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Hypoglycemia and Anemia Associated with Malaria among Pregnant Mothers living with HIV attending Aminu Kano Teaching Hospital, Kano State-Nigeria

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Abstract

Human Immunodeficiency Virus (HIV) and Malaria each interact with the host immune system, resulting in complex activation of immune cells. Human Immunodeficiency Virus (HIV) positive patients are predisposed to severe malaria with marked reduction of CD4 cells count and increase in plasma viral load. An assessment was carried out to examine the relationship between hypoglycemia, HIV infection and malaria prevalence in pregnant mothers as well as parasitemia in relation to severity of infection. A hospital based case-control study was carried out. Screening was done at the antenatal and ART clinics, Aminu Kano Teaching Hospital through routine voluntary and confidential HIV testing. After obtaining ethical approval, a total of 200 HIV positive and equivalent numbers of HIV negative pregnant mothers were selected from whom socio-demographic and biomedical data was collected using structured Questionnaire. Blood samples were aseptically collected in an EDTA container. Blood smears (Thick and thin) for malaria screening, Packed Cell Volume (PCV) and Blood Glucose Level were systematically performed using standard procedure. The results were analyzed using Microsoft excel and OpenEpi statistical software version 2.3 and p-value of ≤ 0.05 was considered significant. Malaria prevalence was 141(70.5%) in HIV positive and 110(55.0%) in HIV negative clients. The severity of infection was 41(29.1%) and 5(4.5%) in HIV positive and HIV negative respectively with significant difference (p <0.05).Cases of hypoglycemia (Blood glucose level ≤ 2.2mmol/L) were observed to be higher among the malaria positive in both the HIV positive and HIV negative clients (100%). There was no significant difference with the severity of infection (p>0.05).The higher prevalence of severe malaria infection among HIV positive clients obtained in this study reveals that HIV positive pregnant mothers had clear evidence of greater exposure to severe malaria in this study area. Therefore strategies to reduce the severity of malaria during pregnancy should be reinforced especially in area of high HIV prevalence by both governmental and non-governmental agencies.

***Key Words**: Hypoglycemia, Parasitaemia, Anaemia, Malaria Human Immunodeficiency Virus, Blood glucose*

INTRODUCTION

The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission and thus with the level of pre-existing immunity already acquired by the pregnant woman. Each year in malaria endemic areas of tropical Africa an estimated 25 million women become pregnant (Whitworth *et al.*, 2000) in these areas, most adult women have developed sufficient immunity such that, even during pregnancy, *Plasmodium falciparum* infection does not usually result in fever or other clinical symptoms. The health of women in malaria-endemic areas is further affected by HIV (Bicego *et al.*, 2002). A meta-analysis of studies on co-infection in pregnancy (Ter Kuile *et al.*, 2004) demonstrates that HIV infection impairs the ability of pregnant mothers to control *P. falciparum* infection. They are more likely to develop clinical and placental malaria; more often have detectable malaria parasitaemia and have higher malaria parasite densities.

Regardless of the progress made in reducing malaria cases and deaths, 97% of Nigeria's populations are at risk while the remaining 3% of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria.

This compares with 215,000 deaths per year in Nigeria among HIV/AIDS (Nigeria Malaria Fact Sheet, 2011). The global burden of mortality is dominated by countries in sub-Saharan Africa, with the Democratic Republic of the Congo and Nigeria together accounting for more than 35% of the global total of estimated malaria deaths (WHO, 2015). This is because of the five *Plasmodium species* that infect humans (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*) (Cox-Singh and Singh, 2008), in Africa, the majority of infections are caused by *Plasmodium falciparum*, the most dangerous of the five human malaria parasites and most virulent (Snow *et al.,* 2003) as it multiplies so fast and is able to sequester in small blood vessels causing damage to the brain and other organs thus responsible for the majority of morbidity and mortality due to malaria (Freimanis *et al.,* 2013). It is also because the most effective malaria vector, the mosquito *Anopheles gambiae* is the most widespread in Africa and the most difficult to control (WHO, 2002).

Malaria in pregnancy being more severe also turns out to be more fatal, the mortality being double (13%) in pregnant compared to the non-pregnant population (6.5%) (Bernard *et al.,* 2008). In Africa, perinatal mortality due to malaria is at about 1500/day while in pregnant women the morbidity due to malaria includes severe maternal anemia, fever illness, hypoglycemia, cerebral malaria, pulmonary edema, puerperal sepsis, 20-40% of all babies born may have a low birth weight in malaria endemic area and mortality can occur from severe malaria and haemorrhage (WHO, 2014). Malaria, therefore, is seriously hindering the achievement of Millennium Development Goal's (MDG) Goal 5 (improve maternal health).

Therefore this study was aimed at detection of malaria parasites in pregnant mothers with and without HIV infection, to determine the density of malaria parasitaemia among the study subjects and also to determine the anemia status and Blood Glucose level among the study subjects.

MATERIALS AND METHODS

Study Area and Study population

The study was carried out at Aminu Kano Teaching Hospital, Kano State North-Western Nigeria located between latitude 11° and 10°N and longitude of 8°E and 8°E of the prime meridian. The state covers a land mass of 499km^2 (FOS, 2006). It has a population of 9,383,682 million people (National Bureau of Statistic, 2007). The hospital is located along Zaria road which served as the Federal Government Teaching Hospital in the state since its establishment in 1988and receives patients from Kano and other neighboring states. The study population comprised of 200 confirmed HIV positive and 200 HIV negative (control) pregnant mothers attending the antenatal clinic (ANC) which received an average 90 client in a week and the S.S. Wali Virology Center has which has over 19,000 enrolled HIV clients.

Ethical Approval and Informed Consent

Ethical clearance was granted by Aminu Kano Teaching Hospital (AKTH) Research Ethics Review Committee. Participants were well informed about the study and it relevance, and each study participants provided informed consent before sampling.

Sample Collection and Processing

Structured questionnaires were used to obtain clinical data such as history of persistent fever, worsening headache and urine color. Five milliliters of blood samples was aseptically collected from each clientinto an EDTA container for malarial screening, Packed Cell Volume determination and Blood Glucose Level test.

Parasitological Examination of Malaria

Presence of malaria parasite and parasitaemia was examined among the study population using microscopic techniques. Thick and thin blood smear were prepared, air dried, thin film was fixed using methanol, and blood smears were stained using 3% Giemsa-stained. Films were examined microscopically under X100 oil immersion objective. Thick blood film was used to calculate the level of parasitaemia in *Plasmodium* parasite positive slide where by infected erythrocytes was counted in relation to a predetermined number of WBCs and an average of 8000/µl was taken as standard. This was carried out in accordance with the method described by (WHO, 2000; NMCP, 2005; IMMC, 2011).

Packed Cell Volume (PCV)

Haematocrit or PCV which is the volume of red cells expressed as a percentage of whole blood was determine using microhematocrit method as described by Purves *et al.,*(2004); Williams *et al.,*(2009). Where by anti-coagulated blood was drawn into a plain capillary tube filled to about 3/4th length. Filled tube was sealed using clay to about 2mm deep and centrifuged using microhematocrit centrifuge at a speed of 10,000 RPM for five minutes. Centrifuged tube was read using haematocrit reader and the percentage of Packed Cell Volume (PCV) was determined.

Packed Cell Volume (PCV) of <25% in the presence of parasite count ≥10000/µl was considered as indicator of severe malaria.

Blood Glucose Level Test

The blood sugar concentration or blood glucose level is the amount of glucose (sugar) present in the blood of human. Oxidase/peroxidase method was used for the test using Glucose liquizyme reagent which gave a concentration of 5.5mmol/L by the manufacturer. After preparation of Test (by adding 10µl of pipette blood and 1000µl of glucose liquizyme reagent into a centrifuged tube); preparation of Blank (by adding 10µl of buffered and 1000µl of glucose liquizyme reagent into a centrifuged tube); and preparation of Standard (addition 10µl of standard reagent and 1000µl of glucose liquizyme reagent into centrifuged tube), the mixture was shaken vigorously and incubated at 37^0c using water bath for 10 minutes. Extinctions were read using spectrometer at 490nm against the reagent blank.

The calculation was based on the formula below:

$$\frac{\textit{Optical Density of the Test}}{\textit{Optical Density of Standard}} \textit{X Concentration of Standard}$$

Yang *et al.*,(2012).

Blood Glucose Level of <2.2mmol/L in the presence of parasite count ≥10,000/µl was considered as indicator for severe malaria (hypoglycemia).

Data Analysis

Open – Epi version 2.3 statistical soft ware was used to calculate the minimum sample size of 400. Relationship between parasitaemia and packed cell volume (PCV), parasitaemia and Blood glucose level and parasitaemia and clinical symptoms were analyzed using chi-square. Significant difference was set at $P<0.05$.

RESULTS

Four hundred pregnant mothers were enrolled for the study. Of the 400 (200 HIV positive and 200 HIV negative), 251(62.8%) were positive for Malaria parasite as determined by microscopy. HIV-positive clients were found to have the highest prevalence 141(70.5%) while 110(55.0%) were malaria positive among the HIV-negative clients (Table 1). Based on the severity of the infection, of the 141(70.5%) HIV positive and 110(55.0%) HIV negative clients with malaria, 41(29.1%) and 5(4.5%) had malaria density of ≥10,000µl respectively (Table 1). Statistical analysis with Chi-square test at 5% level of significances show that there is a significant differences in the rate of infection and the severity between HIV-negative and HIV-positive clients ($p<0.05$) (Table 1).

Severity of anemia based on Packed Cells Volume (PCV) seem to be very high among the malaria positive clients especially those with higher parasitaemia among both the HIV positive and HIV negative clients (100% both). Out of the 41 (100%) of clients examined with PCV≤ 25%, 27(65.9%) had severe parasitaemia among the HIV positive clients and of the 16(100%) HIV negative, 2(12.5%) had higher parasitaemia (Table 2).

Hypoglycemia (Blood Glucose Level ≤ 2.2mmol/L) was very high among the malaria positive in both the HIV positive and HIV negative clients (100%). Result was not significant with the severity of infection ($p>0.05$) (Table 3).

The results on clinical symptoms of malaria presented by the clients is summarizes in Table 4. Of the 172 (86%) HIV positive clients presented with worsening headache, 129 (75%) are malaria positive compared to 98 (71.5%) among the 137 (68.5%) of the HIV negative pregnant mothers and the differences was not significant ($p>0.05$). Persistence of fever even after 24 hours of initial treatment with antimalarial drugs was reported in 77(38.5%) of the HIV positive clients and 76 (98.7%) are malaria positive as compared with 93 (46.5%)among the HIV negative clients with fever, and 72 (77.4%) are malaria positive.The most frequently reported symptoms in both group is colour change in urine (Hyperbilirunemia). For malaria positive, 100% Black for both, 78.0% and 69.6% Brown, 73.2% and 69% and Red colour for HIV positive and HIV negative clients respectively. This shows a significant difference between the HIV positive and HIV negative pregnant mothers ($p<0.05$).

Table 1: Malaria Parasite prevalence in relation to parasitaemia among the study Clients

P - density	HIV Negative Pregnant	HIV Positive Pregnant	*P*- value
Mild	31(28.2)	11(7.8)	< 0.000001*
Moderate	74(67.3)	89(63.1)	
Severe	5(4.5)	41(29.1)	
Total	110(55)	141(70.5)	

Keys:
P-Density = Parasite density /µl
Moderate = Parasite density of 5000-9999
* = Significant difference
Mild = Parasite density of 1-4999
Severe = Parasite density of ≥10000

Table 2: Packed Cell Volume in relation to Malaria Parasite prevalence and parasitaemia

PCV (%)	HIV Negative Pregnant		HIV Positive Pregnant		*P*- value
	No Examined	No positive	No Examined	No positive	
< 25	16	16(100)	41	41(100)	< 0.000001*
26 – 30	66	52(78.8)	62	50(80.6)	
≥ 31	118	42(35.6)	97	50(51.5)	
Total	200	110(55)	200	141(70.5)	

Key:
PCV-Packed Cell Volume in % *= Significant Difference (p<0.05)

Table 3: Blood Glucose Level in relation to Malaria Parasite prevalence and parasitaemia

BGL	HIV Negative Pregnant		HIV Positive Pregnant		*P*- value
	No Examined	No positive	No Examined	No positive	
< 2.2	21	21(100)	34	34(100)	0.6336
2.3 – 5.0	107	73(68.2)	108	88(81.5)	
>5.1	72	16(22.2)	58	19(32.6)	
Total	200	110(55)	200	141(70.5)	

Key:BGL= Blood Glucose Level in mmol/l

Table 4: Prevalence of Malaria in relation to some clinical symptoms among study Clients

Clinical Symptom	HIV negative		HIV positive		*P*- value
	No Examined	No Positive	No Examined	No Positive	
Urine color					0.02243*
Black	6(3)	6(100)	18(9)	18(100)	
Brown	46(23)	32(69.6)	41(20.5)	32(78.0)	
Red	29(14.5)	20(69)	56(28)	41(73.2)	
No color change	119(59.5)	52(43.7)	85(42.5)	50(58.8)	
Worsening headache					0.5214
Yes	137(68.5)	98(71.53)	172(86)	129(75)	
No	63(31.5)	12(19.0)	28(14)	12(42.9)	
Persistence of fever					0.06484
Yes	93(46.5)	72(77.4)	77(38.5)	76(98.7)	
No	107(53.5)	38(35.5)	123(61.5)	65(52.8)	

Key: * = Significant Difference (p<0.05)

DISCUSSION

Malaria and HIV remain a major public health problem in most resource constrained countries and the interaction between the two poses major public health problems (WHO, 2008). This work was carried out to examine indicators of severe malaria (anemia, hypoglycemia and higher parasitaemia) among HIV positive pregnant mothers.

The clients were categorized into HIV positive and HIV negative (control). Our study showed that HIV positive pregnant mothers have higher prevalence of malaria infection 141(70.5%) than the HIV negative pregnant mothers 110(55.0%). This is quite considerable and shows that malaria infection still remains a threat to HIV patients in this region of the country not withstanding all the control methods in place. This is in conformity with the report of WHO, (2011) that the control of malaria is becoming increasingly difficult and Nigeria currently holds the largest share of the world's burden of malaria. The result also agrees with the findings of Gajida *et al.*(2011) in which highest prevalence of 23.3% was observed among the HIV positive as compared to 8.8% among HIV negative clients. Although the percentage was lower but the researcher restricted only to primigravidae been reported from the same AKTH.

The severity of malaria (parasite density of ≥10,000/µl) was 41(29.1%) and 5(4.5%) among the HIV positive and HIV negative clients respectively. Higher prevalence obtained among the HIV clients may be due to their relatively weak immunity status as observed by a cohort study conducted in Kenya by Ayisi *et al.*(2004) that HIV positive pregnant mothers had higher rates of antenatal malaria transmission than the HIV negative pregnant mothers.

The result also agrees with the observation of Whitworth *et al.,*(2000) which says that HIV infection predisposes to more frequent episode of symptomatic malaria and more episode of severe or complicated malaria. Statistical analysis shows that the rate of malaria infection is significantly high (p<0.05).

Maternal anemia is one of the indicators of severe malaria. In this study, using a Packed Cell Volume as a gold standard criterion for the determination of Haematocrit level it was observed that those with PCV≤ 25% (severe anemia) have the highest severe parasitaemia in both the HIV positive and control. Out of the 70.5% parasitaemic HIV positive clients, 29.1% had PCV ≤25% compared to 14.5% of the 55.0% parasitaemic HIV negative clients. These agrees with a cohort studied conducted in western Kenya by Ayisi, *et al.,*(2004) and Malawi by Rogerson, *et al.,*(2004) were they described a synergistic interaction between malaria and HIV such that pregnant women with dual infection are at significantly greater risk of anemia than pregnant mothers with malaria or HIV infection alone. The increased risk of anemia that occurs in co-infected pregnant mothers may be due to the higher parasite densities and longer duration of malaria infection that occurs in HIV positive pregnant mothers.

Hypoglycemia (low Blood Glucose level ≤ 2.2mmol/L) is one of the indicators of severe malarias as suggested by WHO, (2000). From the result obtained it shows that blood glucose level of ≤2.2mmol/L was 34 (100%) in HIV positive pregnant women and 21 (100%) in HIV negative women with malaria. This implies that determination of blood glucose level for the assessment of severe malaria gives a realistic result as suggested by WHO, (2000). Its use should therefore be encouraged especially among HIV positive pregnant mothers.

CONCLUSION

From the study conducted, it has been observed that, HIV positive pregnant mothers had clear evidence of greater exposure to severe malaria 41(29.1%) than the HIV negative 5(4.5%). All the indicators of severe malaria show a significant difference between HIV positive and HIV negative (p<0.05) with the exception of BGL and Fever that shows no significant difference (p>0.05).

Recommendation

In view of the severity of the infection among pregnant mothers living with HIV, the study recommends screening for malarial parasite during antenatal care as well asassessment of parasitaemia, anemia and hypoglycemia levels among malaria positive clientsfor complete management.

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