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Seropositivity of Malaria Parasite and Siphilis among Prospective Blood Donors in Federal Medical Centre Owerri, Nigeria

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ABSTRACT: Several infections have been shown to be associated with blood transmission. Previous studies in many countries of the world (including African Countries) revealED high incidences of blood borne infections such as malaria parasites and siphilis among apparently healthy blood donors. Few of such studies, however investigated the role of the duo in the transmission of infectious diseases via blood transmission in Africa and the author is not aware of such studies in Owerri, South Eastean Nigeria. The present study was therefore undertaken to assess the sero-prevalence rate of malaria parasites and siphilis among one hundred and twenty prospective blood donors seen between the months of January and March,2008 at the Federal Medical Centre Owerri. The subjects were randomly selected from donors who visited the hospital within the period for the purpose of donating blood and screened for seropositivity to malaria parasite and siphylis using anti-malaria monoclonal antibody rapid diagnostic test (RPD) method and Veneral Disease Research Laboratories test (VDRL) kit respectively. Male donors had a higher sero-prevalence rate of malaria parasite (54.16% out of the total malaria prevalence rate of 61.66%) than the females, the sex-related prevalence being statistically different ($P<0.05$). Similarly, more males had siphilis (3.34% out of the overall sero-prevalence rate of 4.16% for siphilis) than females ($P<0.05$). Younger donors (16-45years) had higher prevalence rate (61.66%) for both infections than older (>46 years) ones. ($P<0.05$). There were more single infections with malaria parasite (61.66%) than siphilis (4.16%, $P<0.05$). A weak negative correlation existed between infection with malaria parasite and those of siphilis. (correlation coefficient: 0.167311). Level of parasitaemia as revealed by microscopic examination of Giemsa stained thick blood film was 11-100 malaria parasites per high power field (HPF) in majority of the donors while the mean malaria parasite density was 191+/-25.07malaria parasites /HPF.The study indicates a high risk of transmission of malaria parasite and low risk of transmission of siphilis to susceptible recepients (non-immune and immuno-compromised). Routine screening of blood donors for malaria parasites and siphilis is recommended in Federal Medical Centre Owerri inoder to forstall the transmission of the infections to lives we intend to save by blood transmission.

KEY WORDS: Steropositivity, Malaria parasites, Syphilis, Blood donors, Federal Medical Center, Owerri, Nigeria

INTRODUCTION

Blood, a fluid connective tissue which circulates all over the body through the arteries, veins, and capillaries provides a vehicle by which digested food materials, oxygen, carbon dioxide, nitrogenous wastes, hormones, vitamins among others are transported in the body (Harrison *et al,* 2004). It is composed of a pale yellow fluid called plasma in which are suspended the red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes). Plasma makes up about 55% of the total volume of blood and contains 92% of water. It also contains blood proteins, dissolved mineral slats, hormones, enzymes, and antigens. Plasma contains fibrinogen which is broken down to release energy, and antibodies which destroy bacteria and foreign bodies (Okolie,2004)

Prospective blood donors are people between the ages of 17-65 years who are ready to donate their blood for transfusion to patients that need them. Blood transfusion therapy is a form of treatment based on the use of blood and its products to save human life. It is a very essential part of curative and preventive medicine all over the world. Nutritional anaemias in paediatric cases of haemolytic anaemia, obstetric accident cases are conditions that place great premium in blood transfusion services. Among the criteria for recruiting prospective blood donors are the screening of their blood for the presence of HIV, malaria, microfilaria, syphilis, hepatitis B virus, etc. However, blood can be a dreadful vehicle for the transmission of some infectious agents such as *Treponema palladium*, *Plasmodium falciparum*, and other haemoparasites (Ukaejiofor, 2002).

Alaria is an infectious disease caused by malaria parasite, protozoa of the genus *Plasmodium.* In Nigeria, only three species of *Plasmodium*are known: *P. falciparum* (the dominant strain), *P. ovale*, and *P. Malariae*. It is transmitted from one person to another by female *Anopheles* mosquito. It is one of the commonest and widely spread diseases of the human race (Prescot *et al*, 2002). Malaria presents enormous health problems in Africa and it is possible that over 90% of the 200 million estimated malaria-infected people in the world are from Africa. Of the 110 million clinical cases of malaria reported annually, more than 90 million are in Africa, south of Sahara.

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An estimated 280 million people are carriers of malaria parasite in the region. According to a United Nation population report in 1990 (NIH,1995) malaria is the only disease today, apart from Acquired Immunodeficiency Syndrome (AIDS) that shows a significant rising tendency.

The fight against malaria remains one of the big challenges of public health (Okara *et al*, 2001). It is the most common cause of out patient visit to health facilities and is consistently reported as one of the five leading causes of death. Factors that contribute to the continuous persistence of malaria infection (*P. falciparum*) in Nigeria in spite of instituted control measures include: impoverished economy, spreading resistance to *P. falciparum*, poor environmental conditions, increased urbanization and development of epidemics following natural disasters and social unrest (Adomeh, 1998).

Syphilis, a venereal disease caused by a spiral bacterium, *Treponema palladium* can be transmitted congenitally or by sexual intercourse. It is a slender flexous helix, about 6-14μm long with 6-12 evenly spaced coils. The organism is actively noticed rotating steadily around the endoflagella, often attached to the cells by their tapered ends (Uwaezuoke *et al*, 2003). World Health Organization estimated that there are about 12 million cases of syphilis worldwide each year. Most of these occur in developing countries. (an estimated 3.5 million in sub-Saharan Africa, 1.3 million in Latin America and the Caribbean, 5.8 million in South west and South east Asia.) Infection rate are high among commercial sex hawkers, and long distances drivers. (WHO, 1999). According to the World Health Organization(1999), the prevalence of syphilis infection has increased markedly since 1985. In Nigeria, cases of mistreated and untreated syphilis have led to high rate of infertility. Other health problems associated with syphilis infection include premature delivery, still birth, prenatal death and congenital syphilis.

Transfusing blood infected with malaria parasite can cause malaria in recipients especially those without effective body immunity such as young children and pregnant women. Malaria parasite is associated with high morbidity and mortality rate of malaria infection, still birth, premature delivery, anaemia, placenta malaria, low birth weight, and rarely congenital malaria (Macleod, 1998). On the other hand, *T. palladium*, when transfused in blood can cause syphilis with high mortality rate. Syphilitic pregnant mothers can easily infect their unborn babies with syphilis because treponemes in the blood pass through the placenta, a situation which may lead to abortion, premature delivery, still birth, prenatal death, and congenital syphilis (Uwaezuoke *et al*, 2003).

Several infectious diseases have been associated with transfussion of blood. In Bangladesh, Kharn and Narzul (2000) had reported prevalence rates of 3.5% and 2.3% for hepatitis B and siphilis respectively. Evaluation of HIV infection, hepatitis B and siphilis among blood donors within the period 1986-1990 had been carried out in Haiti. (Larco *et al,* 1991). In India, Gupta and Kaura (2002) reported prevalent rates of 0.095% and 0.086% for siphilis and immuno-deficiency virus respectively. In Africa, the works of Matee *et al* (1999), Adjei *et al* (2003), Okochaa et al(2005), Mbanugo and Emenalo (2004), Umeanaeto *et al* (2006) bore eloquent testimonies of the role of blood transfussion in transmission of pathogens.

According to Matee *et al* (1999), the overall frequency of anti-HIV, anti-Hepatitis C Virus, anti-Hepatitis B surface antigens (HbsAg), Human T-lymphotropic Virustype 1 (anti-HTLV-!) and siphilis antibodies among 300 blood donors in Tanzania, East Africa were found to be 8.8%, 22%, 11%, 0%, and 10.9% respectively. In Ghana, West Africa, Adjei *et al* (2003) found a sero-prevalence rate of 7.5% for siphylis. Similarly, Okochaa *et al* (2005) and Umeanaeto *et al* (2006) reported the prevalence rates of 30.2% and 46% for malaria parasites among blood donors at Nnamdi Azikiwe Teaching Hospital Nnewi. The only published work on transfussion-associated infections in Owerri was that of Mbanugo and Emenalo (2004) who observed that 77.4% of 387 blood donors had malaria parasites.

One of these previous studies investigated the sero-prevalence rate of malaria parasites and siphilis among blood donors. Thus, it has become imperative to embark on the present study which is aimed at evaluation of sero-positiviy of malaria parasites and siphilis among blood donors in Federal Medical Center Owerri, South Eastern Nigeria. The import of the study cannot be overemphasized when one considers the unacceptable high levels of morbidity and mortality associated with malaria parasites and the risk of contacting the duo by immunocompromised recipients of blood products. In most Nigerian hospitals, screening test for syphilis and malaria parasite is often neglected. This study attempts to investigate the prevalence of these life-threatening infectious diseases among prospective blood donors in Owerri.

MATERIALS AND METHODS

Study Area

This research work was carried out at the Federal Medical Centre Owerri, Imo State. Federal Medical Centre is located in Owerri Municipality. The inhabitants of the area are mostly civil servants, traders, and students of the tertiary institutions with varying family backgrounds, life styles and habits.

Study Population

The study population consisted of adult prospective donors who came to donate blood at the Haematology / Blood Group Serology Department of the Federal Medical Centre, Owerri. Ethical clearance was obtained from the hospital Ethical Committee. Using a systemic random sampling, only 120 subjects were selected from all prospective blood donors within the period of study. Their ages ranged between 17 65 years (109 males and 11 females). Exclusion criteria were lactating mothers, menstruating females and pregnant women. Subjects who had skin rashes, purpura and those who had undergone surgery or accident in the last three months were also excluded. Inclusion criteria were subjects who were physically fit.

Collection of Samples

Using a 5ml syringe, 4ml of blood samples were collected from each subject through venipuncture. 2ml of the sample were placed in plain bottles without anticoagulants to obtain serum for syphilis test. The remaining 2ml were placed in EDTA bottles for the detection of malaria parasites.

Serological Methods for Syphilis and Malaria Parasite Detection

A commercially prepared ultra rapid test strip (an immunoassay method) manufactured by Acon Laboratories Inc. USA (Lot No syp 7090010) was used for detecting *T. palladium* antibody in the serum. Producer's standard operating procedures (PSOP) were strictly adhered to. Principle of the quantitative membrane base immunoassay method is as follows: recombinant *T. palladium* antigen is immobile in the test region of the test strip. When the test pad is dipped into the specimen, antigen-antibody reaction occurs. A purple coloured line in the test region indicates a positive result while the control line region remains a negative result.

The proceedure were as follows. Test strip pad was removed from the sealed pouch with the aid of a Pasteur pipette. Two drops of serum were placed on the specimen pad of the strip and 1 drop of clearing buffer was added. The result was read after 10 minutes. Two colour lines (one in control and the other in the test region) indicated positive result, while one coloured line in control region alone indicated negative result.

Detection of malaria parasites using Rapid Diagnosis Test (RDT)-Dipstick Testing

The RDT Kit manufactured by Orchid Biomedical System Inc. India (Lot NO PF51120338) was used to establish the presence of malaria parasite antigen in the blood. The test is based on the principle of antigen-antibody reaction. Malaria antigen from a lyzed blood sample which is reacted with anti-malaria monoclonal antibody conjugated to colloidal gold complex migrated along the nitro cellulose membrane where it is bound by a line of specific monoclonal antibody, producing a pink line in the test region.

The procedure were as follows. Blood sample was collected with a Pasteur pipette. Two drops of the blood sample was blotted into the sample pad on the test strip below the arrows. One drop of the clearing buffer was added. The result was read after 15 minutes.

Two pinkish lines each on test and control regions of the test strip indicated a positive result while one line on the control region indicated a negative result.

Estimation of malaria parasite density using thick film stained with giemsa staining technique

Giemsa stain is based on the principle of the reaction of eosin Y (acidic anionic dye) which stains the basic component of the blood cells and Azure B (methylene blue derived dye) which stain the acidic component of the blood cells. The stain is diluted with buffered water of 7.2 pH to enhance ionization. Thick blood smears were made on clean grease-free microscope slides and allowed to air dry. The smears were stained with 3% dilution of Giemsa stain for 30 minutes. The slides were respectively passed over slow running clean water and allowed to air dry on a staining rack. The back of the slides were cleaned and placed in a draining rack to air dry.

The preparations were examined under the microscope using x100 objective lens with oil immersion. Malaria parasite stained brown-black with different morphology depending on the stage of the parasite.

Parasite load was counted thus:

+	=	1-10 per High Power Fields
++	=	11-100 per High Power Fields
+++	=	101-1000 per every High Power Field.

Chi-Square goodness of fit and pearson's correlation coefficient (r) tests were used to analyze the data.

RESULTS

In the study, data on the sero-prevalence of antibodies to siphilis and antigens to anti-malaria monoclonal antibody among 120 prospective male and female blood donors, aged between 16-65 years in Federal Medical Centre Owerri, Nigeria were provided (tables 1 and 2; figures1-3). The prevalence rate of antibodies to siphylis was 4.17% as against the higher prevalence (61.66%) recorded for malaria parasite antigens ; the difference being statistically significant (P<0.05). It was observed that majority of the prospective donors (64.17%) were males within the age brackets of 16-25 and 26-35 years (table 1. figure 1). The mean age of donors was 30.59 +-10.37. Older donors (>45 years) were relatively fewer (11.67%) than the younger ones and this variation was statistically significant (X^2 Cal.=0.967916; X^2 Critical (Tabulated), d.f:1, P<0.05=5.9969).

The prevalence rate for both organisms showed a significant difference with sex of the donors, the males exhibiting a much higher prevalence rate (54.16% for malaria parasites and 3.34% for siphilis) than the females (7.50% for malaria parasites and 0.83% for siphilis ,table 1). Rate of co-infection with both organisms was significantly lower (4.16%) than single infections (61.66% for malaria parasites and 4.14% for siphilis; table 2, P<0.05). There was a weak correlation between infection with malaria parasite and siphilis (Correlation coefficient=0.167311). Level of parasitaemia was 11-100 malaria parasites per high power field (HPF) in majority of the donors and the mean parasite density was 191.87 +-25.07 malaria parasites per HPF. (figure 2). Males had higher malaria parasite densities than females (figure 3).

Table 2: Co-infection with malaria parasite and siphylis among prospective blood donors

AGE	SEX	FREQUENCY	PERCENTAGE
16-25	Males	2	1.66
26-35	Males	2	1.66
36-45	Females	1	0.83
Total		**5**	**4.16**

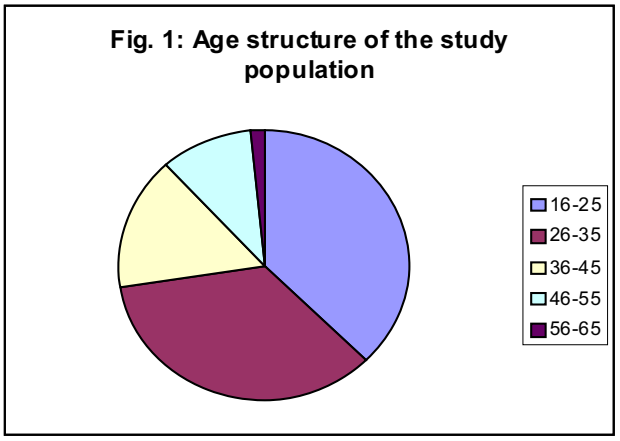
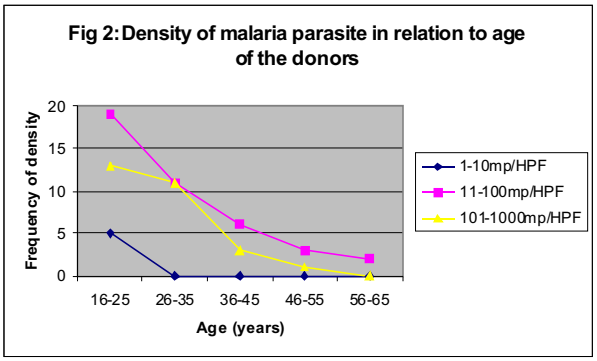
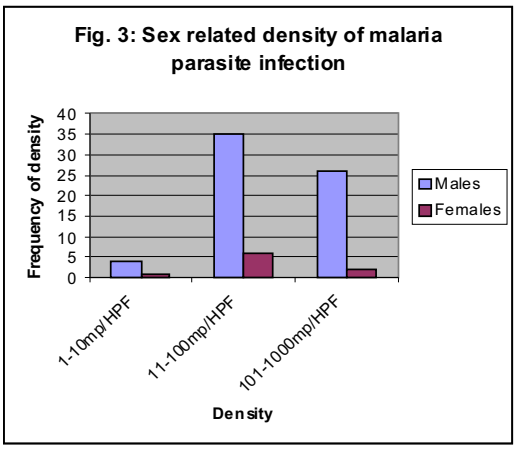


Table 1: Age and sex prevalence of malaria parasite antigens and antibodies to siphylis in the study area.

Age (years)	Males			Females			Total	
	No. Examined(% frequency)	No. Infected with siphylis (%)	No. Nifected with m.p (%)	No. Examined (% frequency)	No infected with siphilis(%)	No. Infected with m.p (%)	**No. Examined**	**No. Infected (%)**
16-25	39 (32.5)	2 (1.67)	27 (22.50)	6 (5.0)	0	5 (4.16)	**45**	**34 (28.32)**
26-35	38 (31.67)	2 (1.67)	21 (17.50)	4 (3.38)	0	3 (2.50)	**42**	**26 (21.66)**
36-45	18 (15.0)	0	12 (10.0)	1 (0.83)	1 (0.83)	1 (0.83)	**19**	**14 (11.67)**
46-55	12 (10.0)	0	4 (3.33)	0	0	0	**12**	**4 (3.33)**
56-65	2(1.67)	0	1 (0.83)	0	0	0	**2**	**1 (0.83)**
Total	**109 (90.84)**	**4 (3.34)**	**65 (54.16)**	**11 (9.16)**	**1 (0.83)**	**9 (7.50)**	**120**	**79 (65.83)**





DISCUSSION

The observation that majority of the donors (64.17%) fall within the age bracket of 16-35years and were mostly males was similar to those of Okocha *et al* (2005) in Nnewi, Nigeria and Adjeja *et al* (2003) in Accra, Ghana,. The predorminance of male donors within that age range could be attributable to the high level of commercial donors in the study population as was previosly reported in Accra, (Adewuyi,2001). On the other hand, the females, apart from being culturally inhibited as far as commercial blood donation is concerned are often scared of donating blood because of the awareness that they often loose blood during the normal monthly physiological process of menstruation; hence low number of female donors were observed in the study.

The rate of sero-positivity for malaria parasites (61.66%) seen in the study is lower than those of Mbanugo and Emenalo (2004) in Owerri (77.4%) and higher than those of Umeanaeto et al (2006) in Nnewi (46%). The present rate could be very worrisome in view of the fact that the would-be blood receipients who are mostly pregnant women and children are highly vulnerable to malaria (Weir and Sewart,1997). The implication of the present finding is that about 2 out of every 3 blood transfusions in the study area carries the risk of transmitting malaria parasites to the receipients. Of a still greater concern is that majority of the donors who were screened had moderate level of parasitaemia and some had concormitant infections with siphilis and malaria parasites. Again, immigrants from outside malarious regions run a risk of being infected with malaria parasites or/ and siphilis when transfussed with the blood from the study area. Although speciation of malaria parasite was not carried out in the present study, previous studies in Owerri had shown that *P. falciparum* is the predorminant species in Owerri. (Okolie, 2006; Mbanugo and Emenalo, 2004).

The results indicated that malaria parasites are prevalent among healthy donors in Owerri. Since transfusion therapy is an indispensable form of treatment and paucity of blood donors exists in Nigeria, the process of donor deferral usually carried out after results of serolgical testing had prooved positive in United states and other non-malaria endemic zones (Mungai et al, 1999) cannot be a practicable alternative in the study area (a developing country). Compulsory screening of all pints for malaria and the result of this post-donor screening (either positive or negative) indicated on the bag is recommended. Patients who must receive malaria-positve pints should be given a curative regimen of anti-malarials especially if they are vulnerable to malaria attack. Administration of curative regimen of anti-malarials prophylactically to all patients transfussed with blood may as well be considered a safer and desireable alternative (Snyder and Dodd, 2001). If this later option is preferable , due cognisance should be taken with pregnant women who receive normal prophylactic intermittent treatment (PIT) to avoid excessive use of anti-malarials which cold possibly increase the risk of emergency of resistant strains of the parasites.

The sero-prevalence rate of siphilis observed in the study is comparatively lower than those of some previous researchers. For example, prevalence rates of 7.5%,and 12.7% were reported in Ghana (Adjei *et al* ,2003) and Tanzania (Matee *et al,* 1999) respectively. Neverthless, the present figure is relativey higher than those of Gupta and Kaur (2002) and Khan and Nazrul (2000) who found prevalence rates of 0.095% in India and 3.5% in Bangladesh respectively. It is suggested that donors who tested positive for siphilis should be excluded from blood donation or be treated and certified to be free from the infection before they are bleed. Fortunately, the sero-prevalence rate for siphilis was found to very low in comparison to that of malaria parasites. Subsequently, pints to be lost due to sero-positivity to siphilis is at a abysmial level. Since males were found to be significantly more infected with both organisms, there is need to exercise caution in transfussing blood collected from male donors. It is also suggested that the sex of the donors should be clearly indicated on the blood bags.

In developed countries of the world, transmission of infections via blood transfusion has been brought to low ebb due to low improved donor selection processes, compulsory serological screening of donors for blood-borne pathogens as well as a drift from transfusion of fresh blood components to transfusion of refrigerated products (NIH,1995; Vos,1998; Adjei, 2003). Developing countries such as Nigeria have not been able to fully implement the above procedures aimed at making blood donation safe as far as transfusion-transmitted infections are concerned. The risk of transmission of infections through blood donations is compounded by the increased demand for blood transfusion occasioned by high incidence of anaemia, malnutrition and surgical emergencies (Mollison *et al*,1993). Hence studies such as the present one has become inevitable in order to proffer solutions to the nagging issue of disease transmission to recipients of blood products particularly immuno-compromised and/or immuno-suppressed patients. But the extent to which this laudable objective can be achieved will depend on the availability of further complementary studies which will seek to address issues such as 1. What is the level of blood-borne infections among asymptomatic blood donors in Nigeria? 2. Whether sero-positivity to these diseases actually provide sufficient grounds for a risk of transmission to patients. 3. if such risks exist, how will it develop in the future and what measures may be kept in place by health authorities in order to tackle the problem?. Nevertheless, the present study, although limited by the small sample size had lend credence to the urgent need for compulsory and universal serological screening of blood donors for transfusion-transmitted infections.

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