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Haemoparasitic Infections: Risk Factors and Effects on Packed Cell Volume of Pregnant Women in Samaru, Zaria (Haemoparasitic Infections in Pregnant Women)

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Abstract: Pregnant women in Nigeria still suffer a great burden of *Plasmodium falciparum* and *Trypanosoma brucei gambiense* infections. However, malaria is one of the dominant diseases during pregnancy. These haemoparasites affect both maternal and foetal health. Structured questionnaires on socio-demographic and risk factors of haemoparasitic infections were administered on 125 consented pregnant women between the ages of 16-45 years in Samaru, Zaria. Each participant's venous blood sample (2ml) was collected and average packed cell volume (PCV) was determined. The samples were examined for haemoparasites on thin and thick blood smears stained with Giemsa dye using oil immersion (100x) objective of the light microscope. Only two types of haemoparasites were detected: *Plasmodium falciparum* (47.2%) and *Trypanosoma brucei gambiense* (4.0%), with a co-infection of 0.8%. *Plasmodium falciparum* infections were found across all the age-groups, but the youngest of pregnant women within 16-20 years and 21-25 years were the most infected. Women in their first trimester of pregnancy were significantly most infected with *P. falciparum* (P=0.047). Women without formal education (75.0%) as well as those that were unemployed (48.1%) had more haemoparasites. Living in houses surrounded by stagnant water and involvement in unprotected farming were risks for haemoparasitic infections. The prevalence of anaemia among the pregnant women was 45.6%. The presence of *P. falciparum* was statistically related to low PCV of 21-30% which was indicative of anaemia (P=0.000). Also, among the women with low PCV, there was higher occurrence of *T. b. gambiense* with a case of co-infection. Pregnant women should be encouraged to enroll for ante-natal checkup at early stage of pregnancy. Parasitological examination during the ante-natal visits should not be limited to malaria alone: other neglected tropical diseases should be checked for and treated adequately.

Keywords— Haemoparasites, *Plasmodium falciparum, Trypanosoma*, pregnant women, anaemia, packed cell volume.

1. INTRODUCTION

Haemoparasites are blood-dwelling parasites. This study focused on *Plasmodium falciparum* (*P. falciparum*) and *Trypanosoma brucei gambiense* (*T. b. gambiense*) which cause malaria and Gambian (or chronic) sleeping sickness in humans respectively. Although different species of *Plasmodium* are capable of causing malaria, *P. falciparum* is most prevalent in sub-Saharan Africa [1, 2]. Malaria is a life-threatening disease (especially in pregnant women and infants) and it is transmitted through the bites of infected mosquitoes [3, 4]. Malaria during pregnancy is an important public health problem, posing great risks on pregnant woman, foetuses, and newborn children [2]. Malaria alone has caused 75,000 to 200,000 infant deaths during pregnancy [3]. Trypanosomiasis is transmitted by infected tsetse fly of the genus *Glossina* [6]. There are 24 countries of west and central Africa known to be endemic for *T. b. gambiense*– responsible for more than 97.6% of reported cases of sleeping sickness [7]. Malaria and trypanosomiasis affect human health due to destruction of red blood cells which finally results in anaemia. Anaemia also leads to high risk of foetal death, low birth weight, poor neuro-development and increased mortality [8, 9, 10]. Infection with *T. b. gambiense* can lead to anaemia, wasting and lethargy. However, in an event where the parasite crosses the brain barrier, it can lead to coma or even death [11]. Pregnant women require full protection from malaria and trypanosomiasis through the 'roll back' malaria program, and aids from the WHO, UNICEF and other non-governmental organizations [4]. Malaria and trypanosomiasis remain threats to health; therefore adequate attention should be given toward their eradication. The study was focused on detection of haemoparasites in blood samples of pregnant women as well as to establish some risk factors and anaemia due to them.

2.0 MATERIALS AND METHODS

2.1 Study area and population

The study was conducted in three selected hospitals in Samaru, Zaria. The hospitals were Ahmadu Bello University Medical Center, Jama'a Clinic and Maternity, and Samaru Primary Health Care Centre. Pregnant women on ante-natal visits who consented to participate in this study were included. Permission and clearance for this study were

obtained from the hospitals and Sabon-Gari Local Area authority where the hospitals are located.

2.2 Questionnaire administration
Structured questionnaires were administered on the consented pregnant women on ante-natal visits to collect data on their socio-demography and exposure to some risk factors.

2.3 Collection of blood samples
Venous blood samples (2ml each) were collected from the 125 consented pregnant women using 2ml sterile syringe and needle by experienced hospital personnel. The samples were emptied into sterile ethylene-diamine-tetra-acetic acid (EDTA) bottles. The samples were taken immediately to the laboratory for examination (within 10 minutes) at the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

2.4 Determination of packed cell volume (PCV)
The PCV for each blood sample was determined. Two microhaematocrit centrifuge tubes were filled with the blood to three-fourth their lengths and sealed by means of Bunsen blue flame to the 2mm red mark [4]. The tubes were arranged and balanced on the microhaematocrit centrifuge and spun at a relative centrifugation force (RCF) of 12, 000 xg for 5 minutes. This was followed by adequate reading of the red packed cell (i.e., PCV) on Haematocrit reader and the average value was taken [4, 12].

2.5 Detection of haemoparasites in blood smears
For each blood sample, thin blood smear was prepared by placing a drop of the blood on a labeled clean, grease-free glass slide. A spreader inclined at an angle of 60^{o} was used to spread the blood to obtain a smooth tail-end. The blood smear was air-dried and fixed in methanol for 2 minutes. The smear was rinsed with water and stained with 10% Giemsa dye for 10 minutes. This was followed by rinsing with water before air-drying. For each sample also, thick blood smear was prepared using 2-3 drops of the blood and stained the same way. Both the thin and thick stained smears were covered with immersion oil and examined for haemoparasites using 100x objective of the light microscopy [4, 12].

2.5 Statistical analysis
Data obtained from administered questionnaires along laboratory results were subjected to statistical analyses by Chi Square (χ^2) and Odd Ratio (OR) at P = 0.05 using IBM SPSS version 21.0. [4].

3.0 RESULTS
Out of 125 blood samples of pregnant women between the ages of 16-45 years, overall prevalence of *Plasmodium falciparum* was 59(47.2%), *Trypanosoma brucei gambiense* was 5(4.0%) and co-infection between these two parasites was 1(0.8%) as shown in Table 1.

Age distribution of haemoparasites in the pregnant women had no statistical significance. Though *P. falciparum* infections occurred across all the age-groups, most of the infections were found in women of between 16-20 years and 21-25 years of age. On the other hand, *T. b. gambiense* infection was absent in women of 16-20 years, 31-35years, and 41-45 years of age. Its highest occurrence was found in women of 26-30 years of age. The single case of co-infection was found within the age group of 21-25 years (Table 2).

Based on some socio-demographic factors of the pregnant women, only the gestational age (also called trimester of pregnancy) was statistically associated with *P. falciparum* infections (P=0.047). Pregnant women in their first trimester were most infected with *P. falciparum* (72.7%) and *T. b. gambiense* (9.1%); while the least infections of *P. falciparum* and *T. b. gambiense* were among those in their third trimester (37.9%) and second trimester (2.1%) of pregnancy. The one co-infection case was in a woman in her third trimester (Table 3).

Pregnant women who had no formal education had the highest occurrence of *P. falciparum* (75.0%) and the least was among those with tertiary education (40.5%). However, *T. b. gambiense* was absent in women with no formal education, but gradually increased in those with higher level of education. The relationship was not statistically significant (Table 3).

Though unemployed pregnant women had slight higher risk of *P. falciparum* (48.1%, OR =1.3) and *T. b. gambiense* (4.7%, OR =1.1) infections than those that were gainfully employed, the relationship was not statistically significant (Table 3).

Secondigravidae (i.e., women in their second pregnancies) had higher occurrence of *P. falciparum* infections. But in the case of *T. b. gambiense*, the highest infection was found among the primigravidae (i.e, women in their first pregnancies). However, the single co-infection case was in a multigravida (i.e., a woman that has had more than two pregnancies). There was no related statistical significance in this comparison as shown in Table 3.

Also in Table 3, women that were married into polygamous homes had more *P. falciparum* infections (52.0%, OR =1.3) than those in monogamy. Contrarily, all the cases of *T. b. gambiense* infections as well as the co-infection occurred among those in monogamy (P>0.05).

The prevalence of anaemia among the pregnant women was 57(45.6%) as shown in Figure 1. Presence of *P. falciparum*

in the blood samples was statistically related to low PCV of 21-30% which is indicative of anaemia (P=0.000). Also, among these women with low PCV, there was higher occurrence of *T. b. gambiense* with a case of co-infection of the two parasites (Table 4).

From Table 5, all the risk factors examined did not show any significant statistical relationship with the haemoparasites. However, pregnant women who used untreated mosquito nets, and had stagnant water bodies around their homes, as well as those who engaged in farming had more *P. falciparum* infections. Similarly, pregnant women who engaged in farming and those whose homes were surrounded with stagnant water had more *T. b. gambiense* infections. But those who claimed to use ITNs were rather more infected with *T. b. gambiense*.

Table 1: Prevalence and co-infection of haemoparasites among pregnant women in Samaru, Zaria

Haemoparasites	Prevalence (%) n=125
Plasmodium falciparum	59(47.2)
Trypanosoma brucei gambiense	5(4.0)
Co-infection	1(0.8)

Table 2: Age-distribution of haemoparasites among pregnant women in Samaru, Zaria

Age (year)	Number examined	*P. *falciparum* Positive (%)	#T. b. *gambiense* Positive (%)	kCo-infection Positive (%)
16-20	9	6(66.7)	0(0.0)	0(0.0)
21-25	32	17(53.1)	1(3.1)	1(3.1)
26-30	37	13(35.1)	3(8.1)	0(0.0)
31-35	26	12(46.2)	0(0.0)	0(0.0)
36-40	14	8(57.1)	1(7.1)	0(0.0)
41-45	7	3(42.9)	0(0.0)	0(0.0)

*χ^2= 4.600, df= 5, P=0.467; #χ^2=3.800, df= 5, P=0.579; kχ^2=1.200, df= 5, P=0.889

Table 3: Haemoparasites in pregnant women in relation to their socio-demographic factors

Socio-demographic factor	Number examined	*P. *falciparum* Positive (%)	#T. b. *gambiense* Positive (%)	kCo-infection Positive (%)
Trimester				
First	11	8(72.7)	1(9.1)	0(0.0)
Second	48	26(54.2)	1(2.1)	0(0.0)
Third	66	25(37.9)	3(4.5)	1(1.5)
Level of Education				
Not formal	8	6(75.0)	0(0.0)	0(0.0)
Primary	27	14(51.9)	1(3.7)	0(0.0)
Secondary	48	22(45.8)	2(4.2)	1(2.1)
Tertiary	42	17(40.5)	2(4.8)	0(0.0)
Gainful Employment				
Employed	19	8(42.1)	0(0.0)	0(0.0)
Unemployed	106	51(48.1)	5(4.7)	1(0.9)
Gravidity				
Primigravidae	33	16(48.5)	2(6.1)	0(0.0)
Secondigravidae	26	15(57.7)	0(0.0)	0(0.0)
Multigravidae	66	28(42.4)	3(4.5)	1(1.5)
Type of marriage				
Monogamy	100	46(46.0)	5(5.0)	1(1.0)
Polygamy	25	13(52.0	0(0.0)	0(0.0)

Trimester: *χ^2= 6.112, df= 2, P=0.047; #χ^2=1.253, df=2, P=0.535; kχ^2=0.901, df=2, P=0.637
Education: *χ^2= 3.515, df= 3, P=0.319; #χ^2=6.406, df=3, P=0.939; kχ^2=1.617, df=3, P=0.656
Employment: *χ^2=0.233, df=1, P=0.629, OR=1.275; #χ^2=0.934, df=1, P=0.334, OR=1.050; kχ^2=1.617, P=0.671
Gravidity: *χ^2=1.774, df=2, P=0.412; #χ^2=1.499, df=2, P=0.473; kχ^2=0.901, df=2, P=0.637
Marriage: *χ^2=0.289, df=1, P=0.591, OR =1.27; #χ^2=1.302, P=0.254, OR=0.91; kχ2=0.252, P=0.616, OR=0.99

Table 4: Effects of haemoparasites on packed cell volume (PCV) of the pregnant women

PCV (%)	Number examined	*P. *falciparum* Positive (%)	#T. b. *gambiense* Positive (%)	kCo-infection Positive (%)
21-30 (anaemic)	57	45(78.9)	3(5.3)	1(1.7)
31-40 (normal)	68	14(20.6)	2(2.9)	0(0.0)

*χ^2= 2.376, df= 1, P=0.000; #$\chi^2$0.435, df= 1, P= 0.509; kχ^2= 1.203, df= 1, P=0.273

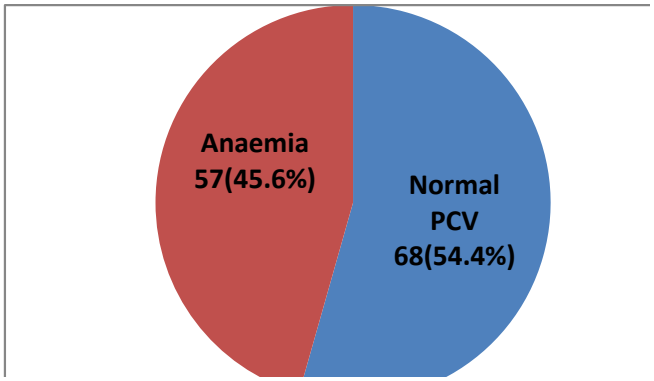

Figure 1: Prevalence of anaemia among pregnant women in Samaru, Zaria.

Table 5: Risk factors of haemoparasitic infections in pregnant women in Samaru, Zaria

Risk factor	Number examined	*P. falciparum* Positive (%)	#T. b. gambiense Positive (%)	kCo-infection Positive (%)
Use of mosquito net				
None	58	27(46.6)	0(0.0)	0(0.0)
Treated	63	29(46.0)	5(7.9)	1 (1.6)
Untreated	4	3(75.0)	0(0.0)	0(0.0)
Stagnant water				
Absent	59	24(40.7)	2(3.4)	0(0.0)
Present	66	35(53.0)	3(4.5)	1(1.5)
Farming				
No	115	54(47.0)	4(3.5)	1(0.9)
Yes	10	5(50.0)	1(10.0	0(0.0)

Mosquito net: *χ^2=1.285, df=2, P=0.526; #χ^2=5.126, df=2, P=0.077; kχ^2=0.992, df=2, P=0.609
Stagnant water: *χ^2=1.907, df=1, P=0.742, OR=1.6; #χ^2=0.108, P=0.742, OR =1.4; kχ^2= 0.90,P=0.342, OR=1.0
Farming: *χ^2=0.034, df=1, P=0.853, OR=1.1; #χ^2=1.019, P= 0.313, OR =1.1; kχ^2= 0.088, P=0.767, OR=1.0

4.0 DISCUSSION

Malaria parasites (*P. falciparum*) and trypanosomes (*T. b. gambiense*) were the only two haemoparasites detected in venous blood samples of pregnant women in Samaru, Zaria. The high prevalence of *P. falciparum* infections indicated a high susceptibility of the women to the parasite and abundance of infected mosquitoes. The WHO has long since advocated for the need of presumptive malaria treatment during pregnancy [13]. The 47.2% prevalence for *P. falciparum* in this study very closely agreed with the findings of Bishop and Aliyu [4] who reported a prevalence of 47.3% in Kano. However, prevalence of malaria ranges between 10.0 - 65.0% [3]. The disparities in all these reports can be explained by differences in ecology and sanitation systems of the various study locations. Though mosquitoes thrive favourably in tropical and sub-tropical countries, their abundance can be influenced or triggered by poor sanitation, stagnant water bodies around residential areas, thick bushes, poor housing and poor drainage system. Samaru in Zaria is a rural area characterized by some of the conditions previously mentioned, which encourage the rapid breeding of mosquitoes among other insect vectors. The low prevalence of *T. b. gambiense* among the pregnant women could be due to less exposure risks to scarce population of *Glossina* flies in the area, as compared to the cosmopolitan mosquitoes. Therefore, co-infection between these two parasites will always be low.

Most of the pregnant women were within the ages of 21-35 years. However, the youngest pregnant women of 16-20 years of age had most of the malaria cases probably due to their inexperience and negligent attitude to fully protect themselves from bites by insect vectors. Age was statistically insignificant in relation to haemoparasitic infections among the pregnant women. Any one exposed to the risk factors can become infected regardless of age. However, there was a report which indicated a significant higher occurrence of *P. falciparum* in pregnant women below 20 years of age [5].

Pregnant women in their first trimester (1-3 months of gestation) were the most infected with haemoparasites. Such women were coming for ante-natal visit for the first time and had been infected already prior to the visit. Majority of the women that were in their second and third trimesters had lesser prevalence of haemoparasites because in their routine ante-natal visits, health professionals educate them on how to prevent the infections; and those clinically detected with any haemoparasites are placed on careful medications. Therefore, haemoparasitic infections in pregnant women occur mostly during the first trimester (P=0.047). Another report on malaria in pregnant women had indicated high infections during their first and second trimesters [3].

Among the pregnant in this study, those that had no any formal education were the most infected with *P. falciparum*. The prevalence decreased as the level of education of the women increased. This had been similarly reported by Bishop and Aliyu [4]. Education is an important factor in the effort toward eradication of malaria among other neglected tropical diseases. Contrarily, *T. b. gambiense* infection was found to increase in women who had attained higher level of formal education. This finding was not supported statistically (P>0.05) as such these women could have gotten the infections due to multifactorial reasons.

Pregnant women that were unemployed had more infections with haemoparasites compared to those that were employed. Those with gainful employment will work in good environments, live in good housing and can afford proper nutrition and health care, with less exposure to risk factors. Unemployed women turn to other alternatives of raising money in order to meet their needs which could expose them more to risks of haemoparasitic infections. Some of them hawk or sell food items in market, streets or road sides until late evenings and nights when mosquitoes are very active; other women are involved in farming—all of which expose them to more contact with parasite-carrying haematofagous flies.

Malaria was higher among women in their second pregnancy (secondigravidae) while *T. b. gambiense* was more among the primigravidae, though this relationship was not statistically significant. Any woman can still get infected with haemoparasites regardless of the number of births, and

previous infection(s) will not confer immunity. It was observed that women from polygamous homes had more *P. falciparum* infections. In such a situation (of a large family size) there is increased demand for income to maintain the family, and the women may go into hawking and unprotected farming activities.

None of the risk factors examined in this study was statistically significant. However, more haemoparasitic infections were observed among those that either do not use mosquito nets during sleep or use untreated ones. The presence of stagnant water around the house and involvement in (unprotected) farming activities exposed the pregnant women to infections with parasites (OR >1). However, significant association between *P. falciparum* and stagnant water had been established [5].

The prevalence of anaemia in this study was relatively very high. There is large evidence of anaemia in pregnant women due to haemoparasites [4]. The occurrence of anaemia during pregnancy is of great public health problem [14], which should be prevented. The cause of anaemia can be multifactorial [9, 10]. However, the presence of haemoparasites in their blood can result in anaemia (P=0.000). Many findings have proved the involvement of *P. falciparum* and *T. b. gambiense* as causes of anaemia [3, 4, 10, 15, 16]. Pregnant women in Nigeria are in danger of multi-parasites burden. Therefore, parasitological screening during ante-natal visits should not be limited to malaria alone: other neglected tropical diseases should be checked for and treated.

5.0 CONCLUSION

Pregnant women in Nigeria still suffer a great burden of haemoparasitic infections. Among the pregnant women in Samaru, Zaria, only two types of haemoparasites were detected in their venous blood samples: *Plasmodium falciparum* (47.2%) and *Trypanosoma brucei gambiense* (4.0%), with a co-infection of 0.8%. *Plasmodium falciparum* infections occurred across all the age groups but the youngest of pregnant women within 16-20 years and 21-25 years of age had most of the infections. Those women in their first trimester of pregnancy were significantly infected with *P. falciparum* (P=0.047). The pregnant women that had no formal education as well as those that were unemployed had more haemoparasites. Living in houses surrounded by stagnant water and involvement in unprotected farming increased the risk for haemoparasitic infections (OR >1). The prevalence of anaemia among the pregnant women was 45.6%, as the presence of *P. falciparum* was statistically related to low PCV of 21-30% which is indicative of anaemia (P=0.000). Also, among these women with low PCV, there was higher occurrence of *T. b. gambiense* with a case of co-infection of the two parasites. Pregnant women should be encouraged to enroll for ante-natal checkup at early stage of pregnancy. Parasitological examination during the ante-natal visits should not be limited to malaria alone: other neglected tropical diseases should be checked for and treated properly.

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Conflict of Interest

The authors hereby declare that there is no any financial interest or conflict of interest in this study.

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