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Detection of Plasmodium Species among Pregnant Women attending Antenatal Care

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Abstract: *The Aim of this study is to detect the presence of plasmodium species in whole blood and antibodies in the serum among pregnant women attending antenatal care in specialist Hospital Sokoto in North Western Nigeria. 5mls of blood samples were collected by vein puncture from three hundred (300) pregnant Women, thin and thick films were made and stained using Giemsa stain for the detection of the ring trophozoite and gametocytes of plasmodium species and parasites were observed microscopically on the films. Then the blood samples were centrifuged at 1500 rpm for 15 minutes and the sera were used for the detection of antibodies using malaria rapid diagnostic kits (ACONS Diagnostic Company USA). Personal data were collected using questionnaires. The results were analyzed statistically using statistical package for social sciences (SPSS). A total of 115(38.3%) pregnant women out of the 300 examined, were infected with plasmodium parasites. Out of 115 infected, 98(85.22%) were infected with plasmodium falciparum, 2(1.74%) were infected with p.malariae ,14(12.17%) were infected with p. vivax, and only 1(0.87%) were infected with p. ovale. The prevalent rates of 48(41.7%), 38(33%), 22(19.1%), 6(5.2%), 1(1%) and 0(0%) were observed for the ages of 15-20, 21-25, 26-30, 31-35, 36-40 and 41-45 years respectively. Primary educated and illiterates pregnant women have high prevalent rates of 40(35%) and 53(46%) than pregnant women with secondary and tertiary education whose prevalent rate is 15(13%) and 7(6%) respectively. Pregnant women in their first, second, and their third trimester had prevalent rates of 60(52%), 34(30 %), and 21(18%) respectively. Prevalent rates for primigravidae, secundigravidae and multiparous women were 47(40.9%), 42(36.5%) and 26(22.6%). The result obtained from this study, indicated that, young maternal age, level of education, and parity greatly contributed to the seroprevalence of malaria parasitaemia among pregnant women.*

I. Introduction

An estimated 30 million women living in malaria endemic areas of Africa become pregnant each year. Pregnant women are particularly vulnerable to malaria because pregnancy reduces immunity to malaria; increases susceptibility to malaria infection, the risk of illness, severe anaemia, and acute pulmonary edema, renal failure, puerperal sepsis, postpartum haemorrhage, and increases the risk of death. Malaria in pregnancy results in adverse pregnancy outcomes, such as spontaneous abortion, neonatal death, and low birth weight. Chronic anemia, due to malaria may also affect a child's growth and intellectual development (12).

Malaria has also serious socio-cultural consequences in families by interfering with farm activities in the rainy season. It is estimated that malaria afflicted families on the average can harvest only 40% of the crops harvested by healthy families, causes absenteeism from school, thus affecting school performance. It is estimated that in endemic areas like Uganda, malaria may impair as much as 60% of the schoolchildren's learning ability. Poor malaria-stricken family may spend up to 25% of income on malaria treatment and prevention, and loose household incomes through absenteeism from work. It is estimated that workers suffering from a malaria bout can be incapacitated for 5-20 days. A study in Apac, Kampala, and Rukungiri Districts showed that malaria was responsible for 54%, 33% and 50% respectively of absenteeism from work per month in the above districts (4).

Yet, malaria transmission is increasing in Nigeria due to deforestation, cultivation of wetlands, poor environmental sanitation, other man made breeding sites such as construction works, brick pits or fish ponds, among others, all of which create breeding sites for mosquito.

II. Materials And Methods

Study Area
Specialist Hospital Sokoto South Local Government of Sokoto State.

Study population
The study included 300 pregnant women attending antenatal care unit at Specialist hospital Sokoto

Ethical consideration
Ethical clearance for the study was sought and obtained from the ethical committee of Sokoto state specialist Hospital.

Study design
The study is a cross–sectional prevalence study determining seroprevalence of *Plasmodium spp*among pregnant women.

Sample size
300 pregnant women.

Eligibility criteria
All pregnant women attending antenatal care unit at Specialist Hospital Sokoto

Exclusion criteria
All non-pregnant women

Laboratory Method
Specimen collections
Blood samples were taken for quantitative, qualitative and serological malaria test.

Procedure for collection of blood
Informed consent was obtained from each participant before sample collection. Using a sterile disposable syringe, 5mls of blood samples were collected by vein puncture from three hundred (300) pregnant Women, immediately thin and thick films were made, then the blood samples were centrifuged at 1500 rpm for 15 minutes within 24 hours of collection, the sera were separated and kept at -20^{o} C until when needed.

Films Making
Principle:
An unfixed dried film, about five to six red blood cells thick, is made and the hemoglobin lysed out during the staining process. The malaria parasites in the film are stained with little interference from the large numbers of red blood cells present.

In this study, both thick and thin films were made on the same slide. The steps involved in making the films are as follows:

- At an angle of 45^{o}, the blood drop was allowed to spread horizontally along the edge of spreader which was used to touch the drop.
- At a decreased angle, the spreader was drawn forward and gently to make a thin film with characteristic head, body and smooth tail.
- Without any delay, the other drop of blood was spread continuously to an area of about 15 x 15mm to make a thick film.
- The slide was labeled.
- It was then allowed to air-dry in a horizontal position.

Staining Techniques
The staining technique employed in this study for the identification of malaria parasite in both the thick and the thin films was Giemsa staining technique. Prior to staining with Giemsa, the thin film was fixed with water free absolute methanol. This was done by placing the thin film portion of the slide into the absolute methanol for 2 minutes, making sure the absolute methanol did not touch the thick film.

Giemsa Staining Technique:

Giemsa stain from the stock solution was diluted only when it was ready for use. 3% Giemsa solution was prepared for this study. The following steps were employed for staining both the thick and the fixed thin blood films:

- The slide was placed horizontally on the staining rack.
- 3% Giemsa solution was used to cover the thick and the fixed thin films.
- The films were allowed to stain for 30 minutes.
- The stain was flushed off with clean water.
- The back of the slide was wiped clean.
- The slide was then placed in a draining rack to drain and air-dry.

Film Reading

The blood films were examined microscopically using the 100x objective of the microscope. A drop of immersion oil was applied to an area of the film. For the purpose of this study quantitatitiveparasitaemia count and species identification was performed.

Immunochromatographic test

Material/reagent: cassette devices, plastic droppers, sample diluents, clock, and serum.

Principle:

Antibodies including IgG, IgM, and IgA against aldolase, generated following the infection by the either form of malaria protozoa if present in the specimen will bind to the pan-malaria conjugates. The immune complex is then captured by the pre-coated aldolase antigen on the membrane, forming a burgundy coloured T bands, indicating a Plasmodium antibody positive result

Procedure:

- The cassette device was placed on a clean, flat surface and labeled with sample ID number
- Using plastic dropper, one drop of serum was dispensed into the sample well without forming air burble
- One drop of sample diluents was dispensed
- The timer was set up, and the result was read in 15 minutes

Interpretation of result

Positive result

- The presence of two colour bands at (C and 1) indicates a positive result for P. *falciparum.*
- The presence of two colour bands at (C and 2) indicates a positive result for P. vivax or other Plasmodium *spp*.
- The presence of three colour bands indicates a positive result for P. falciparum and P. vivax.or other plasmodium spp.

Negative result

- The presence of only one band within the result window indicates negative results.

Invalid

- The test is invalid if the C line does not appear.

Data Analysis

The data collected from this study were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) for windows (version 18.0). Differences were shown to be statistically significant where $P<0.05$.

III. Result.

Table 1 shows the distribution of Malaria Parasites with Age. Highest prevalence was observed in the age group of 15-20, 21-25 and 26-30 years, 48(41.7%) 38(33%) and 22(19.1%) respectively, with least seen in older women 31-35, 36-40 and 41-45 years 6(5.2%), 1(1%), 0(0%). Age was statistically significant ($P<0.05$).

Table 2 shows the distribution of Malaria Parasites with Education ($P<0.05$). The distribution showed that those with Primary Education and without Formal Education have high prevalence accounting for 40(35%) and 53(46%), with least seen in those with Secondary 15(13%) and Tertiary education 7(6%).

Table 3 shows the distribution of Malaria Parasites with Gestation ($P<0.05$). Pregnant women in their First Trimester have the highest rate of Malaria infection 60(52%), followed by second trimester 34(30%), with least seen in their Third Trimester 21(18%).

Table 4 shows the distribution of Malaria Parasites with Parity (P<0.05). The distribution showed high prevalence in Primigravidae 47(40.9%) and Secundigravidae 42(36.5%), with least seen in Multigravidae 26(22.6%).

Table: 1: Age and Malaraia Parasites Distribution

Age			Plasmodium species					
	NE	NI	P.	P.	P.	P.		
	falciparum	malariae	vivax	ovale				
15-20	73(24%)	48(41.7%)		38(38%)	-(0%)		9(60%)	1(2%)
21-25	98(33%)	38(33%)	36(36%)	-(0%)		2(13%)	-(0%)	
26-30	82(27%)	22(19.1%)	18(18%)	-(0%)	4(27%)	-(0%)		
31-35	38(13%)	6 (5.2%)		6(6%)		-(0%)	-(0%)	-(0%)
36-45	7(2%)	1 (1%)	1(1%)	-(0%)		-(0%)	-(0%)	
41-45	2(1%)	0 (0%)	-(0%)	-(0%)		-(0%)	-(0%)	
Total :	**300**	**115**	**99**	**0**		**15**	**1**	

NE= Number Examined, NI= Number Infected.df = 1, P<0.05 (significant)

Table: 2: Education and Malaria Parasites Distribution

Education			Plasmodium species					
	NE	NI	P.	P.	P.	P.		
	falciparum	malariae	vivax	ovale				
Primary	60(20%)	40(35%)	30(75%)	2(5%)		7(18%)	1(2%)	
Secondary	98(32.7%)	15(13%)	13(87%)			- (0%)	2(13%)	- (0%)
Tertiary	42(14%)	7(6%)	7(100%)		- (0%)	- (0%)	- (0%)	
Others	100(33.3%)	53(46%)	50(94.3%)			1(1.9%)	2(3.8%)	- (0%)
9Total:	**300**	**115**	**100**			**3**	**11**	
						1		

NE= Number Examined, NI= Number Infected.df=1, P<0.05 (significant)

Table: 3: Gestation and Malaria Parasites Distribution

Trimester	Plasmodiumspecies					
	NE	NI	P.	P.	P.	P.
	falciparum	malariae	vivax	ovale		
First trimester	98(33%)	60(52%)	55(91.6%)	1(1.7%)	4(6.7%)	- (0%)
Second trimester	97(32%)	34(30%)	28(82%)	- (0%)	5(15%)	1(3%)
Third trimester	105(35%)	21(18%)	21(100%)	- (0%)	- (0%)	- (0%)
Total	**300**	**115**	**104**	**1**	**9**	**1**

NE= Number Examined, NI= Number Infected.df=1, P<0.05 (significant).

Table: 4: Parity and Malaria Parasites Distribution

Parity	Plasmodium *species*					
	NE	NI	P.	P.	P.	P.
	falciparum	*malariae*	*vivax*	*ovale*		
Primigravidae	99(33%)	47(40.9%)	33(70%)	1(2%)	13(28%)	-(0%)
Secundigravidae	96(32%)	42(36.5%)	35(83.3%)	1(2.4%)	6(14.3%)	-(0%)
Multigravidae	105(35%)	26(22.6%)		25(96.2%)	-(0%)	1(3.8%) -(0%)
Total	**300**	**115**	**93**	**2**	**20**	**0**

NE= Number Examined, NI= Number Infected. df=1, P<0.05 (significant)

IV. Discussion

Malaria disease is dangerous especially an infection with *Plasmodium falciparum* is more hazardous during pregnancy. Pregnancy appears to interfere with the immune processes in malaria, a disease which itself alters immune reactivity (1). In highly endemic malarious area where semi-immune adults usually have substantially acquired resistance to local strains of plasmodia, the prevalence of clinical malaria is higher and its severity is greater in pregnant women than non-pregnant women (1). This is also true in this study in which the prevalence of 38.3% of malaria parasites is recorded. This prevalence is higher than that reported by Agboghoroma and colleagues 31% from the National Hospital Abuja (2). It is higher than 23 % reported in Mozambique (7), 26.75% reported in Malawi (6), and higher than that reported by Akum and co-workers in south-western Cameroon, where the prevalence is 32.8% (10). The prevalence is lower than 62% found in Tanzania (11), it is also lower than 41% found in Kenya (8).

Among these parasitaemic women 86 % were infected with P. *falciparum*, 1.3% with P. *malariae*, 12% with P. *vivax* and only 0.7% with P. *ovale*. These findings comfirmed a previous report that P. *falciparum* is the most prevalent species in Nigeria accounting for about 98% of malaria cases in the country (2). Other findings

reported that 80-95% of malaria infection in tropical Africa is caused by P. *falciprum*(10).The infection by P. *vivax* in this study also comfimed the previous report that P. *vivax* is transmitted in 95 tropical, subtropical and temperate countries (8). People living at risk of *P. vivax*malaria infection are 2.85 billion, 91% living in Central and South East Asia region, 5.5% in America and 3.4% in Africa (8). As many as 57.1% of people exposed to *P. vivax* infection lives in unstable malaria areas (6). Stable – unstable classification is another way to determine malaria endemicity (6). Macdonald defined malaria stability on the ground of the number of mosquito's lifetime bites in the human host (6). This vector-based index differentiated stable and unstable malaria. Vector-based classification is less used because of entomological-based metrics complexity; ethical concerns related to exposing human beings to malaria infection and measurement error issues (10).

Distribution Of Malaria Parasites With Age: The result of this study, revealed a significant distribution of Malaria Parasites ($P<0.05$) with Age, implying that pregnant women between the ages of 15-20, 21-25 and 26-30 years, 48(41.7%) 38(33%) and 22(19.1%), were more at risk than older pregnant women of the ages, 31-35, 36-40 and 41-45 years , 6(5.2%), 1(1%), 0(0%). The different malaria prevalent rates observed among these age groups could be attributed to the level of acquired immunity that increases with age, which may also be associated with protection from malaria infection (5). This finding is similar to that reported by other research carried out in Nigeria (5).

Distributionof malaria parasite with education: The result obtained from the study, showed that pregnant women with primary education and illiterates were more infected, with the prevalence of 40(35%) and 53(46%), than those with Seconadary and Tertiary Education, in which the prevalence is 15(13%) and 7(6%) respectively. The prevalent rate seen in illiterate's pregnant women and those with primary education is probably because they are more exposed to malaria parasite due to bad environmental condition and their life styles.

Distribution of malaria parasites with gestation: The high prevalent rate obtained within the first trimester 60(52%) and second trimester 34(30%) in this study, may be due to the attitude of the women of not starting pre - natal care early in pregnancy. Some of the women began pre - natal care either towards the end of first trimester or mid second trimester. Also some avoided antimalaria chemoprophylaxis for fear that the foetus may be affected. The prevalence observed in the first trimester and second trimester may also be attributed to the expression of adherent proteins on the surface of infected red blood cells (IRBCs), enabling the IRBCs to adhere to micro-vascular capillaries of vital organs causing severe pathological condition (8).

Distribution of malaria parasites with parity: Paucigravidae (Primigravidae) 47(40.9%) and Secundigravidae42(36.5%), had a higher infection rate than the Multiparous (Multigravidae) pregnant women 26(22.6%).. The prevalence observed in primigravidae and secundigravidae is lower than 62% found in Tanzania (11).The results were definitely higher than that reported of 26.2 % observed among the Primigravidae in Malawi (6).The different prevalence rate observed, is as a result of acquisition of specific immunity to placental malaria due to previous exposure by Multigravidae, Acquired specific immunity accumulates with subsequent infection and subsequent pregnancies (9).

V. Conclusion

The result obtained from this study, indicated that, young maternal age, level of education, and parity greatly contributed to the seroprevalence of malaria parasitaemia among pregnant women. Pregnancy is one of the factors affecting the rate of malaria parasite infection in women living in malaria-endemic communities.

VI. Recommendation

Antenatal clinic is where most pregnant women attend, therefore is a very good avenue to implement health policies that affects women and their children. Mass enlightenment on the importance of environmental hygiene and local means of vector control has to be intensified. Such campaign should be included during routine antenatal talks by the health personnel. Capacity building of health providers has to strengthen in order to give adequate health talk on malaria, use and application of ITMNs. Government should make ITMNs available to the pregnant women at an affordable price if not free. Vector control including use of aerial spray of insecticides and prompt refuse disposal and wearing of long sleeve shirts by the pregnant women in the evening will greatly reduce the burden of malarial infection. Adequate supply of antimalarial drugs should be included in free antenatal drugs inorder to ensure prophylaxis care to the pregnant women

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