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Malaria in pregnancy; facts from the parasitology laboratory: a ten-year study in Abuja, North Central Nigeria

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ABSTRACT

Malaria, which is transmitted by female *Anopheles* mosquitoes, is the major cause of mortality among the pregnant women in the sub-Saharan Africa. A ten year study of malaria in pregnancy was carried out in Abuja, North Central Nigeria. Thick and thin blood films were stained with the Giemsa methodology. Of the 16760 pregnant women blood samples, 4571 (27.3%) were positive for malaria parasites caused by *Plasmodium falciparum*. Of the 4571 positive cases, 75 (1.7%) had parasite density of >5000 parasites/ μ l of blood; 148 (3.2%) had between 500-5000 parasites/ μ l of blood; 520 (11.4%) had between 50 - 500 parasites/ μ l of blood; while 3828 (83.7%) had between 5-50 parasites/ μ l of blood. With the current estimate of over 4500 deaths of pregnant women in Nigeria due to malaria annually, we must make deliberate efforts to stop these unacceptable and painful losses. The continued use of malaria rapid diagnostic tests (M-RDTs) methodologies should be discontinued because of its negative implications. Therefore, the microscopic laboratory diagnostic component should be included in ANC at all level of health care facility.

Keywords: Malaria, pregnancy, *Plasmodium falciparum*, ANC attendees, women in labour.

1. Introduction

Malaria, a vector borne disease transmitted by female *Anopheles* mosquitoes (*Anopheles gambiae* and *A. funestus* in West African sub region) is the major cause of mortality among the pregnant women in the sub-Saharan Africa. The clinical features of malaria in pregnancy depend to a large extent on the immune status of the woman, which in turn is determined by her previous exposure to malaria. In pregnant women with little or no pre-existing immunity, such as those from non-endemic areas, infection is associated with extremely high risks of both maternal and prenatal mortality.

In the malaria epidemic that occurred in Ceylon in 1934-35, maternal mortality in women treated for malaria with quinine was 13%. Pregnancy failure (foetal loss or neonatal mortality) was 67%^[1]. In sub-Saharan Africa alone, approximately 25 million pregnant women are at risk of *Plasmodium falciparum* infection every year. Approximately one in four women show evidence of placental infection at the time of delivery, with a large fraction of infection remaining undetected and untreated^[2].

Based on documented cases, the WHO estimates that there were 219 million cases of malaria in 2010 resulting in 660,000 deaths^[3]. This is equivalent to roughly 2000 deaths every day^[4]. A 2012 study estimated the number of documented and undocumented deaths in 2010 was 1.24 million. The majority of cases (65%) occur in children under 15 years old^[5, 6]. Pregnant women are also especially vulnerable: 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^[7].

In areas of moderate or high transmission (holoendemic or hyperendemic), including large parts or sub-Saharan Africa, adults usually have a high level of immunity to malaria. During pregnancy, this immunity to malaria is altered. Malaria in pregnancy is a common cause of severe maternal anaemia and low-birth-weight babies, these complications being more common in primigravidae than multigravidae.

What is peculiar to malaria in pregnancy is the sequestration of parasites in the placenta, where infection is often extremely heavy. Pregnant women with little or no previous immunity to

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malaria are two or three times more likely to develop severe disease as a result of malaria infection than are non-pregnant adults living in the same area [8]. In addition, if they develop severe disease, they are at a higher risk of dying than their non-pregnant counterparts. Severe disease in pregnant women has been associated with 20-30% maternal mortality and a very high risk of miscarriage, premature delivery or neonatal death [9].

The health consequences of malaria during pregnancy are large: malaria-induced low birthweight is estimated to account for up to 360,000 infant deaths every year [10], overall, 11.4% of neonatal deaths and 5.7% of infant deaths in malaria-endemic areas of Africa are estimated to be caused by malaria in pregnancy [11, 12].

In 2011, the Nigerian Tribune [13] published a statement credited to the Ogun State Commissioner of health, and that about 4,500 pregnant women die of malaria in the country annually. It is an established fact that the disease remains a great threat to the population particularly expectant mothers. Some pregnant women who spoke to the Nigerian Tribune reporter expressed concerns and fears over the debilitating effects of malaria parasites on their present status, lamenting that the infection had done irreversible damage to them in the past. A 36 year old pregnant woman regretted the loss of her four months old pregnancy to malaria in 2009.

In order to avoid the consequences of malaria during pregnancy, WHO guidelines recommend both preventive and curative measures [14, 15].

The aim of this work is to establish the proper and prompt diagnosis of malaria into the antenatal care of pregnant women to effect appropriate curative measures.

2. Materials and Methods

2.1 Study Area

The Federal Capital Territory (FCT), Abuja is the study area. Abuja is located between latitude 8.25 and 9.20 North of the Equator and longitude 6.45 and 7.39 East of the Greenwich Meridian. The Federal Capital Territory has a total landmass of about 8,000sq kms; located geographically at the centre of the country. The current population size of the FCT according to the 2006 census figures stands at 1,405,201 [16].

Two seasons are experienced in FCT. These are the wet (rainy) season and the dry season. The rainy season lasts between April to October (with a mean total rainfall of 62.8 – 262.7 mm; August being the highest). The dry season is between November and March (with a mean total rainfall of 1.2 to 11.3 mm).

Abuja has a daily temperature range between 20.4–34.7 °C with an average of 27.2 °C/81 °f in January; and between 21.9–29.1 °C with an average of 25.6 °C/78 °f in July [17].

2.2 Study Population

The population studied consisted of 16,760 pregnant women (comprising of 12,946 antenatal care (ANC) attendees and 3,814 women in labour whose blood samples were presented at the Parasitology Laboratory of National Hospital, Abuja for malaria parasite diagnosis. The study was carried out from May 2000 to December 2010.

2.3 Parasitological Techniques

Thick and thin blood films were made on clean grease-free slides and stained appropriately using Giemsa's staining method. Giemsa stain is the most commonly used of the Romanowsky stains and is the best for routine diagnosis because of its applicability to both thick and thin smears, its stability on storage and its constant and reproducible staining quality over a wide range of temperatures. The stained films were then examined microscopically using 100x objective to count the parasites.

Parasites were counted by estimating the parasite numbers/ μ l of blood from the thick film. This was carried out by multiplying the average number of parasites per thick film field (100x objective) by 500. Between 10 -100 fields (depending on parasitaemia) were examined to determine the average number of trophozoites per thick film field. Ten fields are sufficient when the parasite density is high [18].

The factor of 500 was proposed by Greenwood and Armstrong [19]. They calculated that 5 – 8 μ l is the volume of blood required to make a satisfactory thick film and that the volume of blood in one thick film field (100x objective) of a well-prepared thick film is about 0.002 μ l. Therefore the number of parasites per thick film field multiplied by 500 gives the estimated number of parasites/ μ l of blood. This method was found to be more accurate and quicker than counting the parasites against 100 white blood cells in a thick film using WHO method as used by Molineaux and Gramiccia (1980) [20]. In 2002, Ikeh *et al*, used this technique in their study at Jos [21].

For designation of the relative parasite count on a thick film, a simple code from one to four crosses or the plus sign scheme is used to report parasite numbers:

- + (1+) = 1–10 parasites per 100 thick film fields
- ++ (2+) = 11–100 parasites per 100 thick film fields
- +++ (3+) = 1–10 parasites per one thick film field
- ++++ (4+) = > 10 parasites per one thick film field

3. Results

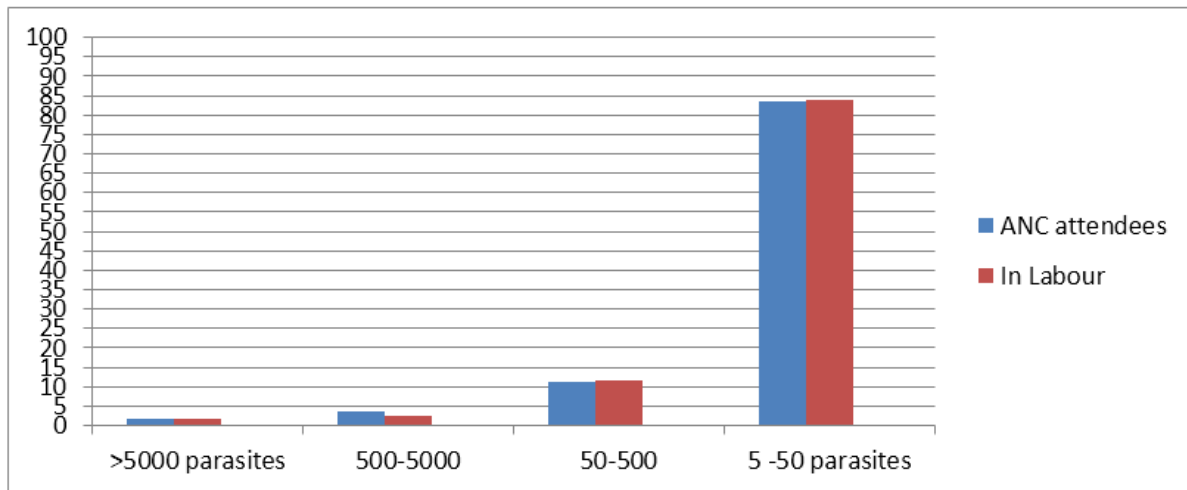
Of the 16,760 blood samples analyzed, 4571 (27.3%) were positive for malaria parasites mainly caused by *Plasmodium falciparum*. 75 (1.7%) had parasite density of >5000 parasites/ μ l of blood (4+); 148 (3.2%) had between 500 – 5000 parasites/ μ l of blood (3+); 520 (11.4%) had between 50 – 500 parasites/ μ l of blood (2+); and 3,828 (83.7%) had between 5-50 parasites/ μ l of blood (1+).

Of the 12,946 antenatal care (ANC) attendees, 3,594 (27.8%) had a positive malaria result. 58 (1.6%) of the positives had parasite density of >5000 parasites/ μ l of blood (4+); 124 (3.5%) had between 500 – 5000 parasites/ μ l of blood (3+); 404 (11.2%) had between 50 – 500 parasites/ μ l of blood (2+); and 3008 (83.7%) had between 5-50 parasites/ μ l of blood (1+).

Of the 3,814 women in labour, 977 (25.6%) had a positive malaria result. 17 (1.7%) of the positives had parasite density of >5000 parasites/ μ l of blood (4+); 24 (2.5%) had between 500 – 5000 parasites/ μ l of blood (3+); 116 (11.8%) had between 50 – 500 parasites/ μ l of blood (2+); and 820 (84%) had between 5-50 parasites/ μ l of blood (1+).

Table 1: Prevalence and Intensity of Malaria Parasitaemia amongst ANC attendees and those in Labour

Stage	>5000/ μ l	500-5000/ μ l	50-500/ μ l	5-50/ μ l	Total Positives	Negatives	Total No Examined
ANC Attendees	58 (1.6%)	124 (3.5%)	404 (11.2%)	3008 (83.7%)	3594 (27.8%)	9352	12946
In Labour	17 (1.7%)	24 (2.5%)	116 (11.8%)	820 (84%)	977 (25.6%)	2837	3814
Total	75 (1.7%)	148 (3.2%)	520 (11.4%)	3828 (83.7%)	4571 (27.3%)	12189	16760

**Fig 1:** Graphical presentation of parasite density among ANC attendees and those in labour

4. Discussion

This study highlights the importance of prompt and proper parasitological diagnosis of malaria amongst pregnant women. Malaria parasites were found in 27.3% of samples analyzed.

From our results, there is no significant difference in prevalence and parasite intensity amongst the ANC attendees (27.8%) from those in labour (25.6%) as stated in Table 1 and Figure 1. What is not clear to us is if the ANC attendees that tested positive for malaria were not on intermittent preventive treatment in pregnancy (IPTp) programme, or that they were not properly treated for malaria before time of delivery or those still positive during labour were unbooked cases or an indication of resistant strains resulting to treatment failure.

Silamut and White, reported that because *Plasmodium falciparum* matures in internal organs ('sequestration'), a severe form of malaria may occur at a time when the parasites in the peripheral blood are scanty or even absent [22]. In order to avoid the consequences of malaria during pregnancy, it behooves on clinicians to commence treatment with appropriate antimalarial drugs on all positive cases and not minding the clinical threshold or critical value. WHO (2000) recommended that it is necessary to examine serial blood films at intervals of 6 – 12 hours to confirm the diagnosis of negative blood films [23].

Since the introduction of Roll Back Malaria (RBM) strategy, several efforts have been on course towards the reduction of the burden of malaria by formulation of strategic plans. The vision of the current five-year strategic plan (2009 – 2013) is to ensure that Malaria no longer becomes a major public

health problem in Nigeria as illness and death from malaria gets significantly reduced. This is to be achieved by ensuring that families will have universal access to malaria prevention and treatment. The ultimate long-term vision is having "A malaria free Nigeria". Consequently, some of the targets is to see that "at least 80% of fever/malaria patients receive appropriate and timely treatment according to national treatment guidelines and all (100%) pregnant women attending ANC receive at least two doses of Intermittent Preventive Therapy (IPT) by 2013" [24].

For Nigeria to achieve this feat, the missing component of the Roll Back Malaria (RBM) strategy – Laboratory Diagnosis should be incorporated if we must succeed. This is to be achieved by ensuring that families will have universal access to malaria prevention, laboratory diagnosis and treatment.

Meanwhile, the continued use of malaria rapid diagnostic tests (M-RDTs) methodologies in Nigeria for individual diagnosis of malaria should be highly discouraged because of its negative implication. The implication of the continued use of these malaria rapid diagnostic tests (M-RDTs) methodologies includes underdiagnosis, misdiagnosis of malaria and mismanagement of non-malarial fever, which wastes limited resources, erodes confidence in the health care system, contributes to drug resistance and eventual administration of unnecessary antimalarial drugs aimed at mopping up all negative test results where patients may still present with symptoms.

Several studies have shown that malaria rapid diagnostic tests (M-RDTs) methodologies have malaria parasites detection limit of 397 – 500 parasites/ μ l of blood and in

some cases even more than 500 parasites/ μ l of blood [25-31] as against the 5 parasites/ μ l of blood detectable with the Giemsa stained slide microscopy examined by microscopists who are experienced and competent in the preparation and staining of blood films, as well as in the recognition and identification of the characteristic stages of malaria parasites usually found in human blood.

There is need to monitor the effectiveness of the Intermittent Preventive Therapy in pregnancy (IPTp). This could be easily done by testing for malaria parasites on all pregnant women attending ANC and those in labour. This aspect will become real with the acquisition/provision of laboratory facilities manned by microscopists who are experienced and competent in the preparation and staining of blood films, as well as in the recognition and identification of the characteristic stages of malaria parasites usually found in human blood by way of capacity building through continuous training.

5. Conclusion

With the current estimate of over 4,500 deaths of pregnant women in Nigeria due to malaria annually, we must make deliberate efforts to stop these unacceptable and painful losses. Therefore, in order to avoid the consequences of malaria during pregnancy;

1. Community participation and health education strategies promoting awareness of malaria and the importance of control measures aimed at reducing the incidence of malaria in our environment should be encouraged through media advocacy and during ANC hours. Relevant government agencies should ensure that insecticide-treated mosquito nets (ITNs) are made available free to every pregnant woman.
2. There is need to evaluate the success rate of the intermittent preventive treatment in pregnancy (IPTp) programme; if found successful, more funds should be made available to increase participation and compliance by pregnant women.
3. The laboratory component should be included in ANC at all level of health care facility (primary – rural settings, secondary and tertiary) manned by microscopists who are experienced and competent in the preparation and staining of blood films, as well as in the recognition and identification of the characteristic stages of malaria parasites usually found in human blood.
4. Appropriate antimalarial drugs for treatment should be given free to all malaria positive pregnant women

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