Seroepidemiology of HIV and Malaria Coinfection Among HIV-Infected Individuals in Ebonyi State, Nigeria

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ABSTRACT: This study assessed the prevalence of HIV/Malaria co-infection and its association with socio-demographic, immunological, and virological characteristics among 200 individuals living with HIV. Overall, 26.0% (52/200) of participants were co-infected with malaria parasites. The prevalence was significantly higher in females (31.5%) than in males (11.2%) (p=0.0035). Age was also significantly associated with co-infection (p=0.0245), with individuals under 30 years recording the highest prevalence (51.1%), followed by those aged 31–40 years (28.5%). Individuals aged 41–50 years and those aged 51 and older had lower rates of 25% and 16.6%, respectively. Educational status and marital status were not statistically significantly associated with co-infection (p=0.1781 and p=0.4422, respectively). However, those with secondary education had the highest malaria co-infection rate (31.8%), followed by tertiary (26.4%) and primary education (10%). Co-infection was more prevalent among married individuals (28%) than singles (23.2%). Immunologically, individuals with CD4 counts <200 cells/µL had a significantly higher co-infection rate (42.2%) compared to those with 201–349 cells/µL (20%) and >350 cells/µL (25%) (p=0.0000). Virologically, participants with viral loads >1000 copies/mL had the highest co-infection rate (60%), compared to those with viral loads of 41–999 copies/mL (19%) and <40 copies/mL (23.1%) (p=0.0011). These findings suggest a strong association between malaria co-infection and immune suppression, as well as high viral replication in HIV-infected individuals. Targeted malaria prevention and control strategies should be prioritised among HIV-positive patients, particularly those with low CD4 counts and high viral loads, to reduce the burden of co-infection and improve health outcomes.

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1. INTRODUCTION

Malaria and HIV-1 coinfection is one of dire public health challenges, most especially within sub-Saharan Africa which has the highest double-burden diseases. There is a bi-directional interaction between the two infections which aggravates the severity and the control of each of them, forming a multifaceted clinical problem. Malaria is caused by Plasmodium parasites mainly by *Plasmodium falciparum* which is transmitted by the female anopheles mosquitoes and ranges from low-grade miliary febrile disease to the worst of scenarios or complications such as cerebral malaria. The most dominant and vicious form of the human immunodeficiency virus is HIV-1 and it causes systemic weakening of the immune system, which badly leads to acquired immunodeficiency syndrome (AIDS) in the absence of appropriate treatment.

The co-existent factors of malaria and HIV-1 infection influence the immune system in a manner which increases the burden of the two diseases affecting the body. On the other hand, the immune suppression caused by HIV-1 lowers the capacity of the body to fight malaria and therefore, there is an increase in the levels of parasitemia and there is also an increase in the chances for the individual to have a serious malaria manifestation. The role of CD4+ T cells in the anti-infection response especially against the Plasmodium parasite is adversely impacted by HIV-1 or AIDS (Roberds et al., 2021). Thus, HIV-positive people have increased risks of developing severe forms of malaria, such as anaemia and cerebral malaria as well as high rates of mortality related to malaria (Roberds et al., 2021).

On the other hand, the existence of malaria will accelerate and increase the progression of HIV-1.

Episodes of acute malaria elicit a strong immune response, resulting in the upregulation of pro-inflammatory cytokines such as TNF-α, IL-6 and IFN-γ. Such inflammatory responses can lead to the activation of dormant HIV-1 reservoirs, enhanced viral turnover and higher reservoir levels increasing the possibility of progression to AIDS (Figueroa-Romero et al., 2024).

Additionally, malaria-induced hemolysis and anaemia can place more strain on an already weakened immune system further exacerbating the HIV-1 clinical picture. The stringency of symptoms in malaria incurred with HIV-1 is worsened more due to the correlation between the two diseases with deformities in the immune system. Malaria, caused by Plasmodium parasites, particularly *Plasmodium falciparum*, is associated with mild fever and chills as well as serious complications such as cerebral malaria, severe anaemia and multi-organ failure. In the presence of interfering infection with HIV-1, the vice versa conflict occurs whereby the onset time and the intensity of the symptoms are greatly accelerated. It is HIV that confers the contradiction as it is capable of immuno-suppression. HIV-1 infects and destroys CD4+ cells, which are important for an appropriate immune response against Plasmodium parasites. Higher parasite load in HIV-infected persons results in greater immune suppression in individuals infected by malaria. Malaria-affected individuals with HIV-positive status are prone to severe malaria indices and some of these extremes include cerebral malaria where victims have seizures, fall into a coma and may eventually die (Figueroa-Romero et al., 2024). Following all these, this study is set to determine the prevalence of malaria and HIV coinfections in people living with HIV in Abakaliki Nigeria a well as the risk factors associated with coinfections.

2. MATERIALS AND METHOD

2.1. Study Design and Population.

A Cross-sectional method was utilized to assess the presence of malaria in plasma of consented HIV patients using a Dia pro Diagnostic Malaria Ab ELISA kit as well as SD detection cassette for rapid screening for *plasmodium falciparum* antibodies in the patient's serum according to manufacturer's instruction

2.2 Study Population

This study consists of HIV-1 seropositive individuals under clinical monitoring in antiretroviral clinic in AEFUTHA and Mile Four specialist hospital, Abakaliki.

2.2.1 Inclusion criteria

All consenting HIV-1 sero-positive individuals.

2.2.2 Exclusion Criteria

Non -consenting individuals as well as those whose HIV status have not been confirmed.

2.4 Ethical consideration

The study was conducted after obtaining ethical clearance from the University of Port Harcourt Research Ethics Committee.

2.5 Sample size determination

Sample size was determined using the formula $N = \frac{Z^2P(1-P)}{d^2}$ (Charan & Biswas, 2013). Where N is the anticipated sample size, P is the expected prevalence in the target population, q is 1-p, Z is 1.96; standard error, d is the level of statistical significance (0.05). A P-value of 0.8% (0.8% reported for Ebonyi State by NAIIS (NACA, 2019) was used representing maximum uncertainty (Awando et al., 2013; Macfarlane,1997).

2.6 Laboratory Analysis

Blood samples were collected from consenting HIV-I patients, thereafter plasma was analysed for the presence of Malaria antibodies in patients' sample. This was carried out at the University of Port Harcourt's Virus & Genomics Research Unit of the Department of Microbiology.

2.6.1. Malaria Ab Analysis

The microwell holder was filled with the necessary number of microwells. Following that, 50 microliters (50 µL) of sample diluent were carefully distributed into each of the meticulously designated microliters except A1 used for blanking. Similarly, 150 µL of the negative control were dispensed in triplicate, 150 µLof calibrator in duplicate, then 150 µLof positive control was added to the single well. Thereafter, 150 µLof patients' serum was added, the plate was sealed using adhesive sealing foil or sealant and thereafter incubated at +37°C for 60 minutes. Following the initial incubation, an ELISA automatic washer was used to wash the microplate in four to five cycles. Except for the A1 well, which serves as a blank, one hundred microliters (150 µL) of Enzyme Conjugate 1 were pipetted into each well, sealed and incubated for another 30 minutes of incubation at +37°C, the microplate was washed as previously mentioned. Thereafter 100 µL of conjugate 2 was added and thus incubated for 30min, then wash. Thereafter,100 microliters (200 µL) of the TMB/H_2O_2 mixture were pipetted into all wells, blank inclusive. After resealing the microplate, it was incubated for 30 minutes at room temperature (18–24°C). To prevent a high backdrop, care was made to avoid exposure to intense direct illumination. After being kept at room temperature for incubation, 100 µL of sulfuric acid was pipetted into each well. This will result in a colour change in positive control and samples from blue to yellow. Each well's colour intensity was

assessed and read using the ELISA reader at a wavelength of 450 nm filter reading.

2.7. Data analysis

In other to summarise the data obtained, descriptive analysis such as the student t-test for continuous variables, Chi-square test for categorical variables and Fisher's exact test for two-by-two contingency tables. P-values of ≤ 0.05 were reported to be statistically significant.

3. RESULTS

3.1. Analysis of Study Participants' Characteristics

The age range of the 200 HIV-1 positive patients who participated in the study was ≤30- 50 years and above with a mean age of 35.3 years. Table 1. indicates by age that the age group 31-40 has the highest number of HIV infections, with 70 cases. This is closely followed by the age group ≤30 with 43 infections. This is followed by age group (41-50) and the elderly group (>50) have lower frequencies of 12 infections, respectively. By sex (Table 1), The data indicates that the number of HIV infections is significantly higher among females (146 cases) representing 73% of the study population compared to males counterpart 54 (27%). This could suggest that based on socioeconomic and biological variations, females are more vulnerable or exposed to factors that increase the risk of HIV infection in this population. As shown in Table 1, the married category has the highest number of HIV infections, with 114 cases representing 57% of the infected study population. This was followed by single Individuals with 86 cases representing 43% of the infected study population. Based on educational background, 68.0% of the study population comprised individuals with tertiary education, followed by 22.0% secondary and 10% with primary education. (Table 1).

3.1.2 Immunological and Virological characteristics Of Study Participants

The results obtained from clinical and immunological parameters from the study population were characterized and are shown in Table 4.2. Their CD4 count ranges from 200- 349 cells/µl (). A high degree of the populates had CD4 ≥350 (83.0%, n=166), followed by folks with CD4 of 201-349 (). Only a few persons had CD4 of less than 200 (7.0%, n=14) as indicated in Table 2. Concerning virological markers, a majority of participants had a viral load of ≤40 copies/ml (69.0%, n=138), followed by 41-999 copies/ml (21.0%, n=42) and least ≥1000 copies/ml (10%, n=20) as displayed in Table 2.

3.2. Overall Seroprevalence of Coinfections

All 200 random samples selected were reconfirmed of their HIV-positive status. Furthermore, serological analysis was conducted to detect coinfections with malaria. The overall viral coinfection was 26.0%. Following the sociodemographic characteristics as illustrated in Table 1, higher seropositivity of HIV/Malaria coinfection occurred in the age group ≤30 (51.1%), followed by the age group 31-40 (28.5%). Seroprevalence of HIV/Malaria coinfection was observed to decrease following an upsurge in ages 41-50 (25%) and age group 50 and above (16%) as displayed in Table 1. The study shows a higher prevalence of HIV/Malaria coinfection among females (31.5%) than in male participants (11.0%). In this study, higher coinfection rates were observed among the married (28.0%) than singles (23.2%) as indicated in as shown in Table 1. For the education and occupation of the participants, higher rates of coinfection were observed in those with secondary education (31.8%) and among those who were teachers by occupation (33.3%) as shown in Table 1.

Table 1: HIV/Malaria Co-infection based on Socio-Demographic features.

Variables	Categories	Tested (%)	HIV/MP (%)	P-Value
Sex	**Males**	54 (27.0)	6(11.2)	0.003507
	Females	146(73.0)	46(31.5)	
Age Groups (Years)	≤30	43 (21.5)	22(51.1)	0.0245
	31-40	70 (35.0)	20(28.5)	
	41-50	32 (16.0)	8(25)	
	≥51	12(6.0)	2(16.6)	
Marital Status	Single	86 (43.0)	20(23.2)	0.4422
	Married	114(57.0)	32(28)	
Educational Level	Primary	20 (10.0)	2(10)	0.1781
	Secondary	44 (22.0)	14(31.8)	
	Tertiary	136(68.0)	36(26.4)	
TOTAL		**200(100.0)**	**52(26.0)**	

This study shows that CD4 cells of less than ≤200 had a higher positivity (42.8%) for MHC followed by those with CD4 cells greater than 350 (25%), as shown in Table 2. The viral load above 1000 had the highest seropositivity of MHC (60%) as shown in Table 2.

Table 2: HIV/MP Co-infection based on Immunological and Virological Markers.

Markers	Categories	No. Tested (%)	HIV/MP (%)	P-Value
CD4 counts (cells/µl)	≤200	14 (7.0)	6(42.2)	0.0000
	201-349	20 (10.0)	4(20.0)	
	≥350	166(83.0)	42(25.0)	
Viral Load (copies/µl)	≤40	138(69.0)	32(23.1)	0.001092
	41-999	42(21.0)	8(19.0)	
	≥1000	20(10.0)	12(60.0)	
TOTAL		**200(100.0)**	**52(26.0)**	

4. DISCUSSION

Both malaria and HIV are two of the world's most deadly diseases, broadly distributed in sub-Saharan Africa. Although different in transmission route, both diseases affect the poorest population segment. The coinfection of malaria and HIV accounts for about 2 million deaths globally every year (WHO, 2020). Following the results obtained from this study, a coinfection prevalence rate of 26% was observed with malaria. This is similar to the rate reported by Jegede et al. (2017) in a study in Kano, where the prevalence of MHC was 27.7%. This also agrees with a coinfection rate of 24.0% reported in Jos (Iroezindu et al., 2012) and a 22.9% prevalence reported by Gumel (2012). This observation contrasts with a lower prevalence reported by Bate et al. (2016), where a 13.04% prevalence was reported in the Buea Health District. This is attributed to multiple breeding sites of mosquitoes in the hospital environment (Isah et al., 2020). In contrast to the prevalence observed in this study, it is higher than the prevalence rate of 0.0% observed in 2019 in a study conducted by Okonko et al. (2019) in Port Harcourt, 5.0% that was discovered in Port Harcourt, South-South Nigeria (Okonko et al., 2021), and the 5.0% that was discovered in Yenogoa, Bayelsa State, Nigeria (Okonko et al., 2023b), also higher than the 10% reported in Akure Ondo state Nigeria by Dada et al. (2016); 18.5% in Osogbo (Ojurongbe, 2014); 14% prevalence in Ruwanda (Habyarimana & Ramroop, 2020), 14.2% and 14.0% as reported by Amadi et al. (2018) among people living with HIV in Oyo state and a 14.3% co-infection recorded in cross river rate Ejike et al. (2020).

Based on gender, many of the participants were females (73%) while 27.0% were males. This agrees with Okonko et al. (2018, 2020a), who also found most HIV subjects to be females. Females are at a higher risk of HIV in developing nations, particularly in Sub-Saharan Africa. Following this study, a higher prevalence of HIV/Malaria parasitemia was discovered among women (31.5%) compared to their male counterparts. This can be attributed to several factors, such as biological and socioeconomic factors. This is in agreement with a report by Sandie et al. (2019) but in contrast to Kimbo et al. (2013) where a higher prevalence was observed among males. As reported in a study carried out in the city of Ondo in Nigeria, HIV-positive females, who are pregnant are prone to having malaria, this is more evident in the first trimester of pregnancy (Olusi et al., 2019).

The findings in this study are consistent with past observations in which suggest women, especially in the reproductive age period, are more prone to the disease due to factors like compromising the immune self-defence during pregnancy as well as exposing themselves to vector-borne infections while engaging in household chores. Pregnant women, especially, are at an increased risk of contracting malaria, which also has an increased potential of leading to maternal anaemia, lower than average birth weights and even death. In a study conducted by Okonko et al. (2021) and Innocent-Adiele et al. (2023b) in Port Harcourt, females were also discovered to have a high prevalence of HIV and malaria Coinfection. Kimbi et al. (2013) also recorded a higher prevalence among males which is attributed to the fact that males are less likely to use preventive measures such as insecticide-treated bed nets or mosquito repellents compared to females (Okiring et al.,2022). This is however in divergence with studies carried out in Bayelsa State, Nigeria, by Okonko et al. (2023b), where HIV/Malaria coinfection was observed to be higher among men than women. Kublin et al. (2005), Tagoe and Boachie (2012), Onankpa et al. (2017), Ejike et al. (2020), and John et al. (2020) all concur with this conclusion. This can be attributed to occupational activities, which keep men outside into the evenings when mosquitoes are more active (Obimakinde & Simon-Oke, 2017).

Relating to age, the populace within the age grade less than and equal to thirty (≤30) had a higher seropositivity of 51.1%, followed by age groups 31-40(28.5%), 41-50(25%) and 16.6% for ages 50 and above. This result is however in line with the 55.0 % recorded in Limbe (Sandie et al., 2019). This agrees with the report by Okonko et al. (2023b) who discovered that HIV/malaria co-infection rate reduces with an increase in age, from 6.0% for those between 21 – 40 years to 4.4% for those 41 years and above. This observation is also comparable to the studies that have been conducted in the past, which

have shown that the rate decreases with age. On the other hand, this study is in agreement with a report by Sarasino et al. (2012), who also recorded a higher prevalence of MHC at the age of 21 as compared to other age groups. Also, following the findings of research by John et al. (2020) and Jegede et al. (2017), in Calabar, Nigeria, higher rates of HIV/malaria co-infection were observed among those under the age of 25, as observed in this study. The findings in this study concerning age group agree with previous research work.

A greater incidence of MHC was also detected among individuals who were younger than 25 or 30 years of age. The result in this study is moreover way higher than the 24.8 % prevalence of malaria among HIV-infected children in Mutengene reported by Bate et al.(2016) as well as the 13.04 % reported by Isah et al. (2020), in Buea Health District and the 14 % malaria parasite prevalence reported in Rwanda in HIV- unexposed children (Habyarimana & Ramroop, 2020), where prevalence was attributed to several mosquito breeding observed throughout the year as well as resistance of mosquitoes to insecticides.

Education level is associated with the risk of malaria infection. Following the educational exposure of participants in this study, the prevalence of malaria and HIV is higher among those who have completed secondary school (31.8%). This is similar to the findings of Alaofin et al. (2020), who also reported a higher prevalence of HIV-malaria co-infection among those who have completed secondary school, and it is the least common among participants with higher/tertiary education. Furthermore, this finding contradicts the results of John et al. (2020) and Ejike et al. (2020a, b), who found a higher rate among individuals with a primary education. Additionally, it was discovered that this finding was also contradictory to the work done by Ibrahim et al. (2022), Nyarko and Cobblah (2014), and Ugwu et al. (2013), all of which showed that those with low literacy levels are more susceptible to malaria. A study by Isaac Isioko et al. (2024) found that children whose moms have either completed basic school or had no education had a greater prevalence of malaria. This supports the idea that education can improve one's understanding of how to utilize and purchase household mosquito nets.

A higher coinfection prevalence rate of 28.0% was observed among the married folks in contrast to the singles 23.2% in the study. Although there was no significance in MHC concerning marital status. Occupation was observed to be a risk factor in the coinfection of malaria and HIV in this current study. The prevalence of MHC was significant among teachers at 33.3% (p-value 0.00007588). This could be due to poor environmental sanitation and adherence to drug use. This is in contrast to a report by Okonko et al. (2023) in Yenegoa, Bayelsa state Nigeria and Oyeniran et al. (2022) where both recorded the highest prevalence among students. This is also in contrast to the findings of Dalu et al. (2022) and Ibrahim et al. (2022), who discovered that farmers were significantly more likely to get malaria than people in other occupational categories.

According to this research about viral load, HIV individuals who also have malaria were associated with high HIV viral loads ≥1000 copies (60%). This may be linked to late therapy presentation, medication resistance or interaction, or poor ART adherence. The risk of illness development, transmission, and death is increased by persistently elevated viral loads.

Children with viral load greater than 1000 copies were more likely to be malaria-infected as reported by Obeagu et al. (2024), the alteration of the immune system by HIV and the presence of the virus in the blood increases the susceptibility to severe malaria (Obeagu et al.,2024) and the number of malaria parasites rises as viral loads do. These results are consistent with several related investigations and hypotheses. In his 2018 systematic analysis, for example, Kwenti found that HIV infection is linked to a higher malaria parasite density and that malaria infection is linked to a higher HIV viral load both in vivo and in vitro. This could point to a potential interaction between the virus and the malaria parasite.

Following this research, a higher prevalence was identified among those with CD4+ (cluster of differentiation) ≤200 (42.8%) This indicated that parasite density rises when the CD4 count falls, and also these groups already have the present an advanced form of the disease which may be due to a delayed diagnosis, therapy failure, or late-stage presentation of symptoms. This agrees with the study of Ngwa et al. (2024) who reported a higher prevalence of malaria among children with CD4+ less than 200. This is also in correlation with studies by Tagoe and Boachie (2012) in Ghana and Jegede et al. (2017) in Nigeria where a higher prevalence of malaria was reported among those with reduced CD4+ count. This percentage is in line with research showing that a lower but noteworthy percentage of individuals present with severe immunosuppression, especially in areas where early HIV identification and access to reliable therapy are lacking. However, the findings in this study are in contrast to Ivan et al. (2015) who recorded a higher risk of coinfection among those with a CD4+ rate greater than 350 cells. Ekere et al. (2020), on the other hand, discovered that the fraction of patients increased as the CD4 count groups increased, with those with CD4 less than 200 cells/µl having the lowest percentage at 14%. Additionally, Okonko et al. (2020a) found that the

smallest proportion of individuals (16.8%) had CD4 counts below 200 cells/µl.

5. CONCLUSION

The study revealed a 26.0% prevalence of HIV/Malaria co-infection among the study population, with significantly higher rates observed in females, younger individuals (particularly those under 30 years), and participants with low CD4 counts and high viral loads. While educational level and marital status were not statistically significant, co-infection was more prevalent among those with secondary education and married individuals. Immunological and virological markers showed strong associations with co-infection, as individuals with CD4 counts below 200 cells/µL and viral loads exceeding 1000 copies/mL had markedly higher co-infection rates. These findings highlight the critical need for integrated malaria prevention and control strategies within HIV care programs, especially for immunocompromised patients and those with uncontrolled viral replication. Strengthening routine malaria screening, prompt treatment, and immune monitoring in HIV-infected populations is essential for reducing morbidity and improving overall patient outcomes.

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Disclosure of conflict of interest

The authors claim that there are no conflicting interests.

Statement of ethical approval

All authors declare that all experiments have been examined and approved by the University of Port Harcourt Research Ethics Committee. Therefore, the study is performed following the ethical standards

Statement of informed consent

All authors declare that informed consent was obtained from all individual participants included in the study.

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