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PREVALENCE OF HEPATITIS B SURFACE ANTIGEN, HEPATITIS C AND HUMAN IMMUNODEFICIENCY VIRUS ANTIBODIES IN A POPULATION OF STUDENTS OF TERTIARY INSTITUTION IN NIGERIA

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ABSTRACT

Objective: Human immunodeficiency virus (HIV), hepatitis B virus, and hepatitis C viruses (HCV) are major causes of mortality and morbidity worldwide. They are also among the commonest transfusion-transmissible infectious agents. Students of higher institutions are often used as voluntary unpaid donors by many hospitals in Nigeria. In this study, the prevalence of HIV and HCV and HBsAg is determined in a population of students attending Ladoke Akintola University of Technology in south west Nigeria, to provide background information on the burden of these infections in this population.

Materials and Methods: Serum samples were obtained from students of the Pre-degree Science programme of Ladoke Akintola University of Technology, Ogbomosho and tested for antibodies to HIV, HCV and HBsAg using the ELISA procedure.

Results: The prevalence rates of antibodies to HIV and HCV in the student population were 0% and 4.8%, respectively and that of HBsAg was 9.5%.

Conclusion: The findings of this study which showed that the prevalence of antibodies to HIV and of HBsAg in this group of students is somewhat similar to those carried out in similar populations. This strongly suggests that the viral burden amongst this population of students is similar and that probably similar factors (demographic) are responsible for maintaining this level of viral load. Further studies would be needed to elucidate the reasons why this is the case. Also it would be necessary to re-emphasize the methods of prevention of transmission of these viruses, and to ensure their implementation in order to reduce the viral levels and therefore avoid the long term sequelae.

Key words: Transfusion, Infection, Blood Donation

INTRODUCTION

Human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus (HCV) are major causes of morbidity and mortality worldwide. Today, HIV is a leading cause of death in many parts of the world, especially in African countries. It is estimated that over 40 million people worldwide are living with the virus with over 75 % of them living in Africa (1). HIV/AIDS is

spreading fast globally and in Nigeria, the virus infection is now endemic in rural and urban areas (2). UNAIDS estimates that in Nigeria around 3.9% of adults between the ages 15-49 years are living with HIV/AIDS (1).

Hepatitis B virus infection is endemic in many developing parts of the world. It is

estimated that globally, there are over 400 million chronic carrier of the virus (3, 4). Studies by several investigators have shown that a large percentage of the Nigerian population has been infected by HBV and that the virus contributes significantly to the aetiology of liver diseases in the country (5, 6).

About 3% of the world's population has been infected by HCV and over 170 million people are chronic carriers (7). Although much is known about the epidemiology of HBV in Nigeria, limited investigation has been carried out on Hepatitis C virus infection.

Among the features common to HIV, HBV and HCV is the transmissibility via blood transfusion from the donor to the recipient of the donated blood. Apart from these three viruses many other infectious agents can be transmitted through blood transfusion. These include *Treponema pallidum*, *Plasmodium* sp., human T-lymphotrophic virus (HTLV), *Babesia*, *Leishmania*, *Trypanosoma cruzi*, variant Creutzfeldt-Jacob Disease (vCJD) agent, cytomegalovirus (CMV) and Epstein Barr virus (EBV). Therefore, the need to render donated blood and blood products safe before they are transfused into a patient has become a major challenge for the blood transfusion services worldwide.

To ensure that donated blood is safe for transfusion, the World Health Organization

(WHO) has put in place strict criteria for safe donation. These include donation by voluntary, unpaid, young, healthy, non-pregnant, adult, low risk and fully counseled donors (8). Unfortunately, this category of donors is scarce in Nigeria; therefore, most blood donations are obtained from replacement and paid donors who are at high risk for these infections.

The future of safe blood donation in Nigeria lies in the ability of the blood transfusion services to recruit and retain voluntary unpaid donors. Enquiries made among students of Ladoke Akintola University revealed many students are willing to serve as voluntary unpaid donors. The aim of this study was to determine the prevalence of HIV infection, HBsAg and HCV antibody in students of LAUTECH at the time of enrollment into the University and compare the results obtained to those from similar studies carried out elsewhere.

MATERIALS AND METHODS

Study Participants

The participants in this study were freshmen enrolled in the Pre-degree Science (PDS) programme of Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State. They were recruited into an on-going longitudinal study of HIV infection in a cohort of students of the institution in 2003. A study proforma was used to obtain demographic data such as

age, sex, state of origin, occupation and state of residence of parents were obtained from the participants. Informed consent was sought at the point of recruitment and blood samples were collected only from the students who consented to participate in the study. Pre-test HIV counseling was also given to all participants tested for HIV antibody.

Specimens

Blood samples were collected by venipuncture from all consenting study participants at the LAUTECH Health Center, Ogbomosho, with sterile 5ml plastic syringes and 22 gauge needles. After collection, blood samples were transported in a cooler containing ice pack to the Virology Laboratory, Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, LAUTECH College of Health Sciences, Oshogbo, Osun State. Serum was separated by low speed centrifugation at 1,500 revolutions per minute (r.p.m.) and stored at -20°C in 2 ml Eppendorf tubes before testing.

Laboratory Tests

Serum samples were tested for antibody against HIV-1, and Hepatitis C virus, and for Hepatitis B surface antigen (HBsAg). All assays were performed using the ELISA procedure.

Assay for HIV antibody was performed using GenScreen ELISA kits, Immunocomb and Genie 2 rapid test kits. (details of test procedure is as described by the manufacturer)

Test for HBsAg was carried out using the Blinotech Diagnostics kit, following the procedure described by the manufacturer

The HBsAg EIA is a solid-phase simultaneous sandwich immunoassay, which employs monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter well is coated with monoclonal antibodies specific for HBsAg. A serum specimen is added to the antibody coated Microtiter wells together with enzyme conjugated polyclonal antibodies. HBsAg, if present, will form an antibody-HBsAg-antibody-enzyme complex. The plate is then washed to remove unbound material. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop in proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is visualized by naked eye or read by EIA plate for absorbance at the wavelength of 450nm.

Hepatitis C antibody was also assayed using the clinotech diagnostics kit, following the procedure described by the manufacturer.

Principle of the Test

This ELISA uses recombinant proteins derived from core region of HCV virus to detect the presence of HCV antibodies in human sera. Multiple epitopes of HCV proteins are bonded to the microtiter wells. When antibodies to HCV are present in the test sample, they react with recombinant proteins and attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with goat-anti-human IgG peroxides conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photometrically quantitated.

RESULTS

A total of two hundred and ninety seven students were recruited into the study. The mean age for males was 19.54 years and for

females was 18.59 years with an age range of 15-26 years. There were 137 males and 160 females giving a male to female ratio of 1:1.19.

Out of all those screened none tested positive for HIV-1 with Genscreen ELISA.

Twenty eight (9.5%) participants tested positive for HBsAg, while fourteen (4.8%) tested positive for HCV antibody. Those who tested positive for HBsAg were found to be older than those who tested negative.

Statistical analysis found this to be statistically significant. ($p < 0.05$). So also with HBsAg there was a significant association between sex and incidence. Males were found to be at higher risk for HbsAg than females. ($p < 0.05$). This was however at variance with incidence of HCV where no significant association between sex and hepatitis C status was found. It was noted though, that those found to be positive for HCV were younger, however this was not statistically significant.

Table 1: Demographic characteristics of Students test for HBsAg, HCV an HIV antibodies

GENDER	HEPATITIS C VIRUS	
STATUS	POSITIVE	NEGATIVE
MALE	8	129
FEMALE	6	154

Table 2: HBsAg, HCV and HIV antibodies among students of a Tertiary Institution

GENDER	HIV	
STATUS	POSITIVE	NEGATIVE
MALE	NIL	NIL
FEMALE	NIL	NIL

GENDER	HEPATITIS B VIRUS	
STATUS	POSITIVE	NEGATIVE
MALE	20	117
FEMALE	8	152

DISCUSSION

Studies carried out by various authors have shown that HIV, HBV and HCV infections are highly prevalent among Nigerians (9,10,11,12). Infection by two of these viruses, HIV and HBV was the leading cause of discarding donated blood at the University of Benin Teaching Hospital (13). This is probably the case in many blood transfusion centers in the country. The financial cost of discarding so many units of blood is enormous, thus constituting a major burden on the health budget.

The present study showed that none of the students tested in this study had HIV infection. The prevalence of HIV infection in the Nigerian population in 2003, the time these blood specimens were collected was 4.5%. The absence of HIV infection in this group of students may be due to the fact that most of the students tested were very young and were from the high socioeconomic group, and were probably well-informed through HIV/AIDS awareness campaigns about how to avoid being infected by HIV.

The prevalence of HBsAg (9.4%) found in this study is similar to findings determined among university freshmen in Ife (8%) (14). However studies in 1985, 17% of antenatal clinic patients were positive for HBsAg (15). In studies carried out among paid blood donors the HIV seroprevalence rate was slightly higher (2.1%) (16) compared to 0%

obtained here probably due to the fact that this group (paid donors) are a well known high risk group.

The 4.7% prevalence of HCV antibody in the student population is a little higher or somewhat similar to reports in previous studies in Nigeria (17). However it has been shown that it has been shown that HCV antibody prevalence is much higher in older adults than in young adults. For example, it was shown that HCV antibody prevalence was much higher (8%) in clergy men aged 30-39 years (18).

Future similar studies would specifically include HIV II , Nigeria being a West African nation.

The clinical implications of the findings is that a significant number of those found positive depending on individual immunity and other factors , are likely to go on to develop the long term sequelae of harboring these viruses including cirrhosis and hepatocellular carcinoma. Demographically it however implies that there are similar social and environmental factors operating to ensure the maintenance of the viral burden .

CONCLUSION

In conclusion, this study showed that the prevalence of HIV and HCV antibodies and HBsAg is generally comparable to results obtained from similar studies carried out elsewhere. From this it can be concluded that among this population of students the

viral burden is similar because there are probably similar factors operating demographically, assisting in the maintenance of this viral load.

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SEROPREVALENCE OF HEPATITIS C VIRUS ANTIBODIES AMONGST BLOOD DONORS IN AHMADU BELLO UNIVERSITY TEACHING HOSPITAL (ABUTH) KADUNA

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ABSTRACT

Background: Hepatitis C virus (HCV) is one of the most common transfusion transmissible infections hence the introduction of routine screening for its antibodies in blood donors in most blood banks.

Methods: This was a retrospective study in which the blood donor screening register for all intending donors were reviewed and analyzed.

Results: There were 4,731 potential donors with age range 20-41years. Eighty six (1.8%) were sero-positive to HCV antibodies with a decreasing incidence over the period under review. The highest incidence was recorded the age group 31-40 years.

Aim: To determine the sero-prevalence of HCV among blood donors in ABUTH Kaduna.

Conclusion: HCV infection is not uncommon in our environment hence the need to emphasize it's routine screening among all potential donors.

INTRODUCTION

HCV is a single stranded RNA virus which until 1989 was named non A, non B hepatitis virus, was responsible for 80% of post transfusion hepatitis (1,2,3). The modes of transmission are sexual intercourse, accidental inoculation (as in intravenous drug use, tattooing, acupuncture) with HCV- contaminated instruments, contact with infected body fluids and vertical transmission (4,5,6). Sickle cell disease (SCD), haemophilia and haemodialysis are risk factors due to the need for repeated transfusion with

blood and blood products (4, 5). The seroprevalence of HCV antibodies ranges from 1.1% to 6.7% (7) in the West African sub-region.

Liver disease of variable course is the most common manifestation of HCV infection (4). Pegylated α -interferon and/or Ribavirin are used for the treatment of HCV with variable outcomes (8).

HCV antibody screening was introduced in ABUTH blood bank in 1997 and since then its prevalence has not been determined among blood donors which this paper seeks to do.

Methods: This was a hospital- based retrospective study. It involved a review of the records of all intending blood donors on whom HCV antibody screening was done over a four-year period, from January 2000 to December 2003. The donors were classified and analyzed

based on age and seropositivity. HCV antibody screening was done with ELISA using Grand Medical Diagnostic (USA) Rapid Test kits.

Results: There were 4,731 blood donors over the study period with an average of 1, 184 donors per year. All donors were males with an age

range of 20-41 years. The highest seroprevalence for HCV was in the age group 31-40 years (41%). Eighty six (1.8%) of donors were seropositive for HCV antibodies (Table 1).

Table 1: HCV Seropositivity among blood donors

Year	NUMBER OF DONORS	SEROPOSITIVE DONORS (%)
2000	914	19 (2)
2001	1,425	29 (2)
2002	970	16 (1.7)
2003	1,422	22 (1.5)
TOTAL	4,731	86 (1.8)

Table 2: Age distribution of seropositive donors

AGE GROUP	YEAR				TOTAL
	2000	2001	2002	2003	
≤ 20	2	3	-	-	5(6%)
21-30	7	13	8	3	31(36%)
31-40	6	9	6	14	35(41%)
≥41	4	4	2	5	15(17%)
TOTAL	19	29	16	22	86(100%)

Discussion: The routine screening of donors is essentially to ensure blood safety, particularly from transfusion transmissible infections such as HIV, HCV and HBV. The seroprevalence of HCV was 1.8% however there was a gradual decline over the study period (Table 1). This may be due to Media health education on the

prevention of viral infections especially HIV which share same routes of transmission with HCV.

The finding of HCV seroprevalence of 1.8% among blood donors in ABUTH Kaduna was remarkably lower than the finding by other workers from other parts of the country. Egah *et*

al (3) reported a HCV seroprevalence of 6% in Jos. Chukwurah *et al*(8) in South Eastern Nigeria found a HCV seroprevalence of 7.6% while Ayolabi (10) *et al* (2006) in Lagos reported a HCV seroprevalence of 8.4% all among blood donors. Halim (10) *et al* (2000) in Benin reported a seroprevalence of 12.3% and Fasola (12) *et al* (2008) in Ibadan reported a seroprevalence of 3.9%. The significantly lower prevalence in our study may be due to differences in the lifestyle of people in different regions of the country.

Vardas² *et al* in Namibia reported a seroprevalence rate of 0.9% which is lower than our findings in Kaduna. Arthur (13) *et al* (1997) in Egypt reported 24.8% HCV seroprevalence a value that is significantly higher than all the Nigerian reports. This may be attributed to method the method of HCV antibody determination (second generation enzyme immunoglobulin G antibody assay by Abbot). In 2003 Etard *et al* (7) reported a seroprevalence of 0.8% in Senegalese blood donors which is remarkably lower than our finding but comparative to the Namibian findings.

Conclusion: The seroprevalence of HCV was 1.8% and was more prevalent amongst the 31-40yrs age group. There is a need for mandatory screening for HCV antibodies in all blood banks and for continuous health education of the donor population in order to ensure the availability of safe blood.

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CLINICAL STUDIES ON SEROPREVALENCE OF RUBELLA VIRUS IN PREGNANT WOMEN OF CAMEROON REGIONS.

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ABSTRACT

A study was conducted to investigate the seroprevalence of the rubella virus amongst pregnant women and the relationship it has with the duration of pregnancy, premature delivery, and past history of abortion in pregnant women visiting the Yaoundé Gynecological, Obstetric and Pediatric Hospital (HGOPY). 211 pregnant women attending the prenatal consultation of mean age 27 ± 5.99 years were randomly selected and screened for rubella IgG antibodies. 39.3% of them were in their third trimester of pregnancy while 25.6% and 35.1% were in their first and second trimester of pregnancy respectively. 11.73% of the women had a history of premature delivery and 40.3% had a history of at least one abortion. Spearman's correlation was calculated between antibody titre and age. 88.6% of pregnant women were seropositive while 9% (susceptible) were seronegative and 2.4% had equivocal results. The most susceptible women to rubella infection were in the age group 26-30 years while women in the age group 21-25 years band were the most seropositive. There was a strong correlation between the antibody titre and age ($r=0.549$ $p<0.01$). There was no statistical difference between the pregnancy in trimesters and antibody titres ($p=0.0926$) as well as between the number of previous abortions and the antibody titre ($p<0.01$, $r=0.246$). No correlations between antibody titre and pregnancy duration, or occurrence of premature births. There was a weak correlation between the antibody titre and number of previous abortions.

INTRODUCTION

Rubella virus is an infection caused by a virus of the genus Rubivirus of the Togavirus family [1]. It has a simple architectural structure of single stranded RNA genome enclosed by an icosahedral nucleocapsid, protected by a lipid bilayer membrane [2-4]. Rubella (which means "little red" and is also known as German measles) was originally thought to be a variant of measles. It is a mild disease in children and adults, but can cause devastating problems if it infects the fetus, especially when the infection occurs during the first weeks of pregnancy [1, 2]. This is known as congenital rubella syndrome (CRS). When a woman is infected

with the rubella virus early in pregnancy she has a 90% chance of passing the virus unto

the fetus [2, 3]. This can cause the death of the fetus, it may cause CRS. The complications include hearing loss, congenital heart defects, neurologic problems (psychomotor retardation), ophthalmic problems (cataract, glaucoma, and retinopathy) intrauterine growth retardation, hepatomegaly, splenomegaly [2, 3, 4]. There may also be variety of other problems including bone lesions [1, 3]. Virus from congenital infections persists after birth and persons with congenital infections has

the potential to infect others after birth for a year or more [2, 5]. The virus occurs in nasopharyngeal secretions, urine and feces. Later on, patients with congenital syndrome may develop additional complications including diabetes mellitus (up to 20%), thyroid dysfunction, growth hormone deficiency; ocular complications [2, 3, 6]. When a woman is infected with the rubella virus, the body produces both immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to fight against infection [2, 4, 7]. Once IgG exists, it persists for a lifetime, but IgM antibody usually wanes over six months [3].

If rubella IgG is present it can be confirmed that a patient has immunity to rubella. Specific IgG determination is performed through enzyme Linked Immunosorbent Assay (ELISA) techniques. The results are expressed in IU/ml. [1, 4, 8]. The microbial world is complex and constantly evolving and despite scientific efforts to contain diseases with microbial etiology, the growth of international travel has increased the ease with which microbes formerly restricted to certain geographical areas are spread across continents [5]. For instance the recent movement of people fleeing the war in Chad to Cameroon is a situation that could trigger the spread of the rubella virus amongst unvaccinated population. Rubella is one of the most common causes of birth defects in the world, resulting in spontaneous abortions, stillbirths, and congenital rubella syndrome (CRS) rubella rashes [2, 3, 8, 9]. The manifestations of CRS include hearing

impairment, blindness, heart defects, and mental retardation. According to the World Health Organization, in 1996, two thirds of the world's population live in countries where rubella vaccination was not practiced routinely, and the number of infants with CRS born each year worldwide was estimated to be 110,000 in 1999 [3]. About 5 to 25% of women of childbearing age lack rubella IgG antibodies and are susceptible to primary infection [2, 7]. Rubella is transmitted by the respiratory route. The incubation period is 13 to 20 days, during which a viraemia occurs and virus disseminates throughout the body [12]. In adults a prodromal phase may be present with fever and malaise for a day or two before the rash develops [13, 14]. The rash is typically a maculopapular rash, which first appears on the face and then spreads to the trunk and the limbs. The rash seldom lasts more than 3 days. The exact mechanism of how the rash is induced is uncertain but an immunopathological mechanism may be present [15]. Lymphadenopathy may precede the rash by up to a week and persists up to 2 weeks after the rash has gone [16].

Rubella has a worldwide distribution. Before the introduction of vaccination outbreaks tend to occur in spring and summer [6]. Infection is uncommon in preschool children but outbreaks involving school children and young adults are common [7, 8]. In general, about 50% of 10 year olds have rubella antibodies. About 80% of women of childbearing age were found to be immune

in the pre-vaccination era [10] Children 3 to 10 years are most frequently affected. Despite the vaccination program 5 to 10 % of women of child bearing age are susceptible to Rubella infection [11]. So far, no vaccination programme has been put in place in Cameroon. Statistics from the World Health Organization (WHO) show that this virus is present in Cameroon with confirmed cases: 83 in 2004, 159 in 2005, 58 in 2006, and 126 in 2007 [4, 7]. These cases were initially suspected cases of measles which turned out negative and rather tested positive for rubella. Considering the fact Cameroon is one of the countries not implementing a vaccination scheme, the danger of an eventual outbreak cannot be over emphasized. There is the need to know the epidemiology of rubella in pregnant women because of the congenital rubella syndrome (CRS), and the *de novo* infection in the first trimester of pregnancy. The purpose of this study was to identify the susceptibility of women to the rubella virus in Yaoundé through the assessment of the Immunoglobulin IgM protective antibody level in Pregnant women at the Yaoundé Gynecology, Obstetric and Pediatric Hospital (HGOPY) in Cameroon. Samples were collected randomly at (YGOPY) Cameroon, so that the data generated from the study would be useful for introducing vaccination in Cameroon.

MATERIALS AND METHODS

Study Design

A Cross-sectional descriptive study was carried out in pregnant, outpatient's women visiting the Yaoundé Gynaeco-Obstetric and Pediatric Hospital (YGOPH). This hospital was chosen because of its high patient's attendance as well as logistic and administrative facilities.

Collected blood specimens were analyzed at the Center for the Study and control of Communicable Diseases (CSCCD), of the Faculty of Medicine and Biomedical Sciences (FMBS), University of Yaoundé. This study was for 3 months and ran from April to July 2008.

The Inclusion Criteria, was basically to be a pregnant woman, sign the consent form as a volunteer, with no cash involvement and the acceptance to participate in the study.

The Exclusion Criteria included the refusal to participate in the study

Sample Size: The minimum acceptable sample size was 207 as calculated using Lorenz formula for two-tailed dichotomous variables.

Where N = sample size, Za= the normal distribution value for which $\alpha=0.05$ (the standard normal deviate=1.96) 95%, confidence interval; α =level of statistical significance ($\alpha=0.05$), p=prevalence (9), $Q=1-p$, D=degree of precision= level of error we want to accept (D=0.05 for a 95% confidence interval)

Using Z $\alpha=1.96$, P = 84 % (9), D = 0.05,

$$N = \frac{(1.96)^2 \times (0.84) \times (0.16)}{(0.05)^2} \sim 207$$

Sampling Method

Consecutive sampling was used whereby subjects who satisfy the inclusion criteria during the study period were included in the study.

Data and blood specimen collection

Each patient was made to sit comfortably, then the arm region intended for the venupuncture was cleansed with an alcohol swab, the selected vein pricked with a sterile needle attached to a syringe (10 ml) and 4-5 ml of blood drawn. The needle was then withdrawn under a dry cotton and brief haemostasis effected by digital pressure with the swab at the puncture site. The blood sample was put in a sterile dry tube. Centrifugation was done at 2000 rotations per minute (rpm) for 5 minutes. Serum was collected in cryotubes and stored in refrigerator at -20 degree Celsius. Cryotubes were put inside a cold box and transported to the CSCCD of the FMBS. Laboratory analysis was done at the end of the month.

Laboratory analysis of specimens:

Reagents and specimens were brought at room temperature before use. Testing for the presence of rubella virus was done using Human-Rubella IgG ELISA (26) this is an enzyme immunoassay for the detection of rubella antibodies in the plasma or serum. 10µl of patient serum were diluted to 1ml of buffer and mixed properly. Well A1 was left blank while B1/C1 100 ml of negative control (NC) was put. D1/E1 100µl of cut off control (CC) and F1/G1 100 ml of positive control (PC). 100µl of each serum to be tested was

added to the microtitre plate. The microtitre plate was covered with adhesive foil and allowed to incubate for 30 minutes at 25 degree Celsius. They were then washed 4 times with 350µl washing solution using an automatic washing device. Each well was filled with 100 µl conjugate solution (Anti-human IgG rabbit, peroxidase-conjugated). Then, the plate was covered, and incubated at 25 degree Celsius for 30 minutes, then washed 5 times as above. Then, each Well was filled with 100µl of substrate reagent (3,3',5,5' tetramethylbenzidine (TMB hydrogen peroxide). The plate was covered and incubated for 15 minutes at 25 degree Celsius in a dark room. 100 µl of STOP solution was added to each well. The Wells were read using a zero-balanced photometer at 450 nm within 30 minutes after termination of the reaction, using a reference wavelength of 690nm.

Calculation of control values and cut-off:

Mean absorbance values of negative control (NC) in wells B1 and C1, mean negative control (MNC) in wells D1 and E1, mean cut-off control (MCC), and Positive control (PC) in wells F1, and G1 mean positive control (MPC) were calculated according to:

$$\text{MNC} = \frac{A_{450}(\text{B1}) + A_{450}(\text{C1})}{2}; \text{MCC} = \frac{A_{450}(\text{D1}) + A_{450}(\text{E1})}{2}; \text{MPC} = \frac{A_{450}(\text{F1}) + A_{450}(\text{G1})}{2}$$

The test was considered valid as the following criteria were met: **Substrate blank in well A1 <0.150; MNC ≤ MCC; MPC >0.750; MPC: MNC >2.5 . Interpretation of results: $A_{450}(\text{patient}) > \text{MCC} + 15\%$ anti RV-IgG-Ab-positive $A_{450}(\text{patient}) > \text{MCC} - 15\%$ antiRV-IgG-Ab-negative**

Due to physiological and analytical variations, patient's results lying at 15% above or below the calculated cut-off were considered equivocal [26].

Quantitative Estimation of rubella IgG in patient samples

Each plate test was validated when the absorbance of the mean cut off control were <10 iu/ml and the absorbance of the positive control >15 iu/ml and values in-between were considered equivocal.

Data Quality Control: To guarantee the authenticity of the information collected, A standardized questionnaire was used to record the information obtained from every patient, to ensure uniformity. The questionnaire were pre-tested during a short pilot study on few (10) subjects before recruitment proper. The questionnaire was then revised following the results of this pilot study before the main study started.

The data was filled by the researcher personally to ensure precision of information.

Data Presentation and Analysis: The data collection forms were first of all cross-checked to make sure all the relevant information was appropriately entered. The EPI INFO version 3.3.2, February 09, 2005 (Centre for the Disease Control and Prevention, Atlanta, Georgia, USA) statistical software was used for the data entry, validation and analysis. To ensure accuracy of entry a CHECK programme was created. This programme ensured that only legal entries and data in specified ranges and codes were entered. Discrepant records

were subsequently reviewed and corrected. All entries on computer were further checked against that on paper, item by item. Finally, frequency tables were generated for variables in order to examine for unusual entries. Spearman correlations were used to calculate the various variables. The prevalence of rubella virus among pregnant women was calculated as:

$P = N1/N2 \times 100\%$: Where P is prevalence; N1 the total number of women presenting antibodies to the rubella virus; N2 the total number of women tested for antibodies.

Ethical considerations

Institutional Ethical Clearance was procured from the Faculty of Medicine and Biomedical Sciences (FMBS) ethical committee. Informed, written and signed consent was obtained from subjects by way of a consent form, after the purpose and the procedure of the study had been explained. Non-consenting individuals were excluded from the study. Records were kept strictly confidential with code numbers used at the registration of each participant and records accessible only to members of the immediate research team. The entire procedure was of minimal risk to the subjects. Each needle was used once and properly discarded after use. The informed consent of each subject was sought systematically before recruitment. The aim and the nature of the study were explained to each patient and her role in the study clarified. Confidentiality was strictly respected and all records were accessible only to members of the immediate research

team. Questionnaires were coded to ensure anonymity.

RESULTS

From April to July 2008, two hundred and eleven (211) pregnant women were recruited in our study population from the Yaoundé Gynecology, Obstetric and Pediatric Hospital.

General Characteristics of the subjects of study

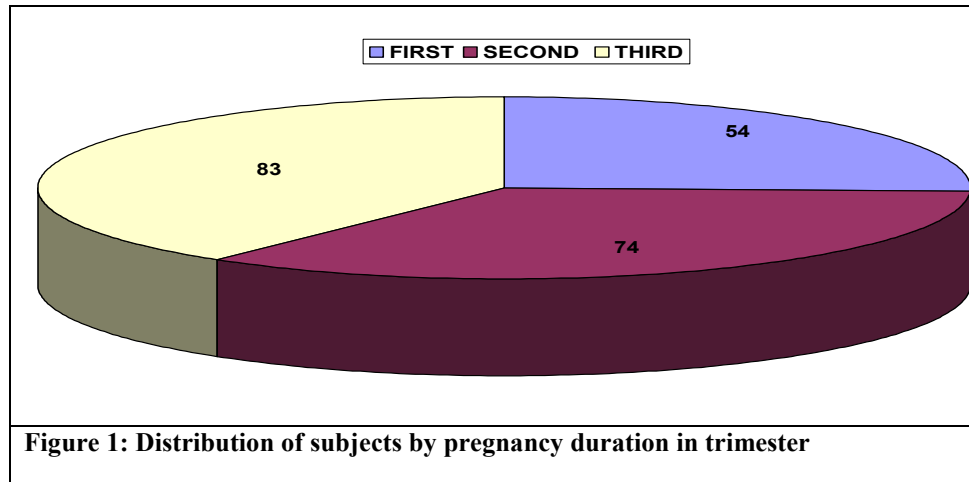
The age of the subjects ranged from 14 to 46 years. The 21-25 years and 26-30 years were the most represented, with 29.4% and 33.6% respectively, as shown in Table 1, the mean age was 27 ± 5.99 years. The subjects were distributed in first, second and third trimesters as shown in figure 1.

Table 1: Distribution of subjects into age groups

Age	Frequency	Mean percentage
<15	1	0.5 ± 0.0
16-20	15	7.1 ± 1.3
21-25	62	29.4 ± 6.0
26-30	71	33.6 ± 5.9
31-35	36	17.1 ± 3.2
36-40	18	8.5 ± 2.2
40+	8	3.8 ± 0.8
Total	211	100 ± 7.6
P-value	0.3922	

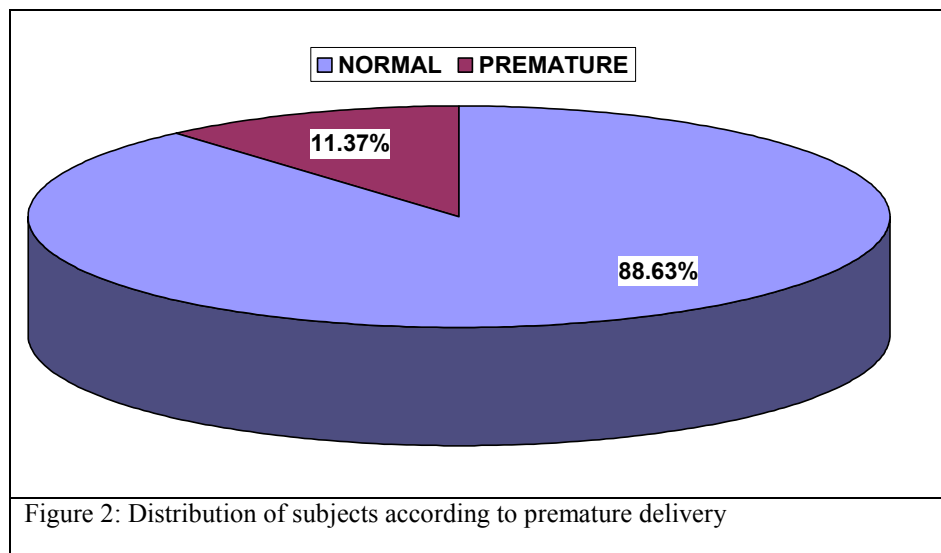
Fifty-four (54) of the subjects were in the first trimester of pregnant. The partition of subjects based on history of premature delivery showed that (Figure 2) showed that a low incidence of subjects 24

(11.37%), with any history of premature delivery. There was a high subject population 187 (88.63) with no history of premature delivery in the study.



The calculated percentage shown is with respect to the Sero status. (Figure 3) The age group 21 to 25 and 26 to 30 years had the highest prevalence of the antibodies

against the rubella virus, with a mean age of 27.0 ± 5.99 years. No statistical difference was obtained between the age groups ($P=0.403$).



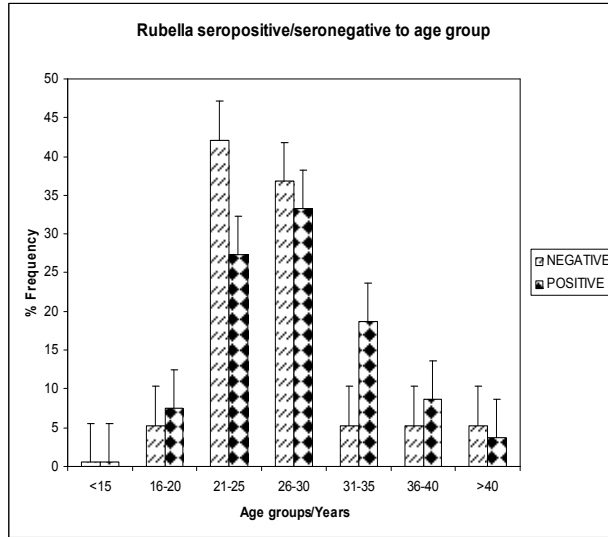


Figure 3: Classification of rubella seropositivity/negativity with respect to age group

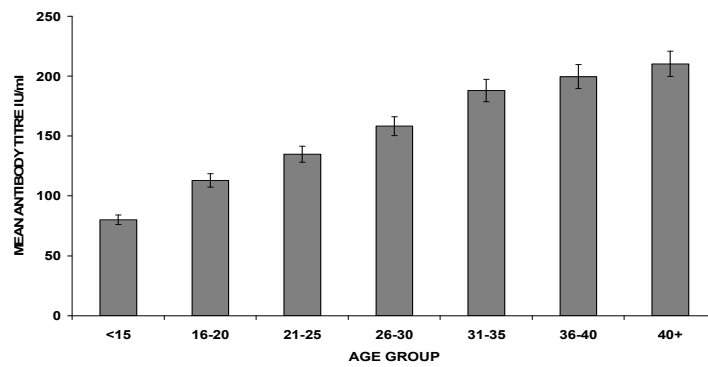
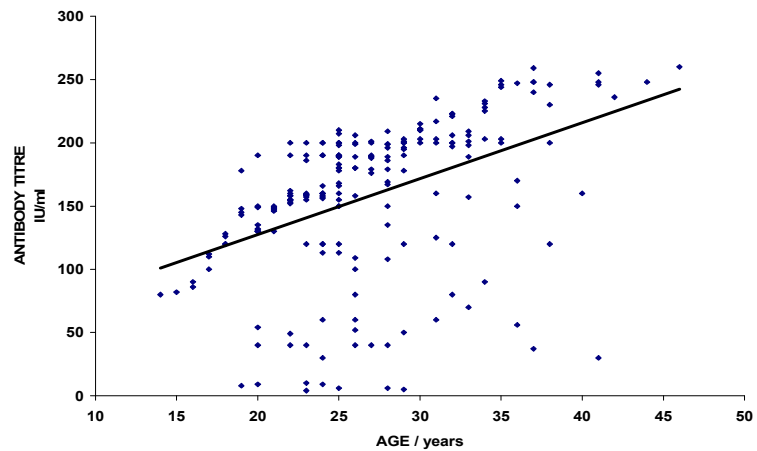


Fig 4: Mean Antibody titre and age distribution

Figure 5: Correlation between age and antibody titre of subjects



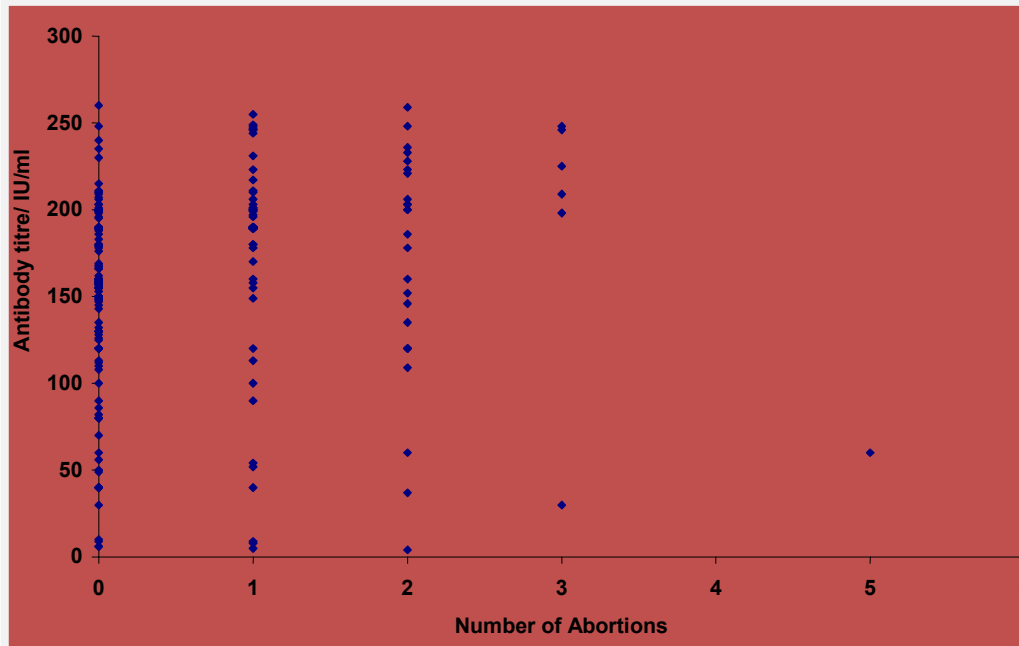


Figure 6: Correlation between antibody titre and number of abortions

There was a steady increase in the mean antibody titre levels with increase in age (Figure 4) There is a significant difference between mean antibody titre of the women age values ($P < 0.0001$). This showed that as the age increases, the antibody titre significantly increased. Investigation of the relationship between age and antibody titres by the Spearman Correlation analysis values showed that there was a significant positive correlation ($p < 0.01$, $r = 0.549$, $N = 211$) between the subject ages and the antibody titre. This means that as the age increases, the antibody titre increases. (Figure 5). Investigation to establish any relationship between antibodies titre and the number of abortions by the Spearman correlation showed that there was a positive but weak correlation ($P < 0.01$, $r = 0.246$

$N = 211$) between the number of abortions and the antibody titre. (Figure 6).

DISCUSSION

The investigation conducted on the seroprevalence of rubella virus amongst pregnant women visiting the Yaoundé Gynecological, Obstetric and Pediatric Hospital (HGOPY) showed that of the 211 pregnant women randomly selected visiting prenatal consultation. The seroprevalence of the rubella virus was found to be 88.6% while 9% were seronegative (susceptible) to the rubella virus. 5 women (2.4%) were found to be equivocal. The latter may be due to re-infection cases, the IgG is highly elevated whilst IgM may be demonstrable, giving equivocal results [8]. For such cases, it is recommended to collect fresh samples taken within 7 to 14 days and repeat the

assay in parallel [25], to confirm these equivocal cases. However, it was not possible to repeat the tests for these samples due to the time allocated for this study and also difficulties involved in scheduling another meeting with the subjects.

The seroprevalence of (88.6 %) recorded in this study is similar to those reported in other African countries in pregnant women, women of childbearing age, women and men [40, 41, 42, 43, 44, 46, 47, 48, 48, 49, 38, 51, 52]. The assays for rubella-specific IgG varied between studies, as did the titre that was considered positive. The Haemagglutination Inhibition (HI) test, which is considered the reference standard was used in most of these studies, but some used Single Radial haemolysis (SRH), latex Agglutination, or Enzyme-based Immunoassay (EIA). Although there is a general agreement between these tests, the results vary between laboratories, and those of different assays or different commercial kits may not be strictly comparable [2, 10, 17].

None of these women had previous history of vaccination of rubella virus. This high prevalence might suggest the presence of the wild type virus [12, 13, 22, 25] also, since it is a hospital based study, and most of the women were living in urban areas, the seroprevalence might be higher than normal due to overcrowding and the ease with which the virus spreads amongst unvaccinated population [16-21, 36]. It might also be as a result of selection bias due to

exclusion of women who did not come for prenatal checks. Previous studies performed in different populations and study zone reported seroprevalences ranging from 59% to 94% [22, 25, 26, 40, 41, 42]. Seroprevalence of up to 90% in countries without any mass vaccination program, are generally a reflection of post-epidemic immunity (37). We cannot conclude that these cases were from post epidemic immunity since no data is available for epidemics in Cameroon Rubella natural infection is followed by a high level of protection from re-infection [19] However, re-infection can occur which is generally asymptomatic and in pregnancy it poses minimal risk to the fetus [18]. Studies to investigate any relationship between maternal age and the mean antibody titre (IU/ml) within the subjects ranging from 14 to 46 years showed that the 21 to 25 years and 20 to 30 years band were the most represented with mean values of 29.4% and 33.6% respectively. An observation of a steady increase in the antibody titres levels and the mean ages was recorded. This increase, was significant ($p < 0.0001$). Also a significant spearman moment product correlation ($P < 0.01$, $r = 0.549$, $N = 211$) was observed between the age and antibody titre levels (iu/ml). This suggests that as the age increases, the antibody titre significantly increases as confirmed by other publications [37, 39].

The majority of pregnant women were in their third trimester of pregnancy (39.3%)

the mean antibody titre was higher in the first trimester no statistical significant difference was observed between the first, second and third trimesters and mean antibody titre ($P=0.0926$) and also no Spearman correlation between antibody titre and pregnancy duration in trimesters ($p=0.07$). This may imply the rubella virus does not affect pregnancy duration. This correlates with previous datum that shows that there was no relationship between pregnancy duration prevalence of rubella [38]. 59.7% of the subjects did not have any history of abortion and 40.3% had previous history of abortion. The higher the antibody titre, the greater the chances of abortion occurring [16, 26]. A look into the variation of number of abortion with antibody titre was necessary. Looking at the relationship between number of abortions and the prevalence of rubella, we observed that the prevalence of rubella significantly increased with the number of abortions ($P<0.05$) furthermore, there was a significant but weak Spearman correlation ($P<0.01$, $r=0.246$ $N=2$.) between the number of abortions and the Antibody titre. This means that the higher the antibody titre, the higher the probability of abortion, implying that those with higher rate of abortions had higher antibody titre. Rubella virus enters the fetus during the maternal viraemic phase through the placenta [3, 21, 29] The damage to the fetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others [22, 48, 49]. However, since the study was cross

sectional, it is difficult to say whether the occurrence of high antibody titre preceded or followed the abortions recorded. Generally, the rubella virus plays a significant role in the occurrence of abortion in the study population. [2, 7, 53]. There was no correlation between the antibody titre and the occurrence of premature births ($P=0.947$, $r=0.012$, $N=24$). This either suggests that there were no enough rubella antigens to induce active immunity against the virus and since it was a cross sectional study, no information was present to say if the occurrence of premature birth was due to high antibody titres. Thus these premature cases might have either resulted from low titre of antibody with encountered with the virus or from other sources.

Rubella virus enters the fetus during the maternal viraemic phase through the placenta [21]. The damage to the fetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others [22]. Chromosomal aberrations and reduced cell division are present. The fetus is almost invariably infected if the mother is infected during the first trimester. After the first trimester, the virus is isolated infrequently from the neonates, probably because fetal immune mechanisms can be activated and infection can be terminated. [23, 45].

Rubella virus is seldom isolated from infants whose mothers acquired rubella after the first trimester. However rubella- specific IgM can be detected in a high proportion of these infants which means that they were infected.

Major abnormalities are very rare because organogenesis is complete by 12 weeks and the immune response may be more developed [29]. Deafness and retinopathy (which does not affect vision), are likely to be the only abnormalities associated with post first trimester rubella. Deafness is usually the sole clinical manifestation of fetal infection occurring between 13 and 16 weeks [30].

Rubella virus specific IgM antibodies are present in people recently infected by Rubella virus but these antibodies can persist for over a year and a positive test result needs to be interpreted with caution [6]. The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis [2, 11, 35]. Serology is the mainstay of diagnosis of rubella infection. A recent rubella infection can be diagnosed by [26] detection of rubella-specific IgM, [8] rising titres of antibody in HAI and ELISA tests, and seroconversion [27]. It is essential to obtain accurate information relating to the date and time of exposure, the date of onset of illness, a history of previous rubella vaccination, as well as previous results of rubella screening tests. Blood should be collected from pregnant women with features of rubella-like illness as soon as possible after onset of symptoms. [26]. A significant rise in HAI antibodies can often be demonstrated. However, rubella-specific IgM is the test of choice for demonstrating patient vaccinated postpartum [1, 5, 8]. This study certainly has certain limitations since it

current infection. It has been shown though that low and transient level of IgM can be detected in cases of reinfection [26, 31, 50]. Furthermore, low levels of rubella IgM may persist for a few months to 4 years following rubella vaccination.

Typical serological events following acute rubella infection [8], note that in reinfection, rubella-specific IgM is usually absent or present at a low level transiently ELISA is now the test of reference in many laboratories but it is considerably more expensive than the SRH. An antibody titre of equal or greater than 15 IU/ml is regarded as being immune to rubella. However, there is some controversy as to the 15 IU/ml cutoff since it was arrived at empirically in the first place. It is quite clear that lower levels of antibody, such as 10 IU/ml would probably be protective as well. HAI is not used for rubella antibody screening because it is not sensitive enough [18].

It is important that women are vaccinated prior to their first pregnancy [12]. United States recommendations are for childhood vaccination to prevent epidemics, combined with vaccination of susceptible, non-pregnant adolescent and adult females [37]. The vaccine is contraindicated for pregnant women, but when unwittingly used, no problems have been seen. If the patient is pregnant and seronegative, the pregnancy should be monitored carefully and the

is a hospital based study selection bias could have occurred due to the criteria of

selection since some women might not have visited prenatal consultation. The study is limited to females visiting the prenatal consultation and it is difficult to extrapolate the results to the general population. There are constraints on the use of data from a cross-sectional survey to estimate the

CONCLUSION:

The majority of pregnant women attending the Gynecology Hospital possess a protective level of Rubella IgG antibodies. However, 9% are susceptible to rubella. Furthermore, rubella antibodies increase with increasing number of previous abortions and with maternal age. Some recommendations to be made is geared towards encouraging the ministry of Public Health in Cameroon the necessity for a

transmission dynamics of rubella. The duration of study was too short to give a strong conclusive finding. However, this preliminary investigation has provided a platform form a wider and long duration project as a follow up by a team of PhD students.

mass vaccination program, Increase awareness through media. There is also the need for the clinician to systematically check rubella serology in all female desiring pregnancy and in women of child bearing age, and also prenatal screening of pregnant women and vaccination of those who are seronegative to reduce the morbidity and mortality related to rubella virus in new born babies.

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DETECTION OF SERUM ANTIBODY LEVELS AGAINST NEWCASTLE DISEASE IN LOCAL CHICKENS IN BAUCHI METROPOLIS, BAUCHI STATE, NIGERIA

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ABSTRACT

Poultry diseases are one of the main factors constraining poultry practice in most developing countries. Newcastle disease (ND) is a highly contagious and commonly fatal viral poultry disease caused by Newcastle disease virus (NDV). Detection of antibodies to Newcastle disease virus in 300 blood samples from local chickens slaughtered at Muda Lawal Market Bauchi was carried out using the haemagglutination inhibition test (HI). This was to determine the immune status of local birds to NDV in Bauchi Metropolis. 169 (56.3%) birds tested positive with antibody titre ranging from 2 to 512. The geometric mean titre was 19.7. This low antibody titre reveals the epizootic nature of the virus in the study area and is suggestive of an inter-epidemic phase or early phase of infection pointing a finger to possible economic losses in the event of an outbreak, alongside the role of local chickens in the transmission cycle of NDV to other avian species. Vaccination of local chickens where possible is advocated for.

Key words: Poultry diseases, Newcastle disease, haemagglutination inhibition, antibody titre

Introduction

Poultry keeping is the dominant form of poultry production in the developing world. The practice of traditional poultry system is higher because it entails less or minimal human involvement with birds scavenging in the backyard for food. It entails no investments beyond the cost of the foundation stock, a few handfuls of local grain, and possibly simple night shades, and little or no veterinary medical attention [1]. As in many tropical and subtropical countries in Asia, Africa and South America, a large population of small traditional chicken flocks exists alongside large industrialized poultry farms. This may mean that chickens in the same vicinity may be in direct or indirect contact with each other [2].

Poultry diseases such as Newcastle disease (ND) are one of the main factors constraining this

poultry practice in most developing countries. ND is the most important infectious disease affecting local chickens. Outbreaks of the disease have a tremendous impact on backyard poultry farming, where these birds are a significant source of protein and this disease is endemic. Its spread is normally either via newly introduced birds, selling or giving away sick and carrier birds. The usual source of infection is usually other chickens.

Newcastle disease (ND) is a highly contagious and commonly fatal viral poultry disease affecting mainly domestic and wild avian species [3]. The disease is caused by Newcastle disease virus (NDV) which belongs to the *Paramyxoviridae* family and genus *Rubulavirus*

[4]. The Paramyxoviruses isolated from avian species have been classified by serological testing into nine serotypes designated; APMV – 1 to APMV-9. NDV has been designated APMV – 1 [5]. APMV -1 strain is classified into three pathotypes based on their virulence in chickens which are: lentogenic, mesogenic and velogenic [6,7]

The disease is characterized by respiratory symptoms such as coughing, gasping, sneezing and rales. Other signs include: dropping wings, dragging legs, swelling of the tissues around the eye and neck, twisting of the neck, circling and cessation of egg production [8]. Human infection via exposure to infected birds can cause mild conjunctivitis and influenza-like symptoms and in severe cases, it can lead to some lasting impairment of vision [9].

Newcastle disease, being an epizootic problem in different parts of Nigeria [10,11,12,13] can have serious negative impact on the economics of these areas and the country at large. These losses will be due to losses in productivity and death of poultry. Its presence can limit trade and the development of intense poultry production resulting to major constraint to the availability of protein for human consumption.

Establishment of the disease status in Bauchi metropolis is therefore of great importance to avoid the economic losses highlighted above. This objective triggered this research which was aimed at detecting antibodies to Newcastle disease virus infection from local chickens at Muda Lawal Central Market, Bauchi (a

converging point for the sale and slaughter of local chickens from different localities in the State).

Methods

Study location

Samples were collected at slaughter house in Muda Lawal Market Bauchi, Bauchi State, Nigeria.

Sample collection and processing

300 blood samples were collected randomly from unvaccinated local chickens at slaughter point. Samples were taken from the wing veins of chickens. They were allowed to clot. Sera was separated, transported in a refrigerated box and subsequently stored at -20°C in Virology laboratory, Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute (NVRI), Vom). The antigen used was obtained in the same laboratory.

Washing of RBC's

5ml of chicken blood was collected aseptically in a disposable syringe and transferred to a sample bottle containing 1 ml of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 minutes. The plasma and buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 10% and 1% suspensions in PBS were made to be used in spot test and HI test.

Spot test was carried out using clean white tile. A drop of viral antigen was placed at the centre of the tile. A drop of 10% chick red blood cell was added to the antigen, mixed together and

rocked gently. The mixture was observed for haemagglutination. This test was used to identify potency of the antigen [7].

Test procedure

The test was performed as described by Allan and Gough [18]. Briefly, after making two fold serial dilution of test serum up to 10th well, 4 HA unit of Newcastle disease virus was added up to 11th well and kept at 25 - 30°C for 25-30 minutes. A 1% chicken RBC's suspension was added into each well. The samples showing peculiar central button shaped settling of RBC's were recorded as positive and the maximum dilution of each sample causing

haemagglutination inhibition was the end point. The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

Results

Results from 300 samples screened show that 169 samples (56.3%) were positive for Newcastle disease virus antibodies while 131 samples (43.7%) were negative as shown in table 1. 130 (77.4%) of samples showed specific immunity levels as presented in table 2. Haemagglutination inhibition antibody titre ranged from 2 to 512. The geometric mean titre was 19.7. These are shown in Table 3.

Table 1: NDV antibody screening results.

Nos of samples	Nos positive	Percentage positive	Nos negative	Percentage negative
300	169	56.3	131	43.7

Table 2: Serum samples showing specific or non-specific immunity to ND by HI test

Positive samples	Specific immunity	% Non-specific immunity	Non-Specific immunity
168	130	77.4	38

Table 3: NDV antibody titres of positive samples

Nos of samples	Titres									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	GMT
168	23	15	31	18	10	16	21	27	7	19.7

GMT- Geometric Mean Titre (Brugh, 1978).

Discussion

ND is regarded as the most economically important disease that devastates village poultry

in Nigeria [14] as it causes death of millions of birds (particularly young birds) and economic losses through the slaughter of sick birds [15]. Mortality rate as high as 80% has been recorded in chickens [16].

Out of 300 samples screened in this study, 169 were positive representing 56.3%. This result indicates considerable presence of Newcastle disease virus among local chickens sampled. As these birds have no history of vaccination against the disease, demonstration of antibodies to NDV in them is an evidence of natural infection with the virus. Our finding only buttresses the findings of other researchers who reported the presence and epizootic nature of NDV in some northern and middle belt States of Nigeria [10,11,13,17]. These similarities in ND prevalence may be due to similarities in live style and poultry practises in these areas. Inter-State trade of poultry and poultry products is also a common feature especially on known market days.

Allan and Gough [18] suggested a ND–HI titre greater than 8 as indicative of specific immunity. This means that 130 (77.4%) birds had specific immunity to NDV. Schmidt and Schmidt [19] considered HI titer of 32 and above to be protective against NDV. In this study, 81 (47.93%) birds had antibody titres greater than

32 but the geometric mean titre was 19.7 which gave a clearer picture of the poor immune protective level against NDV infection. Since [20] low ND-HI antibody prevalence is suggestive of an interepidemic phase or early phase of infection, problems with ND outbreaks in the near future may have to be expected unless the vaccination practice is improved substantially. The wider range of NDV titres in birds is likely due to natural infection which is known to produce higher antibody titres than vaccination [21].

In spite of vigorous vaccination schedules, ND is still a threat to the poultry industry in developing countries [22] especially Nigeria. Vaccination is an effective method of controlling ND in both commercial and village poultry, but is rarely given priority in rural communities of Nigeria where majority of local poultry are kept [13,16]. Limitations to vaccination of local chickens are basically a vaccine dose/vial issue. Most vaccines come in doses for 100 birds or more but local chicken farmers have just a couple of birds. Also, the availability and means of maintenance of vaccines in cold chain in rural communities coupled with poor enlightenment is difficult and most times not available. So ‘why bother’, most of the farmers ask.

However, the advent of the heat stable ND V₄ vaccine [3,23,24,25] offers village poultry keepers the opportunity and hope of still being in business. The V₄ vaccine technology, though not yet fully adopted by village poultry owners in Nigeria, has been tested with good results [15,26]. The cost-benefit analysis following the

use of the V₄ in village chicken in Kaduna State, Nigeria showed that it was highly beneficial to adopt this technology in protecting village poultry flocks than leaving them unvaccinated/unprotected [26].

Conclusion

The high titre of HI antibodies to NDV local chickens recorded in this research is suggestive of a possible epizootic of the disease in Bauchi metropolis. Although the presence of antibody to NDV in unvaccinated local chicken is mostly associated with contact with field strain of the virus which occasionally confers immunity to

them, it may not protect in times of outbreak. Infected birds could also serve as reservoir and source of transmission of the virus to exotic

poultry and other avian species in that area due to their close proximity. Were possible, we

recommend that alternative routes of vaccinating local birds via incorporation of thermostable vaccines in their food [25] should be practiced.

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NOSOCOMIAL INFECTIONS AND THE CHALLENGES OF CONTROL IN DEVELOPING COUNTRIES.

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ABSTRACT

Nosocomial infection is a recognized public health problem world-wide with a prevalence rate of 3.0-20.7% and an incidence rate of 5-10%. It has become increasingly obvious that infections acquired in the hospital lead to increased morbidity and mortality which has added noticeably to economic burden.

However, after about three decades of nosocomial infection surveillance and control world-wide, it still remains an important problem for hospitals today. Studies have shown that most hospitals in developing countries especially Africa, have no effective infection control programme due to lack of awareness of the problem, lack of personnel, poor water supply, erratic electricity supply, ineffective antibiotic policies with emergence of multiply antibiotic resistant microbes, poor laboratory backup, poor funding and non-adherence to safe practices by health workers.

It is recommended that the cost of hospital infection control programme should be included in the health budget of the country and fund allocated for the infection control committee for routine control purposes and to bear the cost of outbreaks. There is need for adequate staffing and continuous education of staff on the principles of infection control, especially hand washing which is the single most important effective measure to reduce the risks of cross infection.

INTRODUCTION

Nosocomial infections, otherwise known as hospital-acquired infections are those infections acquired in hospital or healthcare service unit, that first appear 48 hours or more after hospital admission or within 30 days after discharge following in patient care (1). They are unrelated to the original illness that brings patients to the hospital and neither present nor incubating as at the time of admission. (1, 2). There are several reasons why nosocomial infections are even more alarming in the 21st century. These include hospitals housing large number of people who are sick and whose immune system are often in a weakened state, increased use of

outpatient treatment meaning that people who are in hospital are sicker on average, many medical procedures that bypass the body's natural protective barriers, medical staff move from patient to patient thus providing a way for

pathogens to spread, inadequate sanitation protocols regarding uniforms, equipment sterilization, washing and other preventive measures that may either be unheeded by hospital personnel or too lax to sufficiently isolate patients from infectious agents and lastly the routine use of anti-microbial agents in hospitals creates selection pressure for the emergence of the resistant strains of microorganisms(1). Nosocomial infections may range from mild to severe with an incidence of 5-10 %(2). A WHO prevalence study puts its

prevalence rate at 3.0-20.7%³ Hospital infection control programs can prevent 33% of nosocomial infections (2).

The significance of nosocomial infection lies not only in its ability to substantially alter morbidity and mortality statistics, but also in its economic implications. Nosocomial infection prolongs duration of hospitalization, increases the cost of health care, emergence of multiple antibiotic resistance microorganisms and reduces the chances of treatment for others (4, 5, 6). Nosocomial infections pose a problem of enormous magnitude globally, hospital localities have proven favourable in transmitting infections due to existing suitable pathogen-host-environment relationship. Estimates from various countries show that at any point in time, a significant number of hospitalized patients develop infections which were not present or incubating when the patients were admitted to hospitals. Furthermore, it has become increasingly obvious that such infections acquired in hospital add noticeably to morbidity and economic burden (7). Realizing the importance of this to world health, various international organizations including accreditation and governmental agencies, national associations and organizations, World Health Organization, United Nations Environmental programme, United Nations Children's Fund and various countries have made commendable efforts in checking these infections.⁷ However, after about three decades of nosocomial infections surveillance and control in hospitalized patients world-wide, nosocomial infections remain an important problem for hospitals today (7). This review is important considering the increase in morbidity and mortality due to hospital acquired infections, coupled with the lack of effective infection control programmes in some hospitals especially in developing countries due to poor adherence to safe practices by health workers and also lack of personnel trained in infection control practices. There is need for renewed efforts geared towards education through training and re-training coupled with research to keep nosocomial infections in check (7). The objective of this review is to present an overview of nosocomial infections, identify the major challenges of control in developing countries and make appropriate recommendations aimed at effective control.

HISTORICAL PERSPECTIVE

Nosocomial infections must have existed from the time a number of people with various ailments were brought together for care, but were only readily acknowledged in the middle of the 19th century. During this period, some clinicians notably Oliver Wendel Holmes in Boston and Ignaz Philip Semmelweis in Vienna emphasized on the contagious nature of puerperal sepsis or child – bed fever (8,9). Semmelweis noticed that about 8.3 % of women admitted to maternity services died of nosocomial puerperal sepsis. He was able to demonstrate a dramatic reduction to 2.3 % in the incidence of this disease by insisting on stringent hand washing in a solution of chlorinated lime by students coming from the postmortem room before the women could be examined. Joseph Lister recognized the significance of bacteria in producing postoperative wound infections, and successfully attempted the use of carbolic acid in treating compound fractures and wounds (10, 11). These observations and innovations provided the basis for the epidemiology and control of nosocomial infections and these authors were the forerunners of modern aseptic techniques. The introduction of antimicrobial agents specifically penicillin into chemotherapeutics in 1941 brought a wave of premature optimism that, diseases, especially those acquired in hospital could be readily treated (11). Unfortunately, interest in infection control practices rapidly waned. In the 1940s and 1950s, severe world-wide *Staphylococcus aureus* pandemics caused substantial morbidity and mortality in hospitals, resulting in a rebirth of the need to establish infection control programmes in hospitals (11, 12). The joint commission on Accreditation of Health Care Organization (JCAHO) in the United States of America, in part due to the pandemics, recommended the appointment of infection control committees by hospitals (13).

The field of hospital infection was first conceptualized and implemented in England in late 1950s (1959) when Dr. Brendan Moore, Director of the Public Health Laboratory in Exeter, had a nurse in today hospital appointed as an “infection control sister” (14). In 1960, Dr. Moore still initiated the appointment of a second sister at the Exeter hospital. The mission of the two nurses was to improve efforts in combating and controlling the widespread problem of nosocomial infection. In 1963, the United States followed England's lead into the field of infection control and appointed the first control

nurse, Kathryn Wenzel, a registered nurse at Stanford university, California 11). The appointment of infection control nurses in the United Kingdom and the United States marked the beginning of a new era, the recognition of infection control as a specialty in its own right. The discipline of hospital infection control was thus born out of sheer determination to minimize infections in hospitalized patients and to help optimize patient care. During the 1960s, control procedures in many hospitals were random and non specific in approach. They consisted mainly of routine microbiologic culturing of air and environmental surfaces such as floors, walls and table tops in various areas of the Hospital. In the 1970s, such practices were critically evaluated and emphasis shifted away from the environment to the surveillance of infections in patients (17).

The hospital-wide surveillance method, also known as comprehensive or total surveillance, was used whereby all patients were monitored for nosocomial infections at body sites, and overall rates of infection were calculated (18). This system remained essentially unchanged until 1986 when the need for more precise measurements of nosocomial infection risks and outcome in specific patients groups led to the introduction of three other surveillance methods, viz adult and paediatric intensive care unit, high risk nursery and surgical patients method. The four techniques may be used singly or simultaneously and are designed to form the foundation of a hospital's overall surveillance programme. They also allow flexibility for incorporating other aspects into the surveillance efforts. As part of this revision, definitions of nosocomial infections were expanded to include clinical and laboratory algorithms (19).

EPIDEMIOLOGY

All over the world, nosocomial infection is a recognized public health problem, Surveillance programmes estimate the rate of infection at 5-10 % of hospital admissions (1, 20, 21, 22, 23). Nosocomial infections are responsible for about 90,000 deaths in the U.S. per year and approximately 10% of American hospital patients (about 2 million every year) acquired a clinically significant nosocomial infections²³. Estimates of the annual cost range from \$4.5-11 billion^{4,24}. In France, the prevalence is of nosocomial infections 6.9% to 7.5%. A rate of 5 to 19% hospitalized patients are infected and up to 30 % are in intensive care units (25, 26). In

Italy in 2000s, about 6.7 % of hospitalized patients were infected; that means, between 450,000 and 700,000 patients had nosocomial infections out of which between 4,500 and 7000 died (26, 27). In Switzerland, extrapolations assume about 70,000 hospitalized patients affected by nosocomial infections (between 2 and 14% of hospitalized patients (27). In Nigeria, nosocomial infection rate of 2.7 % was reported from Ife (28), while 3.8 %²⁹ from Lagos and 4.2 % from Ilorin³⁰ The cause of nosocomial infections might be endogenous or exogenous. Endogenous infections are caused by organism present as part of the normal flora of the patient, while exogenous infections are acquired through exposure to the hospital environment, hospital personnel or medical devices (22). Nosocomial infection rates vary substantially by body site, by type of hospital and by the infection control capabilities of the institution (17). The proportion of infections at each site is also considerably different in each of the major hospital services and by level of patient risk. This is exemplified by surgical site infections (SSIs) which are most common in general surgery, whereas urinary tract infections and blood stream infections are most frequent in medical services and nurseries. Rates of nosocomial infection vary by surgical subspecialty, low in ophthalmology and high in general surgery. The differences are largely due to variations in exposure to high risk devices or procedures (7, 31).

Urinary tract infections (UTI) represent the most common (34%) type of nosocomial infections. Indwelling catheters cause the majority while others are caused by genito urinary procedures³¹. Surgical wound infections represent 17 % nosocomial infection and are the second most common hospital acquired infections. The classification of wound infections is based on the degree of bacterial contamination, including clean, clean contaminated and contaminated. Co-morbid and contamination of the surgical site contribute to the infection rate. The risk factors for surgical wound infections include age, obesity, concurrent infection and prolonged hospitalizations. The origin of the bacterial agent is dependent on direct inoculation from a host's flora, cross-contamination, the surgeon's hands, air-borne contamination and devices such as drains and catheters³¹. Lower respiratory infection (LRI) or pneumonia represents 13 % of nosocomial infections⁵. This is the most dangerous of all nosocomial infections with a

case fatality rate of 30 %. It manifests in the intensive care unit or post-surgical recovery room. Endotracheal intubation and tracheostomy dry the lower respiratory tract mucous and provide entry for microbes. Other agents that cause nosocomial LRI are ventilators and nebulizers (31). Nosocomial blood stream infections (BSIs) represent 14 % of nosocomial infections (5). Nosocomial bacteremia can be classified as primary or secondary. Primary nosocomial bacteremia occurs without any infection in other sites. Secondary bacteremia is the presence of infection in a site such as urinary tract, surgical wound or lower respiratory tract which can lead to a blood stream infection with the same organism. Mortality from nosocomial bacteremia is greater than primary bacteremia. It is greater than if it is community – acquired. Primary bacteremia or fungaemia usually occurs due to intravenous catheters, intravenous fluid contamination and multidose parenteral medication lines (31). Most blood stream infections are associated with vascular catheter related infections. These catheters may be infected due to contaminated antiseptics used to clean the skin. Contaminated hands of health care personnel, infections following hematogenous seeding or external colonization¹. The risk factors for peripheral intravenous catheters include duration longer than 72 hours, cut down placement, lower extremity site, urgent placement and poor hand washing. Bacteria pathogens causing community acquired infections differ to some extent from those causing nosocomial infections (1, 31).

ETIOLOGY

Although viruses, fungi and parasites are recognized as sources of nosocomial infections, bacterial agents remain the most commonly recognized cause (32). The offending nosocomial pathogen can depend on the site of the infection. Nosocomial blood stream infections are usually caused by gram-positive organisms including coagulase negative *Staphylococcus*, *Staphylococcus aureus* and *Enterococci* (1). *Escherichia coli* is a very common cause of nosocomial urinary tract infection, but other pathogens including *Pseudomonas aeruginosa*, *Klebsiella spp*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Enterococci* and *Candida spp*. *Legionella pneumophila* may also be responsible for epidemic lower respiratory tract infection in hospitals. *Klebsiella spp*, *Pseudomonas spp*, *Proteus spp*, *Escherichia coli* and

Staphylococcus aureus are common cause of blood stream nosocomial infections in neonates.
³¹

The widespread use of broad spectrum antibiotics has led to nosocomial infections with drug resistant microbes (1). Examples include *Methicillin resistant Staphylococcus aureus* (MRSA) *Penicillin resistant pneumococci*, *Vancomycin resistant enterococci*, (VRE) and *multi drug resistant tuberculosis* (MDR-TB). The risk factors for MRSA colonization are overcrowding of wards with limited nursing staff. VRE blood stream infections have been associated with severe illnesses like haematologic malignancies, AIDS, neutropenia, prolonged hospitalization and vancomycin use. Prior use of broad-spectrum antibiotic appears to be a consistent predisposition for drug resistant bacteria. Thus the control and usage of broad spectrum antibiotic is of major importance. MDR-TB has taken place among prison inmates and health care workers (1, 33). Hepatitis A, B and C have been known to be transmitted from patient to health workers and vice versa. The risk of acquiring HIV from a needle stick injuries is 1/250 (1, 33, 34).

Increasingly aggressive medical and surgical interventions, including implanted foreign bodies, organ transplantations and xenotransplantation, create a cohort of particularly susceptible persons. Renovation of aging hospitals increases risk of air borne fungal and other infections (33).

TRANSMISSION

Microorganisms are transmitted in hospitals by several routes and same microorganisms may be transmitted by more than one route. The five main routes of transmission includes contact, droplet, airborne, common vehicle and vector borne (1).

Contact transmission is the most important and frequent mode of transmission of nosocomial infections. Direct contact transmission involves a direct body surface-to-body surface contact and physical transfer of micro-organism between a susceptible host and an infected or colonized person such as occurs when a health care worker turns a patient in bed, or gives a bath or performs other patient-care activities that require personal contact. There can also be cross-infection between two patients with one serving as the source of infection and the other as the

susceptible host. Indirect contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually an instrument such as needle, dressings, or contaminated gloves that are not changed between patients (1, 33). Additionally the improper use of saline flush syringes, vials and bags have been implicated in disease transmission in the U.S, even when health care workers had access to gloves, disposable needles, intravenous devices and flushes (1).

Droplet transmission occurs when droplets are generated from the source person mainly during coughing, sneezing and talking and during procedures such as suctioning and bronchoscopy. Transmission occurs when droplets containing microorganisms from an infected person are propelled a short distance through the air and deposited on the host's body. Airborne transmission occurs by dissemination of either airborne droplet nuclei (small particle residue 5µm or smaller in size of evaporated droplet containing microorganisms that remain suspended in the air for long periods of time) or dust particles containing infectious agent. Microorganisms carried in this manner can be dispersed widely by air current and may become inhaled by a susceptible host within the same room or over a long distance from the source patient depending on environmental factors. Examples include *Mycobacterium tuberculosis*, *Legionella*, the rubeola and varicella viruses. Common vehicle transmission applies to microorganisms transmitted to the host by contaminated items such as food, water, medications, devices and equipments. Vector borne transmission occurs when vectors such as mosquitoes, flies, rats and other vermin transmit microorganisms (1,31,33).

PREDISPOSITION TO INFECTION

Within hours of hospital admission, colonies of hospital strains of bacteria develop in patients' skin, respiratory and genitourinary tracts.³⁵ Risk factors for the invasion and colonization by pathogens can be categorized into three areas: iatrogenic, organizational, and patient-related factors. Iatrogenic risk factors include invasive procedures (e.g. intubation, indwelling vascular lines, urethral catheterization, blood transfusions) and antibiotic use both active and prophylaxis (35).

Organizational risk factors include contaminated air-conditioning system, contaminated water systems, hospital staff and physical layout of the

facility (e.g. nurse-to-patient ratio, open beds close together) (35). Patients' risk factors include the severity of illness, the underlying immunocompromised state and length of stay. Patients already colonized on admission are instantly put at greater risk when they undergo invasive procedures (35,36).

PREVENTION AND CONTROL

Control measures seek to protect potential sites of infection, interrupt routes of transmission, boost host defenses and discourage selection of hospital strains of organisms²². In the hospital, the first step in setting up a viable infection control programme is to set up an infection control committee, which is an essential administrative requirement for effective control of nosocomial infections. The infection control committee is made up of senior administrative staff, i.e. the Chief Medical Director, the infection control doctor, who is often a clinical microbiologist, an epidemiologist or a physician/surgeon with interest in infectious diseases whose opinion is respected, an infection control nurse, heads of clinical departments or their representatives (surgery, medicine, paediatrics, obstetrics and gynaecology etc), representative of nursing staff, pharmacy, engineering, central sterile services department (CSSD). Other co-opted members include representative from catering department, operating theatre, medical supplies and purchasing. (7,37).

The infection control committee should give authority to infection control policies, and to ensure implementation. The principles of infection control are based on the use of common sense and safe practices that prevent or reduce the likelihood of infections being transmitted from a source to a susceptible host³⁷ and this include hand washing, which remains the single most important effective measure to reduce the risks of transmitting microbes from one person to another or from one site to another on the same patient (3, 22, 29, 33). Others include use of protective clothing, good personnel and hospital hygiene, adequate management of soiled linen, management of inoculation and contaminated incidents, proper specimen handling and transportation, avoiding spillage on specimen container during collection, proper management of waste generated within the hospital, appropriate use of antiseptics,

disinfectants and sterilization processes, aseptic techniques in the operating theatre, delivery suites and in the wards, rationale use of antibiotics, surveillance of infection within the hospital by daily collection of data by the infection control team, isolation of highly contagious patients and health education through refresher courses for health workers (37)

CHALLENGES TO THE CONTROL MEASURES

A well-structured infection control programme plays a vital role in reducing mortality, morbidity and cost resulting from nosocomial infections in hospitalized patients. However, infection control activities in developing countries are severely constrained by numerous problems. Most hospitals in developing countries, especially Africa, have no effective infection control programmes and there is lack both of awareness of the problem and of personnel trained in infection control practices (7). Among the difficulties faced that have also been identified as mitigating against an effective infection control programme in the hospital are inadequate and ageing infrastructure including poor water supply, erratic supply of electricity, irregular supply of essential materials like gloves, masks, disinfectants etc, lack of an effective antibiotic policies which is due to the fact that often times these policies are written without due consultation with those that will prescribe and without taking into consideration the antibiotic resistance profile of local organisms, unfortunately, this information is unavailable in many developing nations due to poor or absent antibiotic resistance surveillance programmes which is part of the functions of the infection control team, poor funding of nosocomial infection programme, poor laboratory backup, lack of awareness of the activities of the infection control team or the cost benefits of an efficient control programme, the presence of family members in hospital, limited isolation facilities and in some cases patient presenting in advanced state of the disease process rendering isolation difficult (7,28,29,33,37,38,39).

Although effective hand washing programme still remains the single most important procedure in reducing cross infection, in developing countries, like Nigeria, this is usually difficult because there is often no running water with which to wash hands such that many facilities resort to a “bucket” and “bowl” with consequent

contamination. Even as simple as it seems to be, it is often not used or performed incorrectly (39) Surveillance is the backbone of a good infection control programme, but studies have shown that nosocomial infection surveillance and control have met little success in developing countries such as Nigeria due to non functional infection control committee or there is no infection control programme (39). In many industrialized countries, apart from the hospital infection control committee, there exist national bodies like Centre for Disease Control(CDC), National Nosocomial Infections Surveillance(NNIS) in the USA ,that collate and analyze data on hospital infection nationwide. No such institution exists in Nigeria though there is an urgent need for it (7). The solutions to these problems will include setting up an infection control committee with the powers to implement infection control policies. In formulating policies, the committee should ensure wide consultations, identify interested ward personnel, study the existing practices, formulate and implement simple, easy-to-follow policies, which should be incorporated in written policies in the infection control manual. The cost of hospital infection control programme must be considered an essential part of the health budget of the country and there should be a fund allocation for the infection control committee for routine control purposes and a contingency fund to bear the costs of outbreak (7, 37).

COST-EFFECTIVE APPLICATION OF MOLECULAR TYPING

The use of phenotypic methods for the characterization of nosocomial pathogens has been useful for the understanding of pathogens,; however, these methods have drawbacks that limit their utility for highly discriminatory typing of microorganisms. Limitations of serotyping include lack of availability of certain antisera and problems with standardization of different methods. Biotyping often lacks discriminatory power because of variations in gene expression and random mutations that may alter biologic properties of microorganisms. Bacteriophage typing is labor-intensive and method often demonstrates poor reproducibility and standardization. Bacteriocin typing may have some utility for organisms not easily typed, such as *Pseudomonas aeruginosa* and *Candida* species. Molecular typing methods including Plasmid Analysis, Southern Blot Analysis-Ribotyping, Pulse-field gel electrophoresis(PFGE), Amplified fragment

length polymorphism (AFLP) and Polymerase Chain Reaction (PCR) are powerful tools in the armamentarium for combating the spread of problematic microorganisms in the hospital environment (41). The integration of molecular typing with conventional hospital epidemiologic surveillance has been proven to be cost-effective due to an associated reduction in the number of nosocomial infections (41,42,43)

development of treatment regimens to manage patients affected by related organisms. Therefore, the use of molecular tests is essential in many circumstances for establishing disease epidemiology, which leads to improved patient health and economic benefits through reduction of nosocomial infections (41). Recent information also suggests the use of an integrated laboratory assessment of drug-resistant pathogens to have an impact on rates of endemic infection and can be cost saving (43)

CONCLUSION/RECOMMENDATION

Nosocomial infections may be contained by having an effective control programme with computer-assisted epidemiological surveillance for monitoring which should be handled as a global project with significant inputs from developing countries.

There is urgent need for review and boost of the prevailing infection control measures in various hospitals particularly in developing countries.

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Molecular techniques can be very effective in tracking the spread of nosocomial infections due to genetically related pathogens, which would allow infection control personnel to more rationally identify potential sources of pathogens and aid infectious disease physicians in the

The continuous education of hospital authorities and health workers on principles of infection control through training and re-training is advocated. Infection control should be incorporated into the curriculum of medical/dental students, student nurses and other paramedical. There is need for adequate employment of health workers especially nurses on hospital wards since overworked staff may become ineffective and this can result in epidemic infections in some situations. Major advances in overall control of infectious diseases have resulted from immunization, improved hygiene, particularly hand washing. Health care settings must continually remind hospital personnel on better implementation of existing infection control programmes especially hand washing so that we will not need to rely solely on technological advances in order to bring nosocomial infections to the barest minimal level.

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IN VITRO SURVEILLANCE OF DRUG RESISTANT FALCIPARUM MALARIA IN NORTH CENTRAL NIGERIA

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ABSTRACT

Background: drug resistant malaria is spreading inexorably to areas with drug sensitive malaria parasites. This study compared the *in vitro* sensitivities of *Plasmodium falciparum* fresh parasite isolates, to some standard antimalarial drugs, in Makurdi and Masaka located over 300 km apart, in north central Nigeria.

Methods: The *in vitro* responses of *P. falciparum* isolates; 43 and 39 in Makurdi and Masaka were evaluated by the standard schizonts growth inhibition assay in children aged 2-14 years.

Results: The geometric mean effective concentration-EC₅₀, EC₉₀ and EC₉₉ of quinine between Makurdi and Masaka differed significantly ($P < 0.05$). A similar difference ($P < 0.05$), was observed with the artesunate antimalarial at EC₉₀ and EC₉₉ levels, but not at EC₅₀. No significant difference ($P > 0.05$) was observed in the EC values of amodiaquine between the two locations. 5.13 % (2/39) of parasites at Masaka were *in vitro* resistant to amodiaquine with EC₅₀ > 80 nM. The rest of the isolates were sensitive to the three antimalarial drugs at both locations.

Conclusion: The results demonstrated low *in vitro* resistance of *P. falciparum* to amodiaquine in the region. Constant monitoring and intervention is needed to curtail the spread of resistance to antimalarials in Nigeria.

KEY WORDS: *Plasmodium falciparum*, Resistance, Antimalarials, Nigeria.

INTRODUCTION

The changing patterns in the epidemiology of malaria worldwide have led inexorably to the spread of resistant strains of malaria parasites, and reduced efficacy to very vital antimalarial drugs such as chloroquine and sulfadoxine/pyrimethamine (1,2). The emergence of drug resistant malaria among countries in sub-Saharan Africa where the impact of malaria due to *P. falciparum* is most felt has created as dire necessity for constant surveillance and monitoring of *P. falciparum* responses to the available antimalarial chemotherapies used in the region, using appropriate measures(3).

Constant monitoring is necessary in order to create a reliable national database which could be relied upon, to design and implement appropriate control measures aimed at preventing the wanton spread of multidrug resistance malaria within and across national borders.

In Nigeria, despite the importance of surveillance data to effective malaria control, the existing data on parasite susceptibility to several antimalarial drugs that are used in the country other than chloroquine are sparse. Only a few reports exist, particularly on the latter (4, 5, 6).

Malaria epidemiology, and indeed the spread of multidrug resistant parasite strains associated with it could vary even within the same border due to ecological, environmental, demographic, and cultural factors associated with a given population. Thus different malaria control strategies may be needed, such as the use of two or more different antimalarial drugs to combat malaria among areas with different levels of malaria parasite sensitivities to a specific antimalarial drug.

It was the aim of this surveillance study to determine and compare the level of *in vitro* sensitivities of *P. falciparum* isolates to some selected standard antimalarial drugs, used for malaria treatment in Makurdi and Masaka, located over 300 km apart within the same region of north central Nigeria, and to generate baseline data for future monitoring of parasite responses to those antimalarial drugs in the region.

METHODS

Study site: the study was conducted at the Bishop Murray Medical Centre Makurdi. Samples were obtained from the Medical Centre as well as the Primary Health Care Centre Masaka, a sub urban area of the Federal Capital Territory (Abuja) in north central Nigeria. Samples collected from Masaka were transported on wet ice to Makurdi and processed within 48 hours. The study protocol was approved by the

local ethics committee of the hospital. It lasted from the malaria transmission season between April to October 2006.

Subjects: enrolled subjects were febrile symptomatic children aged 2-14 years, who reported to the hospital with a history of fever, and whose guardian gave written informed consent. Prior to treatment, 2.5ml of venous blood was collected into heparin treated tubes, for microscopic detection of *P. falciparum* mono infections with Giemsa stain, and *in vitro* drug susceptibility test. Subjects with symptoms of severe malaria infections, a recent history of malaria pre-treatment with antimalarial drugs, and confirmed severe anaemia (PCV \geq 21%) were excluded from the study. Confirmed *P. falciparum* mono infections with parasite density of 2,000 to 80,000 asexual forms per μ l of blood were included in the *in vitro* test.⁷

***In vitro* parasite culture and growth inhibition assay:** the *in vitro* cultivation of *P. falciparum* isolates followed a modification of the standard culture techniques (8,9). Malaria positive blood samples were first washed three times in RPMI 1640 medium, to remove the leukocytes and the buffy coat and the infected erythrocytes re-suspended in the culture medium at 50% haematocrit and stored briefly at 4°C. The parasite growth inhibition assay followed the standard procedure for schizonts

inhibition.⁷ A blood medium mixture (BMM) was prepared from a 1:20 dilution of the re-suspended infected erythrocytes, made in a sterile culture medium consisting of 10.43g RPMI 1640 (Invitrogen), 5.96g HEPES, and 25mM NaHCO₃ (Sigma Aldrich), per litre of double distilled water and 0.5ml of 50mg/ml gentamicin, and supplemented with 5% albuamax II (Gibco).¹⁰ 200µl of the BMM per well was pipetted on to the wells of a sterile flat bottom 96 well micro culture plates, pre-dosed with a serial 2-fold varying concentrations of antimalarial drugs. The range of the final antimalarial drug concentrations on the plates were, amodiaquine: 6.25 – 400 nM; artesunate: 0.34 – 22 nM; and quinine: 50 – 3200 nM. The plates were covered, placed in a humid candle jar, and incubated at 37°C, for 26-30 hours,⁷ at the end of the incubation period, thick films were made, and stained with 2.5% Giemsa stain for 35 minutes. The mean number of schizonts formed in duplicate wells per 200 asexual parasites were counted and recorded.

Determination of effective concentrations (EC) and Potency ratios (PR) of antimalarial drugs: the number of schizont counts was fed into a non linear regression software; HN-NonLin made available free of charge by H. Noedl, at <http://malaria.farch.net> specific for malaria *in vitro* drug sensitivity test. Individual dose

response curves were generated, and their EC₅₀, EC₉₀, and EC₉₉ values determined. Potency ratios of each drug were estimated as EC_x A/EC_x B where x = EC values at 50%, 90% and 99%. Standard drug resistant clones were not included in the assay; however, drug resistant *P. falciparum* parasites were identified as isolates with EC₅₀ values, greater than published threshold values for sensitive parasite isolates. The threshold of resistance were; amodiaquine: EC₅₀ > 80 nM, quinine: EC₅₀ > 800 nM (11,12). Artesunate: values not yet determined (13); estimated EC values were therefore reported as a baseline data for future comparison.

DATA ANALYSIS

Geometric mean EC values and 95 % confidence intervals (CI) of each antimalarial drug were estimated for each site; non paired t – test was used to compare EC values between locations, while potency ratios of drugs were compared by ANOVA. The level of significance was set at $P \leq 0.05$.

RESULTS

The percentages of *P. falciparum* parasite isolates that were successfully tested for *in vitro* drug susceptibility test against the three standard antimalarial drugs at the two sampled locations were 82.69 % (43/52) and 76.47 % (39/51) of the original numbers that were subjected to the tests at Makurdi and Masaka respectively. The geometric mean

EC values of quinine between Makurdi and Masaka differed significantly at EC₅₀, EC₉₀, and EC₉₉ (P < 0.05, table 1). The EC values of statistically different at EC₉₀, and EC₉₉, but not at EC₅₀. Comparable values for amodiaquine against *P.*

the artesunate antimalarial drug, between the two sites were *falciparum* parasite isolates at Makurdi and Masaka were not significantly different (P > 0.05).

Table 1. Geometric Mean EC₅₀ EC₉₀ and EC₉₉, 95% Confidence Interval (CI) of Antimalarial Drugs against *Plasmodium falciparum* Isolates at Makurdi and Masaka.

Drug	Geometric Mean EC, (95% CI) nM		t-test	df	P-values
	Makurdi	Masaka			
AMQ: EC ₅₀	21.64 nM (16.22 – 27.06)	24.89 nM (20.78 – 29.00)	0.68	80	> 0.05
EC ₉₀	61.86 nM (51.46 – 72.26)	76.54 nM (62.43 – 90.65)	1.89	80	> 0.05
EC ₉₉	91.51 nM (74.14 – 108.88)	115.32 nM (94.53 – 136.11)	1.89	80	> 0.05
ART: EC ₅₀	1.06 nM (1.02 – 1.10)	1.05nM (1.05 – 1.08)	0.39	80	> 0.05
EC ₉₀	2.33 nM (2.24 – 2.41)	2.47 nM (2.38 – 2.56)	2.14	80	< 0.05
EC ₉₉	2.97 nM (2.81 – 3.12)	3.25 nM (3.06 – 3.44)	2.37	80	< 0.05
QNN: EC ₅₀	232.63 nM (219.33 – 245.93)	286.64 nM (270.08 – 303.20)	5.07	80	< 0.05
EC ₉₀	650.82 nM (614.96 – 686.68)	791.80 nM (751.89 – 831.73)	5.22	80	< 0.05
EC ₉₉	953.63 nM (911.58 – 1005.68)	1125.79 nM (1083.78 – 1167.80)	4.82	80	< 0.05

AMQ = amodiaquine, ART = artesunate, QNN = quinine.

The potency ratio of the individual antimalarial drugs which measured the different *in vitro* activities of amodiaquine, artesunate, and quinine against *P. falciparum* isolates at the two locations shows that there

was no significant difference in the sensitivities of each antimalarial drug between Masaka and Makurdi, ANOVA (F₂, 8, df) = 3.39 P > 0.05, table 2.

Table 2. Potency Ratios of the Antimalarial Drugs at Masaka and Makurdi

EC _x	Potency ratios of drugs		
	Amodiaquine	Artesunate	Quinine
50%	1.15	0.99	1.23
90%	1.24	1.06	1.22
99%	1.30	1.22	1.18

ANOVA (F_{2,8}, df) = 3.39, P > 0.05

All the parasite isolates (100%) were *in vitro* sensitive to quinine and artesunate, at Makurdi and Masaka as determined by their individual EC responses to the drugs. In contrast, 5.13 % (2/39) of *P. falciparum*

isolates at Masaka had values of EC₅₀ > 80 nM and were classified as *in vitro* resistant to amodiaquine. No isolate at Makurdi exhibited a similar disposition towards the amodiaquine antimalarial drug, table 3.

Table 3. Percentage (%) of *in vitro* Sensitive and Resistant Isolates of *P. falciparum*

Antimalarial Drug	location	n	<i>in vitro</i> sensitive (%)	<i>in vitro</i> resistant (%)
Amodiaquine	Makurdi	43	43 (100.00%)	-
	Masaka	39	37 (94.87 %)	2 (5.13 %)
Artesunate	Makurdi	43	43 (100.00%)	-
	Masaka	39	39 (100.00%)	-
Quinine	Makurdi	43	43 (100.00%)	-
	Masaka	39	39 (100.00%)	-

DISCUSSION

The findings from the present data indicate that, wide differences exist in the effective concentration values of *P. falciparum* parasite isolates to quinine in Makurdi and Masaka. This was in spite of the fact that all the parasite isolates obtained from these locations were *in vitro* sensitive to quinine; isolates at Masaka had consistently higher geometric mean EC₅₀, EC₉₀, and EC₉₉ values compared to those at Makurdi. This

demonstrates that the continued use of quinine in these areas, and possibly, future development of parasite resistance to the drug may evolve at different intervals between these sites. Quinine has remained very useful for the treatment of chloroquine resistant *falciparum* malaria in Nigeria;⁵ and most isolates are highly *in vitro* sensitive to the drug in the country compared to other regions in the world (13, 14). The present

EC₅₀ values of quinine in north central Nigeria are very close to 50% inhibitory concentration (IC₅₀) = 0.25µM previously reported in western Nigeria nearly two decades ago (15), suggesting that the sensitivity of quinine in the country has remained very stable over the years, at least between the west and north central parts of Nigeria.

The differences observed in the artesunate antimalarial drug at EC₉₀, and EC₉₉ levels, at the two sites may reflect inherent differences prevalent in the nature of *P. falciparum* parasites in Makurdi and Masaka. However the geometric mean EC₅₀ values of the drug against parasite isolates at each site were low, not significantly different, and comparably similar to values generally reported for parasite isolates that were susceptible to the drug in other parts of the world.^{13,14} The global importance of the artemisinin derivatives and by extension the artesunate antimalarial drug in the current scheme of malaria treatment is predicated on the ability of the drugs to rapidly kill and eliminate the metabolically active destructive stages of the human malaria parasites (16), and save lives. At the moment there is little evidence of parasite resistance to these drugs as has recently been found with other key antimalarial drugs (12,17). Yet, the danger still looms large as *P. falciparum* has often in the past found a way round to acquire resistance against other antimalarial drugs, the artemisinin

derivatives may not be an exception in the future.

Thus to delay parasite resistance and sustain the prolong use of these useful drugs, their combination with other antimalarial drugs have often been advocated (18). In areas with high sensitivity to artesunate, amodiaquine, and quinine as found in the present survey, a combination of artesunate/quinine for the treatment of severe malaria, and artesunate/amodiaquine for the treatment of acute malaria could prove very useful. Such combinations have been reported to produce high cure rates in the treatment of uncomplicated malaria elsewhere (19, 20), and reduced the high expenditure associated with the treatment of malaria, with ineffective antimalarials (21).

Despite the lack of significance difference in the EC values of amodiaquine in Makurdi and Masaka at all the levels of the effective concentrations analysed, 5.13 % of isolates at Masaka were *in vitro* resistance to the drug, unlike their counterpart at Makurdi. Compare to a recent study in western Nigeria, *in vitro* EC values of amodiaquine against *P. falciparum* parasites were EC₅₀ = 0.06 µM, EC₉₀ = 0.26µM, and EC₉₉ = 0.59µM and were higher than the present values in north central Nigeria (22). Moreover, 39% of a mere 36 parasite isolates observed in that study were *in vitro* resistant to amodiaquine,

which highlights wide spread *in vitro* resistance of *P. falciparum* isolates to amodiaquine in western Nigeria, and a noticeable gap in the *in vitro* susceptibility profiles of *P. falciparum* isolates to amodiaquine between the west and north central Nigeria. Thus isolates in north central Nigeria appear to be more *in vitro* sensitive to amodiaquine than their counterparts in the western part of the country. The present and the previous data also suggest that resistance to amodiaquine has emerged in the country, and may be spreading at an undetermined level. Increased parasite resistance to the drug *in vivo* would progressively limit the relevance of amodiaquine, as a viable option for combination therapy with artesunate in the treatment of uncomplicated malaria in Nigeria. The usefulness of the artesunate/amodiaquine combination in producing high cure rates in clinical malaria has been demonstrated in Nigeria and other countries (23,24,25). Generally, *in vitro* studies provide a clue which may be detectable in the clinical treatment outcome of parasite responses to antimalarial drugs *in vivo*. Although the potency ratios of the three drugs used in the current survey did not suggest any significant *in vitro* differences among the activities of these antimalarial drugs, relying solely on *in vitro* assessment to define possible activity similarities or otherwise of antimalarial drugs may not be sufficient. This is because

in vitro studies do not take cognizance of acquired immunity against malaria parasites, which is promoted by certain antimalarial interventions (26). Further studies combining *in vitro* and *in vivo* as well as molecular characterization may be necessary to determine the actual activities of these antimalarial drugs in the north central region of Nigeria.

In conclusion, the present study has provided evidence of *in vitro* resistance of *P. falciparum* to amodiaquine in north central Nigeria. It might be necessary to constantly monitor and initiate critical control measures to limit the spread of resistance to amodiaquine and other antimalarial drugs in the country, which might develop as a result of cross resistance between drugs.

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RELATIONSHIPS BETWEEN BLOOD CELL COUNTS AND THE DENSITY OF MALARIA PARASITES AMONG PATIENTS AT THE REGIONAL HOSPITAL, LIMBE, CAMEROON.

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ABSTRACT

Malaria is one of the most important infectious disease in Cameroon and throughout the world [1]. Globally it results in an estimated 400 millions cases and about 3 millions deaths each year, most of these deaths in children aged 1 to 5 in Sub-Saharan Africa, making it the biggest single infections killer of children in the world [1]. It is a major public health problem in Cameroon, with its prevalence and incidence appearing to be on the increase owing to the lack of adequate control measures [6]. This study was designed to determine the correlation between blood cell counts and the density of malaria parasitemia amongst patients who presented for consultation at the Regional Hospital Limbe (RHL). A total of 100 consecutive patients suffering from malaria who consented to participating in this study were recruited and venous blood (3-5ml) was collected by venepuncture. Thick and thin blood films were prepared, stained and microscopically examined for the presence of malaria parasites. Total blood cells and differential white cell counts were performed using a coulter counter.

The findings depicted a negative correlation between parasite load and haemoglobin concentration [Hb], mean cell volume (MCV), and mean cell haemoglobin (MCH); a positive correlation of parasite density with white blood cell counts (WBC), red blood cell counts (RBC), and the differential white blood cell counts (lymphocyte, monocyte, and granulocytes); and no correlation was observed with the platelet counts.

INTRODUCTION

Malaria is a killer disease and despite efforts to eradicate malaria prevalence, the weight of malaria has not changed in countries where it is endemic. Although an ancient disease, environmental disturbances, malnutrition and the failure of drugs once used to control the disease, have conspired to make death by malaria more frequent now than at any point in history [3]. Malaria has been brought under control and even eliminated in many parts of Asia, Europe, and the Americas; yet in Africa, with various mosquito vectors, increasing drug resistance, and struggling health systems,

malaria infections have actually increased over the last 3 decades [1], [12].

From recent WHO statistic, about 350 to 500 million cases of malaria occur each year, resulting in 2 to 3 million deaths, 1,5 million of which are children (WHO, 2007; Schulman; 2007); thus, one person dies of malaria each minute. Over 90% of the global effect occurs in sub-Saharan Africa, where malaria dominates the chart of major public health problems, just to compete with HIV/AIDS and tuberculosis today [2], [3]. Although in comparison, malaria kills about as many person a year as AIDS has done in the last 15

years. In additions, malaria is an important cause of still birth, infant mortality, low birth weight and blood transfusions errors [4].

In Africa, at least 24 million pregnancies are threatened by malaria each year and malaria in Africa is estimated to cause up to 45 % of maternal anaemia and 35 % of preventable low birth weights [5], [6], [10], [11]. Experts foresee as much as a 20 % annual increase in Africa's rate of malaria related illnesses and death (Facer, 1997).

In Cameroon, malaria remains the first cause of morbidity and mortality. About 3 million clinical cases occur annually (about 95 cases in every 1 000 inhabitants) (PNTP, 1999) and it accounts for; 40 to 45 % of mortality in children below 5 year, 45 to 50 % of persons presenting complaints and 40 % of hospitalizations [6].

Generally, individuals living in endemic areas of malaria tend to acquire immunity, with age, such that susceptibility to the pathogen and pathology of malaria infections reduces with age [5], [7], [13], [14], [15]. Exceptions are seen in children below 5 years of age and in pregnant women [8] who are more vulnerable to malaria infection than non-pregnant women or adult men of the same age.

Malaria, which had been effectively suppressed in many parts of the world, is undergoing resurgence [8]. In developing countries, the burden of malaria has tremendous social and economic impact on a large scale. This caused the WHO to devise a multi-component eradication program based on providing families with information necessary to prevent, recognise and treat malaria at home. Since the proper

management of malaria is still a problem in Cameroon [8]. Even the use of anti-malaria prophylaxis during pregnancy and the development of a vaccine is still a problem. More centres for the study and control of malaria are being created and modern technique for the diagnosis being embraced [5], [15]. These programs also included vector control, the use of curative and prophylactic chemotherapy and the development of a vaccine.

The African summit on Roll Back malaria, which was held in Abuja, Nigeria on the 25th of march 2000, the Pan-African malaria conference on Roll Back malaria forum held in Yaounde Cameroon from the 13th to the 19th of November 2005 and the conference on Roll Back malaria, in the year 2007, reflects a real convergence of political momentum, institutional synergy and technical consensus on malaria mortality for Africa by the year 2010. This has caused the government of Cameroon to create a national program for the fight against malaria, HIV/AIDS and Tuberculosis under the ministry of public health and initiated by the Roll Back malaria, HIV/AIDS and Tuberculosis program.

Objective of Study

The overall objective of the investigation was to determine the correlation between blood cell counts and the density of malaria infection amongst patients at the Regional Hospital Limbe (RHL).

MATERIALS AND METHODS

Study design and setting

This study was a cross sectional descriptive study. It was carried out at the Regional Hospital in Limbe (RHL), the administrative head quarters of Fako Division. informed consent of the experimental subjects and the approval of the regional ethical committee had been obtained.

The climate of Limbe is characterized by two main seasons; the rainy and the dry season with abundant rains ranging from 5500mm³ to 6500mm³ owing to the fact that Limbe is closer to Debundscha known to be the wettest place in Africa recording rainfalls of about 10.000mm³. The rainy season starts in March to October while the dry season is between October and March. The temperatures are high and fairly constant ranging between 25°C and 30°C on the average that characterize this climate [9].

Study duration and population

This study was conducted from February to March 2008. Case recruitment covered the period from the 14th February to the 10th of March 2008.

The study population constituted patients who came from both the rural and urban settings of Limbe for consultation and diagnosis at the Limbe Regional Hospital. These were patients presenting with one or more of the following signs and symptoms: fever, headache, joint pains, abdominal upset, nausea, vomiting, diarrhoea, and digestive disorders; and whom malaria and full blood counts tests were requested. Venous blood was collected by veno-puncture of the antecubital vein into EDTA tubes.

The Giemsa staining method was used to detect malaria parasites in blood films on slides and the culter counter to determine full blood counts.

However, other processes could also be used to detect malaria parasites such as, the Diff-quick staining technique with the field stains which is not so oftenly used in many diagnostic laboratories.

The Giemsa staining technique involved the staining of two prepared blood films; the thick and the thin blood films.

- **Preparation of thin Blood Film**

The thin blood film was made as follows; a drop of blood was put at one end of a well cleaned and grease-free slide, and a spreader was used to produce a uniform spread of the blood over the slide such that a feathery tail end was achieved. The slide was then kept at a dry surface to air dry after which it was fixed with alcohol (95%) or with May Grunward fixative. The fixed slides were then stained with Giemsa stain made from a concentrated stock solution as follow; 3 drops of the stain to 2 ml of distilled water and allowed for a staining duration of 8 to 10 minutes after which it was washed, air dried and observed using the x100 oil immersion objective [5]. When the thin blood films were examined, the following were seen: RBCs and WBCs .

- **Preparation of thick Blood Film**

Unlike the thin blood films, the thick blood films were made just by spreading a drop of blood at the centre of a clean grease-free slide in order to defibrinize the blood. It was

allowed to air dry after which it was stained with the Giemsa stain for 8 to 10 minutes without fixing with alcohol or may gunwale fixative [5]. When the thick blood film was examined, using the x100 objective and the x7 ocular, the following were seen: remains of red blood cells; white blood cells (Leukocytes) and platelets (thrombocytes).

The thick blood film consisted of many dehaemoglobinized red blood cells packed together in a thick mass since after staining with the Giemsa stain, the water in the stain acted on unpreserved red blood cells that caused the contents of the cells to dissolve in the water; hence dehaemoglobinization.

Examination of Blood Films for Malaria Parasite Recognition

Malaria parasites were found to take up Giemsa stain in a special way in both the thick and thin blood films.

Since the malaria parasites are known to pass through a number of developmental stages; in all the stages however, the same parts of the parasite were stained with the same colour: chromatin (part of the parasite nucleus) that is usually round in shape was stain deep red. The cytoplasm was presented in a number of forms; from a ring shape to a totally irregular shape. It was noticed to stain blue, although the shade of the blue could vary among the malaria species.

Stages of the Malaria Parasite

➤ The Trophozoite Stage

This stage was the most commonly seen and is often called the ring stage, although it sometimes took the form of an incomplete ring

The ring stage of malaria parasite in red blood cells

Owing to the fact that, the trophozoite is the growing stage; the parasite within the red blood cell varied in size from small to quite large. The malaria pigment appeared as the parasite grew. The pigment could be a by-product of the growth or metabolism of the parasite. It did not stain but had a colour of its own, which ranged from pale yellow to dark brown or black.

The Schizont Stage

At the schizont stage the malaria parasite is known to reproduce; a reproduction referred to as asexual because the parasite is known to be neither male nor female but reproduces itself by simple division. There were obvious phases in this stage, ranging from parasites with two chromatin pieces to parasites with a number of chromatin dots and definite cytoplasm [16]. It should be noted that, the parasite could be diagnosed using non-microscopic method as well [17].

Examination of the thick blood film

Routinely, thick blood films were examined. Provided that they had been well-made and stained before autofixation could take place. Occasionally, however, it was difficult to tell the difference between the mature trophozoites and the gametocytes of *P. vivax* and between *P. malaria* trophozoites and rounded *P. falciparum* gametocytes. Also it was not possible to distinguish between the late trophozoites and gametocytes of *P. malaria* in thick films, but the need to know whether

gametocytes could be present in the blood was usually confronted to a *P. falciparum*.

However, the routine examination of a thick blood films was based on the examination of 100 good fields. **That is a slide was pronounced negative only after no parasite was found in 100 fields;** some further 100 fields were examined if parasite were present before a final identification of species made. This ensured there was little or no possibility of a mixed infection when more than one species was present in the blood film being overlooked, though *P. falciparum* is known to be the major cause of malaria infections in Africa. The technique in which the thick film examination was done was as follows:

Step 1: The slide was focused using objective 10 and the reading area was selected. A part of the film that was well stained, free of staining debris, and well populated with white blood cells was selected. The film was well made and of even thickness such that there was no problem but poor quality films needed to be searched extensively.

Step 2: The immersion oil was placed on the thick film.

Step 3: The x100 oil immersion objective was then swivelled over the selected portion of the blood film.

Step 4: The oil immersion objective was then lowered until it touched the immersion oil.

Step 5: The blood film was then examined, by moving along the edges of the thick

film, then moving in a lateral movement and so on.

Step 6: In order to determine whether the blood film was positive or negative for malaria, continued examination for 20 to 30 fields was made. If any doubtful diagnosis were made, more fields (up to 100) were examined. The findings were then recorded at the end of the examination on an appropriate record form and the results were included with a parasite count.

THE PARASITE COUNT

Parasite counts for the blood film were necessary for the following reasons: the physician could want to know how severe the malaria was and whether the malaria parasites were responding to anti-malarial treatment given. The parasite counts were especially important in *P. falciparum* infection as they are known to be potentially fatal. The district health officer could need to know the severity of malaria infection being seen on the local health facilities. Also, the data could be needed for special purposes, such as testing the sensitivity of parasites to anti-malarial drugs. Several methods of determining the number of parasites; among them the “number of parasite per micro litre of blood” was used as described, below:

- **Parasite per Micro litre (ul) of Blood**

This was a practical method of adequate accuracy. It was based on the number of parasites per ul of blood in a thick film; these being counted in relation to a predetermined

number of leukocytes [18]. All the white blood cells (WBC) and parasites were counted on 20 to 30 fields, then the following formula was applied [19]:

$$\frac{\text{Number of parasites}}{\text{Number of WBC}} \times 8000 = \text{Number}$$

of parasites per microlitre of blood (Parasites/ul)

- **Parasites per White Blood Cells on Thin Blood Films**

Here, the number of white blood cells (WBC) and parasites could be counted on 20 to 30 fields, and the following formula used to obtain the number of parasites per WBC (WHO, 2007).

Number of Parasites / Number of white blood cells x 1000

- **Parasites per Red Blood Cell on Thick Blood Films**

All the red blood cells (RBC) and parasites could be counted on 20 to 30 fields and the formula below used to obtain the number of parasites per 1000 RBC (WHO, 2007).

$$\frac{\text{Number of parasite}}{\text{Number of red blood cells}} \times 1000$$

PROCEDURE FOR DETERMINATION OF FULL BLOOD COUNT

This was done using the counter (Abx MICROSOT, 1995 RAC 028 Ind., Montpellier; France). It was a 15 parameters counter which did RBC counts, WBC count, HCT, MCV, haemoglobin, Differential count, platelet count, and the other parameters such as MCH,

MCHC and RDW were calculated automatically. Briefly, the principle of measurement was based on the variation of resistance and impedance induced by the passage of cells through a calibrated micro-orifice. In the process of measurement each sample was diluted with electrolyte diluents (charge conductor) whose conductivity was known to quite different from that of the cells. The diluent was aspirated through a calibrated micro-orifice and the electrical resistance between the 2 electrodes increased proportionally to the volume of the cells. For instance, in RBC measurement;

According to Ohm's law;

$$V=IR \quad \text{where; } V = \text{Voltage, } I =$$

Current and R = Resistance

Since I remained constant, R increased every time a red blood cell passed through the micro-orifice, and V then also increased proportionally to the volume of the voltages requiring the use of an amplification circuit which increased the voltages providing room for the electrical analyser (also needed to eliminate the background noise) to analyse the results.

The dilution used for RBC was 12ul of blood sample mixed with 2.2 ml of diluent giving a dilution fraction of 1/148 or a dilution factor of 184; 27.5ml. The dilution was then mixed further with 3ml of the diluent giving a dilution fraction of 1/20 or a dilution factor of 20 and the microprocessor analysed the cells.

STATISTICAL ANALYSIS

Data generated was analysed manually and differences were considered significant at P-value ≤ 0.05 .

RESULTS

DEMOGRAPHIC DATA

The study was conducted within February and March 2008 at the Limbe Provincial Hospital Laboratory. A total of 100 patients were recruited made up of 33% males and 67% females. The ages of the patients ranged from 0.4 year (4 months) to 85 years with a mean (\pm SD) age of 29.602 (\pm 18.138) years and all the 100 patients recruited were positive for *Plasmodium falciparum* malaria (Table 4.1).

The subjects were stratified into the following age groups; <15 years (25 subjects), 15 to 30 years (29 subjects), 31 to 45 years (31 subjects) and > 45 years (15 subjects) (Table 4.1).

MALARIA PARASITE DENSITY

Parasite load was fairly low, ranging from ≤ 500 parasites per microlitre of blood to >1500 parasites per microlitre of blood with a mean (\pm SD) of 680.40 (\pm 1105.59) parasite per

microlitre of blood. As far as age was concern, it was observed that the age group 31 to 45 years had the highest occurrence of malaria infections with a generally low parasitaemia, whereas, the age group <15 years had the highest occurrence of subjects with the highest parasitaemia of >1500 parasites per microlitre of blood. In addition, the age group >45 years had the lowest occurrence of malaria infections also with a generally low parasitaemia. However, there was no statically significant difference in the occurrence of malaria infection in the various age groups (P- value = 0.19).

DISTRIBUTION OF MALARIA PARASIT DENSITY ACCORDING TO AGE GROUPS

The age group 30 to 45 years had the highest infection rate. Many of the cases had parasitaemia of ≤ 500 parasites/ μ l of blood, and a majority of them (90%) had parasitaemia ≥ 320 parasitaemia/ μ l of blood. There was no statistically significant difference in the parasite density among the different ages (Table 4.1).

Table 4.1: Distribution of Malarial Parasite Density According to Ages

Parasite density (N° of parasites / μ l of blood)	Age groups (years)				
	< 15	15-30	31-45	> 45	Total
≤ 500	10	18	22	11	61
501-1000	10	10	08	03	31
1001-1500	01	00	01	01	03
>1500	00	01	00	00	05
Total	25	29	31	15	100

$\chi^2 = 9.63$ d.f = 9 P=0.108

PARASITE DENSITY BY AGES OF SUBJECTS AT LIMBE PPROVINCIAL HOSPITAL

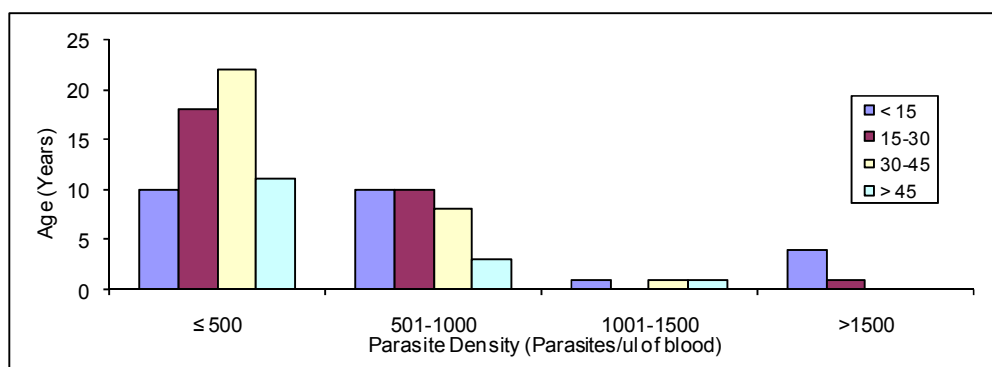


Figure 11. Variation of Parasite Density with Age

4.3 MALARIA PARASITE DENSITY ACCORDING TO SEX

It was observed that, there was generally high occurrence of malaria infection in females than males, leading to a higher prevalence of malaria parasitaemia in females. In addition,

females had the highest occurrence with the highest parasitaemia of >1500 parasites/μl of blood. However, there was no statistically significant difference (Table 4.2).

Table 4.2: Malaria Parasite Density Distribution Influenced by Sex

Parasite density (N° of parasites / μl of blood)	Sex		Total
	Males	Females	
≤ 500	18	43	61
501-1000	10	21	31
1001-1500	03	00	03
> 1500	02	03	05
Total	33	67	100

$$\chi^2 = 6.546 \quad d.f = 3 \quad P = 0.088$$

4.4 DISTRIBUTION OF MALARIA PARASITE DENSITY AS INFLUENCED BY BOTH AGE AND SEX

It was observed that in all the various age groups, females had the highest prevalence of malaria parasitaemia also with the highest

occurrence with the highest parasitaemia >1500 parasites/μl of blood. There was no statistically significant difference (Table 4.3).

4.5 VARIATION OF BLOOD CELL COUNTS FROM THE NORMAL COUNTS BASED ON SEX

Generally, more patients were observed with relatively low red blood cell counts and haemoglobin concentrations with many

females than males. In addition none of the sexes had [Hb] and RBC greater than normal.

Table 4.3: Malaria Parasite Density as Influenced by Both Age and Sex

Parasite density/ μ l of blood	Age								Total
	< 15		15-30		31-45		> 45		
	Sex								
	M	F	M	F	M	F	M	F	
≤ 500	06	04	02	16	06	16	04	07	61
501-1000	02	08	05	05	02	06	01	02	31
1001-1500	01	00	00	00	01	00	01	00	03
>1500	02	02	00	01	00	00	00	00	05
Total	11	14	07	22	09	22	06	09	100

$$\chi^2 = 32.14 \quad d.f = 21 \quad P = 0.057$$

It was observed that, there was an increase in the white blood cell counts in females than in males. Moreover, a normal mean cell volume and mean cell haemoglobin was observed in many of the patients of both sexes, though the

highest occurrence of low mean cell volume count was observed in males and high mean cell volume count in females. However, there was no statistically significant difference in the blood cell indices in the both sexes (Table 4.4).

Table 4.4: Variation of Blood Cell Counts from the Normal Based on Sex

Blood cell Indices	Sex								X ²	d.f	P-value
	Male				Female						
	N	Low	Normal	High	N	Low	Normal	High			
WBC	33	02	25	06	67	09	48	10	1.290	02	0.795
RBC	33	17	16	00	67	34	33	00	0.005	02	0.999
[Hb]	33	20	13	00	67	40	27	00	0.008	02	0.998
(MCV)	33	10	18	05	67	09	45	13	4.093	02	0.351
(MCH)	33	14	16	03	67	19	39	09	2.053	02	0.674

Normal values: WBC $\rightarrow (3.5-10.0) \times 10^3/\text{mm}^3$; RBC $\rightarrow (3.8-5.8) \times 10^6/\text{mm}^3$ [Hb] $\rightarrow (11.0-16.5) \text{ g/dl}$

MCV $\rightarrow (80-97) \mu\text{m}^3$ MCH $\rightarrow (26.5-33.5) \text{ pg}$ N = Number per group

VARIATION OF BLOOD COUNTS FROM THE NORMAL COUNTS BASED ON AGE

It was observed that the age group <15 years had highest white blood cell counts and the age groups 31 to 45 years and >45 years had the lowest white blood cell counts. In addition, all the age groups were observed to have

fairly low red blood cell and haemoglobin concentration. Moreover, the age group <15 years was observed to have low mean cell volumes and mean cell haemoglobin counts, whereas the other age groups had fairly normal counts (Table 4.5).

Table 4.5: Variation of Blood Cell Counts from the Normal Counts Based on Age

Blood cell Indices	Age															
	< 15				15-30				31-45				> 45			
	N	Low	Normal	High	N	Low	Normal	High	N	Low	Normal	High	N	Low	Normal	High
WBC	25	01	13	11	29	05	20	04	31	03	28	00	15	02	12	01
RBC	25	11	14	00	29	18	11	00	31	15	16	00	15	06	09	00
[Hb]	25	19	06	00	29	20	09	00	31	15	16	00	15	06	09	00
MCV	25	11	14	00	29	07	16	06	31	02	19	10	15	01	12	02
MCH	25	17	08	00	29	11	14	04	31	04	19	8	15	01	14	00

N = Number per category

DISTRIBUTION OF BLOOD CELL INDICES WITH THE AGES OF SUBJECTS AT THE LIMBE PROVINCIAL HOSPITAL

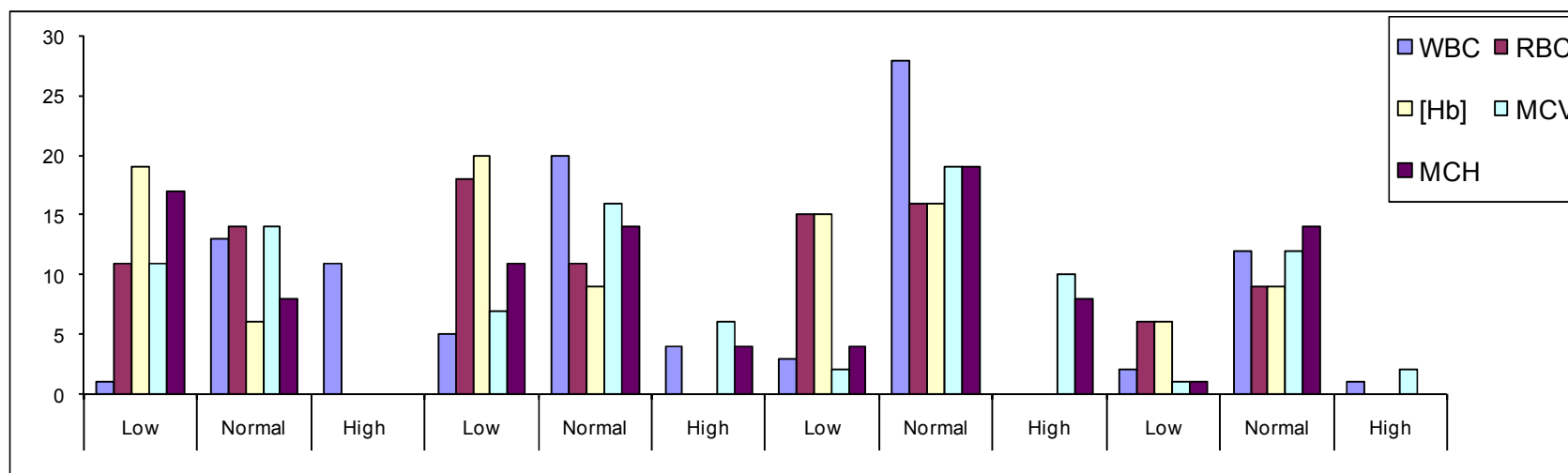


Figure 12. Variation of Blood Cell Counts from the Normal Counts with Age

VARIATION OF THE DIFFERENTIAL WHITE BLOOD CELL COUNTS AND PLATELET COUNTS FROM THE NORMAL COUNTS

From observation, it was found that, more than 70% of the subjects had a fairly normal differential white blood cell counts and platelet counts. However, 25% of the subjects had lower lymphocyte and monocyte counts whereas, 12% showed up with high

granulocyte counts (Neutrophils, Basophiles and Eosinophils). The platelets count was observed to be equally distributed with equal percentages of occurrence with high and low counts (Table 4.6).

Table 4.6: Variation of the Differential White Blood Cell Counts and Platelet Counts from the Normal Counts

Differential white cell count and platelet	N	Low	Normal	High
Lymphocytes	100	21%	65%	14%
Monocytes	100	30%	62%	08%
Granulocytes (Neutrophils, Basophils, Eosinophils)	100	06%	82%	12%
Platelets	100	13%	74%	13%

Normal values: Lymphocytes $\rightarrow (1.2 - 3.2) \times 10^3/\text{mm}^3$

Granulocytes $\rightarrow (1.2 - 6.8) 10^3/\text{mm}^3$

Monocytes $\rightarrow (0.3 - 0.8) \times 10^3/\text{mm}^3$

Platelets $\rightarrow (150 - 390) 10^3/\text{mm}^3$

4.8 HAEMOGLOBIN AND ANAEMIA STATUS

Sixty subjects were observed to have haemoglobin levels $<11\text{g/dl}$, which was an indication of anaemia. Forty subjects were observed to be non-anaemic based on the above classification. Levels of haemoglobin ranged from 3.2g/dl to 15.6g/dl ($\pm\text{SD}$), with a mean ($\pm\text{SD}$) value of $9.962(\pm 2.667) \text{g/dl}$, and the overall prevalence of anaemia was

observed to be 60%. People classified with anaemia had values between 3.2 to 10.8g/dl , which correspond and indicated mild anaemia. The mean haemoglobin concentration ($10.160 (\pm 3.129) \text{g/dl}$) in males ($P\text{-value} > 0.05$) was fairly similar to that of females ($9.566 (\pm 3.432) \text{g/dl}$).

DISTRIBUTION OF HAEMOGLOBIN WITH RESPECT TO PARASITE DENSITY AND BASED ON THE VARIOUS AGE GROUPS

Based on observation, anaemia was mild. Mild anaemia subjects had lower haemoglobin levels than non-anaemic subjects. The mean haemoglobin levels for mild-anaemic subjects was as follows based on the various age ranges: $7.6 (\pm 2.2)\text{g/dl}$ in <15 years subjects; $8.1 (\pm 1.8) \text{g/dl}$ in 15 to 30 years subjects; $9.3(\pm 2.0)\text{g/dl}$ in 30 to 45 year old subjects and $8.1 (\pm 2.0) \text{g/dl}$ in > 45 years subjects. It was

observed that the anaemia status decreased from <15 years to 30 to 45 years old subjects and then increased in >45 years old subjects. In the other hand, it was observed that, parasitaemia decreased from <15 years old subjects. However, in the non-anaemic subjects, it was observed that haemoglobin concentrations were fairly constant and normal, while the parasite density was

observed to be highest in <15 years subjects, 30 years and 30 to 45 years old subjects (Table 4.7).
higher in >45 years subjects and high in 15 to 4.7).

Table 4.7: Distribution of Haemoglobin with Respect to Parasite Density and Based on the Various Age Ranges

Groups	Mean haemoglobin (g/dl)				Mean parasite density/ul of blood			
	< 15	15-30	31-45	> 45	< 15	15-30	30-45	> 45
Anaemic subjects	7.6 (±2.2)	8.1 (±1.8)	9.3 (±1.5)	8.1 (±2.0)	1221.1 (±2315.9)	582 (±495.4)	533.3 (±231.6)	440 (±131.5)
Non-anaemic subjects	12.5(±0.5)	12.3(±0.9)	12.4(±1.2)	12.8(±1.7)	1156.7 (±1301.6)	417.8 (±125.1)	441.3 (±160.4)	533.3 (±243.3)

4.10 RELATIONSHIP BETWEEN PARASITAEMIA AND ANAEMIA STATUS

A majority of the subjects who were anaemic had parasite densities of ≤500 parasites/μl with about 90% of them presenting parasite densities of ≥320 to 640 parasites/μl of blood. Also, the majority of those who were non-anaemic had parasite densities ≤ 500 parasites/μl of blood (Table 4.8).

Table 4.8: Relationship between Parasitaemia and Anaemia Status

Parasite density categorization	Anaemia status		Total
	Anaemic	Non-anaemic	
≤ 500	33	28	61
501-1000	22	09	31
1001-1500	02	01	03
> 1500	03	02	05
Total	60	40	100

$\chi^2 = 2.495$ d.f = 3 P-Value = 0.697

DISTRIBUTION OF PARASITE DENSITY AND BLOOD CELL INDICES ACCORDING TO ANAEMIA STATUS

It was observed that, the mean (±SD) parasite density in the anaemic subjects was higher than in the non-anaemic subjects. The mean (±SD) haemoglobin concentration, mean cell volume, mean cell haemoglobin and red blood cell counts were higher in non-anaemic patients than in anaemic patients. The mean (±SD) total white blood cell counts was higher in the anaemic patients.

Based on the differential white blood cell counts, there was a fairly equal lymphocyte and monocyte counts in both the anaemic and non-anaemic patients, a fairly higher granulocyte (Neutrophil, Basophile Eosinophil) count was observed in the non-anaemic patients. Finally, a higher mean (±SD) platelets count was observed in anaemic patients (Table 4.9).

Table 4.9: Influence of Anaemia Status on Parasite Density and Blood Cell Indices

Parameters	Anaemic		Non-anaemic	
	N	Mean \pm SD	N	Mean \pm SD
Parasite density	60	758 (\pm 1353.6)	40	564 (\pm 554.8)
[Hb]	60	8.3 (\pm 1.9)	40	12.5 (\pm 1.2)
MCV	60	86.2 (\pm 15.4)	40	91.1 (\pm 10.4)
MCH	60	27.5 (\pm 4.6)	40	28.9 (\pm 4.0)
RBC	60	3.1 (\pm 0.8)	40	4.4 (\pm 0.6)
WBC	60	6.8 (\pm 4.5)	40	6.7 (\pm 0.6)
Lymphocytes	60	2.2 (\pm 1.9)	40	2.2 (\pm 1.2)
Monocytes	60	0.4 (\pm 0.4)	40	0.4 (\pm 0.2)
Granulocytes	60	4.0 (\pm 3.1)	40	4.1 (\pm 3.4)
Platelets	60	275.5 (\pm 123.1)	40	257.4 (\pm 85.9)

4.12 CORRELATION BETWEEN PARASITE DENSITY AND BLOOD CELL INDICES

After correlation analysis was performed based on the null (H_0) and alternative (H_a) hypothesis:

- H_0 : Presence of significant correlation between parasitaemia and blood cell indices.
- H_a : Absence of significant correlation between parasitaemia and blood cell indices.

It was observed that, there was a positive correlation between the parasite density and white blood cell count (P -value = 0.317). A positive correlation was also observed with red blood cell count (P -value = 0.05).

A negative correlation was observed with the

haemoglobin concentration, mean cell volume, and mean cell haemoglobin with a very significant correlation with haemoglobin concentration ($P = 0.397$).

There was a significant correlation with the mean cell volume and mean cell haemoglobin ($P = 0.131$ and 0.103) respectively. There was a positive correlation with the lymphocyte, monocyte and granulocyte counts (Eosinophil, Basophil and Nitrophils) ($P = 0.388$, 0.419 and 0.344) respectively.

There was no correlation between malaria parasite density and platelet count ($P > 0.5$). Thus from data in table 4.10, The H_1 Hypothesis was rejected.

Table 4.10: Relationships between Parasite Density and Blood Cell Indices

Parameters	n	Mean \pm SD	ΣX	ΣX^2	ΣX_1X_2	R	r^2	Sr	tcal	d.f	ttab	P-value
Parasite density	100	680.4(\pm 1105.6)	68040	167307200	-	-	-	-	-	-	-	-
WBC	100	6.8(\pm 4.1)	678	6284.96	496307.6	0.08	0.006	0.1	0.769	98	2.000	P=0.429 P>0.025
RBC	100	3.6(\pm 0.9)	360.65	1396.3641	265577.2	-0.19	0.04	1.891	1.891	98	2.000	P=0.058 P>0.025
[Hb]	100	9.9.(\pm 2.7)	996.2	10628.34	665074	-0.04	0.002	-0.432	-0.432	98	-2.000	P=0.693 P>0.025
MCV	100	88.2(\pm 13.8)	8816.6	796076.76	5765256	-0.16	0.03	-1.553	-1.553	98	-2.000	P=0.112 P>0.025
MCH	100	28.1(\pm 4.4)	2808.2	80812.4	1829872	-0.17	0.02	-1.669	-1.669	98	-2.000	P=0.091 P>0.025
Lymphocytes	100	2.2(\pm 1.7)	222	765.86	159718	0.05	0.001	0.473	0.473	98	2.000	P=0.621 P>0.025
Monocytes	100	0.4(\pm 0.3)	41.2	27.68	29268	0.03	0.001	0.340	0.340	98	2.000	P=0.767 P>0.025
Granulocytes	100	6.8.4(\pm 4.1)	404.6	2655.4	298456	0.07	0.004	0.655	0.655	98	2.000	P=0.489 P>0.025
Platelets	100	23.2(\pm 290.1)	2324.2	8385465	15664420	0.4	0.2	4.413	4.418	98	2.000	P=0.0001 P>0.025

$P < 0.025 \Rightarrow$ Reject H_1 : $P > 0.025 \Rightarrow$ Accept H_0

DISCUSSION AND CONCLUSION

There was a generally fair low parasite count observed ranging from ≤ 500 to >1500 parasites per micro litre of blood. This was similar to the findings reported from Yaounde, on low parasitaemia in malaria positive patients at the University Centre Hospital (CHU) [7] and to that reported from Buea on asymptomatic malaria and haemoglobin levels in primary school pupils [18]. The highest parasite rate for the study was observed in subjects of the age group 31 to 45 years made up of 31%, although there was no significant variation in parasite rates in the different age groups, but a significant variation was observed in the parasite density. It was observed that the age group <15 years had the highest frequency, harbouring parasite loads of >1500 parasites per micro litre of blood with the age groups 31 to 45 years and >45 years with a frequency of zero. This could be as a result of low immunity to malaria by the <15 years old children and a stronger immunity in the older age groups.

This study was carried from February to March, a period of transition from the dry to the rainy season in Limbe, though with scanty rainfall, high humidity and high temperatures. During this period, the climatic conditions provide habitats for breeding and the temperature favours rapid growth and development of the *Anopheles* mosquito vectors. The topography of Limbe presents a hilly zone, and lowland with the

presence of the sea and many forest and bushy areas, makes drainage difficult at the road sides, around settlements and houses providing many breeding sites for the vector. These factors probably could be the cause of the high transmission rate in the town. Based on the occurrence and the density of parasite by sex it was observed that the female sex was more exposed than the males. Since the Limbe Regional Hospital provides services to patients from other villages around Limbe, and many women there are mainly farmers, the high occurrence of parasitaemia in women could be as a result of their work exposure to the forest and bush farm lands which are sites which promote the vector breeding.

Based on the haemoglobin levels recorded in patients, the prevalence of anaemia was 60% and anaemia was mild in most cases, though a few cases presented with severe anaemia mainly children and pregnant women. A previous study carried out in Yaounde (CHU) reported a prevalence of 57% with mild anaemia [7]. Another previous study carried out in Buea District reported a prevalence of 78% for anaemia in hospitalized children with acute malaria (unpublished data). In CHU, the mean haemoglobin level was 10.58g/dl and in the Buea District, it was 7.8g/dl. In this study a mean haemoglobin concentration of 9.9g/dl was observed; all indicating mild anaemia.

Anaemia in acute falciparum malaria has been reported to be caused by increased

destruction of both parasitised and non-parasitized erythrocytes and decreased erythropoiesis. In addition, RBC could be increased due to reticulocytosis following haemolysis, whereas the MCH and MCV could be lowered due to an increased ratio of red cell volume in relation to the haemoglobin content (Table 4.10).

As far as white blood cells and platelets are concerned, little was known about the changes that occur in malaria positive patients. A measurement of WBC and platelets of 230 healthy children from a Tanzanian community, 1369 children admitted to hospital with symptomatic malaria and 1461 children with other medical conditions. Children with malaria had higher WBC compared with the community controls and leucocytosis was strongly associated with the younger age, deep breathing anaemia and death. The WBC was not associated with a positive blood culture [19].

In this study, fairly low lymphocyte and monocyte counts were independently associated with morbidity and a fairly high granulocyte count was observed. A platelet count of less than $150 \times 10^3/\text{mm}^3$ of blood was found in 13% of the subjects and was associated with age and parasite density.

Based on observation and basic assumption, the majority of patients and parents of children that took part in this study, could be placed among those who earn above

average income. This is because most of them are civil servants, business men and farmers. This could account for the general well being of the subjects. Furthermore, many patients are familiar with the signs and symptoms of malaria, its treatment, prevention and control.

The introduction of serological methods of testing malaria such as the para sight F test, and the optimal assay for *P. falciparum* now enables patients to get quick results and promptly attack the disease before it reaches the chronic level [20]. Also the use of the arthemisinin combination therapy and impregnated mosquito bed nets have brought in much improvement in the prevention, treatment and control of malaria.

To conclude, from statistical correlation analysis, the following was observed:

A negative correlation between the parasite density and the age of the subjects; the parasite density and the haemoglobin concentration; mean corpuscular volume; and the mean cell haemoglobin.

A positive correlation between the parasite density and the WBC; RBC and the white blood cell differential count.

In addition, it was observed that no correlation exists between the parasite density and the platelets count and the parasite density did not seem to have any significant effect on the blood cell counts.

CONCLUSION

Heavy parasite loads in the body destroy the red blood cells, reticulocytosis and lead to anaemia. The number of eosinophils increased with an increase in the parasite load.

Hence, malaria and anaemia are two important problems facing patients at the Limbe Provincial Hospital especially children <15 years of age. Females were also observed to have a high prevalence of *Plasmodium falciparum* infection especially those pregnant than men, and subjects who presented with anaemia generally had higher parasite load compared to their non-anaemic counterparts.

All anaemic patients should under go check up for malaria parasites and treated promptly when positive.

Control Programme should be organised that would regularly check up children and pregnant women attending the Hospital against malaria to avoid chronic and severe infections which may lead to anaemia.

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