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Prevalence and Types of Anaemia in Malaria Infected Pregnant Women Attending Antenatal Clinic in University of Calabar Teaching Hospital, Calabar, Nigeria

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Abstract
Anaemia is a very common condition during pregnancy. This is particularly so in developing countries where the level of intake of iron rich foods is low; malaria and other intestinal parasites are common. This study was conducted to determine the proportion of pregnant women with malaria parasitaemia and anaemia at booking and also to classify the anaemia using morphologic criteria. A total of 414 pregnant women attending University of Calabar Teaching Hospital in Nigeria antenatal clinic were used as the subjects while 250 age-matched non-pregnant women served as controls. Full blood count was carried out using full automatic cell counter while malaria parasite was determined by thick film method. The prevalence of anemia (61.1%) and malaria parasite (70.1%) was significantly ($p < 0.05$) higher among the pregnant women than in the non-pregnant women (38.3% and 60.8%). Anaemia and malaria parasite was found to be higher in the primigravidae than in the multigravidae. Primigravidae were more susceptible to the parasite especially *Plasmodium falciparum* with mean parasite density of 2112.50 ± 420.90 (parasite/µl) than the multigravidae with parasite density of 446.70 ± 296.90 (parasite/µl). Malaria parasite density increased significantly ($p>0.05$) with gestational age but anaemia was found to be slightly higher in the second trimester (65.8%) than in the first trimester (40.8%) and third trimester (64.5%). Normocytic normochromic anemia was found to be the most prevalent form of anemia in both pregnant and non-pregnant women while microcytic hypochromic anemia and macrocytic normochromic anemia were found to be significantly ($p<0.05$) higher among the pregnant women than in the non-pregnant women There was a negative correlation between haemglobin and malaria parsite density in both pregnant and non-pregnant women ($r = -0.1964, -0.5041$). The results showed that malaria infection caused by *P. falciparum* had serious effect on pregnant women living in the study area. Malaria in pregnancy should be recognized as a global priority in health care services and early malaria prophylaxis among pregnant women in this environment should be encouraged.
Keywords: Malaria, Pregnancy, Anaemia

1. Introduction

Malaria remains the single most important infection causing morbidity and mortality in the world and is second only to Mycobacterium tuberculosis as the single most important infectious agent[1]. It is one of the biggest impediments to progress in Africa and is the biggest killer in Africa, with 90% of the global malaria deaths occurring in this continent[2]. It is responsible for one in four deaths below the age of 5 years and could most times lead to miscarriage at the early stage of pregnancy[2]. In the endemic countries of Africa, children under the age of five and pregnant women bear the brunt of the burden of malaria disease, this is because they have lower immunity to the disease compared to other people in the same environment locations, the malaria situation is deteriorating as a result of environmental changes, including global warming, civil disturbances, increasing travel and increasing drug resistance[1]. According to World Health Organization report, malaria is ranked 7th in the leading selected causes of mortality with fatality rate put at 1.5 to 2.7 million annually while it comes second among the leading selected causes of morbidity with about 300 to 500 million people reporting to hospital due to the infection[3]. Maternal mortality is twice in pregnant malaria women than among non-pregnant patients with severe malaria [4]. Anaemia is the most common symptom of malaria in pregnancy and usually develops during the second trimester[4]. Cerebral malaria is rare in adults except during pregnancy and is responsible for many maternal malaria deaths[5]. Severe *falciparum* malaria may cause deformities in the genital tract to make conception impossible or if conception does occur it may prevent normal implantation and development of the placenta[6].Anaemia is defined as a reduction in the concentration of hemoglobin, packed cell volume or red blood cell count below that which is normal for the age and sex of an individual in a population. Thus according to the World Health Organization Scientific Group, the levels of hemoglobin below which anemia is likely to occur for population living at sea level are: 11g/dl for children aged six months to six years, 12g/dl for children aged between 6 and 14 years, 13g/dl for adult males, 12g/dl for non-pregnant adult females and 11g/dl for adult pregnant females. Anaemia is one of the clinical problems in pregnancy, this is usually caused by increased demand imposed by the growing fetus. Malaria is also a common clinical problem in malaria endemic countries and this is one of the causes of anemia in pregnancy. Association of malaria and anemia will therefore impose

great risk to the health of the mother and fetus. The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission, parasite load and thus with the level of the immunity the pregnant woman has acquire. Anaemia in pregnancy has been associated with maternal morbidity and mortality and is a risk factor for low birth weight.[4] Although so much work have been published on the prevalence of malaria in major cities of Nigeria but little information is available about the prevalence of this disease and anaemia associated with it in the suburbs or outskirts of major cities where transmission is unstable but high as a result of topography, attitude, rainfall, poor drainage system and high human-vector contact to mention a few. This work was therefore aimed at assessing the prevalence of malaria parasite (*P. falciparum)* in pregnant women living in Calabar South, Calabar, Cross River State, Nigeria and also morphologically classify the anaemia that may occur.

2. Materials and Methods

This study was conducted at antenatal clinic of University of Calabar Teaching Hospital, Calabar South, Calbar, Eastern Nigeria. Ethical clearance was obtained from the ethical committee of the hospital and informed consent was also obtained from every participant. The subjects were 414 pregnant women at different gestational period who came for antenantal and were within the age of 15 – 45years. They comprised of women of different socio-economic class who were not suffering from any known disease. Two hundred and fifty apparently healthy non-pregnant women within the same age group were used as controls. They were drawn from female workers in University of Calabar Teaching Hospital and women living within Calabar South Local Government Area. Questionnaires were given to each pregnant woman requesting information on age, parity and gestational age and also to determine those on antimalaria prophylaxis. Gestational age was calculated from the last menstrual period and confirmed by ultrasound scan.

2.1 Method

Four ml of blood was collected into EDTA sample bottles for full blood count. Full blood count was carried out using full automatic blood cell counter, PCE-210 version 5.10 by ERMA INC. Tokyo. For the laboratory diagnosis of malaria parasite infection, thick films were prepared for each of the subject and stained using Giemsa staining method as described by Chessbrough.[8]

2.2 Malaria parasite density determination.

Malaria parasites were counted against white blood cell. A minimum of 1000 white blood cells were counted and the number of malaria parasites counted per the 1000 white cells counted was recorded. The parasite density was then converted to parasite per milliliter of blood according to WHO formula.[9]

$$\frac{\text{Number of parasites counted}}{\text{Number of leucocytes (WBC) counted}} \times 8000 = \text{Number of parasites counted per mm}^3 \text{ of blood}$$

2.3 Statistical analysis

The data generated in this study were analysed for level of significance ($P< 0.05$) using Chi-square test.

3. Results

Six hundred and sixty four subjects made up of 414 pregnant and 250 non-pregnant women were enrolled in the study. Of the 414 pregnant women, 253(61.1%) were anaemic while 290(70.1%) had malaria parasite as shown in table 1. This result showed that the prevalence of anaemia and malaria parasite was statistically significant ($P<0.05$). The table also shows a significance prevalence rate of severe anaemia among the pregnant women. Table 2 shows the morphologic classification of anaemia among pregnant and non-pregnant women. This table showed that microcytic hypochromic anaemia and macrocytic normochromic anaemia were significantly ($P<0.05$) higher in pregnant women than in non-pregnant women. The table also showed that normocytic normochromic anaemia is the most prevalent form of anemia in both groups studied. The distribution of anaemia and malaria parasite among pregnant women according to gravidity was shown in table 3. Primigravidae were found to be susceptible to malaria with a significantly higher parasite density2112.50±420.90 than the multigravidae 446.70±296.90 ($P<0.05$). Anaemia was also found to be higher 89(64.4%) in primigravidae than in multigravidae 164(60.7%) ($P<0.05$). The table also shows that microcytic hypochromic anaemia is higher in multigravidae than in primigravidae. The prevalence of anaemia and malaria parasite among pregnant women according to their gestational age is presented in table 4. Pregnant women appear to be more anaemic in their second trimester 146(65.8%) than the 3rd trimester 78(64.5) although those in their 3rd trimester were more infected with malaria parasite 97(80.2%) than the pregnant women who were in their second trimester 150(67.6%). The pregnant women who were in their 1st trimester were the least infected with malaria parasite 29(40.8%). Figure 1and 2 shows that there was a negative correlation between haemoglobin and parasite density in both pregnant and non-pregnant women (r=-0.1964).

4. Discussion

Malaria infection during pregnancy can have adverse effects on both mother and fetus, including maternal anaemia, fetal loss, premature delivery, intrauterine growth retardation and delivery of low birth weight infants.[4]

This study, has revealed that anaemia, Hb<12g/dl, is present in 38.3% of our non-pregnant women and the same population had malaria parasite prevalence of 60.8%. This agrees with the frequent observation of mild anaemia among healthy population in developing economic conditions in Nigeria which is also malaria endemic area.[10]

The prevalence of anaemia among pregnant women in UCTH calabar was found to be 61.1% in this study. This is slightly lower than 79.1% reported by Usanga *et al*[11] in their work on prevalence of iron deficiency anemia in Nigerian pregnant women and also the work of Achidi *et al.*,[12] who reported a prevalence rate of 68.9%. Severe anaemia was found in 14(5.5%) of anaemic pregnant women, this is significantly higher than 1.3% previously reported by Achidi, *et al.,*[12] and also that of Isibor, *et al.,*[13] who reported no case of severe anaemia in their study. Mild to moderate anaemia is more common in our pregnant first time attenders with a relatively low incidence of severe anaemia. As much as 70.1% of the pregnant women in this study had malaria parasite. This confirms the previous report by Ilona, *et al.,*[14] that malaria infection is one of the major causes of anaemia among pregnant women in malaria endemic area. The 70.1% prevalence rate of malaria parasite (*plasmodium falciparium)* found among the pregnant women in this study is higher than the 32.9% reported in warri Nigeria by Isibor, *et al.,*[13] and also 44% previously reported in south eastern Nigeria by Uko, *et al*.,[10]. Enormous cultural and economic differences may account for this variation.

Normocytic normochromic anemia was found to be the most prevalent form of anemia among the studied groups, this is concordant with the works of Facer[15] and Beales[16] and also with the work of Layla.[17] Anemia in malaria is mostly normocytic normochromic.[18] Since 70.1% of the pregnant women and 60.8% of the non-pregnant women had malaria parasitaemia, the high prevalence of normocytic- normochromic anemia recorded in these groups may result from a combination of haemolysis of parasitized red cells, accelerated removal of both parasitized and innocently unparasitised red cells which may have been opsonised and in the process develop auto-antibodies that make them prone to haemolysis or subsequent sequestration in the reticulo-endothelial system.[18] However a higher prevalence of microcytic hypochromic anemia and macrocytic normochromic anemia were reported in the pregnant women than in the non-pregnant women. This may be due to the increase demand of iron and folate by the growing fetus which is normally common during pregnancy. These findings have emphasized the need for iron and folate supplementation in pregnant women in Calabar.

An analysis of malaria in pregnancy in Africa revealed that parasiteamia is significantly common and heavier in primigravidae than multigravidae [19]. This study showed high level of infection in primigravidae (Table 2). This is because in an area where transmission is high and the level of acquired pregnancy immunity against malaria is expected to be significant, primigravidae is more affected [4,19]. Incidence of anaemia was reported to be slightly higher in the primigravidae (63.6%) than in the multigravidae (59.8%). This agrees with the earlier report by Isibor *et al.,* [13], Nair and Nair,[20] and Brabin[2] that anaemia is a common problem of the primigravidae. Achidi *et al.,*[12] also recorded a higher prevalence of anaemia among the primigravidae (52.1%) than in the multigravidae(47.9%). Normocytic- normochromic anaemia and microcytic hypochromic anaemia were significantly higher in the multigravidae than in the primigravidae.This is presumably because iron deficiency tend to increase as gravidity increases[4]

In this study the prevalence and intensity of malaria parasite infection increased as the gestational age increases, This finding does not totally agree with those of Nair and Nair[20], Menendez[21]; Nosten et al.[22], who reported higher frequency of malaria parasite infection in the second trimester than others. The sequential increase in the intensity of malaria parasite infection as the gestational age increases observed in this study may be due to the depressed immunity usually associated with pregnancy[4]. It was also observed that anaemia is more common in the second trimester than in others, this agrees with the previous report that anaemia due to malaria is more common and severe between 16-29 weeks of gestation.[23] The negative correlation observed in both pregnant and non-pregnant women suggests that malaria parasite infection contributes significantly to anaemia.

5. Conclusion

This research has emphasized that anemia is still prevalent among pregnant women in this environment with malaria parasite (*P. falciparum)* infection as one of the major causes. It has also been able to establish that normocytic normochromic anemia is the most prevalent form of anaemia in this environment and that microcytic hypochromic anaemia is still common among the pregnant women in this environment.

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Table 1 Prevalence of anaemia and parasitaemia among non-pregnant and pregnant women

	Total Number Of Subjects	Number infected (%)	No anaemic (%)	No with Severe anaemia(%)	Mean Parasite ± SEM Den. (par./µl bld.)
Pregnant	414	290(70.1)	253(61.1)	14(5.5)	2112.50 ± 420.90
Non-pregnan	250	152(60.8)	96(38.8)	-	246±320.41
P –Value		P<0.05	P<0.05	P<0.05	P<0.05

Table 2 Morphological Classification Of Anaemia Among Pregnant And Non-Pregnant Women

Morphologic type of anemia	Non-pregnant	Pregnant	P-VALUE
Microcytic hypochromic anemia (MCV <78fl and MCH < 26pg)	28(29.2%)	83(32.8%)	P<0.05
Microcytic normochromic anemia (MCV <78fl and MCH > 26pg)	0	0	
Normocytic normochromic anemia (MCV >78fl but <95fl and MCH>26pg)	68(70.8%)	161(63.8)	P<0.05
Macrocytic normochromic anaemia (MCV > 95fl and MCH > 26pg)	0	9(3.7%)	P<0.05
Macrocytic hypocromic anemia (MCV > 95fl and MCH < 26pg)	0	0	

Table 3 Distribution of anaemia and parasitaemia among pregnant women according to gravidity.

	Primigravidae (n=140)	Multigravidae (n=274)	P-VALUE
Hb < 11g/dl	89(63.6%)	164(59.9%)	P<0.05
Severe anaemia (Hb<7g/dl)	5(3.6%)	9(3.3%)	P>0.05
Microcytic hypochromic anaemia and MCH < 26pg)	24(17.2%)	57(20.8%)	P<0.05 (MCV <78fl
Normocytic normochromic anaemia (MCV >78fl but <95fl and MCH>26pg)	50(35.7%)	10(40.1%)	P<0.05
Macrocytic normochromic anaemia (MCV > 95fl and MCH > 26pg)	6(4.3%)	(1.1%)	P<0.05
No. (%)positive for malaria parasite	100(71.4%)	190(69.3%)	P<0.05

Table 4 Prevalence of anaemia and parasitaemia among pregnant women according to their gestational age

Gestational Age	NO of Subjects	No. anaemic (%)	No. infected (%)	Mean parasite denstiy ± SEM (Parasite/ul of blood)
1st Trimester	71	29(40.8)	43(60.6)	577.40 ± 320.60
2nd Trimester	222	146(65.8)	150(67.6)	885.60 ± 364.10
3rd Trimester	121	78(64.5)	97(80.2)	1913 ± 554.70
P-VALUE		P< 0.05	P< 0.05	P< 0.05

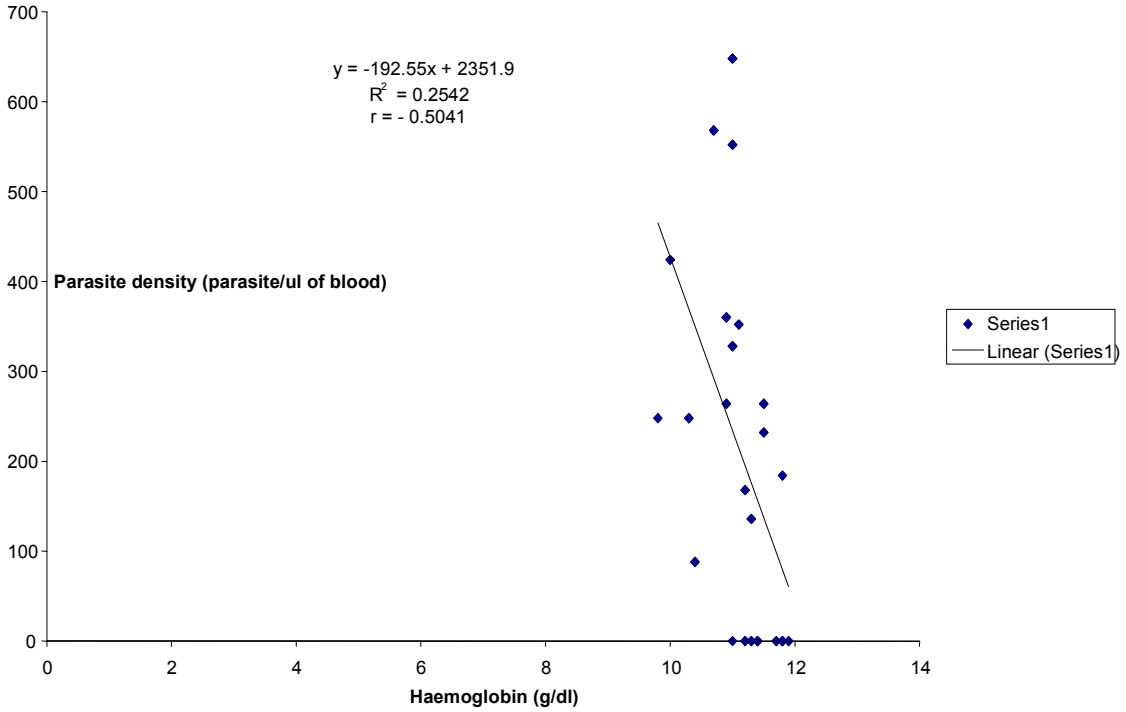

Figure 1- Correlation between haemoglobin and parasite density in non-pregnant women

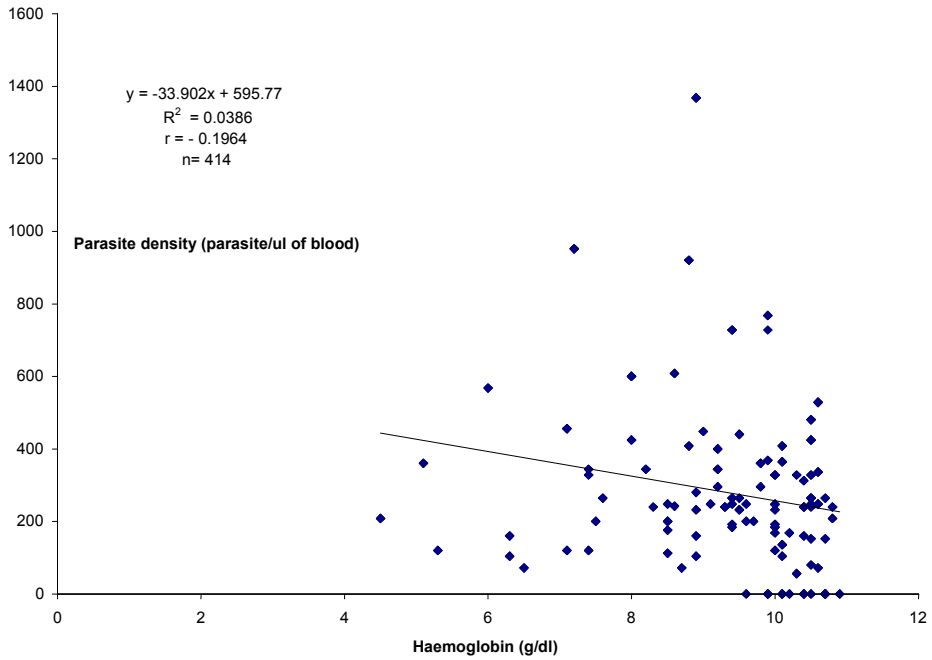

Figure 2 - Correlation graph between haemglobin and parasite density in pregnant women