

Haematological profile, prevalence and intensity of malaria among pregnant women in Awka, Awka South local government area, Anambra State, South eastern, Nigeria.

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Abstract: A survey on malaria parasitaemia and haematological profile of pregnant women in Awka, Anambra State, Nigeria, was carried out between January and July 2013. A total of one hundred randomly selected pregnant women had their blood sample collected by vein-puncture technique. Thick and thin films were made and examined microscopically. Sixty five (65.0%) of them were positive for malaria. Primigravidae women and women in their third trimester (73.7%; 86.7% respectively) had the highest prevalence. Of the 65 positive patients examined, 29 (29.0%) had mild infection of malaria, 28 (28.0%) of them had moderate infection of malaria, while 8 (12.3%) had heavy infection, with women in age group 21-30 years 25 (62.5%) have highest prevalence. Primigravidae women (73.7%) and women in the third trimester (86.7%) had the highest intensity of malaria. Fifty-seven pregnant women (57.0%) had abnormal haemoglobin and were anaemic (0-7g/dl), while 43(43.0%) had normal haemoglobin (8-11g/dl). Women with heavy infection (+++) are more anaemic(%) than non malaria pregnant women(%). nd increase in abnormal range of WBC (<10.5cells/mm cube) while women in the second trimester 15(60.0%) and multiigravidae women 1(33.3%) have lowest malaria and lowest normal range of WBC. Finally differentials were also count with preparation of thin film and viewed microscopically. Pregnant women with heavy infection have below 25% of the count which is abnormal range. The result highlighted the importance of increasing awareness at all levels about integrated strategies for control and prevention of malaria during pregnancy which includes provision of adequate prophylaxis (intermittent preventive treatment drugs), proper and intensive education of the pregnant women, personal protection of the women and provision of nutritional food.

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Introduction

Malaria is a mosquito-borne-infectious disease of humans and other animals caused by protists of the genus *Plasmodium* (Shen *et al*, 2010). It begins with a bite from an infected female *Anopheles* mosquito, which introduces the protists through saliva into the circulatory system of human. In the blood, the protists travel to the liver to mature and reproduce. Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death. The disease is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas (Klebanoff *et al*.2012).

Malaria may cause abortion and premature labor; in tropical areas many pregnant women suffer from severe anaemia due to the infection itself and to the effects of deficiency of iron and folic acid (Adeyemi *et al*, 2007). The results of recent studies indicate that malaria is due to some extent to the immune

destruction of sensitized red cells and a depression of *erythropoiesis* (Akingbola *et al*, 2006). Congenital transmission of malaria may occur, though it is relatively rare; paradoxically, it is more frequent in babies born of non-immune mothers with malaria imported from tropical countries, though massive infections of the placenta with *P. falciparum* are common in immune mothers in highly endemic areas (Akingbola *et al*, 2006).

In 2010 there were estimates of 219 million cases of malaria resulting in 660,000 deaths, equivalent to roughly 2000 deaths every day (WHO,2010). Using a different set of predictive models to estimate mortality, a 2012 study determined the number of documented and undocumented deaths in 2010 to be 1.24 million (WHO, 2010). The majority of cases (65%) occur in children under 12 years old (WHO, 2010). About 125 million pregnant women are at risk of infection each year; in Sub-Saharan Africa, maternal malaria is associated with up to 200,000

estimated infant deaths yearly. Malaria is an annual killer of over 1 million people globally and its essential co-morbidity is anaemia (Bhattacharya, 2006). Anaemia due to Malaria in pregnancy is an important public health concern in developing countries (more pronounced in primigravidae than in multigravidae). However, anaemia due to malaria can be more severe in pregnant women (McCrae *et al*, 2011). Malaria anaemia results from the obligatory destruction of parasitized erythrocytes, the accelerated destruction of normal erythrocytes, and variable dyserythropoiesis. Malaria anaemia can be assessed by the measurement of packed cell volume (haematocrit) and the measurement of Hemoglobin concentration (James *et al*, 2008).

Pregnancy outcome is influenced by many factors some of which include culture, environment, socioeconomic status and access to medical care. The hematological profile of pregnant women also has an impact on pregnancy and the outcome of the pregnancy (Burrows *et al*, 2009). The most common hematological indices are the indicators of hemoglobin concentration. Low hemoglobin in the blood (anemia) is widely identified as a hematologic abnormality and it is associated with adverse pregnancy outcome (Miltchel *et al*, 2006).

Every year, approximately 25 million African women become pregnant in malaria endemic areas. These women are at risk of most especially *Plasmodium falciparum*, the most virulent of the malaria parasites (Aribodor *et al*, 2007). Malaria infection and high-density parasitaemia are more common in pregnant women than in non pregnant women of comparable age, socio-economic status and from the same area. In Nigeria, the disease account for 11% of maternal mortality (Onwukeme *et al*, 2007). In the endemic areas of the world, children under age of five and women in their first pregnancy are more vulnerable to the disease. Anemia due to malaria contributes to low birth weight and miscarriages and it is also a primary cause of low immunity of both the mother and the child, which makes them vulnerable to several infections. Malaria infection especially in the first and second trimesters has been implicated in adverse pregnancy outcomes. It causes 3%-5% of maternal anemia cases. About 50 million pregnant women are exposed to malaria especially in the high endemic regions (Jensen *et al*, 2011).

The hematological status in pregnant women can be analyzed by collection of blood samples during each of the three trimesters, measuring different variables such as packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration,

erythrocyte sedimentation rate (ESR) and platelet count (James *et al*, 2008).

Specific objectives include:

- To determine the prevalence and intensity of malaria in pregnant women in Awka, Anambra state, Nigeria.
- Investigation in the haematological changes in pregnant women infected with malaria parasites.

Materials and Methods

Study Area:

This study was conducted in Awka the capital of Anambra State, southeastern Nigerian, with an estimated population of 301,657 people as of 2006 Nigeria census. Several road networks link Awka to small towns and villages such as Agulu, Nibo, Amawbia, Enugwu-ukwu and Abagana. Awka lies between the latitude 6.2°N and longitude 7.1°E in the rainforest belt of Nigerian. Awka town is situated about 25 miles (40km) Northeast of Onitsha and 45 miles (72km) southeast of Enugu. Awka experience two distinct seasons-a wet season of abundant rainfall which begins in April and ends in October or early November and practically rainless dry season which last from November to March. The temperature ranges between 22°C and 37°C and has an annual rainfall of between 152cm and 203cm. The area is covered with network of other forms of surface water bodies and low level of sanitation which has favored the breeding of mosquitoes which is a contributing factor in high endemicity of malaria.

Awka is made up many rural villages and developed streets. The major occupation of the residents is trading and the minor occupations include farming mainly of the subsistence type; majority of the inhabitants are traders, farmers though of subsistent type while others include civil servants (both active and retired) and students. There are many Nursery, primary and secondary school in Awka owned by government and individuals (private schools). There are two major University in Awka, Nnamdi Azikiwe University and Paul's University. There are several markets in Awka, but there is one major market in Awka called Eke Awka. In Awka there are many borehole owned by government and individuals (private) which provides source of water for drinking and domestic purposes. There are many industries in Awka, but there are two popular industry known by everybody, Juhel Pharmaceutical Industry and Millennium Plastic Industry.

Study Design

This study was carried out to determine the prevalence and intensity and also to investigate the haematological changes in pregnant women, the study was done within a period of six months (January to July 2013) and blood samples collected based on

individual consent, after the ethical permission was granted.

Study Population

The study involves 100 pregnant women. Those included in this study all live permanently in Awka both young and middle age women (less than 20-greater than 40 years of age). Samples were collected during the days of their antenatal clinic.

Out of the 100 pregnant women sampled, 45 were in the first trimester, 25 were in the second trimester, while the remaining 30 were in the last trimester. Also 38 were primigravidae, 32 were secundigravidae while the remaining 30 were multigravidae.

Ethical clearance

An advocacy visit was made to the Chief Medical Director (CMD) of the different hospitals with an introductory letter from the Department of parasitology and Entomology, Nnamdi Azikiwe University, Awka to obtain his permission to work at the hospital. The purpose of this study was explained to them, their blood samples were collected based on their individual consent.

Collection of blood specimen

After cleaning the volar surface of the arm with cotton moistened with methylated spirit, peripheral blood samples were collected, about five milliliters (5ml) of blood from each participant. The blood was dispensed into Ethylene diamine tetra acetic acid bottle (EDTA bottle) to avoid clotting. Thick blood film was immediately be made from the blood collected in the syringe before transferring it into the EDTA anti coagulant bottles for detecting malaria parasite.

Microscopy: (Thick and Thin film) (Cheesbrough, 2000)

Peripheral blood samples were collected in sterile containers. Thin and thick blood smears were made from each of these samples, stained with Geimsa and then examined under the microscope using x100 objective lens in each case. Identification of species was done using the thin blood smear. The parasite density was estimated on the thick smear under oil immersion and viewed using x100 objective lens. The determination of malaria parasites were determined by counting the number of asexual form of *Plasmodium species* parasites against at least 100 leucocytes and 200 leucocytes for definitive count. The number of asexual parasites was calculated using this formula, $\text{Parasites/urL} = \text{No of asexual parasite} \times 8000 \text{ leucocytes}/200 \text{ leucocytes}$. The degree of parasitaemia was graded thus (1-999/urL) as mild or +, (1000-9999/urL) as moderate or ++, and (>10000/ urL) as severe or +++.

A negative result was recorded after thorough examination of 100 fields without any parasite.

Comparison was made with both known positive and negative thin films. Hemoglobin concentration was estimated spectrophotometrically using the cyamethaemoglobin method (Cheesbrough, 2000). The thin film was fixed using methanol for about 2minutes, the film was flooded with 10% Giemsa stain and allow to stain for 10minutes, the stain was washed off with clean water and the back of the slide was wiped and air dry in a draining rack. And view under the microscope total white blood cells was also count with counting chamber; ESR (erythrocyte sedimentation rate) was also stand for one hour undisturbed.

Data analysis

Data collected were analyzed using SPSS version 11 computer software. Results were presented as simple percentages. Test for significance was done

χ^2)

using Chi square (. comparison was made at significant level of $p < 0.05$.

Results

Result revealed that out of 100 pregnant women sampled at the hospital only 65 (65.0%) were positive for malaria. The prevalence and intensity of malaria with respect to age was presented in table 1. The highest prevalence of malaria (75.0%) in women was obtain in those greater than 40 years followed by those in the age group 31-40 years (66.7%). The least prevalence (60.0%) was found in those less than 20 years old. Heavy infection was recorded most in those in the age groups 21-30 years and those greater than 40 years (10.0%) but was completely absent in those in age group 31-40 years old. On the contrary, moderate infection was recorded most among those aged 31-40 years old (40.0%) but least among those in the age group 21-30 years old (22.5%). Statistical analysis showed that there was a statistical difference between age and intensity of malaria.

The prevalence and intensity of malaria with respect to trimester was presented in table 2. The highest prevalence of malaria is found in those in third trimester (86.7%), followed by those in second trimester (60.0%). The least prevalence (53.3%) is found in those in the first trimester. On the intensity of malaria, heavy infection was recorded highest (38.5%) among those in their third trimester, followed by those in their second trimester (33.3%). Conversely, moderate infection was recorded most (45.8%) by those in their first trimester followed by those in their second trimester (33.3%). Statistical analysis revealed a significant difference.

The prevalence and intensity of malaria with respect to gravidity was presented in table 3. The highest prevalence (28:73.7%) was found among the

primigravidae, followed by secundigravidae (22:68.8%) and the least is among the multigravidae (15:50.0%). Heavy infection was recorded most in the primigravidae (46.4%) followed by secundigravidae (33.3%). Moderate and mild infections were recorded highest in multigravidae (46.7% and 40.0% respectively) followed by secundigravidae (40.9% and 36.6% respectively). Statistical analysis showed a significant difference between prevalence and intensity of malaria with respect to gravidity.

Table 4 shows the haemoglobin concentration with respect to age. The result shows that out of 100 women sampled, 57% were anemic. Those within the age group 21-30 years (60.0%) had the highest prevalence of anaemia followed by those in the age group 21-30 years old (57.5%). The least prevalence was found among those less than 40 years (55.0%). Statistical analysis revealed that there was a statistical analysis between prevalence and intensity of malaria and haemoglobin concentration $p < 0.05$.

Table 1: Prevalence and intensity of malaria parasiteamia with respect to age.

Age (%)	Number examined	Number positive (%)	Mild infection(+)	Moderate infection(++)	Heavy infection(+++)
>20	25	15(60.0%)	6(24.0%)	7(28.0%)	2(8.0%)
21-30	40	25(62.5%)	12(30.0%)	9(22.5%)	4(10.0%)
31-40	15	10(66.7%)	4(26.7%)	6(40.0%)	0(0.0)
<40	20	15(75.0%)	7(35.0%)	6(30.0%)	2(10.0%)
Total	100	65(65.0%)	29(29.0%)	28(28.0%)	8(12.3%)

Table 2: Prevalence and intensity of malaria with respect to trimester.

Trimester Examined	Number (%)	No. positive infection+ (%)	No of Mild infection++(%)	No of moderate infection+++ (%)	No of heavy infection++++ (%)
1 st trimester	45	24(53.3%)	7(29.2%)	11(45.8%)	6(25.0%)
2 nd trimester	25	15(60.0%)	5(33.3%)	5(33.3%)	5(33.3%)
3 rd trimester	30	26(86.7%)	9(34.6%)	7(26.9%)	10(38.5%)
Total:	100	65(65.0%)	21(32.3%)	23(35.4%)	21(32.3%)

Table 3: Prevalence and intensity of malaria with respect to gravidity.

Gravidity Examined	Number	No of positive infection+(%)	No of Mild infection++(%)	No of moderate infection+++ (%)	No of heavy infection++++ (%)
Primigravidae	38	28(73.7%)	7(25.0%)	8(28.5%)	13(46.4%)
Secundigravidae	32	22(68.8%)	8(36.6%)	9(40.9%)	5(33.3%)
Multigravidae	30	15(50.0%)	6(40.0%)	7(46.7%)	3(20.0%)
Total:	100	65(65.0%)	21(32.3%)	24(36.9%)	21(32.3%)

Table 4: Hemoglobin concentration of pregnant women with respect to age.

Age group(years) (8-11g/dl)(%)	Number examined (0-7g/dl)(%)	No with normal range	No with abnormal range
>20	25	11(44.0%)	14(56.0%)
21-30	40	17(42.5%)	23(57.5%)
31-40	15	6(40.0%)	9(60.0%)
<40	20	9(45.0%)	11(55.0%)
Total	100	43(43.0%)	57(57.0%)

Table 5: ESR (Erythrocyte Sedimentation Rate) concentration of pregnant women with respect to age group.

Age-group (years)	Number examined 2-7mm/hr (%)	Normal range < 8mm/hr (%)	Abnormal range
>20	25	15(60.0%)	10(40.0%)
21-30	40	23(57.5%)	17(42.5%)
31-40	15	5(33.3%)	10(66.7%)
<40	20	5(25.0%)	15(75.0%)
Total:	100	45(45.0%)	53(53%)

In the table above, out of 100 pregnant women examined, 45 (45%) women have normal range of ESR, which range from 2-7mm/hr in every women, and 53 (53%) women are abnormal and had the highest normal range with the women under the age of 21-30 and lowest with women under the age of 31-40.

Table 6: Total white blood count (WBC) of pregnant women with respect to age.

Age-group (years)	Number examined 4.5-10.5cells/mm cube (%)	Normal range <10.5cells/mm cube (%)	Abnormal range
>20	25	9(36.0%)	16(64.0%)
21-30	40	13(32.5%)	27(67.5%)
31-40	15	5(33.3%)	10(66.7%)
<40	20	7(35.0%)	13(65.0%)
Total:	100	34(34.0%)	66(66.0%)

This shows that out of 100 women sampled 34(34.0%) of them were normal with the highest age group 21-30 years (13:32.5%) and the least age group 31-40 (7:35.0%).

Discussion

Malaria infection represents a major public health problem in the tropical and sub-tropical countries. The result of this study revealed that prevalence (65.0%) of *Plasmodium* infection is very high in Awka and this study have been compare with previous work done in these area, (63.5%) Chukwurah *et al*, 2003). In this study when all the positive cases were pooled together, it become obvious that susceptibility was more in primagravidae. Furthermore, asymptomatic malaria parasitaemia occurred more in the first and second trimesters than third trimester. This is similar to other studies which showed that susceptibility is more marked in the second trimester than during the third trimester (Nwagha *et al*, 2009). The prevalence of Malaria in pregnant Awka women in this study was 65.0% using our local standard (Ogunbode, 1984). Furthermore, hemoglobin concentration, showed a significant decrease with increasing parasite density. This is understandable as increasing parasite density ultimately leads to increase in red cell breakdown and consequently anaemia (loss of blood). ESR concentration (Erythrocyte Sedimentation Rate), WBC (Total white Blood count) showed a significant increase with increasing parasite density.

This is aggravated by the poor socio-economic background of the patient since they are predominantly subsistent farmers and traders. This situation calls for proper evaluation of the nutritional status of our pregnant so as to assist pregnancy care givers double their efforts in malaria prevention. ITN, IPT, integrated vector control and nutritional support as well as health education should be used concurrently in the prevention of adverse effect of malaria in pregnancy. Indeed IPT has been shown to reduce maternal peripheral parasitaemia (Van Ejik *et al*, 2002) placental parasitization (Rogerson, 2000), low birth weight and maternal and neonatal anaemia.

This prospective study was successfully completed irrespective of the difficulties and challenges encountered during the program. However, it would have been more appropriate if I had followed up these patients until delivery to enable me assess the impact of asymptomatic parasitaemia on feto-maternal outcome. This study illustrates a high prevalence of asymptomatic plasmodium parasitaemia and loss of blood in pregnant women in Awka. Apart from IPT and ITN, effort should be made to incorporate other vector control. Awka will benefit from effort at larva control and reduction of breeding sites like bushes near living homes. Concerted effort to reduce poverty, provision of adequate prophylaxis (intermittent preventive treatment drugs), improve nutrition, housing and electricity in rural part of Awka will invariably reduce the malaria burden. This will go a long way in reducing maternal and perinatal morbidity and mortality.

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