physicians. Body mass index and high blood pressure were significantly associated with type 2 diabetes.

Keywords: *Type 2 diabetes, Screening, Pharmacists, Referrals, Nigeria*

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Introduction

The scourge of diabetes is assuming epidemic dimensions[1-3]. It is currently estimated that over 171 million people worldwide are affected by the disease, and the figure is expected to rise to 220 million by 2020.[4-5]. The prevalence rate in the United States of America alone is 8.3%.[6]A few decades ago,

diabetes was virtually unknown in Africa but prevalence is on the rise in developing countries today.[7] This increase in incidence of diabetes in developing countries follows the trend of urbanization and life style changes, perhaps most importantly a "Western- style" diet.[7]

It is estimated that over 1.5 million people in Nigeria are living with diabetes .The national prevalence is estimated to be about 2.2%. [8] . An estimated 183 million people –about half of people with diabetes – are unaware that they have the disease.[9] Therefore screening for diabetes, early detection and prompt intervention can not only prevent development of serious long term complications but can also be life saving[10]. This is especially so in view of reports of sudden death among apparently healthy highly placed staff of the university in recent times. Diabetes and hypertension coexist in more than 40 to 60% people with diabetes;[11] thus increasing the risk of cardiovascular complications in the diabetic population. objectives of this study were to determine the prevalence of undiagnosed diabetes among senior staff in the university community, to explore the relationship between diabetes and various variables such as Body Mass Index (BMI), Fasting Blood Glucose (FBG), Blood Pressure, and Waist Circumference and to determine the percentage of screened subjects that require referral to physicians.

Materials and Method

Setting

The study was carried out in Delta State University, Abraka, a government owned facility with about 3000 staff located in the Niger delta region of Nigeria. Staff of the university have access to one tertiary healthcare institution, and a university health center.

Study design

This is a cross sectional design study.

Population/Sample size

A sample frame consisting of a comprehensive list of senior staff of the university obtained from the establishment department was constructed. 312 subjects were selected for screening by systematic random sampling from the total number of 1418 senior staff. The sample size was calculated using Yamane formula [12-13]

$$n = \frac{N}{1 + N(e)^2}$$

Where: nv = the sample size
N = the population size
e = the level of precision (0.05)

$$n = \frac{1418}{1 + 1418(0.05)^2}$$

$$n = \frac{1418}{1 + 1418(0.0025)}$$

$$n = \frac{1418}{4.55}$$

n = 311.65
n = 312 persons

Ethical approval was obtained from the Health Research Ethics committee, Delta State University Teaching Hospital, Oghara. Informed consent was obtained from each of the subjects in writing.

Data collection process

Sample collection

1. **Blood Glucose Sample:** A sterile finger prick sample was collected from each subject. Fasting Blood Glucose was

measured using a glucometer (On-Call Redi). The glucometer was calibrated daily using control solution (On-Call Redi) supplied with the glucose meter. Readings were recorded in a logbook and a personalized record of the test results was provided for each subject.

2. **Body Mass Index (BMI):** a meter rule was used to obtain the height of the subjects. Weight was measured using a digital weighing scale (Camry EF902 Electronic Scale calibrated to 0.1kg). All participants were required to remove their shoes before the height and weight measurement. The Body Mass Index was calculated as the ratio of weight (kg) to the square of the height (m).
3. **Blood Pressure:** The test was performed using a sphygmomanometer and a stethoscope. The patient was seated properly with the arm at the same level with the heart. The stethoscope was properly placed under the blood pressure cuff wrapped 2 to 3 cm above the brachial artery. The sphygmomanoter was inflated to approximately 30 mmHg higher than the point at which the brachial artery can no longer be palpated this value was determined prior to the actual BP measurement by inflating the cuff while palpating the radial artery and noting the mm Hg at which the pulse disappears; this was the number above which the sphygmomanometer was inflated. Then the cuff was slowly deflated at a rate of 2 to 3 mm Hg per heartbeat or per second by gently turning the air valve of the inflator, while watching the pressure drop. The first korotkoff sound that is heard is the systolic blood pressure. As the pressure continued to drop, the first time the korotkoff sound is no longer heard, the diastolic blood pressure was recorded. Two readings were taken, the average recorded.
4 **Waist Circumference:** A measuring tape (in inches) was used to measure the waist circumference of the subjects and recorded. Measurement was done midway between the lower rib margin and the iliac crest

Documentation

Each subject was given a Diabetic Health Check Card where relevant test values were recorded.

Data analysis

Data were loaded into Excel Spread sheet. Descriptive statistics on sample characteristics and questionnaire items were computed using SPSS Version 16.0.[14] Possible association between demographic variables and the outcome of screening were explored using a two way anova and the pearson correlation test. Inferential statistical analysis were conducted using graph pad instat Version 2.0 which reports exact P values and $P < 0.05$ was interpreted as significant.

Results

Three hundred and twelve subjects were screened. There were 177 males (57%)- and 135 females (43%). Mean age of the subjects was 46 (39-52). About three quarter (236,73%) were found to be either overweight or obese (BMI >25). Ninety seven(31%) of the females had waist circumference above normal (>35),159(51%) of the males had waist circumference of normal values (<40). 106(34%) of subjects screened had high systolic blood pressure, while 73(23%) had high diastolic blood pressure. The prevalence

of undiagnosed diabetes was 9.3%. The proportion of screened subjects referred to the physician for expert management was 12.5%.

Table 1: *The demographic and clinical characteristics of the screened subjects.*

Variables	**Number**	**%**
Sex		
Male	177	57
Female	135	43
Marital status		
Married	310	99
Single	2	1
Cadre		
Academic	103	33
Non-Academic	209	67
Age (years)		
30 – 35	21	7
36 – 40	43	14
41 – 45	71	22
46 – 50	85	27
51 – 55	50	16
56 – 60	24	8
Body Mass Index (BMI)		
Less than 20(Underweight)	8	2
20 – 25 (Average)	76	24
Over 20 – 30(Overweight)	146	47
Over 30 (Obese)	84	27
Waist Circumference		
Women 35 or less(Average)	39	13
Women above 35(Overweight)	97	31
Men 40 or less (Average)	159	51
Men above 40 (Overweight)	17	5
Blood Pressure		
Systolic		
Below 140	206	66
Above 140	106	34
Diastolic		
Below 90	239	77
Above 90	73	23

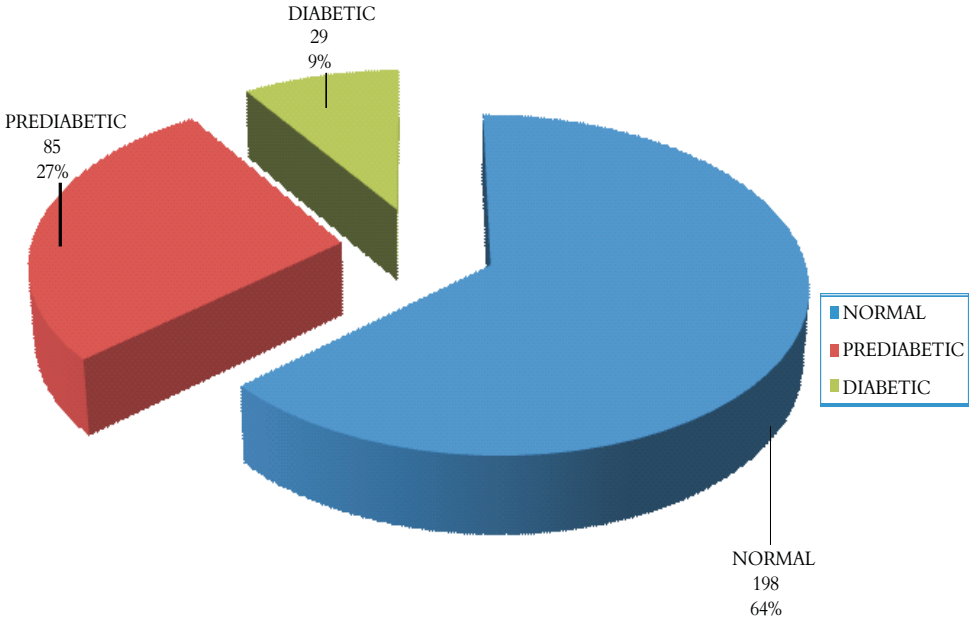

Fig 1: Diabetic status of subjects

Obesity, high blood pressure, and a large waist circumference are known risk factors for diabetes.

Fig 2 Action taken by researcher

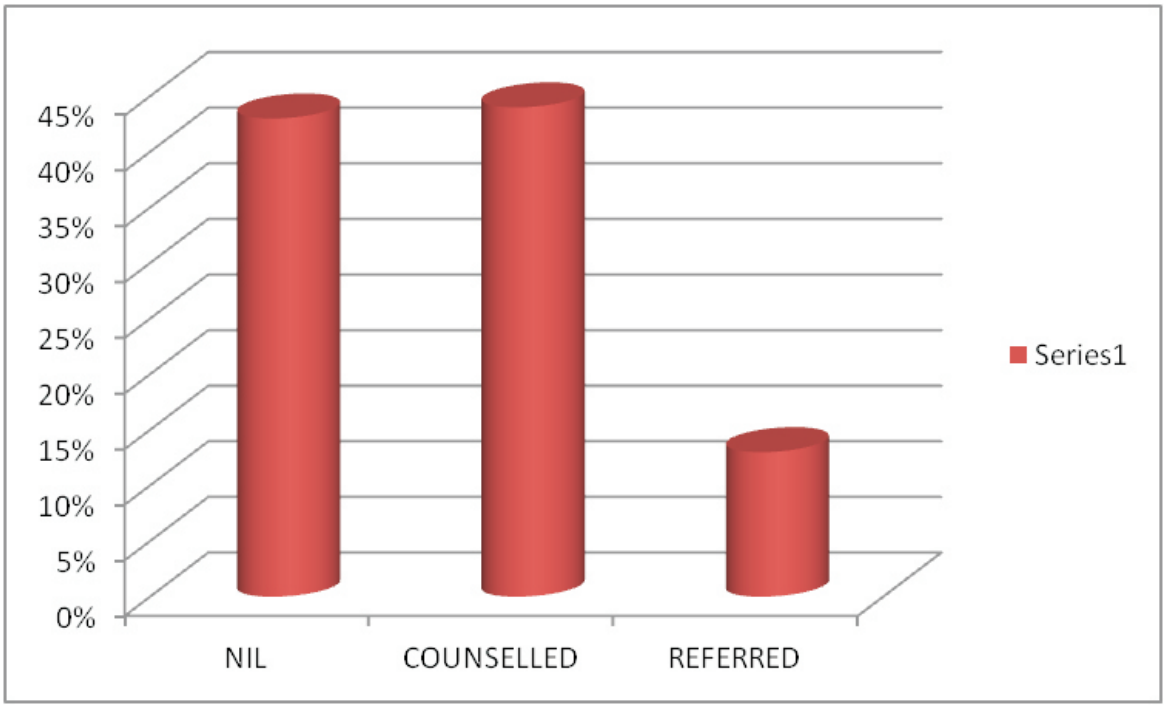

Table 2: *Presence and correlates of Diabetes Mellitus among screened subjects*

Variable		Freq(%)	Mean	SD
Body Mass Index (BMI)				
Less than 20	(Underweight)	8 (2)	100.13	30.81
20 – 25	(Average)	76 (24)	100.96	30.79
> 25 -30	(Underweight)	146 (47)	103.12	34.12
> 30	(Obese)	84 (27)	100.89	30.71
F =0.5137 P =0.6729 Not Significant				
Waist Circumference				
Women 35 or less	(Average)	39 (11)	100.76	30.89
Women above 35	(Overweight)	97 (31)	107.40	36.69
Men 40 or less	(Average)	159 (51)	100.31	28.87
Men above 40	(Overweight)	17 (5)	106.01	35.55
F =3.698 P =0.0115 Significant				
Blood Pressure				
Systolic				
100 – 140	(Average)	206 (66)	100.89	30.74
Above 140	(HBP)	106 (34)	105.73	34.63
Diastolic				
60 – 90	(Average)	239 (77)	100.95	30.79
Above 90	(HBP)	73 (23)	106.15	36.54
F =2.833 P =0.0372 Significant				

Action taken by researcher include counseling patients about diet, drug therapy and the need for adherence to medication regimen. Figure 2 depicts action taken by the researcher following screening

Discussion:

The prevalence of undiagnosed diabetes among senior staff of Delta State University, Abraka was 9.3%. Similarly high prevalence rates have been reported in studies carried out in Warri and Sapele [15] and in in-patients in the University of Nigeria Teaching Hospital, Enugu[16]

Even though other workers have been able to demonstrate significantly positive association between Diabetes and known risk factors such as BMI, hypertension, waist circumference,[17-18] we were only able to establish an association between waist circumference, high blood pressure and type 2 diabetes in the study population. The failure to establish significant association between all variables known to influence blood glucose level may be due to the fact that such correlations are more feasible in large epidemiological studies.

More than ten percent of the screened subjects were referred to the physician for further management. This highlights the importance of regular screening exercise in the university community and the society at large. It also emphasizes the need for collaborative working relationships between other health care professionals and physicians. Such collaboration would facilitate prompt referral and life saving expert intervention.[19-21] For example our intervention enabled a number of persons to seek medical help which would not have been possible if this exercise was not carried out in the university community.

Nearly half of the subjects screened were counseled on the need for adherence to medication regimen and proper diet. Patient counseling by healthcare professionals increases patient knowledge of disease condition and ultimately improves patient adherence to medication, resulting in better quality of life.

Even though the setting of this study was an enlightened university environment, recruiting subjects for this study was a real challenge and some subjects were very reluctant to divulge personal information despite assurances of confidentiality. There is a need for ongoing educational initiatives aimed at helping the populace appreciate the importance of cooperating with researchers.

Conclusion

The prevalence of undiagnosed diabetes among senior staff of Delta State University is 9.3%. There is significant association between waist circumference, blood pressure and blood glucose levels. 12.8% of subjects screened were referred to the physician for prompt treatment, which would not have been possible if the screening exercise wasn't carried out.

Recommendation.

The University authority should make efforts to enlighten staff about the desirability of health checks.

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Antibacterial Activity Against Clinical Isolates from a Tertiary Hospital in Nigeria Exhibited by Methanol and Aqueous Extracts of Root and Stem Bark of *Persea Americana* (Laureaceae)

Ching FP, Josephs GC** & Nwaeze N****

ABSTRACT

Introduction: Various parts of *Persia americana* are used in ethnomedicine in Nigeria to treat microbial infections. We investigated the antibacterial activities of its aqueous and methanol stem bark and roots extracts against clinical bacterial isolates.

Materials and methods: The minimum inhibition concentration (MIC) assay was employed using the agar dilution method. Final nutrient agar extracts concentrations of 25, 50, 75 and 100mg/ml were used. In the zones of inhibition assay 0.2ml of the MIC exhibited by each extract against each bacterium and ciprofloxacin (20μg) was used.

Results: The MICs of the aqueous and methanol extracts of the stem bark against *Staphylococcus aureus* and *Escherichia coli* were 75mg/ml and 50mg/ml respectively with 100mg/ml of either the aqueous or methanol extract against the other isolates. The MIC of the aqueous and methanol extracts of the root against the bacteria tested was 100mg/ml except *Staphylococcus aureus* in which the MIC of the methanol extract was 50mg/ml. The zones of inhibition ranged from 15.0 ± 0.12 mm to 21.0 ± 0.02 mm for the extracts and 10.5 ± 0.21 mm to 44.0 ± 0.03 mm for ciprofloxacin. The extracts produced the largest zone of inhibition (21.0 ± 0.02 mm) against *Staph. aureus* and relatively comparable zones of inhibition against *Escherichia Coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae* . Comparatively, the methanol extracts exhibited a better antibacterial activity than the aqueous extracts.

Conclusion: The results have shown that *Persia americanan* root and stem bark extracts possess antibacterial activity against pathogenic bacteria.

Keywords: *Persia americana, stem bark, roots, microbal infections, antibacterial activity.*

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Introduction

Infectious diseases are amongst the world's leading cause of premature death, killing almost 50,000 people every day[1]. Resistance to antibiotics by human pathogenic bacteria has already been reported from most parts of the world[2]. These reports are on the increase in developing as well as developed countries due to indiscriminate and miss use of antibiotics[1]. The treatment of infectious diseases in immunocompromised, HIV/AIDS and cancer patients has been complicated by drug resistant bacteria[3,4]. The emergence of multiple drug resistance pathogenic organisms necessitates the continued search for new antimicrobial agents from other sources. Globally, there is renewed interest in the search for new drugs from medicinal plant sources and the search for new antimicrobial agents to combat drug resistant bacteria will not be an exception. Plants used for the treatment of microbial infections in human traditional medicine are in exhaustible. *Persia americana* (avocado pear) is one of such plants. The various parts of *Persia americana* have been evaluated for various biological activities. The leaf extract has been reported to possess analgesic and antiinflammatory activity[5], hypoglycemic activity[6] , hypocholesterolemic potential[7], body weight and liver lipids effects[8], antimicrobial activity[9,10], anticonvulsant activity[11], hypotensive activity [12], antiulcer activity[13] and vasorelaxant activity[14]. The seed extract has also been reported to possess antihypertensive effect[15], hypolipemic effect[16], hypoglycemic effect[17], antimicrobial activity[18], larvacidal and antifungal activity[19], antioxidant and antibacterial activity[20], serum lipid and cholesterol level effects[21]. The bark possesses anticancer activity against human lung and breast carcinoma and human colon adenocarcinoma [22]. Similarly *Persia americana* fruit have been reported to exhibit cytotoxic and insecticidal activity[23]. The root and stem bark have been evaluated for cytotoxic and antiproliferative effects[24]. There is however, no scientific report on the antibacterial activity of the root and stem bark of *Persia Americana*. The present study is aimed at the evaluation of the antibacterial activity of the root and stem bark of *Persia americana* against clinical bacterial isolates from patients with bacterial infections who consulted in the University of Benin Teaching hospital, Nigeria.

Materials and Methods

Drugs and Chemicals

The reference drug, ciprofloxacin was in safe conditions for use while the chemicals and solvents were of analytical grade. These included ciprofloxacin, nutrient agar and nutrient broth (Sigma Aldrich Laborchemikallien, GmBH, Germany) and methanol (Scharlau Chemie S.A. Spain).

Clinical Bacterial Isolates

The clinical isolates which consisted of *Staphylococcus aureus* (*Staph. aureus*), *Escherichia Coli* (*E. Coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*), *Enterobacter aerogenes* (*E. aerogenes*), and *Klebseilla pneumoniae* (*K. pneumoniae*) were obtained from the University of Benin Teaching Hospital Medical Microbiology Laboratory stock Unit. They had been isolated from samples of patients being investigated for infections. They were authenticated using standard morphological and biochemical assays[25] in the Department of Pharmaceutical Microbiology, Faculty of

Pharmacy, University of Benin.

Plant Materials

The fresh stem bark and roots of the plant were collected in June, 2011 from a mature tree in Ekosodin, in the environs of University of Benin, Nigeria. The preliminary identification was in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. Identification and authentication was at the Forestry Research Institute of Nigeria (FRIN), where a voucher specimen number (FHI 108336) was obtained for plant specimen.

Extraction of Plant Materials

The stem bark was carefully separated from the woody part, cut into small bits, shade dried and pulverized using a grinder (Lab. Mill serial NO. 4745, Christy and Norris Ltd, England) and stored in dry airtight glass jar. The roots were washed to remove any soil debris, cut into small bits, shade dried, pulverized and stored in dry airtight glass jar and labelled accordingly. Each of the powdered plant materials (500g) were macerated separately in 98% methanol (2L) in glass jars for 72 hours and were shaken intermittently throughout the period. The extracts were filtered separately. Another portion of each of the plant materials (500g) were macerated separately in distilled water (2L) for 24 hours and were shaken intermittently. The extracts were filtered separately. Each of the methanol filtrates was evaporated to dryness at reduced pressure using a rotary evaporator to obtain a dark brown residue (root) and brown residue (stem bark) until a constant weight was obtained and the yield with reference to the powdered material in each case noted. Each of the aqueous filtrates was evaporated to dryness in an hot air oven set at 40 °C to obtain a dark brown residue (root) and brown residue (stem bark) until a constant weight was obtained and the yield with reference to the powdered material in each case noted. The extracts obtained were stored in the refrigerator until when required for experiments reported in our study.

Phytochemical Screening

The powdered stem bark and roots were separately screened for the presence of bioactive constituents using standard phytochemical techniques[26].

Preparation of Concentrations of Extracts and Ciprofloxacin

From a stock solution of 500 mg/ml of either methanol or aqueous extracts of the stem bark and roots of *Persia americana,* final concentrations of 25, 50, 75 and 100 mg/ml in equivalent volumes of 1ml, 2ml, 3ml and 4ml respectively made up to 20 ml with nutrient agar were used for antibacterial assay.

From a stock solution of 100 μg/ml of ciprofloxacin, 20μg of ciprofloxacin in an equivalent volume of 0.2 ml was used as standard for antibacterial assay.

Antibacterial assay

Effects of various concentrations of extracts against the isolates and determination of Minimum Inhibitory Concentrations (MIC).

The agar dilution method described by George and Robert was used[27]. The minimum inhibitory concentrations of the extracts of stem bark and roots were evaluated using final concentrations of 25, 50, 75 and 100 mg/ml in equivalent volumes of 1ml, 2ml, 3ml and 4ml

respectively made up to 20 ml with nutrient agar. In the first set of assays the methanol extracts of the stem bark and roots were used and the minimum inhibitory concentrations were determined against *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa* , *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae*. Each concentration of the extracts was in triplicate plates. Each plate was inoculated with 0.1ml (10^6 CFU/ml) of overnight nutrient broth culture of each of the clinical bacterial isolates. The inoculums were spread uniformly on the surface of the agar with the aid of a sterile glass spreading rod. The plates were incubated at 37°C and observed for growth after 24 hours. In the second set of assays the aqueous extracts of the stem bark and roots were used and the minimum inhibitory concentrations were determined against *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae*. Each concentration of the extracts was in triplicate plates. Each plate was inoculated with 0.1ml (10^6 CFU/ml) of an overnight nutrient broth culture of each of the clinical bacterial isolates. The inoculums were spread uniformly on the surface of the agar with the aid of a sterile glass spreading rod. The plates were incubated at 37 °C and observed for growth after 24 hours.

Evaluation of antibacterial activities against clinical bacterial isolates using zones of inhibition

The agar well diffusion method as described by Perez *et al*. was used [28]. The minimum inhibitory concentration (MIC) against each bacterium exhibited by each extract was used to evaluate antibacterial activity. In the first set of assays the aqueous and methanol extracts of the stem bark of *Persia americana* were evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia Coli* at the minimum inhibitory concentration of 75mg/ml and 50mg/ml respectively while for *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae*, the concentration was 100mg/ml. In the second set of assays the aqueous and the methanol extracts of the roots of *Persia americana* were evaluated for antibacterial activity against *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae* at the minimum inhibitory concentration of 100mg/ml except *Staphylococcus aureus* in which 50mg/ml of the aqueous extract was used . Petri dishes in triplicates were labelled with each of the six clinical bacterial isolates and 25ml of nutrient agar pipetted into them aseptically. The nutrient agar was allowed to set and was inoculated with 0.1ml (10^6 CFU/ml) of an overnight culture of each of the clinical isolates, in separate petri dishes. The inoculums were spread uniformly on the surface of the agar with the aid of a sterile glass spreading rod. Using a sterile cork borer, five wells of 6mm in diameter were made in the nutrient agar of each plate and labelled accordingly with the extract type and ciprofloxacin, (100μg/ml) to which the bacterial isolates were sensitive. The standard drug, ciprofloxacin (0.2ml equivalent to 20μg) was aseptically pipetted into the well at the center while 0.2 ml of each of the minimum inhibitory concentrations of the extracts tested was aseptically pipetted into the appropriately labelled well. The plates were left for a while for the drug and extracts to diffuse into the agar. The plates were incubated at 37 °C for 24 hours and examined

for zones of inhibition around the wells. The zones of inhibition were measured and recorded. The mean and standard error of mean (Mean ± SEM) was determined for the triplicate plates in each case.

Results

The maceration of the powdered stem bark (500g) in distilled water and methanol yield 24g (4.8%) and 26g (5.2%) respectively while those of the root yield 14g (2.8%) and 16g (3.2%) respectively. The preliminary phytochemical screening of the root and the stem bark revealed the presence of saponins, tannins, cardiac glycosides, flavonoids, phlobatanins, terpenoids carbohydrates and reducing sugars (Table 1). In the minimum inhibitory concentration assay, the aqueous extract of the stem bark inhibited the growth of *Staph. aureus and E. coli* at the concentrations of 50mg/ml and 75mg/ml with 50mg/ml as the minimum inhibitory concentration (Table 2). The aqueous stem bark extract minimum inhibitory concentration for *Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae and Serratia marcescens* was 100mg/ml (Table 2). The methanol extract of the stem bark inhibited the growth of *Staph. aureus and E. coli* at the concentration of 50mg/ml, 75mg/ml and 100mg/ml, with 50mg/ml as the MIC (Table 2) while its MIC for *Pseudomonas aeruginosa, Enterobacter aerogenes and Serratia marcescens* was 100mg/ml (Table 2). The stem bark extract MIC for *Klebsiella pneumoniae* was 75mg/ml (Table 2). The minimum inhibitory concentration of the aqueous and methanol extracts of the root against *Escherichia Coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae* was 100mg/ml (Table3). The minimum inhibitory concentration of the methanol extract of the root against *Staph. aureus* was 50mg/ml (Table 3). In Tables 4 and 5 are presented the zones of inhibition against the clinical bacterial isolates exhibited by the stem bark and root extracts. The zones of inhibition ranged from 15.0 ± 0.12 mm to 21.0 ± 0.02 mm for the extracts and that of the reference drug, ciprofloxacin ranged from 10.5 ± 0.21 mm to 44.0 ± 0.03 mm. The extracts produced the largest zone of inhibition (21.0 ± 0.02 mm) against *Staph. aureus* and relatively comparable zones of inhibition against *Escherichia Coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae*. Comparatively the methanol extracts of the stem bark and roots exhibited a better antibacterial activity in the zones of inhibition assay than their aqueous extracts. In the zones of inhibition assay *Serratia marcescens* was the most sensitive organism to ciprofloxacin with a zone of inhibition of 44.0 ± 0.03mm and *Pseudomonas aeruginosa* was the least sensitive with zone of inhibition of 10.5 ± 0.21mm.

Table 1: *Phytochemical constituents of the extracts of stem bark and root of Persia americana*

Constituents	Stem Bark Extract	Root Extract
Tannins	+	+
Alkaloids	-	-
Saponins	+	+
Flavonoids	+	+
Reducing sugars	+	+
Cardiac glycosides	+	+
Phlobatanin s	+	+
Terpenoids	+	+
Anthraquinones	-	-

+, constituents is present and -, constituent is absent.

Table 2: ***Minimum inhibitory concentration (mic) of the aqueous and methanol extracts of stem bark ofPersia americana against pathogenic bacterial isolates***

Pathogenic bacteria	Aqueous stem bark extract				Methanol stem bark extract			
	Final concentration in nutrient agar (mg/ml)				Final concentration in nutrient agar (mg/ml)			
	25	50	75	100	25	50	75	100
Staph. aureus	++	+	-	-	+	-	-	-
E. coli	++	+	-	-	+	-	-	-
P. aeruginosa	++	+	+	-	+	+	+	-
E. aerogenes	++	+	+	-	+	+	+	-
K. pneumoniae	++	+	+	-	+	+	-	-
S. marcescens	++	+	+	-	++	+	+	-

-, No growth of colonies; +, scanty growth of colonies observed; and + +, intense growth of colonies observed. Staphylococcus aureus (Staph. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Serratia marcescens(S. marcescens), Enterobacter aerogenes (E. aerogenes) and Klebseilla pneumoniae (K. pneumoniae).

Table3: *Effects of various concentration of the extracts and determination of minimum inhibitory concentration (mic) of the aqueous and methanol extracts of root of Persia americana against pathogenic bacterial isolates*

Pathogenic bacteria	Aqueous root extract				Methanol root extract			
	Final concentration in nutrient agar (mg/ml)				Final concentration in nutrient agar (mg/ml)			
	25	50	75	100	25	50	75	100
Staph. aureus	++	+	+	_	+	_	_	_
E. coli	++	+	+	_	+	+	+	_
P. aeruginosa	++	+	+	_	+	+	+	_
E. aerogenes	++	+	+	_	+	+	+	_
K. pneumoniae	++	++	+	_	+	+	+	_
S. marcescens	++	+	+	_	++	+	+	_

-, No growth of colonies; +, scanty growth of colonies observed; and + +, intense growth of colonies observed. *Staphylococcus aureus (Staph. aureus*), *Escherichia coli (E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*), *Enterobacter aerogenes* (*E. aerogenes*) and *Klebseilla pneumoniae* (*K. pneumoniae*).

Table 4: *Antibacterial activity of stem bark extracts of Persia americana against clinical bacterial isolates using zones of inhibition*

Pathogenic bacteria	Methanol extract		Aqueous extract		Ciprofloxacin	
	Amount used (mg)	Zone of inhibition(mm)	Amount used (mg)	Zone of inhibition(mm)	Amount used (μg)	Zone of inhibition(mm)
Staph. aureus	10	21.0 ± 0.02	15	20.0 ± 0.22	20	26.5 ± 0.04
E. coli	10	16.5 ± 0.14	15	15.0 ± 0.12	20	19.0 ± 0.20
P. aeruginosa	20	18.0 ± 0.07	20	17.5 ± 0.14	20	10.5 ± 0.21
E. aerogenes	20	20.0 ± 0.02	20	19.0 ± 0.23	20	16.5 ± 0.11
K. pneumoniae	20	19.0 ± 0.11	20	18.5 ± 0.12	20	17.8 ± 0.03
S. marcescens	20	19.5 ± 0.03	20	18.5 ± 0.10	20	44.0 ± 0.30

Zones of inhibition are Mean + SEM of measurements for triplicate plates. *Staphylococcus aureus (Staph. aureus*), *Escherichia coli (E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*), *Enterobacter aerogenes* (*E. aerogenes*) and *Klebseilla pneumoniae* (*K. pneumoniae*).

Table 5: *Antibacterial activity of root extracts of Persia americana against clinical bacterial isolates using zones of inhibition*

Pathogenic bacteria	Methanol extract		Aqueous extract		Ciprofloxacin	
	Amount used (mg)	Zone of inhibition(mm)	Amount used (mg)	Zone of inhibition (mm)	Amount used (μg)	Zone of inhibition(mm)
Staph. aureus	**10**	**21.0 ± 0.02**	**20**	**19.8 ± 0.14**	**20**	**26.5 ± 0.04**
E. coli	**20**	**18.0 ± 0.13**	**20**	**17.5 ± 0.08**	**20**	**19.0 ± 0.20**
P. aeruginosa	**20**	**19.5 ± 0.11**	**20**	**17.8 ± 0.04**	**20**	**10.5 ± 0.21**
E. aerogenes	**20**	**18.5 ± 0.03**	**20**	**17.5 ± 0.02**	**20**	**16.5 ± 0.11**
K. pneumoniae	**20**	**20.0 ± 0.22**	**20**	**19.0 ± 0.01**	**20**	**17.8 ± 0.03**
S. marcescens	**20**	**19.5 ± 0.30**	**20**	**17.8 ± 0.09**	**20**	**44.0 ± 0.30**

Zones of inhibition are Mean + SEM of measurements for triplicate plates. *Staphylococcus aureus (Staph. aureus*), *Escherichia coli (E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*), *Enterobacter aerogenes* (*E. aerogenes*) and *Klebseilla pneumoniae* (*K. pneumoniae*).

Discussion

The emergence of multiple drug resistance in human pathogenic organisms against the currently used chemotherapeutic agents is on the increase and is now a cause for global concern.

Although a few chemotherapeutic agents have been produced to combat the problem of multiple drug resistance, they are either unaffordable or unavailable in most developing nations of the world. In several of these nations there have been renewed interest in traditional health care systems which rely mostly on medicinal plants to treat bacterial infections. Scientific information on the biological activities of some of these plants is scanty. Research on these plants does not only authenticate their traditionally acclaimed uses but also provide future promise in the search for new drugs with antimicrobial actions[18]. Decoctions and extracts of the stem bark and roots of *Persia americana* are reputed for their use in the treatment of infections of microbial origin in Nigeria and other African countries. Our findings in this study revealed that the extracts of the stem bark and roots of *Persia Americana* possessed *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and *Klebseilla pneumoniae* isolated from patients investigated in the University of Benin Teaching hospital in Nigeria. The pathogens were sensitive to the reference drug, ciprofloxacin as shown in the results of our study. Comparatively, the methanol extracts exhibited a better antibacterial activity than the aqueous extracts against the pathogenic bacteria in this study as seen in the zone of inhibition assays. *Staphylococcus aureus* and *Escherichia Coli* showed more sensitivity to extracts compared the other bacteria as evidenced with the lower minimum inhibitory concentrations against them. The stem bark and roots extract possess saponins, tannins, cardiac glycosides, flavonoids, phlobatanins, terpenoids some of which have been reported to possess

antibacterial activities[29,30,31]. The presence of these bioactive constituents in the plant parts may have contributed to the spectra of antibacterial activity observed in this study. The findings of our study on the root and stem bark extracts of *Persia americana* along with other studies carried out on the seed extract[18] and the leaf extract[9] indicate the potential of *Persia. americana* as a source for chemotherapeutic agents, which may provide leads in the ongoing search for antimicrobial agents from plants. The activity exhibited by these extracts against the clinical bacterial isolates that are associated with various infections, may provide scientific justification for the ethnomedicinal uses of the stem bark and roots of the plant.

In conclusion the study has shown that *Persia americana* root and stem bark extracts have antibacterial activity against the clinical pathogenic bacteria and could be beneficial in the treatment of various bacterial infections caused by the organisms used in our study.

Conflict of interest : We declare that there is no conflict of interest.

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Family Size Preferences Among Married Men in A Semi-urban Community in Delta State, South-south, Nigeria

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Abstract

Introduction: Men's reproductive motivation to a large extent affects the reproductive behavior of their wives rather than vice versa. This study was conducted to assess the family size preferences among married men in a semi-urban community in Delta State, south-south, Nigeria.

Materials and methods: This study was a community-based descriptive cross-sectional study conducted in September 2013. A total of 275 married men were selected by a multistage sampling technique for the study. All the participating men were presented with a pre-tested semi-structured questionnaire which was interviewer administered.

Results: Majority of the respondents were in the age group 35-44 years (37.5 %), had at least secondary education (46.9 %), were unskilled workers (38.5 %) and were in a monogamous marital union (75.6 %). The mean desired family size and the mean number of living children among the respondents were 5.6 and 4.6 respectively. Over half of the respondents (60.0 %) had more than four living children. The binary logistic regression analysis indicated that no formal education, primary education status, polygamous marital union and disapproval to family planning were determinants of large family size (more than four children) among the respondents.

Conclusion: The results of this study reveals that majority of the respondents had a large family size. There is need for a conscious effort to motivate men towards having less number of children as recommended by the National Population Policy framework.

Key words: *Preferences, family size, married men*

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Introduction

Interests in men's involvement in reproductive health has been on the front burner since the 1994 International Conference on Population and Development (ICPD) and the 1995 United Nations World Conference on women.[1,2] The program of action of the 1994 International Conference on Population and Development (ICPD) clearly set a new agenda when it stressed male responsibilities and participation in

reproductive health.[1,2] There has also been a goal to achieving gender balance or equality in men's and women's reproductive rights and responsibilities. Though there is the tendency to overlook the relevance of men in matters relating to reproductive health, they have substantial reproductive health influence.

The assumption of women's primacy in fertility and family planning practice has led to a general down play and often neglect of men's roles in fertility and family planning.[3] Men in Africa are dominant and are the major decision makers in family affairs including reproductive matters. The view of men are more influential in family planning decision making in developing countries. [3,4] Men as husbands and heads of households in developing countries control the sexuality of their wives. [4] Their general knowledge and attitudes regarding the ideal family size, sex preference of children, ideal spacing between child births and contraceptive utilization greatly influence women's preferences and opinions.[5] Therefore, men's reproductive motivation to a large extent affects the reproductive behaviour of their wives rather than vice versa. [4,6,7]

Male dominance in this respect is reinforced by the cultural institution of patriarchy, religion and the economic power that men wield. In Nigeria, one of the major reasons why women do not adopt a modern contraceptive method is the husband's resistance. Thus women wishing to practice family planning may fail to take the initiative if they understand the negative disposition of their husbands to family planning. [3,4] Considering the significant role that men play in fertility decision in Nigeria and sub-Saharan Africa at large the national population policy in Nigeria advocates that special emphasis should be given to reaching men with messages on social and economic implication of having "too many children." [8,9]

Nigeria is the tenth most populous country in the world and the largest in sub-Saharan Africa, with an estimated population of over 140 million from the 2006 census.[10,11] Nigeria's population growth rate has been driven by high fertility. The persistence of high fertility has been the subject of considerable investigation during the past decades. [10,11]Desired family size or ideal family size is a good indicator of men's attitude towards childbearing, even though actual reproductive behavior may differ from stated desires. Furthermore, family size is an important reproductive health and socio-economic issue globally.[1] The National Population Policy recommends a family size of not more than four children.[11] It is against this background that this study was conducted to assess the family size preferences among married men in a semi-urban community in Delta State, south-south, Nigeria.

Materials and Methods

This study was conducted in Oghara, a semi-urban community in Ethiope West Local Government Area (LGA) of Delta State in September 2013. Oghara is home to the Delta State University Teaching Hospital, Delta State Polytechnic and Western Delta University. The common language of the people is Urhobo and their major occupation is farming.

A minimum sample size of 196 was obtained using the Fischer's formula[12] with an approval to practice family planning rate of 15.2 %[3]

from a previous study. Although the computed minimum sample size was 196, a multistage sampling technique was however used to select a total of 275 married men for the study. In the first stage, a simple random sampling technique was used to select one clan (Ogharefe) out of the two clans in Oghara. In the second stage, a simple random sampling technique was used to select three quarters out of the five quarters in Ogharefe. A systematic sampling technique (sampling frame of 1:6) was used in the third stage to select households from which the study respondents were selected. In each of the selected quarters, the first house in all the streets was randomly selected and thereafter every sixth house in all the streets was selected. Eligible participants were then recruited for this study from the selected houses. The study instrument was a pretested semi-structured interviewer administered questionnaire which elicited information on socio-demographic characteristics of the respondents, their desired family size, their present family size, their disposition and practice of family planning. Data generated was analysed using SPSS version 16.0 statistical software. Statistical analyses included simple frequency tables, chi-square analysis and binary logistic regression. Statistical significance was set at $p < 0.05$. Ethical approval for this study was obtained from the Ethics and Research Committee of the school of public health, Texila American University, Guyana. Permission for this study was also duly obtained from the community leaders of Ogharefe. All participants consented to the study before recruitment.

Results:

Two hundred and seventy-five respondents were surveyed. Of this number, 103 (37.5 %) were in the age group 35-44 years, over a third of the respondents (46.9 %) had at least secondary education with most of the respondents (38.5 %) being unskilled workers. Over three quarter of the respondents (75.6 %) were in a monogamous marital union, while 24.4 % of them were polygamous (see Table 1). The mean desired or ideal family size among the respondents was 5.6 and it was highest among men in the older age groups, men without formal education and polygamous men (see Table 2). Over half of the respondents (60.0 %) had a family size of more than four living children. The association of age, educational status, type of marriage, religion and disapproval to family planning with large family size was statistically significant ($p < 0.05$)(see Table 3).The binary logistic regression analysis indicated that no formal education, primary education status, polygamous marital union and disapproval to family planning were factors which favoured a large family size (more than four children) among the respondents (see Table 4).

Table 1: *Socio-demographic characteristics of the respondents*

Characteristics	Frequency (N = 275)	Percentage (%)
Age group (years)		
25-34	76	27.6
35-44	103	37.5
45-54	57	20.7
55-64	24	8.7
65-74	11	4.0
75-84	4	1.5
Educational status		
Nil formal education	25	9.1
Primary education	72	26.2
Secondary education	129	46.9
Tertiary education	49	17.8
Type of marriage		
Monogamous	208	75.6
Polygamous	67	24.4
Occupation		
Professional	52	18.9
Skilled workers	51	18.5
Semi-skilled workers	58	21.1
Unskilled workers	106	38.5
Unemployed	8	2.9
Religion		
Christian	235	85.5
Muslim	4	0.01
African traditional religion	36	13.1

Table 2: *Mean number of living children (MNOLC) and Mean desired family size (MDFS) in relation to the socio-demographic characteristics of the respondents*

Characteristics	Mean number of living children (MNOLC)	Mean desired family size (MDFS)
Age group (years)		
25-34	3.3±1.56	4.0±1.32
35-44	3.4±1.13	4.0±0.55
45-54	3.7±1.06	4.2±1.01
55-64	5.8±0.62	7.4±1.75
65-74	5.8±0.58	7.2±1.86
75-84	4.8±1.13	6.5±1.04
Educational status		
Nil formal education	4.8±1.24	7.7±1.27
Primary education	4.3±1.78	5.9±1.19
Secondary education	4.1±1.76	4.8±1.04
Tertiary education	3.7±1.50	4.0±0.76
Type of marriage		
Monogamous	3.4±1.63	4.6±0.81
Polygamous	5.8±0.76	6.6±1.45
Occupation		
Professional	3.3±1.26	4.3±1.16
Skilled workers	4.3±1.47	5.8±0.90
Semi-skilled workers	4.2±1.48	5.8±0.74
Unskilled workers	4.6±1.64	6.4±1.16
Unemployed	4.6±1.44	5.9±1.17
Religion		
Christian	4.1±1.73	5.4±1.04
Muslim	3.6±1.64	5.8±1.45
African traditional religion	5.0±1.35	5.6±1.28

Mean desired number of children = 5.6±1.56; Mean number of living children = 4.6±1.56

Table 3: *Number of living children (NOLC) in relation to the characteristics of the respondents*

Characteristics	Number of living children		Total n (%)	P-value
	Less than 5 n (%)	5 and above n (%)		
Age group (years)				
25-34	56 (73.7)	20 (26.3)	76 (27.6)	**X^2= 77.8 (p=0.0001)**
35-44	42 (40.8)	61(59.2)	103 (37.5)	
45-54	11 (19.3)	46 (80.7)	57 (20.7)	
55-64	-	24 (100.0)	24 (8.7)	
65-74	1 (9.1)	10 (90.9)	11 (4.0)	
75-84	-	4 (100.0)	4 (1.5)	
Total	**110 (40.0)**	**165 (60.0)**	**275**	
Educational status				
Nil formal education	4 (16.0)	21 (84.0)	25 (9.1)	**X^2= 16.8 (p=0.001)**
Primary education	19 (26.4)	53 (73.6)	72 (26.2)	
Secondary education	63 (48.8)	66 (51.2)	129 (46.9)	
Tertiary education	24 (49.0)	25 (51.0)	49 (17.8)	
Total	**110 (40.0)**	**165 (60.0)**	**275**	
Type of marriage				
Monogamous	105 (50.5)	103 (49.5)	208 (75.6)	**X^2= 39.1 (p=0.001)**
Polygamous	5 (7.5)	62 (92.5)	67 (24.4)	
Total	**110 (40.0)**	**165 (60.0)**	**275**	
Occupational status				
Professional	23 (44.2)	29 (55.8)	52 (18.9)	**X^2= 9.2 (p=0.057)**
Skilled workers	20 (39.2)	31 (60.8)	51 (18.5)	
Semi-skilled workers	32 (55.2)	26 (44.8)	58 (21.1)	
Unskilled workers	33 (31.1)	73 (68.9)	106 (38.5)	
Unemployed	2 (2.5)	6 (75.0)	8 (2.9)	
Total	**110 (40.0)**	**165 (60.0)**	**275**	
Religion				
Christian	104 (44.3)	131 (55.7)	235 (85.5)	**X^2 (Fischer Exact test) = 18.6 (p=0.001)**
Muslim	3 (75.0)	1 (25.0)	4 (0.01)	
African traditional	3 (8.3)	33 (91.7)	36 (13.1)	

Table 4: *Determinants of large family size (more than 4 children)among the respondents*

Determinants	Odds ratio (OR)	95 % C.I	p-value
Nil formal education	0.65	0.43 – 0.97	0.031
Primary education	6.92	1.14 – 42.21	0.036
Polygamous marriage	0.13	0.04 – 0.44	0.001
Disapprove and not practicing a method of family planning with wife	0.195	0.066 – 0.577	0.003

Discussion:

Majority of the respondents in this study were found within the age groups of 35-44 and 25-34 years respectively. This suggests that most men in the study area were still within their active reproductive years. This observation is in keeping with the findings from the 2008 National Demographic and Health Survey which revealed that the population structure in most Nigerian communities reflects a preponderance of young persons with only a small proportion being in the elderly and aged groups; indicative of a population with high fertility.[11]The mean desired or ideal family size among the respondents in this study was found to be 5.6.This is comparable to the mean desired or ideal family size of 5.8 and 6.0 reported in Nigeria by Odu et al and Isiugo-Abanihe respectively among men in their studies.[1,4] The mean desired or ideal family size observed in this study was however higher than the mean desired or ideal family size of 3.3, 4.1 and 4.3 reported among men in Egypt, Morocco and Ghana respectively.[13] The mean desired or ideal family size among respondents in this study was highest among men in the older age groups, men without formal education and men in polygamous marital union. Among the occupational groups, unskilled workers had the greatest desire for children with a mean of 6.4, while professionals had a mean desired or ideal family size of 4.3. This is not surprising as men who are professionals are more educated than those who are unskilled workers and education has been shown to have a positive effect on small family size and a positive disposition to the adoption of modern methods of family planning.[1]

The mean number of living children for men in this study was 4.6; similar studies among men in Nigeria[4] and Kenya[14] estimate the mean number of living children to be 4.5 and 4.2 respectively. In Africa, average family sizes are lowest in Ghana (West Africa) and Egypt (North Africa) where the mean number of living children is 3.8 and 3.6 respectively.[13] Globally, the mean number of living children is lowest in developed and high income countries where the mean number of living children is generally below 2.[13] The mean number of living children among the respondents in this study was highest among men who were in the age group 55 – 64 and

above. The fact that the mean number of living children increased with age among the respondents is not surprising because in Africa, as men get older, they marry more wives and have more children to satisfy their reproductive desires.[1] The mean number of living children among the respondents in this study was lowest among men who were in the age groups. This could reflect a generation shift in desired family size with younger couples desirous of smaller family size following their adoption of western culture.[4]In spite of the National Population Policy recommends a family size of not more than four (4) children,[11]over half of the respondents in this study had more than four (4) living children. The factors that favored a large family size (more than 4 living children) among the respondents were having no formal education, having no primary education, practice of polygamy and disapproval to family planning. On the average educated men tend to have smaller families because they tend to marry later and also tend to adopt the western culture which favors a small family size.[4] Men in polygamous marriage have larger family size because there is the competition among wives as to who would produce the greatest number of children. In addition, men in polygamous marriages are unlikely to adopt modern methods of family planning.[3,9]

In conclusion, the results of this study indicates that majority of the respondents have a large family size (more than four living children). There is need for a conscious effort to motivate men in the study community towards having less number of children as recommended by the National Population Policy framework. This can be achieved via increasing the general educational status (including reproductive health matters) of men in the community and advocating on the need for the adoption of modern methods of family planning.

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An Estimation of the Some Antioxidant Vitamins in the Serum of Diabetes Mellitus Patients

*Mordi JC

Abstract

Introduction: Diabetes mellitus and other pathological events such as atherosclerosis and inflammation processes are associated with the generation of reactive oxygen species. Accumulating evidences suggest that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus. The human cells have numerous defense systems to counteract the deleterious effects of ROS and free radicals. Deficiencies of these antioxidant vitamins may increase susceptibility to this disease and the associated complications. This research estimates the level of some antioxidant vitamins in the serum of diabetes mellitus patients in Asaba and environs.

Materials and methods: Serum antioxidant vitamins (Vitamin A, C and E) were estimated in 50 (25 males and 25 females within the ages of 35-65 years) diabetic patients using standard procedures and the results obtained were compared with those of apparently healthy, non-diabetic subjects of comparable age and social status.

Result: Serum glucose level of the diabetic subjects was significantly higher ($p<0.05$) than the value obtain for the non-diabetic subjects. Furthermore, the level of vitamins A, C and E concentration obtained from this study were significantly lower ($p<0.05$) in the diabetic patients relative to the levels of these vitamins in the control subjects.

Conclusion: The antioxidant status of diabetic patients was lower when compared to non diabetic subjects (control). These findings are suggestive of the current antioxidant status of diabetic patients in Asaba and environs with the hope that efficient and dynamic management strategy could be properly initiated especially when the patient at high risk is diagnosed.

Keyword: Diabetes Mellitus, Free radicals, Antioxidant and Glucose

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Introduction

Diabetes mellitus is a chronic metabolic disorder affecting carbohydrate, lipid and protein metabolism. It is a heterogeneous group of disorder characterized by hyperglycemia due to impaired glucose utilization, resulting from defects in insulin

secretion, insulin action or both[1]. Along with hyperglycemia and abnormalities in serum lipids, diabetes is associated with micro vascular and macro vascular complications which are the major causes of morbidity and death in diabetic subjects[2]. In African communities, the prevalence is increasing with ageing of the population and life style changes associated with urbanization[3]. It is known to affect 3%, on the average of adult Nigerians[4]. There are basically two types of clinical diabetes mellitus: Type 1, also known as insulin dependent diabetes (IDDM) and type 2, otherwise known as non-insulin dependent diabetes (NIDDM). NIDDM is the most common form accounting for about 90% of all cases. It occurs frequently in people who are overweight, inactive and usually older than 40 years of age.

Diabetes mellitus being a degenerative disease therefore may be initiated as a result of peroxidation caused by free radicals[5,6]. Since free radical production is increased whereas antioxidants system capacity is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidant compared with healthy individuals[5,6]. Quite a number of antioxidants participate in the protection of human body against free radical pathology and its consequences[7]. They include vitamins A, C, and E. Other micronutrients include Zinc, Chromium, Selenium and Vanadium. This study therefore attempts to examine and evaluate changes in the levels of antioxidant vitamins (A, C and E) as well as possible relationship among these antioxidants in patients with diabetes mellitus in the Southern part of Nigeria.

Materials and Methods

By means of a hypodermic syringe and needle, fasting blood samples were collected from 50 diabetic patients of both sexes (within the ages of 35-65 years) who were attending the outpatients' clinic of the Federal Medical Centre Asaba, Nigeria. Fifty apparently healthy non-diabetic subjects of similar socioeconomic status with normal fasting blood sugar, who were members of the hospital community, were recruited to serve as control. The consent of all subjects were sought and obtained with approval from the Institution's Ethics and Research Committee. Five milliliters of whole blood collected from each subject were centrifuged at 2000 rpm for 10 minutes using a desktop centrifuge. The serum was separated and transferred into a sample labeled bottle at 20°C until required for analysis. The amount of glucose in the sample was determined using the glucose oxidase method described by Trinder, (1969)[8]. The method of Vitamin E estimation was carried out as described by Martinek (1964)[9] while the determination of vitamin A was performed by high- speed liquid chromatography (HSLC) as described by De-Ruyter and De Leenheer, (1976)[10]. Determination of serum Vitamin C was done using spectrophotometric method of Liu *et al*. (1982)[11]. The reagents used were supplied in commercial kits.

Data Analysis

The data obtained were presented as mean± SD. Serum levels of the three antioxidants vitamins were correlated with serum glucose level of both the diabetic and control subjects and the correlation determined. The student t-test was used to determine the level of significance which was set at $P < 0.05$.

Results

Results from this study (Table 1) revealed an

increase BMI in the diabetic subjects which was significantly higher ($p<0.05$) than the normal group. Concentration of vitamin A in NIDDM subjects were significant $p<0.05$ lower than the values obtained for the control subjects (Table 2). Furthermore, the results from this present study showed that the mean values obtained from the vitamin C and vitamin E concentrations were significantly lower ($p<0.05$) from that of the control subject.

Table 1: *Some anthropometric measurement of diabetes mellitus and normal subjects*

Subjects	N	Age(30-60yrs)	Weight(kg)	Height(m)	BMI(kg/m^2)
Normal					
Male	25	49.32±8.8	66.65±11.33	1.69±0.30	23.54±0.13
Female	25	48.29±9.1	65.28±15.55	1.52±0.17	25.34±0.93
Total	50	49.50±9.7	66.96±14.41	1.57±0.32	24.24±1.43
NIDDM					
Male	25	47.12±9.2	79.77±7.28	1.66±0.18	29.79±1.58
Female	25	50.64±6.6	73.11±13.73	1.57±0.31	28.77±1.72
Total	50	48.63±8.9	76.44±10.22	1.60±0.13	29.24±2.34

Values are expressed as mean ±SD for "n" subjects. BMI= Body Mass Index. NIDDM= Non-Insulin Dependent Diabetes Mellitus.

Table 2: *Serum glucose and antioxidant vitamin levels of normal and diabetes mellitus subjects*

Subjects	N	FBS (mg/dl)	Serum VitaminA (μg/dl)	Serum VitaminC (mg/dl)	Serum VitaminE (mg/dl)
Normal					
Male	25	76.04±6.34	43.03±12.30	0.97±0.16	0.67±0.13
Female	25	73.51±8.55	46.23±12.53	0.98±0.24	0.68±0.14
Total	50	74.96±7.47	44.12±11.79	0.97±0.12	0.68±0.13
NIDDM					
Male	25	184.32±8.04	14.42±7.24	0.66±0.13	0.53±0.21
Female	25	227.39±9.20	14.11±7.98	0.64±0.15	0.49±0.17
Total	50	206.67±8.67*	14.37±7.59*	0.66±0.17*	0.51±2.34*

Values are expressed as mean ±SD for "n" subjects. *Value differs significantly ($P<0.05$) from comparable control value. FBS= Fasting Blood Sugar. NIDDM= Non-Insulin Dependent Diabetes Mellitus.

Discussion

The primary need of a diabetic patient is to attain and sustain normoglycemia. This potential problem is largely the complications that could develop as a result of poor management of the disease. In developing countries, management and treatment of diabetes is difficult due to poor level of education, diet and health care facilities[12]. Therefore, reports have shown increase of diabetes in Nigeria and this has been of great concern[12]. Anthropometric variables such as body mass index (BMI) and waist hip ratio (WHR) can be clinically used as a substitute to measure fat distribution in the body[13]. Results from this study (Table 1) revealed an increase BMI in the diabetic subjects which was significantly higher ($p<0.05$) than the normal group. The prevalence of diseases associated with insulin resistance (diabetes and CHD) increases as BMI increases due to increase in adiposity[14].

WHO/MONICA, 1994[15] dual studies of antioxidants and that of Cunningham indicated that normal serum concentration of vitamin A range from 40 to 70μg/dl[16]. Serum concentration of less than 15μg/dl for adults is indicative of deficiency and severe depletion of liver stores of vitamin A[17]. Concentration of vitamin A in NIDDM subjects were significantly ($p<0.05$) lower than the values obtained for the control subjects (Table 2). This is an indication that the NIDDM subjects in the study area are vitamin A deficient. Vitamin A activity is very important for maintaining health thus humans and other animals have developed the capacity to store it. Storage lessens the need for regular intake of the vitamin, since it can be mobilized from these stores as retinal bound to retinol binding protein[18].

An individual is considered vitamin C deficient when his or her serum vitamin C concentration is less than 0.8μg/dl. Results from this present study showed that the mean values obtained from the vitamin C and vitamin E concentrations were significantly lower ($p<0.05$) from that of the control subject. The acceptable normal range for vitamin E is 0.8 to 1.2 mg/dl[16]. It is apparent from this current study that subjects with type 2 diabetes in the study area have low levels of serum antioxidant vitamins. The deficiencies of these vitamins have been implicated in the development of diabetic late complications such as cataract, nephropathy and neuropathy[7]. Some studies suggest that people with diabetes have elevated levels of free radicals and lower levels of antioxidants[7,9]. Other studies suggest that vitamin supplements may improve symptoms of diabetes and reduce the risk of associated complication[7].

In view of the significantly reduced antioxidant concentrations in NIDDM subjects obtained in this study and significant negative correlations between serum antioxidant vitamins and fasting blood glucose level of the subjects, it may not be out of place to recommend the inclusion of antioxidant vitamins in therapeutic regimens for the management of diabetes mellitus in the study area. This could assist in reduction or delaying the risk of diabetes late complications. Therefore the effect of supplementing the therapeutic regimens for NIDDM management with antioxidants vitamins on diabetic complications needs to be studied and facts documented so that health care providers could be convincingly advised.

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Relationship between Obesity indicators and Reduced Renal Function in a Nigerian Rural Population

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Abstract

Introduction: Obesity is defined as a body mass index (BMI) 30kg/m^2 or body fat percentage (BF) 25% for males and 32% for females. It is a growing global epidemic and a risk factor for several disease conditions including, insulin resistance, diabetes mellitus, hypertension, and dyslipidemia amongst others. Indicators of obesity include: waist circumference (WC), waist-hip ratio (WHR), BMI and BF. Though obesity has been associated with chronic kidney disease, there is no consensus on the relationship between obesity indicators, CKD and other metabolic risks. The aim of this study was to determine the prevalence of obesity using various obesity indicators including: WC, WHR, BMI and BF and determine the relationship between obesity indicators and reduced renal function.

Material and Methods: A community-based cross-sectional study. Five hundred and twenty consenting adults were recruited by cluster sampling. Data on their socio-demographic characteristics, health status and laboratory indices were collated.

Results: The sex ratio of participants was 1:1.9 (M:F) while the mean age was 46.7±17.8 years. The overall prevalence of Obesity defined by abnormal BMI, BF, WHR and WC was 14.1%, 57.4%, 91% and 30% respectively. 24.3% of the population had reduced renal function. Increased WHR was significantly associated with reduced renal function. Surprisingly, a reduced BMI was a significant risk factor and predicted reduced renal function.

Conclusion: The prevalence of obesity was high among the population studied. It was much higher when defined using abnormal WHR and BF. Increased WHR and reduced BMI were significant risks for reduced renal function.

Keywords: Relationship Obesity Indicators, Reduced Renal function Nigerian Rural Population.

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Introduction

Obesity is defined as an excess of adipose tissue[1]. The Body mass index (BMI) closely correlates with excess adipose tissue and quantitatively evaluates body fat. The National Institutes of Health

(NIH) define obesity as a BMI of 30 and above[2]. Other factors such as waist circumference, waist hip ratio and body fat percentage are also important in defining obesity. Patients with truncal obesity are known to have greater risk for insulin resistance, glucose intolerance, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, sleep apnoea, arthritis, hyperuricemia, gall bladder disease, and certain types of cancer[3].

Obesity/overweight has been strongly associated with CKD[4, 5, 6, 7]. It is associated with glomerular hyperfiltration and proteinuria both of which are risks for progression of CKD. In a large cohort study done on initially healthy men, increased BMI was associated with significantly increased risk for CKD after 14 years[4]. The association of obesity with CKD has also been shown to be independent of HTN, DM and metabolic syndrome [6].

A study conducted in England, Scotland and Wales involving 4,584 people found that being overweightin early adulthood is strongly associated with reduced renal function at age 60-64 years. Furthermore, WHR was found to affect kidney function, such that people with an apple shape are at increased risk for CKD [8]. Other studies also advocate the use of waist to hip ratio or waist to height ratio in the assessment of obesity in relation to CKD and not BMI, as the obesity associated with CKD is mostly truncal[6,7] .

The drawbacks of using BMI as an indicator for obesity are well documented. It generally does not reveal much about the body composition in terms of muscle mass vs. body fat. Ethnicity also influences the BMI, for instance, African- Americans have a deceptively high BMI that may not translate to higher risk[9]

In developing countries and indeed in Nigeria, obesity is common. Data vary according to the region studied and methods used e.g. definition /indicator of obesity. A review by Chukwuonye[10] in Nigeria reported the prevalence of overweight individuals as ranging from 20.3-35.1%, while obesity ranged from 8.1-22.2%. However, he only reviewed four studies done in Nigeria all using BMI as the indicator of obesity.

The prevalence of Obesity among the CKD population in Nigeria has also been documented. Afolabi et al [11]reported a prevalence of obesity of 33% among a CKD population in an outpatient clinic however, abnormal WHR and not BMI was significantly associated with abnormally increasedurine albumin: creatinine ratio. Ulasi et al [12]as well as Egbi et al [13]reported a surprising negative correlation between BMI and estimated glomerular filtration rate (eGFR) in studies carried out in Southern Nigeria.

A recent study investigating the clinical impact of plasma leptin levels in a CKD cohort reported a positive correlation between leptin and BMI as well as waist circumference but a negative correlation with glomerular filtration rate[14].

Majority of studies investigating the relationship between obesity and CKD have often times used BMI alone as the indicator of obesity. Considering the known drawbacks of BMI, it has become necessary to investigate obesity in the CKD population using other indicators of obesity.

This study aims to determine the prevalence of obesity among the study population using

obesity indicators such as BMI, BF, WC and WHR, and the relationship between obesity indicators and reduced renal function.

Methods

This study was carried out in Ogbona community, a rural village in the Etsako Central Local government area of Edo State in South-South region of Nigeria. Its geographical coordinates are lat. 7° 7'0" North and 6^0 27'0" East. They are predominantly farmers. The village has a community health centre.

A minimum sample size of 246 was determined for the study however a total of 52 zero consenting individuals were studied.
All adults aged > 18years, residing in the community was eligible for inclusion, however only consenting adults were recruited. Participants with any of the following characteristics were excluded; Menstruating females, pregnant females, subjects who had performed arduous physical exercise up to 24hours before, subjects on medications that inhibit creatinine secretion e.g. cimetidine, trimethoprin and febrile subjects.

The Medical Officer in charge of the Ogbona Comprehensive Health Centre (CHC) was informed and the assent of the traditional ruler was obtained in order to facilitate access to community members.

Questionnaires were interviewer administered. Demographic data such as height, weight, hip and waist circumference were recorded in the data sheet.).

5ml of blood was collected and serum creatinine assayed using the Modified Jaffe's Method[15] while eGFR was calculated using Cockcroft-Gault (CG) formula.

Early morning urine was tested using dipsticks and subjects who had urinary abnormalities were re-assessed 3 months later for persistence of abnormalities.

Clinical definitions:

- Body fat percentage was calculated using the Deurenberg's [16]equation-**Body fat percentage = 1.2(BMI) + 0.23(age) - 10.8(sex) - 5.4.**
- BMI was calculated using the equation, **weight (Kg)/height^2 (m).**
- Obesity was regarded as BMI > 30kg/m^2, Body fat percentage of > 32%, for females and >25% for males, waist circumference > 102cm in males, 88cm in females, WHR > 0.7 in females and > 0.9 in males. BMI was further classified according to the WHO classification[17] into underweight (BMI<18.5 kg/m^2), normal (18.5-24.9kg/m^2), overweight (25-29.9 kg/m^2), obesity I (30-34.9 kg/m^2), obesity II (35.9-39.5 kg/m^2) and extreme obesity ($\geq$40 kg/m^2).

Statistical Method: Data entry and management were performed using SPSS statistical software package version 16(SPSS, inc., Chicago, IL). The socio- demographic characteristics, health status and biochemical measurement of the sample were presented as tables. Data were presented as mean SD for continuous variables and as frequency and percentages for categorical variables.

The main statistical analysis involved the estimation of the crude prevalence rates of obesity for the sample; the specific prevalence rate of obesity according to age, sex and renal function. For nominal data, the Chi-square test was used to determine the difference between groups while for numerical data the

Student t-test was used. Yates correction was used in cases where a cell had value less than 5 and Fishers exact test was used when the expected value was less than 10 and marked as †. All p value < 0.05 was regarded as significant and marked with asterix within tables for ease of recognition.

The unadjusted odds ratio (OR) between the indicators of obesity and kidney function (i.e. eGFR) was determined by simple logistic regression analysis. This was followed by multiple logistic regression analysis, where potential predictor variables identified were included in a logistic regression to see what variables were associated with reduced kidney function (i.e. eGFR<60ml/min/1.73m^2).

Results

Response rate for the study was 91.5%. The sex ratio of participants was 1:1.9 (M:F). Age range was 18-90 years while the mean age was 46.7±17.8 years. Across all age groups, the proportion of females was significantly higher than males.(table 1).

The overall prevalence of Obesity defined by abnormal BMI, BF, WHR and WC was 14.1%, 57.4%, 91% and 30% respectively. 24.3% of the population had reduced renal function. Mean weight reduced with increasing age above 34 years. BF, WC and WHR increased with increasing age while weight decreased with increasing age. The was no significant correlation between age and BMI (Table 2)

The mean BF and WC were significantly lower in participants with reduced renal function compared to normal (see table 3). The prevalence of all classes of obesity was significantly higher among individuals with normal GFR (>60ml/min) compared to those who had low GFR and 58.8% of underweight participants had low GFR. (Table 4)

Increased WHR was significantly associated with reduced renal function (P=.040). Surprisingly, a reduced BMI was a significant risk factor and predicted reduced renal function. (P=.001)

Table 1:*Age and sex distribution of subjects.*

Age group (yrs)	All n(%)	Female n(%)	Male n(%)
18-27	83(100.0)	48(57.8)	35(42.2)
28-37	87(100.0)	56(64.6)	31(35.6)
38-47	75(100.0)	57(76.0)	18(24.0)
48-57	78(100.0)	48(61.5)	30(38.5)
58-67	81(100.0)	68(84.0)	13(16.0)
≥ 68	72(100.0)	38(52.8)	34(47.2)
Total	476(100.0)	315(66.2)	161(33.8)

X^2=23.900, df=5, P=<0.0001

Table 2: *Correlation of Anthropometric indices of subjects with Age*

Indicators	r	r^2	CI	P-values
Weight	-0.095	0.009	-0.184,-0.005	.032*
BF	0.475	0.226	0.403, 0.542	<0.0001*
BMI	0.024	0.000	-0.114,0.065	.593
Waist Circumference	0.171	0.029	0.083, 0.257	.0002
Waist: Hip Ratio	0.121	0.014	0.032, 0.209	.007*

BF = Body Fat Percentage BMI = Body Mass Index SD= standard deviation

Table 3: *Mean anthropometric Indices of the Subjects according to renal function*

	ALL Mean ± SD	CKD Mean ± SD	NO CKD Mean ± SD	Mean Difference 95%CI
Weight(kg)	65.2 ±12.3	68.9 ±11.5	63.3 ± 12.3	5.6 (3.3, 7.9)
BF(%)	38.6 ± 8.6	30.6 ±9.1	32.4 ± 8.4	-1.8 (-3.4, -0.2)
BMI(kg/ m^2)	25.0 ±4.7	25.2 ±3.9	25.0 ± 5.1	0.2 (-0.7, 1.1)
Waist Circumference(cm)	86.2 ± 11.1	84.7 ±9.1	86.9± 12.1	-2.2 (-4.3, -0.1)
Waist: Hip Ratio (WHR)	0.9 ± 0.1	0.9 ±0.1	0.9 ± 0.1	0.0 (0.0, 0.0)

Table 4: Distribution of Classes of BMI according to Renal function

Classes of BMI	All	CKD (GFR<60ml/min) n(%)	No CKD (GFR>60ml/min) n(%)
Under weight (<18.5 kg/m^2)	17 (3.6)	10 (8.6)	7 (1.9)
Normal (18.5-24.9 kg/m^2)	233 (48.9)	71 (61.2)	162 (45.0)
Overweight (25-29.9 kg/m^2)	159 (33.4)	26 (22.4)	133 (37.0)
Obesity I (30-34.9 kg/m^2)	50 (10.5)	7 (6.0)	43 (12.0)
Obesity II(35-39.9 kg/m^2)	12 (2.5)	2 (1.7)	10 (2.7)
Extreme obesity (≥ 40 kg/m^2)	5 (1.1)	0 (0.0)	5 (1.4)
TOTAL	476 (100)	116 (100)	360 (100)

X^2= df = 5; P = < 0.0001

Table 5: *Obesity indicators and their association with CKD*

Risk factors	n (%)	Simple logistic regression			Multivariate logistic regression		
		OR	95% CI	p-value	OR	95% CI	p-value
Obesity (↑BMI ≥ 30)	67 (14.1)	4.58	1.84-11.43	0.002★	4.99	1.94-12.51	0.001★
Obesity (↑WHR)	433(91.0)	0.38	0.15-0.99	0.040★			
Central obesity	140(29.4)	0.84	0.75-2.23	0.465			
Obesity (↑BF)	273(57.3)	1.17	0.77-1.79	0.446			

R^2= Goodness of fit for multiple regression analysis = 0.85.
BF = Body Fat Percentage, BMI = Body Mass Index , WHR= Waist Hip Ratio, n(%)= distribution in total population.

Discussion

It is clear from this study that the various indicators studied assess obesity differently. There was a wide variation in the prevalence of obesity when defined by the various indicators; it was 14% when defined with BMI and as high as 91% when defined with abnormal WHR. This could mean that the WHO definition of obesity using the WHR may over diagnose obesity in our population and may require some adjustment, while there is also a possibility that BMI also under-diagnose obesity. The prevalence of obesity (14.1%) obtained in this study compares with the previously reported-8-22%[10,] probably because these studies used BMI as the sole indicator of obesity.

The BF is a better indicator of body fat, for this study a prevalence rate of 57.4% was obtained when obesity was defined with BF. However one needs to recall that the Deurenberg's [16] equation used in this study has not been validated in our population and it also slightly overestimates body fat percentage in the obese. Some more ideal methods of measuring the BF such as skin fold thickness, bioelectrical impedance, hydrostatic weighing, air plethysmography and dual energy X-ray absorptiometry (DEXA) amongst others[18]. However, these will be cumbersome for the purpose of research. Regardless of the method used obesity is prevalent in the African population and this has been ascribed to an increasing intake of western-like diet with reduced or lack of exercise.

This study showed a higher prevalence of CKD among those with BMI< 30kg/m^2 CKD. In a study by Afolabi et al[11], obesity was not significantly associated with proteinuria or low renal function. In the Framingham study [19] the association between obesity and CKD was found to be indirect, after adjusting for other cardiovascular risks. This study identifies a BMI< 30kg/m^2 as a strong predictor of CKD both in univariate and multivariate analysis, however not all cardiovascular risks where considered. Ulasi et al[12] in reported a similar relationship in Southern Nigeria.

It is important to recall that the CG equation which was used in this study factors in the weight of the patient and is known to overestimate GFR in very obese individuals[20], this may have contributed to the higher GFR (and therefore lower prevalence of CKD) among obese compared to non-obese individuals. This is one of the drawbacks of the CG equation.

A high proportion of underweight participants (58.8%) had chronic kidney disease. Low birth weight and reduced kidney mass are known initiating factors for CKD [21]. The participants with reduced BMI in this study may have had low birth weight or are malnourished; either of these can result in reduced kidney mass and therefore a higher risk of CKD.

In recent times there have been a number of reports[22, 23] concerning an entity called metabolically healthy obesity (MHO)in which a BMI above normal ranges does not seem to translate to any health risks. Ortega et al found that 46% of an obese population was MHO and they had low risks of all cause mortality when compared to the rest of the obese population[22]. Hwang et al[23]however reports that compared to the non-obese, The MHO are at increased risk of HTN, Type 2 DM.

This study confirms the high prevalence of obesity in an African population and the variability of the prevalence based on the indicators used for assessment. There is no doubt an interesting relationship between obesity and metabolic risks, which includes CKD. Further research that will accommodate ethnic differences and utilize several obesity indicators is recommended.

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Assessment of Needle Stick Injuries and Post Exposure Prophylaxis Utilization among Doctors in Benin City, Edo State

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Abstract

Introduction: Globally, Needle Stick Injuries (NSIs) are a common source of occupational exposure to blood-borne infections such as human immunodeficiency viruses; hepatitis B and Hepatitis C. Health care workers globally incur over two million needle stick injuries (NSIs) per year. This study was conducted to ascertain the pattern of needle stick injuries and post exposure prophylaxis (PEP) utilization among doctors in Benin City, Edo State with a view to improving early reporting of NSI incidences and PEP utilization in health care facilities.

Materials and methods: A descriptive cross sectional study design was utilized for this study involving self-administration of semi-structured questionnaires to consenting resident doctors. The data collected was analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 with statistical significance set at $p < 0.05$ and 95% confidence interval.

Results: The prevalence of needle stick injuries (NSIs) among doctors was 60.3% with a statistically significant association (fishers exact= 6.168; $p = 0.044$) existing with decreasing age grouping of respondents in years. The level of post exposure prophylaxis (PEP) utilization was 13% among those who sustained NSIs, with male doctors having a significantly higher($\chi^2 = 6.683$; $p = 0.035$) uptake of PEP than female doctors following needle stick injuries.

Conclusion The prevalence of needle stick injuries (NSIs) among doctors was high, withlow level of Post Exposure Prophylaxis (PEP) utilization. There is need to strengthen training and re-training program on the causes of NSIs with sustained sensitization of the health workforce on the importance of PEP for better utilization following NSIs

Keywords: *Benin City, Doctors, Needle stick injuries, Post Exposure Prophylaxis*

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Introduction

Globally, Needle Stick Injuries (NSIs) are the most common source of occupational exposures to injuries and the primary cause of blood-borne infections such as human immunodeficiency viruses, hepatitis B and Hepatitis C to health care workers in health care settings [1]. Patient safety is a global public health concern and health care workers are not left out, since they are continually exposed to risk factors in their work environment.

The healthcare workforce, comprising about 35 million people worldwide, represents about 12% of the working population.[2] The occupational health of this significant group has long been neglected both organizationally and by Governments.[3] The World Health Organization estimates the global burden of disease from occupational exposure to be 40% for hepatitis B and C infections and 2.5% for HIV infections among Health Care Workers (HCWs) due to exposures at work especially from (needle stick injuries) NSIs.[1,4-6] An immediate consequence of unsafe injection safety practices is needle stick injuries.[1] *Some key determinants of needle stick injuries include the following*[7-8]*;*

1. Overuse of injections and unnecessary sharps
2. Lack of supplies such as disposable syringes, safer needle devices, and sharps-disposal containers (sharp boxes)
3. Recapping of needles after use
4. Lack of awareness of hazard and lack of training on injection safety.

The most effective means of preventing the transmission of blood-borne pathogens is to prevent exposure to NSIs. Primary prevention of NSIs is achieved through the elimination of unnecessary injections and elimination of unnecessary needles which are important variables in assessing unsafe injection safety practices. The implementation of Health education measures, standard precautions, elimination of needle recapping and appropriate collection and disposal of sharps coupled with the introduction of safer needle devices can help reduce the incidences of NSIs by 80% [8-9].

Literatures have shown high rates of NSIs in Germany (31.4%) and South Western Nigeria (90.3%) [10,11]. Though, varying levels of reporting of such incidences exists from as low as 14.9% - 19% [12-13] to as high as 78% inJamaica[14]. But in relation to the level of utilization of PEP low levels have been reported in both developing and developed countries [11,15] with a high (59.5%) rate of PEP utilization reported in Jamaica.[14]

This study was therefore conducted to ascertain the pattern of NSIs and the level of PEP utilization among doctors in Benin City, Edo State with a view to improving early reporting of NSI incidences and PEP utilization in health care facilities.

Materials and Methods

A descriptive cross sectional study was conducted involving 320 consenting resident doctors working in a tertiary health facility in Benin City. The hospital provides primary, secondary and tertiary health care services to clients within and outside Edo State. It also has a work force structure comprising doctors, nurses, pharmacists, laboratory scientists, social workers, record officers, administrative Staffs etc and offers residency training in various medical specialties.[16]

The health facility is located in Ugbowo, Egor Local Government Area of Edo State. The study was conducted between July to November, 2010. The sample size was calculated using Cochran's formulae for descriptive study [17] based on a 19%reported prevalence of NSIs from a previous study[13]. Respondents were selected using stratified sampling technique across the various medical and dental departments in the Hospital with final respondents recruited by

simple random sampling technique using a table of random numbers. Self administered closed and open ended semi-structured questionnaires modified from the Safe Injection Global Network (SIGN) research tool for assessing Injection safety among Injection providers[18] was utilized for data collection, following institutional approval from University of Benin Teaching Hospital and informed consent from respondents. Data collected was subsequently coded, entered and analyzed using SPSS version 16.0, association and statistical tests of association were carried with level of significance set at $p < 0.05$.

Results

The mean age of respondents studied was 30.90 ± 4.49 years. One hundred and seven (33.4%) of the respondents were house officers while 102(31.9%), 75(23.4%) and 36 (11.2%) were registrars, senior registrars and medical officers respectively. In relation to duration of professional experience 171 (53.4%), 133(41.6%) and 16(5.0%) were in the 6-10 years, ≤ 1-5 years and ≥ 11 years categories respectively. In relation to duration of work experience in years 203(63.4%), 100 (37.2%) and 17(5.3%) of respondents interviewed were in the ≤ 2 years, 3 -5 years and 6-9 years category respectively.

Table I shows that 176(55.0%) of doctors had received training on Injection safety while 144(45.0%) had not. In relation to the prevalence of needle stick injuries, 193 (60.3%) of respondents sustained needle stick injuries while 127(39.7%) had no such exposure.

Table1: *Injection Safety Training among Doctors (n=320)*

Training Received	Frequency	Percent
Yes	144	45.0
No	176	55.0
Total	320	100

In relation to factors associated with NSIs, Table II shows that age group of respondents influenced the prevalence of NSIs. The prevalence of NSIs decreased with increasing age grouping of respondents, being 66.9% for age group 31-40 years and 37.5% in age group 41 -50 years, and this association was found to be statistically significant ($p=0.044$). One hundred and forty one (62.9%) and fifty two (54.2%) male and female doctors respectively experienced needle stick injuries and this association was not found to be statistically significant ($p=0.141$). Furthermore, in relation to marital status 106 (62.7%) married and 87 (58.0%) single respondents experienced needle stick injuries, but this association was not statistically significant ($p=0.293$).

In terms of designation of respondents 25(69.4%), 51(68.0%), 63 (61.8%) and 54(50.5%) of medical officers, senior registrars, registrars and house officers

Table II: *Factors Associated with Needle Stick Injuries among Doctors (n=320)*

	Experienced Needle Stick Injury.				
Variables	Yes Freq (%)	No Freq (%)	Total Freq (%)	Test	*p*
Age					
21-30years	89 (55.3)	72(44.7)	161(100.0)	fishers	
31-40years	101 (66.9)	50(33.1)	151(100.0)	exact	0.044
41-50years	3 (37.5)	5(62.5)	8(100.0)	= 6.168	
Sex					
Male	141(62.9)	83 (37.1)	224(100.0)	χ^2=2.164	0.141
Female	52 (54.2)	44 (45.8)	96 (100.0)		
Marital Status					
Single	87 (58.0)	63 (42.0)	150 (100.0)	fishers	
Married	106(62.7)	63 (37.3)	169 (100.0)	exact	0.293
Co-habiting	0 (0.0)	1 (100.0)	1 (100.0)	= 2.185	
Designation					
House Officer	54 (50.5)	53 (49.5)	107 (100.0)		
Medical Officer	25 (69.4)	11 (30.6)	36 (100.0)	χ^2=7.529	0.057
Registrar	63 (61.8)	39 (38.2)	102 (100.0)		
Senior Registrar	51 (68.0)	24 (32.0)	75 (100.0)		
Duration of Professional Qualification					
≤ -5 1years	74 (55.6)	59 (44.4)	133 (100.0)	χ^2=2.086	0.352
6-10	109 (63.7)	62 (36.3)	171 (100.0)		
≥ -11 15 1	10 (62.5)	6 (37.5)	16 (100.0)		
Length of work (years)					
≤ y2ears	114 (56.2)	89 (43.8)	203 (100.0)		
3-5 years	67 (67.0)	33 (33.0)	100 (100.0)	χ^2=4.082	0.130
6 - 9 years	12 (70.6)	5 (29.4)	17 (100.0)		

experienced needle stick injuries respectively but these association were not statistically significant (p=0.057). In addition, with increasing duration of professional

qualification in years there was a corresponding increase in the proportion of respondents that experienced needle stick injuries with those in category ≤ 5 years and ≥ 11 years having 74(55.6%) and 10(62.5%) NSIs respectively and 6-10 years category having the highest prevalence 109(63.7%) of NSIs, but these association were not statistically significant (p=0.352). Similarly, 114(56.2%), 67(67.0%) and 12 (70.6%) of respondents in the ≤2 years, 3-5 years and 6–9 years length of work experience experienced NSIs respectively but the association was not statistically significant (p=0.130)

Table III shows that training received on injection safety did not significantly (p=0.158) influence the occurrence of NSIs among doctors who received 100 (56.8%) and 93 (64.6%) did not receive training on injection safety respectively.

Table III *Association between Training received on Injection Safety and Needle Stick Injuries among Doctors (n=320)*

	Experienced Needle Stick Injury				
Received Training	Yes Freq (%)	No Freq (%)	Total Freq (%)	Test	p
No	93 (64.6)	51 (35.4)	144(100)	$\chi 2=1.995$	
Yes	100 (56.8)	76 (43.2)	176(100)		0.158

Twenty Five (13.0%) of respondents who sustained NSIs received Post Exposure Prophylaxis while 168 (87.0 %) did not receive post exposure prophylaxis. In relation to factors associated with the level of utilization of Post Exposure Prophylaxis (PEP) following needle stick injuries Table IV shows that there was an increase in the level of utilization of PEP with increasing age grouping of doctors in years, which was12 (11.9%) and 12(13.5%) for age group 31-40 and 21 -30 years respectively, and 1(33.3%)for doctors in age group 41 -50 years but these differences were not found to be statistically significant (p=0.420).

Table IV *Factors Associated with Level of Post Exposure Prophylaxis Utilization among Doctors(n=193)*

	Post Exposure Prophylaxis Utilization				
	Yes	No	Total		
Variables	Freq (%)	Freq (%)	Freq (%)	Test	*P*
Age					
21-30years	12 (13.5)	77 (86.5)	89(100.0)	Fishers	0.421
31-40years	12 (11.9)	89 (88.1)	101(100.0)	Exact	
41-50years	1 (33.3)	2 (66.7)	3(100.0)	= 1.800	
Sex					
Male	23 (16.3)	118 (83.7)	141 (100.0)	χ^2=5.236	0.022
Female	2 (3.8)	50 (96.2)	52 (100.0)		
Marital Status					
Single	11 (12.6)	76 (87.4)	87 (100.0)	χ^2=0.013	0.908
Married	14 (13.2)	92 (86.8)	106 (100.0)		
Co-habiting	0 (0.0)	0 (0.0)	0 (0.0)		
Designation					
House Officer	8 (14.8)	46 (85.2)	54 (100.0)		
Medical Officer	4 (16.0)	21 (84.0)	25 (100.0)		
Registrar	8 (12.7)	55 (87.3)	63 (100.0)	χ^2=0.824	0.856
Senior Registrar	5 (9.8)	46 (90.2)	51 (100.0)		
Duration of Professional Qualification					
≤ 15 years	11(14.9)	63(85.1)	74(100.0)		
6-10	13(11.9)	96(88.1)	109(100.0)	χ^2=0.419	0.811
≥ 1 1	1(10.0)	9(90.0)	10(100.0)		
Length of work (years)					
2years and below	16(14.0)	98(86.0)	114(100.0)		
3-5 years	7(10.4)	60(89.6)	67(100.0)	χ^2=0.638	0.727
6 - 9 years	2(16.7)	10(83.3)	12(100.0)		

In relation to gender of respondents, 23(16.3%)male and 2(3.8%) female doctors respectively received post exposure prophylaxis following NSIs and this difference was statistically significant (p=0.022). Furthermore, in relation to marital status 14 (13.2%) and 11 (12.6%) married and single doctors respectively received PEP but this difference was not statistically significant (p=0.908). In terms of designation of doctors4(16.0%), 8(14.8%), 8(12.7%) and 5(9.8%) medical officers, house officers, registrars and senior registrars respectively received PEP but these differences were not statistically significant (p= 0.856). With increasing duration of professional qualification in years there was a corresponding decrease in the proportion of respondents that received PEP. One (10.0%), 13 (11.9%) and 11(14.9%) of doctors in ≤ 5

years, 6-10 years and ≥ 11 years professional qualification group received PEP but this association was not statistically significant (p=0.811). In relation to length of work experience, the proportion of doctors receiving PEP increased with increasing length of work in years. Sixteen (14.0%), 7(10.4%) and 2(16.9%) doctors in the ≤ 2 years, 3-5 and 6 – 9 years work experience group but these differences were not statistically significant(p=0.727).

Finally, Table V shows that PEP utilization among doctors who received training and those who did not receive training was 14(14.0%) and 11(11.8%) respectively but this difference was not statistically significant (p=0.653).

Table V *Association between Training Received on Injection Safety and Level of Post Exposure Prophylaxis Utilization among Doctors (n=193)*

	Post Exposure Prophylaxis Utilization				
Received Training	**Yes Freq (%)**	**No Freq (%)**	**Total Freq (%)**	**Test Statistic**	***P***
No	11(11.8)	82 (88.2)	93(100)	χ^2=0.202	0.653
Yes	14(14.0)	86 (86.0)	100 (100)		

Discussion

This study revealed a high prevalence of NSIs among Doctors with low utilization of post exposure prophylaxis following NSIs. The implication of this is that doctors who are key players in the health care industry are exposed to the risk of blood borne viral infections such as HIV, Hepatitis B and C following NSIs with dare health and socio-economic implications.

This study revealed further that a higher proportion of younger respondents (i.eage group 21-30 years) than the older (i.eage group 41 -50 years) respondents experienced needle stick injuries. Training on injection safety did not significantly influence the prevalence on NSIs nor enhance uptake of PEP in this study, although a higher proportion of respondents that received training experienced less proportion of NSIs and had a higher uptake of PEP following NSIs than those who did not receive training. Although this finding is in contrast to a recent study which identified training received as a significant factor that influenced injection safety practices among doctors[16], this observation in a way points to the importance of training as a possible factor that could influence health practices.

This study further shows the possibility that older respondents are more experienced and may be more careful in health matters due to trainings received and years of on the job experience, as such may be less adventurous than younger colleagues. Also younger doctors are usually given more tasks to

perform with little or no allowance to complain even when fatigue sets in due to work over load and mental exhaustion. The medical program is such that doctors can be placed on call and still expected to resume work the next day (this may continue for days to weeks) and must carry out expected duties and if found wanting can be disciplined by senior colleagues. This development can be further compounded by other social demands that predispose doctors to the risk of injuries at work. It is important to note that though this explanation appear tenable, more senior colleagues in this study experienced a higher rate of NSIs but the difference was not statistically significant. The low availability of the new engineered 'safer syringes' and the unpopularity of auto-disabled and auto retractable syringes among doctors as at the time of study, who may prefer the use of older although less safer model of syringe may also explain the high rate of needle stick injuries among doctors.

Furthermore, the long incubation periods of blood borne viral infections a potential risk factor from needle stick injuries are more often overlooked and under estimated by doctors, who may count on the negative laboratory results for blood borne viral test of persons receiving treatment who may be in their window period. This may explain the possible reason for under reporting of needle stick injuries and low utilization of PEP.

The findings from this study is similar to that from a study conducted in a German Teaching Hospital[10]among health care workers which revealed that physicians had the highest NSI rate of 55.1%, also doctors in age group less that 25 years and between 25 and 35 years were more at increased risk of needle stick injuries than those older than 45 year[10].

Similarly, other studies in London[15] and Nigeria[19] revealed low utilization of PEP despite high reporting of NSIs among doctors. Another study in Saudi Arabia[12] revealed that in addition to the high NSI rate of 63% among HCWs, doctors had the lowest reporting rate of 19% for such incidences when compared to other health workers. In addition, high NSI rate of 90.3% has been reported in south western Nigeria[11].

Based on level of utilization of PEP, a low rate of utilization was identified among doctors who sustained needle stick injuries (NSIs) in this study. It also showed that a higher proportion of respondents in age group 21-30 years received PEP than age group (31-40 years), the higher proportion observed in the 41-50 years age group can be due to the fact that only three doctor's experienced NSI and one reported the incident. Also, a higher proportion of males than females received PEP, which was highest among House Officers and least among Senior Registrars. Finally, respondents who received training had a higher uptake of PEP than those without training. This low level of utilization of PEP raises issues about the high level of disconnect between knowledge and practice in relation to the prevalence of NSIs and poor utilization of PEP, and may require further study to ascertain possible explanation for these differences.

This study also showed that more males than females received PEP possibly because a higher proportion of them had sustained NSIs. This is unusual considering the fact that female are known to have better usage of health interventions than male, also the fear of the possible side effects from the

antiretroviral drugs used for PEP might have contributed in the low level of utilization of PEP among respondents.

This research finding, agrees with the result of some previous studies conducted among doctors in Saudi Arabia[12] and in two teaching hospitals in London[15]that had reporting rates between 14.9-19% with a lower rate identified from another study conducted in South Western Nigeria[11] which gave high reporting of needle stick injuries and utilization rate of 9.2% for post exposure prophylaxis. Finally, the low utilization of PEP identified in this study is at variance with a study carried out in West Indies, Jamaica[14] which gave a higher reporting rate for needle stick injuries and utilization rate of 59.5% for post exposure prophylaxis.

Conclusion

This study identified a high prevalence of needle stick injuries among doctors which was significantly more prevalent among younger doctors than older ones. Finally, there was a low level of post exposure prophylaxis utilization among doctors who sustained NSIs with male doctors having a significantly higher PEP uptake than female doctors.

Recommendation

There is need to strengthen training and re-training programs on the causes of NSIs on regular basis (pre-service and in-service trainings) with sustained sensitization of the heath workforce through appropriate and adequate IEC materials on the importance of PEP for better utilization following NSIs.

Limitation of Study

The findings were based on self-report as it was not possible to validate claims made by respondents.

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Lower Extremity Amputation Surgeries in a Sub-urban Teaching Hospital in Nigeria

* *Odatuwa-Omagbemi DO*

Abstract

Introduction: Amputation surgeries involving the lower limbs are common. Indications for these surgeries vary. The effect of major lower limb amputation on the individual and family could be devastating. The aim of this study is to highlight the pattern and burden of lower limb amputations in our environment and make suggestions on the way forward.

Patients and Methods: A 36 month retrospective review of patients who had lower limb amputations in my centre. Data were collected from ward and theatre records of patients in addition to that obtained from case notes and analysed accordingly.

Results: Eighty one patients had 86 lower limb amputation surgeries during the study period. The male to female ratio was 2.5:1. The average age of patients was 56.7$\pm$18.9 years. Most of the patients (58%) were in their 6th to 8th decades of life. The commonest indication for amputation was diabetic foot disease in 55.8% of cases. Below knee amputation was the commonest procedure carried out (67.44%). Wound infection was the commonest post-operative complication occurring in 28.9% of wounds.

Conclusion: Diabetic foot disease was the commonest indication for amputation in our centre. Appropriate measures including patients' education on foot care, good glycaemic control and prompt treatment of foot injuries would help reduce the incidence of lower limb amputations due to diabetes mellitus. In addition efficient social support system, rehabilitation and the right prosthetic fitting where necessary is mandatory for lower limb amputees if they are to function optimally and independently after surgery.

Keywords: *Amputation, Lower limb, Diabetic foot disease.'*

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Introduction

Lower limb amputation remains one of the commonest surgical procedures. The incidence is on the rise in Nigeria due mainly to increasing motorcycle accidents and the prevalence of uncontrolled diabetes complicated by neuropathy, vasculopathy, foot ulcers and gangrene[1].

The commonest indications for amputation appear to vary from region to region and to be changing with time even within the same region[2,3].

Different authors have reported these variations in their studies. In some studies, trauma was observed as the commonest indication,[1,4,5,6] while in another group of studies, diabetic foot complications ranked the commonest indication[3,8,9]. In yet another study, gangrene resulting from traditional bone setters' treatment of limb injuries was reported as the commonest indication by Umaru et al[10]. Most of the studies mentioned above, were from Nigeria and other developing countries.

In England, peripherial vascular disease is said to account for most of the leg and foot amputations and up to 80% of these patients are diabetic[11].

The burden of lower limb amputation is a global one. It is estimated that in England, the cost of lower limb amputation ranges from 10,000 to 15,000 pounds sterling per procedure accounting for about 0.5% of the annual National Health Service budget. This excludes cost of rehabilitation, prosthesis ad social care.

In Nigeria although, there is no definite statistics on cost since most of the patients have to pay out of their pockets, the cost implications may even be more staggering.

On the part of the amputees and their families, the psychological and socio-economic impact of the loss of a lower limb cannot be overemphasised[3,8,11,12].

The aim of this study is to look into the pattern and burden of lower limb amputations in our centre with a view to recommend and institute policies and strategies for prevention of conditions that predispose to the amputations and to properly rehabilitate and fit prosthesis for those that must have their limbs amputated.

Patients And Methods

This was a retrospective descriptive cross-sectional study of patients who had lower limb amputation surgeries at the Delta State University Teaching Hospital, Oghara, Delta State. Nigeria. between January 2011 and December 2013 (36 months). Sources of data were theatre records, ward records and case notes of patients retrieved from the records department of the hospital. Data collected on patients who had lower limb amputation surgeries, included that on age, sex, indications for amputation, level of amputation, post-operative complications and length of hospital stay. Patients whose records were not complete were excluded from the study.

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 17, Illinois, Chicago.

Results

A total of 107 amputation surgeries were carried out in the hospital during the study period out of which 86 (80.4%) involved the lower limbs. The 86 lower limb amputations were carried out on 81 patients made of 58 males and 23 females giving a male to female ratio of 2.5:1.

The average age of the patients was 56.7 $\pm$ 18.9 years ranging from 15 to 90 years. The highest number of patients were in their 8th decade of life (22.2%, n=18) followed by those in their 7th decade (18.5%, n=15). Figure 1 shows further details.

The commonest indication for lower extremity amputation was diabetic foot disease in 48 patients (55.8%). This was

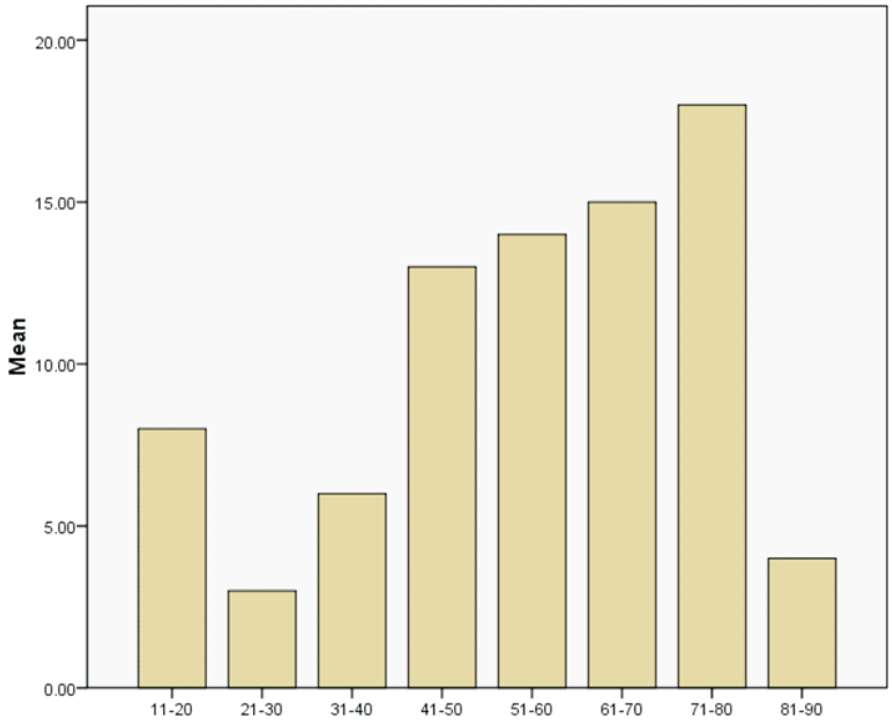
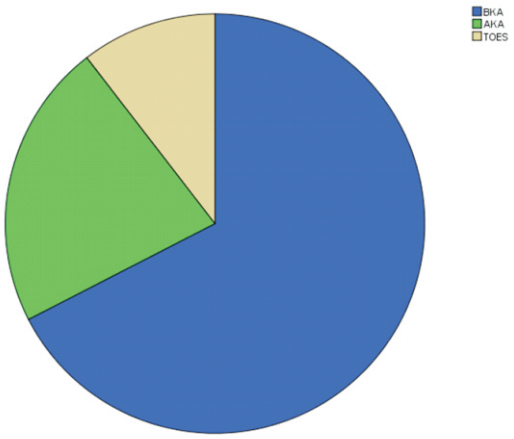


Figure 1. Age Distribution of Patients

The most frequently performed amputation procedure was below knee amputation (BKA) in 67.44% (n=58) of cases, followed by above knee amputation (AKA) in 22.09% cases (n=19).

Table 1.indications For Lower Limb Amputations.

INDICATIONS	NO. OF PATIENTS	PERCENTAGE OF TOTAL
DIABETIC FOOT DISEASE	48	55.81%
TRAUMA	13	15.12%
PVD IN NON-DIABETICS	11	12.79%
TBS GANGRENE	5	5.81%
INFECTION	4	4.65%
CHRONIC LEG ULCERS	3	3.49%
MALIGNANCIES	2	2.33%



AKA= Above knee amputation; BKA= Below knee amputation.
Figure 2. Levels of Lower Limb Amputation Surgeries.

Thirty eight wounds (42.22%) healed primarily. The commonest post-operative complication was wound infection in 28.8% of case (n=26). Six patients (6.67% died in the wards post-operatively, 5 from complications of diabetes and 1 from complication of multiple trauma (see table 2).

Table 2. Outcome Of Lower Limb Amputation Surgeries

OUTCOME	NO. OF PATIENTS	PERCENTAGE
PRIMARY WOUND HEALING	38	42.22
WOUND INFECTION	26	28.89
FLAP NECROSIS THAT NEEDED REFASHIONING	6	6.67
WOUND DEHISCENCE THAT NEEDED RESUTURING	9	10.00
ASCENDING GANGRENE THAT NEEDED PROXIMAL RE-AMPUTATION (BKA to AKA)	3	3.33
POST-OPERATIVE DEPRESSION	2	2.22
DIED	6	6.67

Two patients had bilateral lower limb amputations (one had left AKA and right BKA about one year after due to peripherial vascular disease, while the other patient had

bilateral BKA at 15 months interval due to diabetic foot disease in a patient with chronic renal impairment. Two patients had their wounds reviewed from BKA to AkA due to flap necrosis and infection/gangrene while another had toe amputation reviewed to a BKA.

The average length of hospital stay of the patients was 38.8 $\pm$23 days.

Discussion

A major amputation is a surgical procedure that is invariably followed by different degrees of permanent disability[13].

This is even more serious in Nigeria where major amputees often become permanently handicapped and disadvantaged as a result of the paucity of rehabilitative and prosthetic facilities.[3,8,13.]

Virtually all amputation studies in the literature have recorded the involvement of more males than females generally and in studies involving only the lower limbs.[1-3,5-11,13-16]. However, the ratio of male to female involvement tend to vary from study to study. About 72% of lower limb amputees in this study were males and the rest females. The male to female ratio was about 2.5:1. This is close to ratios of 2.28:1 and 2.8:1 reported by Ofeali[13] and Dada and Awoyomi[8] respectively here in Nigeria. In contrast, a lower male to female ratio of 2:1 was recorded by Chalya et al[12] in Tanzania while a higherone of 6.6:1 was observed by Jawaid et al[9] in Karachi, Pakistan. The frequent exposure of men to risky activities at work and recreation may be partly responsible for this predominance. The negative socio-economic impact of amputations is as a result more drastic on the family in particular and the society at large, the male being the bread winner in most families. The mean age of lower limb amputees in this study was about 57$\pm$18.9 years with about 58% of the patients falling between the 6th and 8thdecade. This mean age is similar to a mean age of 54.4$\pm$18 years reported by Ofeali[13]. It is however higher than the average age of 47.49$\pm$13.20 years and36.0$\pm$16.2 years observed by Jawaidet al[9] and Obalum and Okeke[1] respectively for lower limb amputees in their studies. It is important to note that in the studies with relatively higher mean ages[9,13] mentioned above including the present one, the commonest indication for amputation was diabetic foot disease. In contrast, in the study by Obalum and Okeke[1] which reported a much lower average age of 36 years, the commonest indication for amputation was trauma. The most plausible explanation for this observation is that the type of trauma that would normally lead to amputations, tend to be more common in younger persons while type II diabetes which is responsible for most of the foot complications, usually occur at a later age.

About 56% of amputation surgeries in this study were due to foot complications of diabetes mellitus. Several previous studies have similarly recorded diabetic foot disease as the most common indication for amputation[3,8,9,11,12,13]. In contrast, some other group of authors have reported trauma,[1,4,6,7,15,16] as the most common indication for amputation in their studies. Malignancies and limb gangrene from traditional bone setters' treatment of injuries have also been reported by Yakubu et al[14] and Umaru et al[10] respectively as the commonest indications for limb amputations in different studies. As a result of this obvious variations, that occur in

the relative importance of these indications for amputation even within the same environments at different points in time[7,8.] there is need for regular studies and reviews in order to possibly institute measures and strategies aimed at prevention where necessary. For instance, where traditional bone setting is a problem there would be need to engage in public enlightenment and education/training of the traditional bone setters on the implications of their activities.[17]

Below knee amputation was the commonest procedure carried out during the period of this study making up over 67% of the lower limb amputation surgeries. This agrees with findings by several previous researchers[1,3,7,-8,13,16]. Umaru et al[10] in contrast, reported above knee amputation as the commonest procedure in their study. With good rehabilitation and prosthetic fitting, below amputees usually should function optimally with minimal impairment. This is not however the case in our environment where good rehabilitative and prosthetic facilities are lacking[8,13.].For example the distance between this centre and the nearest prosthetic centre (Enugu or Lagos) is over 300 kilometres. Even where available, the cost of good prosthesis is often beyond the reach of the average amputee and their families. As a result, these set of amputees still suffer significant handicap in a country like Nigeria with virtually no social support system. In fact many of them hop around with wooden crutches begging on our road sides[13]. There is thus, need for government, good spirited individuals, non-governmental organizations and religious bodies to rise up to the challenge of providing good, affordable and subsidised prosthesis to these amputees in order to reduce their level of dependence and improve their function and quality of life.

Post-operative wound infection is the commonest complication reported in most previous studies on amputation[1,8,9,13,17]. The percentage of wound infected nevertheless varies and is usually affected by pre-operative conditions of the patient, the indication for the amputation, co-morbid conditions, condition of local tissues, surgical techniques and tissue handling, post-operative wound care and antibiotic usage. About 29% of wounds were infected in this study. Dada and Awoyomi[8] reported a fairly close figure of 31.4% wound infection rate while Obalum and Okeke[1] observed a lower rate of 26.6%. Higher infection rates of 35.8%, 36.4% and 68.6% have been reported respectively by Jawaid et al[9,] Ofeali[13] and Akinyoola et al[18].

Six of the lower limb amputees died post-operatively in the ward during the study period giving a post-operative mortality of about 6.7%. Lower mortality figures of 1.9% and 4.7% have been reported by Jawaidet al[9] and Obalum and Okeke[1] respectively. In contrast, higher figures of 8.5% and 15.2% respectively were reported by Olasinde et al[8] and Ofeali[13]. All the mortalities in this study were due to complications of diabetes mellitus except one which was due to complications of severe multiple trauma. Meticulous optimization, monitoring and control of co-morbid conditions of patients such as strict blood sugar control in diabetics will go a long way in reducing peri-operative mortality in these patients.

The average hospital stay of our patients was about 39 days. This is longer than the means of 17.3 days, 26.6 days and 32 days reported by Jawaid et al[9], Dada and Awoyomi[8] and

Ofeali[3] respectively. The length of hospital generally gives an indication of peri-operative morbidity. The longer hospital stay in our patients in this study may not be unconnected with co-morbid conditions prominent among which is diabetes mellitus the major indication for most of the amputation surgeries here.

In conclusion, diabetic foot disease was the commonest indication for amputation in this-centre. Appropriate measures including patients' education on foot care, good glycaemic control and prompt treatment of foot injuries would help reduce the incidence of lower limb amputations due to diabetes mellitus. In addition efficient social support system, rehabilitation and the right prosthetic fitting where necessary is mandatory for lower limb amputees if they are to function optimally and independently after surgery.

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Pattern of Tooth Loss in a Niger Delta Region of Nigeria

Etetafia MO[1] Okoro SI[2]

Abstract

Introduction:
The study was carried out to determine the pattern of loss of teeth in Delta State, a region in the Niger Delta. The design was a retrospective review of medical records of patients who visited the hospitals and had dental extractions. The setting is the four dental centres located in the four largest state government hospitals namely, Warri, Ughelli, Sapele and Agbor. All patients that had dental extractions between January 2003 and December 2005 were selected and included in the study.

Materials and methods:
A three year retrospective review of patients' records from four dental centres in Delta State was conducted. Data were entered into SPSS statistical software and results presented as simple tables and proportions.

Results: From the four dental centres in the State a total of 8,066 teeth were extracted from 6,053 patients aged 3 to 100 years giving a mean tooth loss of 1.4. The age group 21-30 constituted 1,880(31.1%) of the patients followed by age group 31-40 with 1,069(17.7%) and then age group 11-20 with 908(15.0%) patients. Lower molar teeth (42.4%) were lost most often followed by upper molars (22.7%), upper anteriors (11.1%) and upper premolars (6.4%).

Conclusion: Molar teeth were lost more often than any other teeth in the mouth. Patients in the third decade of life had more dental extractions than other age groups. The pattern of tooth loss in this study is in line with other similar studies within and outside the country.

Key words: Tooth Loss, Pattern, Extraction.

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Introduction

An understanding of the patterns of and reasons for tooth loss in a population is important for the planning of dental health services[1]. The dental surgeon is trained primarily to preserve teeth in good functional state in the mouth. The attitudes of patients to oral health in our environment have led to

loss of many teeth that could have been conserved. Many patients visit the dentist only when there is severe pain or when the teeth are not amenable to conservative procedures thereby necessitating extractions. Even when conservative options are available, some patients still opt for tooth removal as the immediate relief of pain or discomfort. Although the decision to preserve the teeth or not is considered in the light of the disease affecting the teeth in question, its onset, cause, termination and the likelihood of its response to treatment the contribution of a positive attitude towards conservation cannot be ignored. The type of food intake and the life style are also factors that would affect the tooth condition and influence pattern of bone loss and tooth loss[2]. Studies[3, 4, 5] have shown that molars are the most vulnerable teeth for extraction due to caries, periodontitis and other indications in respect of age and gender. Therefore, measures taken to reduce the susceptibility of these teeth should be targeted to all age groups.

Materials and Methods:

This was a retrospective review of medical records of all patients with adequate documentation that had dental extractions between January 2003 and December 2005 at the central hospitals located in Warri, Ughelli, Sapele and Agbor respectively. These hospitals are all situated in the oil rich Delta state in south south Nigeria with an estimated population of over four million people. There are various ethnic groups in the state, with the major ones being the Urhobos, Itsekiris, Ijaws, and Ibos with diverse religious and cultural backgrounds. Patients whose medical records had inadequate documentation were excluded. A proforma designed for this review was used to collect the study data. The following information were extracted from the records of the patients: age, sex, date of extraction(s), indication for extraction, tooth type extracted and complication associated with the procedure in each patient if any. Permission to assess the hospitals' records and data for this study was obtained from the respective heads of the hospitals and dental centres. The data obtained was coded and fed into the computer using SPSS statistical software (version 16) and same was used for the analysis. The envisaged limitation of this study was the anticipated challenges with retrieval of medical records and likely inadequate documentation of details of patients' management. However, the challenges with retrieval of medical records was minimized as the dental centers had their records domiciled with their respective units different from the general hospital medical records and the information sought were concise and therefore retrievable from the patients' records

Results:

A total of 8,066 teeth were extracted from 6,053 patients who visited the dental centres at Central Hospitals Warri, Sapele, Ughelli and Agbor during the period of review. The mean teeth extracted/loss was 1.4 (Table 1). Majority of patients who had their teeth extracted were in the age group 21 – 30 years (30.0%) while 17.7%, 14.7% and 10.6% of the patients who had tooth extraction were in the age group 31 – 40, 11 – 20 and 41 – 50 years respectively in descending order. Table 2 indicates the number of teeth extracted at each dental centre in relation to age distribution. From the four centres, shown in table 3, the tooth mostly involved in extraction was the

lower molar (42.4%), followed by the upper molar (22.7%), the upper anterior (11.1%), upper premolar (6.4%) and deciduous molar (5.6%) respectively. Table 4 shows the pattern of the teeth extracted at each dental centre.

With reference to deciduous teeth, the molars were extracted twice more (66.2%) than the deciduous anteriors (33.8%). A total of 82.9% of the deciduous teeth were extracted from age group 0-10 years, 13.2% from 11-20; 3.4% from 21-30; 0.3% from 31-40 and 0.2% from 41-50 years. Mandibular anteriors (5.1%) and premolars (3%) were extracted twice less than their maxillary counterparts (11.1%) and (6.4%) respectively. The reverse was the case in molars in which lower molars (42.4%) were extracted almost twice the maxillary molars (22.7%).

Table 1: *Mean teeth extracted in relation to age distribution*

Age group (years)	Number Examined	Number of teeth extracted N (%)	Mean teeth extracted
Less than 10	523 (8.6)	793 (9.8)	1.5
11 - 20	908 (15.0)	1186 (14.7)	1.3
21 - 30	1880 (31.1)	2416 (30.0)	1.3
31 - 40	1069 (17.6)	1431 (17.7)	1.3
41 - 50	696 (11.5)	853 (10.6)	1.2
51 - 60	453 (7.5)	608 (7.5)	1.3
61 - 70	315 (5.2)	457 (5.5)	1.5
71 - 80	151 (2.5)	233 (2.9)	1.5
81 - 90	45 (0.7)	68 (0.8)	1.5
91` - 100	13 (0.2)	21 (0.3)	1.6
Total	6053	8066	1.4

Table 2: *Number of teeth extracted at each dental centre in relation to age distribution.*

Age group (years)	Warri n (%)	Sapele n (%)	Agbor n (%)	Ughelli n (%)	Number of teeth extracted N (%)
0 – 10	340 (4.2)	337 (4.1)	50 (0.6)	66 (0.8)	793 (9.8)
11 – 20	461 (5.7)	517 (6.4)	117 (1.5)	91 (1.1)	1186 (14.7)
21 – 30	1021 (12.7)	927 (11.5)	262 (3.2)	206 (2.5)	2416 (30.0)
31 – 40	583 (7.2)	586 (7.3)	143 (1.8)	119 (1.5)	1431 (17.7)
41 – 50	329 (4.1)	359 (4.5)	90 (1.1)	75 (0.9)	853 (10.6)
51 – 60	219 (2.7)	251 (3.1)	88 (1.1)	50 (0.6)	608 (7.5)
61 – 70	146 (1.8)	229 (2.8)	41(0.5)	41 (0.5)	457 (5.5)
71 – 80	74 (0.9)	108 (1.3)	22 (0.3)	29 (0.4)	233 (2.9)
81 – 90	23 (0.3)	29 (0.4)	11 (0.1)	5 (0.1)	68 (0.8)
91 – 100	4 (0.1)	6 (0.1)	4 (0.1)	7 (0.1)	21 (0.3)
Tot al	3200 (39.7)	3349(41.5)	828(10.3)	689 (8.5)	8066

Table 3: *Pattern of teeth extracted in relation to age distribution*

Age group (years)	Lower Anterior	Upper Anterior	Lower Premolar	Upper premolar	Lower molar	Upper molar	Deciduous Anterior	Deciduou s molar	Total teeth N (%)
Less than 10	40	78	3	1	90	17	166	398	793 (9.8)
11 - 20	32	204	23	47	591	199	46	44	1186 (14.7)
21 - 30	83	286	62	125	1306	531	15	8	2416 (30.0)
31 - 40	49	139	69	121	652	399	2	-	1431 (17.7)
41 - 50	62	76	60	92	325	237	1	-	853 (10.6)
51 - 60	44	55	45	59	220	185	-	-	608 (7.5)
61 - 70	61	35	33	39	135	154	-	-	457 (5.7)
71 - 80	23	17	16	20	80	77	-	-	233 (2.9)
81 - 90	15	6	4	5	19	19	-	-	68 (0.8)
91 - 100	1	1	2	3	4	10	-	-	21 (0.3)
Total	410 (5.1%)	897(11.1%)	317(3.9%)	512(6.4%)	3422(42.4%)	1828(22.7%)	230(2.8%)	450(5.6%)	8066

Table 4: *Pattern of teeth extracted at each dental centre*

Dental centre	Lower Anterior	Upper Anterior	Lower Premolar	Upper Premolar	Lower Molar	Upper Molar	Deciduous Anterior	Deciduous Molar
Warri	110 (26.8)	221 (24.6)	93 (29.4)	181 (36.3)	1469 (43.0)	744 (40.7)	135 (58.7)	214 (47.6)
Sapele	236 (57.6)	480 (53.5)	129 (40.7)	235 (46.0)	1276 (37.3)	725 (40.0)	78 (40.0)	190 (42.0)
Agbor	36 (8.8)	105 (11.7)	38 (12.0)	38 (7.4)	368 (10.8)	223 (12.0)	6 (2.6)	3 (0.6)
Ughelli	28 (6.8)	91 (10.1)	23 (7.3)	47 (9.2)	309 (9.0)	136 (7.4)	11 (40.9)	43 (9.6)
Total	410	897	317	512	3422	1828	230	450

Discussion:

The causes and pattern of dental extractions have been studied in different parts of the globe. The reasons for extracting teeth can be based on local pathology, feasibility of restorative procedures, function of the dentition as a whole and the patients' attitude[6]. Using patients perception of the decision making process leading to the extraction of permanent teeth in Norway[7], 33% of the respondents stated that it was their decision to extract while about two-third said the dentist had suggested extraction. The commonest indications for extracting teeth are severe caries[8] and advanced periodontal disease[6,9] though many recent studies[2,10,11] have indicated caries. Although some studies stated that the prevalence of periodontal diseases and caries were responsible for the increased rate of edentulism or total loss of teeth in developing countries [6,12] a study in a rural area of Kenya[13] showed the prevalence of edentulousness to be less than 0.3% even up to 65 years of age.

The age group 21-30 being mostly involved in extraction is well documented. Some authors [14, 15,] attributed this to be a reflection of the increasing consumption of sugars especially among the affluent young population. A recent study in Nigeria[16] showed that age group 21-40 demanded more for removable partial denture than any other age group. This further confirms their increased involvement in loss of permanent teeth.

The pattern of loss of permanent molars in relation to other tooth types in this study agreed with a study in Lagos[17], Benin-City[18] and United Arab Emirate[2] which found that molars were more vulnerable to extraction than premolars and anterior teeth in both mandible and maxilla. Earlier studies[12,19] in Nigeria also indicated the loss of more permanent molars than other teeth. Reports from USA[20] Libya[21], Italy[3], Kenya[22] and Bangladesh[23] showed similar pattern of loss of more molars than the teeth in the frontal region.

The reason for more molars being extracted than other tooth type has been studied[24], most of which lie on the pit and fissure nature of the teeth thereby creating stagnation areas for

caries to develop. Measures geared at increasing the resistance of the molars will reduce the rate of loss to a considerable degree. Some studies consider the first permanent molars to be the most susceptible tooth to caries attack[18,25] and were more consistently extracted than the second and third molars. Although this study did not consider the causes of loss in relation to individual tooth type, the reason for the susceptibility of the first molar must be based on its being usually the first tooth to erupt into the oral cavity. The remarkably high percentage of deciduous molars extracted before the age of ten years can be attributed to the shedding of deciduous teeth associated with that age group. The loss of upper anterior teeth was mostly due to traumatic episodes which constituted the second most common cause of tooth loss in the study.

In our study, increase in age did not influence the pattern of dental extractions since the molar teeth were still mostly involved in extractions even up to age 90.

A study[26] attributed loss of teeth to non-disease factors like attitude, behavior, dental attendance, characteristics of health care system and socio-demographic factors. Another study[27] of a rural setting showed that poverty, lack of proper education and inadequate diet contributed to widespread premature and heavy losses of permanent teeth. A recent research[28] found out that respondents who had low income, low education, unhealthy behaviors, chronic conditions or disabilities, and no dental insurance coverage were more likely to have fewer teeth compared with their referent groups.

More dental health enlightenment campaigns are required to educate the populace on the importance of maintaining good oral hygiene in order to preserve natural teeth.

Conclusion

This study found that molar teeth were lost much more frequently than other teeth in the mouth. Since caries is the most implicated cause of tooth loss, it is essential to increase the awareness of people through the media and oral health campaigns in order to reduce caries induced tooth loss. More studies to find out reasons for patients negative attitude to tooth conservative procedures is advocated.

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Review Articles

HIV Associated Neurocognitive Disorders Among Adult Nigerians in the Era of Highly Active Anti-retroviral Therapy

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Abstract

Introduction: Human Immunodeficiency Virus (HIV) causes a multi-systemic disease. The brain is substantially affected throughout the course of the infection from the seroconversion period to the advanced stage. The aim of this paper was to review the literature and present an updated knowledge of the epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of HIV associated neurocognitive disorders.

Materials and methods: A careful search of literature was done in both local and international book and journal publications using web-based search engines such pubmed, medscape, hinari, google and visit to libraries in the country and in outside the country.

Results: Prior to the advent of highly active antiretroviral therapy (HAART), HIV associated dementia (HAD) was seen in nearly 50% of those infected often with rapid progression carrying a poor prognosis with a median survival of six months. With ART and sustained virological control the outlook has changed with patients stabilizing with improved immunological and neurocognitive functions and surviving for many years.

Conclusion: HIV is neuro-invasive and neuro-tropic causing a wide spectrum of neurocognitive disorders.

Keywords: : *HIV associated dementia, neurocognitive disorders, neuroAIDS, HIV encephalopathy, antiretroviral therapy*

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Introduction

In sub-Saharan Africa, over 22 million people are infected with human immunodeficiency virus (HIV) accounting for 67% of those affected worldwide. Nearly 54% of those affected are women[1]. HIV prevalence in Nigeria had been recorded from 1991, showing a peak at 5.8% in 2001, then a decrease to 5.5% in 2003, 4.4% in 2005[2] and currently 3.1%.[1] HIV transmission in Nigeria occurs mainly through heterosexual contact, with 10% of infections due to mother-to-child transmission and 10% resulting from use of unsterilized medical equipment, infected blood transfusions and occupational

accidents.[2] HIV infections is a leading cause of ill health with an increasing burden on the limited medical resources in Nigeria.[3]

Neurological illness is one of the common spectra of associated disorders seen in HIV infected patients. Thirty to forty percent of those with acquired immunodeficiency syndrome (AIDS) have some neurological complaint, 40-60% present with neurological signs or symptoms during the course of the infection and 85% of the autopsies have evidence of neurological disease.[4] HIV can cause a range of cognitive and behavioral symptoms that become more frequent and severe as the immune system declines and symptomatic illness and AIDS ensue.[6,7,8,9] The prevalence of HIV dementia in developing countries such as those in sub-Saharan Africa was previously unknown,[10] however, scanty studies have emerged on this in the last decade.[3,8-15] Ogun *et al* in a ten year retrospective study at Lagos University Teaching Hospital, of HIV patients found a prevalence of 5.2% among 362 HIV/AIDS patients[3]. World Health Organization epidemiological studies done in sub- Saharan Africa revealed a range of 4.4% to 6.9%.[10-15] In the U.S. the prevalence of HAD is 10-15%.[16] Epidemiologically, the HAART era has seen a dramatic decline in the incidence of HAD from approximately 7% per year in AIDS patients in the pre HAART era to 2% to 3% per year currently. Despite this decrease in the incidence, the prevalence of HAD is increasing with longer survival offered by HAART.[17] The rate of progression of HIV associated dementia (HAD) is variable, but the average from first symptoms to death in the pre-HAART era was six months.[18] The prevalence of HIV-related minor cognitive motor disorder (MCMD) is less clearly documented but may remain as high as 20% to 50% in symptomatic HIV illness. Changes in diagnosis and clinical management altered the face of HIV infection, such that what was once an almost uniformly fatal illness is now a chronic disease requiring long term medical management.

Materials and Methods:

A careful search of literature was done in both local and international book and journal publications using web-based search engines such as pubmed, medscape, medline, hinari, google**,** google scholar and ebooks. Assess to hard copies of some articles and books was made possible through visit to libraries at Delta state university teaching hospital , Oghara; College of Medicine, University of Lagos ,Queen Square Hospital for Neurology and Neurosurgery, London and Department of Neurology library, Cerahpassa College of Medicine, Istanbul University, Istanbul. The search was carried out for all articles related to HIV and AIDS and neuro-AIDS since the disease was discovered. The search words include 'human immune deficiency virus infection and neuropathogenesis', 'epidemiology of HIV associated dementia', 'HIV dementia', 'neuro-AIDS', 'dementia' , 'HIV and the brain in the era of HAART', 'neurocognitive performance and HIV/AIDS', 'AIDS dementia complex', 'HIV encephalopathy', 'diagnosis of HIV dementia', 'treatment of HIV associated dementia", update on HIV associated dementia'. About seventy articles and fifteen books were retrieved and used. Articles that have to do with functional disorders such as depression, substance abuse and cognitive dysfunction related to liver and renal impairment in HIV

were however, excluded.

HIV Infection of the CNS (Neuro-AIDS)

HIV-1 is both neuro-tropic and neuro-invasive (infects the nervous system).[4, 5, 18] The nervous system is infected immediately after primary HIV infection with rapidly ensuing subclinical neurological injury. The severity and frequency of HIV induced neurological disorders are influenced by the host neurosusceptibility, which are determined by host age, genetic polymorphisms, level of immunosuppression and concurrent infections, together with the host ability to contain the primary infections.[5,6]

Numerous neurologic complications occur in HIV-infected patients, but only a few of these complications are directly related to HIV infection of the central nervous system.[6] HIV related immune dysfunction and CD4 + cell depletion increase host susceptibility to neurological opportunistic diseases and neoplasm. Chronic hyper immune activation and over expression of inflammatory cytokines in response to HIV infection of the CNS also contributes to progressive cellular and tissue dysfunction, including damage to the blood- brain barrier and the surrounding nervous tissues(i.e. glial cells and neurons).[6]

Initial neurological interest in HIV infection was focused on opportunistic infections and tumors that affect the CNS. At the time, the CNS was considered to only be indirectly involved in the progression of HIV disease. Studies have now shown that the CNS and the immune system are major targets of HIV infection.[7,8,20] HIV has been isolated from the CNS tissue and intra-thecal synthesis of anti-HIV antibodies have been found in patients with AIDS and neurological illness.[19,21]

The neurological manifestations directly related to HIV are acute viral meningitis, chronic meningitis, vacuolar myelopathy, involvement of the peripheral nervous system and HIV-associated dementia (HAD). Different types of peripheral neuropathies can be distinguished on the basis of when they occur with respect to the stage of HIV disease, the clinical course, major symptoms, electrophysiological and neuropathological features. These include acute inflammatory demyelinating polyneuropathy (AIDP), Guillain-Barré syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), vasculitic neuropathy, diffuse infiltrative lymphocytosis syndrome (DILS) and distal symmetrical sensory polyneuropathy (DSSP). [6,22,23]

HIV Associated Neurocognitive Disorder (HAND)

Human immunodeficiency virus-type-1 (HIV-1) infection is the most common preventable and treatable cause of cognitive impairment in individuals under age 50. The primary cause of HIV encephalopathy (HIVE) is the infection of the CNS caused by HIV. Other terms used to describe this condition with largely the same significance are AIDS dementia complex (ADC), AIDS dementia, HIV dementia, and HIV associated cognitive/ motor complex.[24] The American Academy of neurology (AAN) and the World Health Organization (WHO) recommended the terms "HIV- associated dementia" and "HIV-1 associated minor cognitive-motor complex" to designate the major and minor forms of cognitive impairment in HIV infection.[25]

Recently the HIV Neurobehavioral Research Center (HNRC) at the University of

California, San Diego (UCSD) proposed a new working research criterion for HIV - related neurocognitive disorders (HAND) which were intended to represent a refinement of the AAN criteria. These criteria recognize three conditions: (i) Asymptomatic neurocognitive impairment (ANI), (ii) HIV-associated mild neurocognitive disorder (MND), and (iii) HIV-associated dementia (HAD) (iv) Mixed ~ compounding factors or confounding illnesses present.[26]

These modified criteria were developed by starting with the existing AAN criteria, and introduced changes based on research and observations made at HNRC, and other published sources. The most notable change is the addition of the category of *asymptomatic neurocognitive impairment*, based on the observation that some individuals have demonstrable (and usually mild) cognitive impairment demonstrated by formal neuropsychological tests without any observed abnormality in everyday functioning. The caveat to this is that the assessment of functional capacity is difficult and frequently requires third-party report, or prolonged observation.

Risk factors for HIV associated dementia

Risk factor for AIDS dementia are older age at diagnosis of AIDS, elevated plasma viral load (>50,000 copies per ml), elevated cerebrospinal fluid (CSF) viral load (>200copies/ml), CD4 count less than 100/μl; low hemoglobin concentration (less than 15g/dl), and systemic disease progression.[7,8,16,27,28]

Neuropathogenesis of HIV-associated dementia

HIV infection of the CNS may occur almost simultaneously with systemic infection. The penetration of HIV into the CNS through neurons by axonal transport, as occurs with herpes virus and rabies virus, is less probable because the CD4 receptor, the main receptor that enables HIV to infect a cell, is absent on neurons.[5,29,30,31] A more likely possibility is penetration through a hematogenic pathway. This occurs through free particles in the CNS crossing the microvessels that compose the blood brain barrier (BBB) or the blood CSF barrier (BCB), or through infected cells such as lymphocytes or monocytes. Possibly all of these forms of penetration are involved, acquiring greater or lesser importance depending on the phase of the infection. The penetration through infected monocytes/ macrophages, as propagated by the "Trojan Horse" theory (where by the HIV and other lentiviruses enter the CNS as passengers in cells trafficking to the brain) is currently considered to be the main form of HIV entrance into the CNS.[20,29-33]

The disrupted BBB during HIV infection allows free particles and infected monocytes to penetrate into the CNS. These cells can enter through tight junctions or through the endothelial cells of the capillaries, with the latter being more likely than the former. In addition to this, higher levels of matrix metalloproteinases (MMPs) in the CNS have been found. MMPs can weaken the basal membrane, facilitating the migration of leukocytes across the BBB.[32-34] Although there is evidence that cell-free HIV virions can penetrate the BBB, it is more likely that HIV enters the CNS by infecting monocytes, which are capable of crossing the BBB (i.e. the "Trojan horse" model). Excessive cytokine expression, cellular proteins, and other toxic by-products of HIV infection of the CNS

may damage neurons and glial cells and weaken the integrity of the BBB. As HIV infection progresses, the number of circulating, activated, HIV-infected monocytes increases, leading to more trafficking of infected cells into the CNS.[33,34]

Two types of CNS cells can be infected by HIV, these are cells derived from monocytes (microglia and macrophages) and astrocytes. These cells differ from other HIV-infected cells in some biological aspects, particularly in the way they express HIV products. The cellular surface CD4 receptor and the receptors of chemokines CCR5 and CXCR4 are involved in the penetration of the virus in the cells of the microglia. The microglia develops a productive infection, leading to syncytial formation and cell death; however, the possibility of chronic infection also exists.[33]

Alternatively, the astrocytes are infected predominantly by lymphocyte strains; however, infections with monocytotropic strains have also been described. Currently, the entrance mechanism of HIV into astrocytes remains unknown. It has been clearly shown that astrocytes do not have CD4 receptors on their surface. The chemokine receptors involved in HIV infection are also not expressed; however, some authors suggest that CXCR4 can be expressed following the activation of astrocytes.[33,34]

After entering the CNS, monocytes differentiate into microglia (perivascular microglia) and macrophages (e.g. meningeal and choroid plexus macrophages), all of which are capable of supporting continued HIV growth and replication. As the BBB deteriorates and endothelial junctions weaken, entry of cell-free HIV virions into the CNS may increasingly occur. These HIV-infected monocytes that penetrate the BBB and enter the CNS and subsequently differentiate may then disseminate virus throughout the brain and spinal cord.[33] There is no consensus on the timing of HIV entry into the CNS, both early (perhaps simultaneously with systemic infection) and late (advanced immunodeficiency) stages of HIV infection have been proposed and are not mutually exclusive.[29-34]

Several models attempt to explain how HIV damages neurons. One model suggests that neurotoxic viral proteins are released in the CNS by HIV-infected microglia and brain macrophages, resulting in neuronal injury or death. Neurotoxic viral components include HIV envelope glycoprotein 160 (gp160) and its cleavage products gp120 and gp41 and HIV regulatory proteins Tat, Nef, and Vpr. In vitro evidence indicates that HIV gp120 indirectly exerts its cytotoxic effect through neurotoxic intermediates, namely inflammatory cytokines and arachidonic acid metabolites, although more recent evidence suggests that gp120 may act directly as a neurotoxin.[4,6,23,29] HIV may also induce nitric oxide synthase, which would prevent astrocytes from performing their neuronal protective function. HIV Tat and Vpr can induce neurotoxicity directly or indirectly through the induction of apoptosis. The direct effect is mediated by reactive oxygen intermediates and caspase activation, whereas the indirect effect appears to be mediated by matrix metalloproteinase produced by stimulated macrophages. Both HIV Tat and Nef increase production of quinolinic acid, a neurotoxic glutamate receptor agonist.[6]

Other models of HIV neuropathogenesis

attribute neuronal impairment and death to the indirect effects of HIV-infected microglia and macrophages.[4,20,29-31] Infected microglia and macrophages in the CNS release cytokines and other soluble molecules as part of the host response to HIV infection. These cytokines and soluble HIV proteins activate uninfected microglia and macrophages, which in turn produce and release additional cytokines and other potential neurotoxins that include the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1-β, chemokines such as MIP-1α, MIP-1β, and RANTES, the α-chemokine interferon-gamma-inducible protein(IP)-10, arachidonic acid, platelet-activating factor, quinolinic acid, nitric oxide, and superoxide anions.[6,19,32-35] TNF-α activates the macrophage/monocytes/microglia leading to release of other cytokines. Acting synergistically with the macrophages it enhances HIV replication and also induces apoptosis with consequent oligodendrocyte and myelin injury and loss.[35]

Glutamate-calcium NMDA mediated nitric oxide synthase activation leads to release of nitrous oxide and peroxynitrite all these working in concert leading to inhibition of methionine synathase and depletion of methionine as well as accumulation of superoxide anions and other free radicals hence oxidative cellular and membrane damage occurs with neuronal loss and axonal degeneration. Methionine depletion leads to SAM depletion with subsequent impairment of methylation hence disordered myelin repair, decrease membrane fluidity and neurotransmitter dysfunction.[36]

These viral proteins presumably operate by interfering with signal transduction pathways that growth factors activate. Generally speaking, the collective and cumulative effect of this now very unstable environment is that these irregular levels of cytokines, chemokines, and viral proteins may eventually activate various cytotoxic pathways and, overall, induce other irregular cellular immune functions and host organ complications.

Pathology of HIV-associated dementia

HIV encephalitis is a diagnosis made by neuropathological evaluation of brain tissue. It is characterized by a number of features that are individually nonspecific (white matter pallor, microglial nodules, multinucleated giant cells, and gliosis), but when they occur as a constellation in the setting of a known HIV infection, they produce a characteristic neuropathologic signature.

The pathological findings are typically mild, even in cases where dementia is severe. Furthermore, encephalitis is neither necessary nor sufficient for dementia. Thus, although the two frequently coexist, each may be present independent of the other. Pathological abnormalities, identified in approximately 90% of patients dying from AIDS, include demyelination, accumulation of mononuclear cells, microglial nodules and multinucleated cells in the sub-cortical gray and white matter. Thus it falls in the general category of sub-cortical dementia, in this regard HIV encephalopathy or dementia is similar to the dementia seen in patients with Parkinson's disease and Huntington's chorea and distinct from that seen in those with Alzheimer's disease. [16]

HIV antigens are demonstrable in macrophages, monocytes, microglia,

oligodendrocytes, astrocytes and capillary endothelial cells but not in neurons. Atrophy typically is in a fronto-temporal distribution. Diffuse myelin pallor may be present but is due more commonly to changes in the blood brain barrier than to demyelination. Vacuolation may be observed. Reduction in synaptic density and dendritic aborisation is also a feature. Some neurons and astrocytes appear to die by apoptosis.[6,16,32,33]

Clinical features and course of HAD

The clinical features of HIV dementia were first described by Snider[22] and later defined in greater details by Navia.[37] HIV-associated dementia should be suspected when a HIV-seropositive patient presents with insidiously progressive cognitive decline occurring over a period of weeks or months. The classical triad is cognitive, behavior and motor symptoms. In early stages the neurological examination is typically normal or shows only slowing of repetitive movements or increased tendon reflexes. Also affected are patients' abilities to maintain and shift attention and to sustain a line of reasoning to solve a problem. These features are characteristic of executive cognitive dysfunction. The early symptoms are often subtle and may be confused with psychiatric complaints or overlooked. Typically symptoms described by the patient include: increasing forgetfulness, difficulty with concentration, apathy, slowness, waning interest in work and hobbies resulting in social withdrawal and loss of libido. Impaired short term memory causes failure in remembering appointments, medications and telephone numbers.[7,8,9,22,26,37]

Motor complains include: poor handwriting, insecure balance and a tendency to drop things easily. Gait difficulty may be similar to impairment of postural reflexes as in patients with other extra-pyramidal disease, including Parkinson's and Huntington's.[37] In Nigerian African patients investigators have demonstrated a range of cognitive impairments including slow reaction timed tasks, reduced attention, concentration, psychomotor speed and memory impairments.[7,8,9]

The neurological examination is often normal in early stages of HIV dementia. Neuropsychological tests may help to detect subtle changes of memory, concentration and frontal lobe function such as speed of processing information and sequential complex tasks in early stages. With advancing dementia, new learning and memory deteriorate. There is a further slowing of mental processing, some language impairment, dysomnia, reduced spontaneity, and abulia becomes more obvious. The later phases of the syndrome are characterized by global impairment with severe psychomotor retardation and mutism. Terminally, the patient is bed bound, incontinent, abulic or mute with decorticate posturing.[39-42]

The clinical features may be unmasked or accelerated in response to worsen systemic disease or metabolic abnormalities, including anaemia and hypoxia but is often reversible following treatment of these disturbances. The patients are remarkably sensitive to sedative, dopaminergic antagonists, and other drugs with CNS effects.

A staging scheme of ADC severity was empirically developed for the purposes of monitoring functional performance by Price and Brew in 1988.[38] The staging system depicts a continuum of neurologic dysfunction ranging from no impairment (ADC stage 0) and subclinical disease (ADC

stage 0.5) to global dysfunction, leaving the patient essentially vegetative (ADC stage 4). This scheme is distinct from the recently updated nosology for HIV-associated neurocognitive disorders by Antinori et al.[26]

Diagnosis of HIV Dementia

The diagnosis of HIV dementia and milder cognitive impairment are made by clinical criteria, after exclusion of other potential causes.[26] No single laboratory test establishes the diagnosis, but ancillary studies are useful for severity of pathology. Alterations in the balance of cytokines, neurotrophic factors, and neurotoxins, including glutamate receptor active compounds, have been found. An important unanswered question is why some patients with HIV infection develop dementia before death while others do not.
Standardized neuropsychological testing is required to assess cognitive functions. To meet the diagnostic criteria for ANI, MND, or HAD, other aetiology of dementia and confounding effect of substance use or psychiatric illness must be excluded.

AAN definitional criteria for HAD

The 1991 AAN criteria defined two levels of neurological manifestations of HIV infection: HIV-associated dementia (HAD) and minor cognitive motor disorder (MCMD). The AAN criteria for HAD are: (1) an acquired abnormality in at least 2 cognitive (non motor) areas causing impairment in work or activities of daily living (ADLs), and (2) either an abnormality of motor function or specified neuropsychiatric or psychosocial functions (e.g. motivation, emotional control, social behavior). The patient must have sufficient consciousness for cognitive abilities to be assessed, and should not have other etiologies that might explain the disorder. The AAN diagnostic scheme defined 3 subtypes of HAD: (1) HAD with motor symptoms (criterion 1 met fully, but only motor symptoms meeting criterion 2), (2) HAD with behavioral or psychosocial symptoms (criterion 1 met fully, but only behavioral symptoms meeting criterion 2), and (3) HAD with both motor and behavioral/psychosocial symptoms (criteria 1 and 2 met fully).

The AAN criteria also defined a less severe condition called MCMD. The essential features of MCMD according to the AAN criteria were (i) a history of impaired cognitive/behavioral function in 2 areas (e.g., impaired attention-concentration, mental slowing, abnormal memory or other cognitive functions, slowed movements, in-coordination, personality change, irritability, lability) and (ii) these abnormalities cause mild impairment in work or activities of daily living (ADL), do not meet criteria for HAD or HIV-associated myelopathy, and cannot be attributed to other etiologies.[25]

The diagnosis of asymptomatic neuro-cognitive impairment (ANI) is made if the patient has one standard deviation (SD) below the mean of demographically-adjusted normative scores in at least 2 cognitive areas of at least 5 tested domains (either attention-information processing, language, abstraction executive, complex perceptual motor skills, memory, including learning and recall, simple motor skills or sensory perceptual abilities) without impairment of activities of daily living (ADL). The impairment does not occur solely as part of a delirium (i.e. a confusional state secondary to opportunistic CNS disease, vascular insult, metabolic derangement, drug effects, or other causes of systemic toxicity)

and, as in all AAN criteria, the diagnosis is possible only if the cognitive impairment cannot be explained by other co-morbidities.

Minor neurocognitive disorder (MND) is diagnosed if a patient meets the criteria for ANI, but also has impairment with ADL as reported by patient or a corroborator. The *mild neurocognitive disorder* (MND) defined by HNRC is similar to the mild cognitive motor disorder (MCMD) previously defined by AAN. Specifically, MND is defined by the following features: (i) an acquired mild-to-moderate impairment in cognitive function documented by a score of at least one standard deviation below demographically-corrected norms on tests of at least 2 different cognitive domains, (ii) the cognitive impairment interferes, at least mildly, with daily functioning, (iii) the functional impairment has been observed for $\geq$ 1 month, (iv) the impairment does not meet criteria for delirium or dementia, and (v) the impairment is not fully explained by co-morbid conditions.

HIV associated dementia (HAD) is diagnosed if patients score two standard deviation below the mean in at least two cognitive domains and have marked impairment in ADL as a result of cognitive decline. The criteria for *HIV-associated dementia* (HAD) include: (i) acquired moderate-to-severe cognitive impairment, documented by a score of at least two standard deviation below demographically corrected normative means in at least two different cognitive domains, (ii) marked difficulty in ADL due to the cognitive impairment, (iii) a duration of impairment $\geq$ 1 month, (iv) the impairment does not meet criteria for delirium or dementia, and (v) the impairment is not adequately explained by co-morbid conditions.[26]

Differential diagnostic considerations

The principal differential diagnostic considerations in HIV patients with cognitive changes are encephalopathies (delirium) due to drugs and metabolic derangements, chronic brain syndromes, due to substance use and head injury, CNS opportunistic disease and severe primary psychiatric disturbances. Renal or hepatic insufficiency is capable of causing encephalopathy directly, or through diminished clearance of CNS- active drugs. In toxic and metabolic encephalopathies, delirium with variable attention and arousal represents the most typical presentation.[7,8,9,16]

Investigations

Neuropsychological tests: Standardized neuropsychological (NP) testing is very helpful in diagnosis. It provides clear documentation of cognitive impairment and assists in differentiating HIV dementia from other disorders that may cause cognitive impairment. Some useful ones are test of verbal and nonverbal learning (e.g. the Hopkins verbal learning Test) and sustained attention (Paced Auditory Serial Addition Test), Iron psychology (FePsy), International HIV Dementia Scale (IHDS) ,Community Screening Instrument for Dementia (CSID) among others. Such tests are administered by an experts and interpreted by comparison to normative data with appropriate demographic corrections, including age, gender, education, and in some cases, ethnicity.[39-44]

Viral load of HIV in the CSF

In the peripheral blood there is no relationship between viral load and neurological symptoms during primary HIV

infection. However, HIV RNA viral load in the CSF has been found to be related to the severity of neurological dysfunction. As HIV RNA in the CSF increased, neurological dysfunction increases. The clinical importance of the determination of the viral load in the CSF is to monitor the therapeutic effect of HAART, to identify patients with CNS escape and to prevent misdiagnosis of psychiatric symptoms.[45]

Among patients with CD4 + > 200 cells/μl, the viral load in the CSF is positively correlated with the viral load in blood, but it is not correlated with neurological alterations. After the institution of HAART, patient viral load decreases in the CSF in parallel with blood level. Among patients with CD4+ < 200 cells/μl, the viral load in the CSF correlates with the neurological alteration. However, there is no correlation between the viral load in the CSF and the viral load in the peripheral blood; after HAART there is a slower decrease in CSF viral load in comparison to blood.[45]

Molecular markers

Molecular markers such as β_2-microglobulin, quinolinic acid, neopterin, PGE_2 and PAF studied in CSF could help in the diagnosis, mainly neopterin and β_2-microglobulin, but they are not sufficiently sensitive or specific to assist in clinical diagnosis.

Imaging studies

HIV infection is associated with structural and metabolic changes of the brain. These abnormalities have been depicted by anatomic and functional neuro-imaging studies. Cerebral atrophy, often accompanied by ventricular enlargement represent the most common structural changes seen on either computerized tomography (CT) or magnetic resonance imaging (MRI). It has been observed in the majority of patients with HAD and a smaller percentage of patients at earlier stages of infection suggesting that a critical loss of volume may antedate the appearance of cognitive symptoms.

Functional imaging studies show that HIV infection is associated with metabolic dysfunction both at the regional and cellular level. Positron emission tomography with fluorodeoxyglucose has shown two general patterns of metabolic alteration in the HIV brain; hypermetabolism in the basal ganglia and thalamus, possibly characteristic of early stages of HAND and hypometabolism in the cortex that likely correlates with the severity of dementia. Focal areas of reduced cerebral perfusion were demonstrated by single photon emission tomography in patients with HAD as well as in asymptomatic subjects with CD4+ cell counts greater than 500/mm [46] suggesting that these defects appear during the early stages of infection.

Magnetic resonance spectroscopy (MRS) has shown a reduction in N-acetyl-aspartate, a marker of mature neurons in patients with advanced dementia in addition, significant elevations in brain choline and myoinositol have been found in all stages of infection.[47] These early changes in choline and myoinositol provide a marker of early HIV brain infection. Study of cerebral blood volume changes by functional nuclear magnetic resonance (NMR) imaging has revealed significant increases in the deep gray matter, in patients with HAD further supporting the idea that sub-cortical regions bear the brunt of primary injury.[47]

Treatment of HIV Associated Dementia

A substantial amount of literature has accumulated in support of highly active antiretroviral therapy as the mainstay of treatment for HIV associated neurocognitive disorders. Highly active anti- retroviral therapy (HAART), which is a combination of anti- retroviral drugs from two or more classes, was introduced for treatment of AIDS in 1996.[4]The effective treatment of cognitive disorders related to HIV is most likely dependent on the complete suppression of the replication of HIV in the CNS. The lack of complete suppression of replication in the CNS related to factors such as lack of antiretroviral drug penetration into CNS can facilitate the development of resistance to antiretroviral drugs or permit mutations in the virus.[48]

The impact of ARV therapy on HIV-associated dementia and other primary neurologic manifestations of HIV infection are difficult to assess. Obiabo *et al* in a prospective randomized interventional study among adult Nigerian African patients with AIDS recently showed the benefit of anti-retroviral therapy on cognitive performances with significant improvement in their psychomotor speed, memory and concentration abilities except in motor speed.[7] Improvement in cognitive performance is strongly related to immune reconstitution effected by HAART. The benefits of HAART has also been documented by other researchers in Sub-Saharan Africa as well as other parts of the world.[49-52] The benefits accrued to HAART have been substantiated by the demonstration of improvements of cognition in the presence of HIV RNA decline in cerebrospinal fluid (CSF) by Marra and colleagues[53] and this decline has been shown to be an independent predictor of cognitive improvement.[53] Furthermore, it has also been shown that CNS penetrating capacity of the anti-retroviral drug is a strong predictor of cognitive improvement supporting the use of neuro-penetrating ARV drugs to treat patients with HAND.[54] Among the ARVs, zidovudine has the highest CSF penetration level, followed by abacavir, nevirapine and stavudine. As a class, the protease inhibitors (PIs), most of which are highly protein bound and relatively large in size, have low CSF penetration levels. Very few studies have compared the different ARV combinations in the treatment of HIV-associated cognitive disorders, investigators have inferred the importance of CNS-penetrating ARVs predominantly from retrospective studies, comparing the rate of cognitive decline or CSF HIV viral load with the use of different regimens with conflicting reports.[54-56] In a recent study by Obiabo *et al* the effects of five combination ARV drugs with at least one CNS-penetrating ARV on cognitive functions in advanced HIV infection were compared and efavirenz-based combination were found to be associated with insignificant improvements in neurocognitive performance.[7] This may be related to the CNS toxicity of this drug or the patient to patient variation in the cognitive benefits that may be derived from HAART. Key factors that affect entry of a drug into the brain are related to the drug's physiochemical properties: lipid solubility, size, and degree of ionization, the degree of protein binding and cerebral blood flow.

Cognitive functions deteriorate with treatment failure. To prevent this, antire-troviral therapy should be continued

indefinitely and in severely ill patients a third party should be involved in administration of medications. The HAART era has drastically reduced the mortality associated with brain manifestations of the HIV infection and increased prevalence of neurocognitive disorders as a consequence of long survival. The incidence of HAD, a common terminal presentation in the pre-HAART era, has continued to decline with use of ART. HAART remains therapy of choice for those with neurocognitive disorders. No significant data in support of other intervention measures. Patients with HAND tend to have enhanced susceptibility to sedatives and with unpredictable effects and these should be avoided. The patients should be followed up with neuropsychological testing.

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Molecular Techniques: Their Role in Microbial Diseases and Antibiotic Resistance Detection

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Abstract

Introduction
Advancement in molecular biology has led to the development of new tools for detection of microbial diseases and antibiotic resistance. Nucleic acid hybridization and amplification techniques are exploited to detect the DNA or RNA sequences unique to a particular microorganism. This review aims to explore the role of molecular techniques in microbial diseases and antibiotic resistance detection.

Materials and method: A review based on theoretical and empirical literature was done through internet search engines such as Google, Pubmed, Medline, journals and books. The literature search spans from 1990 - 2013.

Results: A total of 61 publications are reviewed. Most of them are international articles. The review reveals that since the advent of conventional polymerase chain reaction, other molecular methods utilizing diverse labels, formats and strategies have been described by various researchers. These techniques have been applied for detecting various microbial, diseases and antibiotic resistance gene .

Conclusion: Huge information is available on molecular methods using different labels, formats and strategies in the internet. Simpler methods need to be devised by manufacturers using information available so that resource constrained countries can be exposed to the knowledge and method of use. Staff should be trained on both conventional and molecular techniques. Molecular biology should be introduced as a course of study in life sciences. Method cannot be recommended for monitoring the effectiveness of therapy but may be used to improve/augment the quality of diagnosis of diseases and antibiotic resistance for effective health care delivery and management of diseases.

Keywords: *Molecular techniques, microbial diseases, antibiotic resistance gene detection.*

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Introduction

The discovery of molecular structure of nucleic acid by Watson and Crick in the 1950s and understanding of this macromolecule by advancement of molecular biology has led to the development of new tools and therefore new methods for microbial and antibiotics resistance detection. Molecular methods may

be an improvement over conventional microbiological diagnosis in many ways. Currently, their most practical and useful application include, detection and identification of infectious agents for which routine growth based culture and microscopy may not be adequate for detection[1-2] or confirmation or both of antimicrobial resistance[3]. The phenomenon of antibiotic resistance has been known since almost the beginning of antibiotic use. As early as 1943, Alexander Fleming who rediscovered penicillin observed that some bacteria were resistant to the drug and warned that indiscriminate use of penicillin would lead to the proliferation of resistant pathogenic bacteria[4-5]. After more than 50 years of widespread use, however, many antibiotics are not as effective as they used to be.

Materials and Methods

The articles used for this review span from 1990 to 2013. A total of 115 articles were retrieved from literature search. A total of 61 articles were used for this review, others were excluded due to fact that they were very old publications and in some cases only abstracts were available and full text of article were not available. Article based on both empirical and theoretical approach was adapted for this review. The search engines included used Google, Yahoo, Medline, Pubmed and MSN. Molecular techniques, microbial diagnosis, molecular diagnosis, antibiotic resistance detection, bacterial, fungal, parasitic, viral diseases nucleic acid hybridization, polymerase chain reaction, real-time polymerase chain reaction were search words. Relevant textbooks on molecular biology were also consulted.

Traditional or Conventional Method of Microbial Diagnosis

Traditional microbial detection relies solely on phenotypic features such as morphologic features, growth variables and biochemical utilization of organic substrates[6]. Viral cytopathic effect, fungal conidiogenesis and parasitic morphology are other aspects of phenotypic features that are exploited in conventional microbiology diagnosis of disease[7]. Traditional methods have been in use for over a hundred years. In fact, it is still considered as the "gold standard" of detecting certain diseases[8]. Traditional culture methods show the whole picture of the organisms. They are quantitative, isolates can be typed or finger printed (by phenotypic or genotypic methods), antibiotics sensitivity test can be done, isolates can be archived and these methods have stood the test of time. The disadvantages are that they are slow required skilled, experienced personnel and only cultivable bacteria can be detected which is not possible if the culture medium does not support their growth or if antibiotics have been used[9].

Molecular Methods of Detecting Microbial Diseases and Antibiotic Resistance Genes

Molecular techniques for microbial and resistance gene detection can be divided into hybridization techniques and amplification techniques though most amplication technologies are also partly based on hybridization technology[1]. Only nucleic acid hybridization techniques and polymerase chain reaction (PCR) will be discussed in this review.

Nucleic acid hybridization methods exploit

the speicificity of base pairing between two strands of nucleic acid to detect certain sequence while nucleic acid amplification techniques selectively amplify specific targets present in lower concentration. They offer superior performance in terms of sensitivity over direct probe test. Only polymerase chain reaction will be discussed among the nucleic acid amplification techniques.

Hybridization is a chemical reaction between probe and the deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) to be detected. Methods that utilize nucleic acid hybridization include colony blotting, southern blotting, fluorescence in situ hybridization, nucleotide array technology and molecular beacons[5].

In situ Hybridization

In situ hybridization also known as hybridization histochemistry was introduced in 1969[10]. When the hybridization is performed on actual tissue sectioned cells or isolated chromosomes, to detect the site where the DNA or RNA is located, it is said to be done "in situ". Molecular hybridization and simultaneous viewing of the histopathological structure of specimen is possible using this method[7].

This method is useful in detecting intracellular parasites like viruses and malignancies. Small probes are used since the probe has to reach its target inside the cells and be able to penetrate tissue. Sensitivity is limited to accessibility of the target to the cell. In situ hybridization may be divided into fluorescent in situ hybridization (FISH) where the oligonucleotide probe is labelled with a fluorophore that is detected by direct fluorescence microscopy and chromogenic in situ hybridization (CISH) where the nucleotide probe is labelled in a way that an enzymatic reaction generates a colour that can be viewed using traditional light microscopy. Peptide nucleic acid (PNA) was developed in the 1990s and have been successfully used as hybridization probe in FISH assays. The advantages of PNA-FISH probes over DNA probes include the stability of the PNA-RNA hybrid due to the uncharged nature of DNA. In addition, the relative hydrophobic nature of PNA compared to DNA oligonucleotides allow PNA probes to penetrate the hydrophobic cell wall of bacteria following mild fixation conditions that do not lead to disruption of cell morphology[11].

In situ hybridization is similar to Northern and Southern blots in that all detect the presence of DNA or RNA sequences in the cell, but differs in the sense that insitu hybridization reveals the actual location of the sequences in the cell[12].

Application in Microbial Diseases and Antibiotic Resistance Detection

Fluorescence in situ hybridization has been reported by various researchers as useful for microbial and antibiotic resistance detections. Fastidious organisms such as *Bartonella spp* and *Yersinia pestis* have been detected by FISH[13]. Family, genus and species-specific FISH probes have been developed and published for detection of *Chlamydia spp*[14] and *Helicobacter spp*. Yinaz and Demiray used FISH method on paraffin embedded tissue to detect *Helicobacter pylori*[15] and clarithromycin resistance within three hours according to gold standard as a non-culture method[12]. FISH method maybe applied on cultured *H. pylori* colonies and also used directly on biopsy specimens for histological and microbiological examination[16,17]. A few FISH nucleic acid tests

are commercially available including those from microscreen Ribotechnologies (Groningen, the Netherlands) for detection of human intestinal bacteria such as *Lactobacillus* spp, *Streptococcus* spp, *Bifidobacterium* spp, *Escherichia coli* and *Clostridium* spp[13]. Ribosomal RNA (rRNA) occur much in number in the cell. rRNA sequence are commonly used as targets for fluorescent, labelled probes PNA are the most advanced technology in respect to applications in rRNA-FISH. Commercially diagnostic tests have been developed by Advan Dx Inc. (Woburn, MA USA). Ribosomal based probes are more sensitive than DNA based probe due to the fact that there are more copies of rRNA in the cells than there are copies of genes for the ribosomes[12,18] which increases the sensitivity of the assay. Secondly the presence of rRNA indicates the presence of a viable organism while the detection of DNA by amplification methods such as polymerase chain reaction, may occur even after the organism is dead. The limitations of ribosomal RNA targeted FISH include detection of ribosomal RNA sequence of closely related strains, subspecies or even different species as identical and cannot therefore be used as differential marker. Ribosomal inquiry cannot be used to characterize antimicrobial resistance.

Frank-Kamemetskii and workers proposed a novel FISH type genomic sequenced-based molecular technique which was based on a synergistic combination of PNA-based technology and signal enhancing rolling circle amplification reaction. This technique was able to detect and discriminate methicillin sensitive strains of *Staphylococcus aureus* (MSSA) from methicillin-resistant staphylococcusaureus (MRSA)[18].

In situ hybridization has been used to detect trypanosomes in patients with African sleeping sickness and to differentiate mycobacteria in culture of histologic sections[19,20] *Aspergillius fusarium* and *Pseudallescheria* species has been detected in tissue section using in situ hybridization techniques[21]. Chromogenic in situ hybridization has been used to differentiate morphologically similar microorganisms. Yeast and yeastlike fungi have been differentiated using this method[22]. *Staphylococcus*, *Enterococcus*, *Klebsiella* and *Candida* species have been identified directly from positive blood cultures in less than two hours using PNA-FISH techniques[23-25].

DNA Microarray

DNA microarrays are based on the principle of hybridization, which allow the mass screening of sequences[13,26]. The method rely on gene-specific probes deposited on a solid surface like glass or a silicon chip. Hybridization of the DNA fragments to fluorescently labelled probe is detected by advanced instrumentation and software[13]. Large number of resistant genes can be detected on a single experiment using this method. Significant automation in a microchip format is also possible. Compared with traditional nucleic acid hybridization with membranes, microarrays offer additional advantages of high density, high sensitivity, rapid detection, low cost, and low background levels[27]. Microarrays provide a better option for a large scale detection of pathogens and resistance genes. It is also possible to survey a sample for a multitude of sequences simultaneously[28]. Microarray technology is costly[19]. Inconsistent reactions

in some system and data management, interpretation make it difficult to apply test to routine use[13,28].

Inspite of the limitation of method, some manufacturers provide off the shelf assays which are reported to be sensitive, specific and easy to interprete[13]. Microarray has been used to detects *Staphylococcus* species, Staphychip which detected 15 species of *Staphylococcus* including methicillin resistance *S. aureus* (MRSA)[29]. Microarray have been designed and used to detect *Salmonella enterica* and gram positive bacteria[30,31]. Based on the possibility that horizontal gene exchange is possible between distantly related bacteria, Frye et al developed DNA microarray for the detection of multiple antimicrobial resistance genes in a variety of diverse bacteria. The oligonucleotide microarray developed by them was able to detect control strains obtained from National Centre for Biotechnology Information database and 51 distantly related bacterial resulting in identification of 61 different antimicrobial resistance genes in the bacteria tested[32]. In another research, Frye et al also constructed a glass slide microarray for detection of 775 antimicrobial resistance genes or resistance associated genes[33].

Microarray analysis is not as cheap as PCR detection for an individual gene but can be used to assay many genes simultaneously which make it cheap at the long run. Targeting the internal transcribed spacer (ITS1 and ITS2) sequences of the rRNA genes, oligonucleotide array was developed to identify 77 species of clinically relevant yeast belonging to 16 genera. A collection of 452 yeast strains were tested and a sensitivity of 100% and a specificity of 97% was obtained by the array[36]. Microarray has been applied to parasite research. *Entamoeba histolytica, E. dispar, Giardia lambi*a assemblages A, B and C parvium types 1 and 2 were detected in a single assay with high specificity and sensitivity[34].

Molecular Beacons

Molecular beacon was introduced few decades ago[35]. Molecular beacons are hair-shaped oligonucleotide probes that become fluorescent upon hybridization to a RNA or DNA target sequence. The loops of the beacons serve as probes and about 15 to 25 nucleotide long. The stems serve to bring the two ends of the molecules that are usually linked to a fluorophore and a quencher, in close proximity[36]. Although the stems are only 5 to 7 nucleotide long, they keep the label in close proximity so that the fluorescence of the fluorophore is quenched in the free probes. However, upon binding to their target, they undergo spontaneous conformational reorganization that remove the fluorophore from the vicinity of the quencher and restores its fluorescence. As at the time of writing this review, only few reports using molecular beacons were detected. Molecular beacons was used to detect *Chlamydia trachromatis* in urine. HCV, HBV, and HIV have been detected using molecular beacon in real time assay to detect and quantify viral nucleic acid[26].

Southern Blots, Northern Blots and Colony Blots

Southern blots was named after its inventor Edward Southern, a British biologist at Edinbough University in the 1970s[37]. DNA is extracted from the organisms and purified. The DNA is cut into smaller fragments using

restriction enzymes. The resultant fragments are separated on agarose or gel by electrophoresis. Southern blots is designed to locate a particular sequence of DNA within a complex mixture. For example, southern blotting could be used to locate a particular gene within an entire genome. Colony blots are used to detect a given nucleotide sequence in a crude preparation of genomic DNA obtained from colonies grown on an agar plate. The technique can be used to determine which cell in a collection of colonies contains the DNA of interest[5]. Northern blots is similar to Southern blots only that in Northern blots, RNA is immobilized on the membrane.

The Southern blot techniques was used to detect *MecA* gene in *Staphylococcus aureus* and to evaluate the efficiency of the technique as PCR[38].

Advantage of Nucleic acid Hybridization Techniques

- `Nucleic acid probe techniques are more sensitive compared to culture for fastidious microorganism but less sensitive than the nucleic acid amplification assay.
- Direct DNA test, though are expensive than culture, DNA test may save money for the laboratory, when cost of both materials and labour are considered.
- The use of Direct nucleic acid test for microbes saves time.

Polymerase Chain Reaction (PCR)

Developed by Kary Mullis who shared the 1993 Nobel prize in Chemistry with Michael Smith[39-40]. PCR does not require bacterial vectors to amplify the target. PCR supercedes bacterial cloning because of its greater sensitivity, selectivity and speed. PCR involves cycles of heating the sample for denaturing, annealing of the primers and elongation of the primers. Application in Microbiology and infectious disease include disease agents that cannot be cultured easily and rapid identification of antimicrobial resistance[41] The advantage of PCR include high sensitivity, specificity, good reproductibility and rapidity. Ability to detect the presence of infecting microorganism that cannot be detected by conventional methods are among the advantages. The limitation of PCR methods are contaminated from prior sample testing by autoanalysers and amplicon from previous amplification of the same target. Aliquoting error during result and record keeping, technical complexity of procedures, expensive equipment and reagent are other aspects of limitation of methods[42]. Other types of PCR methods have been devised to overcome the limitation of PCR. These include nested PCR, multiplex PCR, competitive PCR, reverse transcriptase PCR and real-time PCR. Of all types mentioned, only real time will be discussed.

Detection of PCR products

Methods by which amplification products are detected include Agarose gel electrophoresis, polyacrylamide gel electrophoresis, DNA separated by size, visualized with intercalation dye such as ethidium bromide and syber green.

Real Time PCR

Unlike conventional PCR, real time PCR allows continual monitoring of accumulating amplification in real time by labeling primers, oligoprobes or amplicons with molecules capable of fluorescing. Speedily designed

thermocyclers, attached optical systems and labelled probes, makes the amplification process to be monitored in each tube as it occurs. A labelled probe added to the PCR mix confirms and quantifies the PCR product as it is being generated (in real-time). No additional post-amplification detection or conformation system are required thereby shorten the assay time[8-,43].

Amplification of PCR in Microbial diseases and Antibiotic Resistance Detection

Most microorganisms of clinical intrest have been detected and studied by PCR. Antimicrobial resistance genes have also been detected by PCR. Fredricks et al[44] used PCR confirmed with FISH to detect agents of bacterial vaginosis. These include Gadnerella spp, *Atopobium* spp, *Clostridium* spp, *Tropheryma whippeli* were detected by PCR. *Trypanosoma brucei*, *Leishmania* spp[45-46] are among parasites that have been detected using PCR. Targeting the internal transcribed spacer, (ITS) regions, *Aspergillus* spp was detected using repetitive sequenced based PCR[47]. Other moulds such as *Fusarium* spp, *Penicillum* spp, using DNA sequencing of the internal transcribed spacer were detected[27] methicillin resistant genes (*MecA*) have been detected using PCR for coagulase-negative *Staphylococcus* spp[48]. *TetM*, *ermB*, and *errnTr*, *MecA* have been detected by method in human and bovine isolates of *Streptococcus agalactic*[49]. There are many reports on detection of *Mycobacterium tuberculosis* using PCR. Resistance of Rifampin, Ethambutol, Streptomycin have been reported by various researchers[50].

PCR, apart from detection, provides the researcher with a tool to examine the presence or absence of particular genetic elements within populations and when coupled with DNA sequencing allow researchers to examine the composition and variability of genes of interest.

Application in Microbial Disease and Antibiotic Resistance Detection using realtime pcr method

Most microorganisms that were detected in the past using conventional PCR have been detected more recently using real-time formats. Fastidious organisms which had formerly been detected with conventional PCR, have also been detected. Numerous assays have been described that detect bacteria and the genes associated with resistance. Two real-time PCR assays have received FDA approval for use on the smatcycler system. Assays for group B streptococcus for prenatal screening during pregnancy and assay for the detection of methicillin resistance *Staphylococcus aureus* (MRSA) colonization kits are also available for the detection of bacteria. *Enterococcus* (specifically vancomycin resistant enterococci VRE), *Staphylococcus* and MRSA. *Pseudomonas* and *Bacillus anthracis*. Many assay are available that target microorganism which are very difficult to culture such as *T. whippelii*, *Rickettsia* and *Bartonella* specimen have been detected [51-52]. Slow growing fungi are difficult to cultivate in the routine laboratory; *Pneumocystis jirovecii* and *Histoplasmas capsulatum* have been identified[53]. Smartcycler and Tagman have developed assays for Mycobacterium tuberculosis detection and resistance gene detection for Isoniaziid and rifampin[54]

Assays also exist for detection and identification of stool parasites, such as

Cryptosporidium and *Micorsporidia* and tissue parasites such as *Leishmania* and *Trypanosoma* and detection and differentiation of *Plasmodium* species[57-58.]

Limitations of Molecular techniques as a means of detecting disease and antibiotic resistance

Molecular techniques may be of higher quality in terms of rapidity, specificity and sensitivity in diagnosis of disease, thereby facilitating effective patient management in Health care delivery. However, there are drawbacks in the method: the assays are very difficult to perform. That is, the accuracy and reproducibility of methods depend on the technical expertise and experience of operator. The methods are very expensive. Reagents and other equipments for nucleic acid detection techniques are very expensive[41] hence, the techniques have not found wide usage in resource constrained countries except in special and research laboratories[42]. Molecular methods for resistance detection may be applied directly to the clinical specimen, providing simultaneous detection and identification of the pathogen plus resistance characterization.

Due to their high specificity, molecular method will not detect newly emerging resistance mechanism. Besides, the methods may not to be useful in detecting resistance genes in species where the genes have not been observed previously. Unless unknown, mechanisms share sufficient DNA homology to allow annealing of primers or probes: genuinely novel mechanisms or high divergent resistance genes will be missed[2]. Because of this fundamental shortcoming, it is highly unlikely that molecular tests for resistance will ever replace traditional phenotypic test. Phenotypic antibiotic susceptibility testing methods allow laboratories to test many organisms and detect newly emerging as well as established resistance patterns,[58] Molecular technique cannot detect clinically relevant 1 % proportion of resistant bacteria compared to conventional microbiology techniques.[59] In most cases, it is difficult to interprete results obtained from molecular methods due to the broad range nature. A positive nucleic acid detection results may indicate the presence of viable disease-related organisms or simply the detection of transient colonizers or nucleic acid that is persisting from old disease[62]. For example, samples from a patient who is being successfully treated with anti-tuberculosis drugs often test positive in DNA tests, but negative in cultured-based test that rely on growing the organism. These are problems that must be considered in designing new genetic tests and using them in clinical practice.

Conclusion

Molecular techniques have advanced since its inception in 1983 by Cetus scientists. The conventional PCR techniques have been modified from the open system prone to contamination and hours of turn around time to real-time technology; which is an improvement over the conventional PCR method. Turn around time for new technologies takes about few hours. These techniques have been applied for disease and antibiotic resistance genes detection. The expectation that molecular techniques will replace traditional cultural techniques for microbial and resistance gene detection is yet to be achieved. Techniques can augument conventional traditional/cultural techniques

for effective management of infectious diseases. There is need for manufacturers to harness available information provided by researchers to devise techniques that will take into cognizance, sensitivity, specificity, cost, laboratory staff resource and training, the number and type of specimens that can be tested at one time, thereby democratizing techniques, especially in resource constrained countries where they are most needed.

While molecular techniques are widely used in the developed countries, it is yet to find wide usage in resource constrained countries. However, when techniques are simplified, they can be applied in the developing world. This will reduce morbidity, mortality and incidence of antibiotic resistance.

Staff and students in life sciences should be exposed to molecular biology. This in effect will boast research. Funds should be made available by relevant bodies to equip laboratories. This in effect will lead to rapid diagnosis of disease, thereby controlling microbial disease and antibiotic resitance leading to better health care delivery.

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Case Reports

Vitamin K Deficiency Bleeding Masquerading as a Malignant Disease

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Abstract:
Introduction
Vitamin K deficiency causes bleeding disorder in neonates. It is a clinical condition, where a newborn develops bleeding disorder usually from the second day of life. Vitamin K which is required for the activation of clotting factors 5, 7, 9 and 10 is produced by the gut flora. At birth, the gut of the newborn is sterile, thereby causing a deficiency in vitamin K production. This prompted the WHO to recommend that every newborn should be given vitamin K at birth to cover for the period of apparent deficiency.
Case report
This case study, describes an infant who was not given the recommended prophylaxis and the infant presented with bleeding disorders in the newborn period. The symptoms were not typical of vitamin K dependent bleeding of the newborn.
Discussion
Vitamin k deficiency disease is most often a clinical diagnosis with support and confirmation by radiological and laboratory investigations respectively. Ultrasound may only be helpful in the diagnosis, if there is a high index of suspicion of this condition. Therefore vitamin k deficiency should always be considered when dealing with bleeding disorder in the newborn period.

Keywords: *Bleeding, vitamin K deficiency, newborn*

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Introduction

Vitamin K represents a group of lipophilic and hydrophobic vitamins which are relevant in the activation of clotting factors 5, 7, 9 and 10. Vitamin K is an essential cofactor for the γ-glutamyl carboxylase enzymatic activity that catalyses the γ-carboxylation of specific glutamic acid residues in a subclass of proteins.[1,2] These vitamin K-dependent proteins are known as Gla-proteins.[1-3] Newborn infants, apart from the risk of developing vitamin K deficiency because of sterility of their gut and as such no gut flora,[1-3] transplacental transfer of vitamin K is very limited during pregnancy and breast milk contains low quantity of vitamin K. These result in low vitamin K stores in the neonatal

liver and subsequently in the blood.[1-4] This makes the newborn vulnerable to bleeding unless exogenous vitamin K is given as prophylaxis immediately after birth.[5-7] Vitamin K deficiency bleeding although not a common cause of bleeding, may result in very serious bleeding disorder due to inadequate activity of vitamin K-dependent factors, this condition was formally called haemorrhagic disease of the newborn. Since this is not the only cause of bleeding in the newborn period,[4-7] it is preferable to mention the causes of this particular type of bleeding hence the vitamin K deficiency bleeding.[1-4] Common causes of bleeding in the newborn period include children with haemophilia, immune thrombocytopenic purpura, leukaemia amongst others.[5-7] Basically, vitamin K deficiency bleeding may present as early onset vitamin K deficiency bleeding, the classical vitamin K deficiency bleeding and late onset vitamin K deficiency bleeding.[1,2,5-7] While the early onset usually occurs during the first 24 hours after birth, the classical vitamin K deficiency bleeding starts after 24 hours and the late is usually 2 to 12 weeks of birth.[5-7] In terms of aetiology, the different types of vitamin K deficiency bleeding also varies. Usually, the early onset is mainly due to maternal drugs/medications especially anticonvulsants like carbamazepine and phenytoin.[5-7] The classical onset is mainly seen in healthy infant who have not received prophylaxis vitamin K at birth, and the late onset is usually seen in exclusively breastfed infants who did not get vitamin K prophylaxis at birth.[1-7]

Generally, the most common sites of bleeding are the umbilicus, mucus membrane, the gastrointestinal (GI) tract, circumcision and venipuncture sites. Hematomas frequently occur at the sites of trauma (i.e. large cephalohematomas, scalp bruising related to instrumentation used at delivery and rarely, intracranial haemorrhage).[8-10] Most of the affected newborn infants are healthy upon examination. Internal haemorrhage of organs other than the brain as a method of presentation is not common.[8-11]

Case Report

From information contained in the referral note, baby was an exclusively breastfed male neonate delivered in a government hospital in Edo State, Nigeria at 40 weeks gestation by Spontaneous Vertex Delivery to a 37-year old para $5+^{2}$ (five alive) mother. Birth weight was 3kg and had a good APGA score, there was no peripartum pyrexia and no prolonged rupture of membrane, no history of maternal illness during pregnancy and maternal HIV screening and hepatitis B surface antigen were negative.

The baby was apparently well from birth. Baby was put to breast immediately after delivery and sucked well. There was no fever, there was no sign of illness in the baby. Baby passed meconium on the second day of life. The meconium was described as greenish and not fowl smelling and volume was adequate, bowel motion remain normal afterwards. On that same day, baby was noticed to become markedly pale and weak, this prompted a packed cell volume (PCV) check which was 9%. Baby also noticed to have marked abdominal distension despite the fact that the baby had passed meconium, no vomiting, no fever and was sucking well from the breast, bowel sounds were heard and were normal, no skin discolouration in the baby.

There was no history of bleeding from the

umbilical cord or any other orifice, jaundice or maternal drug use during pregnancy. Other male siblings are alive and well, maternal uncles were also alive and well, no history of bleeding in any of them. The cord was treated with methylated spirit, no history of native medication for dressing cord, no history or record of vitamin K prophylaxis.

Following the PCV of 9, it was documented in the referral note that the patient was transfused with whole blood in two aliquots, this was said to have raised the PCV to 51%. The abdominal distension was considered to be due to necrotising enterocolitis by the clinicians at the referring centre and abdominal ultrasound scan and x-ray were ordered and patient was also commenced on IV Ceftriaxone, IV Gentamicin and IV Metronidazole.

The ultrasound reported peritoneal thickening with generalised ascites and was suggestive of a mitotic right kidney upper lobe mass with right adrenal gland and right liver lobe involvement. Following this report, the patient was referred from the initial hospital to a paediatric surgeon in the teaching hospital. However, considering the fact that the said private clinic had offered surgical services to the family of the index patient in the past, the parents decided to take the neonate there, for possible surgical intervention.

At presentation in the private health facility on the fourth day of life, patient was active, sucking well from the breast, not acutely ill-looking and no fever (36.8^0C). Patient was pink in room air, not jaundiced, there was no bleeding from the cord, which was clean and not fowl smelling, there was no inflammation around the cord, there were no rash on the skin and no bleeding under the skin. Occipitofrontal circumference (OFC) was 34cm, weight 3kg and length 50cm. Anterior fontanel was normotensive, tone and deep tendon reflexes were normal, the heart rate at this point was 158/min, was regular, heart sounds were 1 & 2 only, blood pressure 80/45 mmHg and respiratory rate was 38/min, was regular and patient was not dyspneic. Abdomen was distended, cord was clean and dry no bleeding, abdominal girth was 42cm, 5cm above the umbilicus, fluid thrill was positive, organs were not palpable, a tap of the abdomen yielded frank blood. Bowel sounds were heard and normoactive.

Samples were immediately sent for prothrombin time (PT) and activated partial thromboplastin time (APTT), urine microscopy, stool for occult blood, blood culture, full blood count including PCV, other invasive procedures including lumbar puncture were suspended. However, before the results were gotten, considering the fact that the patient never had fever or subnormal temperature, constipation or a history of vomiting, septicaemia and enterocolitis were low on the list and also the researcher did not think the baby had a malignancy since the baby was not ill-looking. Moreso, considering the history that the baby never had vitamin K prophylaxis at birth, vitamin K deficiency bleeding was considered and the baby was immediately commenced vitamin K at 1mg/kg, that is, 3mg of vitamin K (IV daily). The antibiotics commenced at the referring hospital was continued. Vital signs were closely monitored. PT = 14 seconds (upper limit of normal) and APTT = 48 seconds (prolonged) normal range being 15 – 45 seconds. WBC count 6000/cm^3 (fourth day), neutrophils 49%, lymphocytes 51%,

eosinophils, 0%, monocytes 0%, basophils 0%. Nomocytic, nomochronic, platelet count 252,000. No occult blood seen in stool, urinalysis and urine microscopy normal, done on the third day. Vitamin K assay could not be done due to lack of facilities and secondly, because of recent transfusion. Blood culture was however sterile and the abdominal girth was monitored by doing daily measurements. On the 6th day of treatment in the private health centre (which is the tenth day of life), patient was still active and pink in room air, there was no fever and all findings on physical examination were normal. PCV had remained 37% and abdominal girth had gone down to 35cm. A repeat ultrasound reported that the ascites had disappeared although there was thickening of the peritoneal wall. It also reported right upper pole kidney mass with contiguous peritoneal thickening and which could be mitotic or dysplastic. At this stage, there were no liver or adrenal abnormalities as reported in previous ultrasound report.

The antibiotics were discontinued on the tenth day of admission, PT = 10 seconds (normal) and APTT = 15 seconds (normal). All the haematological, biochemical and clinical parameters were all normal, weight at discharge was 3.5kg, baby was active and sucking well, OFC was 34cm, length 50cm, abdominal girt had come down to 30, pulse ranged between 135 – 155Bpm throughout admission and respiratory rate 38 – 45cpm throughout admission. There was no labile temperature and no sub-normal temperature, temperature range was 36.8 – 37^0C, blood pressure was normal throughout admission ranging from 80/45 to 85/55 mmHg, except for the ultrasound report, the baby was then discharged home. Parents were to report at the hospital if they notice any problem, but if not, patient is to visit the hospital every week for follow-up, patient was on follow-up for 6 months when a third ultrasound was done and reported a normal general abdominal scan without adrenal or renal mass lesion nor collection. Baby was well grown for age, now weighing 7kg at 6 months, there is no complaint at all since the baby had been on follow up for 6 months.

A detailed report of the serial ultrasound scan done reflects thus:

There is a fairly well defined solid non-homogeneous mass involving the right adrenal fossa and upper pole of the right kidney measuring in part 4.3cm x 3.3cm from which the right liver lobe cannot be separated. We note contagious right liver lobe nodular areas, but there are no signs of liver abscess or biliary dilatation. There are no signs of bowel masses or mesenteric masses.

There was severe generalised ascites. The gall bladder, common bile duct, pancreas, left kidney and spleen are of normal configuration. There are no signs of stones in the gall bladder or in the kidneys and neither were there signs of obstructive uropathy. The urinary bladder is normal.

Sonogram signs suggestive of mitotic right kidney upper pole mass with right adrenal gland and right liver lobe involvement.

Ultrasound result done on the tenth day of life shows that the liver, gall bladder, common bile duct, pancreas, left kidney and spleen are of normal configuration.

There are no demonstrable hepato-biliary mass lesions or obstructions.

There is a hypo-echogenic now well defined

solid mass lesion in the upper pole of the right kidney with right adrenal fossa involvement separate from the right liver lobe measuring 4.3cm x 3.9cm. There is no sign of calcification within this mass lesion.

There are no signs of stones in the gall bladder or in the kidneys or obstructive uropathy.

There are no signs of ascites or sub-phrenic collection, but we note mild peritoneal thickening in the right hepato-renal fossa. There are no signs of bowel mass.

The urinary bladder and other pelvic organs are normal

Right upper pole kidney mass with contiguous peritoneal thickness and which could be mitotic or dysplastic.

On six months follow up, a comparison was made with the previous sonogram of this patient.

There are no signs to suggest an adrenal area or upper renal mass/collection.

The liver, gall bladder, common bile duct, pancreas, both kidneys and spleen are of normal configuration. There are no demonstrable hepato-biliary mass lesions or obstructions.

There are also no signs of stones in the gall bladder or in the kidneys nor obstructive uropathy.

There are no signs of ascites or sub-phrenic collections.

There are no signs of bowel mass or mesenteric mass nor peritonitis.

The urinary bladder is normal.

Normal general abdominal scan without adrenal or renal mass lesion nor collection. It is likely that the previously noted lesion was inflammatory.

Discussion

Vitamin K deficiency bleeding is a disease of the newborn that is caused by deficiency of vitamin K in an individual hence the need for prophylactic vitamin K. In the case of the above presented patient, vitamin K was not given to the baby, which may be due to lack of awareness of such policy or nonavailability of vitamin K. The presentation above is a very unusual and bizarre pattern. The patient only showed anaemia, there was no external blood loss, there was no GIT bleeding, there was no bleeding from the cord. The referral centre never suspected vitamin K deficiency bleeding because of this unusual manner of presentation, they however attributed the abdominal distension to nechrotizing enterocolitis despite the fact that fluid thrill was positive due to massive ascites. Although the ascites prompted the doctors in the referring centre to order a scan, which contributed to derailing the line of thinking of the doctors. The ultrasound scan report as shown above, done on the third day of life clearly concluded that the patient had a possible mitotic renal mass that may require surgical intervention. This was sustained in the second scan report (done on the tenth day of life). Although, some of those mitotic growth as claimed by the ultrasound report had started disappearing on the tenth day of life. It was strongly believed that these so-called mitotic growths were haematomas in the various organs and the massive ascites most likely a haemoperitonium (positive bloody tap). This pattern of presentation despite wide literature search is uncommon.[5-7,12-15] Interestingly, despite the widespread

internal haemorrhage, the patient never exhibited any sign of intracranial haemorrhage. At discharge, patient was quite active and sucking well, no adverse effect had been documented, no neurological manifestations.[7,8,12-15] PCV remain stable at 37% and at that point, patient obviously had stopped bleeding and there was no obvious residual damage to the kidney or the adrenals as this patient never experienced any blood pressure abnormality or urinary abnormality. By the sixth month on follow up, the parents had not reported any adverse effect in the patient, and patient has growing well. There was also no evidence of any organ dysfunction. The only intervention medically that was done in the private health facility following the diagnosis or suspicion of vitamin K deficiency bleeding was the administration of vitamin K1, this also reverted the haematological parameters (PT and APTT) which were initially prolonged (despite the transfusion) to become normal. Despite the inability of the medical team in the private health facility to exhaustively investigate the patient by way of vitamin K assay, MRI and CT scan, it could be inferred from the response to vitamin K that this was most likely a case of vitamin K deficiency bleeding. Inflammatory cause may not have been likely since the patient never had fever and was never acutely ill-looking. This unusual and bizarre pattern of presentation is important and worth reporting so as to increase our index of suspicion and the need for prophylactic vitamin K administration in the newborn infant and also the need for maternal supplementation.[16-19]

Conclusion

This case report is important to care givers especially neonatologist. It shows that except we have a high index of suspicion, cases of vitamin K deficiency disease may be overlooked, misdiagnosed and mismanaged. It is also important to emphasise the need for prophylactic vitamin K administration in every newborn in our environment ,since a single dose of vitamin K may be all that is needed to save the baby from the surgeon's knife with all its attendant risks and complication .

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Erratum

The name of Dr. Otene was wrongly spelt as Oteni on page 56 of the African Journal of Tropical Medicine and Biomedical research Vol. 1 No. 4 September 2012 issue. So it should be Otene CI.