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Studies on Parasitaemia, Immunologic and Virologic Indices among Pregnant Mothers Living with HIV Attending Aminu Kano Teaching Hospital, Kano State-Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NMS and AUM designed the study. Author AUM participated in data collection and designed the manuscript while author MY provided critical advice on data analysis and manuscript writing. Author NMS reviewed files and participated in design and critical review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the presence of malaria parasite and also examine the relationship between HIV infection and the severity of malaria.
Study Design: A hospital based prospective cohort study was carried out among 200 pregnant mothers living with HIV at Aminu Kano Teaching Hospital through routine voluntary and confidential HIV screening.
Place and Duration of Study: The study was conducted at Aminu Kano Teaching Hospital, Kano between July and December, 2016.
Methodology: Biomedical data was obtained and blood samples were aseptically collected in an

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EDTA container. Malaria screening, CD4 - T cell Count and Viral load were systematically performed using standard procedures. Chi-square test was used to establish statistical association between study variables where p-value of ≤ 0.05 was considered significant.

Results: Malaria prevalence was 141(70.5%) with 41(29.1%) having parasitaemia of ≥ 10,000/µl. Primigravidae had 69(48.9) and Multigravidae had 72(51.1) prevalence rates. The severity of infection was 27(65.9%) among those with CD4 count ≤ 200 cells/mm^3. On the basis of viral load estimation, severity of the infection was higher 28(68.3%) among those with viral load ≥ 10000 copies/ml.

Conclusion: In view of the higher prevalence of malaria infection among pregnant mothers living with HIV, there is need for Antenatal care service to necessitate routing screening of malaria parasite and level of parasitaemia along with CD4 cells count and viral load test since the severity of malaria infection increased due to immunosuppression.

Keywords: Plasmodium; Primigravida; human immunodeficiency virus; parasitaemia.

1. INTRODUCTION

Human Immunodeficiency Virus and malaria have similar global distributions, with the majority of those affected living in sub-Saharan Africa, the Indian subcontinent, and Southeast Asia. Given the overlap of their geographic distribution and resultant rates of co-infection, interactions between the two diseases pose major public health problems. Together they accounted for over 3 million deaths in 2007 [1], and millions more are adversely affected each year. Malaria and HIV/AIDS are both diseases of poverty and contribute to poverty by affecting young people who would otherwise enter the workforce and contribute to the local economy [2]. Dual infection therefore, makes life difficult to the mother, the infant and the treating physician due to higher frequency (parasitaemia and viral load) and with resulting outcome of low birth-weight, prenatal death and maternal anemia [3].

Malaria is a devastating mosquito-borne febrile illness which is caused by the intracellular protozoan's parasite of the genus Plasmodium that invades and multiplies in the liver and red blood cells (RBCs) during its life cycle in man [4]. The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and progress to acquired immunodeficiency syndrome (AIDS) [5]. Infection with HIV affects vital cells in the human immune system such as CD4+ T cells [6]. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections [7]. As HIV reproduces within the body, viral load increases and HIV destroys the CD4+ T-cells and thus lowers the amount of cells present. Generally, the higher the HIV viral load, the more CD+ T-cells is being destroyed [8].

Most women in their first or second pregnancy are at higher risk of severe or complicated malaria than during subsequent pregnancies. However, this protective effect is diminished in HIV positive pregnant mothers who, irrespective of the number of pregnancies remain susceptible to the negative consequences of malaria infection. However, malaria complication associated with HIV positive pregnant mothers are rarely investigated during antenatal care In order to attain complete management and also to prevent vertical transmission. An assessment was carried out to determine the relationship between HIV infection and malaria prevalence in pregnant mothers, parasitaemia in relation to severity of infection as well as relationship between HIV and severe malaria across gravidies.

2. MATERIALS AND METHODS

2.1 Study Design and Study Area

This was a hospital- based prospective study conducted between July and December, 2016 at Aminu Kano Teaching Hospital due to the client flow of this facility which is situated in Kano State North-Western Nigeria. The area is known for malaria endemicity due to the large number of malaria vectors found. The hospital is located along Zaria road and receives patients from Kano and other neighboring states. Patients from far states are often received as well. The hospital operates antenatal clinic (ANC) attending to an average 90 clients in a week. The S.S. Wali Virology Center has over 19,000 enrolled HIV clients while prevention of mother-to-child transmission of HIV (PMTCT) programme has been fully integrated into the hospital obstetrics service.

2.2 Study Population and Sample Collection

The study was carried out among pregnant mothers living with HIV attending the Prof. S.S. Wali Virology center of Aminu Kano Teaching Hospital and antenatal clinics (ANC). Two hundred participants were randomly selected based on a systematic random sampling method to avoid bias. Socio-demographic data and biomedical history were collected using a structured questionnaire which includes information on gravid status, parity, previous antimalarial use in pregnancy and other research-related data such as ART and level of ART. About 5ml of blood sample was aseptically collected into EDTA (Ethyl diamine tetracetric acid) for HIV Tests, malaria parasite screening, CD4 cell count and viral load test.

2.3 Ethical Consideration and Informed Consent

The study was conducted according to ethical standards for human studies and approved by the Aminu Kano Teaching Hospital Research Ethics Review Committee (AKTH/MAC/SUB/12A/P-3/VI/1488). Informed consent was obtained from all participants to take part in the study, filled questionnaire and to give blood, and for the data to be analyzed and published. The purpose, risk, method and benefit of the research were fully explained to the clients.

2.4 Laboratory Analysis

2.4.1 HIV screening

All blood samples collected were re-screened for Human Immunodeficiency Virus (HIV) test using national guidelines for rapid-test as outlined in the UNAIDS/WHO, 2004 using serial algorithm [9].

Determine was used for initial screening while UNI-GOLD was used as confirmatory test. Sample that yielded discordant results in the two tests were resolved using stat- Pak as the tie breaker.

2.4.2 Microscopic examination of malaria parasite

In order to determine the prevalence of malaria parasite and parasitaemia among the study population, microscopic examination of blood films was performed. Blood films (thick and thin) were immediately prepared, dried, fixed (thin film), peripheral blood smears were stained with 3% Giemsa reagent and The slide was examined microscopically under X100 oil immersion objective. Parasitaemia was determined by counting the asexual parasites against 200 white blood cells (WBC's) assuming a count of 8,000 leukocytes per microlitre of blood. This was carried out in accordance with the method describe by [10,11,12]. Malaria slides read were checked by an experienced laboratory scientist. Parasite counts of 10,000 or more per mm^3 were considered as indicators of severe malaria.

2.4.3 CD4 T – Cells count determination (Pertec ® Cyflow (Flow Cytometry Method)

In order to assess the density of malaria parasite in relation to immune status (CD4 cell Count), the determination of CD4 T Cell count was carried out for all the HIV positive pregnant mothers in this study using Flow Cytometry Method based on whole blood lysis method for the analysis of lymphocyte populations a product of Pertec ® Made in Germany was used. Results obtained were subsequently categorized into three groups, namely; immunocompetent (CD4 ≥ 500 cell/mm3), Asymptomatic (CD4 count 200-<500cell/mm3) and AIDS (CD4 ≤ 200 cell/mm3) [8].

2.4.4 Viral load estimation (COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) automated real time PCR)

In order to assess the density of malaria parasite in relation to plasma viral load, the determination of plasma viral load estimation was carried out for all the HIV positive pregnant mothers using fully automated Real Time PCR in the COBAS AmpliPrep/COBAS TaqMan 48 (CAP/CTM) by the use of AMPLILINK software as described by the manufacturer. Results obtained were subsequently categorized into three groups, namely; low plasma viral load (<20 RNA copies/ml), moderate plasma viral load (21-10000 RNA copies/ml) and higher plasma viral load (>10000 RNA copies/ml).

2.5 Statistical Analysis

Data generated were compiled in Excel spread sheet and were analyzed using OpenEpi statistical software version 2.3. Statistical association or a lack thereof between participant variable and prevalence rates of severe malaria were determined using Chi square to estimate p-

value with 95% confidence interval. A p value of ≤0.05 was set as indicator of statistical significance.

3. RESULTS AND DISCUSSION

3.1 CD4 Cell Count in Relation to Prevalence of Malaria and the Severity of Infection

Table 1 summarizes the finding on malaria prevalence and level of the infection in relation to CD4 cell count.

On the basis of prevalence of malaria in relation to CD4 cells count, out of the 200 HIV positive clients with 141(70.5%) malaria prevalence rate, 44(22%) were categorized as AIDS patients with CD4 cells count of ≤200 cell/ml and malaria prevalence rate was 40(90.9%), followed by 136(68%) clients categorized as asymptomatic with CD4 count above 200 but below 500 cell/ml and malaria prevalence of 96(70.6%). The last category with CD4 count of ≥500 cell/ml were found to be 20(10%) and considered as immunocompetent with only 5(25%) malaria prevalence rates.

Among the AIDS patients (those with CD4 count ≤200 copies/ml), higher percentage was obtained among those with higher parasitaemia 27(67.5%), followed by moderate 12(30%) and only one 1(2.5%) had mild infection. With regards to asymptomatic clients, higher percentage was obtained among those with moderate parasitaemia 72(75%), followed by severe 14(14.6%) and 10(10.4%) had mild infection. All immunocompetent clients had only moderate infection 5(100%). Result shows significant difference (p <0.05) when subjected to chi-square analysis.

3.2 Viral Load in Relation to Prevalence of Malaria and the Severity of Infection

Table 2 shows results of the viral load in relation to malaria prevalence and level of the infection. Of the 141(70.5%) malaria positive clients, most of the clients 37(100%) had viral load ≥ 10,000 RNA copies/ml followed by 55(76.4%) with moderate plasma viral load (21-10000 RNA copies/ml) and 49(54.4%) had viral load of <20 RNA copies/ml.

Among clients with low plasma viral load, higher percentage was obtained among those with moderate parasitaemia 40(81.6%), followed by mild 7(14.3%) and only few 2(4.1%) had severe infection. With regard to clients with moderate plasma viral load, higher percentage was obtained among those with moderate parasitaemia 41(74.5%), followed by severe 11(20%) and 3(5.54%) had mild infection. Based on clients with higher plasma viral load, prevalence rate was higher among those with severe parasitaemia 28(75.7%) followed by moderate 8(21.6%) and the least 1(2.7%) had mild infection. Result shows significant difference (p <0.05) when subjected to chi-square analysis.

Table 1. Prevalence of malaria parasite in relation to CD4 Cells/mm^3 & Parasitaemia/mm^3

CD4 count	No examined	No positive	Parasite density (mm^3)		
			Mild	Moderate	Severe
≤ 200	44	40(90.9)	1(2.5)	12(30)	27(67.5)
201-499	136	96(70.6)	10(10.4)	72(75)	14(14.6)
≥ 500	96	5(25)	0(0)	5(100)	0(0)
Total	200	141(70.5)	11(7.8)	89(63.1)	41(29.1)

Key: Mild = Parasite density of 1-4999 Moderate = Parasite density of 5000-9999 Severe = Parasite density of ≥10000; P-value = <0.00000001

Table 2. Prevalence of malaria parasite in relation to viral load & Parasitaemia/mm^3

Viral load	No examined	No positive	Parasite density (mm^3)		
			Mild	Moderate	Severe
< 20	91	49(53.8)	7(14.3)	40(81.6)	2(4.1)
21-10000	72	55(76.4)	3(5.5)	41(74.5)	11(20)
>10000	37	37(100)	1(2.7)	8(21.6)	28(75.7)
Total	200	141(70.5)	11(7.8)	89(63.1)	41(29..1)

Key: Mild = Parasite density of 1-4999 Moderate = Parasite density of 5000-9999 Severe = Parasite density of ≥10000 P-value = 0.0096

3.3 Prevalence of Malaria and the Severity of Infection in Relation to Clients Receiving ART Regimen and the Level of ART

Based on ART regimenn, malaria prevalence was found to be highest among clients not on drugs at all 10(83.3%) followed by clients receiving ART 131(69.7%). Severity of malaria was 6(60%) among clients not on ART regimen followed by moderate infection 4(40%). On the basis of clients receiving ART, prevalence rate was found to be highest among clients with moderate infection 85(64.9%) followed by clients with severe infection and was least among clients with mild infection 11(8.4%). Result shows no significant difference (p >0.05) (Table 3).

On the level of ART (Antiretroviral therapy), malaria prevalence was highest among clients receiving 2nd line regimen 29(90.6%) followed by client without ART regimenn 10(83.3%) and then clients receiving 1st line regimen 102(65.4%). Severity of malaria (Parasite density of ≥10000) was higher among clients on 2nd line regimen 19(65.5%), followed by clients on no ART regimen 6(60%) and the least among clients on 1st line regimen 16(15.7%). This shows significant difference (p <0.05) (Table 4).

3.4 CD4 Cell Count in Relation to Prevalence of Malaria among Gravidity

Based on gravidity, malaria prevalence was 69(48.9) among primigravidae and 72(51.1) among multigravidae HIV positive pregnant mothers. On the basis of prevalence of malaria in relation to CD4 cells count among the gravidity, Multigravidae had 24 (60%) malaria prevalence rates as compared to 16(40%) primigravidae among the AIDS patients with CD4 cells count of ≤200 cell/ml. Among the asymptomatic clients with CD4 count above 200 but below 500 cell/ml, primigravidae had 49(51.0%) and multigravidae had 47 (49.0%) malaria prevalence rates.

The last category with CD4 count of ≥500 cell/ml (immunocompetent), highest prevalence rate was obtained among primigravidae 4(80%) and the least among the multigravidae 1(20%). The result was not significant when subjected to chi-square analysis (p <0.05) (Table 5).

Table 3. Prevalence of malaria parasite in relation to art & parasite density (/µl)

ART	No examined	No positive	Parasite density (mm^3)		
			Mild	Moderate	Severe
Yes	188	131(69.7)	11(8.4)	85(64.9)	35(26.7)
No	12	10(83.3)	0(0)	4(40)	60(60)
Total	200	141(70.5)	11(7.8)	89(63.1)	41(29.1)

Keys: ART =Antiretroviral therapy No = Patients not taking ART

Table 4. Prevalence of malaria parasite in relation to level of art & parasite density/µl

Level of art	No examined	No positive	Parasite density (mm^3)		
			Mild	Moderate	Severe
No	12	10(83.3)	0(0)	4(40)	6(60)
1st line	156	102(65.4)	10(9.8)	76(74.5)	16(15.7)
2nd line	32	29(90.6)	1(3.4)	9(31.0)	19(65.5)
Total	200	141(70.5)	11(7.8)	89(63.1)	41(29.1)

Keys: ART =Antiretroviral therapy First line = 1st line regimen 2nd line = Second line regimen
P-value = 0.0096

Table 5. Distribution of malaria positive cases based on CD4 Cell counts among primigravidae and multigravidae pregnant mothers living with HIV

CD4 cells count	No examined	No positive	Gravidity		
			Primigravidae	Multigravidae	*P*- value
<200	44	40(90.9)	16(40)	24(60)	0.1846
200-499	136	96(70.6)	49(51.0)	47(49.0)	
≥500	96	5(25)	4(80)	1(20)	
Total	200	141(70.5)	69(48.9)	72(51.1)	

3.5 Viral Load in Relation to Prevalence of Malaria among Gravidity

Based on gravidity, malaria prevalence was 69(48.9) among primigravidae and 72(51.1) among multigravidae HIV positive pregnant mothers. On the basis of prevalence of malaria in relation to viral load among the gravidity, primigravidae had 30 (61.2%) malaria prevalence rates as compared to 19(38.8%) multigravidae among the clients with viral load <20 copies/ml. Among clients with viral load 21-10000 copies/ml, primigravidae had 24(43.6%) and multigravidae had 31(56.4%) malaria prevalence rates. The last category with viral load >10000 copies/ml, highest prevalence rate was obtained among multigravidae 22(59.5%) and the least among the primigravidae 15(40.5%). The result was significant when subjected to chi-square analysis (p <0.05) (Table 6).

This study has revealed a 70.5% prevalence of malaria among 200 randomly selected pregnant mothers living with HIV and the severity of the infection was 29.1%. This indicates that malaria infection is endemic in the study area, and this may be attributable to lack of antimalarial drugs, low CD4 cells count and higher plasma viral load. The prevalence reported in this study is higher than 23.3% prevalence reported in a previous study conducted by [13] conducted from the same hospital (AKTH) but the researcher restricted only to primigravidae.

HIV RNA (viral load) and CD44 T lymphocyte (CD4) cell count are the two surrogate markers of antiretroviral treatment (ART) responses and HIV disease progression that have been used for decades to manage and monitor HIV infection according to [14]. CD4+ cells are white blood cells that are an essential part of the human immune systems. They are the primary receptor used by HIV to gain entry into the host cells as citied by [15]. Having CD4 cells counts of ≤ 200 cells/mm^3 showed a strong association with malaria parasite infection compares to those with CD4 counts ≥ 500 cells/mm^3. This is because analysis of our result showed that out of the 44 participants with CD4 count ≤200 cells/mm^3, 40(90.9%) were malaria positive with 67.5% prevalence rates of severe parasitaemia while only 5(25%) those with CD4 counts ≥ 500 have malaria with 0% severity of the infection.

This confirms that pregnant women with HIV have higher rates of symptomatic malaria and malaria infection decrease the level of CD4 cells count in HIV positive individual as reported by [16]. This also showed that CD4 cells count is an excellent indicator of HIV positive pregnant mothers risk of developing severe malaria infection.

According to [17], HIV viral load test measures the amount of HIV particle in an individual which is usually reported as copies of HIV in one milliliter of blood. With regard to viral load, the higher percentage of malaria 37(100%) was observed among those with viral load ≥10000 copies/ml, with 28(75.7%) having parasitaemia of ≥10,000/µl. This finding also agrees that malaria infection increase plasma viral load as reported by [16]. Dual infection therefore, makes life difficult to the mother due to higher frequency (parasitaemia and viral load) as reported by [3].

Based on antiretroviral drugs (medications used for the treatment of HIV), the highest prevalence was observed among those not using antiretroviral drugs 10(83.3%) than those using the drugs 131(69.7%). Higher malaria parasitaemia was observed among those without antiretroviral drugs at all 6(60%). This finding reveals that, even though the drugs do not kill or cure the virus, but when taken in combination they can prevent the replication of the virus and when the virus is slowed down, so is HIV disease which is aimed at preventing a person more susceptible to opportunistic infections like malaria as reported by [14]. It also shows that, delay in ART initiation may prove less effective in reducing infection transmission as reported by [18].

Table 6. Distribution of malaria positive cases based on viral load among primigravidae and multigravidae pregnant mothers living with HIV

Viral load	No examined	No positive	Gravidity		
			Primigravidae	Multigravidae	*P*- value
< 20	91	49(53.8)	30(61.2)	19(38.8)	0.02078*
21-10000	72	55(76.4)	24(43.6)	31(56.4)	
>10000	37	37(100)	15(40.5)	22(59.5)	
Total	200	141(70.5)	69(48.9)	72(51.1)	

The result of the present study showed that clients receiving second line regimen (combination of two drugs from NRTIs and one PIs drugs), have higher prevalence of malaria infection 29(90.6%) followed by client without receiving ART regimen 10(83.3%) and the least was observed among clients receiving 1st line ART regimen (consist of two NRTIs and one NNRTs) 102(65.4%). Severity of malaria (Parasite density of ≥10000) was higher among clients receiving 2nd line regimen 19(65.5%), followed by clients not taking antiretroviral drugs 6(60%) and the least was observed among clients on 1st line regimen 16(15.7%). This shows significant difference (p <0.05). The higher prevalence observed among clients on second line regimen may be due to immune suppression, since second line ART regimen are stronger than first line regimen where medication switch when the patient fails the first line regimen as the HIV strain has become resistance to the course of drugs. This can occur as a result of drug resistance, poor drug absorption or a weak drug combination and increased viral load as reported by [19]. Antiretroviral drugs on second line regimen may have more side effects also.

The prevalence of malaria was seen across all gestational ages. Results obtained showed that multigravidae have higher malaria infection 72(51.1%) than the primigravidae 69(48.9) among HIV positive pregnant mothers. Similar observation had also been made by [20] who reported higher prevalence of malaria of 74.7% in multigravidae compare to 72.7% among the primigravidae. The difference in the prevalence might be due to the fact that HIV increases the risk of malaria in adults, especially in those with advanced immunosuppression as seen in this present study multigravidae had 24 (60%) malaria prevalence rates as compared to 16(40%) primigravidae among the AIDS clients with CD4 cells count of ≤200 cell/ml and also on the basis of viral load, multigravidae had 22 (59.5%) malaria prevalence rates as compared to 15(40.5%) primigravidae among clients with viral load >10000 copies/ml. Our result is also in conformity with the report of [21] who said that, multigravidae in holoendemic areas for malaria develop parity-specific immunity following recovery from first pregnancy Parasitaemia however, and HIV infection impairs immunity and lead to an increase in susceptibility to malaria in the higher parity groups.

4. CONCLUSION

The prevalence of malaria infection was high among pregnant mothers living with HIV and the severity of the infection increased due to immune suppression ($P < 0.05$) i.e. low CD4 count and higher plasma viral load. Also significant association was found between primigravidae and multigravidae malarial positive in relation to plasma viral load ($P < 0.05$). While no association between primigravidae and multigravidae parasitaemia in relation to CD4 cell count ($P > 0.05$).

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper.

ETHICAL APPROVAL

The study was conducted according to ethical standards for human studies and approved by the Aminu Kano Teaching Hospital Research Ethics Review Committee (AKTH/MAC/SUB/12A/P-3/VI/1488).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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