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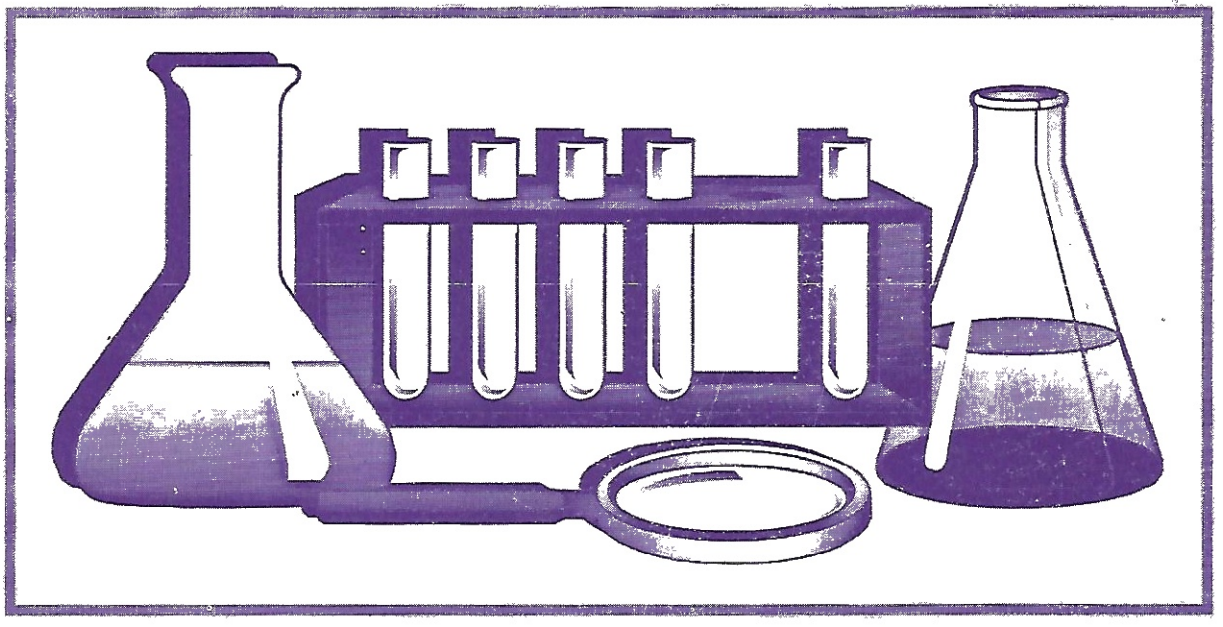

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JOURNAL OF BIOMEDICAL INVESTIGATION, DECEMBER, 2008, Vol. 6, No. 1

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Journal of Biomedical Investigation, 2008; 6(1):1-5.

Original Article

Prevalence of malaria parasites, Hepatitis B and C viral infections in pregnant women attending ante-natal clinic.

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ABSTRACT

The prevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV) and malaria parasites (MP) was studied amongst pregnant women. One hundred pregnant women on routine visit to the ante-natal clinic were randomly recruited into the study. Analysis of their blood samples showed that 2(2%) out of the 100 pregnant women were sero- positive for HBV and 77(81%) out of 94 of pregnant women were positive for malaria parasites. However, all of the pregnant women were HCV sero-negative. The alanine amino transferase (ALT) values are not definitive for any particular infection. This study revealed that malaria parasites were the most prevalent infection among pregnant women. HBV infection in pregnant women is a cause for concern as it may result in congenital infection.

Keywords: Hepatitis B and C, Malaria, Pregnant women

INTRODUCTION

Nigeria is one of the countries situated in malaria endemic area of Sub-Saharan Africa. The transmission of malaria is believed to be stable throughout the year. Therefore report of symptomatic and asymptomatic cases of malaria infection is not uncommon finding in pregnant women[1]. However, the emergence of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in an area of malaria endemicity calls for concern since any of these infections have high mortality and morbidity rate,[2,3,4]. In our earlier report, we observed 7% and 3% prevalence rates of HBV and HCV respectively amongst the blood donors[5]. In continued search to actually know the prevalence of these infectious agents in different population groups, we decided to screen pregnant women for HBV and HCV and possible role of alanine amino transferase (ALT) in such infections in malaria endemic area of south Eastern Nigeria.

MATERIALS AND METHODS

Subjects: One hundred pregnant women aged between 18-39 years, with no history of HBsAg vaccination, attending ante-natal at Nnamdi Azikiwe University Teaching Hospital, Nnewi, were recruited for this study. A structured standard questionnaire was used to collect medical and maternal data such as; fever, malaise, nausea, chills, fatigue or weight-loss, headache, and history of previous blood transfusion, abortion and still-birth and current age and gestational age were taken. Blood samples were collected for determination of serum alanine amino transferase (ALT) activity, Hepatitis B surface antigen (HBsAg), and Malaria Parasites and Hepatitis C virus (HCV) screening. The serum samples were stored at -20°C until analyzed. The participants in the present study gave informed consent before being recruited for the study.

METHODS

Hepatitis B surface antigen (HbsAg) screening:

The principle is based on detection of Hepatitis B surface antigen (HbsAg) antibodies in the serum using one step HbsAg strip (ACON

Laboratories incorporated USA). One step HbsAg strip is a qualitative, lateral flow immunoassay. The membrane is pre-coated with anti-HbsAg antibodies on the test line region of the strip. The procedure for the detection of HBV was as described by the manufacturer (ACON Laboratories, Incorporated, USA). The test strip was immersed vertically in the serum specimen for 15 seconds, thereafter; the test strip was placed on a non-absorbent flat surface, and allowed to incubate for 15 minutes. This allows for reaction between the pre-coated anti-HbsAg antibodies and the HbsAg antibodies present in the serum and generate a coloured line. For HBV sero-positive sera, two distinct red lines appeared; one line on the test region (T) and the other on the control region (C). However for HBV sero-negative, one red line appeared on the control region (C) but no red line appeared on the test region (T). The appearance of red line at the control region validates the result and test strip.

Hepatitis C Virus (HCV) Screening

The principle is based on detection of HCV antibodies in the serum using HCV rapid test device (Core Diagnostics UK). HCV rapid test device is a qualitative membrane based antigen-antibody immunoassay. The membrane is coated with anti-HCV antibodies on the test line region of the device. The procedure is as described by the manufacturer (CORE Diagnostics United Kingdom) Two drops (0.1ml) of serum sample was added using pasture pipette, into appropriately labeled sample well of the rapid test device and allowed to incubate for 10 minutes. This will allow for complete reaction between the pre-coated anti-HCV antibodies and the HCV antibodies if present in the serum will generate a coloured line in the test region. For HCV sero-positive sera, two distinct lines appeared, one on the control region (c) and the other on the test region (T), while only one red line appeared on the control region (C) and non on the test region (T) for negative sera. The appearance of red line at the control region validates the result and test strip.

Determination of Alanine Amino transferase (ALT) activity: ALT was determined as described by Reitman et al[6]. The principle of the test was based on the measurement of pyruvate hydrazone formed by the reaction between alpha oxoglutarate and L-alanine in the presence of alanine amino transferase. The procedure followed was as supplied by the manufacturer of the kit (Randox Laboratories limited. UK.). In brief, 0.1ml of sera were added into appropriately labeled test tubes containing 0.5ml of solution-1 (comprising phosphate buffer, L-alanine, α-oxoglutarate). The reaction mixtures were allowed for incubation for 30 minutes at 37°C before the addition of 0.5ml of 2, 4-dinitrophenylhydrazine into the respective sample test tubes. The tubes were allowed to incubate for further 20 minutes at 20°C. Subsequently, 0.5ml of sodium hydroxide was then added into the respective test tubes and mixed. After 5 minutes, the contents of test tubes were mixed and the absorbance of the samples read against the reagent blank at 530nm wavelength. For the reagent blank similar procedure was followed except that 0.1ml of distilled water replaced the 0.1ml of sample. A calculating chart that accompanied the kit was used to deduce the serum activities of ALT using the observed absorbance.

Detection of Malaria Parasites:

Thick blood films were made on clean grease free slides. The films were air dried and allowed to stain in 1/20 diluted Giemsa stain for 30 minutes. The blood stained films were screened for *P. falciparum* malaria parasites.

STATISTICAL ANALYSIS

The quantitative variables were expressed as mean (±SD) while the incidences of the various infections were expressed in percentage. The student t-test was used to determine significance difference in mean.

RESULTS

Out of the one hundred pregnant women recruited for this study, 2 (2%) were sero-positive for Hepatitis B virus (HBV), while 98 (98%) were sero-negative. No sero-positive

result was recorded for Hepatitis C virus (HCV). However, 77 (82%) of the pregnant women had positive malaria parasites blood smear while 17 (18%) had no detectable malaria parasites in their blood smears. See table 1

The two subjects who were HBV sero-positive as well as MP positive , had mean Alanine amino transferase (ALT) activity of 68.5 ± 25.5 (iu/l) [(a)], while those who were MP positive but HBV sero-negative had mean ALT value of 50.3 ± 24.8 (iu/l) [(b)]. Whereas subjects who where HBV sero-negative as well as MP negative, had ALT value of 75.5 ± 18.8 (iu/l) [(c)]. The difference in mean ALT between HBV sero-positive and MP positive subjects vs. MP positive and HBV sero-negative[(ab)] subjects was significantly different (P<0.05). No significant difference in mean ALT values was observed between HBV sero-positive and MP positives vs. HBV sero-negative and MP negatives[(ac)] (P>0.1) or between MP positive and HBV sero-negative vs. MP negative and HBV sero-negative[(bc)] subjects (P>0.1). See table 2

Out of 77(82%) subjects who where MP positive, 41(53%) were asymptomatic while 18(23.3%) had symptoms and 18(23.3%) had their history not recorded. Among 22 subjects with raised ALT, 15(68%) were asymptomatic, 5(22.7% had history of previous abortion and 1(4.5%) had malaise. The two pregnant women who were positive to HBV were asymptomatic. A total of 18(18%) had history of previous abortion, 2(2%) had history of previous blood transfusion. See table 3.

Table 1: Prevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Malaria parasites (MP) amongst pregnant women attending antenatal clinic

Variables	Positive (%)	Negative (%)	Total
HBV	2(2%)	98(98%)	100
HCV	0 (0%)	100(100%)	100
MP	77(82%)	17	94

Table 2: ALT levels in pregnant women with different status of HBV, HCV and MP.

Variables	ALT iu/ul	P-Value
HBV and MP positive (n = 2)	68.5 ± 25.5 a	P > 0.1[(ac)]
Positive MP & HBV negative (n=46)	50.3 ± 24.8 b	P< 0.05[(ab)]
HBV & MP negative (n=8)	75.5 ± 18.8 c	P >0.1[(bc)]

Key:
ac = HBV & MP positives vs. HBV & MP negatives
ab = MP Positive & HBV negative vs. HBV & MP positives
bc = MP positives & negative HBV vs. MP negatives & HBV negatives
MP = Malaria Parasite
HBV= Hepatitis B virus
ALT = Alanine aminotransaminase
n = number of subjects.

Table 3: presentations of pregnant women during recruitment at the ante-natal clinic.

Variables	Vomiting	Malaise	Fever	+Abortion	Still Birth	Headache	Chills	*Transfusion	Asymptomatic	No History
MP positive	5	6	6	10	2	9	2	2	41	18
HBV pos	-	-	-	-	-	-	-	-	2	-
HCV	-	-	-	-	-	-	-	-	-	-
ALT raised	-	1	-	5	-	-	-	-	15	2

+previous abortion
*previous blood transfusion

DISCUSSION

The result of this work showed low prevalence of HBV amongst pregnant women in Nigeria. The 2% positive cases of HBV infection amongst pregnant women may be considered a significant report considering that it also represents 20 out of every 1000 pregnant women in Nigeria. In the largest study performed so far, investigating more than 15 000 pregnant women from northern Italy over a period of four years, a prevalence of 2.4% was found[7]. This gives a count of 360 sero-positive pregnant women detected within 4 years. The zero prevalence of hepatitis C viral infection in pregnant women in this study calls for further investigation with higher number of pregnant women participating. Although with the same sample size we have reported incidences of HCV infection amongst apparently healthy blood donors[5]. Since our previous study and this present study were conducted within same locality, it could be that blood donors might be active source of reservoir for HCV transmission. Report elsewhere, has shown that the prevalence of HCV specific antibodies varies geographically and in different population groups[8]. For instance, 5% cases of HCV have been observed in another study in another location in Nigeria[9]. Although the population used for the study were normal blood donors and multiple transfused sickle cell aneamia patients.

The essence for using ALT was to see if there could be any evidence of active hepatitis. However, only 1 out of the 2 HBV positive pregnant women had increased serum activity of ALT above the confidence interval for the study population. Conclusion may be difficult to draw from the present study with regard to the number of patients positive for HBV. Hence we could not attribute increased ALT activity as a marker for HBV infection. In their study, Acquave and Tetty[8] could only detect raised ALT activity in 23 (33.85%) out of 68 serum samples of subjects with positive Hepatitis B viral infection. Thus suggesting that raised ALT activity may not be a consistent finding in Hepatitis B viral infection.

In the present study, the two pregnant women positive for HBV on presentation were asymptomatic. Report has shown that 15% of asymptomatic carriers exist in developing countries, and HBV is frequently associated with asymptomatic conditions[2]. It is true that asymptomatic conditions can escape diagnosis and then increase the chances of congenital infection and infection of unsuspecting health workers in cases of exposure.

Because most pregnant women were asymptomatic, the possibility of missing the diagnosis may portend grave consequences to their children who if congenitally infected may present with hepatocarcinoma in future. The strong possibility of vertical transmission lends importance to diagnosing acute or chronic HBV infection in pregnant women and justifies mandatory ante-partum serum HBsAg screening. By doing so, previously unsuspected chronic HBV infection is diagnosed in young otherwise healthy individuals[10,11]. This has the added benefit of making it possible to refer them for appropriate antiviral therapy before

development of significant liver damage and associated functional insufficiency. The infants of potentially infectious mothers are treated with HBV Human Hyperglobulin (HBIG) at delivery and simultaneously active immunoprophylaxis is initiated[10]. This approach is effective in preventing chronic HBV in approximately 85% of neonates[10]. It has been reported that childhood infection can easily lead to chronic stage of the HBV and HCV infections[12]

82% positive cases of malaria parasite infection in pregnant women showed high prevalence. High prevalence of malaria parasites infection was previously reported among pregnant women in Nigeria[1]. This is possible because the study location is endemic to malaria. Maternal anemia contributes significantly to maternal mortality and causes an estimated 10.000 deaths per year most of which are as a result of infection with *Plasmodium falciparum*[13]. Thus Pregnant women in malaria endemic areas may experience a variety of adverse consequences from malaria infection including maternal aneamia, placental accumulation of parasites, low birth weight (LBW) from prematurity and intrauterine growth retardation (IUGR), foetal parasite exposure and congenital infection, and infant mortality (IM) linked to preterm-LBW and IUGR-LBW[13].

Considering the grave consequences of HBV infection on pregnant women and pregnancy outcomes, we are advocating for routine screening for all individuals within the reproductive age as this will help identify asymptomatic cases and proper management instituted. Malaria parasite screening should also be routinely performed on pregnant women in this part of the world.

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Original Article

Prevalence of Malaria and Splenomegaly in children under 13 years in Awka South Local Government Area of Anambra State, Nigeria.

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ABSTRACT

The present study was designed to assess the prevalence of malaria and splenomegaly in children under the age of 13 years. A total of 720 children who presented with clinical signs and symptoms of malaria in the study area were recruited. Blood samples were collected in EDTA tubes. Giemsa stained thick and thin smears were microscopically examined to confirm malaria diagnosis. Haemoglobin (Hb) genotype was determined by alkaline cellulose acetate electrophoresis to exclude sickle cell anaemia as possible cause of splenomegaly. Splenic enlargement was determined by palpation. Out of the 720 subjects, 600 (83.3%) were positive for *P. falciparum* malaria while 114 (15.8%) of these children with malaria presented with splenomegaly. The result of the Hb genotype showed 576 (80%) of the children were HbAA while 144 (20%) were HbAS. No child with HbSS was seen. The finding showed that malaria is a major cause of splenomegaly in the study area which is an urban setting. The public health implication of the observation is discussed.

Keywords: Genotype, Children, Malaria, Spleen.

INTRODUCTION

Most of the victims of malaria reside in the tropics and subtropics with children and pregnant women forming the high risk groups [1]. High malaria prevalence has been reported in the above mentioned groups in the South Eastern Nigeria[2,3]. Malaria induces inflammatory response from the host and one of the body organs responsible for such inflammation is the spleen – a lymphoid organ which functions include among other things, the filtration of blood to remove deformed constituents and foreign antigens and as reservoir of immunocompetent cells [4]. The spleen gets easily enlarged in situations that provoke antigen-antibody reaction especially where such reactions are frequent and chronic. This condition is referred to as splenomegaly. Apart from inflammatory causes, so many other conditions have been associated with splenomegaly [5]. In endemic areas, malaria is believed to be one of the commonest causes of splenomegaly, ranking first beforevisceral leishmaniasis (kala-azar) and intestinal schistosomiasis. In children, splenomegaly or spleen rate is used as an indicator of malaria endemicity and is defined as the percentage of children aged 2-10 years showing palpable enlargement of the spleen [6,7]. Each attack of clinical malaria is accompanied by a degree of enlargement of the spleen due to congestion. The spleen regresses as soon as the attack resolves. In endemic areas, repeated attacks leads to a slowing down of the regression process leading to persistent splenomegaly [8]. A condition known as functional hypersplenism occurs in children with sickle cell anaemia as a

result of repeated vaso-oclusion of the splenic tissues attributable to the sickling process[9]. Spontaneous regression leading to a condition known as autosplenectomy has been reported in subjects with sickle cell anaemia [10]. The epidemiological value of splenomegaly in malaria endemic areas cannot be over emphasized. Decreasing spleen rates may indicate a reduction in the general immunity and this may predispose to the risk of epidemics. The present study therefore seeks to assess the prevalence of splenomegaly in a malaria endemic area with stable transmission, where other causes such as schistosomiasis and leishmaniasis are not endemic.

MATERIALS AND METHODS

Study Area: The study was conducted in Awka South Local Government Area (L.G.A.), one of the 21 L.G.A that make up Anambra State in the South-Eastern part of Nigeria, between January and September 2007. Awka South L.G.A. consists of nine autonomous communities with an estimated population of 180,000 [11]. It has a tropical rain forest climate with humid vegetation, which makes it a breeding ground for anopheles mosquitoes hence malaria transmission takes place through out the year and is stable. Most of the component communities are agrarian in nature. The local government area is not usually prone to schistosomiasis [12].

Subjects: A total of 720 children aged less than 13 years who presented with clinical signs and symptoms of malaria were recruited for the study from four different hospitals and clinics located at Awka, Nibo and Nise all within Awka and it's environ.

Sample Collection: The above hospital/clinics were first written to obtain their permission and co-operation. Consent was sought for and obtained from the parents or guardians. 1ml of whole blood was collected from each participant through venipuncture under sterile conditions and the specimen was placed in a tube containing EDTA, which was properly covered and labeled.

METHODS

Parasitological Examination

Giemsa staining technique of thin and thick smears as described by Cheesbrough [13] was used. On a single slide two blood films (thin and thick) were prepared and stained with 10% Giemsa for 10 minutes. The slide was washed with clean water and air-dried. The blood films were microscopically examined using 100x objectives (oil immersion) and 7x eyepiece as recommended by World Health Organization [14]. Malaria parasites and pigments were identified using standard charts [12].

Determination of Hb Genotypes

The alkaline cellulose acetate electrophoresis as described by Cheesbrough [13] was used. This method employs a cellulose acetate membrane. The materials used included a genotype machine, Tris-EDTA borate buffer (PH 8.5) and standard Hb genotype samples. The blood samples were first haemolyzed by applying drops of distilled water (in a ratio of 1 drop of blood to 2 drops of water). The heamolysates and the standard Hb genotypes were then applied on the cellulose acetate membrane using an applicator. This was then placed in the electrophoretic chamber and ran under stable electric current for 5 minutes to obtain Hb genotype variants.

Examination for Splenic Enlargement

This was by palpation according to Hamilton Bailey [15].

RESULTS

Out of 720 subjects that participated in the study 600 (83.3%) were positive for malaria parasite and *P. falciparum* was the only parasite species identified. A total of 114 children had enlarged spleen representing 15.8%. All the children with splenomegaly had positive malaria parasite while none of the children with negative malaria result presented with splenomegaly. See table 1. Out of the 720 subjects, 576 (80%) were HbAA, 144 (20%) were HbAS. There was no HbSS encountered in the study. See table 2.

Table 1: Prevalence of Malaria and Splenic Enlargement in Children Under 13 Years.

Total No. Screened	No. Positive for *P. falciparum*	No. children with both P. falciparum and enlarged spleen
720	600 (83.3%)	114 (15.8%)

Table 2: Determination of Genotypes in Children under 13 years.

Total No. Screened	Hb Genotypes		
	AA (%)	AS (%)	SS (%)
720	576 (80%)	144 (20%)	0 (0%)

DISCUSSION

The high prevalence rate of *P. falciparum* malaria (83.3%) observed in this study could be attributed to the fact that all the children were symptomatic hospital based subjects as opposed to studies in Ghana and Uganda [16,17] respectively where asymptomatic subjects had been used. However, similar reports of high prevalence of malaria in children in the study area have been documented previously by Mbanugo and Ejims [2]. Lower values have been reported in asymptomatic children in Northern Nigeria [18]. The high level of malaria attacks means that the spleen, which is involved in clearing the malaria parasites is constantly challenged. In the present study splenic enlargement was observed in only 15.8% of the children with *P. falciparum malaria*. This finding possibly suggests that the children with splenic enlargement may be presenting with multiple re-infections at the rate the spleen is overwhelmed, thus resulting in enlargement. This suggests that the study area is mesoendemic. The fact that no sickler participated in the study ruled out the possibility of sickle cell anaemia (SCA) being responsible for the observed splenomegaly. Schistosomiasis and leishmaniasis are not common in the study area [12] either.

Splenomegaly has also been reported in normal new born and 10% of children may have a palpable spleen [19]. However none of the children who participated in this study was less than 6 months old. A spleen rate of 60% has been reported in Lagos Nigeria[1] where the study population had consisted of people with normal HbAA, HbSS and HbAS. Spleen rate is of much epidemiological value where malaria chemoprophylaxis is not practiced. The public health importance of the present study lies in the fact that most children get exposed to malaria transmission very early in life leading to early development of splenomegaly. This trend needs to be checked by environmental measures like improved housing and environmental sanitation to reduce the incidence of mosquito bites and malaria transmission.

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Original Article

Isolation of Dematiaceous Fungi from Soil and their Pathogenic Potentials.

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ABSTRACT

The presence of dematiaceous fungi in 200 samples of soil collected from 4 different villages located in South-Eastern Nigeria were investigated. Using the pour plate method, the samples where cultured in Saboraud dextrose agar with 0.05mg/ml of chloramphenicol and incubated at room temperature. Identification of isolates was by slide culture technique and the following dematiaceous fungi were identified at different proportions; *Madurella grisea* 20 (43%), *Fonsecaea dermatitidis* 10 (21.7%), *Cladosporium werneckii* 10 (21.7%), *Madurella mycetomi* 5(10.8%), and *Leptosphaeria senegalensis* 1 (2.17%). Loamy soil yielded the highest number of varients of dematiaceous fungi. *Madurella grisea* and *Fonsecaea dermatitidis* were pathogenic for laboratory mice when injected subcutaneously. The findings of several dematiaceous fungi in soil samples in these villages may represent possible pattern of infections in humans since people residing in the study area were rural farmers.

Keywords: Mycoses; Soil; Infection.

INTRODUCTION

The demonstration of saprophytic existence of pathogenic and non pathogenic fungi in a particular region serves as reliable index of endemicity of infections caused by them. Soil that serves as home for many organisms also serve as reservoir of infections they cause1,2 Dematiaceous fungi are heterogeneous group darkly pigmented fungi widely distributed in the environment that occasionally cause infections in humans, animals and plants3. When grown on agar, colonies are dark grey, brown and importantly have a black reverse when the bottom of the agar plate is examined. This characteristic dark colour is due to the presence of melanin pigment contained in their cell wall, which are known to be the virulent factor for these organisms4. These fungi can be isolated form soil, air, rotten wood, plants and plant roots, bird nest, staw, water, cereals in tropical and subtropical region of Africa, India, South America, Latin America and Japan2. There are more than one hundred types of dematiaceous fungi in nature. Some of the common species are *Cldosporium, Curvuleria, Madurella, Alternaria, Wangiella, Fonsecaea, Phialophora. Fonsecaea, Phialophora, Madurella, Cladosporium, Exophiala* are pathogenic to humans and animals causing rarely fatal infections in those who have normally intact host defense mechanism. Life threatening illness occur more often in immuncompromised patients. These dematiaceous fungi also contribute significantly to the morbidity and mortality of solid organ transplant recipient[6,7]. With these reports of the pathogenic potentials of dematiaceous fungi in humans and because the people of this town are largely peasant farmers, the focus of this study is to determine the prevalence of these organisms in soil samples and determine the pathogenicity of the identified isolates.

MATERIALS AND METHOD

Soil samples collected from 4 villages in Ebenebe town, Awka North L.G.A of Anambra

State, South-Eastern Nigeria were used. The 200 soil samples were randomly collected from the following towns: Umuji, 50 soil samples, Umuogbuefi 50 soil samples, Umuajani 50 soil samples and Umuoye 50 soil samples. The soil samples were collected in sterile cellophane bags using sterile spoons and processed within 24 hours of collection.

PROCESSING OF SAMPLE

One gram of each soil sample was weighed out and put into bottles containing 100mls of sterilized water. The bottles were shaked for 10 minutes and allowed to stand for 20 minutes. 1ml of the supernatant was used for the 10 folds serial dilution as previously described[8]. 1 ml each of the serial dilutions were dispensed into test tubes containing the 10^{-4}, 10^{-5} and 10^{-6} dilutions and cultured on Sabouraud dextrose agar (SDA) using the pour plate method. The plates were incubated at room temperature. The isolated colonies were then sub-cultured on agar plates.

IDENTIFICATION OF ISOLATES

The matured fungal growths were examined macroscopically and microscopically. Macroscopically established colonies were evaluated on characteristic like texture, size of colony and pigmentation if any. Also the colour of the reverse side or bottom of the plate or bottle was noted. For detailed study of the morphology and natural arrangement of the macro-conidia and conidia or arthrospores on the mycelium, slide cultures of the isolates were prepared. In the microscopic evaluation, the appearance observed was matched against those contained in colour atlases of pathogenic fungi[9,10].

PATHOGENICITY TEST

One isolate each of the dematiaceous fungi; *Fonsecaea dermatitidis, Madurella mycetomi Madurella grisea, Cladosporium werncki and Leptosphaeria senegalensis* isolated from the soil sample were used for the pathogenicity test in the study. 4 weeks old mice (body weight 15 to 20g) were housed in wooden cages and fed for two weeks. A loopful each of the test isolates were dispensed into a sterilized bottle containing 5mls of sterile water and homogenized with glass beads. Then 0.5ml of this homogenized solution was used to inoculate the mice subcutaneously using 1ml syringe as described previously[11]. The cell suspensions were also cultured on sabouraud dextrose agar (SDA) which showed growth after 4 days. The mice were observed for up to 5 weeks for the presence of lesions at the inoculation sites. The mice were sacrificed at the end of the 5th week and samples collected from the subcutaneous lesion were cultured and observed for possible recovery of the organisms.

RESULTS

Five species of dematiaceous fungi were recovered form the 200 samples of soil investigated. *Madurella grisea* was the most frequently recovered 20 (43%) followed by *Fonsecaea dermatitidis* 10 (21.7%), *Cladosporium werneckii* 10 (21.7%), *Madurella mycetomi* 5 (10.8%) and *Leptospaeria senegalensis* 1 (2.17%). Table 2 shows that most of the isolates were recovered from loamy soil (21 (50%). Table 3 shows that the samples collected from Umuji village yielded most of the dematiaceous fungi 19 (14.3%) while the samples from Umuogbuefi yielded the lowest number of organism 8 (17.3%). There were no mortality in the inoculated mice. However, *Madurella grisea and Fonsecaea dermatititidis* produced induration at the site of inoculation on the experimental mice. The organism were recovered from the subcutaneous tissue taken from the site of lesion.

Table 1: Fungal Isolates from soil and their frequencies of occurrence

Dematiaceous fungi	Frequency	% of spp isolated
Madurella grisea	20	43.4
Madurella mycetomi	5	10.8
Leptospaeria senegalensis	1	2.17
Fonsecaea dermatititidis	10	21.7
Cladosporium werneckii	10	21.7
Total	46	100%

Table 2: Distribution of dematiaceous fungi isolated according to different soil types

Isolates	Loamy soil	Clay soil	Sandy soil
Madurella grisea	10	4	6
Madurella mycetomi	5	-	-
Leptospaeria senegalensis	1	-	-
Fonsecaea dermatititidis	3	5	2
Cladosporium werneckii	6	2	2
Total	21 (50%)	11(26.26%)	10 (23.8%)

Table 3: Distribution of dematiaceous fungi isolated according to area of collection

Isolates	Umuji	Umuogbefi	Umuonye	Umuajana
M. grisea	10	4	3	3
M. mycetomi	4	-	-	1
L. senegalensis	1	-	-	-
F. dermatititidis	2	2	5	1
C. werneckii	2	2	2	4
Total	**19 (41.3%)**	**8 (17.3%)**	**10 (21.7%)**	**9 (19.5%)**

DISCUSSION

The study showed that soil serve as important natural reservoir of dematiaceous fungi. This is in conformity with the report of isolation of these fungi from soil in other countries[4,6,8,12]. The study also showed that dematiaceous fungi were more frequently isolated form loamy soil than sandy and clay soil. This is due to the more organic nature of loamy soil[13]. The only isolate of *L. senegalensis* was from loamy soil.

The five species of dematiaceous fungi isolated where more frequently isolated from Umuji village than from the other three villages. This may be due to the differences in the microbial habitat or microbial ecology of the samples. Microbial habitat is the physical location in the environment to which an organism has adapted and this depends on the climate, temperature, moisture, microbial action, pH and mineral content of the soil[4]. Although *L. senegalensis* was isolated only at one site in this study, but have been isolated more frequently from Senegal and Chad and this is why mycetoma, the infection caused by these fungi are common in the area.[15] The possible reason could be due to difference in climatic condition. This finding indicates that inhabitant of Umuji village that are mostly farmers have a high risk of contacting the infection caused by these fungi than the inhabitants of the other three villages.

The pathogenicity of the species of dematiaceous fungi isolated was confirmed in this work by the ability of *M. grisea and F. dermatitidis* to produce subcutaneous lesion in mice. Similar report has been made by Okeke et al[14] in their isolation of dematiaceous fungi *F. pedrosoi, Cladosporium carrionii, Phialophora verrucosa* from soil samples collected in Nsukka South Eastern Nigeria that produced similar lesion on mice. This shows that the microbial ecology of the South Eastern Nigeria may be similar. Gugnani also has reported

isolation of *M. mycetomi* from soil in India and which also was pathogenic to laboratory mice[8]. This support the information from literatures of the pathogenicity of dematiaceous fungi in humans[2,3,4,5,6].

These findings indicate that the study area habour dematiaceous fungi, therefore the inhabitant of the three villages have a high risk of contacting the infections caused by these fungi. There is need therefore for an epidemiological study to be carried out on the population to determine the prevalence rate of the cutaneous, subcutaneous and systemic infections caused by these pathogenic fungi.

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Original Article

Red Cell Parameters in Nigerian Women Using Oral Contraceptives (Lo-Femenal).

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ABSTRACT

The study was designed to assess the Red Blood Cell parameters in Nigerian women using oral contraceptives (Lo-Femenal). 50 women practicing the oral contraception method for varying length of time were recruited for the study. They comprise 22 women who had been on Lo-femenal for < 1 year 9 women who had been on Lo-femenal for 1 year and 19 women had been on Lo-femenal for > 1 year. Similarly 50 women not practicing contraception were recruited and served as control. Blood samples collected from all participants were used for the analysis of some haematological parameters using standard haematological techniques. The result showed that the Haematocrit, haemoglobin and red blood cell count dropped significantly in the Lo-femenal users compared with non-users ($p < 0.001$). The drop in blood levels of these parameters was much profound in users of < 1 year duration. On the other hand, red cell indices (MCHC, MCV and MCH) were significantly raised in users compared with non-users ($p<0.001$). These elevations were more in users of < 1 year duration. Blood films from Lo-Femenal users predominantly showed mild macrocytosis and anisocytosis. The reversibility of haemoglobin and packed cell volume with time in women who initiate Lo-Femenal warrants further study.

Keywords: Oral contraceptives, Lo-Femenal, Red cell parameters.

INTRODUCTION

The continuous growth of the world population and the recognition of gender equity, equality and women empowerment has necessitated the introduction of oral contraceptives (pills) as one of the family planning regimen for the prevention of unwanted pregnancies and abortions as well as to improve the timing of child birth in women of child bearing age [1, 2]. Oral contraceptives containing synthetic oestrogen and progestogen have been shown to be efficacious in preventing pregnancy with only about 0.34 pregnancies per 100 women occurring in OCs users[2]. In addition to prevention of pregnancies, oral contraceptive may affect many organ systems. For example, it has been found that OCs containing oestrogen decrease bile flow and bromosulphtalein excretion while progestin containing OCs have the propensity to cause intrahepatic cholestasis and decrease in bilirubin conjugation[3]. An increased risk of thromboembolic diseases [4], myocardial infarction[5], migraine[6] and circulatory disease and carcinogenicity[7] have been formally demonstrated in women taking oral contraceptive, although the risk tended to diminish with the use of low doses of hormonal steroids. It has been found that oral contraceptives alter some biochemical laboratory values [8] depending not only on the dose and choice of oestrogenic and progestional components but also on the interactions between the two. For instance, a review of nutritional aspect of OCs has shown that vitamin A is increased during oral contraceptive use in contrast to decreases in serum vitamins B_1, B_2, B_6 and B_{12}, ascorbic acid and zinc [9]. Available data on haemostatic studies in oral contraceptive

users, mostly from developed countries have produced divergent results [10-13]. There is paucity of information on the effect of oral contraceptives especially, Lo-Femenal, on the haematology of users in developing countries. The aim of the present study is to ascertain the changes, if there is any, in the red cell parameters in Nigerian women using Lo-Femenal brand of oral contraceptives.

MATERIALS AND METHODS

Our subjects were recruited from women (aged 21-38 years) attending Family Planning Clinic in the Department of Obstetrics and Gynaecology of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The Research Ethical Committee of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife approved the protocol for this study. The purpose of the study was explained to the participants after which willing volunteers gave their consent to participate in the study. Women were included if they were on Lo-Femenal brand of oral contraceptives and were willing to participate in the study. Those excluded were smokers, users of other brands of oral contraceptives or have haematological disorders that may interfere with the outcome of the study. In all, a total of fifty (50) women comprising users of Lo-Femenal for < 1 year (n = 22), 1 year (n = 9) and > 1 year (n = 19) respectively were enlisted for participation. Sociodemographic data such as age, duration of Lo-Femenal use, level of education, occupation and parity of the participants were obtained by oral interview. Weight (in Kg using a standard hospital balance) and height (in m using a metal rule) were measured (in light clothing, without shoes). Fifty (50) apparently healthy non-pregnant, non-contraceptive users matched for age, weight, height and socioeconomic status and who do not smoke comprising staff of Obafemi Awolowo University Teaching hospital Complex, Ile-Ife served as controls. Two millilitres (2ml) of blood were collected at 08.00 hours from the antecubital veins of participants into EDTA bottles and thoroughly but gently mixed. Haematocrit, haemoglobin concentration and red cell indices were calculated [14]. Thin blood films made were stained by standard Leishman techniques, dried and viewed under oil immersion for the morphology of red blood cells. All samples were analysed within 2 hours of collection. Body mass index (BMI) was calculated using the formula: BMI = weight (Kg)/ height (m^2) [15] while level of education and occupation were used to assess the socio-economic status of the participants.

DATA ANALYSIS

Data were analysed for mean and standard deviation. Significant difference between groups was determined by students't-test at $p < 0.001$.

RESULTS

The baseline characteristics of the subjects and controls are shown in table 1. Twenty two (22) women had been on the pills for less than one year, 9 for one year, and 19 for more than one year. Although there was no significant age, parity and BMI differences between Lo-Femenal users and non-users, the users were heavier, older and had more deliveries than the non-users. Lo-Femenal users had significantly ($p < 0.001$) lower haematocrit, Hb and red cell count, which were much lower in those who had been on the pills for a period of less than one year than the non-users (table 2). However, the decreases in haematocrit and Hb concentration in users tend to normalise with increasing duration of Lo-Femenal use such that the mean haematocrit and Hb in women who had been on the pill for greater than one year were comparable to non-users (table 2). On the other hand, red cell indices (MCHC, MCV and MCH) were significantly ($p < 0.001$) higher in women who use Lo-Femenal than those who do not and this elevations were more in those who had used the pill fro less than one year (table 2). Blood films of Lo-Femenal users predominantly showed mild macrocytosis and anisocytosis (data not shown).

DISCUSSION

Most studies on the effects of hormonal oral contraceptives have concentrated on the biochemical and haemostatic parameters [11-13, 16]. In the present study, red cell indices among oral contraceptive (Lo-Femenal) users varied significantly from non-users. For instance, the mean haematocrit, Hb and red cell count were significantly depressed in users in corroboration with the study of Frassinelli-Gunderson *et. al.* [17]

Table 1: Baseline characteristics of Lo-Femenal users and non-users.

Duration	N	Mean age ± SD (yrs)	Mean parity ± SD	Mean BM (Kg/m^2)
Controls	50	27.9 ± 3.3	1.6 ± 1.8	25.7 ± 1.7
< 1	22	29.2 ± 3.4	2.7 ± 1.0	26. 2 ± 2. 4
1	9	31. 1 ± 4.5	3.2 ± 2.0	26.6 ± 2.6
>1	19	31.7 ± 2.1	4.1 ± 1.2	27. 1 ± 1.9

BMI (Body mass index); **SD** (Standard deviation).

Table 2: Red cell parameters in Lo-Femenal users and non-users.

Duration (yrs)	Hct (L/L)	Hb (g/dl)	Rbc x 10^{12}/L	MCHC(gHb/dl rbc	MCV (fl)	MCH (pg/cell)
Controls (n = 50)	0.39±0.02	13.1±0.68	5.3±0.43	32.5±0.54	75±4.40	24.8±1.88
<1(n =22)	0.36±0.02*	11.8±0.40*	3.6±0.40*	33.5±0.91*	102±10.34*	35.1±5.95*
1 (n = 9)	0.37±0.02*	12.3±0.36*	3.7±0.33*	33.1±0.93	99±6.98*	32.9±2.30*
>1(n= 19)	0.39± 0.02†	12.8±0.58†	3.9±0.52*†	32.9±0.31*	101±11.71*	33.1±3.9*

Legend: *p < 0.001 from controls; †p < 0.001 from < 1 year; **Hct** (Haematocrit); **Hb** (Haemoglobin); **Rbc** (Red blood cell count); **MCHC** (Mean corpuscular haemoglobin concentration); **MCV** (Mean corpuscular volume); **MCH** (Mean corpuscular haemoglobin).

except that in their study, the difference in Hb between the users and non-users was not statistically significant. It however contrasts the findings of Prasad *et. al.* [18] where no effect of oral contraceptive agents was seen on Hb, haematocrit and erythrocytes count. The discrepancies in the findings may be attributed to the study population. While Prasad and colleagues [18] conducted their study in America (developed country) the present study was done in developing country where sub-clinical anaemia is not uncommon probably due to latent micronutrient deficiencies [19]. Although the cause of decreases in Hct, Hb and red cell count in women who initiate Lo-Femenal is yet unknown, it may be related to haemostatic adjustment to the drug as study has shown that women initiating hormonal oral contraceptives reported more bleeding-spotting days [16]. On the other hand, investigation has shown decreased serum and red cell folate concentrations in women taking oral contraceptives, and cases of megaloblastic anaemia related to folate deficiency have been reported [10]. Additionally, OCs and other drugs have been shown to change the requirements for folic acid [20]. Interestingly however, the lower Hb and Hct observed in patients initiating Lo-Femenal

tended to normalise with increasing duration of OCs use as evidenced by lack of significant difference in Hct and Hb in women who had used the pill for greater than one year and non users. This finding suggests that the initial decreases in Hb and Hct may be partly attributed to maternal depletion syndrome.

Although, we could not establish the nutritional status of our subjects as this was not included in the original design of the present study, maternal depletion of iron, ferritin, folic acid and vitamin B_{12} may account for the initial decreases in Hb and Hct observed. This may probably be a consequence of inadequate child spacing as women in this group have had approximately 3 (2.7) deliveries before initiation of OCs. On the other hand, OCs use might have improved the haematological parameters in users as previous *in vitro* [21] and *in vivo* [22] studies showed evidence of inhibition of lipid peroxidation by oestrogen, suggesting that OCs may have antioxidant activity. This antioxidant protection may account for the higher values of Hb and Hct in women who had been on Lo-Femenal for greater than one year than those who had been on it for shorter duration in the present study thus reaffirming the safety of prolonged use of Lo-Femenal as a combined oral contraceptive [23]. The significantly raised levels of MCHC, MCV and MCH observed in the present study are in accord with earlier reports by Frassinelli-Gunderson *et. al.* [17] and may be due to difference in iron stores between the OCs users and non-users. We conclude that Lo-Femenal brand of combined oral contraceptive produces reversible depression of haemoglobin and haematocrit during initiation. Further study is needed to clarify the mechanism underlying these effects and to elucidate the effect of Lo-Fermenal in anaemic women.

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Original Article

Effect of Short Term Antiretroviral Therapy on CD4+ Cells and Immunoglobulins in HIV Seropositive Subjects.

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ABSTRACT

The study was designed to assess the effect of short-term antiretroviral therapy (ART) on IgA, IgG and IgM and CD4 $^{+}$ T cell counts in HIV seropositive subjects. 20 confirmed HIV seropositive subjects, aged between 15-65 years were recruited for the study. They were on triple combinations therapy consisting zidovudine, lamivudine and nevirapine. 20 HIV seronegative subjects were used as control. Blood sample was collected from the participants for the determination of the above parameters. The CD4 $^{+}$ T cell counts show no significant difference between pre-ART and 2 months post-ART ($P>0.05$) but was significantly higher by 4 months post-ART compared with the pre-ART ($p <0.05$). IgG and IgM serum levels showed significantly high values by 2 and 4 months post- ART compared with the pre-ART value ($p <0.05$ in each case). However, the serum IgA level by 4 months post-ART showed no significant difference compared with pre-ART value ($p >0.05$). Meanwhile there were no significant differences in CD4 count, IgM, IgA, and IgG levels between 4 months post-ART values compared with the corresponding values in HIV seronegative control subjects. The present study showed an improvement in the blood concentration of CD4 cell by 4 months post-ART administration, which suggests possible recovery of cellular immunity. The insignificant difference in IgA concentration within the study period possibly suggests non-progressive mucosal or sub mucosal infections. Similarly the raised IgM and IgG concentrations within the study period may be an indication of existing infections and signifies possible potentials towards short-term recovery. This shows that with the use of these drugs prognosis seem good for the short term.

Keywords: ART, RVD, human.

INTRODUCTION

HIV infection is wide spread in the sub Saharan Africa1[1,2]. The viral infection has been shown to affect both the number and functions of the CD4+ T cells, thus causing impaired T cell functions[3,4]. At the same time hypergammaglobulineamia have be1en noticed in patients with human immunodeficiency virus (HIV) infection. They have been shown to exhibit a generalized non–HIV specific polyclonal B–cell activation with increased production of HIV specific antibodies[5,6,7,8,9]. However, the use of ART has shown good promise because it effectively suppresses the HIV in vivo replication, restoring CD4+T cell number and functions. Consequently the use of ART has reduced both HIV morbidity and mortality rates. Presently, there is coordinated use of combined ART in Nigeria. The present study was thus designed to understand the recovery of CD4+ T cell and total immunoglobulin A,G,M concentration before and during commencement of the combined ART.

MATERIALS AND METHODS

Subjects:

20 confirmed HIV seropositive subjects (male = 5 and female = 15), aged between 15 – 65 years were recruited for the study and followed up bimonthly for 4 months. 5mls of blood was collected from the participants at each visit and dispensed into EDTA tubes for CD4+ T cell count and into plain tube for serum extraction for immunoglobulin (G, M, A) determinations. 20 HIV seronegative subjects were used as control. The participants gave informed consent and the study design was approved by Nnamdi Azikiwe University Teaching Hospital (NAUTH) Board of Ethical Committee.

METHODS

HIV Screening by Immunochromatographic Method: The procedure was as described by the manufacturers of the kit (CHEMBIO diagnostic system, Inc. New York USA). In brief, 5μl of serum samples was dropped into the "specimen pad" of the test strip. Then 80μl of buffer was then added. The reaction was allowed for 10 minutes, the appearance of distinct red line at the test region and control region of the kit suggest positive HIV test while one distinct red line in the region of the control suggest HIV seronegativity. The appearance of the distinct red line at the control region validates the result without which the kit is assumed to have deteriorated.

Cyflow Counter Automated CD4+ Count: 20μl of EDTA whole blood was collected into Partec test tube (Rohren tube). Then 20μl of CD4+T antibody was added into the tube .The contents were mixed and incubated in the dark for 15 minutes at room temperature. 800μl of CD4 buffer was gently added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the CD4+T cells were displayed as peaks and interpreted as concentrations.

Immunoturbidimetric Method of Immunoglobulin - A,G, M, Estimations

Protocol for Determination of IgA:

The procedure was as described by the manufacturer of the kit (Human, Germany). 900ul of the phosphate buffer (PH 7.2) was delivered in a test tube and 3ul of sample added into the tube. The same procedure was performed for the standard IgA. The reaction was mixed and incubated at 37^0c for 5 minutes in water bath. It was read as A1 spectophotometrically. Subsequently, 80ul of anti-IgA reagent was added into the same tube, mixed and incubated at 37^0C in water bath for 5 minutes. It was then read spectophotometrically and recorded as A2. The result was read off the standard immunoglobulin graph plotted with concentration against the absorbance using various dilutions of the standard immunoglobulin.

Method for Determination of IgG by Spectrophotometry

The procedure was as described by the manufacturers of the kit (Human, Germany). 400ul of the phosphate buffer PH 7.2 was delivered in a test tube and 4ul of sample was added to the tube. The same procedure was performed for the standard IgG serum. The reactions was mixed and incubated at 37^0c for 20 minutes in water bath. It was read as A1 spectrophotometrically. Subsequently, 15ul of anti IgG reagent was added into the same tube, mixed and incubated at 37^0c in water bath for 20 minutes. It was then read and recorded as A2. The result was read off the standard immunoglobulin graph plotted with concentration against the absorbance using various dilutions of the standard immunoglobulin.

Method for Determination of IgM by Spectrophotometry.

The procedure was as described by the manufacturer of the kit (Human, Germany). 900ul of phosphate buffer PH 7.2 was delivered

in a test tube and 4ul of the sample was added to the tube. The same procedure was performed for the standard IgM serum. The reaction was mixed and incubated at 37^0c for 20 minutes in water bath. It was read as A1 spectrophotometrically. Subsequently, 150ul of anti IgM reagent was added into the same tube, mixed and incubated at 37^0c in water bath for 20 minutes. It was then read spectrophotometrically and recorded as A2. The result was read off the standard immunoglobulin graph plotted with concentration against the absorbance of the standard dilutions of the immunoglobulin.

RESULTS

There was no significant difference in mean CD4+ T cell counts (/mm^3) in HIV positive subjects between pre–ART value of 348±216 and 2 months ART value of 408±227) ($p>0.05$). similarly, no significant difference in mean value of serum IgA was seen between the pre-ART era (240.2±82.1) and 2 months post–ART era (220.7±46.9) $p>0.05$. This was also the case for both IgG and IgM during the same period of evaluation. See table 1.

By 4 months post ART era, no significant difference in mean value of CD4+ T cells (484±225) was observed compared with value during pre-ART era ($p>0.05$). there was also no significant difference in mean serum IgA concentration by 4 months post ART era (212.5±56.0) compared with pre-ART value ($p>0.05$). However, the mean serum IgG concentrations (mg/dl) by 4 months post–ART era (784.4±183.1) was significantly higher compared with the pre-ART value (568.1±339.0) ($p<0.05$). Similarly, the serum IgM concentration (mg/dl) by 4 months post–ART (151.9±49.7) was significantly higher than the corresponding value pre-ART (108.0±35.0) ($p<0.05$). See table 2.

The mean CD4+T cell count (/mm^3), and mean serum concentrations of IgA, IgG and IgM by 4 months post ART era were similar to their corresponding values in control HIV seronegative subjects ($P>0.05$ in each case). See table 3.

Table 1: The mean blood CD4+ T cell count (/mm^3), serum concentrations (mg/dl) of IgA, IgG and IgM in HIV seropositive subjects pre–ART and 2 months post– ART

Parameter	HIV Patients pre – ART (n = 20)	HIV Patients 2 months post – ART (n = 20)	P – value
CD4 count (per ul)	348.2±216	408.0±227	>0.05
IgA (mg/dl)	240.2±82.1	220.7±46.9	>0.05
IgG (mg/dl)	568.1±339.0	732.1±220.1	>0.05
IgM (mg/dl)	108.0±35.0	119.4±32.0	>0.05

Table 2: The mean blood CD4+ T cell count (/mm^3), serum concentrations (mg/dl) of IgA, IgG and IgM in HIV seropositive subjects pre–ART and 4 months post– ART

Parameter	HIV Patients pre – ART (n = 20)	HIV Patients 4 months post – ART (n = 20)	P – value
CD4 count (per ul)	348.2±216	494.2±225	<0.05
IgA (mg/dl)	240.2±82.1	212.5±56.0	>0.05
IgG (mg/dl)	568.1±339.0	784.4±183.1	<0.05
IgM (mg/dl)	108.0±35.0	151.9±49.7	<0.05

Table 3: The mean blood CD4+ T cell count (/mm^3), serum concentrations (mg/dl) of IgA, IgG and IgM in HIV seronegative subjects and HIV seropositive subjects at 4 months post- ART.

Parameter	HIV negative control subjects (n = 20)	HIV Positive Patients 4 months post – ART (n = 20)	P – value
CD4+ T cell (per ul)	537.4±116	494.2±225	>0.05
IgA (mg/dl)	183.7±52.8	212.5±56.0	>0.05
IgG (mg/dl)	699.7±343.0	784.4±183.1	>0.05
IgM (mg/dl)	125.4±34.5	151.9±49.7	>0.05

DISCUSSION

The present study observed an improvement in the blood concentration of CD4 +T cell by 4month post-ART administration. This suggests possible recovery of cellular immunity within short term ART combination therapy. CD4 + T cells are cells known to control both the cellular and humoral immune responses. However, these immune cells are the principal target of the invading HIV.

The invasion of these immune cells by the HIV causes CD4 dysfunction and death. This will subsequently result in reduction of CD4 + T cell population. In the present study, the administration of ART combination for short term led to the recovery of the CD4 + T cell population this might be an indication of possible impact of the ART at limiting the viral replication processes in the infected hosts. Similar finding has been reported independently by Resino et al.[10] and neckolic et al.[11].

The serum IgA concentration showed no significant different within the study period. The non significant different in IgA concentration observed within the study period possibly suggest, non-progressive mucosal or submucosal infection. The observed similar concentration between the Pre-ART treatment and 4-month post-ART treatment may also suggest non-deterioration in mucosal and sub-mucosal immunity and integrity upon commencement of ART. The non-progressive or drop in mucosal infection may mean an index of good prognosis in HIV infected individuals on ART.

However, this study observed raised IgM and IgG concentration within the study period. This might be possibly due to the opportunistic infections, usually associated with HIV infection. The existing systemic infection may have activated B cell proliferation and differentiation into plasma cells, and shedding of IgM immunoglobulin against the antigens. Similarly, the raised IgG shows that there was effective immunoglobulin class switching leading to the production IgG that is mainly involve in secondary immune response. The high production of the immunoglobulin M and G may signify possible potential towards short term ART administration. Studies elsewhere have shown that HIV causes hyper activation of B cells[5,6,12]. The finding of the present study suggest that the hyper gammaglobulineamia encountered in HIV subjects on ART are mainly IgG and IgM induced but not IgA induced.

The present study thus concludes that there is possible recovery of cellular immunity and progressive recovery of humoral immunity in HIV subjects following short term ART combination administration.

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Original Article

Hypertension prevalence and awareness amongst a group of women attending 'August' meeting.

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ABSTRACT

The present study was designed to assess the prevalence of hypertension, awareness and control on a group of women attending an annual meeting in the South Eastern Nigeria popularly called 'August' meeting. A total 164 women (mean age: 54.4±12.7 yrs.) attending 'August' meeting had their BP measured and history of hypertension and/ or history of anti-hypertensive drug use taken. Hypertension was defined as systolic blood pressure≥ 140mmHg and diastolic blood pressure ≥ 90mmHg. The prevalence of hypertension was 44.5% while 43.7% were aware they were hypertensive. 90.3% of those aware of being hypertensive were on anti-hypertensive drugs of which 13% had BP ≤ 140/90mmHg. There is a high prevalence of hypertension in our women and efforts should be made to ensure that their blood pressure is well controlled.

Keywords: women; blood pressure; history

INTRODUCTION

Hypertension is a disease without symptoms, frequently referred to as a "silent killer". It is a condition of great Public Health importance being a major risk factor for cardiovascular disease, renal disease and cerebral vascular accidents. It causes a large burden of ill-health and premature death [1, 2] much of which is preventable with effective treatment [3] It is becoming particularly an important public health problem in Sub-Sahara Africa.[4, 5, 6] Previous estimates of hypertension prevalence from Nigeria [7, 8, 9, 10, 11] were based on older definition of Systolic Blood Pressure (SBP) ≥ 160mmHg and Diastolic Blood Pressure (DBP) ≥ 95mmHg and thus had lower prevalence rates. The higher the blood pressure (BP) the greater the risks are for heart attack, heart failure, stroke and kidney disease[12, 13, 14]. Conversely lower BP was associated with greater probability of survival to age 85 yrs. free of major co morbidities[15]. Latest surveys show that by age ≤ 50 yrs. prevalence is approximately 30%[16] in rural Africa but > 50% in semi-urban West Africa and ≈ 50 – 60% in a mixed South African population. Findings in Sao Paolo Brazil showed a prevalence of 55.9% in women 60 yrs. and above.[17] In addition, prevalence rates are said to be rising. For example, in Ghana a prevalence of 28.3% was found in 2003[18] and 3yrs. later in 2 rural communities in the same Ghana another group found a prevalence of 32.8%[19]. This rising prevalence provided the need for the study to determine the prevalence amongst a group of women and also to determine the level of awareness and control of hypertension amongst them.

SUBJECTS AND METHODS

Women attending "August" meeting at Naze in 2005 were recruited for the study. Naze is one of the communities that make up Owerri- North Local Government Area, Imo State of Nigeria. It is a suburb of Owerri, the capital of Imo State which is one of the five states that make-up the South East Zone of Nigeria, inhabited by Igbo speaking people. In the month of August every year, women married to men of the South East origin usually return to the towns and villages of their husbands irrespective of where they reside

in Nigeria to attend the "August" meeting. Initially it used to be mass return but later it became a delegate's conference. During these meetings various development projects are discussed as well as how to achieve the desired goals. Decisions taken at these "August" meetings play important roles in the lives of these towns and villages. It was therefore considered opportune by the authors when the executive of the Naze group approached the authors for a health talk during the 2005 "August" meeting. The topic Hypertension was chosen for discussion and in the course of the discussion it was discovered that participants were eager and interested requesting for measurement of their blood pressure and this gave birth to the study. A total of 164 women attending the 2005 "August" meeting at Naze, Owerri North Local Government Area, Imo State, were admitted into the study. These attendees represented all the socio- economic classes as amongst them were medical doctors, lawyers, teachers of secondary and primary schools, senior civil servants, market women etc, though not in equal proportions. Included in the sample were those who gave informed consent. Those who withheld consent were excluded from the study. Each participant was asked about history of hypertension and or antihypertensive drug use and age, followed by BP measurement. The BP was measured using a Standard Mercury Sphygmomanometer with appropriate cuff size, in a well ventilated room in the sitting position by nurses who are also attending the "August" meeting and had earlier been shown by the authors what is expected regarding BP measurement. Two BP readings were obtained at intervals of 2 minutes and the mean was used for analysis. Korotokoff I, V were taken as systolic and diastolic BP respectively. Hypertension was considered to be present if the participant gave a history of hypertension and or at the time taking medication or had Systolic BP (SBP) or Diastolic BP (DSP) $\geq$ 140/90 mmHg.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS.

RESULTS

A total of 164 women attending 2005 "August" meeting at Naze were examined. The mean age ($\pm$ S.d.) was 54.36 $\pm$ 12.73 yrs. The mean systolic blood pressure (SBP) was 141.63mmHg $\pm$23.42. The mean diastolic blood pressure (DBP) was 92.67mmHg $\pm$ 14.67. The prevalence of hypertension defined as Blood pressure $\geq$ 140/90mmHg was 44.5%. Only 43.7% were aware of being hypertensive 90.3% of those who are aware of their hypertension are on drugs. 13% of those on drugs have BP $\leq$ 140/90. Age correlated with SBP P>0.003 but did not correlate with DBP P>0.306.

DISCUSSION

Our finding of a prevalence of 44% provides evidence of high prevalence of hypertension in a group of women attending "August" meeting in South East Nigeria. The observed prevalence of 44% is lower than the 54.6% recorded in 2007 in Ghana[20] Comparison with previous surveys [7, 8, 9, 10, 21] are problematic since these surveys occurred in different study samples with different age structures and diverse definitions of hypertension. The earlier studies in Nigeria used SBP $\geq$ 160 and DBP $\geq$ 95mmHg while newer studies used SBP $\geq$ 140 and DBP $\geq$90. This being so it is impossible to compare the result obtained using these cut off points. The difference in prevalence rates may be partly explained by the sampling methods. Studies [16, 19, 22] have shown that prevalence of hypertension in Sub-Saharan Africa is significantly higher in the urban population compared to the rural population. Some of the studies were on urban dwellers, some on rural while others were on both urban and rural dwellers. Those recruited for this study it should be remembered were urban dwellers who had come for 'August' meeting. However, hypertension prevalence appears to be increasing worldwide. In 2003 a prevalence of 28.4% was found in a Ghanaian population[18]

which increased to 32.8% in 2006 [19]. In the same Ghana in 2007 a prevalence of 54.6% was found in a population of women[20]. The prevalence of hypertension in our study is higher than most comparable studies in Nigeria. However recent studies elsewhere showed prevalence rates in women of 38.6% [23] in one population and 55.4% [17] in another population. The basis for observed increase in hypertension prevalence is not well known. However considering the socio-economic challenges facing families in Nigeria, there are indications that Psychological Stress has been related to higher BP and unfavorable cardiovascular profile [24]

Our finding of high prevalence of hypertension provides evidence of the increasing cardiovascular burden due to hypertension in Sub-Saharan Africa. 43.7% of the study population was aware of their hypertension. This compares favorably with the study which showed an awareness of 46.1%[25] and better than the study in Ghana with an awareness of 34%[18] and that in Korea with an awareness of 22.9%[26]. This level of awareness is encouraging as hypertension is diagnosed by measuring blood pressure which is a simple, in-expensive, rapid and non-invasive procedure.

Of those who are hypertensive 90.3% are on anti-hypertensive drugs. This is rather a high proportion when compared to other studies [20]. The population it ought to be remembered is not a rural population but rather from various urban centers of Nigeria. It is a literate group as they were delegates representing their members at the 'August' Meeting and this may explain the high awareness rate. However about 13% of those on drugs have BP ≤ 140/90mmHg. This is better than the studies from Ghana of 4%[18] and 4.4%[20] and compares favorably with the study from Korea with 10.7%[26] control rate. The overall health impact of low control rates is significant. Patient non compliance with treatment is common in hypertension therapy. Various reasons have been adduced for non compliance among which are lack of education, lack of awareness of hypertension and its risks for cardiovascular diseases in the lay population, misguided drug use, availability and costs [27, 28, 29]. Anecdotally long-term drug therapy is not a welcome concept. The authors hold that proper education through programs directed at such meetings will heighten the success of management strategies in hypertensive patients.

The mean age of the study population is 54.36±12.73yr and thus an older age group. The higher prevalence could partly be explained by the age of the population as it is known that Blood Pressure correlated positively with age [10]. It has been said that loss of estrogen, which has been shown to improve endothelial function [30] occurs postmenopausal and increases the risk of cardiovascular disease in women [31, 32]. Even though prevalence of hypertension increases dramatically with age, it is important to realize that the burden of hypertension is occurring in the middle age group and therefore efforts aimed at reducing the burden should be directed to those of middle age.

The study has shown a high prevalence of hypertension in our women and thus a significant public health problem. Identification of women with hypertension is essential as elevated blood pressure can be controlled by taking prescribed medication thereby reducing cardiovascular burden.

Fig. 1: The mean, standard deviation, minimum and maximum for age, SBP and DBP.

	Age yrs	SBP mmHg	DBP mmHg
Mean	54.36	141.68	92.67
S.d.	12.73	24.42	14.67
Minimum	26.00	100.00	60.00
Maximum	88.00	230.00	140.00

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Original Article

Prevalence of Trichomonas vaginalis among pregnant and non pregnant women in Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria.

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ABSTRACT

Vaginal trichomoniasis is a recognized Sexually Transmitted Infection (STI) with reproductive health complications as well as causing an increase in susceptibility to HIV infection. However few data exist in our environment concerning the pathogen in pregnant and non pregnant women since the HIV epidemic. In this study, 2720 women comprising 1420 pregnant and 1300 non pregnant women were screened for Trichomonas vaginalis. Vaginal swabs of these women were processed using three diagnostic methods viz: - Saline wet preparation, Giemsa staining and culture method using modified chocolate agar. The prevalence of *T. vaginalis* was 9.3% in both pregnant and non pregnant women. The highest diagnostic yield of 246 (9.04%) was from the culture method while the least 182 (6.7%) was obtained with the Giemsa staining. The women were also screened for HIV infection and 480 (17.7%) were HIV sero-positive with 182 of them having *Trichomonas vaginalis* infection. At $P \leq 0.05$. It was found that there is a significant relationship between trichomoniasis and HIV infection. The culture technique though costly and time consuming is the most sensitive among the three methods used and in view of the relationship between STIs especially Trichomoniasis and HIV infection it becomes pertinent to use a sensitive method to diagnose Trichomonas infection with subsequent proper treatment.

Keywords: Trichomonas vaginalis: Pregnant and non-pregnant women.

INTRODUCTION

The parasite protozoan *Trichomonas vaginalis* is a common pathogen that causes trichomoniasis and has been linked to preterm birth acquisition of human immunodefiency virus, infertility and non gonococcal uretritis[1-3]. Inspite of the knowledge, Trichomoniasis is probably the most neglected sexual infection not only in Nigeria but Worldwide[4]. Due to recognized anatomical and physioimmunological factors, this infection is known to be worse during pregnancy or immediately after menstruation. Studies on prevalence of *T. vaginalis* have been in the past in Ibadan[5], Lagos[6] and Owerri[7] These studies are relatively old, were done before the HIV epidemic and the patients were not screened for HIV and none has been done in Nnewi. This study was designed to provide an up to date knowledge on the prevalence of *T.vaginalis* in Nnewi among pregnant and non pregnant women and the relationship between HIV infection and Trichomoniasis.

MATERIALS AND METHODS

Between October 2002 and April 2003, 1420 pregnant women attending antenatal clinic at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi were selected for the study as well as 1300 non pregnant females attending the Gynaecology out patient clinic in the same hospital. The ages ranged between 11 and 50 years. These patients were examined after their consent was obtained. Pre test counselling was done by trained councellors prior to HIV test and post test counselling before HIV screening result was revealed to patient. Data on sexual history, clinical signs and symptoms were

recorded. Three samples of vaginal fluid were taken from the posterior fornix of the vaginal wall using sterile cotton swabs, after the introduction of a sterile vaginal speculum without antiseptics or lubricants. Using the first swab, the organism was detected by microscopic examination of a saline mount [8,9]. The second swab was inoculated onto a plate containing 25ml of Ewang's modification of chocolate agar[10]. The third swab was used to make a smear on a clean grease free slide fixed with methanol and then stained with Giemsa. Blood was collected, allowed to clot and the serum used for HIV1 and 2 Bispot screening.

RESULTS

Of the 2720 women studied, 252 were found to be infected with *T.vaginalis* giving a prevalence of 9.3%. Out of the 252 positive cases, 150 were pregnant while 102 were non pregnant. Table 1 shows the comparison of the three diagnostic methods used. Using a chi square (x^2) test at $P \leq 0.05$. It was found that there was a significant difference between the three methods employed, with the culture method being the most sensitive and the Giemsa staining being the least. The distribution of *Trichomonas vaginalis* infection among different age groups is shown in Table2. The ages mostly affected are those between 21 and 30 years and the least affected are those between 41-50 years. The HIV screening of the 2720 women revealed that 480 were HIV seropositive while 2240 were negative. Of the 480 HIV seropositive cases, 182 were infected with *Trichomonas vaginalis* of which 150 were pregnant and 32 were non pregnant while in 2240 HIV negative women, only 70 were found with *T. vaginalis* infection. Using a chi square χ^2 to test for significance at $P \leq 0.05$, shows a statistical difference between *T.vaginalis* infection in pregnant and non pregnant women. Table 3. Table 4 shows *Trichomonas vaginalis* infection in relation to clinical signs and symptoms.

DISCUSSION

The prevalence of *T.vaginalis* infection in the study is 9.3%. This conforms with previous work by Acholonu[11], Rotimi and Somorin[12] in Lagos which gave prevalence rates of 8.21% and 10.5% respectively but differs remarkably with the report of Anosike et al, [7] where students of higher institution who are known to be very sexually active were used as the study population. Hart [13], in 1993 showed that pregnancy is an independent predictor for trichomoniasis.

Table 1: Comparison of the saline wet preparation, culture, and Giemsa stain technique for the detection of *Trichomonas vaginalis*.

Diagnostic methods	Number examined	Number positive	Number Negative
Culture	2720	246	2474
Wet mount	2720	218	2502
Giemsa Stain	2720	182	2538

$X^2 = P \leq 0.05$

Table 2: Distribution of *Trichomonas vaginalis* infection among different age groups.

Age group in years	Number examined	Number positive(%)
11-12	720	62 (24.6)
21-30	1,004	124 (49.2)
31-40	802	58 (23.0)
41-50	194	8 (3.2)
Total	2,720	252 (100%)

Table 3: Distribution of *Trichomonas vaginalis* infection among HIV sero-positive and HIV sero-negative individuals.

HIV serostatus	HIV positive (%)	HIV negative (%)	Total number
T. vaginalis	182 (37.9%)*	70 (3%)	252
Absence of *T. vaginalis*	298 (62.1%)	2170 (97%)	2468
	480(100%)	2240 (100%)	

( )* = 31.3% are pregnant women

Table 4: Symptoms associated with *Trichomonas vaginalis* infection in the patients studied.

Symptoms	Number examined	Number with *T vaginalis* infection	Number without *T. vaginalis* infection
Itching/Burning sensation	680	96 (14.1%)	584
Discharge	520	65 (12.5%)	455
Itching/Burning sensation and vaginal discharge	618	116 (18.7%)	502
No symptoms	1940	28 (1.4%)	1912

The majority of the infected women were within the age ranges of 21-30 years, followed by 11-20 years in the present study. These correspond with the second and third decades of life which Anosike[7] noted as being the most affected group as they are very sexually active at this stage. The incidence of *Trichomonas vaginalis* in the last reproductive age range was the least. This could be attributed to the decline in sexual activity and also alteration in pH due to lower or at times non secretion of reproductive hormones[14]. The distribution of *T.vaginalis* between HIV seropositive and sero negative individuals revealed that a significant statistical relationship existed between trichomoniasis and HIV infection. The works of Shutter[14], Ugo and Acholonu[15], and Cameron et al[16] also buttress that fact. *T.vaginalis* enhances the transmission of HIV[17] and symptomatic *T.vaginalis* infection significantly increases the amount of HIV shed in semen [18]. Moreover treatment of *T.vaginalis* infection significantly lowers the vaginal and seminal HIV viral load in dually infected subjects [19,20,21]. Given the high prevalence of *T.vaginalis* infection and the inter relationship with HIV/AIDS, its control will have a significant impact on the HIV epidemic in Africa, and may reduce the incidence of adverse pregnancy outcome.

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Original Article

Detection of Anti-HBV and Anti-HCV in sera of subjects with evidence of normal or abnormal liver assessment results.

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ABSTRACT

The study was designed to evaluate the prevalence of HBV and HCV infections in cases referred for biochemical laboratory evaluation of liver function status. For this study, 50 serum samples with evidence of liver impairment based on biochemical evaluation and other 50 serum samples without any biochemical evidence of liver impairment, were randomly selected and called 'impaired liver function and normal liver function groups respectively. The sera were screened for the presence of anti-HbsAg, anti-HCV and in-vivo antibody sensitization. The result of the present study showed that Anti-HbsAg was detected in 3 (6%) of the sera of normal liver function group and 5(10%) of the sera of impaired liver function group. However sensitized antibodies and anti-HCV were not present in any of the sera in both groups. The present study revealed an over all 16% prevalent rate of possible HBV infections in cases referred for biochemical evaluation of the liver function. The study showed that hepatitis B viral infection may be responsible for 1 out 10 cases of hepatitis in this area. The incidence of hepatitis B viral infection in cases of normal liver function possibly suggest that when clinical indications suspects hepatic involvement in a subject inclusion of HBV screening in addition to the normal biochemical evaluation may be necessary. The lack of evidence for HCV infection is discussed.

Keywords: Hepatitis B virus, Hepatitis C virus, impaired liver.

INTRODUCTION

Viral hepatitis is a major health problem causing liver disease in most parts of the world where the prevalence rate is high. We have previously reported a prevalence rate of 7% for HBV, 3% for HCV and 2% for co-morbidity amongst blood donors[1]. We have also shown a prevalent rate of 2% for HBV amongst pregnant women[2]. However, HCV infection was not detected among pregnant women in the study. Hence, from our study of both HBV and HCV infections in this locality, it is possible to say that blood donors seemed high risk for reservoir of both infections. It has been estimated that there are more than 350 million infected carriers of Hepatitis B virus (HBV) and about 170 million people are infected with Hepatitis C virus (HCV)[3]. Majority of the cases of the viral infections remains asymptomatic and invasion of the liver by these agents leads to a wide range of symptoms[4,5]. Since infections with HBV and HCV could present with no specific early signs and symptoms, it could be possible to miss such diagnosis especially when the index for hepatic assessments had shown no evidence of impairment. Hence the present study was designed to evaluate the prevalence of HBV and HCV infections in cases referred for biochemical laboratory evaluation of liver function status.

MATERIALS AND METHODS

Samples:

One hundred serum samples referred to the chemical pathology laboratory for the biochemical evaluation (such as alanine and aspartate aminotransferase and bilirubin) of the liver function were randomly selected for the study after all the results for the original requests have been generated. The left over samples were then screened the same day of collection for anti- HbsAg, anti-HCV and antibody sensitization. based on the biochemical results for liver functions tests, the sera were group into "impaired liver function group (ILFG) (n=50) and normal liver function group (NLFG) (n=50). In using the left over samples for the present study, the WHO ethical guide for use of left over sample was applied in the present study.

Hepatitis B surface antigen (HbsAg) screening:

The principle is based on detection of Hepatitis B surface antigen (HbsAg) antibodies in the serum using one step HbsAg strip (Acon Laboratories incorporated USA). One step HbsAg strip is a qualitative, lateral flow immunoassay. The membrane is pre-coated with anti-HbsAg antibodies on the test line region of the strip. The mixture migrates upwards on the membrane chromatographically by capillary action to react with anti-HbsAg antibodies on the membrane and generate a coloured line. The presence of this coloured line in the test region indicates positive result, while its absence indicates a negative result. The procedure is as described by the manufacturer. In brief, the test strips were immersed vertically in the respective sera for 15 seconds. The test strips are then placed on non-absorbent flat surface allowed to incubate for 15 minutes. This allows for reaction between the pre-coated anti-HbsAg antibodies and the HbsAg antibodies present in the sera. For HBV sero-positive sera two distinct lines at the test and control regions of the test strip will appear. However, for HBV sero-negative sera only one distinct line at the control region of the test strip will appear.

Hepatitis C Virus (HCV) Screening:

The principle is based on detection of HCV antibodies in the serum using HCV rapid test device (Core Diagnostics UK). HCV rapid test device is a qualitative membrane based antigen-antibody immunoassay. The membrane is coated with anti-HCV antibodies on the test line region of the device. The procedure was as described by the manufacturer. In brief, 0.1ml of serum sample was added into appropriately labeled sample well of the rapid test device and allowed to incubate for 10 minutes. This allowed for complete reaction between the pre-coated anti-HCV antibodies and the HCV antibodies in the serum. For sero-positive HCV samples two distinct lines appeared on the control and test regions respectively while only one distinct red line at the control region is seen for sero-negative samples.

The Antihuman Globulin Screening Test:

Blood group O Rhesus positive red cells from seven different blood bags were pooled. This was washed several times with normal saline and 5% suspension made. 4 volumes of the test serum were mixed with one volume of the 5% suspension of red cells. The set up was incubated for two hours at 37°C. After incubation, the cells were washed four times with normal saline and re-suspended to 5% strength. One volume of this 5% suspension of cell was mixed with one volume of the anti-human globulin and incubated for 5minutes at room temperature. The result was then read both macroscopically and microscopically. For the validation of the test reaction control tests were set up. This consists of weakly reacting positive cells, strongly reacting positive cells and non reacting cells.

Statistical Analysis: The variables were expressed in percentage.

RESULTS

Anti-HbsAg was detected 3(6%) of the sera of normal liver function group and was detected in 5(10%) of sera of impaired liver function group. No anti-HCV and no sensitized antibodies were detected in both groups of sera. See table 1.

Table 1: result of screening tests for anti- HbsAg, anti-HCV and antibodies sensitization in sera of NLFG and ILFG.

Screening	n	positive	negative
Anti- HbsAg		( )*	
NLFG	50	3(6)	47(94)
ILFG	50	5(10)	45(90)
Anti-HCV			
NLFG	50	0(0)	50(100)
ILFG	50	0(0)	50(100)
Antibodies sensitization			
NLFG	50	0(0)	50(100)
ILFG	50	0(0)	50(100)

Key: NLFG = normal liver function group
ILFG = impaired liver function group
( )* = percentage in parenthesis

DISCUSSION

Viral hepatitis is a primary infection of the liver commonly caused by Hepatitis B virus (HBV) and Hepatitis C virus (HCV). In the present study we did not observed HBV and HCV co-infections in any of the groups studied. However, the detection of anti- HbsAg in both groups represents an interesting finding that showed 16% prevalence in patients with high clinical indication for hepatic involvement.

The study showed that 1 out of 10 cases of possible hepatitis are caused by HBV infection in this locality. However, the 6% prevalence of possible HBV infection in cases of normal liver function suggest level of asymptomatic infections that could be missed out if clinical conditions suggest hepatitis function evaluation but excludes HBV screening.

The detection of anti- HbsAg may not only suggest recent infections which could be asymptomatic[4] or progressive infection especially in the presence of hepatitis but may also suggest burden of infection in the locality since specific antibodies could still be detected few months after infection[6].

The prevalent rate of HBV in these cases referred for biochemical evaluation of liver functions was high than those previously reported in this locality among pregnant women and blood donors[1,2]. However, unlike the study involving the blood donors where HBV and HCV co-infections were observed, this was not the case in the present study, although one would have suspected that there will be high prevalence of HBV and HCV co-infections in the present study.

Elsewhere co-infection of HCV and HBV has been reported among drug addicts, patients on heamodialysis, patients undergoing organ transplantation and HIV infected subjects[7-10]. This possible suggest that factors such as the above if present may predispose to consistent incidences of co-infections involving both viral infections.

The present study thus concludes that it may be necessary to include HBV screening in requests for biochemical evaluation of the liver when there is high indication for it.

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