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Obstetric and Haematological Determinants of Malaria Parasitaemia among Pregnant Women in Kano State, Nigeria

Dantata, A.A.
Department of Biological Sciences, Bayero University, Kano
aidanta@yahoo.com

Abstract
Malaria during pregnancy has serious health consequences, and poses substantial risk to the mother, her fetus, and the new born. This study was carried out to assess the relationships between malaria parasitaemia and some obstetric, haematological and clinical characteristics of pregnant women of various parities in the study area, with the hope to better understanding the local malaria situation. The laboratory techniques employed were: preparation of thick and thin blood smears, by Giemsa staining, for malaria parasite screening; measurement of blood haemoglobin, for detection of anaemia by Cyanmethaemoglobin method; alkaline pH electrophoresis of haemoglobin, for detection of haemoglobin variance. Parity was found to be significantly associated with malarial infection, with high prevalence seen in nulliparous and primiparous women, and the rate of infection decreasing with increasing parity. Likewise the sickle cell trait was found to influence the prevalence of malaria infection, with pregnant women who had the sickle cell condition (Hb SS) and those with normal haemoglobin (Hb AA) being more at risk for malaria infection than those individuals with the sickle cell trait (Hb AS and SC). A strong association was also found between anaemia and malaria parasitaemia, with individuals that were anaemic being more infected than those that were not anaemic. However, history of fever and previous chemoprophylaxis were not found to have any impact on the rate of malaria infection. Health education programs on the management of malaria in pregnancy should be intensified.
Key words: Obstetric, Haematological, Determinants, Malaria, Pregnancy, Kano

INTRODUCTION

Malaria during pregnancy remains a major public health problem in malaria-endemic areas of sub-Saharan Africa (WHO, 2005b).It has serious health consequences, and poses substantial risk to the mother, her fetus, and the neonate (newborn child). In particular, it increases the risk of maternal anaemia (reduced haemoglobin levels) or death; spontaneous abortion (fetal death/miscarriage), stillbirth, premature delivery and low birth weight (Brabin, 1991; WHO 2005b; WHO, 2013c). Studies suggest that in areas of Africa with stable malaria transmission, *P. falciparum* in pregnancy contributes to 2 – 15% of maternal anaemia annually, which translates to an estimated 400,000 cases of severe maternal anaemia, a potentially fatal condition (Steketee *et al.*, 2001). In Nigeria malaria during pregnancy causes up to 10,000 maternal deaths each year and contributes to high rates of maternal morbidity including fever and severe anaemia (Ekejindu*et al.*, 2006).

Maternal factors that are considered to be the principal influences or the risk factors of malaria in pregnancy include maternal age, parity (the number of times that a woman has given birth) or gravidity (the number of times that a woman has been pregnant) and gestational age (Takem and D'Alessandro, 2013). The harmful effects of malaria are more pronounced among pregnant women in their first and second pregnancies (Marchesini and Crawley, 2004; Desai *et al.*, 2007, Takem and D'Alessandro, 2013).

It has been well established that the sickle gene (S) provides resistance to individuals living in malaria – endemic regions, against the malaria parasite (Ringelhann *et al.*, 1976; Luzzatto, 2012).

People with sickle cell condition (Hb SS) can get malaria just like anyone else; however, people with sickle cell trait (Hb AS) are less likely to get malaria compared to the normal-haemoglobin counterparts. The sickle haemoglobin (Hb S) is considered to be protective against malaria. However, the sickle gene (S) offers some protection against malaria in the heterozygotes (AS), which surprisingly, may not necessarily apply to homozygotes (SS) (Bouyou-Akotet *et al.*, 2003; Luzzatto, 2012).

Although malaria infections have been associated with symptoms of fever, fever has not been found to be a good indicator of clinical malaria, as in endemic areas asymptomatic *P. falciparum* infections are frequent in adults; also, malaria is not the main aetiology of fever during pregnancy.

Other causes like urinary and genital infection could be the cause (Bouyou-Akotet *et al*., 2003). Parise *et al*. (2003) found that although fever was associated with placental parasitaemia (i.e. presence of asexual blood stages of *Plasmodium* parasite), substantial numbers of women with placental parasitaemia will not be identified if fever history is used to predict parasitaemia. Approximately 25-30% of infected women reported no fever at any time during their pregnancy – and would not be receiving treatment if the decision to treat were based on symptoms.

Chemoprophylaxis (the attempted prevention of disease by means of drugs) with antimalarial drugs such as chloroquine or pyrimethamine, has been associated with fewer malaria parasite infections in pregnant women (Mockenhaupt *et al*., 2000; Akanbi *et al*., 2005). However, Bouyou-Akotet *et al*. (2003) found that previous chloroquine prophylaxis had no effect on susceptibility to *P. falciparum* infection. Although the efficiency of chloroquine as a prophylactic is questionable, since it is known that its efficacy in curative treatment in children and adults is less than 20%, Chloroquine is still recommended as the first-line anti-malarial drug in preventive treatment for pregnant women in some areas such as Gabon, where malaria is hyperendemic (Bouyou-Akotet *et al*., 2003). Of great interest, is a study carried out by Adefioye *et al*. (2007) in Osogbo, southwest Nigeria, in which it was found that the patients who refused to take drug due to their religious belief had the highest prevalence rate of 89%, followed by those on prophylaxis (68.4%) while those who used to take herbs (indigenous drug) had no malaria attack. Thus, the use of herbs by some pregnant women proved 100% active against *P. falciparum*, in other words herb is highly effective for treating malaria. The authors suggested that this calls for more research in local herb in order to develop new and more effective drug for prevention and control, particularly in view of the rapid spread of drug resistance.

This study therefore assessed the relationships between malaria parasitaemia and some obstetric, haematological and clinical characteristics of the study population in the study area. It is hoped that the information obtained will help in better understanding the local malaria situation, and will be useful in the planning and evaluation of services for malaria prevention in Kano State.

MATERIALS AND METHODS

Selection of the Study Sites and Study Design

The study was carried out in three selected public State and Federal Government healthcare facilities, within Kano metropolis, which have antenatal clinics (ANCs) and were fully patronised. One healthcare facility was selected from each of the three levels of public healthcare facilities (i.e. Primary, Secondary and Tertiary), to ensure full and adequate representation. A facility survey was carried out to determine the healthcare facilities to be used as sample study sites from each of the three categories of healthcare facilities. Criteria used in the selection include the availability of facilities for managing malaria in pregnancy and the facility with the highest ANC attendance was chosen from each level of health care, using the average of monthly ANC attendance for the previous one year in each of the facilities, as suggested by Akinleye *et al*.(2009). The three healthcare facilities selected as the study sites are as follows: (1) Gwagwarwa Primary Health Centre (PHC), a primary health centre in Nassarawa Ward, Nassarawa Local Government Area (LGA); (2) Murtala Mohammed Specialist Hospital, a secondary specialist hospital in Zango Ward, Kano Municipal LGA; and (3) Aminu Kano Teaching Hospital (AKTH), a teaching hospital in Darmanawa Ward, Tarauni LGA.

A descriptive cross-sectional study design was used to assess the distribution of malaria parasitaemia in relation to some obstetric, haematological and clinical characteristics of the Antenatal Care (ANC) clients.

Study Population and Minimum Sample Size Estimation

The study population comprised of pregnant women of various parities (primigravida and multigravida), selected by systematic random sampling as suggested by Araoye (2003) and Akinleye *et al*.(2009), from those attending the selected antenatal clinics on their first booking clinic day (i.e. when they come for their first antenatal visit), from July 2012 to March 2013.

The minimum sample size for the antenatal clients required was calculated using Fisher's formula ($n = z^2 pq / d^2$) for estimating minimum sample size for descriptive studies, where n = minimum sample size required (when population is greater than 10,000); z = the standard normal deviate, corresponding to the 95% confidence level; p = prevalence of malaria parasitaemia at booking among antenatal clients obtained from a previous similar study; q = complementary probability of p = (1 – p); d = degree of precision at 95% confidence limit (Araoye, 2003).

The minimum sample size that was calculated was 329, however, the sample size was raised to 600; thus 200 antenatal clients were sampled from each of the 3 study sites.

Data Collection and Laboratory Investigations:-

Bio-data of each ANC client with respect to parity, Hb genotype, Hb level, history of fever prior to the first ANC visit and exposure to antimalarial drug (previous chemoprophylaxis), were collected using data collection forms as suggested by Parise *et al*. (2003) and Bouyou-Akotet *et al*. (2003).

Malaria Parasite Screening

Capillary blood samples were collected from each of the study participants through finger pricking, using disposable lancets (Cheesbrough, 2000; Parise *et al*., 2003). These were used to prepare thick and thin blood smears by Giemsa staining technique and were examined microscopically for the presence of malaria parasites.

Measurement of Blood Haemoglobin (for Detection of Anaemia)

In order to assess the impact of malaria in pregnancy, haemoglobin (Hb) levels were measured using Cyanmethaemoglobin (HiCN {i.e. Drabkin's solution/reagent}) method as an indicator of anaemia, as described by Dacie and Lewis (2006).In order to assert the association between anaemia and malaria parasitaemia, the infection rates in women from the three different categories of Hb level were compared.

Investigation of Blood for Determination of Genotype

Haemoglobin electrophoresis at pH 8.5 (i.e. alkaline pH), using cellulose acetate membrane was carried out for the detection of clinically important haemoglobin variants, e.g. sickle cell trait, as described by Wild and Bain(2002). This was done in order to compare the vulnerability of clients with different Hb genotypes to malaria infection.

Data Analysis

Data obtained with the data collection forms were entered in a database and analyzed using a modern analytical software package, PASW (Predictive Analytical Software), version 20, formally known as SPSS (Statistical Package for Social Sciences). Differences between means were tested using one-way analysis of variance (ANOVA), and the extent to which some variables were related was tested using cross-tabulation. Statistical significance was achieved when $p<0.05$ (Parise *et al*., 2003). The results were summarized using a frequency table.

RESULTS

Relationship between Malaria Parasitaemia and Some Obstetric, Haematological and Clinical Characteristics of the ANC Clients

The prevalence of malaria in relation to parity, genotype, haemoglobin level, history of fever and previous chemoprophylaxis is shown in the Table.

Relationship with Parity

With respect to parity, higher rates of infection were seen among the nulliparous (women who have not given birth previously) and primiparous (women who have given birth once), with 62.4% and 77.6% respectively. With increasing parity, the rate of infection decreased to 54.6% and 56.5%, for the multiparous (women who have given birth more than once) and grandmultiparous women respectively. An analysis of variance (ANOVA) showed a significant difference ($p < .05$) in the infection rates between the different groups of parities, with the nulliparous and primiparous groups being more affected by malaria compared to the multiparous and grand multiparous groups.

Relationship with Hb Genotype

With respect to the effect of haemoglobin (Hb) genotype on malaria infection, a large proportion (62.3%) of women with haemoglobin (Hb) genotype AA (i.e. normal genotype) and 66.7% of women with Hb genotypes SS (i.e. those with sickle cell condition) were infected with malaria parasite; whereas, the infection rate was found to be much lower (50%) for those individuals with Hb genotype AS (i.e. carriers of the sickle cell trait). An analysis of variance shows a significant difference in the rate of infection between the different genotypes at $p<0.05$. The AA and SS genotypes were more affected by malaria.

Relationship with Haemoglobin Level

A total of 81 clients had anaemia (i.e. Hb < 10.0g/dl). Out of these 53(65.4%) were parasitaemic. Almost all (7{87.5%}) of the women that had severe anaemia (Hb <7g/dl) were found to be parasitaemic; likewise, a significant proportion of the women (46{63%}) with mild to moderate anaemia (Hb 7-9.9g/dl) were also parasitaemic. However, a large proportion (58.8%) of the women that had normal haemoglobin ($\geq$10g/dl) were also parasitaemic. Nevertherless, the difference in infection rates between these three groups was statistically significant ($p<0.05$). The result indicated that anaemic women were more affected with malariathan non-anaemic women.

Relationship with History of Fever

With respect to history of fever, one hundred and eight (108{60%}) out of the 180 ANC clients, who reported that they had fever within the past three weeks before being enrolled for the study, were found to have malaria infection. This was slightly higher than the proportion of those that did not report fever, but were also parasitaemic (250 out of 421 ANC clients {i.e. 59.4%}). However, the observed difference between these groups was statistically insignificant ($p > .05$).

Relationship with Previous Chemoprophylaxis

Most women who reported having fever recently (i.e. in the past three weeks before enrollment for the study) had taken some medication. Ironically, high prevalence of malaria infection was observed among those that admitted to taking some form of chemoprophylaxis. Out of 98 women that took pain reliever, 55(56.1%) were parasitaemic; all 4(100%) women that took antibiotic were parasitaemic; while 27(562%) out of 48 women who had taken antimalarial (treatment) drugs, were parasitaemic; all 4(100%) women who reported taking flu medicine were parasitaemic. On the other hand, 18(69.2%) out of 26 women who reported to have had fever, but did nothing (i.e. did not take any medication) were found to be parasitaemic; while 250(59.4%) out of 421 who did not have fever, were also parasitaemic. The difference in infection rates was not statistically significant ($p >0.05$).

Prevalence of Malaria in Relation to Parity, Hb Genotype, Haemoglobin Level, History of Fever and Previous Chemoprophylaxis

Variables	Frequency (Percentage	Parasitaemic (Positive cases) Frequency (Percentage within groups)	Aparasitaemic (Negative cases) (Percentage) N=600 within groups)	Total Frequency
Number of previous deliveries (births)/Parity				
None (Nulliparous)		78(62.4)	47(37.6)	125(100.0)
1 (Primiparous)		59(77.6)	17(22.4)	76(100.0)
2 - 4 (Multiparous)		125(54.6)	104(45.4)	229(100.0)
5 or More (Grand Multiparous)		96(56.5)	74(43.5)	170(100.0)
Significance			***p* < .05**	
Total		**358(59.7)**	**242(40.3)**	**600(100.0)**
HB Genotype				
AA (Normal)		289(62.3)	175(37.7)	464(100.0)
AS (Carrier of sickle trait)		63(50.0)	63(50.0)	126(100.0)
SS (Sickle cell condition)		6(66.7)	3(33.3)	9(100.0)
SC (Sickle carrier, but haemoglobin C)		0(0.0)	1(100.0)	1(100.0)
Significance			***p* < .05**	
Total		**358(59.7)**	**242(40.3)**	**600(100.0)**
Haemoglobin Level				
Severe anaemia (<7g/dl)		7(87.5)	1(12.5)	8(100.0)
Mild to Moderate anaemia (7 – 9.9g/dl)		46(63.0)	27(37.0)	73(100.0)
Normal Hb (10g/dl or more) (i.e. Not anaemic)		305(58.8)	214(41.2)	519(100.0)
Significance			***p* < .05**	
Total		**358(59.7)**	**242(40.3)**	**600(100.0)**
History of fever in the past 3 weeks				
Had fever		108(60.0)	72(40.0)	180(100.0)
Did not have fever		250(59.4)	171(40.6)	421(100.0)
Significance (*n.s*)			***p* > .05**	
Total		**358(59.7)**	**242(40.3)**	**600(100.0)**
Previous Chemoprophylaxis				
Pain reliever		55(56.1)	43(43.9)	98(100.0)
Antibiotic		4(100.0)	0(0.0)	4(100.0)
Antimalarial drugs		27(56.2)	21(43.8)	48(100.0)
Nothing		18(69.2)	7(26.9)	26(100.0)
Flu Medicine		4(100.0)	0(0.0)	4(100.0)
Not relevant (Did not have fever)		250(59.4)	171(40.6)	421(100.0)
Significance (*n.s*)			***p*> .05**	
Total		**358(59.7)**	**242(40.3)**	**600(100.0)**

n.s=Not Significant

DISCUSSION

Parity was found to be significantly associated with malarial infection, with high prevalence seen in nulliparous and primiparous women, and the rate of infection decreasing with increasing parity. Many researchers have reported similar findings, among them are Bouyou-Akotet *et al.*(2003), Takem and D'Alessandro(2013), and it has been attributed to cellular immune responses. Anti-adhesion antibodies against chondroitin sulphate A-binding parasites are said to be associated with protection from maternal malaria, but these antibodies develop only over successive pregnancies, accounting for the susceptibility of primigravidae (i.e. nulliparous women) to infection (Duffy and Fried, 1999; WHO, 2002; Marchesini and Crawley, 2004; Takem and D'Alessandro, 2013).

The sickle cell trait was found to influence the prevalence of malaria infection; the pregnant women who had the sickle cell condition (i.e. with Hb SS) and those with normal haemoglobin (i.e. Hb AA) were more at risk for malaria infection than those individuals with the sickle cell trait (i.e. with Hb AS and SC). This is in line with what has been reported by other researchers such as: Ntoumi *et al.* (1997) and Luzzatto (2012), who reported that people with sickle cell condition (Hb SS) can get malaria just like anyone else; however, people with sickle cell trait (Hb AS) are less likely to get malaria compared to the normal-haemoglobin counterparts. The sickle haemoglobin (Hb S) is considered to be protective against malaria. The sickle gene (S) offers some protection against malaria in the heterozygotes (AS), which surprisingly, may not necessarily apply to homozygotes (SS). Some explanation given, being that the protection offered by the S gene is not through failure of infection, but is based on something that takes place subsequently. Experimental work is consistent with a plausible mechanism: namely, that in AS heterozygotes, *P. falciparum*-infected red cells sickle preferentially and are then removed by macrophages. The clinically relevant consequence of this is to keep parasitaemia relatively low in AS heterozygotes. On the other hand, in individuals that are homozygous for the sickle gene (i.e. SS) there is often hyposplenism (a disorder which causes the spleen to rapidly and prematurely destroy blood cells), which reduces clearance of parasites. In addition to this, there are other protective mechanisms at work: for instance, it has been found that AS parasitized red cells have impaired adherence to endothelial cells, which could decrease the risk of cerebral malaria (Luzzatto, 2012).

Results from the study indicated that there is a significant association between anaemia and malaria parasitaemia, with individuals that were anaemic being more infected than those that were not anaemic. Also, the level of parasitaemia varied with the degree of Hb level, with individuals having severe anaemia having the highest level of parasitaemia (87.5%), those with mild to moderate anaemia having a moderate level of parasitaemia (63%), while individuals that were not anaemic were less affected than the first two categories, with (58.8%) level of parasitaemia. These results are in agreement with findings by other researchers, where it has been shown that *P. falciparum* infection is usually associated with anaemia or reduced haemoglobin levels (Nair and Nair, 1993; Bouyou-Akotet *et al.*, 2003; Takem *et al.*, 2010; Takem and D'Alessandro, 2013).

In this study there was no significant association between past history of fever and malaria parasitaemia. Although a relatively high proportion (60.3%) of the ANC clients who recently had fever were found to be parasitaemic, there were also an almost equally high percentage (59.4%) of those who did not report fever (i.e. were asymptomatic), but were also found to be parasitaemic. Surprisingly, in a similar study carried out by Bouyou-Akotet *et al.*(2003), the prevalence of plasmodial infection was found to be significantly higher in women without fever. Thus, history of fever has been regarded not to be a good indicator of clinical malaria, as a substantial proportion of malaria infection may be symptomless (Bouyou-Akotet *et al.*, 2003; Parise *et al.*, 2003), while malaria may not also be the main aetiology of fever during pregnancy (Bouyou-Akotet *et al.*, 2003).

The results showed that previous chemoprophylaxis did not have any statistically significant effect on susceptibility to malaria infection, and it can thus be assumed that in this study there was no association between prevalence of malaria infection/parasitaemia and previous chemoprophylaxis of any kind (be it antipyretic or antimalarial). This result agrees with findings from a previous study by Bouyou-Akotet *et al.* (2003). However, it is not consistent with the observations by other researchers (Mockenhaupt *et al.*, 2000; Akanbi *et al*, 2005), where it was shown that the prevalence of infection and the mean parasite density was significantly lower in those individual who took antimalarial drugs prior to booking at the antenatal clinic than in those that did not.

It was thus ascertained that antimalarial drugs are associated with fewer malaria parasite infections and the reason offered is that the antimalarial drugs were effective in killing the malaria parasite. In this study the antimalarial drugs taken by the ANC clients seemed not to have been very effective in clearing the assumed malaria parasite infection; possible reasons may be that those that took antimalarial drugs may not have taken the correct doses, or may not have completed the treatments.

CONCLUSION AND RECOMMENDATIONS

This study showed that parity affects the risk of malaria infection; nulliparous and primiparous women were more affected compared to multiparous and grandmultiparous women. The study has also shown that malaria infection is associated with significant increase in anaemia (i.e. reduced haemoglobin level); anaemia was more common in the infected than non-infected women. There was also a relationship between malaria and Hb genotype, with Hb AA and Hb SS being more vulnerable for malaria infection than Hb AS. However, history of fever and previous chemoprophylaxis did not have any impact on the rate of malaria infection in the study.

It was observed from interaction with the study subjects that majority of them were not aware of the risk factors involved with malaria infection. It is therefore recommended that health education programs on the management of malaria in pregnancy should be intensified.

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