IMPACT OF *PLASMODIUM FALCIPARUM* ON SOME HAEMATOLOGICAL PARAMETERS OF PREGNANT WOMEN ATTENDING ANTENATAL CARE IN UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL (UPTH), RIVERS STATE, NIGERIA.

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ABSTRACT

A study of the Impact of *Plasmodium falciparium* on some Haemoglobin levels of pregnant women attending antenatal care in UPTH was carried out between July and September, 2015 in Rives state. Blood samples were collected and examined for malaria parasite using thin and thick films stained with giemsa and Haemoglobin level (Hb) was estimated using the cyanomethaemoglobin method. Of the 200 sampled, 105 (52.5%) were infected with malaria parasite. More infection 65(72.2%) was recorded among primigravidae than in the multigravidae 40(36.4%) ;these differences were statistically significant ($P < 0.05$, df =1, X^2cal= 26.2, X^2tab= 3.851). Highest prevalence was recorded among those in their first trimester 55(61%) and lowest among those in the third trimester 20(44%), these differences were also statistically significant ($P < 0.05$, df =3, X^2cal= 5.3, X^2tab= 5.991). Pregnant women with AA genotype recorded the highest prevalence of 77.3% while AS had 22.2%; this difference was significant at 5% level ($P < 0.05$, df =3, X^2cal= 59.1, X^2tab= 5.991). No SS individual was encountered. 53 (72.60%) of primigravid mothers, 20 (27.40%) of the multigravidae recorded heamoglobin levels lower than the World Health Organization benchmark (11g/dl). Anaemia was to be dependent on infection status, pregnancy stage and parity ($P<0.05$). The effects of malaria and its clinical features on the mother and foetus was again re-stressed with emphasis on availability, affordability and sustainability of malaria control efforts.

KEYWORDS: Malaria, anaemia, genotype, blood group,Portharcourt.

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INTRODUCTION

Malaria infection during pregnancy is a major public health problem in the tropics and subtropics, affecting approximately 24 million pregnant women (Skeketee, *et al*, 2001). The burden of malaria in pregnancy is caused chiefly by *Plasmodium falciparum* the most virulent of the *Plasmodium* parasites, especially in the sub-Saharan Africa (Wedie, 2002). Malaria infection during pregnancy can have adverse effects on both the mother and the foetus. The effects on maternal haematological parameters, particularly haemoglobin concentration (HbC), ABO blood group and haemoglobin genotype on pregnancy outcome have not been adequately evaluated in many malarious areas of the sub-Saharan Africa. It is well established that anaemia is the most common consequence of *P. falciparum* malaria infection and it is generally accepted that in malaria – endemic areas, *P, falciparum* is a major contributor to anaemia in pregnancy (Menendez, *et al*, 2000). It has been suggested that the ABO system has evolved under a positive selection pressure in both humans and other primates (O'hlligin, *et al,* 1997). The implication is that certain ABO groups appear to provide a selective vulnerability to individuals possessing a particular ABO blood group. Whether ABO system 'influences susceptibility of pregnant women to malaria is yet to be fully ascertained (Mbanefo, *et al*, 2009).

Epidemiological and clinical studies have indicated that malaria susceptibility and severity are influenced by haemoglobin genotype with haemoglobin HbAS individuals having a selective advantage in malarial environments (Colombo, *et al*, 1985). Thus, the high frequency of HbAS in human populations has been attributed to the decreased malarial morbidity and mortality experienced by HbAS heterozygote individuals (Eteng, 2002; Williams, *et al*, 2005).However, the extent of the influence of haemoglobin genotype on the susceptibility and severity of malaria in pregnancy is yet to be clearly established (Mbanefo, *et al*, 2009).

Anaemia in pregnancy is an important public health concern in developing countries and it is usually more pronounced in primigravidae than in multigravidae (Flemming, 1989; Jackson, *et al*, 1991; Matteelli, *et al* 1994 and Shulman, *et al,*1996). The predisposing factors of malaria in pregnancy are multifactorial and it includes poor nutrition, malaria parasite, haemoglobinopathies, advanced HIV infections and infection with other parasites (Shulman,1999; Mahomed, 2000 and Brabin, *et al*, 1997). Though most of the factors are preventable, the overall prevalence of anaemia in pregnancy continues to be a common clinical problem in the third world (Harrison, 1988).

Anaemia has been reported to contribute significantly to both maternal and foetal morbidity (Lassey, *et al*, 1999) and it is also found to have a serious effect on neonatal birth weight. (Aribodor, *et al,* 2007). Different studies have confirmed that majority of pregnant women in developing countries are anaemic (Singh, *et al*, 1999; Matteelli, *et al*, 1994). Pregnant women especially the primigravidae, represent the

most important risk group of malaria among the adult population.

The influence of ABO blood group, haemoglobin concentration and haemoglobin genotype on the susceptibility of pregnant women to malaria has not been previously studied in Port Harcourt. This study therefore seeks to investigate malaria infection and its effects on haemoglobin concentration of pregnant women attending antenatal clinic in UPTH.

MATERIALS AND METHODS

STUDY AREA

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, a government tertiary health care institution with a total of 500 bed spaces and outpatients clinics. Port Harcourt is a cosmopolitan city with a population of about 2 million inhabitants (NPC, 2006). The geographical location is latitude 4°31'-5°31' and longitude 6°30'-7°21'. It is the state capital of Rivers State and Nigeria's second largest commercial and industrial centre and has the second busiest seaport in Nigeria. Thus, apart from the indigenes, there are various ethnic groups living in Port Harcourt.

The subjects therefore, represent subgroups of Nigerian pregnant women. The majority of the inhabitants are civil servants, traders, students and other professionals.

STUDY POPULATION

The study population was drawn from both primigravid and multigravid women, attending antenatal clinic at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt. Systemic sampling technique was used to select 200 consented pregnant women out of the registered pregnant women who were on antenatal visits. The study lasted for three months between July to September, 2015 on every antenatal visit day. They were all out patients, residing permanently in their respective homes within Port Harcourt.

SAMPLE COLLECTION

Blood samples were obtained from the pregnant women by venipuncture. A tourniquette was tied around the upper arm in order to make the veins prominent as well as increase blood pressure in the vein. The area from where the needle was introduced into the body was cleaned thoroughly with a spirit swab. The needle was then inserted into the vein and 5ml of blood drawn into the syringe. The tourniquette was loosened before the needle was pulled out from the vein; the blood was transferred into a sterile EDTA (Ethylenediamine Tetra Acetic Acid) container, mixed thoroughly to avoid clotting and then labeled.

PARASITOLOGICAL TECHNIQUES

Both thick and thin blood films were made for the detection of malaria parasites and identification of the *Plasmodium species* present.

STAINING OF THE BLOOD FILMS

10% Giemsa stain was used to stain the blood films. Prior to that, the thin blood film was fixed using methanol for 2 minutes. The diluted stain which was diluted by measuring 50ml of buffered water and adding 1.5ml Giemsa stain and it was mixed gently, was then placed on the slide until it covered the two blood films. This was allowed to stand for 10 minutes. After that, the stain was washed off the slide using clean water. The back of each slide was cleaned with cotton wool and placed in a draining rack for the preparation to dry (Cheesbrough 1998).

Examination of the Blood Films

Both blood films were examined microscopically using 100x oil immersion objective lens. The thick blood film was examined first in order to detect the presence of malaria parasite. This is followed by the examination of the thin blood film for identification of the *Plasmodium species* present according to (Cheesbrough 1998).

HAEMOGLOBIN (HB) ESTIMATION

This was done using cyanmethaemoglobin method (Baker, *et al*, 1995). Blood samples in EDTA were mixed with blood mixers. Four mililitre of Drabkin's solution were dispensed into a 75×10mm test tube, using volumetric pipette. 20µL of blood was dispensed into the same tube using haemoglobin micropipette.The contents of the tube were mixed and allowed to stand for 10mins at room temperature. The spectrophotometer was blanked with drabkin's solution. The test and standard were then read and the result calculated thus;

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard} = \text{Haemoglobin in g/dl.}$$

The World Health Organization benchmark for pregnant women is 11g/dl (Cheesbrough, 1998).

DATA ANALYSIS

Data obtained were analyzed using chi-square (X^2) to determine the significance of the observed differences in the study population.

RESULTS

Of the 200 pregnant women whose blood samples were examined, 105 (52.5%) had malaria parasites while 95 (47.5%) tested negative for malaria (table 1). All identified parasites were of the *Plasmodium falciparum* species.

TABLE 1: PREVALENCE OF MALARIA PARASITE AMONG PREGNANT WOMEN ATTENDING CLINIC BY AGE (IN YEARS).

Age	No Examined	No Infected (%)	(%)
<19	4	1(25.0)	3(75.0)
20 – 24	31	20(64.5)	11(35.5)
25 – 29	80	41(51.3)	39(48.8)
30 – 34	50	28(56.0)	22(44.0)
35 – 39	24	10(41.7)	14(58.3)
>40	11	5(45.5)	1(54.6)
Total	**200**	**105(52.5%)**	**95(47.5%)**

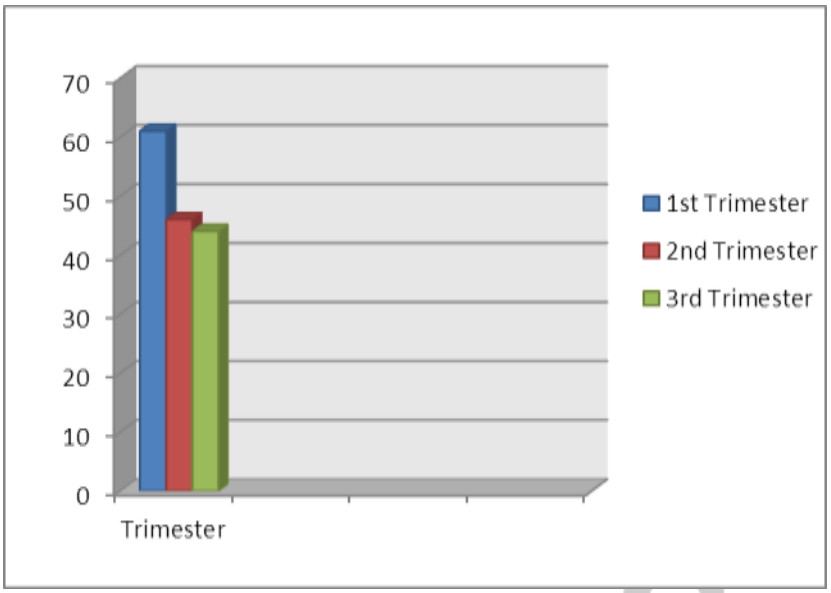
($P < 0.05$, d.f =5, X^2cal= 4.5, X^2tab= 11.07).

TABLE 2: PREVALENCE OF MALARIA PARASITE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC ACCORDING TO NUMBER OF PREGNANCIES.

Gravids	No Examined	No Infected	%Infection
Primigravidae	90	65	72.2
Multigravidae	110	40	36.4
Total	**200**	**196**	**52.5**

($P < 0.05$, df =1, X^2cal= 26.2, X^2tab= 3.841)

FIG.1 PREVALENCE OF MALARIA PARASITE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC BY TRIMESTER.



($P < 0.05$, df =2, X^2cal= 5.4, X^2tab= 5.991).

% Infection

FIG 2: PREVALENCE OF MALARIA PARASITE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC BY GENOTYPE.

($P < 0.05$, df =2, X^2cal= 59.1, X^2tab= 5.991).

TABLE 3: COMPARISON OF HAEMOGLOBIN LEVELS (g/dl) OF MALARIA PARASITE POSITIVE AND NEGATIVE CASES AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC BY GRAVID.

Haemoglobin	Primigravidae (%)	Multigravidae (%)	Total(%) Level g/dl
9.0 – 10.9			
Malaria +ve	53(72.60)	20(27.40)	73(36.5)
Malaria –ve	12(70.60)	5(29.41)	17(8.5)
11.0 – 12.9			
Malaria +ve	8(40.00)	12(60.0)	20(10.0)
Malaria –ve	5(10.64)	42(89.40)	47(23.5)
13.0 – 14.9			
Malaria +ve	2(16.67)	10(83.33)	12(6.0)
Malaria –ve	1(3.23)	30(96.77)	31(15.5)
Total	**81**	**119**	**200**

TABLE 4: HAEMOGLOBIN LEVELS OBSERVED AMONG THE PREGNANT WOMEN ACCORDING TO MALARIA STATUS AND PREVALENCE RATE.

Haemoglobin Level g/dl	Positive cases (%)	Negative cases (%)	Total (%)
9.0 – 10.9	73(81.1)	17(8.9)	90(45)
11.0 –12.9	20(29.9)	47(70.2)	67(33.5)
13.0 –14.9	12(27.9)	31(72.1)	63(21.5)
Total	**105(52.2)**	**95(47.5)**	**200**

($P < 0.05$, df =2, X^2cal= 54.9, X^2tab= 5.991).

TABLE 5: PREVALENCE OF MALARIA PARASITE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC BY ABO BLOOD GROUPING.

Blood group	No Examined	No Infected(%)
A	50	20(40)
B	40	10(25)
AB	10	2(20)
O	100	73(73)
Total	**200**	**105(52.5)**

($P < 0.05$, df =3, X^3cal= 47.18, X^2tab= 7.815).

TABLE 6: PREVALENCE OF MALARIA PARASITE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC BY GENOTYPE.

Genotype	No Examined	No Infected	%Infection
AA	110	85	77.0
AS	90	20	22.2
SS	0	0	0.0
Total	**200**	**105**	**52.5**

($P < 0.05$, df =2, X^2cal= 59.1, X^2tab= 5.991).

DISCUSSION

The prevalence rate of 52.5% found in this study is relatively high. There are results higher than the values obtained such as that of Akinboroye *et al*., (2008); Aribodor, *et al*., (2007) with 74%, 66.0%, respectively. However Mvondo *et al*., (1992) obtained lower values of 45.0%. The high prevalence rate recorded in this study area (Port Harcourt) may be due to the fact that the climate and vegetation are consistent with

tropical rainforest. The seasonal rainfall is higher and longer which gives rise to much surface water to support the breeding of vectors. The period of study which is the rainy season also may have contributed to the high infection rate. High rate of malaria transmission during rainy season has been reported by (Ekwunife *et al*., 2011 and Uneke *et al.,* 2006).

There was a higher prevalence of malaria among younger pregnant women between the ages of 25-29 years than older women. This agreed with the findings of Dicko *et al*., (2003) who opined that adolescents and young adult pregnant women were more susceptible to malaria than older pregnant women, because of continuous development of malaria immunity in older women.The Prevalence rate was higher among primigravidae pregnant women than the multigravidae. This agrees with the work of Stekette, *et al*., (2001) which suggested that multigravidae pregnant women acquire immunity from previous infections and may have also experienced physiological changes caused by pregnancy. Onwere *et al*., (2008) in Aba also found highest prevalence in the primigravidae. Cell-mediated immune responses to malaria antigens are more markedly suppressed first pregnancy than in subsequent ones (Brabin, 1996). High plasma corticosteroid levels may have an immunosuppressive effect on cell mediated immune responses. The multigravidae are presumably less affected because immunological memory from first pregnancy is retained. In first and second pregnancies women are especially vulnerable. McGregor (2004), identified the factors responsible for susceptibility of primigravidae to malaria as inhibition of type 1 cytokine responses (interferon, interleukins 2 and 12 and TNF).

Pregnant women in their first trimester had the highest prevalence than those in

second and third trimesters. This correlated with the work done by Brabin, (2003) in western Kenya that prevalence was highest at 13 – 16 weeks gestation (1st trimester), and found similar number of recoveries in both groups during the 2nd and 3rd trimesters. The loss of immunity in early pregnancy was equivalent to an 11-fold decrease in the rate of recovery from infection. The recovery seen in the late pregnancy suggests that the women mount a satisfactory immune response to malaria infection, re-acquiring their pre-pregnancy immune status at about the time of delivery (Saute *et al*., 2002). The observation could also be as a result of constant intermittent preventive treatment (IPT) given to these women during antenatal care visit which usually commence during second trimester.

Pregnant women with AA genotype had the highest prevalence of malaria parasite than women with AS. No sickle cell (SS) pregnant women were encountered in the course of this study. Although studies that investigated the relationship between malaria and heamoglobin genotypes in pregnancy are not many, malaria has been shown to be consistently higher in individuals with AA genotype compared to those with AS (Eteng, 2002). Resistance to malaria infection has been found to be associated with certain genetic factors. The heamoglobin S is known to interfere with the growth and replication of *Plasmodium falciparum* (Akinboroye *et al*., 2008). People with AA genotype are more susceptible to malaria because their red blood cells are conducive for the growth and development of *Plasmodium falciparum* (Williams, *et al*., 2005).

About 73% of malaria positive primigravid mothers and 27% malaria positive multigravid mothers have heamoglobin levels below the World Health Organization benchmark for pregnant women (11g/dl). Even among the uninfected group, the primiparae still recorded a greater prevalence of low haemoglobin levels. The pregnant women especially the primiparae recorded relatively lower heamoglobin levels as opposed to the multigravidae. This study confirms that anaemia is more intrinsic feature common among primigravidae compared to multigravidae as also recorded by Nagaraj, (2003) and Mbanefo, *et al*., (2009). This is because malaria, a major cause of anaemia in pregnancy in endemic areas is known to be more severe among primigravidae (Miaffo, *et al*, 2004). This is an indication that primigravidae are more at risk of maternal death as a result of severe anaemia. This observation is attributable to the increased susceptibility to malaria and other infections during pregnancy, due to the suppression of the immune system to ensure the establishment and non-rejection of the foetus as a foreign allograft (Akanbi, *et al*, 2004). Reasons for this might also be that most of these women who are supposed to be on routine drugs i.e. folic acid, iron don't take their medications and malnutrition may also pay a part.

The prevalence of malaria among ABO blood groups pregnant women was high among the O group. The high prevalence number recorded among O blood group may be due to the fact that more people in this group were sampled. Findings from studies evaluating the relationship

between malaria and ABO blood group are contradictory (Uneke, 2007). Some reports however vary with this (Facer and Brown, 2009; Martin *et al.,* 2009; Montoya *et al*., 1994). However, the high infection rates observed among all blood groups suggest that they are all susceptible to malaria. In fact, there is evidence that the ABO histo-blood group is not correlated to the incidence of malaria (Fischer and Boone, 1998; Ekwunife *et al*., 2011), but it has been linked as a co-receptor in parasite and vascular cytoadherence, with higher rosette rates among non-group O compared to group O erythrocytes (Cserti and Dzik , 2007).

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