pISSN:2384-5805
eISSN:2536-6645

WORLD JOURNAL OF BIOMEDICAL RESEARCH

April 2017 **Vol. 4 No. 1**

An Official Journal of Faculty of Clinical Sciences, College of Health Sciences, University of Uyo, Uyo - Nigeria

pISSN:2384-5805
eISSN:2536-6645

WJBMR Journal Secretariat:
Faculty of Clinical Sciences, College of Health Sciences
University of Uyo, Uyo - Nigeria

E-Mail Address: wjbmr2014@gmail.com
Website: www.wjbmr.org

pISSN:2384-5805
eISSN:2536-6645

Subscription Information:
World Journal of Biomedical Research is published bi-annualy by the Faculty of Clinical Sciences, University of Uyo, Nigeria. The 2017 subscription price is N1000/copy, N2000/ issue (Nigerian Institutions), rest of Africa US $50/ issue, the rest of the world US$100/issue including postage free. Journals are dispatched within Nigeria by surface mail and to the rest of Africa and other continents by various forms of air speeded delivery.

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pISSN:2384-5805
eISSN:2536-6645

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Physical Signs and Disease Control among Asthma Patients Attending a Respiratory Diseases Clinic in Nigeria

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ABSTRACT

The clinical manifestation of asthma results from variable abnormalities of airway function. The control of asthma is usually assessed subjectively by the presenting symptoms and objectively by the assessment of lung function. In the absence of objective assessment of lung function, the presence of physical signs of air flow limitation may be useful in evaluating disease severity. Asthma patients referred to a respiratory diseases clinic were recruited for this study. The asthma control test (ACT) was administered on the patients and physical examination was carried out by senior respiratory physicians. Seventy patients were recruited for this study. The average age of the patients was 46 ± 18 years. The average ACT score was 14.4 ± 4.8. Fifty eight (82.9%) of patients had poorly controlled asthma. Fourteen of the patients had tachypnoea, 6 had barrel shaped chest wall, 20 patients had expiratory wheeze on auscultation of the chest, 18 had fine crepitations on chest auscultation while two patients had hyper-resonant percussion notes. Twelve patients presented with one physical sign while six patients presented with two physical signs. Three physical signs were found in another 12 patients. In all 30 patients presented with any physical sign consistent with asthma. Multiple physical signs had a significant relationship with low ACT score after controlling for other variables. The presence of abnormal physical signs of asthma may be an indicator of severe asthma and the presence of multiple physical signs has a good correlation with ACT score.

Keywords: Bronchial asthma, pathophysiology, disease control, complications, physical signs

INTRODUCTION

Asthma is a chronic inflammatory disease of the airway[1]. The clinical manifestations of this condition result from abnormalities of airway function characterised by long and short-term variations in airflow resistance in the intrapulmonary airways[2]. The extent to which asthma is controlled is measured by the presenting clinical features in each patient which is a function of the extent of intrapulmonary airflow limitation[3]. There are several tools for measuring asthma control such as the asthma control test (ACT)[4] and the asthma control questionnaire (ACQ)[5]. These tools generally rely on patient reported symptoms and sometimes the lung function for assessing control.

Physical signs are generally not included in these tools for measuring asthma control. These signs are non-specific and may occur in respiratory or non-respiratory conditions associated with or without airway obstruction[6].These signs are easy to elicit by most physicians and in a resource limited environment where lung function tests are not readily available, their detection may indicate loss of asthma control and severe disease[7].

Based on evidence from previous studies[8,9] and anecdotal observations of asthma patients referred to our clinics it appears that there is generally poor control among asthma patients. This study was carried out to determine the profile of physical signs and its implication to control among asthma patients.

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METHODOLOGY

Setting

This study was conducted in the respiratory diseases clinic of UUTH, a public hospital located in the capital of Akwa Ibom state, South-Eastern Nigeria. This hospital provides secondary and tertiary health care services for the people of Akwa-Ibom and the neighbouring states. The respiratory diseases clinic receives referrals from the Hospital's general outpatient clinics, the general medical out-patient clinic and from other primary and secondary health care facilities within and around the state.

The survey was conducted over a 12 month period (June 2011-May 2012). During this period 511 patients were referred to the respiratory diseases clinic of which 102 (5.0%)

had asthma . All consecutively newly referred asthma patients who consented to participate were recruited for this study. Patients were subjected to a physical examination after administration of a questionnaire by the investigators.

Questionnaire

The questionnaire consisted of two parts; the first part documented information relating to patients demography. The second part was made up of the asthma control test (ACT). This is a five question test. Each question has five options with scores from one to five. The total score ranges between 5 and 25 with a higher score indicating better control. Good asthma control is determined by a score of ≥ 20. The ACT assesses asthma control over the previous four weeks. Translations of ACT into local languages have been verified for assessment of asthma control in previous studies[10,11]. The questionnaire was translated into the local language (Ibibio) and back translated for consistency. The local language version was administered on patients who had a poor understanding of the English version.

Physical examination

Two senior physicians in the respiratory diseases unit were enlisted to perform physical examinations. They examined all the patients recruited for this study independently one after the other and recorded their findings in a prepared proforma.

All patients were initially examined while reclining on a couch with the back rest raised to 45^0 for evidence of cardiac failure which was an exclusion criteria for this study. In the absence of clinical evidence for heart failure a respiratory examination was conducted in the supine position. The patients were examined for the presence of the following physical signs

1. Tachypnoea; respiratory rate above 25 cycles per minute,
2. Hyperinflation of the lungs;
   a. Increased antero-posterior (AP) diameter (AP diameter devided by transverse diameter greater than 0.5 as reported by Sharp *et al.*[12]
   b. Increased resonance on chest percussion,
3. Wheezing on chest auscultation
4. Crepitation on chest auscultation.

Each examiner recorded the presence or absence of each physical sign. Where there were discrepancies in their findings the observers re-examined the patient together and arrived at a consensus. Anthropometric parameters of height and weight were also measured. Height was measured using a stadiometer (SECA Gmbh and co. Germany ser no. 570005609867). Each participant stood up straight against the backboard of the stadiometer barefoot, with the heels together and wearing light clothing. The back of the head, shoulder blades, buttocks and the heel made contact with the backboard. The stadiometer head piece was lowered to firmly rest on top of the participant's head with pressure enough to compress the hair. The height was then recorded to the nearest 0.1 metre and the weight was recorded in kilograms (kg) to the nearest 0.5kg. The Body mass index (BMI) was calculated by dividing the weight in kg by the square of the height in metres (in kg/m^2).

The presence of a physical sign and the total number of signs were recorded for each patient. A total ACT score was calculated for all the patients. The patients were divided into 2 categories based on the ACT score: good control of asthma i.e. ACT score 20-25, and poor control of asthma ACT score ≤ 19[4]. Good asthma control was further categorized into well controlled asthma; ACT score 20-24 and totally controlled asthma; ACT score of 25

Statistical analysis

Data was analysed using SPSS statistical software version 18. Categorical variables were presented on frequency tables and the relationship between the categorical variables and the presence of physical signs was examined using cross-tabulation. The relationship between the presence each physical signs and total ACT score was determined using the Student's t-test for independent samples while a one way analysis of variance test (ANOVA) test was used to determine the relationship between groups of physical signs and total ACT scores. A regression model was constructed to evaluate the relationship between physical signs and total ACT score after controlling for other variables. Significant association between variables was set at a p value of 0.05.

RESULTS

Characteristics of respondents Demographics

Seventy patients with asthma were recruited for the survey. There were 38 (54.3%) females and 32 (45.7%) males. The age of the respondents ranged between 20 and 87 years with an average of 46 ± 18 years(mean±SD). The males were slightly older than the females (50.3±19.0 vs. 43.7±16.9 years) but this was not significant. About half (51.4%) of the patients were above 45 years in age. A majority of the respondents (48.6%) had tertiary education and most of them (68.6%) were public servants.

Risk factors for poor control

There was a high frequency of emergency room visits with 38(54.3%) of the respondents making at least one emergency room visit within the last 12 months. A majority of the respondents 46(65.7%) reported persistent exposure to possible allergens and pollutants with dust and kerosene fumes being the commonest pollutants. Twenty six (37.1%) of the respondents used their rescue medications frequently. Sixteen patients had normal BMI with another 16 being obese (Table 1).

Physical signs

Table 1: Characteristics of asthma patients

Parameter	N = 70 (%)
20-30 years	14(20.0)
31-40 years	18(25.7)
41-50 years	14(20.0)
51-60 years	6(8.6)
>60 years	18(25.7)
Female	38(54.3)
Male	32(45.7)
No education	4(5.7)
Primary	12(17.1)
Secondary	20(28.6)
Tertiary	34(48.6)
Unemployed	8(11.4)
Unskilled worker	4(5.7)
Semi-skilled worker	6(8.6)
Public servant	48(68.6)
Business man/woman	4(5.7)
Normal BMI	16(22.9)
Overweight	26(37.1)
Mild obesity	12(17.1)
Moderate obesity	4(5.7)
Emergency room visit	38(54.3)
Allergen exposure	46(65.7)
Cigarette smoke	2(2.9)
Kerosene fumes	34(48.6)
Firewood smoke	4(5.7)
Dust	36(51.4)
Frequent use of reliever medications	26(37.1)

Fourteen of the patients had tachypnoea, eight patients showed evidence of hyper-inflation of the lungs, 20 patients had expiratory wheeze on auscultation of the chest, 18 had fine crepitation on chest auscultation. Twelve (12) patients presented with one physical sign while six patients presented with two physical signs and another 12 patients with three physical signs. In all 30 patients presented with any physical sign consistent with asthma (Table 2).

Table 2: Frequency of clinical sings among

asthma patients

Asthma control

Clinical sign	N = 70 (%)
Tachypnoea	14(20.0)
Barrel shaped chest	6(8.6)
Hyper-resonant percussion notes	2(2.9)
Wheeze	20(28.6)
Crepitations	18(25.7)
Frequency of clinical signs	
1	12(17.1)
2	6(8.6)
3	12(17.1)

The total ACT scores ranged from six to twenty five with an average of 14.4 ± 4.8 (mean ± SD). Fifty eight 82.9% of patients had poor control, 14.3% were well controlled and only 2.9% had total control of their asthma symptoms (Table 3). Thus 17.2% of patients had good control of their asthma (total score ≥20).

Table 3: Control of asthma symptoms among asthma patients using the ACT

Distribution of physical signs

Level of control	N = 70 (%)
Not controlled	58(82.9)
Well controlled	10(14.3)
Total control	2(2.9)

A cross-tabulation table was constructed to examine the association between some patient parameters and physical signs of asthma using the Chi-square test. Poor asthma control, frequent rescue medication use and prior unscheduled emergency room visit in the previous 12 months were significantly associated with the presence of physical signs of asthma ($p<0.05$). There was no significant association between gender, education, continuing allergen exposure and obesity with the presence of physical signs. In predicting poor asthma control, the presence of any physical sign had a sensitivity of 51.7% and a specificity of 100%. This indicates that all the patients with any physical sign of asthma have poorly controlled asthma while the absence of a physical sign would indicate good control in only about half of the patients (Table 4).

Table 5 shows the relationship between age, asthma control and the nuber of physical signs consistent with asthma. There was a significant moderate negative correlation between age and ACT score ($r = -0.426$, $p < 0.001$) and a positive correlation with the number of physical signs ($r = 0.486$, $p < 0.001$). ACT score had a significant negative correlation with the number of physical signs ($r = 0.607$, $p < 0.001$).

Relationship between physical signs and total

Table 4:Cross-tabulation of clinical features and some variables

Parameter	*Any clinical sign* Absent N =40 (%)	Present N =30 (%)	X^2	P
Female	22(57.9)	16(42.1)	0.019	1.0
Male	18(56.2)	14(43.8)		
No education	2(50.0)	2(50.0)	0.439	0.933
Primary	6(50.0)	6(50.0)		
Secondary	12(60.0)	8(40.0)		
Tertiary	20(58.8)	14(41.2)		
Continuing allergen exposure	28(60.9)	18(39.1)	0.38	0.54
Obesity	30(53.6)	26(46.4)	0.82	0.37
Poor asthma control	28(48.3)	30(51.7)	8.8	0.003
Frequent medication use	10(38.5)	16(61.5)	4.74	0.029
Prior emergency room visit	8(21.1)	30(78.9)	41.0	<0.0001

Table 5: Physical signs and total ACT score

	Age	ACT score	Physical signs
Age	1	-0.426**	0.486**
ACT score		1	-0.607**
Physical signs			1

*Correlation at 0.05 level, ** Correlation at 0.001 level,

ACT score

The impact of the presence of any clinical sign of asthma on the ACT score was assessed using the independent t-test. Patients with physical signs consistent with asthma significantly scored lower than patients without signs apart from patients with hyper-resonant percussion notes. A one way between groups ANOVA was conducted to explore the impact of physical signs on the average ACT score as a measure of asthma control. Patients were divided into four groups depending on the number of physical signs they presented with. There was a statistically significant difference in ACT scores for the four groups: F (3, 70) = 20.2 p<0.001. The presence of physical signs had a large effect on the distribution of ACT scores (eta squared = 0.39). There was a progressive decline in ACT score with an increase in number of physical signs. Post-hoc comparison of the groups using Tukey HSD showed that there was a significant difference between patients with no clinical signs and patients with any clinical sign. There were significant differences in ACT scores between patients with one, two or three clinical signs (Table 5).

In Table 6 a linear regression model was used to evaluate the relationship between the number of physical signs and the ACT score after controlling for age, education emergency room visits and BMI. The model explained between 39% and 43% of the variance in asthma control; $R^2 = 0.433$, F (4, 65) = 9.7; P <0.0001. Age, BMI and physical signs made significant contributions to the model, with physical signs recording the highest beta value (beta = 0.427, P = 0.007).

Table 6: Average ACT score and clinical feature comparison

Clinical sign	Present *Mean (SD)*	Absent *Mean (SD)*	Student's t-test	P-value
Tachypnoea	9.43(2.7)	15.61(4.44)	-6.59	<0.0001
Barrel shaped chest	9.0(2.36)	14.88(4.69)	-3.01	0.004
Hyper-resonant percussion notes	8.0(0.0)	14.56(4.77)	-1.93	0.058
wheeze	11.3(2.97)	15.6(4.89)	-4.45	<0.0001
Crepitation	11.1(2.76)	15.5(4.89)	-4.66	<0.0001
Any clinical sign	11.1(2.8)	16.8(4.6)	-6.4	<0.0001
Frequency of signs				
0	16.8(4.6)		15.6	<0.001
1	12.7(2.1)*			
2	11.7(2.3)*			
3	9.3(2.8)*			

Table 7: Predictors of ACT score

	B	Beta	T	P	95%CI for B	
					Lower	Upper
Age	-0.07	-0.27	-2.1	0.03	-1.38	-0.007
Education	-0.42	-0.08	-0.68	0.5	-1.6	0.79
Emergency room visits	-0.59	-0.06	-0.44	0.66	-3.2	2.1
BMI	0.22	0.21	2.4	0.045	0.005	0.43
Clinical features	-1.8	-0.427	-2.7	0.007	-3.05	-0.51

DISCUSSION

This study has demonstrated that the control of asthma is sub-optimal among our patients and falls short of the guideline recommendations. Using the ACT Over 80% of our patients had poorly controlled asthma with only 2.9% achieving total control. Previous studies in Nigeria have made similar observations; Desalu *et al.*[8] in a survey of asthma patients in Ilorin, North-Central Nigeria observed poor control among 69% of the patients. Poor control of asthma has also been reported by investigators from other parts of the world; In Oman, Al-Busaidi *et al.*[13] observed poor control among 54% of asthma patients while Rabe *et al.*[9] in the Asthma Insights and Reality in Europe (AIRE) study observed that 46% of asthma patients reported daytime symptoms at least once a week. The frequency of poorly controlled asthma among patients in this study is higher than observations by other investigators despite the relatively high level of education and socioeconomic status of the patients. Logically one would have expected a much better level of control from this cohort considering that a higher socioeconomic status should lead to better understanding of the disease and adherence to therapy,[14] but local studies have shown no correlation between asthma control and socioeconomic status.[8] This trend may be due to the fact that asthma is associated with a higher socioeconomic status in developing countries in contrast to what obtains in the developed countries[15].

In this study 65% of the patients reported continuing exposure to indoor allergen and air pollution. This may partly explain the frequent rescue medication use and high rate of emergency room visits as well as the poor control of asthma. Studies have shown that continuing exposure to indoor allergen and pollutants is associated with poor asthma control and frequent exacerbation[16] and improvement in indoor air quality may lead to a reduction in asthma symptoms and health care utilisation[17].

Abnormal physiology of lung function which can be detected as physical signs during physical examination of asthma patients is largely confined to asthma exacerbation[18]. The physical signs of asthma are non-specific[6] and generally reflect a worsening of the condition[7]. In this study over 40% of our patients presented with physical signs associated with asthma which are indicative of airflow limitation. Godfrey *et al*.[19] in a study to determine physical signs of airway obstruction observed that 70% of patients with severe airway obstruction (FEV1<1L/s) had wheezing compared to 36% with less severe obstruction. McFadden I[7]. compared the degree of airways obstruction with the clinical manifestations of asthma in 22 patients with acute exacerbation of asthma. All the patients had physical signs of asthma. During a subsequent bronchodilator therapy, the absence of symptoms reported by the patients did not correlate with normal lung function. When 90% of the patients reported normalcy, 40% still had physical signs and their average FEV1 was less than 50% of predicted value and when there were no objective physical signs, the FEV1 was only 63% of predicted value

The high frequency of physical signs among our patients may suggest poor management and late presentation when they are almost overwhelmed by the condition. Patients generally tend to underestimate the severity of their asthma [20,21] and over-estimate their control[22]. The presence of physical signs of asthma in apparently stable asthma patients signifies poor control with reduced lung function. Observations over the last few decades suggest that active asthma has a negative impact on the longitudinal changes in lung function[23]. Some patients with asthma, especially those with more severe disease, are at risk of impaired growth of lung function, a lower maximally attained level of lung function and excessive decline in lung function in adulthood,[24] which may lead to life-threatening lung function impairment[25]. Clinical markers of poorly controlled airway inflammation appear to have a negative impact on the longitudinal changes in lung function[26]. Disease progression to non-reversible airflow obstruction may be observed in some patients with poorly controlled asthma[27]. All the patients with any physical sign had poor asthma control but the absence of physical signs was not necessarily associated with good asthma control due to low sensitivity of clinical signs.

In this study, older age was significantly associated with the presence of physical signs and loss of asthma control. Asthma is often considered a disease of younger people, but the high prevalence of asthma in the community indicates that many older people suffer from asthma with its associated impact on morbidity and mortality. Current statistics suggest that older people suffer disproportionately from the burden of asthma

with the majority of those dying from asthma being older patients[28]. Clinical evidence suggests that asthma in older age-groups has different characteristics from that in younger groups with a lower lung function and greater symptom severity[29]. In Australia out of 411 asthma related deaths in 2009 over 60% occurred in older patients[30]. There are several reasons for age related increased severity of asthma; older patients tend to have a poorer perception of asthma symptoms[31] and broncho constriction[32]. Even when they recognise the symptoms, they tend to associate it the normal consequence of aging[33]. Other markers of poorly controlled asthma such as frequent rescue medication use and prior unscheduled emergency room visit were significantly associated with physical signs of asthma in this study. Similar observations have been made other investigators. In a case-control study to investigate the association between frequent rescue medication use and adverse outcome among asthma patients by Spitzer *et al*.[34] the investigators observed that the frequent use of β2 agonist by asthma patients was associated with an increased risk of death or near-death (OR 6.1 95%CI 3.1-12.2). Similarly Turner *et al.*[35] in a study to evaluate risk factors for severe asthma observed that a prior admission in the emergency room was an independent predictor for near-fatal asthma (OR 19.3 95% CI 3.5-105.7).

The presence of multiple physical signs of airflow limitation should indicate a more severe disease. Using univariate analysis patients in this study with more than one physical sign had significantly lower ACT scores indicating more severe disease and the higher the number of physical signs, the lower the total ACT score recorded by the patient. Physical signs of asthma had an inverse relationship with the total ACT score and contributed the most to the variation in total ACT score after controlling for the influence of other variables (B-1.8, 95% CI -3.05 to -0.51, Beta 0.427). This is similar to observations made by Fisch *et al*.[36] in a study to determine the ability of some variables such as pulse rate ≥120 per minute, respiratory rate ≥30 per minute, pulsusparadoxus ≥18 mm Hg, peak expiratory flow rate ≤120 litres per minute, moderate to severe dyspnea, accessory-muscle use, and wheezing to predict severe asthma among 205 asthma patients presenting in the emergency room. In that study, the authors reported an inverse relationship between the number of physical signs and a favourable outcome.

CONCLUSION

Most of our asthma patients are poorly controlled and a lot of them present late in the outpatient clinics with severe disease and accompanying physical features. Physical signs of asthma are largely non-specific and are not very useful in making a diagnosis of asthma. However they form an important part of patient evaluation as their presence usually signifies a more severe disease with adverse outcome. We would recommend that to improve the level of asthma control among our patients there is a need to improve the knowledge and attitude of the managing physicians because poor asthma control is a reflection of poor management.

Patients access to health services should be improved as a lot of these patients with physical signs of asthma should ordinarily be seen in the emergency room and not in the outpatient clinic. This is a reflection of limited access to health care facilities for the patients as they tend to tolerate more severe illness without medical help[37].

Finally, we want to advocate an improvement in the quality of care provided for asthma patients in terms of education about the condition including the symptoms and signs of asthma, regular monitoring, use of appropriate medications and the establishment of functional patient physician partnership.

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Correlates of Anxiety and Depression among Caregivers of Cancer Patients at the Department of Radiotherapy, University College Hospital (UCH), Ibadan, Nigeria

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ABSTRACT

There is a near absence of studies on psychological morbidities among cancer informal caregivers in Nigeria. Consequently, this study was carried out to determine the prevalence of anxiety and depression (and the variables associated with them) among caregivers of cancer patients at the University College Hospital, Ibadan, Nigeria. The study also investigated out the socio-demographic and characteristics of the caregivers. One hundred and thirty five consecutive caregivers attending the Radiotherapy Clinic completed questionnaires after ethical approval was obtained from the Joint Ethical Review Committee of the University of Ibadan/University College Hospital, Ibadan. Data was collected using a questionnaire that consisted of socio-demographic and other variables of caregivers and the Hospital Anxiety and Depression Scale. The mean age of the participants was 45 years while the age range was 22-76 years. The prevalence rate of depression was 50.7% while that of anxiety was 40.0%. One hundred and seventeen (86.7%) of them had helper to support with care giving and almost all (94.1%) of them were concern about the cost of treatment. Ninety six (71.1%) enjoyed financial support from family members. More than three quarters of the participants reported their care receivers' functioning to be fair to very good. Age above 40 years, fear of having to carry on with care much longer and absence of feeling that care receiver is not getting proper treatment significantly differentiated caregivers who had depression and anxiety from those that did not have. The high prevalence rates of depression and anxiety may constitute a major setback for management of cancer patients as caregivers need to enjoy mental health to be able to play their roles.

Keywords: Anxiety, depression, caregivers, cancer, Nigeria

INTRODUCTION

Depression in informal caregivers of cancer patients may contribute more to the pain, emotional and behavioral disturbances of cancer patients compared to the physical impairment of the patients[1-4]. Some cancers show variation in prevalence according to geographical location and race. Cancer of the cervix is extremely seldom in Malaysia[5]. Stomach cancer has decreased incidence among the Japanese; but they experience increase incidence of breast, colon and prostate cancers. The highest incidence of colorectal cancer is found in the United States of America. Liver cancer is common in Sub Saharan Africa, Indonesia and the Philippines[6]. Meanwhile, pancreatic and testicular cancers are reported to be rare among the blacks[6].

Caregivers (also known as informal or family caregivers) carry out complex tasks for cancer patients that include physical, spiritual and psychological support[7]. They play significant role in the treatment of cancer patients and involving them in the management at the outset is critical. They are involve in the planning of treatment, making decisions and even in implementation[8]. These roles carried out by the caregivers are not without consequences on them. They experience powerful feelings[9,10]. While meeting the needs of the patients, they simultaneously try to meet the need of family and work. The imperative to arrange logistics such as transportation, booking of hospital appointments/visits and home care may be physically and psychologically unwieldy for caregivers of cancer patients.[11]The immediate post-hospitalisation may be a most traumatic period for caregivers. They are concerned about managing the patient at home[12], medication, food supply and managing adverse effects and emergencies[13,14]. The caregiver may have to forgo social activities and neglect work in a bid to attend to the patient.

It is significant to observe that the wellbeing of cancer patients and that of the caregivers is mutually related/linked.[15]This underscores the relevance of the health of cancer caregivers to that of the patients. Caregivers have

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been reported to be at increased risk of psychological morbidity owing to personal physical, social, psychological factors and factors associated with the cancer patients they are caring for[16,17]. Attitude of medical staff has also been reported as a factor that determines depression symptoms among caregivers[18].

Unfortunately, there is a near absence of studies on depression and anxiety among cancer caregiversin Nigeria. Consequently this study was carried out to determine the prevalence of anxiety and depression and the variables associated with them among caregivers of cancer patients attending the Department of Radiotherapy, University College Hospital, Ibadan, Nigeria. The study also sought to determine the characteristics of the caregivers.

MATERIALS AND METHODS

The study was carried out at the clinic of Department of Radiotherapy of University College Hospital (UCH), Ibadan, Nigeria. UCH is located in Ibadan North Local Government area of Oyo State. Oyo state is one of the 36 states in Nigeria.

The Caregivers of 135 consecutive cancer patients attending the Radiotherapy Clinic were recruited for the study after sample size was calculated using the formula, $N=Z^2pq/d^2$.[19] Nine point seven percent was used as the best estimate of prevalence of depression[20,21]. The inclusion criteria were that the caregiver must be 18 years or greater and there must be histological evidence of the cancer, while the exclusion criterion was refusal to give consent.

Data was collected from the participant susing a questionnaire that consisted of demographic and other variables of caregivers and the Hospital Anxiety and Depression Scale (HADS). The participants completed the questionnaires. The Hospital anxiety depression scale which was developed by Zigmond and Snaith in 1983, is used to screen for anxiety and depression[22]. It has 14 items, 7 items each for depression and anxiety. It is a likert scale and each item of the questionnaire is scored from 0 to 3. The cut off for caseness for anxiety and depression is 8; such that 0-7 is described as non-case, 8-10 borderline or possible case while greater than or equal to 11 is described as a case[23]. The instrument has been validated in Nigeria[24]. The data from the questionnaires was entered into the Statistical Package for Social Sciences (version 20) by the researchers. Descriptive inferential statistics was done and the level of significance was set at $p<0.05$.

Ethical approval was obtained from the Joint Ethical Review Committee of the University of Ibadan/University College Hospital, Ibadan. Confidentiality (participant "A" did not know or see the responses of participant "B") and anonymity (participants did not write their names on the questionnaire) were observed. The questionnaires were kept in a locked cupboard (so that only the researchers could have access) and the computer data file was pass-worded (the data entered from the questionnaires into the computer had a file created for it and the file was given a pass word so that only the researchers could have access). The study was non-invasive and those who refused to participate were not discriminated against. The participants were assured that they were free to withdraw their consent at any point and that the treatment of their loved one or patient would not be affected in any way.

RESULTS

The mean age of the participants was 45 years while the age range was 22-76 years. The prevalence rate of depression was 50.7% while that of possible depression was 28.1%. On the other hand the prevalence rates of anxiety and possible anxiety were 40.0% and 17.0% respectively. Other aspects of the results are shown in tables 1 through 3.

DISCUSSION

Table 1: Caregivers characteristics

Characteristics		Frequency	Percent
Age	18-25 years	5	3.7
	26-40 years	48	35.6
	41-60years	67	49.6
	Above 60 years	15	11.1
Sex	Male	50	37.0
	Female	85	63.0
Marital status	Single	31	23.0
	Married	92	68.1
	Divorced	7	5.2
	Separated	2	1.5
	Widowed	3	2.2
Level of education	No formal education	15	11.1
	Primary school	19	14.1
	Secondary education	22	16.3
	Tertiary education	64	47.4
	Post tertiary education	15	11.1
Religion	Christianity	106	78.5
	Islam	29	21.5
Ethnicity	Yoruba	78	57.8
	Igbo	43	31.9
	Hausa	3	2.2
	Others	11	8.1
Place of residence	Urban	105	77.8
	Rural	30	22.2
Relationship to patient	Parent	24	17.8
	Spouse	32	23.7
	Sibling	35	25.9
	Extended family member/friend	44	32.6
Availability of helper	No	18	13.3
	Yes	117	86.7
Concern about cost of treatment	No	8	5.9
	Yes	127	94.1
Financial support from family members	No	39	28.9
	Yes	96	71,1
Caregivers perception of patients functioning	Very poor	3	2.2
	Poor	20	14.8
	Fair	41	30.4
	Good	62	45.9
	Very good	9	6.7

Table 2: Association between participants' characteristics and anxiety

Characteristics ++	Absence of Anxiety no.(%)	Possible anxiety no.(%)	Anxiety no.(%)	p-value
Gender				
Male	24(48.0)	7(14.0)	19(38.0)	
Female	34(40.0)	16(18.8)	35(41.2)	0.61
Age in years				
18-25	2(40.0)	3(60.0)	0(0.0)	
26-40	24(50.0)	5(10.4)	19(39.6)	
Above 40	18(22.0)	16(19.5)	48(58.5)	0.04**
Fear of having to carry on with care much longer				
No	38(55.9)	9(13.2)	21(30.9)	0.01**
Yes	20(29.9)	14(20.9)	33(49.2)	
Fear of having to deal with uncooperative medical staff				
No	33(37.9)	16(18.4)	38(43.7)	0.28
Yes	25(52.1)	7(14.6)	16(33.3)	
Feeling that patient is not getting proper treatment				
No	14(28.5)	9(18.4)	26(53.1)	0.02**
Yes	44(51.2)	14(16.3)	28(32.5)	
Lack of understanding of the disease				
No	26(44.8)	11(19.0)	21(36.2)	0.75
Yes	32(41.6)	12(15.6)	33(42.8)	

** = statistically significant

Table 3: Association between participants' characteristics and depression

Characteristics	Absence of depression no. (%)	Possible depression no. (%)	Depression no.(%)	p-value
Gender				
Male	16(32.0)	10(20.0)	24(48.0)	
Female	21(24.7)	28(32.9)	36(42.4)	0.25
Age in years				
18-25	4(80.0)	0(0.0)	1(20.0)	
26-40	15(31.2)	12(25.0)	21(43.8)	
Above 40	10(12.2)	26(31.7)	46(56.1)	0.03**
Fear of having to carry on with care much longer				
No	27(39.7)	22(32.4)	19(27.9)	0.00**
Yes	10(14.9)	16(23.9)	41(61.2)	
Fear of having to deal with uncooperative medical staff				
No	23(26.5)	27(31.0)	37(42.5)	0.60
Yes	14(29.2)	11(22.9)	23(47.9)	
Feeling that patient is not getting proper treatment				
No	9(18.4)	10(20.4)	30(61.2)	0.01**
Yes	28(32.6)	28(32.6)	30(34.8)	
Lack of understanding of the disease				
No	19(32.8)	15(25.9)	24(41.3)	0.48
Yes	18(23.4)	23(29.9)	36(46.7)	

** = statistically significant

The examination of the socio-demographic characteristics of the participants shows that the ages of the caregivers appear to cut across a wide range. It appears that practically any age group could be a cancer caregiver. This finding is not unique to the study[25,26]. However, the age range of caregivers for children with cancer has been reported to be lower than that of those who care for adult patients[27]. This may not come as a surprise. Caregivers may usually be parents and siblings. As such if a child has cancer the caregivers may be expected to be younger than when an adult has cancer. Majority of the caregivers were 41-60 years old[28,29]. This is within the productive age and it may have a serious economic implication for the caregiver, patient and the society in terms of loss of productivity.

A higher proportion of the caregivers were females (63%). This is in tandem with what has been found by other researchers[30]. Akpan-Idiok and colleague reported that females made up 62.9% of caregivers[25]. Understandably, a study that examined gynaecological cancers reported more caregivers to be males[31]. Sixty eight point one percent of the caregivers were married[25,30].The implication of this is that the caregivers also had their family needs to meet. Majority attained tertiary education and majority resided in urban centres. The level of educational attainment may be on the rise in Nigeria. Every state has a tertiary education facility. Indeed some states even have plural of such institutions. Majority were Yoruba, this may mean they are likely to be mainly from the south-west, which appears to be a geopolitical zone with several tertiary institutions. The fact that majority of the participants resided in urban centres may imply that they may have relatively easy access to tertiary education.

Most of the caregivers were extended family members/friends of the patients or care receiver. This is at variance with what other researchers have found. Parents, siblings and spouses have been reported to constitute the majority of caregivers[25,31]. The difference may be because of the difference in the type of cancer patients the various caregiver catered for. The current study considered caregivers of all types of cancer patients as long as they were seen during the study period and they gave consent.

Virtually all the participants expressed concern about the cost of treatment. This may bea reflection of the economic reality of Nigeria. The cost of cancer treatment and indeed any chronic medical condition may be expected to be expensive. The National Health Insurance Scheme appears to be at its infancy, many services may not be covered and the informal sector has not been properly incorporated. Therefore, what obtains largely is out of pocket payment for services. However, it is gladdening to note that almost three quarters of the participants reported that family members were supporting financially. It would appear that despite the hard times, Nigerians may still care for one another. Second, almost 90% of the participants reported the availability of helpers. This no doubt will help to cushion the pressure on the caregivers.

It is interesting to note that more than 80% perceived the care receiver's functioning as fair to very good. This probably was not expected considering the chronicity and the general prognosis of cancer. Clipp and George who carried out their study at the Duke University medical Center, North Carolina, United States in 1992reported that almost all their participants perceived their care receiver functioning negatively[32]. The fact that the current study considered different categories of caregivers while the study just cited considered spouse caregivers may underlie this difference.

The prevalence rate of depression was 50.7% while that of anxiety was 40.0%. Ambigga and colleagues who did a study in Malaysia in 2005 reported prevalence rate of depression and anxiety among caregivers of cancer patients to be 29.4% and 48.6% respectively[33]. The prevalence rate of depression is very much comparable to the 50% reported by Carter and his colleagues[34]. These prevalence rates are quite high. Meanwhile, a meta-analysis carried out on 211 studies showed that the prevalence of depression among cancer patients ranged from 3% to 31%[35]. It has been reported that cancer caregivers were significantly more anxious and depressed than patients[36,37]. Despite this, caregiving may have rewards. Greater self esteem, personality growth, fulfilment and an opportunity to reciprocate care may be benefits of caregiving[38,39].

Participants' characteristics were compared with regard to anxiety and depression. Age, fear of having to carry on with care much longer and feeling that patient is not getting proper treatment significantly differentiated participants who had anxiety from those who did not have. Gender, fear of having to deal with uncooperative medical

staff and lack of understanding of the disease did not. Fifty eight point five percent of the caregivers who were above 40 years had anxiety, whereas none of the participants aged 18 to 25 years had anxiety. A higher proportion of those who had fear of having to carry on with care much longer had anxiety compared to participants who did not have such fear. A higher proportion of those who felt the care receiver were getting proper treatment had anxiety compared to those who felt the care receiver was not getting proper treatment. Those who felt the patient was getting proper treatment may be more distressed if the patient is not improving while those who feel the patient is not getting proper treatment may be optimistic that the patient would improve or do well once proper treatment commences. The implication is that, caregivers who are above 40 years and those likely to carry on treatment for much longer need to be sorted out by clinicians for psychological evaluation and support. There is need for clinicians to properly carry caregivers along in the course of treatment. Caregivers should be given opportunity to express their worries and questions.

Similarly, caregivers' characteristics were compared with regard to depression. Like it was found for anxiety, participants who were above 40 years, those who feared having to carry on with care much longer and those who felt the care receiver was getting proper treatment significantly had more depression. This is what may be expected since anxiety and depression are very much related constructs and anxiety may actually progress to depression.

Many researchers have compared the prevalence of depression and anxiety between cancer caregivers and non-caregivers. The finding is that caregivers suffer depression and anxiety significantly more than non-caregivers. The feelings they may have include fear and hopelessness.[40-42]

CONCLUSION

The prevalence rates of depression and anxiety among caregivers of cancer patients is alarmingly high. This will constitute a major setback for management of cancer patients as caregivers need to enjoy mental health to be able to play their roles; for a better outcome for the patients and the society. This study has brought to the fore the imperative to establish cancer caregivers support schemes at all levels, beginning from oncology units. There is need for liaison between Oncologist and Psychiatrists

The study is limited owing to the fact that it was a cross-sectional one and so cause and effect relationship could not be established. Past or present history of mental illness or significant life events was not ruled out in the caregivers. HADS is a screening instrument and thus may have over-diagnosed anxiety and depression.

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Oxidative Stress Indices among Patients with Sickle Cell Anaemia in Kano, Northwestern Nigeria

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ABSTRACT

Pro-oxidant-antioxidant imbalance mediates the oxidative damage of critical biomolecules such as lipids, proteins and nucleic acids in living organisms. The reactive oxygen species (ROS) produced in the cells has been implicated in the Sickle Cell Anaemia (SCA) complications. Improved antioxidant status minimizes oxidative damage and this may delay or prevent pathological changes. This study conducted a preliminary comparative analysis of enzymatic antioxidants, non-enzymatic antioxidants, essential trace metals and oxidative stress status in patients with SCA and healthy controls attending Aminu Kano Teaching Hospital, Kano, Northwestern Nigeria. Serum catalase (CAT), glutathione peroxidase (Gpx), malondialdehyde (MDA), copper (Cu), Iron (Fe) and vitamins A and C were determined in 40 patients with SCA and 20 healthy control subjects. The results showed that MDA level was statistically higher ($p<0.05$) in patients with SCA than in controls. Serum catalase activity in the control group was however, not statistically different ($p>0.05$) compared to patients with SCA. Serum Gpx activity was statistically lowerin patients with SCA than in control group. The serum levels of antioxidant vitamins (A and C), Cu, Zn and Fe were found significantly lower ($p<0.05$) in patients with SCA than in the control group. This study revealed that the decrease in both enzymatic and non-enzymatic antioxidant levels in patients with SCA might be due to the compensatory antioxidants measures against excessive free radicals generation in patients with SCA as indicated by the high levels of MDA. It might be due to this increase of oxidative stress that complications are observed in SCA. Since the risk of oxidative cell damage is increased in SCA, periodic antioxidant status assessment and therapy is justified to reduce the risk of long term complications that may arise due to pro-oxidant-antioxidant imbalance.

Keywords: Antioxidants vitamins, Sickle cell anaemia, Malondialdehyde, Oxidative Stress

INTRODUCTION

Oxidative stress (OS) is generally associated with molecular alterations in reactive oxygen species (ROS), reactive nitrogen species (RNS), and nitric oxide (NO) signalling pathways, thus, decreasing bioavailability of NO while ROS and RNS production is increased[1].In normal physiological conditions, there is an equilibrium between the ROS level and the defense systems of antioxidant enzymes and non-enzymatic antioxidants, which prevents or minimizes oxidative damage. Intracellular metabolic activities produce ROS even in healthy individuals[2].

Many antioxidant molecules have been found in blood and they are responsible of preventing and/or inhibiting harmful free radical reactions that is associated with lipid peroxidation[3]. Exogenous antioxidants (e.g. vitamins C, A and E) and endogenous antioxidants (albumin and uric acid) are the radicals scavenger antioxidants protecting the cell against the potentially harmful effects of oxidative agents[4]. The cell is naturally blessed with several antioxidant systems to counteract the extent of lipid peroxidation, but under certain conditions, especially diseases state, this protective mechanism can be overwhelmed, leading to increased tissue levels of peroxidation products[5].

Sickle Cell Disease (SCD) is classified as haemoglobinopathy, which results from a single point mutation in the β-globin chain of hemoglobin, inducing the substitution of a hydrophobic amino acid(valine) for hydrophilic one (glutamic acid) at position sixth of the chain[6].This change leads to the formation of abnormal haemoglobin (HbS) and eventually sickled RBCs. Apart from homozygous sickle cell disease(HbSS), other forms of abnormal haemoglobin such as HbSC and HbSb-thalassemia are also found[7].The sickle haemoglobin is known to interact with diverse genes and environmental factors, producing a multi systemic disease with several phenotypes[8,9].

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SCD is also associated with increased risk of developing stroke, frequent hospitalization of patients, pulmonary hypertension, acute chest syndrome, priapism, nephropathy, retinopathy, leg ulcers and avascular necrosis and eventually limiting life expectancy[10-15].

Several studies have demonstrated the role of ROS in the pathophysiology of SCD[15-20].The SCD results in an increased production of ROS from chronic inflammation, ischemia-reperfusion, hemolysis and auto-oxidation of sickle haemoglobin[21-23].Increased oxidative stress worsens SCD steady state by accelerating rate of haemolysis, depleting nitric oxide, endothelial cell activation, evoking inflammation and coagulation.

Increasing life expectancy of SCD patients through improved care is accompanied by increased chronic organ complications in which ROS are major key player. Therefore, measures that will reduce ROS, such as many potential antioxidants, are being explored as a possible therapeutic option[24]. N-acetylcysteine has shown some promise in this regard[25]. The main aim of this study was to determine the level/status of enzymatic, non-enzymatic antioxidants, oxidative stress markers and some essential trace metals in SCD patients.

MATERIALS AND METHODS

Study Site

Kano state is the most populous commercial state in Northwestern Nigeria with a population of 9,383,680 according to 2006 National Population Census. However, currently it is estimated that Kano state has a population of about 14,000,000 people. The state lies between latitude 13^0N in the North and 11^0N in the South and longitude 8^0W in the West and 10^0E in the East. It is the centre of North-west geopolitical zone. The North-west composed of Hausa/Fulani tribe and majority depend largely on subsistence farming, commercial activities and domestic animal rearing. The participants in this study were recruited from Aminu Kano Teaching Hospital (AKTH). The facility is situated at the heart of the densely populated areas of Kano state, serving majorly as referral centre for the Primary and Secondary Healthcare facilities in the entire Northwestern part of the country. Patients from Jigawa, Katsina, Kaduna, Sokoto, Kebbi, and Zamfara States, apart from the host state, patronize the hospital on daily basis.

Research Design and Study Subjects

This was a case control study comprising subjects grouped into two: Group I: Forty (40) known sickle cell aneamia patients (SS-genotype) in steady state attending Haematology Clinic of Aminu Kano Teaching Hospital (AKTH), Kano, North-western, Nigeria. Group II (controls): Twenty (20) apparently healthy persons(AA-genotype) presenting to blood donor Clinic of AKTH for blood donation.

Sampling Method

Systematic sampling was used to recruit SCA patients from the list of those scheduled for routine follow-up while controls were recruited consecutively until the desired sample size was obtained.

Inclusion Criteria

Subjects confirmed HbSS (SS-genotype) positive (male and female) and scheduled for routine follow-up were included in this study while the healthy controls also (male and female) were recruited from Blood Donor clinics and confirmed HbAA (AA-genotype).

Exclusion Criteria

Individuals with AS-genotype, thalassemia, hypertensive, Alcoholic, smokers Pregnant and diabetes and those who refused to give their consent to participate in the study were excluded.

Sample Collection and Preparation

After obtaining a written consent, a total of 5ml f blood was withdrawn aseptically from the anti cubital veins from each patient. In preparation of erythrocyte hemolysate, 2ml of blood was used, which was subsequently used for the determination of enzyme activity (Catalase, Glutathione peroxidase) and malondialdehyde while $3cm^3$ was centrifuged for 10minutes at 3000rpm and the serum was subsequently used for redox metals (Cu, Fe, Zn) and vitamins A and C analyses.

Ethical Consideration

Approval of the study protocol was obtained from the Ethical Research Committee (ERC) of Aminu Kano Teaching Hospital Kano-Nigeria. Informed written consent from all participants in the study after a verbal explanation to each participant was obtained.

Socio-demographic data of all the participants were obtained through semi-structured questionnaire. All data collected from the participants were kept confidential. The provision of the HELSINKI declaration was respected at every step of this study.

Laboratory Methods

Vitamins A and C were determined by the spectrophotometric method as described by Rutkowaski and Grzeggorezyk[26] and Keitaro *et al.*,[27] respectively. Atomic Absorption spectrophotometry was used to measure the serum levels of redox active metals: copper, iron and zinc. Malondialdehyde was determined by a modification of the thiobarbituric method described by Ohkawa *et al.*[28] Gluthathione peroxidase (GPx) was determined by inverse photometry[29] while catalase enzyme activity was determined using the peroxide coupled oxidation method as described by Van lente and Pepoy[30].

Statistical Analysis

Data generated were analysed using GraphPadInStat® statistical software (2000), version 3.05 from GraphPad Incorporated and limit of significance of $P<0.05$ was considered.

RESULTS

Serum enzymatic activities of catalase, glutathione peroxidase **(**Gpx) and level of malondialdehyde (MDA), Essential trace metals and antioxidant vitamins (A and C) in patients with SCA and healthy control were investigated using standard procedures. The results are presented in Tables 1, 2 and 3, respectively.

Table 1: Serum Enzymatic Antioxidant Activities and Malondialdehyde Level in Patients with SCA and Healthy controls

Group	Catalase (µmol/L)	GPx (EU/ml)	MDA (µmol/L)
Control (n=20)	3.50 ±2.75	260.60 ±39.18[a]	0.85±0.57[b]
SCA(n=40)	2.77 ±2.34	134.15±25.98[a]	1.38±0.56[b]

Results are presented as mean ± SD, values on the column having the same superscript are significantly different($p<0.05$).

Enzymatic antioxidants in patients with SCA were determined. Serum catalase activity was 2.77±2.34 µmol/L which is lower than the control subjects (3.50±2.75 µmol/L). However, the result was not statistically significant ($p>0.05$). Glutathione peroxidase activity was found statistically ($p<0.05$) lower (134.15±25.98 EU/ml) in patients with SCA than the control subjects (260.60±39.18 EU/ml). Malondialdehyde (MDA), a marker of oxidative stress was also found to be significantly higher ($p<0.05$) in patients with SCA (1.38±0.56 µmol/L) compared to control subjects (0.85±0.57 µmol/L).

Table2: Serum Levels of Some Essential trace Metals in Patients with SCA and Healthy Controls

Group	Iron (Fe)(µg/dl)	Copper (Cu)(µg/dl)	Zinc(Zn)(µg/dl)
Control (n=20)	156.64 ± 22.99[a]	136.5 ± 0.60[b]	127.85 ±14.25[c]
SCA(n=40)	96.00 ± 11.29[a]	103.5 ± 7.35[b]	120.85 ±10.29[c]

Results are presented as mean ± SD, values in the same column bearing the same superscript are significantly different($p<0.05$).

The levels of serum essential trace elements, iron (Fe, 96.00±11.29 µg/dl), Copper (Cu, 103.50±7.35 µg/dl) and Zinc(Zn, 120.85±10.29 µg/dl) were all lower in patients with SCA compared to control subjects. The differences were statistically significance at $p<0.05$ (Table 2).

Table 3: Levels of Serum Vitamin A and C of Patients with SCA and Healthy Control

Group	Vitamin A (µmol/l)	Vitamin C (mg/dl)
Control (n=20)	96.22 ± 11.02[a]	0.74 ± 0.85[b]
SCA (n=40)	87.23 ± 11.60[a]	0.30 ± 0.20[b]

Results are presented as mean ± SD, values in the same column bearing the same superscript are significantly different ($p<0.05$).

Serum level of antioxidant vitamin A in patients with SCA was lower (87.23±11.60 µmol/l) than the control subjects (96.22±11.02 µmol/l). Vitamin C level was found to be higher in control subjects(0.74±.85mg/dl) compared to patients with SCA (0.30±0.20mg/dl). The levels of antioxidant vitamins in patients with SCA were significantly different compared to control subjects ($p<0.05$).

DISCUSSION

The main function of RBC as gas transporters renders them specifically vulnerable to oxidation by Oxygen molecule and free radicals such as OH radical. Several oxidants or ROS [superoxide ($O_2^{\cdot-}$) radicals, Hydrogen peroxide (H_2O_2), peroxyl radicals (ROO·-) and Hydroxy radical (OH-)] are produced during metabolic activities in both RBCs and most other cells of the body. These may disturb the prooxidant-antioxidant balance thereby causing oxidative damage. To defend against oxidative damage, the system is generously equipped with endogenous enzymatic and non-enzymaticantioxidant mechanisms[31].

Increased levels of malondialdehyde (MDA), a marker of oxidative damage *invivo* indicated increase lipid peroxidation which is a hallmark of increased free radical generation. In this study, there was increased MDA levels in patients with SCA compared to normal control ($p<0.05$). This finding is likely due excessive free radicals generated and also may be as a result of the effect of repeated transfusions these patients undergo as demonstrated in a previous study[32].Malondialdehyde (MDA) interacts with several nucleic acid bases to form several different adducts. This potentially genotoxic activity of MDA may lead to mutation and subsequently to cancer,[33] and may also triggers several complications associated with SCA. Indeed, the oxidative stress of SCA-RBC could also be attributable to the inherent instability of HbS[34] that the RBC of patients with SCA produces large quantities of free radicals than normal RBC.

In normal physiological system, antioxidant enzymes and free radical scavengers (such as vitamins) ensure that basal fluxes of ROS do not damage or injure the organism[35]. It has been reported by Rusonova *et al*.[36]that sickled RBCs are more prone to oxidative damage than normal RBCs and the treatments currently for SCA focus on applying chemistry of free radical to sickled cells[36] leading for the creation of the hypothesis that the complications and symptoms of SCA are caused by extensive free radical damage and oxidative stress. In turn, oxidative stress is associated with chronic RBCs hemolysis. Sickle RBCs have a high frequency of phosphatidyl serine exposure, which is because of oxidative stress, suggesting that oxidative stress might play a significant role in intravascular hemolysis. High Blood Pressure in patients with SCA was found to be associated to increased ROS, which in turn, deactivate endothelial nitric oxide synthase (eNOS) directly and thus, reducing NO levels, which is an important vasodilator,[37] and hence increase the risk of hypertension.

The increased generation of lipid peroxidation products induces compensatory changes expressed by enhanced utilization of antioxidants, decreased serum activity of antioxidant enzymes (GPx, and CAT) and non-enzymatic antioxidants (Vitamin A and C) in SCA patients as was observed in our study.

The serum catalase activity in patients with SCA was lower than the normal control subjects' though not statistically significant ($p>0.05$). This finding confirms the previous study of Aslan *et al.*[35]; Kling and Farber[18] that patients with SCA have lower CAT levels than in normal controls. This could be due to the increased production of lipid peroxidation products which induces compensatory changes expressed by enhanced utilization of non-enzymatic antioxidants and decrease serum activity of antioxidant enzymes (GPx, and CAT).

Catalase is involved in the antioxidant defenses against reactive oxygen species (ROS), by conversion of hydrogen peroxide (H_2O_2)to water (H_2O) and oxygen (O_2), in which the enzyme also functions as peroxidase enzymes[38]. Therefore, the reduced activity of CAT in patients with SCA suggests the accumulation of toxic H_2O_2in the SCA subjects. The H_2O_2 is regarded as a powerful reactive oxygen metabolite that can injure or damage body cells at a relatively low concentration. Direct damaging activities of H_2O_2 includehaem proteins degradation, release of iron, enzymes inactivation and peroxidation of lipid components and DNA oxidation[39].

Serum glutathione peroxidase (Gpx) activity in patients with SCA was statistically lower than the control group ($p<0.05$). This decrease in the activity of Gpx in SCA could be attributable to the strategic confinement of this enzyme intracellularly burdened with its responsibility of destroying intracellular peroxide rather than superoxide dismutase (SOD) or catalase. It was also reported by Aslan *et al.*[35] and Kling and Farber[18] that in SCA patients, Gpx and catalase levels are lower than in normal control subjects as subsequently confirmed by this study. This could results in abnormal hydrogen peroxide accumulation in the cell and causes increase in oxidative damage in sickle cells,[18,35] as indicated by the high level of MDA observed in this study, and thus, probably sickle cell complications. This enzyme is induced by oxidative stress and it plays important role in the peroxyl radicals scavenging mechanism and also in maintaining functional integrity of the cellular membranes[40].

The serum levels of the vitamins (A and C) were significantly lower in patients with SCA compared with normal controls ($p<0.05$). The reduced levels of antioxidant vitamins (A and C) observed may be related to increased oxidative stress in SCA patients, resulting in higher utilization of these vitamins and consequently leading to their decreased serum levels. Indeed, the synergistic antioxidant activity of vitamins C and E assist in sparing vitamin E, and vitamin C utilized during this process, thus, resulting in lower level of serum vitamin C [41]. This vitamin is generally considered the most powerful natural antioxidant[42]. Vitamins A and C are thought to increase the activities of antioxidant enzymes, free radicals scavenging, oxidative damage prevention and thus, protecting the membrane lipid components of the cells against lipid peroxidation[43,44,45].

The serum levels of zinc in patient with SCA was found significantly lower ($p<0.05$) than the normal subjects. This decreased serum zinc level could be attributed to its utilization as cofactor by some enzymes such as superoxide dismutase (SOD), which its activity is elevated in patients with SCA.[18,35,46] The low serum zinc level observed was in conformity with previous findings by Prasad[47,48] that zinc deficiency in SCA is associated with certain manifestations such as growth retardation in sicklers, hypogonadism, high blood ammonia, and disorder of cell mediated immunity. It also functions as cofactor for many enzymes such astranscriptases and some pyrimidine nucleotide dehydrogenases[49]. RBCs from zinc-deficient rats showed increased level of osmotic fragility associated with decreased concentration of plasma membrane sulfhydryl. This can be reversed by dietary zinc supplementation[50]. The metabolism and bioavailability of vitamins A and E are also dependent on zinc status of an organism.

Copper and iron are important trace elements for normal cell functions; they are components of several proteins and enzymes involved in variety of metabolic pathway. In this study, Serum copper and iron level in patients with SCA was significantly lower ($p<0.05$) than the normal subjects. It has been reported that copper can lead to reduction of defense system enzymes (selenium-dependent glutathione peroxidase (Se-GPx) and catalase)[51]. Iron (Fe) is an essential cofactor of catalase, hemoglobin and myoglobin, but is also a prooxidant capable of generating more free radicals (via Fenton reactions) when it is present in excess. Probably, this may account for the decrease in the serum activities of Gpx and catalase observed in sickle cell patients. It has been reported that during oxidative stress, the hypothalamus, adenohypophysis, and adrenal cortex are activated, regardless of the source, and this leads to plasma iron decrease[52]. This may probably account for the observed decrease of serum iron in patients with SCA as justified by increased level of MDA. Other possible mechanisms of serum iron decrease in patients with SCA may include nutritional inadequacy and increased urinary excretion of iron.

Many studies have provided humanity

with reliable data on the deficiencies of antioxidant and metals some of which are exacerbated by sickling episode. Some of these antioxidant and metals are iron, copper, zinc, vitamin A and vitamin C resulting in many pathophysiological complications of these syndrome[53,54]. This may result into an increased utilization of the antioxidant vitamins (A and C) in scavenging the excess amount of free radicals produced which probably, explain the decrease serum levels of the vitamins during period of sickling crises. The exposure of SCD patients to antioxidants such as N-acetylcysteine, vitamin C and vitamin E may decreases their oxidative stress and hence the complicating sickle cell crises.

CONCLUSION

From the results of this study, low level of antioxidant vitamins A and C and essential trace metals (Fe, Cu and Zn) are (were) observed in patients with SCA. This might be due to increase in oxidative stress which may be responsible for the complications observed in SCA. Oxidative modifications to proteins and lipids disrupt RBC membrane stability and function. Thus, results of this study may necessitate the importance of monitoring the levels of antioxidants and other biochemical parameters in sickle cell patients before and after therapy of the complications. Therefore, SCA patients should be given food, fruits or drugs containing antioxidants (Supplementation) to improve their health status and also as protection of RBCs membranes from ROS-mediated oxidative damage, which is important in SCA management.

ACKNOWLEDGMENTS

We sincerely thank members of staff and management of Aminu Kano Teaching Hospital (AKTH) and particularly, Department of Biochemistry, Bayero University, Kano for providing us with enabling environment to conduct this study. Our profound gratitude also goes to those SCA patients and controls who voluntarily donate their blood samples for this preliminary study.

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Prevalence and Perceived Risk Factors Associated with Road Traffic Crashes among Commercial Drivers in a City in South-South Nigeria

Johnson OE

ABSTRACT

Road traffic crash is a major but often neglected public health problem which affects both the occupants of affected vehicles and other road users. This study was carried out to determine the prevalence and perceived risk factors associated with road traffic crashes among commercial drivers in Uyo, Nigeria. This was a descriptive cross-sectional study conducted in May, 2016 among commercial motor vehicle drivers in Uyo, Nigeria. Data was collected using interviewer administered semi-structured questionnaire and analysed with IBM SPSS version 20. Level of significance was set at 5%. A total of 205 respondents participated in the study: 130 (63.4%) of them were aged less than 40 years. Only 34.6% reported learning how to drive in driving schools. Ninety four (45.9%) respondents admitted to driving even when tired, while 25 (12.2%) reported driving more than 100km/hour on highways. The lifetime prevalence of road traffic crashes was 46.8%, with 25% reporting 3 or more occurrences since onset of driving for commercial purposes. Over speeding, 43.4%, substance use 19% and bad road 15.6% were identified by respondents as the commonest risk factors of road crashes. The drivers who closed later than 8pm were more likely to have accidents compared to those who closed by 5-6pm (OR=3.56; CI=1.36-9.34, p<0.05). Road crashes were common among commercial drivers in this study.Over speeding, late driving and substance use should be discouraged among commercial drivers. Road repairs would also help in reducing road traffic crashes among this group of road users.

Keywords: Road traffic crashes, commercial drivers, prevalence, risk factors, Uyo, Nigeria

INTRODUCTION

Road traffic crash is a major but neglected public health challenge with a global estimate of 1.2 million lives lost and 50 million injured yearly[1]. Deaths from road traffic injuries have increased substantially in low and middle income countries since the 1960s and 1970s[2,3]. The global burden of disease study predicted that from 1990 to 2020 road traffic injuries would rise in rank to sixth place as a major cause of death worldwide[4]. While Kopits and Cropper predicted 27% decrease in annual road traffic deaths in high income countries, an increase of 83% was expected in low and medium income countries from year 2000 to 2020[5].

In low-income countries, including Nigeria, the majority of road deaths are among pedestrians, passengers, cyclists, users of motorized two-wheelers and occupants of buses and mini-buses[2].Nigeria remains one of the worst hit countries with a high level of vehicular population estimated at over 7.6 million[6]. The Federal Road Safety Corp reported that 12,077 road crashes were recorded in 2015 which led to 5,400 fatalities[7]. Among the different types of road accidents, crashes involving commercial motor drivers are of great concern as they not only put the drivers at risk but also have direct impact on the other vehicle occupants[8]. A review of literature to ascertain the road safety situation in Ghana reported that buses and mini buses caused 35% of fatal crashes, while cars were responsible for 32%[9]. In a retrospective study among road traffic injury victims in Olabisi Onabanjo University Teaching Hospital, minibuses which are a popular mode of commercial transportation were involved in 63.9% of the crashes, whereas cars, motorcycles and bicycles were involved in 14.8%, 6.6% and 0.6% respectively[10]. Commercial buses have also been implicated in road traffic crashes in a Lagos study[11].

The three elements involved in road traffic crashes are human, vehicle and road related factors[12]. Many times, a combination of these factors are involved in crashes. Human factor contributes immensely to any crash. Among the human factors, excessive speeding and reckless driving have been implicated in road traffic crashes in many studies in developing countries[11,13]. Statistics show that about 30% of accidents on the roads are due to excessive speeding[14,15] . Speeding contributes more to the risk of traffic injury than do other risk factors with available population attributable risk[16]. Excessive speeding was among the six variables identified as possible significant contributors to road traffic

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crashes in a study among commercial drivers in Lagos, Nigeria[17].

In terms of human factors, tiredness and sleepiness at the wheel among drivers also play important roles in the occurrence of accidents. Sleepiness at the wheel, sleep restriction and nocturnal driving have been implicated in 7-20% of traffic crashes[18,19]. In a study among bus drivers in Peru, the leading cause of road accidents identified was tiredness[20]. Other factors which have been identified include use of phones and driving under the influence of alcohol and other substances[21]. These also impair the ability of the driver to concentrate and contribute to loss of control by drivers.

In addition to human factors, mechanical faults and road defects have also been identified as causes of road crashes in several studies[13,22,23]. It is a driver's responsibility to ensure that a vehicle is in good order before using it. Evidence of road traffic injuries caused by burst tyres has been reported in a study among accident victims in a teaching hospital[10].

Many crashes among commercial drivers are largely preventable. There is paucity of data on studies which focus on the prevalence and perceived risk factors of road traffic crashes among commercial drivers in Uyo. A similar previous study on commercial drivers in Uyo excluded drivers who had no licenses and those who had never been involved in accidents[23]. This study therefore set out to determine the prevalence and perceived risk factors associated with road traffic crashes among all categories of commercial drivers in Uyo, Nigeria with the intention of communicating findings to the drivers' association and other stakeholders.

METHODOLOGY

This study was conducted among commercial motor vehicle drivers in Uyo, a city in south-south Nigeria with a projected population of 413,381 in 2015[24]. A common means of transportation within the city is the tricycle, while commercial vehicles mainly provide intercity transportation between Uyo and other towns in Akwa Ibom State and beyond. These vehicles are highly patronized by passengers who need their services to get to those destinations. There are four major parks in Uyo metropolis[25].

This study was a descriptive cross sectional study conducted among commercial motor drivers in Uyo. The sample size was calculated using the formula for estimating single proportion for cross sectional studies, using a prevalence of road traffic crash of 0.15 from a previous study[26], z of 1.96, sampling error set at 5%. A sample size of 198 was obtained and adding 10% to accommodate for non response increased it to 218.

The four major parks in Uyo metropolis have a total of 365 registered commercial drivers operating in them. The names of the parks and the number of commercial drivers in each park as obtained from the chairmen of the commercial drivers in the respective parks were Ekom Iman junction 40, Nung Oku 35, Mbiabong Etoi 80 and Central Itam 210. Using proportionate sampling technique, the number of respondents enrolled from the consecutive parks in the city to make up the sample size were 24, 21, 48 and 126, respectively. This gave a total of 219 respondents. Selection of study participants was subsequently done using simple random sampling method using table of random numbers

Data were collected using a self-designed interviewer administered semi-structured questionnaire which assessed the respondents' socio demographic characteristic, number of accidents since commencement of commercial driving and perceived risk factors of road traffic crashes. The questionnaire was pretested on 10 commercial drivers in Abak, a nearby town, about 20km from Uyo. Ambiguous questions were subsequently rephrased to ensure better comprehension. Five male Community Health Officers who were previously trained served as research assistants

The data obtained was analysed using IBM SPSS version 20. Data analysis was done using descriptive (frequency and proportion to summarize variables) and inferential statistics (chi square and logistic regression to test the significance of association between categorical variables). The level of significance was set at 5%.

Ethical approval to conduct the study was obtained from Akwa Ibom State Health Research Committee. Also, permission was obtained from the chairman of the commercial drivers' association in each park. The purpose and content of the study were adequately explained to the respondents after which either written or verbal consent was obtained from each of them depending on their level of literacy. Participation was entirely voluntary. The interviews were anonymous to ensure confidentiality.

RESULTS

In all, 218 respondents were eligible to

participate in the study, but 13 refused to participate thus giving a response rate of 94%. A large proportion of the commercial drivers, 130 (63.4%) were less than 40 years old, 118 (57.5%) were married, while 57 (27.8%) were single and 66 (32.2%) had 4 or more children.

Only 74 (34.6%) reported learning how to drive in driving schools. Though 95.6% of the respondents claimed ownership of license, only 47.8% had the license with them. Majority, 175 (85.4%) reported using seat belt while driving. Reasons given by those who did not use included absence of functional seatbelt (66.7%) and discomfort (33.3%).Concerning driving habits, 94 (45.9%) respondents admitted to driving even when tired, while 25 (12.2%) reported driving greater than 100km/hour on highways. (Table 1).

Table 1: Driving history of respondents

Variable	Frequency N=205	Proportion (%)
Learned driving from		
Family and friends	111	54.1
Driving school	74	34.6
Self taught	20	9.8
Ownership of license		
Yes (seen)	98	47.8
Yes (not seen)	98	47.8
No	9	4.4
Seatbelt use		
Yes	175	85.4
No	30	14.6
If No, why not?	**N=30**	
No functional seatbelt	20	66.7
Discomfort	10	33.3
Driving when tired		
Yes	94	45.9
No	111	54.1
Maximum speed limit (km/hour)		
<100	90	43.9
100	90	43.9
>100	25	12.2

DISCUSSION

Road traffic crash is a major but often

Ninety six (46.8%) of the commercial drivers admitted to ever being involved in road traffic crashes, with 25% reporting 3 or more occurrences since onset of driving for commercial purposes. Over speeding was perceived by 43 (44.7%) to be the cause of the most recent crash. Objects collided with included tricycles/motorcycle, 35 (36.5%) and other vehicles, 15(22.9%). Fifty eight (60.4%) sustained injuries during the most recent accident out of which 28 (48.3%) had bruises/abrasions, while13 (22.4%) had fractures. (Table 2).

Table 2: History and characteristics of road traffic crashes among respondents

History of crash	Frequency N=205	Proportion (%)
Ever being involved in road traffic crash		
Yes	96	46.8
No	109	53.2
Frequency	**N=96**	
1-2	72	75.0
3-4	17	17.7
>4	7	7.3
Perceived cause of most recent crash	**N=96**	
Over speeding	43	44.7
Bad tyres /brake failure	18	18.8
Substance use	16	16.7
Lack of concentration	15	15.6
Bad roads	4	4.2
Object collided with		
Tricycles /motorcycle	35	36.5
Stationary object	24	25.0
Vehicles	22	22.9
Pedestrian	15	15.6
Sustaining of injuries in most recent crash		
Yes	58	60.4
No	38	39.6
Most severe type of injuries sustained	**N=58**	
Bruises/abrasions	28	48.3
Laceration	17	29.3
Fracture	13	22.4

Over speeding was identified by 89 (43.4%) respondents as the single commonest risk factor of road crashes. Speed reduction and provision of good roads were the top preventive measures recommended by 29.3% and 23.9% of respondents respectively. (Table 3).

Table 3: Respondents' perception of the single commonest cause of road traffic crashes and preventive measures

Variable	Frequency N=205	Proportion (%)
Perceived commonest risk factor of road traffic crashes		
Over speeding	89	43.4
Substance use	39	19.0
Bad road	32	15.6
Poor vehicle condition	20	9.8
Lack of concentration	19	9.3
Poor driving skills	6	2.9
Most important perceived preventive measures		
Speed reduction	60	29.3
Provision of good roads	49	23.9
Discontinuation of substance use	35	17.1
Health education	28	13.6
Concentration/rest	17	8.3
Vehicle maintenance	11	5.4
Prayer	5	2.4

Though not statistically significant, history of crashes was highest among respondents aged 30-39 years,42 (50.0%) and least among those <30 years. Also, crashes were least among those who learnt to drive in driving schools, 30 (40.5%)and highest among those who were self taught, 11 (55%) (p>0.05).There was no statistically significant association between speed/ driving when tired and occurrence of road crashes (p>0.05).Significantly, history of crashes was highest among those who were divorced, widowed or separated, 20 (66.7%) and lowest among those that were single, 20(35.1%)(p<0.05). The proportion of respondents who reported ever having road crashes was highest among those with more than 6 children, 5(83.3%), and least among those with no children, 17(32.1%)(p<0.05). A higher proportion of those who closed later than 8pm reported ever having accidents, 58 (55.8%) compared to those who closed by 5-6pm (p< 0.05)(Table 4).

Table 4: Association between selected variables and being ever involved in road traffic crashes

Variable	Ever involved in RTA		Total	Statistics	p-value
	Yes N=96	No N=109		X^2	
Age (years)					
<30	14 (43.7)	18 (56.3)	32	0.59	0.75
30-39	42 (50.0)	42 (50.0)	84		
≥40	40 (44.9)	49 (55.1)	89		
Learned driving from					
Driving school	30 (40.5)	44(59.5)	74	2.04	0.36
Family and friends	55 (49.5)	56 (50.5)	111		
Self taught	11 (55.0)	9 (45.0)	20		
Speed					
<100	43 (47.8)	47 (52.2)	90	2.60	0.27
100	45 (50.0)	45 (50.0)	90		
>100	8 (32.0)	17 (68.0)	25		
Driving when tired					
Yes	46 (41.4)	65 (58.6)	111	2.82	0.09
No	50 (53.2)	44 (46.8)	94		
Marital status					
Single	20 (35.1)	37 (64.9)	57	7.92	0.02*
Married	56 (47.1)	62 (52.9)	118		
Others**	20 (66.7)	10 (33.3)	30		
No. of children					
Nil	17 (32.1)	36 (67.9)	53	Fishers exact=	0.03*
1-3	43 (50.0)	43 (50.0)	86		
4-6	31(51.7)	29 (48.3)	60		
>6	5 (83.3)	1 (16.7)	6		
Closing time					
5-6 pm	13 (27.1)	35 (72.9)	48	10.86	0.00*
7-8 pm	25 (47.2)	28 (52.8)	53		
>8 pm	58 (55.8)	46 (44.2)	104		

Multivariate logistic regression showed that drivers who had more than 4 children were almost three times more likely to have accidents compared to those with 0-4 children (OR= 2.79, CI= 1.21-6.48). Also, those who closed later than 8pm were three and a half times more likely to have accidents compared to those who closed by 5-6pm (OR=3.56; CI=1.36-9.34).(Table 5).

Table 5:Multivariate Logistic Regression in Response to ever having RTA among respondents

Variable	Odds ratio	95% Confidence interval	p value
Marital status			
Single	Ref		
Married	1.10	0.49-2.49	0.82
Others**	2.30	0.85-6.28	0.10
No. of children			
0-4	Ref		
>4	2.79	1.21-6.48	0.02*
Closing hour (pm)			
5-6	Ref		
7-8	2.88	1.01-8.26	0.05*
>8	3.56	1.36-9.34	0.01*

*Significant **Separated, divorced, widowed

neglected public health problem which affects both the occupants of affected vehicles and other road users. This study was carried out to determine the prevalence and perceived risk factors associated with road traffic crashes among commercial drivers in Uyo, Nigeria.

The lifetime prevalence of road traffic crashes among the commercial drivers in this study was 46.8%. This was higher than a prevalence of 24.7% reported among commercial drivers in Uyo in 2010[27]. A prevalence of 45.5% which is similar to that in the present study was reported by Pepple and Adio in Port Harcourt, Nigeria in 2014[28]. Many of the drivers in these studies were involved in road crashes more than once. In the present study, a quarter reported three or more episodes since the onset of commercial driving. In a similar study, 36.02% reported being involved in road crashes three or more times[23]. About a third of the drivers in the present study reported learning how to drive in driving schools. The implication of this is that majority did not have the opportunity to acquire proper driving skills and may not have had adequate exposure to traffic regulations. This could result in risky driving. It is worthy of note that history of crashes among commercial drivers in the present study was least among those who learnt to drive in driving schools. A study in south eastern Nigeria reported that driver's inexperience accounted for 21.5% of the road crashes recorded[13].

A lower prevalence of road crashes of 16.2% was reported among drivers of public institutions in Ibadan[22]. A possible reason for this lower figure could be the fact that certain pre-employment screening processes are usually carried out in institutions to ensure selection of better skilled-drivers compared to those driving for commercial purposes. The institution drivers for instance would be required to have licenses which may not be a prerequisite for commercial driving. Also, the institution drivers in that study were quite mature with a mean age of 50.1 years compared to the present study where close to two-thirds were less than 40 years old. Maturity is likely to lead to less recklessness on the road[29]. History of crashes in the present study was highest among respondents aged 30-39 years. In a study in Ghana, road users aged 16-45 years were the most vulnerable group and accounted for 58% of total crash fatalities from 2002-2006[9]. Another possible reason for the lower prevalence of road crashes in the Ibadan study could be the fact that since the institution drivers were paid monthly salaries, they were not anxious to make money on daily basis compared to the commercial drivers.

Another study among commercial drivers in Lagos reported a prevalence of road traffic crashes of 15.2% in the previous 5 years prior to the interview[26]. This was also much lower than the prevalence reported in the present study, probably because it considered a five year period while the present study looked at lifetime prevalence of road crashes. These crashes often result in varying degrees of injuries among the car occupants.

Apart from personal injuries sustained by commercial drivers during road traffic crashes, the passengers in their vehicles were also at risk. A study carried out in a teaching hospital in Sagamu, Nigeria reported that the victims of road traffic injuries included 102 drivers, 811

passengers and 42 pedestrians[10]. Other road users are at risk as the commercial vehicles often collide with other vehicles or pedestrians in the event of a crash. In the present study, over a third collided with tricycles/motorcycles, while up to one fifth collided with other vehicles. Thus a single crash can lead to a chain of events leading to multiple casualties.

Though majority in the present study claimed ownership of licenses, less than half showed the licenses on request. It is possible that many of those who could not produce theirs did not possess any. A study carried out to determine the level of compliance with driver's license laws[30] among commercial bus drivers in Lagos reported that formal drivers' training was low (26.1%). Only 9.3% fulfilled all the pre license obligations before obtaining their first driver's license[31]. The adequacy of the driving skills of such drivers is in doubt with negative implication on the safety of their passengers and other road users.

More than eight out of every ten respondents in the present study reported using seat belts. This was lower than findings in a study among commercial drivers in Lagos, where 97% reported fastening their seatbelts[26] but higher than a similar study in Makurdi, where 27.3% reported regular use of seatbelts[32]. In all the studies, there were still some drivers who did not use seatbelts. Reasons given for non-use in the present study were absence of functional seatbelts and discomfort. The use of seatbelt significantly reduces injuries and fatalities in the event of a crash. In the present study, six out of every ten respondents sustained injuries during the most recent road crashes, of which a fifth even had fractures. This outcome may have been different if all the drivers had used seatbelts.

In terms of perceived causes, excessive speeding was identified by more than 40% of respondents in the present study as the commonest perceived risk factor of road crashes. Speeding reduces the ability of a driver to avoid a crash in the event of an unexpected occurrence on the road. It also increases the impact on the object of collision during a crash, thus increasing the likelihood of fatalities[33]. Speeding by motorist is a common phenomenon reported in many studies. An observational study in Egypt on motorists driving on a highway and two urban roads reported that 11.8%-39.4% were driving above the speed limit[34]. In the present study, about one-tenth of the respondents reported driving above 100km/hour on highways. The maximum speed recommended on the highway for buses and taxis by the Federal Road Safety Corp in Nigeria is 90 km/hour[35]. Since many of the commercial drivers do not go through training in driving schools, many are ignorant of several traffic regulations including speed limits. A study carried out among commercial drivers in Lagos State reported that 100% of them had poor knowledge of the maximum speed limits[11]. A previous study among commercial drivers in the present study location also reported excessive speeding (36.02%), as the top perceived behavioral cause of road traffic crashes[19].

Several studies have also identified tiredness and lack of adequate sleep as contributory factors to road traffic crashes[18-20]. In the present study, close to half admitted to driving even when tired. In a study among 238 bus drivers in Peru, 55% slept less than 6 hours a day and 80% had the habit of driving more than 5 hours without stopping, 32% admitted to sleeping while driving and 55% opined that tiredness was the leading cause of RTA. Having an accident or near accident by drivers in that study was strongly associated with tiredness [20]. In a study in southern Nigeria, one of the top perceived behavioral causes of road traffic crashes among drivers was fatigue (31.18%)[23]. The desire to make as much money as possible could be the driving force behind these dangerous habits.

In the present study, the commercial drivers who had above 4 children were almost three times more likely to have accidents compared to those with 0-4 children. Since this was a life time study, the number of children may be related to years in driving which obviously will increase life time road traffic crashes. However, those drivers with large family sizes were also more likely to make efforts to earn more money to meet the demands of the larger family size. This could make them work for longer hours than those with fewer children. Moreover, it was observed that those who closed later than 8pm were three and a half times more likely to have accidents compared to those who closed by 5-6pm. Tiredness which could result from long hours of driving may increase proneness to accidents. Among those who admitted to ever having accidents in the present study, about one sixth mentioned the use of psychoactive substances as a perceived cause of the most recent road crash. One of the possible reasons for such substance use may be the need to stay alert.

Bad road was another perceived risk factor of road traffic accidents mentioned by 15.6% of the drivers in the present study. This was higher than findings of 12.5% among drivers of public institutions in Ibadan[22] and lower than findings of 29.03% among commercial drivers in another study[23]. A driver is expected to drive with a speed that is appropriate for a specific type of road on all occasions. However, many commercial drivers want to make several trips in one day and could be tempted to ignore the road conditions which may result in road crashes.

Up to 50% of drivers in an institution based study in Ibadan identified mechanical fault as a cause of road traffic crashes[22]. A study in south eastern Nigeria reported that mechanical fault and road defects accounted for 21.1% of the road crashes recorded[13]. Only about one out of every ten respondents in the present study considered vehicular issues as a risk factor of road crashes. Ensuring that a car is in good condition is a key responsibility of all drivers. All faults in a car ought to be attended to promptly. Some drivers are known to "manage" vehicles with faulty brakes until a crash occurs. Speed reduction and provision of good roads were the top preventive measures recommended in the present study by about one third and more than a fifth of the respondents, respectively.

CONCLUSION

Road crashes were common among the commercial drivers in this study. Excessive speeding and bad roads were the topmost identified contributory factors to road traffic crashes among commercial drivers. Large family size and long hours of driving significantly contributed to road crashes among commercial drivers. Over speeding, late driving and substance use should be discouraged among commercial drivers. Road repairs would also help in reducing road traffic crashes among this group of road users. Moreover, regular education of commercial drivers on safer driving habits during their association meetings would be of benefit to these drivers.

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Urine Dipstick Abnormalities in Asymptomatic Adolescents in Sokoto Metropolis, Sokoto State, North-Western Nigeria

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ABSTRACT

Urinary tract disorders are usually asymptomatic in early stages, and may remain undetected until severe or irreversible kidney damage has occurred. Dipstick urinalysis provides a simple cost effective way for screening of urinary abnormalities, thus enabling early diagnosis and management of such disorders. The aim was to determine the pattern of abnormal urinalysis and associated factors amongst asymptomatic adolescents in Sokoto metropolis. Four hundred and twenty-six (426) students aged 10 to 18 years were selected through multi-stage sampling in a descriptive cross sectional study from April to July 2014. Three hundred and ninety-three (92.3%) had their urine tested with dipstick test strips by Bayer, Plc. Their blood pressure, body mass index and socioeconomic status were documented. There were 166 males and 227 females (M: F = 0.73:1). Their mean age was 14.6 ± 2.0 years. Abnormal urinary findings occurred in 117 (29.8%) of the subjects which were proteinuria in 30 (7.6%), haematuria in 16 (4.1%), combined proteinuria/haematuria in 24 (6.1%), nitrituria in 47 (12%), and leucocyturia in 12 (3.1%) subjects. The prevalence of urine abnormality was higher in the 14-16 year age group though not statistically significant (p = 0.96). Proteinuria and nitrituria were higher in females while hematuria was more in males (p = 0.16). All urine abnormalities were significantly higher in the low socioeconomic class (p < 0.01). It was concluded that nitrituria and proteinuria were the commonest abnormalities in adolescents in Sokoto metropolis. Also urine abnormalities were significantly higher in subjects of low socioeconomic class.

Keywords: Prevalence, urinary abnormalities, adolescents, Sokoto

INTRODUCTION

Kidney and urinary tract diseases are often diagnosed in patients who are asymptomatic[1]. These disorders are usually asymptomatic in early stages and urine analysis by dipstick method is the first step to detect some of them. Dipstick urinalysis is a simple inexpensive test which has been employed in screening for urinary tract disorders for decades worldwide[2]. The disorders include post infectious glomerulonephritis, IgA nephropathy, membranous nephropathy, urinary tract infections and congenital disorders of the kidney and urinary tract. The ultimate end point of these disorders is chronic kidney disease (CKD) [1].

CKD is a worldwide public health problem with increasing prevalence[3]. Its adverse outcomes include progressive loss of kidney function, cardiovascular disease, and premature death[4]. Urine dipstick can be used for early detection of dysfunctions of the kidney and urinary tract which will ensure prompt treatment and prevent development of complications. There has been some controversy on the cost effectiveness of urinalysis screening programs in preventing CKD in children[5,6].

Widespread school screening programs have been ongoing for about three decades in Asian countries like Japan, Korea and Taiwan where it is believed that early detection of renal disorders in childhood will lead to effective interventions and reduction in the number of individuals who develop end-stage renal disease (ESRD)[1,5]. However, investigators from Europe and North America, do not share this view.[7] This can be seen in the changing position of the American Academy of Paediatrics (AAP) on recommendations for screening urinalysis in children over the years. The 2007 AAP recommendation states that healthy asymptomatic children should not be screened except those with risk factors for CKD[8].

There are considerable differences in the pattern of renal disease around the world due to racial variation and socioeconomic factors[1]. Infectious or acquired causes predominate in developing countries, where patients are referred in the later stages of kidney disease[1]. In Nigeria, there is no guideline on urine screening programs for children. Most studies on urine abnormalities have been carried out in younger age group below 12 years[9,10]. During adolescence, there is double burden of childhood problems and increase in

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adult onset diseases.[11] Even though orthostatic proteinuria which is benign is common in adolescents, other abnormalities in the urine may warrant further investigation, hence necessitating targeted screening programs. This study was carried out to assess the pattern of abnormal urinalysis and the associated factors amongst asymptomatic adolescents in Sokoto metropolis.

METHODOLOGY

Study area: The study was carried out in Sokoto metropolis which is the capital city of Sokoto State of Nigeria. The metropolis consists of three Local Government Areas (LGAs). It has a population of 427,760 based on 2006 census figures with a projected population of 541,874 in 2014 at an annual growth rate of 3%[12]. The study population comprised students (male and female) aged 10 to 18 years from selected secondary schools in the three LGAs within Sokoto Metropolis.

Study design: The study was descriptive and cross-sectional in design, conducted between April 28th, 2014 and July 27th, 2014.

Sample size determination: The minimum sample size was determined with a standard formula[13]. A prevalence of 50% was assumed since there was no previous study in the area[13]. The minimum sample size was 384. With addition of non-response (n^s/0.9; 384/0.9), it amounted to 426.

Eligibility criteria: Inclusion criteria were apparently healthy students aged 10 to 18 years who had informed written consent by the parents or guardians (and assent by the students).
Exclusion criteria included those involved in vigorous exercise such as racing, trekking long distances about 24 hours prior to the urine sample collection, febrile illnesses within 2 weeks prior to and at the time of the study, symptoms of urinary tract infection such as dysuria, frequency, urethral discharge, use of antibiotics and non-steroidal analgesics (NSAIDS) 2 weeks prior to the study, female students who were menstruating or menstruated within 2 weeks prior to the study, any child whose genotype was known and had sickle cell disease, history of cigarette smoking, use of oral contraceptives among the females, and alkaline pH (>7) which may affect the dipstick results. Those subjects who had taken drugs known to affect the results such as radio-opaque dye and nitrofurantoin within the previous 4 weeks[14].

Sample selection: The participating schools were selected by multi- stage random sampling. The first stage involved selecting all the co-educational schools in the three LGAs. There were 47 secondary schools in Sokoto metropolis and 27 of them were co-educational. Twenty-eight of the schools were public schools (government-owned) while 19 were private schools. Co-educational schools were used to ensure adequate gender representation.

The second stage involved selecting a public and private school from each of the LGAs. This was done to ensure equal representation of public and private schools in each of the three LGAs in the Metropolis. Two schools (1 public and 1 private school each) were sampled from each of the three LGAs by balloting. This amounted to a total of six schools. The total sample size of 426 was proportionally divided amongst the six schools based on their population.

In the third stage, one class each was selected from each level (JSS 1 to 3 and SSS 1 to 3) giving six classes. The total number of students for each school was divided proportionally amongst the six classes identified. Students from each class were selected by systematic random sampling based on a regular interval from the class list.

Structured questionnaires were used to record the subjects' biodata (age, gender, address, and tribe), parental occupation, educational levels, and family history of hypertension or diabetes mellitus. The subjects' weight, height, body mass index and blood pressure were recorded. Blood pressure was classified by the National High Blood Pressure Education Program (NHBPEP) charts. Body mass index was determined according to US CDC charts. This was carried out by the investigator and two properly trained research assistants who were medical interns.

Procedure of urine dipstick testing

Each subject recruited was given a sterile universal bottle which was properly labeled with his/her serial number to take home. They were

instructed on how to carefully void 10mls of their first morning urine (collected at mid-stream) into the bottle the following morning at home then bring same to school that morning. One of the researchers collected the urine samples from the subjects as they arrived into a cool box which was transported to the laboratory in Usmanu Danfodiyo University Teaching Hospital. In the laboratory, dipstick urinalysis was performed on the bottle of urine, using 10SG Multistixstrips by Bayer Plc. The reagent strips were completely immersed in the urine for 2-3 seconds. The test pad colour was compared visually with colour chart on the bottle containing the strips after 30 seconds for all the urine samples and the results were recorded.

Ethical issues

Ethical approval for the study was sought and obtained from the Ethics Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto. Written approval was sought and obtained from the Ministry of Education of Sokoto State. Written informed consent and assent was obtained from the parents or caregivers and the participating students respectively.

Data analysis

Data entry and analysis was done using statistical package for social sciences (SPSS) version 20.0. Data entered was checked for outliers, multiple and wrong entries. The results were illustrated using frequency tables and appropriate charts/ diagrams. Continuous variables were presented as means and standard deviations and comparison between the two groups was by student's t-test. Categorical variables were presented as percentages and compared using Chi square test or by Fisher's exact test where figures were small. All statistical analysis were done at 5% level of significance, that is, p-value < 0.05 was considered statistically significant.

RESULTS

Out of the 426 subjects sampled, 393 (92.3%) had their urine samples analysed. Twelve of them withdrew from the study and another 21 had provided contaminated urine samples and were excluded from the analysis.

Demographic characteristics of the study subjects

There were 166 males and 227 females (M: F = 0.73:1) students aged between 10 to 18 years. Their mean age was 14.6±2.0 years. One hundred and eighty-seven (47.6%) were aged 14-16 years. One hundred and eighty-six (47.3%) were of lower socioeconomic class as seen in Table 1.

Table 1: Socio-demographic characteristics of the study population (n = 393)

Characteristics	Gender (%)		Total (%)	χ^2	df	p-value
Age group (yr)	**Male**	**Female**				
10-13	43 (10.9)	84 (21.4)	127 (32.3)			
14-16	80 (20.4)	104 (27.2)	187 (47.6)	8.5		2
0.014						
17-18	43 (10.9)	36 (9.2)	79 (20.1)			
School type						
Private	38 (9.7)	58 (14.8)	96 (24.4)	0.367		0.5
Public	129 (32.6)	169 (43)	297 (75.6)			
Socio-economic status						
Upper	25 (6.4)	57 (14.5)	82 (20.9)	6.8	2	0.03
Middle	52 (13.2)	7 2 (18.3)	124 (31.6)			

Pattern of urinary dipstick abnormalities

Urinary dipstick abnormalities were found in 117 subjects (29.8%) comprising 53 males and 64 females (M: F= 0.83:1). Nitrite was the commonest urine abnormality seen in 47 (12.0%) of the subjects followed by proteinuria in 30 (7.6%). Others were combined proteinuria/ haematuria in 24 (6.1%), haematuria in 16 (4.1%) and leucocyturia in 12 (3.1%) subjects. All the subjects with leucocyturia also had nitrite in their urine. The pattern of urinary abnormalities is shown in Figure 1 below.

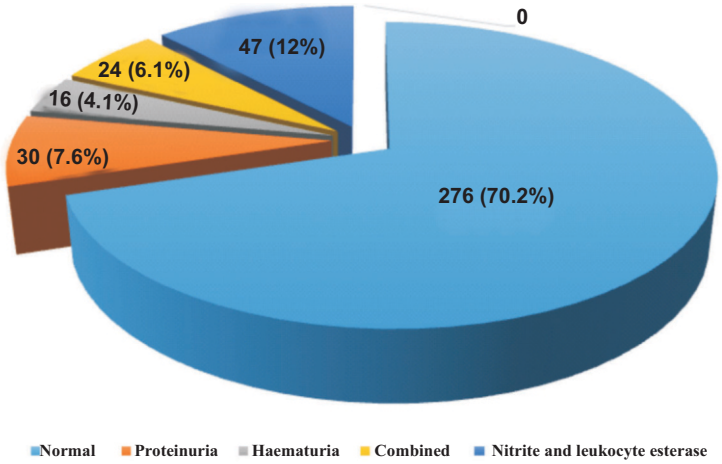

Figure 1: A pie-chart showing the different urine abnormalities in the adolescent study population.

Age and gender distribution of subjects with urinary dipstick abnormalities

Occurrence of urinary dipstick abnormalities was higher in the 14 to 16 year age group when compared to other age groups but not significant. Proteinuria and nitrite were higher in females while hematuria was more in males though none was significant (p = 0.169). The age and gender distribution of the findings is shown in Table 2 and Figure 2, respectively.

Table 2: Age distribution of subjects with urinary dipstick abnormalities (n = 117)

	Age Group (yrs)			
	10-13(%)	14-16(5)	17-18(%)	Total(%)
Urine dipstick findings				
Protein	10 (8.5)	14 (12.0)	6 (5.1)	30 (25.6)
Blood	5 (4.3)	6 (5.1)	5 (4.3)	16 (13.7)
Protein and Blood	8 (6.8)	12 (10.3)	4 (3.4)	24 (20.5)
Nitrite	15 (12.8)	23 (19.7)	9 (7.7)	47 (40.2)
Total	38 (32.5)	55 (47.0)	24 (20.5)	117 (100)

χ^2= 1.53, df = 6, p-value = 0.95

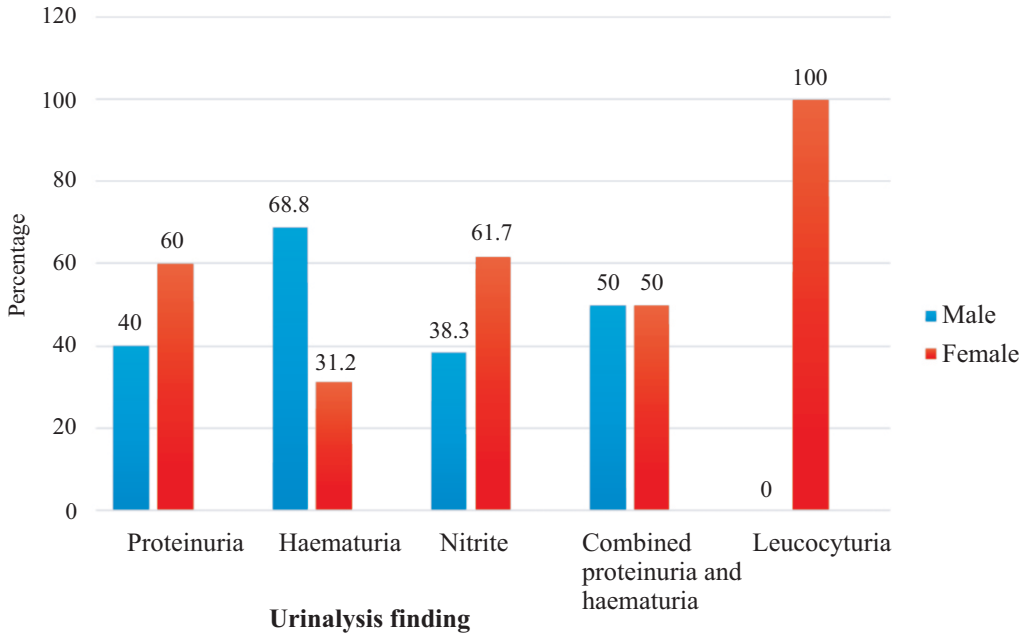

Figure 2: Gender distribution of urinary dipstick abnormalities

Socio-economic class of subjects with urinary dipstick abnormalities

All the urine abnormalities were more frequent in those of lower socioeconomic class III ($p < 0.01$) who accounted for 55.6% of those with abnormal dipstick findings (Table 3).

Blood pressure and body mass index of subjects with urinary dipstick abnormalities

Of those with abnormal urinalysis results, five subjects (4.3%) were overweight (BMI > 85th percentile) while 24 (20.5%) had blood pressure above the 90th percentile for age, gender and height. There was no significant association between the presence of proteinuria and haematuria with the blood pressure levels or body mass index of the subjects as shown in Tables 4 and 5 below.

Table 3: Socio-economic status distribution of subjects with urinary dipstick abnormalities (N = 117)

	Socio-economic Class			
	[a]SEC I	SEC II	SEC III	Total
Urine dipstick findings				
Protein	10 (8.5)	6 (5.1)	14 (12.0)	30 (25.6)
Blood	2 (1.7)	1 (0.9)	13 (11.1)	16 (13.7)
Protein and Blood	4 (6.8)	3 (2.6)	17 (14.5)	24 (20.5)
Nitrite	6 (5.1)	20 (17.1)	21 (7.9)	47 (40.2)
Total	22 (18.8)	30 (25.6)	65 (55.6)	117 (100)

$\chi^2 = 18.6$ df = 6, p-value = 0.005 [a] SEC= Socio-economic Class

Table 4: Urinary dipstick abnormalities and body mass index (BMI) category (N = 117)

	Body Mass Index (percentile)			
	< 5th	5th - 85th	> 85th	Total
Urine dipstick findings				
Protein	7 (6.0)	21 (17.9)	2 (1.7)	30 (25.6)
Blood	6 (5.1)	10 (8.5)	0 (0)	16 (13.7)
Protein and Blood	9 (7.7)	15 (12.8)	0 (0)	24 (20.5)
Nitrite	10 (8.5)	34 (29.1)	3 (2.6)	47 (40.2)
Total	32 (27.4)	80 (68.4)	5 (4.3)	117 (100)

$\chi^2 = 5.2$ df = 6, p-value = 0.51

Table 5: Urinary dipstick abnormalities and blood pressure (BP) category (N = 117)

	Blood Pressure (percentile)		
	< 90th	≥ 90th	Total
Urine dipstick findings			
Protein	22 (18.8)	8 (6.8)	30 (25.6)
Blood	15 (12.8)	1 (0.9)	16 (13.7)
Protein and Blood	19 (16.2)	5 (4.3)	24 (20.5)
Nitrite	37 (31.6)	10 (8.5)	47 (40.2)
Total	93 (79.5)	24 (20.5)	117 (100

$\chi^2 = 2.7$ df = 3, p-value = 0.43

Follow up of subjects with urinary dipstick abnormalities

The turn-out of subjects to produce a second sample of urine for testing two weeks after the first test was poor. Only 44 (37.6%) of the 117 subjects with urine abnormalities submitted samples for a second test. The samples were tested at the laboratory in UDUTH. Eight (18.2%) of the 44 subjects still had proteinuria, while 10 (22.7%) had haematuria. Four (9.1%) had combined haematuria and proteinuria, 3 (6.8%) had nitrite and 19 (43.2%) had normal findings. Those with positive results were referred to the UDUTH, Sokoto for other diagnostic tests. Of those with haematuria, four of them were males who on further tests were diagnosed and treated for Schistosomiasis, while one male had urinary tract infection. One of the female subjects with combined hematuria and proteinuria was diagnosed with pyelonephritis.

DISCUSSION

The prevalence of urinary dipstick abnormalities amongst adolescent school students in this study was 29.8%. This is comparable to the prevalence of 26.7% observed by Ekunwe[15] in Lagos amongst children aged 2 to 17 years, but higher than the prevalence of 5.25% previously reported amongst adolescents in Benin and a prevalence of 2.7%, among preschoolers aged between 5 and 7 years in Enugu by Oviasu[9] and Odetunde[10], respectively. Studies from India, Egypt, Nepal, Lebanon and Iran have reported lower prevalence of urinary abnormalities when compared to this study[1,16-19].

The reason for these variations may be due to differences in methodology and sociodemographic characteristics of the study cohort, or due to epidemiologic variations between the study areas.[20] As observed by Utsch and Klaus, causative factors of renal diseases may vary in different study area,[20] and this can have implication in the pattern and prevalence of urinary abnormalities.

For the age distribution of urinary abnormalities, some authors[16, 21] have reported among their subjects that the prevalence increased with age. In this study, the prevalence of urinary abnormalities was highest at the 14 to 16 year age group but lower at 17 to 18 years, similar to the patternseen by Vinoth[16] in Chennai. Possible reasons in studies where proteinuria is commoncould be orthostatic proteinuria, since this finding is common in the adolescent age group[22]. However, we tried to minimize this factor by asking for the early morning urine sample of the subjects.

With regards to the gender distribution of dipstick abnormalities, findings were similar to what was reported by Parakh *et al.*[18] amongst school children aged 5 to 15 years where male to female ratio was 0.84:1. Female subjects in this study had more proteinuria and nitrite in their urine, while the males had more of hematuria than the females. This is similar to what was reported by Jiya *et al*[23] in a study among asymptomatic primary school pupils where males had a higher prevalence of hematuria than females. This was unlike the findings by Oviasu[9] in which proteinuria and hematuria were more frequent in the female subjects, though this was not significant as also in this study. El Sharif[24] in Sudan found males had more of proteinuria and females more of hematuria but equal numbers had combined hematuria and proteinuria as this study. Vinoth *et al.*[16] and other authors[25] also found all the urinary abnormalities were more frequent in the males.

The different gender prevalences of haematuria and proteinuria in these studies could be due to varying pattern of environmental exposure to disease agents in different populations. The higher prevalence of haematuria in males in this study could be due to schistosomiasis infestationwhich is prevalent in the area[26] and occurs more in males because they have more contact with infested water during activities like farming and fishing[27].

Nitrite was the commonest urine abnormality in this study. It was the second commonest in the studies by Hajar in Lebanon and Nodoshan in Iran[1,19]. It was commoner in females in this as in other studies[1,19]. Leukocyte esterase in this study was found exclusively in the femalesubjects and it was also common in female adolescents as reported by Hanif[28]. These gender differences may reflect susceptibility to prevalent diseases in different locales and other confounding factors. For instance, false positives may be due to exposure to cold, prolonged recumbence, andcontamination of urine samples with menstrual bloodin females[18].

It was found in this study that all urinary abnormalities occurred predominantly in the lower socio-economic class III. The reason for this may be due to exposure to a higher level of agents that can cause urinary tract infection in the study area. Most studies have not empasized on the socio-economic levels of those found with urinary abnormalities and this is a possible factor to assess when developing targeted screening programmes in different locales. However, in a study amongst primary school children in Egypt, Bakr[17] did not find any relationship with socio-economic status.

Kidney diseases may be more prevalent in those with cardiovascular risk factors including hypertension, diabetes, and obesity[29]. In this study, there was no significant relationship between the markers of these diseases which include proteinuria/ hematuria, elevated blood pressure and overweight. In the study carried out by Vinoth[16], there was a significant relationship between these abnormalities and blood pressure but not with the weight. In fact, some studies have reported that a lower weight may be associated

with predisposition to proteinuria.[14] The reason given for this was fewer nephronsin those with low weight especially if they had low birth weight. This will induce hyperfiltration mechanism in the fewer than normal nephrons leading to proteinuria. In this study about 25% of those with low BMI for age had proteinuria.

CONCLUSION

Urinary dipstick abnormalities are common in school adolescents in Sokoto Metropolis, with nitrituria, proteinuria and haematuria being the most prevalent. Low socioeconomic class is significantly associated with these abnormalities. It is recommended that urinary screening programmes should be instituted in schools, as this will enable early detection and treatment of renal diseases.

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Childhood Nephrotic Syndrome in Uyo, Nigeria: Challenges of Management in a Resource Limited Setting

***Ikpeme EE, Dixon-Umo OT**

ABSTRACT

Childhood nephrotic syndrome is a common glomerular disorder with massive proteinuria. Interplay of socio-demographic factors, variation in clinical presentation, steroid responsiveness and access to steroid-sparing therapy has remarkable impact on its outcome. The presentation and management outcome of nephrotic syndrome in a resource limited setting were reviewed. Data was obtained from the renal register of the paediatric renal unit of University of Uyo Teaching Hospital, Uyo, Nigeria from January 2007 to December 2016. Fifty-five children aged 9 months to 17 years with a mean age of 10.1±4.7 years formed the study population. Peak age was 14-15 years with a gender distribution of 36 males (65.5%) and 19 females (34.5%) giving a M:F ratio of 1.9:1. Sixty percent of the patients belonged to the low socio-economic class. Significant clinical features were: generalised oedema (94.5%), hypertension (67.3%), oliguria (65.5%) and microscopic haematuria (50.9%). Complications included pulmonary oedema and encephalopathy (9.1% each), severe anaemia (5.5%) and primary peritonitis (2.6%). Serum creatinine could be done in 89.1% and estimated glomerular filtration rates were as low as ≤15mls/1.73m^2/min in 10.9% of the patients. Possible secondary aetiologies were Hepatitis B and C {(6/34) 17.6%} and {(4/34) 11.8%} respectively), sickle cell anaemia {(3/20) 15%} and human immunodeficiency virus {(2/35) 5.7%}. Renal biopsy was indicated in all the 17 steroid resistant patients but was not affordable. Overall, favourable steroid response (remission) was documented within 7-42 days in 21 (38.2%)of the patients and 17 (30.9%) were steroid resistant. The remaining 17 (30.9%) did not continue long enough in care to ascertain their response to steroid therapy. Further remission with cyclophosphamide occurred in 7/17 (41.2%) of the steroid resistant group. Thirteen patients (23.6%) of the total study population required renal replacement therapy. Only 5/13 (38.5%) could afford between two and eight sessions of haemodialysis, out of which 2/5 (40%) recovered renal functions. Financial constraint was a prominent factor in the poor outcome of our study population. Subsidised treatment of childhood nephrotic syndrome in our locale is therefore recommended.

Keywords: Nephrotic syndrome, children, resource limited setting, management

INTRODUCTION

Nephrotic syndrome is a common chronic renal disorder, occurring fifteen times more in children than in adults[1]. It is characterised by massive proteinuria of dipstick ≥3+ or 40mg/m^2/hr in children or a spot early morning urine protein: creatinine ratio of > 200mg/mmol. Other characteristics include: hypoalbuminaemia (serum protein < 25g/l), generalised oedema, and hypercholesterolaemia (serum cholesterol > 5.2 mmol/l)[2,3].

The incidence is estimated at 2-7 per 100,000 children annually in children less than 16 years old in the United States of America, and a cumulative prevalence of 16 per 100,000 individuals[4]. An annual incidence rate of 2-7 new cases per 100,000 children and 11.6 per 1,000,000 children have also been reported in India[5] and Libya[6] respectively. In Nigeria, childhood nephrotic syndrome accounted for 1.34% of all paediatric admissions in a tertiary health facility in Enugu, eastern Nigeria[7] and 5.6 per million in the south-south region[8]. In Uyo, south-south Nigeria, it was the commonest childhood renal disease with a prevalence of 38.3%[9].

The syndrome could be secondary to various causes including, but not limited to, immunologic disorders, infections/infestations, metabolic derangements, and neoplastic diseases or it could be idiopathic. Different histological types have been described from renal biopsy studies[1]. The major determinant of its outcome is steroid responsiveness, but second line immunosuppressive and steroid-sparing drugs for treatment of steroid-resistant cases have been reported to increase response by 20-50%[10].

Interplay of widespread poverty and low literacy levels also pose additional challenges to the management of this chronic renal syndrome[11]. The negative impact of late presentation, limitations of investigations, and inability of utilising current treatment options have significant effects on management outcome[12-14]. Outcome may further be worsened in settings where renal replacement therapy is not sufficiently available or affordable. We therefore

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studied the presentations and management of this important chronic paediatric renal disorder, in a typical resource limited setting.

PATIENTS AND METHODS

A ten-year study was carried out in the paediatric renal unit of the University of Uyo Teaching Hospital (UUTH) in Akwa Ibom State, Nigeria, from January 2007 to December 2016. The state has a population of about 3.9 million,[15] majority of the people being civil servants and predominantly small-scale business owners. The hospital is the only facility rendering specialist paediatric renal treatment/services to children within the state and neighbouring states of Cross River, Abia and Imo states. The hospital has a growing haemodialysis unit with 5 functional haemodialysis machines for both adult and paediatric renal patients. Children younger than 5 years are offered peritoneal dialysis when feasible.

A review of the paediatrics renal register was undertaken for children 0-18 years from January 2007 to December 2016. Patients with a diagnosis of nephrotic syndrome were studied. Diagnostic criteria were generalized oedema, massive proteinuria (dipstick ≥3+ or 40mg/m^2/hr or a spot early morning urine protein: creatinine ratio of > 200mg/mmol), hypoalbuminaemia (serum protein <25g/l), and hypercholesterolaemia (serum cholesterol > 5.2 mmol/l). Data extracted included age, gender, educational level and occupation of parents/caregivers, from which their social classes were determined using Oyedeji's criteria[16]. Also, the presenting complaints, examination findings like pallor, blood pressure, abdominal organ enlargement were noted. Blood pressure recordings between 95th and 99th percentile plus 5 mmHg for 3 consecutive times was regarded as stage 1 hypertension while above 99th percentile plus 5 mmHg for 3 consecutive times was stage 11 hypertension. Investigation results including urinalysis, full blood count, serum electrolytes, urea and creatinine, serology for Human Immunodeficiency virus (HIV), Hepatitis B and Hepatitis C viruses, malaria parasites, haemoglobin electrophoresis, total serum protein and albumin, serum cholesterol, urine culture (where indicated) were also retrieved from the renal register. Estimated glomerular filtration rate was calculated using the Schwartz formula. Renal biopsies for histopathological studies, were not done.

The treatment protocol undertaken was prednisolone tablet at 60mg/m^2/day in 3 divided doses, to a maximum of 60mg/day, for all presumed idiopathic nephrotic syndrome cases. Those with secondary nephrotic syndrome were also treated for the underlying diseases like use of Highly Active Anti-Retroviral Therapy (HAART) for HIV infected patients and routine management of patients with sickle cell anaemia. Daily prednisolone was given for 6 weeks followed by 40mg/m^2/day (maximum 40mg/day) on alternate mornings for 6 weeks. Remission (trace or absence of dipstick proteinuria for 3 consecutive days) on prednisolone therapy within the first 6 weeks of treatment was regarded as steroid sensitive nephrotic syndrome (SSNS). Persistence of massive proteinuria despite 6 weeks of daily prednisolone was regarded as steroid resistant nephrotic syndrome (SRNS) and this was treated with oral cyclophosphamide at 2-3mg/kg/day for 12 weeks. Recurrence of massive proteinuria or any proteinuria with recurrence of oedema was defined as a relapse. Steroid dependent nephrotic syndrome (SDNS) cases were those with 2 consecutive relapses on tapering or cessation of steroid therapy. Frequent relapsing nephrotic syndrome (FRNS) was defined as 2 or more relapses within the first 6 months, or 4 or more relapses in a year after an initial episode of remission.

Treatment of relapse was recommencement of prednisolone with addition of alternate day levamisole at 2.5mg/kg at a maximum dose of 150 mg/day for 12 months. Steroid was reduced to low dose of 1mg/kg on alternate days after remission but when there was steroid toxicity, cyclophosphamide at 2-3mg/kg/day and levamisole were used. In the last 2 years of the study, from January 2015 to December 2016, cyclosporine A was prescribed for frequent relapses with steroid toxicity although most times it was not affordable.

RESULTS

Fifty-five children with nephrotic syndrome whose case files were retrieved constituted the study population. They were 36 males (65.5%) and 19 females (34.5%) giving a M:F ratio of 1.9:1. The ages ranged from 9 months to 17 years with a mean age of 10.1 ± 4.7 years (median 10 years), and a peak age of 14-15 years. The age distribution is shown in Figure 1. Majority of the children 33 (60.0%) belonged to the low socio-economic class while 13 (23.6%)

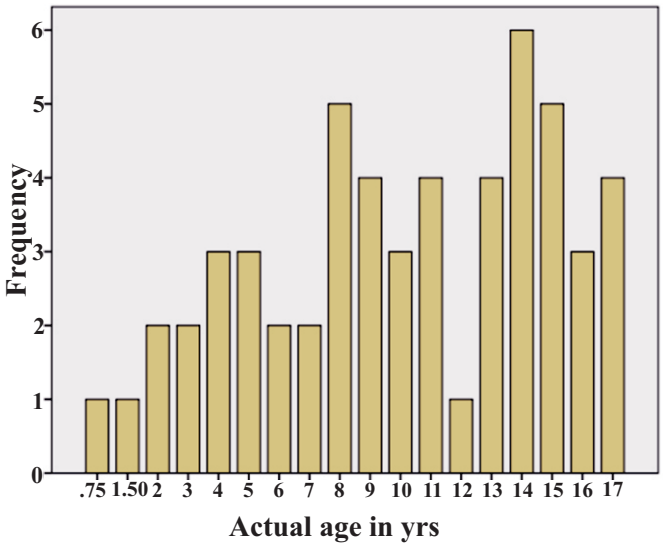

Figure 1: Age distribution of the study population

and 9 (15.4%) were of the middle and upper socioeconomic classes respectively. The age, gender and social classes of the patients are shown in Table 1.

Table 1: Socio-demographic parameters of patients with nephrotic syndrome. N=55

Parameter		Frequency	Percentage
Age (years)	< 5	12	21.8
	6-10	16	29.1
	11-15	21	38.2
	>15	6	10.9
	Total	55	100.0
Sex	Male	36	65.5
	Female	19	34.5
	Total	55	100.0
Social class	I	4	7.3
	II	5	8.1
	III	13	23.6
	IV	26	47.3
	V	7	12.7
	Total	55	100.0

The major clinical features were generalized oedema 52 (94.5%), oliguria 36 (65.5%), fever 19 (34.5%) and microscopic haematuria 28 (50.9%). Hypertension was documented in 37(67.3%), of which 21(38.2%) were in stage 1 and 16 (29.1%) were in stage 11. Sixteen (29.1%) patients presented with complications: pulmonary oedema 5 (9.1%), encephalopathy 5 (9.1%), severe anaemia 3 (5.5%), peritonitis 2 (3.6%) and pleural effusion 1(1.8%). This is shown in Table 2.

Forty-nine (89.1%) patients had their serum electrolytes, urea and creatinine tested and their glomerular filtration rate (GFR) calculated for assessment of their renal status. Six (10.9%) had GFR≤15mls/min/1.73m^2. The mean serum protein was 42.5g/l (62-82g/l), mean serum albumin was 19.2g/l (36-52g/l) and mean cholesterol was 8.3mmol/l (3.1-6.5mmol/l).

Table 2: Pattern of clinical presentation of nephrotic syndrome

Clinical presentation	Frequency	Percentage
Generalized oedema	52	94.5
Facial puffiness only	3	5.5
Oliguria	36	65.5
Anuria	5	9.1
Microscopic haematuria	28	50.9
Gross haematuria	7	12.7
Hypertension stage 1	21	32.7
Hypertension stage 11	18	29.1
Primary peritonitis	2	3.6
Pleural effusion	1	1.8
Fever	19	34.5

Thirty five (63.6%) had HIV serology done, out of which 2 (5.7%) were positive. Thirty four (61.8%) were tested for HBsAg and anti HCV, with 6 (17.6%) and 4 (11.8%) being positive for HBV and HCV respectively. Malaria parasitaemia was present in 12 (44.4%) out of 27 (49.1%) of patients who were tested. Only one of the 12 positive malaria parasitaemia was *Plasmodium malariae,* and 11 were *Plasmodium falciparum* species. Three (15.0%) patients out of 20 (36.4%) who had haemoglobin electrophoresis done were HbSS. Renal sonography showed loss of corticomedullary differentiation in 24 (75.0%) out of 32 (58.2%) patients. Five (17.2%) patients out of 29 (52.7%) had urinary tract infections caused by *Escherichia coli in* 80.0% and *Staphylococcus aureus* in 20.0%. Renal biopsy was not done although it was indicated in all the steroid resistant cases, mostly because of lack of funds. Details of the laboratory investigations are shown in Table 3.

Remission was achieved in the steroid sensitive patients within 7-42 days with 66.7%

Table 3: Investigation results of 55 patients with nephrotic syndrome

Parameters		Number	Percentage
Serum creatinine	53-115 µmol/l	33	60.0
	>115 µmol/l	16	29.1
	Not done	6	10.9
Serum urea	≥2.1-7.1 mmol/l	32	58.2
	mmol/l	17	30.9
	Not done	6	10.9
Haemoglobin	< 10g/dl	15	27.3
	≥10g/dl	34	61.8
	Not done	6	10.9
HIV serology	Reactive	2	3.6
	Non-reactive	33	60.0
	Not done	20	36.4
HBV serology	Positive	6	10.9
	Negative	28	50.9
	Not done	21	38.2
HCV serology	Positive	4	7.3
	Negative	30 5	4.5
Malaria parasite	Not detected	15	27.3
	P. falciparum	11	20.0
	P. malariae	1	1.8
	Not done	28	50.9
Hb electrophoresis	AA	16	29.1
	AS	1	1.8
	SS	3	5.5
	Not done	35	63.6

Key: HIV: Human Immunodeficiency Virus
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus

responding within 15-28 days. Six of the steroid sensitive cases did not have any relapse in 2 years of follow up, but 15 of them had 1 to 4 relapses within 12 months and cyclosporine A could only be commenced in one frequent relapser. This patient could no longer afford cyclosporine A after 6 months of starting the medication. Seven (41.2%), out of the 17 steroid resistant patients achieved remission with cyclophosphamide and levamisole, but 3 (17.6%) absconded. The remaining 7 were to commence cyclosporine A but could not afford it and therefore continued on cyclophosphamide, four (23.5%) of which were DAMA and 3(17.6%) were discharged for outpatient treatment on alternate day prednisolone and levamisole. Thirteen (23.6%) of the study population had clinical and laboratory indications for dialysis but 8/13 (61.5%) of them could not afford it and were DAMA. The remaining 5 (38.5%) had between 2 to 8 sessions of haemodialysis of which 2/5 (40.0%) recovered renal function and are on follow up. A total of 31 patients were discharged, fifteen (48.4%) were lost to follow up, but 4 (12.9%), 5 (16.1%) and 7 (22.6%) have been on regular follow up for 1year, 2years and more than 2years respectively.

Overall, twenty-one (38.2%) were steroid sensitive, 17 (30.9%) were steroid resistant. Outcome could not be ascertained in 17 (30.9%) patients because of 12 Discharges Against Medical Advice (DAMA), 1 referral and 4 deaths.

DISCUSSION

The manifestations of childhood Nephrotic Syndrome has global variations from region to region, even within the same country.[7-8,17-28] This study revealed a male preponderance as reported by other studies[7,17-18,23-24] but contrasts equal gender distribution reported by Anochie *et al.*[8] in southern Nigeria and Doe *et al.*[31] in Ghana. The study population comprised older children with a peak age of 14-15 years, the highest reported so far in Nigeria. Comparable peak ages of 12 years has been reported in USA,[29] Pakistan[30] and Ghana[31]. In contrast, earlier studies reported peak ages of < 5years[7-8,17-18,28] and 7-8 years[20,32-36] from different countries and regions of Nigeria.

Socioeconomic class has been implicated in nephrotic syndrome, although its exact role is not clear. Majority of our study population belonged to the low socioeconomic class and this has also been reported in south-west Nigeria[28] and India[37]. This explains the high level of poverty manifested in the study population. A high proportion also had infectious agents isolated which probably played an aetiological role in the development of the syndrome.

Generalized oedema as seen in this study continues to be the commonest presenting clinical feature as also reported by other authors[7-8,17]. Most of the patients (74.6%) presented with oliguria/anuria as against 42.9% and 10% reported by Anochie *et al.*[8] and Okoro *et al.*[7] respectively. Also, a high proportion of our patients, 67.3%, presented with hypertension, compared to only 23.0% from Enugu,[7] 28.6% from Port-Harcourt[8] and 35.0% from Kano[35]. Microscopic haematuria was also a significant finding occurring in 50.9% of our patients. This finding is comparable to 53.6% reported in Port-Harcourt[8] but higher than 26.0% from Enugu[7]. These features strongly suggest that our study population is more likely to be a predominant non-minimal change nephrotic syndrome, as older age, hypertension and haematuria have been reported to be associated with membrano-

proliferative glomerulonephritis (MPGN) and focal segmental glomerulosclerosis (FSGS).[17,35,38]

About 30-50% of our patients could not afford to pay for the numerous basic investigations usually outlined in search of secondary aetiologies of nephrotic syndrome. One third of those who presented with some complications would rather channel their limited resources towards treatment than for investigations. This could have a negative impact on the outcome as also reported by other authors.[12-14] Despite the low access to investigations, there was a high yield of probable aetiologic agents for childhood nephrotic syndrome in this study. Quartan malaria nephropathy has been documented to be caused by *Plasmodium malariae* and *Plasmodium falciparum*[39]. About 45.0% of our patients had malaria parasitaemia: *Plasmodium malariae* was isolated in one patient and *Plasmodium falciparum* in 11. This is lower than 60% reported in the Port-Harcourt[8] study. However, the later study and that from Ghana[31] did not report the isolation of *Plasmodium malariae*. Only 2 of our patients tested positive for Human immunodeficiency virus (HIV), although Akwa Ibom State had a seroprevalence rate of 8% - 9.8% during the study period.[40-41] The widespread use of Highly Active Antiretroviral Therapy may have delayed the glomerular damage by this virus. Hepatitis B (HBV) and C (HCV) viruses are known causes of childhood nephrotic syndrome. Seropositivity for these viruses was 17.6% and 11.8% for HBV and HCV respectively. These values are comparable to but lower than 24.0%[34] and 30.0%[35] from northern Nigeria. This may be a reflection of the seroprevalence rates of the individual communities. Conversely, studies from Pakistan[30] reported 6.5% while both Iran[16] and south south Nigeria[8] reported zero per cent. Sickle cell anaemia (HbSS) is also an important aetiological factor in childhood nephrotic syndrome. Fifteen per cent of our patients had HbSS. This is higher than 3.3% and 3.57% reported by Okoro *et al.*[7] and Anochie *et al*.[8] Repeated sickling and microinfarcts cause renal papillary necrosis and glomerulosclerosis.

This study showed a low steroid responsiveness of 38.4% in contrast to several other studies from the same country who reported high steroid sensitivity.[8,22,25-26,28] This difference may be explained by the afore-mentioned features suggestive of a non-minimal change pattern in the study population. Steroid resistance is more associated with secondary nephrotic syndrome. This shows the role of poverty, poor and delayed access to health care as important negative determinants of this chronic disease. Okoro *et al.*[7] and Olowu *et al.*[27] in south-east and south-west Nigeria reported similar low rates of steroid responsiveness of 30.0% and 45.2% respectively and higher mean ages of 7.9±3.4 years and 9.95± 3.15 years respectively.

Steroid sparing agents are available for the treatment of steroid resistant cases but were not affordable by the cohort of patients. Financial challenges were the underlying factors for the high rate of patients discharging against medical advice 16 (29.1%) or absconding 3 (5.5%). This rate of DAMA is very high compared to 7.4% reported by Okechukwu[42] and a specific 4.9% for paediatric renal disorders. However, low socioeconomic status contributing to financial challenges was the commonest reason for DAMA (42.2%). The low mortality rate of 7.3% may not be a true picture of our overall mortality rate because majority of DAMA were moribund and probably would have contributed to mortality if they stayed longer. The low access to dialysis in this study of 38.5% compares favourably with an earlier study in the same centre of 26.9% when outcome of all the paediatric renal diseases was studied[43]. This uptake is much lower than the experience in Libya[44] and United States of America[45] where good outcome was obtained as a result of high access to dialysis.

CONCLUSION

Nephrotic syndrome in our environment is more likely to be secondary Nephrotic syndrome. Inability to afford the cost of investigations, steroid sparing drugs and renal replacement therapy, pose a huge challenge to its management, resulting in an attendant relative poor outcome. Substantial subsidy of the cost of management of this disease would improve its outcome.

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Extracellular Hydrolytic Enzymes and Location of Multidrug Resistance Markers in Urinary Bacterial Isolates

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ABSTRACT

The extracellular hydrolytic enzymes and location of multidrug resistance markers in urinary bacterial isolates were determined using appropriate culture media, disc diffusion technique and acridine orange. The percentages of occurrence of the isolates were: Escherichia coli (28.1 %), Staphylococcus aureus (16.1 %), Pseudomonas aeruginosa (14.3 %), Klebsiella pneumoniae (8.9 %), Enterococcus faecalis (7.1 %), Proteus spp. (7.1 %), Coagulase negative (CoN) Staphylococcus spp. (7.1 %), Citrobacter freundii (5.4%) and Streptococcus spp. (5.4 %). The antibiogram results evidently showed ciprofloxacin as the most effective drug against the uropathogens. All the Streptococcus spp. were sensitive to Gentamycin; between 70.0 % and 75.0 % S. aureus, E. faecalis, P. aeruginosa and K. pneumoniae were sensitive to Ceftriaxone; = 40.0% Streptococcus spp and CoN Staphylococcus spp. were resistant to Nalidixic Acid, while resistance of the isolates to Penicillin, Cotrimoxazole and Augmentin ranged from 16.7 to 50.0%. The results showed that 29 (25.9%) of the isolates were resistant to at least one antibiotic and = 42 (37.5 %) isolates exhibited multiple antibiotic resistance. Of 42 multi drug resistant (MDR) isolates, 6 (14.3%) and 5 (11.9%) isolates had their entire antibiotic resistance markers located on the chromosomes and plasmids, respectively, while 31/ 42 (73.8 %) isolates had their antibiotic resistance markers residing both on the plasmids and chromosomes. Of the 23 streptomycin resistant isolates, 14 (60.9%) showed resistant to streptomycin in spite of using different volumes of acridine orange. The occurrence of the antibiotic resistance markers on plasmids can bring about the horizontal transfer of antibiotic resistance genes among the uropathogenic bacterial isolates through conjugation process.

Keywords: Hydrolytic, Enzymes, Plasmid, Chromosome, Resistance, Antibiotic

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections in humans, affecting people of all ages, from the pediatric to the geriatric age groups[1,2,3]. Worldwide, about 150 million people are diagnosed each year with UTIs, costing in excess of 6 billion dollars[4,5]. The UTIs may involve only the lower urinary tract or both the upper and the lower tracts, and encompasses a spectrum of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urethritis[6]. The urinary tract consists of various organs of the body involved in the production, storage and excretion of urine[7,8]. The prevalence of UTI is higher in women than in men, owing to several clinical factors such as hormonal effects, physiological, anatomical and behavioural patterns[9]. *E. coli*, *Pseudomonas* spp, *Proteus* spp., *Enterobacter* spp. *Enterococci* spp., *Klebsiella* spp., group B *Streptococci* and *Staphylococcus* spp are often implicated as causative agents of clinical or subclinical UTIs[6,10].

The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world with high level of poverty, ignorance, poor hygienic practices, inappropriate use of antimicrobial agents, and high prevalence of fake and spurious drugs of questionable quality in circulation[11,12]. The changing patterns in the causative agents of UTIs and their sensitivities to commonly prescribed antibiotics have been reported[13]. Bacterial isolates demonstrate a high frequency of antimicrobial resistance due to the presence and heterogeneous nature of plasmids[14,15,16]. Plasmids can be either conjugative or non-conjugative, depending on whether or not the plasmid carries a set of transfer genes, which perform the complex process of conjugation. Some microorganisms possess virulence factors that enhance or contribute to their pathogenicity[17,18]. These virulence factors such as toxins, cell surface protein and extracellular hydrolytic enzymes (protease, amylase, neuraminidases, phospholipases, urease and lipase) are frequently involved in the direct interaction with the host tissues or in concealing the bacterial surface from

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the host's defense mechanism[19,20]. Virulence factors can alter host cellular receptors in a manner that can subvert the binding of their usual ligands and alter microbial behaviour to promote invasiveness and serum resistance[21]. The extracellular hydrolytic enzymes also damage host cells and provide nutrients by digesting substrates into smaller components that can be assimilated by microbes[22]. Thus, this study aimed to determine the extracellular hydrolytic enzymes (virulence factors) and location of multidrug resistance markers in urinary bacterial isolates.

MATERIAL AND METHODS

Collection of Samples

Mid-stream urine (MSU) samples were aseptically collected using sterile, wide-necked, leak-proof universal bottles from students of University of Uyo between June and September, 2016, after obtaining their informed consent. The samples were collected from the students who were not on antimicrobial therapy or who had not taken antimicrobials for at least one week prior to sampling. Samples were kept on ice immediately after collection and transported to the microbiology laboratory for bacteriological analyses.

Bacteriology of Mid-stream Urine Samples

One microlitre (µl) of each uncentrifuged, uniformly mixed, mid-stream urine sample was aseptically inoculated onto plates of Cysteine Lactose Electrolyte Deficient (Oxoid, UK) and incubated aerobically at 37ºC for 24 hrs. After incubation, the colonies obtained were subcultured onto nutrient agar plates, incubated for 24 hrs at 37ºC, and streaked onto nutrient agar slant. The isolates were characterized and identified using standard conventional microbiological techniques.

Detection of Deoxyribonuclease (DNase) Producing Bacterial Isolates

The DNase producers were determined using Deoxyribonuclease agar[18,23]. The DNase agar plates were spot inoculated with the bacterial isolates using sterilized inoculating loop and aerobically incubated at 37°C for 24 hr. The growths on the agar plates were flooded with 1N HCL. Isolates were considered positive for DNase production if there was formation of clear zones around the colonies.

Detection of Thermostable Deoxyribonuclease (TNase) Producing Bacterial Isolates

The TNase producers were detected using Toluidine Blue DNA agar[24]. Toluidine Blue DNA agar plates were spot inoculated with the *bacterial* isolates using sterilized inoculating loop and aerobically incubated at 37 °C for 24hr. Formation of pink halos around the colonies indicated the production of TNase[18].

Detection of Lipase Producing Bacterial Isolates

The lipase producing isolates were detected using tributyrin agar. The plates of tributyrin agar were spot inoculated with the isolates using sterilized inoculating loop and incubated for 24-48 hr at 37 °C. Clear zones around the colonies indicated the production of lipase.

Detection of Gelatinase Producing Bacterial Isolates

The gelatinase producing bacterial isolates was detected using gelatin agar. The isolates were streaked on gelatin agar plates and incubated for 24-48 hr at 37 ºC. Zone of clearance around the bacterial colonies indicated gelatinase production.

Detection of Caseinase Producing Bacterial Isolates

Caseinase producing bacterial isolates was detected using skimmed milk agar. The isolates were streaked on skimmed milk agar plates and incubated aerobically for 24 hrs at 37 ºC. Transparent zones around the bacterial colonies indicated caseinase production.

Detection of Amylase Producing Bacterial Isolates

Amylase producing bacterial isolates was detected using starch agar. The bacterial isolates were streaked onto starch agar plates and aerobically incubated for 24-48 hr at 37 ºC. After incubation, 3 drops of 10 % Lugol iodine was put on the culture plates and allowed to react for 10 min. Clear zones around the bacterial colonies indicated amylase production.

Detection of Lecithinase (Phospholipase) Producing Bacterial Isolates

Lecithinase producing bacterial isolates was detected using egg yolk agar. The isolates were streaked on egg yolk agar plates and

aerobically incubated for 24 hrs at 37 ºC. Opaque zones around the bacterial colonies indicated positive lecithinase reaction.

Detection of Haemolysin Producing Bacterial Isolates

The haemolytic activities of the bacterial isolate were identified by the presence of clear or greenish colouration halos around the colonies on blood agar. The bacterial suspensions were streaked onto blood agar plates and aerobically incubated for 24 hr at 37 ºC. Haemolytic zone around colonies after incubation were made and recorded.

Antibiotic Susceptibility Testing

In vitro antibiotic susceptibility of bacterial isolates was determined using Kirby-Bauer disk diffusion technique. Ten microlitres of each bacterial isolate prepared directly from an overnight agar plate adjusted to be equivalent to 0.5 McFarland Standard was inoculated onto each of the Petri dishes containing Mueller-Hinton Agar (Oxoid, UK). The antibiotics tested were: Penicillin (PEN,10µg), Streptomycin (STP, 10µg), Ofloxacin (OFL, 5µg), Ceftriaxone (CEP, 30µg), Nalidixic Acid (NA, 30µg), Gentamycin (GEN, 10µg), Pefloxacin (PEF, 5µg), Augmentin (AUG, 30µg), Ciprofloxacin (CIP, 5µg) and Cotrimoxazole (COT, 25µg) (Oxoid, UK) and were aseptically placed onto the surfaces of the culture plates with a sterile forceps, gently pressed to ensure even contact and incubated aerobically at 37°C for 18 hr. After incubation, the diameters of inhibitory zones around the antibiotic discs were observed and measured in millimeters using a ruler. The interpretation of the measurement as sensitive, intermediate and resistant was made according to the Clinical and Laboratory Standards Institute's Guidelines[25].

Determination of Multiple Antibiotic Resistance Index (MAR)

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=*x/y,* where *'x'* was the number of antibiotics to which test isolate displayed resistance and *'y'* was the total number of antibiotics to which the test isolates has been evaluated for sensitivity[26,27]. Isolates that were resistant to three or more antibiotics were taken to be multiple antibiotic resistant[28].

Presumptive Detection of Location of Antibiotic Resistance Markers in the Isolates

Plasmid elimination method was used to determine the location (chromosome-encoded or plasmid - borne) of the antibiotic resistance marker(s)**.** The elimination (curing) of the resistant plasmids of the MDR bacterial isolates was done using sub-inhibitory concentration of acridine orange with slight modifications[27,29]. Each of the bacterial isolates was grown for 24 hr at 37 °C in a test-tube containing 9 ml of nutrient broth (pH; 7.6) and 1 ml of acridine orange ($1g.1000^{-ml}$). After overnight incubation, the broth culture was shaken and loopful subcultured onto Mueller-Hinton Agar (MHA) plates and antibiotic sensitivity testing was carried out as previously described. Cured markers were determined by comparison between the pre-curing and post-curing antibiograms of the isolates. Loss of resistance markers was indicative of plasmid-mediated resistance (antibiotic resistance markers located on the plasmids), while resistance markers expressed after curing were regarded as being chromosome-mediated (antibiotic resistance markers located on the chromosome).

Effect of Different Volumes/Concentrations of Acridine Orange on Streptomycin Resistant Urinary Bacterial Isolates and the Inhibitory Zone

The effects of different volumes and concentrations of acridine orange ($1g.1000^{-ml}$) on streptomycin resistant (STP^r) bacterial isolates and the inhibitory zones were determined using the method of[27] with slight modification. Each of the STP^r bacterial isolates was

grown at 37°C for 24 hr in each test tube "A" containing 9 ml of nutrient broth and 1 ml of acridine orange. Also, each of the STP^r bacterial isolates was grown for 24 hr at 37°C in each test tube "B" containing 9 ml of nutrient broth and 2 ml of acridine orange, while STP^r broth culture in each test tube "C" contained 9 ml of nutrient broth and 4 ml of acridine orange, and was incubated at 37°C for 24 hr. After overnight incubation, each of the broth cultures was shaken and 0.1 ml was sub-cultured onto each MHA plate. Streptomycin disc was aseptically placed onto the surfaces of each MHA plate with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37°C for 18-24 hr. Inhibitory zones after incubation were observed and measured in millimeters using a ruler.

RESULTS

The morphological and biochemical characteristics of nine species of bacteria, comprising four (4) Gram positive and five (5) Gram negative bacteria obtained from the MSU samples and their acridine orange treated derivatives are shown in Table 1. One hundred and twelve (112) bacterial isolates were obtained from the MSU samples. The Gram positive bacteria isolated were *Staphylococcus aureus*, Coagulase negative (CoN) *Staphylococcus* spp, *Streptococcus* spp and *Enterococcus faecalis*, while the Gram negative bacteria were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus* spp. and *Citrobacter freundii* (Table 1). The percentages of occurrence of bacterial isolates from MSU samples are shown in Fig 1. The bacterial isolate with the highest percentage of occurrence was *E. coli* having 28.1 %; followed by *S. aureus*, *P. aeruginosa* and *K. pneumoniae* with 16.1 %, 14.3 % and 8.9 %, respectively. *E. faecalis, Proteus* spp. and CoN *Staphylococcus* spp had 7.1 % each, while the bacterial isolates with the lowest percentage of occurrence were *C. freundii* and *Streptococcus* spp with 5.4 % each (Fig. 1).

Table 1: Morphological, Biochemical Characteristics of Bacterial Isolates and Their PlasmidCured Derivatives (Acridine- Orange Treated Bacterial Isolates)

	Uncured Bacterial Isolates									Acridine Orange Treated (Cured) Bacterial Isolates								
Tests	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
Morphology	cocci	rod	rod	cocci	cocci	rod	rod	cocci	rod	cocci	rod	rod	cocci	cocci	rod	rod	cocci	rod
Gram Staining	+	-	-	+	+	-	-	+	-	+	-	-	+	+	-	-	+	-
Catalase	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+	-	+
Citrate Utilization	+	-	+	-	+	+	+	-	+	+	-	+	-	+	+	+	-	+
Oxidase	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Coagulase	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Indole	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Urease	+	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+
Motility	-	-	+	+	-	+	+	+	-	-	-	+	+	-	+	+	+	-
Methyl Red	+	-	-	+	+	-	-	+	-	+	-	-	+	+	-	-	+	-
Vogues Proskauer	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+
Glucose	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Lactose	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	+	+

Key: - : Negative; + : Positive; a : *S. aureus*; b : *E. coli*; c : *P. aureginosa*; d : *Streptococcus* spp; e : CoN *Staphylococcus* spp; f : *Proteus* spp; g : *C. freundii* ; h : *E. faecalis*; i : *K. Pneumoniae.*

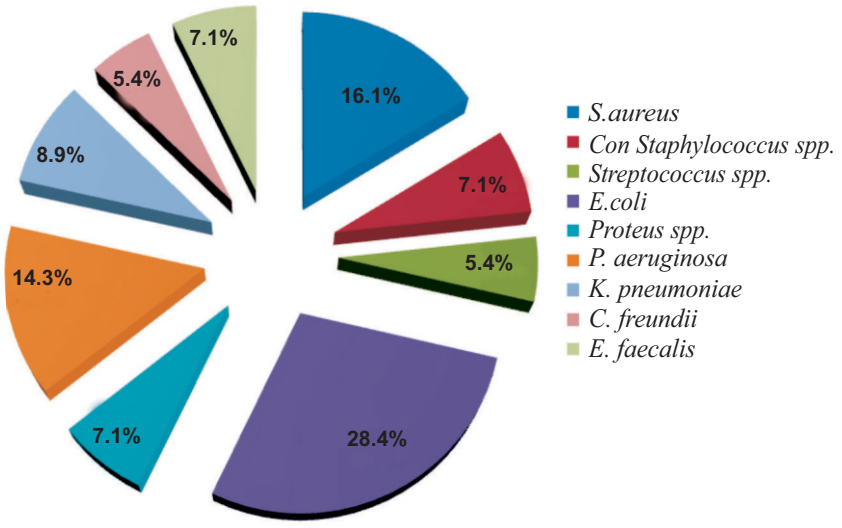

Fig 1: Percentage of Occurrence of Bacteria Isolated from Urine Samples

The results showed marked variabilities of antibiotics sensitivity among the bacterial isolates from the MSU samples. These bacterial isolates were highly sensitive to CIP, CEF and STP (Tables 2 and 3). All the *Streptococcus* spp isolated were sensitive to GEN, while between 70.0 % and 75.0 % *S. aureus, E. faecalis, E. coli, P. aeruginosa* and *K. pneumoniae* were sensitive to CEP (Tables 2 and 3). More than 40.0% *E. faecalis, Streptococcus* spp and CoN *Staphylococcus* spp were resistant to NA, while = 25 % of the bacterial isolates were resistant to PEN, OFL, NA, COT and AUG (Table 2). Of the 32 *E. coli* isolated, 10 (31.3%), 14 (43.8%), 11 (34.4%) and 9 (28.1%) were resistant to PEF, AUG, COT and PEN, respectively (Table 3).

Table 2: Antibacterial Susceptibility of Gram Positive Bacterial Isolates

	***S. aureus* (n=18)**			**CoN *Staphylococcus* spp. (n=8)**			***Streptococcus* spp (n= 6)**			***E. faecalis* (n= 8)**		
Antibiotics	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
	No (%)	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**
Penicillin	10(55.6)	3(16.7	5(27.8)	4(50.0)	0(0.0)	4(50.0)	2(33.3)	1(16.7)	3(50.0)	3(37.5)	2(25.0)	3(37.5)
Ceftriaxone	13(72.2)	1(5.6)	4(22.2)	3(37.5)	1(12.5)	4(50.0)	3(50.0)	1(16.7)	2(33.3)	6(75.0)	0(0.0)	2(25.0)
Ofloxacin	10(55.6)	5(27.8)	3(16.7)	4(50.0)	1(12.5)	3(37.5)	3(50.0)	0(0.0)	3(50.0)	2(25.0)	3(37.5)	3(37.5)
Nalidixic Acid	12(66.7)	2(11.1)	4(22.2)	2(25.0)	2(25.0)	4(50.0)	2(33.3)	1(16.7)	3(50.0)	4(50.0)	0(0.0)	4(50.0)
Gentamycin	9(50.0)	5(27.8)	4(22.2)	5(62.5)	1(12.5)	2(25.0)	6(100)	0(0.0)	0(0.0)	3(37.5)	1(12.5)	4(50.0)
Pefloxacin	11(61.1)	1(5.6)	6(33.3)	3(37.5)	1(12.5)	4(50.0)	4(66.7)	0(0.0)	2(33.3)	1(12.5)	1(12.5)	6(75.0)
Augmentin	10(55.6)	4(22.2)	4(22.2)	2(25.0)	2(25.0)	4(50.0)	2(33.3)	2(33.3)	2(33.3)	4(50.0)	0(0.0)	4(50.0)
Ciprofloxacin	13(72.2)	2(11.1)	3(16.7)	6(75.0)	0(0.0)	2(25.0)	2(33.3)	2(33.3)	2(33.3)	5(62.5)	1(12.5)	2(25.0)
Cotrimoxazole	9(50.0)	4(22.2)	5(27.8)	4(50.0)	0(0.0)	4(50.0)	2(33.3)	1(16.7)	3(50.0)	5(62.5)	1(12.5)	2(25.0)
Streptomycin	9(50.0)	3(16.7)	6(33.3)	2(25.0)	2(25.0)	4(50.0)	4(66.7)	0(0.0)	2(33.3)	6(75.0)	0(0.0)	2(25.0)

Key: S: Sensitive; I: Intermediate; R: Resistant; CoN: Coagulase negative.

Table 3: Antibacterial Susceptibility of Gram Negative Bacterial Isolates

	***E. coli* (n = 32)**			***Proteus* spp. (n= 8)**			***P. aeruginosa* (n = 16)**			***K. pneumoniae* (n = 10)**			***C. freundii* (n = 6)**		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Antibiotics	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	No (%)	**No (%)**
Penicillin	20(62.5)	3(9.4)	9(28.1)	4(50.0)	0(0.0)	4(50.0)	10(62.5)	1(6.3)	5(31.3)	6(60.0)	1(10.0)	3(20.0)	2(33.3)	2(33.3)	2(33.3)
Ceftriaxone	23(71.9)	3(9.4)	6(18.8)	5(62.5)	1(12.5)	2(25.0)	12(75.0)	2(12.5)	2(12.5)	7(70.0)	1(10.0)	2(20.0)	4(66.7)	0(0.0)	2(33.3)
Ofloxacin	19(59.4)	5(15.6)	8(25.0)	4(50.0)	2(25.0)	2(25.0)	8(50.0)	2(12.5)	6(37.5)	6(60.0)	2(20.0)	2(20.0)	4(66.7)	1(16.7)	1(16.7)
Nalidixic Acid	20(62.5)	4(12.5)	8(25.0)	2(25.0)	2(25.0)	4(50.0)	12(75.0)	0(0.0)	4(25.0)	5(50.0)	1(10.0)	4(40.0)	3(50.0)	1(16.7)	2(33.3)
Gentamycin	18(56.3)	5(15.6)	9(28.1)	3(37.5)	1(12.5)	4(50.0)	9(56.3)	3(18.8)	4(25.0)	7(70.0)	1(10.0)	2(20.0)	2(33.3)	2(33.3)	2(33.3)
Pefloxacin	19(59.4)	3(9.4)	10(31.3)	6(75.0)	0(0.0)	2(25.0)	7(43.8)	3(18.8)	6(37.5)	5(50.0)	2(20.0)	3(30.0)	3(50.0)	0(0.0)	3(50.0)
Augmentin	18(56.3)	0(0.0)	14(43.8)	6(75.6)	0(0.0)	2(25.0)	9(56.3)	1(6.3)	6(37.5)	5(50.0)	1(10.0)	4(40.0)	3(50.0)	1(16.7)	2(33.3)
Ciprofloxacin	25(78.1)	2(6.3)	5(15.6)	4(50.0)	1(12.5)	3(37.5)	10(62.5)	3(18.8)	3(18.8)	5(50.0)	3(30.0)	2(20.0)	4(66.7)	1(16.7)	1(16.7)
Cotrimoxazole	15(46.9)	6(18.8)	11(34.4)	2(25.0)	2(25.0)	4(50.0)	7(43.8)	2(12.5)	7(43.8)	4(40.0)	3(30.0)	3(30.0)	3(50.0)	2(33.3)	1(16.7)
Streptomycin	20(62.5)	4(12.5)	8(25.0)	6(75.0)	0(0.0)	2(25.0)	9(56.3)	2(12.5)	5(31.3)	6(60.0)	0(0.0)	4(4 0.0)	2(33.3)	1(16.7)	3(50.0)

Key: S: Sensitive; I: Intermediate; R: Resistant.

The MAR indices of the isolates are shown in Table 4. The results showed that 29 (25.9%) and 41(36.6%) isolates were resistant to at least one antibiotic and 2 antibiotics, respectively, while 42 (37.5 %) isolates exhibited multiple antibiotic resistance. The antibiotic resistant *S. aureus*, *Streptococcus* spp., *C. freundii* and *Proteus* spp had MAR indices ranging from 0.3 to 0.6. The antibiotic resistant *E.coli* had the widest range of MAR indices of 0.4 to 0.9 (= 9 antibiotics) (Table 4).

Table 4: Multiple Antibiotic Resistance (MAR) Indices of Bacterial Isolates

MAR Index	Number / Percentage (%)									Total No (%)
	SA	CS	SS	EC	EF	CF	PA	PS	KP	
0.1	8 (44.4)	0 (0.0)	0 (0.0)	13(43.8)	0 (0.0)	1 (16.7)	5 (31.3)	0 (0.0)	2 (20.0)	29 (25.9)
0.2	4 (22.2)	3 (37.5)	2 (33.3)	13 (40.6)	3 (37.5)	2 (33.3)	6 (37.5)	4 (50.0)	4 (40.0)	41 (36.6)
0.3	1 (5.6)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	1 (10.0)	4 (3.6)
0.4	1 (5.6)	1 (12.5)	1 (16.7)	2 (3.1)	3 (37.5)	0 (0.0)	1 (6.3)	1 (12.5)	1 (10.0)	11 (9.8)
0.5	3 (16.7)	1 (12.5)	1 (16.7)	0 (0.0)	0 (0.0)	1 (16.7)	1 (6.3)	1 (12.5)	1 (10.0)	9 (8.0)
0.6	1 (5.6)	1 (12.5)	1 (16.7)	1 (3.1)	0 (0.0)	1 (16.7)	1 (6.3)	2 (25.0)	0 (0.0)	8 (7.1)
0.7	0 (0.0)	2 (25.0)	0 (0.0)	1 (3.1)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	6 (5.4)
0.8	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	2 (12.5)	0 (0.0)	0 (0.0)	3 (2.7)
0.9	0 (0.0)	0 (0.0)	0 (0.0)	1(3 1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)

Keys: SA: *S. aureus;* CS: CoN *Staphylococcus* spp; SS: *Streptococcus* spp; EF: *E. faecalis;* EC: *E. Coli*; CF: *C. freundii*; PS: *Proteus* spp; PA: *P. aeruginosa;* KP: *K. Pneumoniae*

Table 5 shows the pre-curing and post-curing antibiotic resistance profiles of bacterial isolates from MSU samples. Of 42 multidrug resistant (MDR) bacterial isolates, 6 (14.3%) bacterial isolates, comprising of *S. aureus* SA-06, *Streptococcus* spp SS-06, *E. faecalis* EF-01, *P. aeruginosa* PA-14, *Klebsiella* spp KP-09 and *Proteus* spp PS-03 had their entire antibiotic resistant markers located on the chromosomes. Five bacterial isolates, consisting of three Gram positive bacteria (*S. aureus* SA-01, *Streptococcus* spp SS-03 and *E. faecalis* EF-05) and two Gram negative bacteria (*E. coli* EC-06 and *Klebsiella* spp KP-08) had all their antibiotic resistant markers located on the plasmids, while 31/ 42 (73.8 %) of the MDR bacterial isolates had their antibiotic resistant markers residing both on the self-replicative extra-chromosomal plasmids and the chromosomes. Among these bacteria were: *S. aureus* SA-03; CoN *Staphylococcus* spp CS-01, *C. freundii* CF-04, *P. aeruginosa* PA-06 and *Streptococcus* spp SS-05 (Tables 5 and 6). Although, the pre-curing antibiotic resistant profile of *S. aureus* SA-14 and *S. aureus* SA-17 was PEN-CEF-OFL-PEP-STP, there was slight difference in the their post - curing antibiotic resistant profiles, indicating that both organisms (*S. aureus* SA-14 and *S. aureus* SA-17) might be of different strains (Table 5a). *P. aeruginosa* PA-01 and *P. aeruginosa* PA-06 had the same pre-curing and post - curing antibiotic resistant profiles of PEN-OFL-NA-GEN-AUG-CIP-COT-STP and PEN-OFL-NA-CIP-STP, respectively, indicating that both organisms (*P. aeruginosa* PA-01 and *P. aeruginosa* PA-06) might be of the same strains (Table 6).

Table 5: Pre-curing and Post-curing Antibiograms of Gram Positive Bacterial Isolates

Bacterial Isolates	Isolates Code	Antibiotic Resistant Profile	
		Pre-curing	Post-curing
S. aureus	SA-01	PEN-GEN-PEF	---
	SA-02	CEF-GEN-AUG-COT	GEN-AUG
	SA-03	PEN-CEF-OFL-PEF-STP	PEN-OFL-PEF-STP
	SA-06	PEN-NA-GEN-PEF-AUG-STP	PEN-NA-GEN-PEF-AUG-STP
	SA-14	PEN- CEF-OFL-PEF-STP	PEN-OFL-PEF-STP
	SA-17	PEN-CEF-OFL-PEF-STP	PEN-OFL-PEF
Con *S.* spp	CS-01	PEN-CEF-NA-GEN-AUG-COT-STP	CEF-NA-GEN-COT
	CS-04	PEN-CEF-NA-GEN-AUG-COT-STP	PEN-CEF-NA-GEN-COT
	CS-05	PEN-CEF-OFL-PEF-AUG-COT	CEF-PEF-AUG
	CS-07	CEF-PEF-AUG-CIP-STP	PEF-AUG-CIP-STP
	CS-08	NA-PEF-CIP-COT	NA-PEF-CIP
Streptococcus spp	SS-02	PEN-CEF-OFL-NA-COT-STP	CEF-OFL-NA
	SS-03	PEN-PEF-STP	---
	SS-05	OFL-NA-AUG-CIP-COT	OFL-NA-CIP
	SS-06	CEF-NA-PEF-COT	CEF-NA-PEF-COT
E. faecalis	EF-01	CEF-PEF-COT-STP	CEF-PEF-COT-STP
	EF-02	PEN-OFL-NA-GEN-PEF-AUG-CIP	PEN-NA-PEF-AUG-CIP
	EF-04	PEN-OFL-NA-GEN-PEF-AUG-CIP	PEN-NA-PEF-AUG-CIP
	EF-05	CEF-NA-PEF-STP	---
	EF-08	PEN-PEF-AUG-COT	PEF-AUG

Key: CoN *S*: Coagulase negative *Staphylococcus*; PEN: Penicillin; CEF: Ceftriaxone; OFL: Ofloxacin; NA: Nalidixic Acid; GEN: Gentamycin; PEF: Pefloxacin; AUG: Augmentin; CIP: Ciprofloxacin; COT: Cotrimoxazole; STP: Streptomycin

Table 6: Pre-curing and Post-curing Antibiograms of Gram Negative Bacterial Isolates

Bacterial Isolates	Isolates Code	Antibiotic Resistant Pattern	
		Pre-curing	Post-curing
E. coli	EC-01	PEN-OFL-NA-GEN-AUG-CIP-COT-STP	GEN-AUG-CIP-STP
	EC-02	PEN-OFL-AUG-CIP-COT-STP	PEN-OFL-CIP-COT
	EC-06	OFL-NA-GEN-PEF	---
	EC-18	PEN-CEF-OFL-NA-GEN-PEF-AUG-CIP-COT-STP	PEN-OFL-NA-PEF-CIP-STP
	EC-22	CEF-OFL-NA-GEN-PEF-AUG-CIP-COT-STP	OFL-NA-PEF-AUG-CIP-COT-STP
	EC-27	CEF-OFL-NA-PEF-AUG-CIP-COT	NA-PEF-AUG-CIP-COT
P. aeruginosa	PA-01	PEN-OFL-NA-GEN-AUG-CIP-COT-STP	PEN-OFL-NA-CIP-STP
	PA-06	PEN-OFL-NA-GEN-AUG-CIP-COT-STP	PEN-OFL-NA-CIP-STP
	PA-09	PEN-OFL-GEN-PEF-AUG	GEN-PEF
	PA-10	CEF-OFL-PEF-AUG-COT-STP	OFL-PEF--STP
	PA-14	NA-PEF-CIP-COT	NA-PEF-CIP-COT
K. pneumoniae	KP-03	OFL-NA-PEF-AUG-CIP	OFL-NA-CIP
	KP-05	PEN-CEP-OFL-NA-GEN-CIP-COT	PEN--NA-CIP-COT
	KP-08	PEN-AUG-STP	---
	KP-09	CEF-NA-COT -STP	CEF-NA-COT -STP
C. freundii	CF-01	CEF-NA-CIP	CEF-NA-CIP
	CF-03	PEN-CEF--PEF-AUG-STP	PEN-PEF
	CF-04	PEN-OFL-GEN-AUG-COT-STP	PEN-OFL-COT-STP
Proteus spp	PS-01	PEN- CEF-NA-GEN-COT-STP	CEF-COT-STP
	PS-03	NA-GEN-AUG-CIP	NA-GEN-AUG-CIP
	PS-06	PEN-OFL-GEN-PEF-COT	PEN-OFL--PEF-COT
	PS-07	PEN-CEF-OFL-NA-GEN-COT	PEN--OFL-NA

Keys: PEN: Penicillin; CEF: Ceftriaxone; OFL: Ofloxacin; NA: Nalidixic Acid; GEN: Gentamycin; PEF: Pefloxacin; AUG: Augmentin; CIP: Ciprofloxacin; COT: Cotrimoxazole; STP: Streptomycin

The effect of different volume / concentrations of acridine orange on streptomycin resistant (STP^r) bacteria and inhibitory zones are shown in Table 7. The results showed that the higher the volume of the acridine orange used for curing the streptomycin resistance in the bacteria isolated from MSU samples, the wider the zone of inhibition obtained. Of the 23 STP^r bacterial isolates, 14 (60.9%) were still resistant to streptomycin (plasmid not cured of streptomycin resistance) despite using different volumes of acridine orange (Table 7).

Of the 112 bacterial isolates tested, 27 (24.1%), 21 (18.8%), 25 (22.3%) and 25 (22.3%) produced Dnase, TNase, caseinase and gelatinase, respectively, while between 39(34.8%) and 52 (46.4%) bacterial isolates were lipase, amylase, lecithinase and haemolysin producers (Table 8).

Table 7: Effect of Different Volumes / Concentrations of Acridine Orange on Streptomycin Resistant Bacteria and Inhibitory Zones

Bacterial Isolates	Isolate Code	InoculumSize (cells / ml) mm ± S.D x 10^6	Pre-curing Inhibitory Zone (mm)	Post-curing Inhibitory Zone (mm) using Acridine Orange (1g /1000 ml) 1 ml	2 ml	4 ml	Inference
S. aureus	SA-03	1.3 ± 0.6	7	7	8	8	PNC
S. aureus	SA-06	1.5 ± 1.5	NZ	NZ	NZ	NZ	PNC
S. aureus	SA-14	2.2 ± 0.6	NZ	NZ	7	10	PNC
S. aureus	SA-17	1.7 ± 1.0	9	15	18	22	PC
Streptococcus spp	SS-02	1.4 ±1.0	11	17	18	20	PC
Streptococcus spp	SS-03	2.0 ± 0.6	7	14	16	19	PC
CoN *S.* spp	CS-01	1.9 ± 1.5	11	16	21	24	PC
CoN *S.* spp	CS-04	1.1 ± 0.6	9	16	17	19	PC
CoN *S.* spp	CS-07	1.7 ± 1.5	NZ	NZ	NZ	NZ	PNC
E. coli	EC-01	1.5 ± 1.0	NZ	NZ	NZ	NZ	PNC
E. coli	EC-02	2.2 ± 0.6	8	15	19	22	PC
E. coli	EC-18	2.4 ± 0.6	NZ	NZ	NZ	NZ	PNC
E coli	EC-22	1.7 ± 1.5	NZ	7	7	7	PNC
P. aeruginosa	PS-01	1.3 ± 1.0	10	10	10	10	PNC
P. aeruginosa	PS-06	1.7 ± 0.6	9	9	9	10	PNC
P. aeruginosa	PS-10	2.4 ± 1.5	NZ	NZ	NZ	NZ	PNC
K. pneumoniae	KP-08	1.9 ± 1.5	11	15	17	20	PC
K. pneumoniae	KP-09	1.6 ± 1.5	NZ	NZ	NZ	NZ	PNC
E. faecalis	EF-01	2.0 ± 0.6	10	10	10	10	PNC
E. faecalis	EF-05	2.1 ± 1.0	8	16	19	24	PC
C. freundii	CF-03	1.8 ± 0.6	11	14	15	17	PC
C. freundii	CF-04	1.8 ± 0.6	NZ	NZ	NZ	NZ	PNC
Proteus spp	PS-01	2.3 ± 1.5	7	7	7	9	PNC

Keys: CoN *S*: coagulase negative *Staphylococcus*; NZ: No inhibitory zone; PC: Plasmid cured; PNC: Plasmid not cured; mm: mean; S.D: Standard Deviation

Table 8: Extracellular Hydrolytic Enzymes in Urinary Bacterial Isolates

Bacterial Isolates	No of Isolates	Extracellular Hydrolytic Enzymes DNase No (%)	TNase No (%)	Lipase No (%)	Amylase No (%)	Caseinase No (%)	Gelatinase No (%)	Lecithinase No (%)	Haemolysin No (%)
S. aureus	18	15(83.3)	8(44.4)	10(55.6)	11(61.1)	7(38.9)	6(33.3)	11(61.1)	14(77.8)
E. coli	32	6(18.6)	4(12.5)	4(12.5)	10(31.3)	0(0.0)	0(0.0)	2(6.25)	13(40.6)
P. aureginosa	16	0(0.0)	6(37.5)	10(62.5)	6(37.5)	5(31.3)	6(37.5)	12(75.0)	3(18.8)
Streptococcus spp	6	2(33.3)	0(0.0)	3(50.0)	2(33.3)	3(50.0)	3(50.0)	3(50.0)	4(66.7)
CoN *S.* spp	8	2(25.0)	2(25.0)	5(62.5)	0(0.0)	1(12.5)	0(0.0)	2(25.0)	6(75.0)
Proteus Spp	8	0(0.0)	0(0.0)	3(37.5)	4(50.0)	3(37.5)	3(37.5)	2(25.0)	2(25.0)
C. freundii	6	0(0.0)	0(0.0)	2(33.3)	2(33.3)	1(16.7)	0(0.0)	0(0.0)	0(0.0)
E. faecalis	8	2(25.0)	1(12.5)	3(37.5)	3(37.5)	4(50.0)	4(50.0)	1(12.5)	5(62.5)
K. pneumoniae	10	0(0.0)	0(0.0)	5(50.0)	4(40.0)	1(10.0)	3(30.0)	6(60.0)	5(50.0)
Total	112	27(24.1)	21(18.8)	45(40.2)	42(37.5)	25(22.3)	25(22.3)	39(34.8)	52(46.4)

Key: CoN *S*: coagulase negative *Staphylococcus*

DISCUSSION

Mid-stream urine samples are among the numerous specimens frequently sent for analysis so as to reduce the morbidity and mortality attributed to UTIs[30]. The isolation of *E. coli, E. faecalis*, *K. pneumonia, Proteus* spp., *P. aeruginosa* and *S. aureus* from the MSU samples in our study is in consonant with other researchers who reported the occurrence of these uropathogens in the MSU samples[31,32]. *E. coil* had the highest occurrence in this study and this agrees with the reports of Lowy[31] and Gupta *et al.*[32] but differs from the reports of[33]. A number of studies have established the geographical variability of occurrence of uropathogens among populations with the predominance of Gram-negative bacteria especially *Enterobacteriaceae* such as *E. coli* and *Enterobacter* spp[34,35], while in some regions of the world, *S. aureus* had the highest prevalence and followed by *E. Coli*[36].

Effective management of patients with bacterial UTIs generally depends on the identification of aetiologic organisms and the choice of effective antibiotics[10]. The results of the antibiotic susceptibility profiles of the bacterial isolates from MSU samples of the subjects showed varied percentages of sensitivity and resistance. The high sensitivity of *S. aureus* and *E. coli* to Ciprofloxacin in this study is similar to the results of Nester *et al.* (1998). The moderately high sensitivity of the uropathogens to Gentamycin corroborates the previous reports of[37]. The resistance of ≥ 33.3% *S. aureus* and ≥ 25.0% *E. coli* to Streptomycin in this study is in agreement with the results obtained by Nester *et al.*[38]

The MAR indices of these uropathogens ranged from 0.3 to 0.9 and MAR index higher than 0.2 has been said to be an indication of isolates originating from an environment where antibiotics were often used[26,39]. Antimicrobial resistance can arise through acquisition of genetic material encoding enzymes that inactivate a particular antibiotic. In our study, the antibiotic resistance markers of the MDR uropathogens were located on plasmids, chromosome or both. Plasmid replication is inhibited by various agents principally acridine orange that intercalates between the bases of DNA, without inhibiting the chromosomal DNA replication[27]. The loss of antibiotic resistance markers in the MDR uropathogens in this study using acridine orange is in agreement with the previous results of similar studies[27,40,41]. The occurrence of the antibiotic resistance markers in chromosomes observed in this study also agrees with the results of Yah *et al.*[40]

Extracellular hydrolytic enzymes are frequently involved in the direct interaction with the host tissues or in concealing the bacterial surface from the host's defense mechanism[19,20]. These extracellular hydrolytic enzymes specifically favour the development of pyelonephritis, cystitis, and asymptomatic bacteriuria[42]. In our study, *S. aureus* isolated from MSU samples produced DNase and this is in concordance with[18,23]. Isolation of amylase producing *Streptococcus spp and Lipase producing Pseudomonas spp from MSU samples in this study corroborates the previous reports of*[19,20].

CONCLUSION

The observed resistance of the uropathogens to antibiotics is a probable indication of earlier exposure of these uropathogens to antibiotics and/or unselective use of antibiotics among the subjects, which has favoured the emergence of resistance bacterial strains. The occurrence of the antibiotic resistance markers on plasmids can bring about the horizontal transfer of antibiotic resistance marker genes among the bacterial isolates through conjugation process.

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Socio-demographic Determinants of Patients Accessing Free Cataract Surgical Services in Uyo, Nigeria

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ABSTRACT

Globally, cataract blindness is the commonest, yet surgical intervention as a means of treatment is one of the most rewarding surgical interventions known because of the expected good visual outcome. Sadly, there are certain undetermined factors militating against accessing cataract surgeries. In our locality, no study has explored these factors. The objective of the study was to determine socio-demographic factors common to patients who had free cataract surgeries during an Eye Camp in Uyo, Nigeria. The study design was prospective and non-randomized of individuals who had free cataract surgeries during the period. During the one month program, Two Hundred and Fifty Three patients (n = 253) were operated upon; 146 (57.7%) were males and 107 (42.3%) were females with a mean age 59.50±14.1. Most participants were rural dwellers (152; 60.1%). Financial handicap was the commonest reason for delay in accessing cataract surgical services. In conclusion: Poor financial status of the participants was the main reason for delay in accessing treatment for cataract. Other identified barriers to cataract service uptake were fear of surgical outcome and non-maturity of cataracts.

Keywords: Socio-demographic determinants, Cataract-blindness, Uyo

INTRODUCTION

Cataract is any opacity of the crystalline lens with or without associated visual disturbances. Blindness from cataract can be described to the layperson as "white" blindness as against "black" of glaucoma to which it might be confused. This apt aphorism is derived from pupillary appearance of individuals with cataract and glaucoma respectively. Cataract blindness sharply contrasts that of glaucoma being reversible with a surgical procedure that has evolved to maximize visual outcome while shortening recovery time.

Cataract is responsible for 33% of the visual impairment worldwide and is the single most important cause of blindness affecting 51% of the global population[1]. Available data from the Nigerian National Eye Survey[2] for Blindness and Visual Impairment showed cataract as the commonest cause of severe visual impairment and blindness being responsible for 45.3% and 43.0% of cases respectively. Surveys in Latin America and the Caribbean islands showed that cataract is the leading cause of blindness and visual impairment, with 47%-87% of individuals being bilaterally blind[1,3].

Cataract surgical rate (CSR), the number of cataract operations performed per year per million population, is not widely reported among Nigerian population and as such it has been difficult to establish what factors influence cataract service uptake. Unless regional CSR increases considerably, the VISION 2020 goal of eliminating avoidable vision loss from cataract will not be met. Based on studies from India, China and Paraguay, CSR is not wholly based on the socio-economic development of a country[4-7]. Across Latin America[8,9], the CSR ranges from approximately 900-6,000, with an average of 2,672. This surpasses the World Health Organization (WHO) recommendation of 2,000 per million population set to clear cataract backlog[10]. In many regions of Africa, however, cataract surgical rates are less than 500 per million population despite availability of quality cataract services[10-12]. Therefore, there is a need to determine factors that influence uptake of available health care to enhance policy making and program planning. With dwindling Nigerian government earnings from petroleum products, limited health resources will be properly channeled if necessary information is available to aid allocation. This study's aim was to determine the socio-demographic parameters among a group of participants who accessed a free cataract surgical outreach program. The knowledge from this study will aid Government, individuals or groups who might want to provide similar services in future.

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MATERIALS AND METHODS

This was a cross-sectional non-randomized study conducted among individuals who voluntarily sought free cataract surgical services after a state-wide media advertisement of a free Eye camp in January 2016. The location of the study was in the state capital. For the purpose of categorization, individuals from the state capital, Local Government Headquarters and a few designated towns based on availability of social amenities, were considered urban dwellers. Other people outside these definitions were categorized as rural dwellers.

All attendees of the screening program were registered. Thereafter, there was identification of those with operable cataracts and referrals of those with complicated cataracts for further assessments in the base hospital of the researchers. The diagnosis of cataract was based on torchlight exam and distant direct ophthalmoscopy. Vision was assessed with Snellen's E chart at 6 meters in a well lite environment. The inclusion criteria were individuals with VA of 6/60 or worse traceable to cataract. Those with ocular co-morbidities such hypertensive retinopathy, and glaucoma having significant cataract were also operated upon having been told that prognosis was guarded. Patients were explained to in the local language about the purpose and procedure of the study.

Due to non-availability and prohibitive cost of hiring, biometry was not done. Choice of intraocular lens (IOL) was based on the available IOL and age of patient. For older patients overall hypermetropia was the target to enable distant vision for the mostly uneducated population. Myopia was the optical aim for the younger participants who might want to read without optical aids. Cataract surgeries were done by 4 surgeons with varying degrees of competences.

Questionnaire to answer research questions on socio-demographic parameters was designed and data collected included age, gender, rural or urban residence, education, and occupation. A verbal section surveying the barriers to access or reasons that delayed access to cataract surgery was devised from the existing literature. It was developed in English and a translator for the local tribal language was used when necessary. The questionnaire was administered in interviews conducted by ophthalmic nurses or ophthalmic assistants who have been involved in similar studies.

Ethical clearance was not obtained from any institutional Ethical Review Board but Informed written consents were obtained from all subjects and study complied with the tenets of the Helsinki Declaration on research in Human Subjects. Data obtained was coded and fed into Statistical Package for the Social Sciences version 20.0 (SPSS, IBM Corp., Armonk, NY, USA). For descriptive statistics, frequencies and percentages were used for categorical variables in univariate analyses. In bivariate analyses, using p value as inferential statistics, value less than 0.05 at 95% confidence interval was considered statistically significant.

RESULTS

A total of 253 patients had free cataract surgery of whom 146 (57.7%) were males and 107 (42.3%) were females. The youngest participant was 14 years and the oldest 83 years giving a mean age of 59.50±14.1 (Table 1). Most participants were rural dwellers (152; 60.1%). Figures 1 and 2 show monthly earnings and duration of visual impairment, respectively before cataract surgery was received. Tables 2 to 4 show reasons for delay in being operated upon, educational and occupational distribution of study subjects respectively.

Table 1: Age and Sex Distribution of Subjects

Age Groups (Years)	Gender			
	Male		Female	
	Frequency	%	Frequency	%
Less than 20	2	0.8	1	0.4
20 to less than 40	20	7.9	7	2.8
40 to less than 60	49	19.4	35	13.8
60 and above	75	29.6	64	25.3
Total	146	57.7	107	42.3

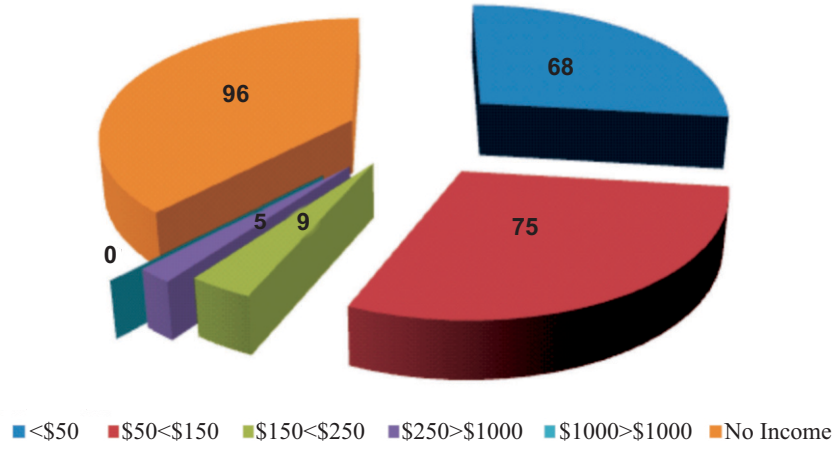

Figure 1: Monthly earnings of the study population

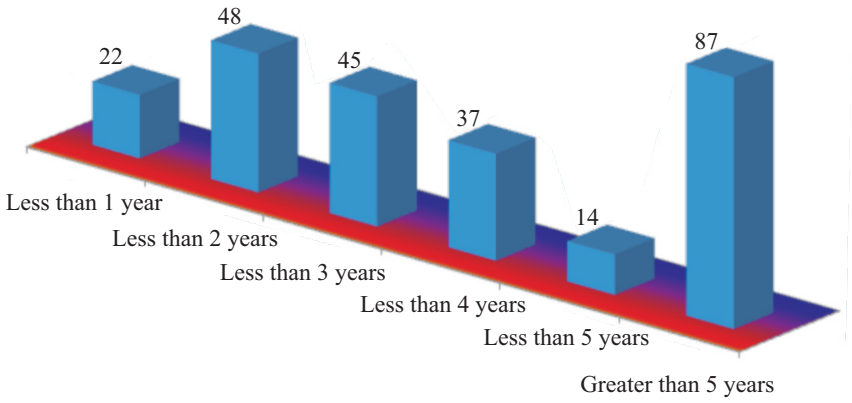

Figure 2: Average duration of visual impairment from cataract

Table 2: Reasons for delayed presentation

Reasons for delayed presentation	Frequency	Percent
There was no money	216	85.4
I was afraid of surgery	10	4.0
Doctor said it was not mature	17	6.7
Was initially painless but now painful	01	0.4
There is no reason	01	0.4
I was preparing to come to hospital	01	0.4
I had high blood pressure	02	0.8
I did not know I had cataract	03	1.2
I live far from hospital	01	0.4
I had thought vision would improve	01	0.4
Total	253	100.0

Table 3: Distribution based on level of education

Education status	Frequency	Percent
Primary	118	46.6
Secondary	56	22.1
Tertiary	27	10.7
None	52	20.6
Total	253	100.0

Financial handicap was the commonest reason for not going early to hospital for treatment. Over 90% of the patients waited for more than one before receiving cataract surgery. This corresponds to the income of the study population as over 94% earned less than $250 or no income at all. About a quarter had no formal education with another 47% having only elementary education. Petty trading and subsistence farming were the commonest forms of occupation.

Reasons for surgery delay was cross tabulated with other independent variables in bivariate analyses. There was statistically significant association with age (P<0.002 at 95% confidence interval). All p values were greater than 0.05 with monthly income, sex, occupation, education and residence, meaning there was no statistically significant association with these variables.

DISCUSSION

In developed countries, visual impairment due to cataract is not a major issue[13-17]. However, the same cannot be said of developing countries where the burden of cataract blindness is an ever present challenge despite concerted efforts by government and various Non-governmental organizations to improve on a very low CSR. Factors such as affordability, far-to-reach communities and poor access to information are reasons for cataract blindness[18-20].

Surgical fees are often reported as barriers to undergo cataract surgery. Similar to other findings from Nigeria[21], The Gambia[22],Nepal[23], and India[24], financial constraints ranked first, constituting over 85% of responses as the reason for not having or delaying cataract surgery in study participants. The average cost of Extra-Capsular Cataract Extraction (ECCE) in Nigeria is Fifty thousand Naira ($150) in government hospitals. Private Eye care facilities on the other hand charge higher to break even in running costs. Where phacoemulsification is offered, the cost is often prohibitive. These expenses are out of reach to the generality of the populace. In addition, poor access roads and lack of access to educative information on treatment options for cataract, also contribute to patients not seeking treatment early.

Table 4: Distribution based on occupation

Occupation	Frequency	Percent
Civil Servant	23	9.1
Trading	67	26.5
Farming	62	24.5
Artisan	20	7.9
Minor	01	0.4
Retiree	44	17.4
Dependant	22	8.7
Student	05	2.0
Clergy	3	1.2
Unemployed	06	2.4
Total	253	100.0

While cost has been a major reason why individuals do not access cataract services even when available, population-based studies from India indicated that cataract acceptance rates were low even when surgery was free and transportation was provided[6,25-27]. In this study, there was no statistically significant relationship between the reason for not accessing cataract surgical services and “There was no money” or literacy level. Similar findings were reported in a recent study in Ghana[12].

Studies have suggested that fear of surgery and a low perceived need for better sight contribute more significantly to not accessing cataract surgical services. Even when free surgical services are offered, there can be a lack of demand and low utilization[28-30]. Only few subjects in the current study were deterred by fear and perceived little need for improved vision. Good surgical outcomes with improved vision may have doused doubts leading to less fear. Manual Small Incision Cataract Surgery (MSICS) is widely used in our locality with significant success rates. It is possible that those who have been previously operated upon with good outcomes serve as counselors and encouragement to friends and acquaintances.

Accessibility to health facilities was not a significant cause of delay in service uptake in this study. Thus, far distance from a hospital or residence away from a main road was cited infrequently as barriers to early uptake of cataract surgical service. This has also been reported by other authors[6,25].

Age as a barrier to cataract service uptake has not been reported by available population studies. This study found a statistically significant association in a bivariate analysis of age and reasons for delay in uptake of surgery. This may not reflect the true situation in the community as statistical analysis may have skewed the outcome, the study being done in a relatively homogenous age group of geriatrics (88.1% being 40 years and above). On the hand, it is possible that certain age-related co-morbidities such as lack of family support, and psycho-social factors may have hindered service uptake.

Non-randomization and purposive sampling technique used may have constituted some limitations to this study. However, the study was conducted among the desired population it was intended being individuals that have not accessed available healthcare services. It is concluded that a hospital-based study to answer similar research questions may supply additional information for the purpose of increasing CSR and clearing cataract backlog in underserved communities of developing countries. Based on the findings, therefore, adequate interventions could be offered to improve the situation in the locality, its environs and similar settings in developing nations.

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Prevalence of Parasitic Infections and their Influence on the Haemoglobin of Pregnant Women Attending Ante-Natal Clinics in two Secondary Health Facilities in Akwa Ibom State

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ABSTRACT

Parasitic infections and their effects on both nutritional and health status of pregnant women are of serious global concern because of their high propensity in causing maternal deaths, still birth, low birth rate and fertility impairment. A survey of parasitic infections was carried out on pregnant women who attended maternity clinics at Mary Slessor General Hospital, Itu and St. Luke's Hospital, Anua-Uyo in Akwa Ibom State of Nigeria to determine the parasite burden of the pregnant women. Samples of skin snips, blood, stool, vaginal swabs and urine were collected from the pregnant women at the maternity sections of the hospitals, over a period of 10 months, and analyzed using standard parasitological methods. Of the 572 pregnant women screened, 79(13.8%) were infected by one or more of the identified parasites. The parasites identified include Plasmodium falciparum (4.7%), Ancylostoma duodenale (3.5%), Ascaris lumbricoides (2.3%), and Trichomonas vaginalis (2.6%). Multigravid Pregnant women who had multi-infections were found to have haemoglobin levels <11.0g/dl (anaemia in pregnancy) and delivered babies with very low birth weights (<3.0kg). There was no adverse effect on the pregnant women associated with Ascaris lumbricoides except where there were multiple infections with other parasites like hookworm and Plasmodium falciparum. There were no cases of Onchocerca volvulus among the pregnant women. Control measures should be put in place to sensitize pregnant women on the possible preventive measures against malaria, other protozoan parasites, and helminthic parasites in the study areas.

Keywords: parasites, pregnant women, maternity, haemoglobin

INTRODUCTION

Parasitic infections constitute a global health problem causing clinical morbidity in over 450 million people worldwide, with many of them being women of reproductive age (pregnant women) in developing countries[1]. Elevated parasitic infections have been recorded in developing countries as a result of low literacy level, poor hygiene, lack of safe drinking water, malnutrition, hot and humid tropical climate and poverty[2,3].

Millions of pregnant women which represent a significant number in the society are affected by parasitic infections which directly or indirectly lead to a spectrum of adverse maternal and fetal/placental defects[4]. Pregnant women often experience more severe infections than their non-pregnant counterparts[4]. Parasitic infection could occur at any stage of the trimesters during pregnancy but infections during the first trimester is associated with more severe fetal and placental pregnancy[5]. Furthermore, the infection becomes more severe in women who are pregnant for the first time (primigravidae) compared with other gravidae[6].

Parasites such as *Ascaris lumbricoides, Ancylostoma duodenale, Entamoeba histolytica, Balantidium coli*, among others, cause intestinal obstruction, and malabsorption of folic acid which are essential during pregnancy cannot be manufactured by the body[8]. This condition becomes life threatening to the expectant mother harboring the parasite, and such parasitic effect is said to be indirect[9]. In a direct parasitic effect, parasites such as *Toxoplasma gondii, Leishmania donovani, Plasmodium falciparum,* among others penetrate the cells and cross the placenta, causing implicit damage to the fetus which leads to growth retardation, abortion and still birth[10].

Parasitic infections and their effects on both nutritional and health status of pregnant women are of serious global concern because of their high propensity in causing maternal deaths, still birth, low birth weight and fetal impairment[11]. Infections with most of these parasites are generally associated with poor hygiene and can be transmitted from one person to another oro-

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faecal, vertically or sexually[12]. High parasite burden in pregnant women results in so many clinical consequences including anaemia in pregnancy and low neonatal birth weight among others[13]. Anaemia, as defined by WHO refers to a haemoglobin level of less than 12.0g/dl in non-pregnant women and less than 11.0g/dl in pregnant women. This occurs in 40-80% of pregnant women in Africa[14]. The prevalence varies considerably with differences in socio-economic conditions, life style changes, cultural settings, recurrent parasitic infections and gravidity[15,16].

Presently, there is a dearth of public data on the prevalence of parasite burden among pregnant women of varying demographic backgrounds in Akwa Ibom State, South-south region of Nigeria. In this study, the various parasites which could possibly colonize the system of pregnant women due to immune suppression were identified and their colonization rate determined in two secondary health facilities in Akwa Ibom State of Nigeria.

MATERIALS AND METHODS

Study area: This study was conducted in Itu and Uyo Local Government Areas of Akwa Ibom State, South-South region of Nigeria. The two areas lie between latitude 5.5°N and 6.0°N, and longitude 6.0°E and 6.5°E of the Greenwich Meridian. They are located in the rain forest belt with elevation of less than two feet above sea level. The maximum temperature experienced is between 26-28°C and annual rainfall of about 362.5mm (Canback Dangel Database). The climate present two distinct seasons; a rainy season (May to October), and a dry season (November to April).

Study design: This is a descriptive cross-sectional study carried out between June 2012 and April 2013, involving pregnant women attending maternity clinics at the Mary Slessor General Hospital, Itu and St. Luke's Hospital Anua, Uyo. No sample from non-pregnant women was included in the analysis.

Ethical Consideration: Ethical approval was obtained from the Ethical Review Board of both hospitals and presented to the Consultants in charge of the Obstetrics and Gynaecology Departments of the two hospitals before the study was carried out.

Participant informed consent: The consent of the pregnant women was obtained before they were enlisted to participate in the study. Those women that declined or showed no interest in the study were not enlisted.

Questionnaire administration: Socio-demographic data of the pregnant women were obtained using a preformed questionnaire designed for such purpose.

Sample collection: Samples examined include stool, urine, blood, vaginal swab and skin snips. Samples were collected from women on their first and last antenatal visits. Samples were taken to the Parasitology laboratory of the two secondary hospitals, where they were analysed following standard Parasitology procedures.

Stool samples: Labeled sterile containers were given to mothers and they were instructed on how to collect the stool based on parasitological standard.

Urine samples: Mothers were instructed to obtain their mid-stream urine at the point of urination, using a labeled sterile container given to them.

Blood samples: Five (5) millilitres of blood was obtained from mothers and transferred into an EDTA bottle. This was taken to the Parasitology laboratory for further analysis.

Skin snip: skin snips were collected only from mothers presenting symptoms of Onchocerciasis.

High Vagina Swab: This was done using a sterile swab stick, with the aid of a sterilized speculum.

Laboratory Analysis

Stool analysis

- **Macroscopic examination:** Each of the stool samples were examined for whole parasites of *Ascaris lumbricoides, Ancylostoma duodenale, Taenia solium* and *Taenias aginata*.
- **Microscopic examination:** The stool samples were examined on a wet mount preparation using normal saline, iodine and the Kato flotation method for the presence of cysts and ova of parasites.

Urine examination

- **Urine analysis:** This included physical examination involving colour and turbidity and further analysis to check for the presence of blood, protein, glucose, nitrite, pH, ketone, bilirubin, ascorbic acid and urobilinogen in the urine of the pregnant women using a Medi-Test Combi-9 kit.
- **Urine microscopy**: The supernatant of the centrifuged urine spun at 3000 rpm for 5 minutes was discarded and the sediments examined under a microscope for the ova of parasites.

High Vagina Swab: The obtained vagina discharge was examined by wet preparations made using a drop of physiological saline on a microscopic slide, covered with a cover slip and examined using x40 objective lens for the presence of *T. Vaginalis*.

Blood samples: Obtained blood samples were used to make thick and thin films; they were stained with a Giemsa stain and viewed under x100 oil immersion lens. The haemoglobin level was determined using a micro-heamatocrit reader (Sysmex Haematology Analyzer). Pregnant women with Haemoglobin values less than 11.0g/dl were said to be anaemic.

Skin snips: A Walter type cornea sclera punch was used to obtain a 2mm of skin snip, thereafter the instrument was cleaned with absolute alcohol, and the sample placed in a drop of physiological saline contained in a micro-titer plate for 30 minutes, and examined.

Weight of Babies: The weight of babies were obtained and recorded immediately after delivery. This information was obtained from the babies'/mothers' cards provided by the mid-wives.

Data analysis: Socio-demographic variables and frequency of parasites occurrence were analyzed using SPSS version 17. Data were analysed using descriptive statistics and Chi-square, values at $p<0.05$ were considered statistically significant.

RESULTS

Table 1 shows the Demographic Variables and parasite burden of Subjects at Mary Slessor General Hospital, Itu. From the table, it can be deduced that the age group 16-20 had the highest burden of *Ancylostoma duodenale* (8.1%), *Ascaris lumbricoides* (8.1%) and *Plasmodium falciparum* (13.5%). There was no record of *Trichomonas vaginalis* and *Ascaris lumbricoides* within the age range 41-45. Single pregnant ladies had a higher infection load of *Ancylostoma duodenale* (5.0%), *Ascaris lumbricoides* (3.8%), *Plasmodium falciparum* (7.5%) and *Trichomonas vaginalis* (6.3%) than the married ladies. A high parasite burden of *Ancylostoma duodenale* (5.5%) was observed among Civil servants, while a high burden of *Ascaris lumbricoides* (5.7%), *Plasmodium falciparum* (11.4%) and *Trichomonas vaginalis* (5.7%) was observed in students.

DISCUSSION

Table 1: Demographic variables and parasite burden of subjects at Mary Slessor General Hospital, Itu (N=233)

Demographic Information	No. Sampled	*Ancylostoma duodenale*	*Ascaris Lumbricoides*	*Plasmodium falciparum*	*Trichomonas vaginalis*	Total
Age-groups(years)						
16-20	37	3(8.1)	3(8.1)	5(13.5)	1(2.7)	13(35.1)
21-25	41	1(2.4)	2(4.9)	3(7.3)	3(7.3)	9(22.0)
26-30	55	2(3.6)	1(1.8)	4(7.3)	2(3.6)	9(16.4)
31-35	49	2(4.1)	1(2.0)	2(4.1)	3(6.1)	8(16.3)
36-40	38	1(2.6)	1(2.6)	1(2.6)	1(2.6)	4(10.5)
41-45	13	1(7.7)	0	1(7.7)	0	2(15.4)
Marital Status						
Married	153	6(3.9)	5(3.3)	10(6.5)	5(3.3)	26(17.0)
Single	80	4(5.0)	3(3.8)	6(7.5)	5(6.3)	18(22.5)
Occupation						
Business(traders)	80	3(3.75)	2(2.5)	5(6.3)	3(3.75)	13(16.3)
House wives	63	3(4.8)	3(4.8)	3(4.8)	2(3.2)	11(17.5)
Civil servants	55	3(5.5)	1(1.8)	4(7.3)	3(5.5)	11(20.0)
Students	35	1(2.9)	2(5.7)	4(11.4)	2(5.7)	9(25.7)

Table 2 shows the infection rate of mothers in respect to haemoglobin level and weight of babies at Mary Slessor General Hospital, Itu. Mothers without any infection had an average haemoglobin level greater than 12.0±0.13, their babies had an average weight of 3.78±0.11 and an average haemoglobin level greater than 16.73±0.1. The overall average weight of babies born to these pregnant women was 3.00±0.05kg.

Table 2: Infection Rate of Mothers in Respect to Haemoglobin Level and Weight of Babies at Mary Slessor General Hospital, Itu (N=233)

Types of Infection	No. sampled	Mean Hb. of Mothers (g/dl)	mean Wt. of Babies (kg)	mean Hb. of Babies (g/dl)
Without Infection	199	12.0±0.13	3.78±0.11	16.73±0.1
With Single Infection	21	11.0±0.24	2.70±0.02	14.70±0.02
With Multiple Infection	13	10.6±0.08	2.54±0.03	13.01±0.11

Key: Wt = weight, Hb = haemoglobin, Av= Average

Table 3 shows the Demographic Variables and parasite burden of Subjects at St Luke's Hospital, Anua, Uyo. The age group 16-20 had the highest infection of *Ascaris lumbricoides* (5.4%), the age group 41-45 had the highest *Plasmodium falciparum* (12.5%), the age group 21-25 had the highest *Trichomonas vaginalis* infection (3.6%) while the age group 31-35 had the highest burden of *Ancylostoma duodenale* (6.1%). Married pregnant ladies had a higher infection of Hookworm (2.9%) and *Plasmodium falciparum* (3.6%), while pregnant but single ladies had a higher infection of *Ascaris lumbricoides* (4.8%) and *Trichomonas vaginalis* (3.3%) than the married ladies. Students had the highest Hookworm infection (5.0%), *Ascaris lumbricoides* (5.0%), *Plasmodium falciparum* (7.5%), while there was no presence of *Trichomonas vaginalis* in any of the student's samples.

Table 3: Demographic Variables and parasite burden of Subjects at St Luke's Specialist Hospital, Anua, Uyo (N=339)

Demographic Information	No. Sampled	*Ancylostoma duodenale*	*Ascaris lumbricoides*	*Plasmodium falciparum*	*Trichomonas vaginalis*	Total No. (%)
Age-group(yrs)						
16-20	37	1(2.7)	2(5.4)	3(8.1)	1(2.7)	7(18.9)
21-25	55	3(5.5)	1(1.8)	2(3.6)	2(3.6)	8(14.5)
26-30	114	2(1.8)	2(1.8)	1(0.9)	0	5(4.4)
31-35	66	4(6.1)	1(1.5)	2(3.0)	1(1.5)	8(12.1)
36-40	59	0	3(5.1)	2(3.4)	1(1.7)	6(10.2)
41-45	8	0	0	1(12.5)	0	1(12.5)
Marital Status						
Married	276	8(2.9)	6(2.2)	10(3.6)	3(1.1)	27(9.8)
Unmarried	63	2(3.2)	3(4.8)	1(1.6)	2(3.2)	8(12.7)
Occupation						
Business(trading)	60	3(5.0)	2(3.3)	4(6.7)	2(3.3)	11(18.3)
House wife	72	2(2.8)	2(2.8)	2(2.8)	1(1.4)	7(9.7)
Civil Servant	167	3(1.8)	3(1.8)	2(1.2)	2(1.2)	10(6.0)
Students	40	2(5.0)	2(5.0)	3(7.5)	0	7(17.5)

Table 4 shows the infection rate of mothers in respect to haemoglobin level and weight of babies at St. Luke's Hospital, Anua, Uyo. Mothers without any infection had the highest average haemoglobin (g/dl) level of 12.0±0.02, their babies had an average weight of 3.79±0.11 and an average haemoglobin (g/dl) level of 17.3±0.1. The overall average weight of babies born to these pregnant women in this hospital was 3.07±0.11kg.

Table 4: Infection Rate of Mothers in Respect to Haemoglobin Level and Weight of Babies at St Luke's Hospital, Anua, Uyo (N=339)

Types of Infection	No. sampled	mean Hb. of Mothers (g/dl)	mean Wt. of Babies (kg)	mean Hb. of Babies (g/dl)
Without Infection	304	12.0±0.02	3.79±0.11	17.3±0.01
With Single Infection	20	11.6±0.16	2.80±0.09	14.6±0.02
With Multiple Infection	15	10.20±0.24	2.62±0.14	13.4±0.21

Key: Wt=weight, Hb=haemoglobin, Av. = Average

Table 5 shows the prevalence of the parasites among subjects from the two hospitals. Higher prevalence of parasitic infections was recorded in Mary Slessor Hospital, Itu. Having a prevalence rate of 18.9% compared to 10.3% obtained in St. Luke's Hospital, Uyo. The overall prevalence rate of parasitic infections recorded in the area was 13.8%.

Table 5: Prevalence of the Parasites among Subjects from the two hospitals

Parasites	Mary Slessor Hospital, Itu N=233 (%)	St. Luke's Hospital Anua, Uyo N=339 (%)	Total N=572 (%)	Chi square
A. duodenale	10(4.3)	10(2.9)	20(3.5)	
A. lumbricoides	8(3.4)	9(2.7)	17(2.3)	
P. falciparium	16(6.9)	11(3.2)	27(4.7)	1.62*
T. vaginalis	10(4.3)	5(1.5)	15(2.6)	
Total	44(18.9)	35(10.3)	79(13.8)	

Key: * There is no significant statistical difference at P >0.05

Table 6 shows the effects of gravidity and parasitaemia on haemoglobin levels of the pregnant women. Primigravid women had a higher haemoglobin level of 11.0±0.11g/dl and lower infection rate 10.0% than the multigravid women.

Table 6: Effects of Gravidity and Parasite burden on Haemoglobin Levels of the Pregnant Women (N=79)

Gravidity	No. Sample	No. of +ve Parasite infection (%)	Av. Haemoglobin values (g/dl)
Primigravidae	180	18(10.0)	11.0±0.11
Multigravidae	392	61(15.6)	10.7±0.14
Total	572	79(13.8)	21.7±0.25

The prevalence of parasitic infections among different groups of people, particularly among pregnant women, is regarded as an important health problem both in the tropical and sub-tropical countries of the world. Of the 572 pregnant women screened for various parasitic infections at Mary Slessor General Hospital, Itu and St. Luke's Hospital, Anua, Uyo, 79 (13.8%) were found to be infected by one parasite or the other. Thus, the prevalence rate of parasitic infection among pregnant women in the study areas was 13.8%. The various parasites isolated include *Plasmodium falciparum* with prevalence rate of 4.7%. However, the rate of *P. falciparum* infection was higher in the hospital in Itu (6.9%) compared to that in the hospital in Uyo (3.2%). This could be as a result of the level of sanitation and high awareness of the method of transmission and control of the parasite among the inhabitants of Uyo. These results were low when compared to 7.7% obtained from pregnant women in Lagos, South-west Nigeria by Chimere *et al.*[17] and 26% obtained from pregnant women in Port Harcourt, Niger-Delta region of Nigeria by Wogu *et al.*[18]. This could be as a result of the active detection and treatment programme implemented by the Government and other NGO's in the State. Low prevalence rate of *P. falciparum* to an extent can be attributed to good hygiene measures and monthly environmental sanitation exercise enforced by Government that cuts across all the major towns of the State, including the study areas. These sanitation exercises in which gutters are drained, cleaned and surroundings are cleared ensure absence of breeding sites for malaria vectors.

The prevalence rate of *Ancylostoma duodenale* obtained from this study was 3.5%. This is very low when compared to 53.4% obtained from similar study in South Eastern Nigeria[19]. The possible reason for this high prevalence could be as a result of the use of human faeces and animal dungs as organic fertilizers for vegetables, which when under-cooked or not properly washed can lead to faecal-oral ingestion of the parasite eggs, thus, increasing the risk of infection [20.]. However, the prevalence rate of *A. duodenale* obtained from the hospital in Itu was 4.3%, while that obtained from the hospital in Uyo was 2.9%. The variation in the prevalence rate could be as a result of the rural nature of Itu and its environs where the planting of vegetables is done in a much commercial quantity using animal dungs, and the low awareness level of the parasites' transmission among the dwellers, when compared to Uyo.

The prevalence rate of *Ascaris lumbricoides* obtained from this study was 2.3%. This is low when compared to 55.0% obtained from a study carried out in Eku, Delta State of Nigeria [21] and 52.0% reported from studies conducted in Abia and Imo States of Nigeria[22]. The reason for the high prevalence of this parasite could be due to unhygienic practices by some individuals engaged in certain occupations in these regions. However, the prevalence of the parasite in the hospital in Itu was 3.4%, while that of the hospital in Uyo was 2.7%. This slight difference could be as a result of poor environmental and unhygienic practices among Itu dwellers.

The prevalence rate of *Trichomonas vaginalis* obtained from this study was 2.6%. This was low when compared to the 20% obtained from a study in Abeokuta, Ogun State of Nigeria[23]. The possible reason for this low prevalence rate obtained in this study could be as a result of early diagnosis and periodic examination of pregnant women in the study areas.

In this study, there was no marked effect on the babies delivered as the average weight was 3.04±0.08kg. This was probably due to progressive increase in immunity during the course of pregnancy since exposure and incubation rates are unlikely to alter greatly if appropriate ante-natal measures are taken during pregnancy [24]. The mean haemoglobin values of healthy pregnant women, infected mothers with single and multiple infections in the hospital in Uyo are 12.0±002g/dl, 11.6±0.16g/dl and 10.20±0.24g/dl, respectively while that in the hospital in Itu was 12.0 ± 0.13g/dl, 11.0± 0.24g/dl and 10.6±0.08g/dl. Low level of haemoglobin or anaemia is an important risk factor for low birth rate, maternal death, still birth and fertility impairment[11]. This could be caused by high level of parasitic infections as seen among these subjects. The low haemoglobin values obtained in this study could also be due to the influence of malaria parasite infection on immunity of pregnant women. *Plasmodium falciparum* was the only haemo-parasitic isolate capable of causing clinical evidence of the disease-

parasitaemia, in both the mother and the foetus in the study. Pregnant mothers from Itu, even though they reside where the Creek water is very rich in protein foods like periwinkles and snails, are prevented from consuming them due to local customs relating to food taboos[8].

The prevalence of parasitic infections was also studied using different demographic variables in the study areas. The prevalence rate of parasitic infection among the married and unmarried pregnant women in Itu was 26(17.0%) and 18(22.5%), respectively, while that in Uyo was 27(9.8%) and 8(12.7%), respectively. Students had the highest parasitic infection in Itu, 9 (25.7%), while business women had the highest parasite burden in Uyo, 11(18.3%). However, it is pertinent to note that variation observed among pregnant women in the study areas in respect of parasite load is dependent on a series of factors including the afore mentioned factors. The prevalence of parasitic infection among pregnant women varies considerably due to differences in socio-economic conditions, health seeking behaviours and cultural norms of the people, especially, in rural communities[25]. The prevalence rate of parasitic infections in relation to occupation from Uyo study area although lower than that in Itu, was similar to that obtained from a related study in the University College Hospital, Ibadan, Nigeria[26], who reported highest prevalence rate of 65.6% among pregnant business women (Traders).

The age bracket of 16-20 years had the highest prevalence rate of parasitic infection in Itu study area (35.1%), followed by the age range 21-25 years with 22.0% prevalence rate, while the least prevalence rate was obtained in the age group 36-40 years with 10.5%. In Uyo study area, the age range 16-20 years had the highest parasitic burden (18.9%), followed by the age range 21-25 years with 14.5% while the least prevalence rate was observed in the age range 26-30%. These prevalence rates obtained within these age groups are similar to that obtained in a study carried out at the University College Hospital, Ibadan, Nigeria[26]. The high prevalence rate among this age group is usually attributed to gestational age which is always related to high level of hormonal activity with concomitant decrease in immunity of pregnant women resulting in increased risk of infection; as it has been proven that intestinal colonization of parasites among pregnant women is dependent on age[12].

The effects of gravidity and parasite load on haemoglobin levels of the pregnant women showed that the primigravidae had a higher haemoglobin level of 11.0±0.11g/dl than the multigravidae with 10.7±0.14g/dl. Also, the multi-gravid women had more parasite burden of 15.5% than the primigravid which had 10.0%. Anaemia in pregnancy has been defined as a haemoglobin level (Hb) <11.0g/dl, in accordance to WHO guidelines[16]. Studies have shown that low level of haemoglobin is associated with increasing number of previous pregnancies. This is consistent with the finding of this study which revealed multigravid women having an average haemoglobin level lower than 11.0g/dl. A similar observation was made by Ivoke *et al.*[12] who reported higher prevalence of anaemia among multigravid women having multiple parasitic infections. Research over the years have shown that menstrual blood loss and repeated pregnancy, as well as increased burden of parasitic infections in pregnant women contribute significantly to iron imbalance and folate deficiencies which impact upon the haemoglobin level of pregnant women in developing countries[13,15].

CONCLUSION

The overall prevalence of parasitic infection among pregnant women obtained in this study was 13.8%. There was no significant statistical difference in the prevalence rates of parasitic infection in Itu and Uyo study areas (X^2= 1.62; P>0.05). The prevalence of parasitic infection was highest among multigravid women who also had low levels of haemoglobin thus; increased prevalence of anaemia in pregnancy was recorded among these subjects. Predisposing factors to parasitic infections among pregnant women are multi-factorial, including socio-economic status, local customs and lifestyles, hygiene and number of pregnancies. In view of this, it is advisable that measures to control parasitic infections among pregnant women such as sensitization on the possible preventive measures against malaria, other protozoan parasites, and helminthic parasites be put in place in the study areas, in particular and Akwa Ibom State, at large.

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Biologic Subtypes of Breast Cancer in Sokoto, Nigeria

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ABSTRACT

Most recently published works from across Nigeria and Africa point to a rising prevalence of breast cancer in these hitherto low incidence areas. The literature is also replete with studies on molecular markers of breast cancer amongst pre and post- menopausal women especially in the more advanced countries. Even though our centre has also witnessed a rising trend in the incidence of breast cancer, not much work has been done to elucidate the molecular biology of the disease. This study was therefore undertaken to determine the predominant receptor status of breast cancer among patients in Sokoto, North-western Nigeria. A one year retrospective study in which the case note of patients with histologically confirmed diagnosis of breast cancer between January and December, 2015 were reviewed. Parameters studied included demographic characteristics and receptor status of biopsy specimens. A total of forty six patients were seen in this study and all were females. The age range was 25-75 years. The mean age was 44.65 years. The median age was 45.00 years while modal age was 35.00 years. Seventy two percent (33) of the women with breast cancer were pre-menopausal while 28.26% (13) were post-menopausal. Immunohistochemical analysis shows that 47.8% (22) of cancers were ER positive, 41.3% (19) PR positive, 39.1% (18) ER/PR positive, and 43.5% (20) were HER 2/neu positive. Immunohistochemical classification based on ER, PR and HER2 gene expression showed that 24%(11) were Luminal A(ER+, PR+,HER2-), 22%(10) Luminal B(ER+,PR+,HER2+), 26%(12) HER2 type(ER-,PR-,HER2+) while 28%(13) were Basal-like(Triple negative) tumours. There was no statistically significant relationship between receptor status and age of patients. It was concluded that majority of the tumours in this study exhibited triple negative (basal-like) receptor characteristics in a predominantly premenopausal patient population.

Keywords: Breast cancer, receptor status, luminal, immunohistochemistry

INTRODUCTION:

Breast cancer is now the most common female malignancy in Nigeria, Africa and globally[1-5]. Reports from Nigeria have shown a consistent rise in the incidence of the disease[6,7]. Although there is a rising trend of breast cancer across Nigeria, overall incidence in black Africa and Asia is considered low in comparison to Western Europe and North America where incidence ranges between 50 to 100 per 100 000 women[8,9].

Breast cancer is classified into four groups based on immunohistochemical profile of ER/PR and HER2/neu expression, which correlates well with intrinsic gene expression microarray categorization as: ER/PR+, HER2-corresponding with Luminal A; ER/PR+, HER2+ corresponding with Luminal B; ER/PR-, HER2+ corresponding with HER2 over-expressed or enriched and ER/PR-, HER2 - corresponding with triple-negative/basal-like tumours[10-12].

In Nigeria as in most developing countries, late presentation of breast cancer with unfavorable prognosis is the norm[13-15]. More than two thirds of breast cancer patients in Nigeria present with advanced disease at the time of diagnosis[1-3,16]. The African patient is likely to present with a more aggressive and advanced tumor than her Western counterpart and is likely to die from the disease[15] Several works have shown that basal-like tumours occur significantly higher among pre-menopausal African-American and African women than Caucasian women[17-20]. Ikpatt *et al.* conducted a detailed pathological analysis to evaluate apoptotic activity in breast cancer from Nigerian (n = 300) and Finnish (n=285) women and concluded that tumours of Nigerian women had less tubular differentiation and higher mitotic to apoptotic index compared to Finnish women[21].

Patients with ER/ PR negative tumours are less likely to respond to endocrine therapy and more likely to have a poorer overall survival than women with ER/PR positive tumours[20,22]. Similarly, patients with HER2/neu positive tumours have been shown to have significant survival advantage with targeted therapy using the humanized monoclonal antibodies against HER2/neu[23]. Studies have also shown that advanced tumours are more likely to be hormone unresponsive compared to early tumours[24].

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METHODS

This was a one year retrospective study at the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Northwest Nigeria between January and December 2015 in which the case note of patients with confirmed diagnosis of breast cancer were retrieved and reviewed. Parameters studied included demographic and receptor characteristics.

Inclusion/Exclusion criteria: All histologically confirmed malignant breast lesions from both sexes with receptor status were included**.** Histologically confirmed malignant breast lesions without receptor status were excluded.

Receptor characteristics: Our center started routine immunohistochemistry to determine receptor status of breast cancer in 2015. All consecutive breast specimens received in 10% buffered formalin were processed with auto processors. Paraffin-embedded sections at 3-4 ìm were routinely stained with haematoxylin and eosin (H & E) stains to determine their suitability for immunohistochemistry. Immunohistochemical assays were performed on representative paraffin embedded blocks by deparaffinizing and rehydrating the section with xylene. This was followed by heat-induced epitope retrieval where slides were heated in a buffer for 40 minutes to unmask antibodies and antigen. The slides were subsequently incubated in 0.03% hydrogen peroxide for 5minutes to block endogenous peroxidase activities, followed by incubation for 20 minutes in a protein-blocking solution (Protein Block Serum-Free Solution) to reduce nonspecific background. Slides were then incubated for 10 minutes with 3, 31 di-aminobenzidinechromogen (for enzymatic immunodetection) and counter stained with haematoxylin and covered. Results of the immunostaining were scored semi-quantitatively and presented as receptor status. Comparison of patient's age with receptor status was carried out for any association using the Pearson's Chi square test. A *P-value* of less than 0.05 was considered significant.

Data Analysis: Results were analyzed using the Windows statistical package for Social Sciences (SPSS) version 20.

RESULTS

A total of forty six cases were seen and analysed in this study and all were females. The age range was 25-75 years. The mean age (±Standard Deviation) was 44.65 (±12.55) years. The median age was 45.00 years while modal age was 35.00 years (Table 1). Seventy two percent (33) of the women with breast cancer were pre-menopausal while 28.26% (13) were postmenopausal. Immunohistochemical analysis shows that 47.8% (22) of cancers were ER positive, 41.3%(19) PR positive, 39.1%(18) ER/PR positive, and 43.5%(20) HER 2/neu positive (Table 2). Immunohistochemical classification based on ER, PR and HER2 gene expression showed that 24%(11) were Luminal A (ER+, PR+,HER2-), 22%(10) Luminal B (ER+,PR+,HER2+), 26%(12) HER2 type (ER-,PR-,HER2+) and 28%(13) were Basal-like (Triple negative) tumours. Table 3 showed that there was no statistically significant relationship between receptor status and the age of patients.

Table 1:Age distribution of patients with breast cancer

Age group (Years)	Frequency	Percentage
20 - 29	2	4.35
30 - 39	16	34.78
40 - 49	15	32.61
50 - 59	4	8.70
60 -69	6	13.04
≥70	3	6.52
Total	46	100

Table 2: Receptor status of breast cancer in Sokoto in 2015

Status	ER	PR	ER+PR	HER2/ NEU
N	46	46	46	46
Positive	22 (47.8)	19 (41.3)	18 (39.1)	20 (43.5)
Negative	24(52.2)	27(58.7)	28 (60.9)	26 (56.5)

Key: ER: Oestrogen Receptor, PR: Progesterone Receptor, HER2/NEU: Human Epidermal growth factor Receptor 2.

Table 3: Relationship between receptor status and age at diagnosis

Variable	ER Status		χ^2	*p* value
	Negative	Positive		
Age Group(years)				
< 50	17 (50.0)	17 (50.0)		
≥50	10 (83.3)	2 (16.7)	2.139	0.144
	PR Status			
	Negative	Positive		
< 50	20 (58.8)	14 (41.2)		
≥ 50	12 (100.0)	0 (0.0)	3.617	0.057
	HER2/NEU Status			
	Negative	Positive		
< 50	19 (55.9)	15 (44.1)		
≥ 50	12 (83.3)	2 (16.7)	1.638	0.201

DISCUSSION

The study shows that breast cancer in our women is predominantly pre-menopausal (71.74%) in contrast to Caucasian women where the disease is mainly post-menopausal[25]. Most published reports from Nigeria and America revealed that breast cancer in African women occur a decade earlier than the Western average[25-27]. African-American women are also known to present at significantly younger age than their Caucasian counterparts[28,29]. Similarly, black British women presented significantly younger (median age of 46 years), than white patients (median age of 67 years)[30]. The factors responsible for this are not fully understood although it may be due to mutations in the breast cancer genes (BRCA 1 and 2) their variants[29].

Studies have shown that pre-menopausal African-American women have higher prevalence of triple negative (basal-like) breast tumours, than their white counterparts [31] and this trend has also been seen among indigenous African women[13,20,26].

Our study showed a preponderance of triple negative (basal like) tumours at 28% (13). Reports from Jos, (North Central Nigeria); Ile-Ife, (South-Western Nigeria); Aba, (South-Eastern Nigeria) and other parts of Africa also showed similar pattern[13,32-36].

In Ile-Ife, Omoniyi-Esan *et al.* in a 5-year prospective study of 136 cases of breast cancer specimens showed that 45 cases (33.1%) of tumours were Basal-like (Triple negative), 30 cases (22.1%) were Her2 over expressing, 21 cases (15.4%) were Luminal B type and 20 cases (14.7%) were luminal A type[32]. The peak age group was 40-50 years in agreement with our study[32].

Adisa *et al.* also carried out a detailed analyses of tumour receptors and infiltrating macrophages (TAM) on 17 breast cancer specimens from Aba, South-East Nigeria and concluded that majority of the tumours were high grade (100% were grade III), triple-negative (65%), and occurred more commonly in young women (mean age 47 years)[33].

Similarly, Gukas *et al.* from Jos, North Central Nigeria in a review of 36 consecutive patients with breast cancer reported that there was a predominance of high grade, invasive ductal carcinomas which were likely to be ER/PR negative but *P*53 positive. They concluded that these features suggested a biologically aggressive form of breast cancer in Nigerian women with possibility of poor response to both hormonal therapy and chemotherapy[13]. Even though majority of our patients were premenopausal with predominant triple negative

receptors a comparison of age at diagnosis with receptor expression did not show any significant statistical relationship perhaps because a good proportion of the tumours were also ER and PR positive. In contrast to our study however, Nwafor and Keshinro in a retrospective analysis of 48 breast cancer specimens from Lagos, South-West Nigeria reported a higher proportion of luminal A (ER+/PR+,HER?), 19 (39.6%), tumours compared to other subtypes with majority of the patients aged 50 years and above[37]. Triple negative/basal-like cancers were the second most common type of cancer in their series accounting for 29.2% (64.3% of which were seen in age groups 30-49 years and 78.6% with tumour grades 2 and 3)[37]. There was however no statistical correlation between the triple negative groups and their tumour grades or age distribution[37]. Also, McCormack *et al.* in a case series report on breast cancer receptor status and stage at diagnosis concluded that although a greater proportion of black than non-black South African women had ER-negative or triple negativebreast cancer, majority in all racial groups were still predominantly ER-positive[38].

Previous reports have shown that women with luminal A tumors have better recurrence-free and overall survival than women with other molecular subtypes[38,39]. Similarly, Luminal tumors are known to have better survival outcome compared to HER2+ or triple negative tumors[39,40].

CONCLUSION

It was concluded that majority of the tumours in this study exhibited triple negative (basal-like) receptor characteristics in a predominantly premenopausal patient population. These features showed a biologically aggressive form of breast cancer in our women with possibility of poor response to both hormonal therapy and chemotherapy.

Limitations of the study: The manual system of storage of information by the medical records department makes retrieval of case files a challenging task. A two-year study period would have meant a larger sample size and longer period of evaluation.

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Multifocal Avascular Necrosis Complicating Delivery. A Report of two Cases

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ABSTRACT

Avascular necrosis (AVN) is characterized by bone death. It affects bones with precarious blood supply. Trauma with disruption of blood supply is a common cause. The aim of this case report is to highlight the difficulty in managing patients with AVN. We present the cases of two young mothers who presented with pains in the shoulder and hip joints. The first is a 22year Para 1+ 0 housewife referred to our centre with pains in both shoulders and hips of 14 months duration, six months following her last confinement. The Pain was severe, non-radiating, aggravated by movements and relieved with intake of analgesics. She was said to have been in labour for over 24 hours following which she was delivered of a baby boy through spontaneous vertex delivery (SVD). She however had a retained placenta that was removed piecemeal after several attempts at removal failed. The second patient is a 25year old Para 1+0, none alive woman presented with 8years Complaint of pains in both hips with limitation of movements following a difficult delivery 10 months earlier. Both patients were from a low socioeconomic economic background. They were managed with analgesics and physiotherapy and counselled for total joint replacement. Avascular necrosis and osteoarthritis of major joints poses a major challenge in management in a poor resource setting without health financing and insurance.

Keywords: Avascular necrosis, osteoarthritis, hip, shoulder

INTRODUCTION

Avascular necrosis (AVN) also known as osteonecrosis or ischaemic necrosis affects bones with precarious blood supply and disruption of the vascular supply results in necrosis of the marrow, medullary bone, and cortex[1]. Individual patients developing avascular necrosis usually have more than one risk factor; this indicates that the pathogenesis of AVN is likely multifactorial. Vascular occlusion can occur from trauma involving extra-osseous blood supply as in fracture or dislocation. Altered lipid metabolism can lead to lipid deposition in the femoral head causing femoral hypertension and ischaemia[1,2]. Corticosteroid administration was associated with fat emboli in the femoral heads of rabbits. Other risk factors include intravascular coagulation as seen in familial thrombophilia, hypercholesterolemia, infection, malignancy and pregnancy[3]. Dead bone sets up a process of repair that involves osteoclasts, osteoblasts, histiocytes, and vascular elements. New bone is formed on top of the dead bone, leading to sclerosis that prevents revascularization of the necrotic bone, with resultant abnormal joint remodelling and joint dysfunction.

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Ficat and Arlet staged AVN based on radiologic features between early pre-symptomatic stages and late stages where there is collapse and secondary osteoarthritis[4]. Management of early stages will involve use of analgesics and physiotherapy while surgery is indicated once there is structural damage and distortion of the head. We present two cases of radiologically established avascular necrosis and osteoarthritis involving both heads of humerus and femur in order to enrich our knowledge.

CASE PRESENTATION

Case 1

We report the case of a 22year Para 1+ 0 housewife referred to our centre with complaints of pains in both shoulders and hips of 14months duration following her last confinement. There was also history of difficulty in walking of the same duration. The patient problems started after her last delivery when she experienced pains in both shoulders and hips. The pain was severe, non-radiating, aggravated by movements and relieved by intake of analgesics. She was said to be in labour for over 24 hours following which she was delivered of a baby boy through spontaneous vertex delivery (SVD). She however had a retained placenta that was removed piecemeal after several attempts at removal failed. There was much bleeding and patient went into shock

and was resuscitated and transfused with three pints of whole blood. Her pregnancy was booked and she attended ante-natal care regularly and was immunized. Patient is not a known sickle cell disease patient and was confirmed by haemoglobin electrophoresis and has no previous history of hand and foot pain. There was no history of use of contraceptive or prolong steroid. Patient was commenced on analgesics and physiotherapy at the referring hospital and was able to walk with the aid of a walking stick. Examination revealed a young lady, not pale, afebrile and not dehydrated. The respiratory rate was 18 cycles per minute and the chest was clinically clear. The pulse rate was 90 b/min with a blood pressure 100/60mmHg. The abdomen was full and no masses felt. Musculoskeletal examination showed that patient walks with an antalgic gait and the left shoulder was wasted with restriction of movement; active abduction was 0 degree while passive abduction was 20 degrees. The other shoulder and hip joints also were tender during passive movement with restriction of both internal and external rotation movements. She had haematological and radiological investigations with the x-rays confirming Ficat and Arlet stage 3 AVN for the humerus and stage 4 for the hips [Figure 1, 2]. She was counselled on the condition and continued on physiotherapy and possible joint replacement before she was lost to follow up after discharge.

Figure 1. AVN involving both heads of humerus

Figure 2. AVN involving both femoral heads

Case 2

She was a 25 year P1+0 non alive divorced young lady who presented with an 8years history of pains in both hips. Pain was insidious in onset and is mild to moderate in severity and has gradually been associated with limitation of movements. Pain is relieved by analgesics and worsened by activities. Pain started following her last delivery from a prolonged labour for which she eventually lost the child. She is not a known sickle disease patient and was not on any family planning or steroids. The restriction of hip movements has affected her activities of daily living. Examination revealed a young woman with loss of abduction, abduction, internal and external rotation movement in both hips. Haemoglobin electrophoresis showed genotype AA. Radiographs showed avascular necrosis with secondary osteoarthritis [Figure 3]. She was placed on analgesics while she awaits bilateral total hip replacement.

Figure 3. Osteoarthritis secondary to AVN involving both femoral heads

DISCUSSION

There are several causes of avascular necrosis of bone and pregnancy related cases are rare [5]. But many cases are classified as idiopathic because of the difficulty in determining the cause. The intraosseous microcirculation is as susceptible to a prothrombotic state as any other part of the circulation[4,5]. Taking Virchow's triad as an example, this may be the result of one or a combination of factors, including endothelial damage, circulatory stasis, or a hypercoagulable state. Endothelial damage may be related to trauma, atherosclerotic lesions, or autoimmune inflammatory connective tissue disorders that may affect the endothelial lining.[5,6] Osteonecrosis of the femoral head is a rare manifestation of pregnancy, especially in healthy women who have no known risk factors for the development of this disorder. The cause of osteonecrosis in these patients is unknown, and the pathogenic mechanism is multifactorial[6,7].

Different hypotheses have postulated the possibility of amniotic fluid emboli, a relative hypercoagulable state, excessive mechanical strain, and an increase in endogenous steroid production[7,8]. The possibility of amniotic fluid embolism is further enhanced by the difficult delivery with retention of placenta that was removed piecemeal. In pregnancy, the elevated levels of oestrogens and progesterone could induce the development of osteonecrosis by fatty embolism[8]. Pregnancy produces a hypercoagulable state and also causes mechanical stress as a result of the weight gain during pregnancy. Hypercoagulability can cause vascular occlusion and ischaemia and death of bone from thromboembolism[9,10]. To date, the largest case series was described by Montell and associates. In this case series, 13 women developed hip pain late in the second or in the third trimester of their pregnancy. In general, these women tended to have a small body habitus, and during their pregnancy, they had gained excessive weight, indicating that this may have a pathogenic role in the development of osteonecrosis[11]. All the affected women in their series had involvement of their left hip, and four of them had bilateral involvement. Involvement of the both shoulders and hips as seen in our first case is quite rare as most cases reported in the literature involved the hips.

Early diagnosis is important in determining the course of the disease as both patients presented late. The natural history of AVN shows that bone death is an irreversible process leading to secondary osteoarthritis. Management of this condition is quite challenging as both patients are poor with no healthcare financing. The first patient was lost to follow-up while it took the second patient 8 years after developing symptoms to present in hospital. She lacked social support as she was divorced during the course of her illness.

CONCLUSION

Avascular necrosis should be considered as differential diagnosis of pains involving the large joints during pregnancy and the postpartum period.

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pISSN:2384-5805
eISSN:2536-6645

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