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**RESEARCH ARTICLE**

# CO-INFECTION OF *PLASMODIUM FALCIPARUM*, HEPATITIS B AND C VIRUSES AMONG HIV INFECTED PATIENTS ATTENDING A TERTIARY HEALTH FACILITY IN RIVERS STATE

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***Authors' contributions***
EW and EC conceptualized and designed the topic, EW did the field sampling and drafted the manuscript, WN, paid attention to CD4 analysis, and EC supervised and read the work to fullness in making sure that all the necessary things are put in place while OI took a look on the diagnostic techniques used in detecting *Plasmodium falciparum* in patients. All authors read and approved the manuscript.

## ABSTRACT

Co-infection is the simultaneous infection of host by multiple pathogenic species, which may co-exist together or not. The study aimed at ascertaining current prevalence of *P.falciparum* and other diseases among HIV infected patients. The result of this study shows that the prevalence of malaria in male was (89.7%), out of 378 respondents and women (78.3%), with a P-value of 0.012. The triple infection rate for HIV/HBV/HCV *Plasmodium falciparum* was 3.7%, 0.26% and 7.1%; other co-infection were 9.14% and 8.6% for malaria alone, 60%, 26.7% and 13.3% for malaria and HBV, malaria and HCV had 1(100%) between (10-19) medium, (3-10) low and (19.30) high with the P-value of (0.508). Higher HIV/MPF triple infection of CD4+ T cell count level occurred in 272 male respondents and 106 female respondents. In male (801-1000µl, 405-800µl, 400µl – 0 and 930-1120µl, female had 800 - 1110µl, 400 - 800µl and 920 - 1120µl with P-value of 0.020, 0.504 and 0.405, showed significant differences among the study subjects. The present study has further confirmed the low occurrence (0.26%) of HBV/HCV/ *Plasmodium falciparum* among HIV-infected individuals in Port Harcourt, Nigeria. Co-infection of malaria with HBV/HCV and HIV suggests that malaria can exist in the same host. This calls for prompt malaria treatment among the immune compromised patients. Occurrence of HIV/HCV/HBV and malaria exist in Port Harcourt Rivers State, Nigeria. Therefore, the study suggests proper routine screening of HIV-infected patients for simultaneous infection with HBV, HCV and malaria. The study will bring to light the current prevalence of *P.falciparum*, Hepatitis B and C among HIV co-infected individuals in Rivers State as well as correlation of co-infection with associated factors such as age, sex and $CD4^+$ count. There is need to investigate the effect of co-infection of liver infection in HIV patients including liver enzyme levels and liver fibrosis.

**Key Words***: Malaria, virus, Anopheles, Plasmodium falciparum, Triple infections*



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Malaria has plagued man since early times and it is a parasitic disease caused by *Plasmodium* species mainly via the blood feeding of infected female *Anopheles* species (Joy *et al.,* 2003). Other forms of transmission include sharing contaminated needles/sharp objects, congenitally – acquired malaria (mother to foetus), transfusion of infected blood and organ transplantation (Filler *et al.,* 2003, Owusu-Ofori *et al.,* 2013). Malaria infection is a global health problem with an estimated 429,000 deaths in 2015; about 95% of these deaths occurred in Africa and 86% of the victims were children below 5 years (World Health Organization, 2016). Nigeria and Democratic Republic of Congo jointly account for 44% of the total malaria cases in Africa (Federal Ministry of Health, 2010). The major challenge faced by people living with HIV are opportunistic infection caused by viruses, parasites bacteria, fungi and a range of other pathogenic infections resulting in significant mortality (Feyisayo *et al.*, 2020). The potential consequences of both disease interaction including understanding their reciprocal effects on host immune response and their combined effect on host response to other pathogens is important (Woke *et al.*, 2022). HIV and Malaria co-infection particularly *Plasmodium falciparum* species are both pathogens that induces significant perturbation and activation of immune system, both pathogens (HIV and Malaria) may be contributing factors in the modification of each other's development, disease severity and disease progression rate (Tavares *et al.*, 2013) Such co-infection have been reported as the two most important health problems of developing countries including Nigeria, accounting for more than 4 million death globally (Feyisayo *et al.,* 2020). These synergistically resulted to health outcomes Ejele *et al.,* 2004).

The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life – threatening opportunistic infections (Froebel, *et al.,* 2004). Infection with HIV occurs by the transfer of blood, pre-ejaculated semen, vaginal fluids or breast milk. Within their bodily fluids, HIV is present as both free virus particles and virus within infected immune system such as help T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through number of mechanisms, including pyroptosis of abortively infected T cells, apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected $CD4^+$ T cells of CD8 Cytotoxic lymphocytes that recognize infected cells (Garg *et al.,* 2012). When $CD4^+$ T Cell numbers decline below a critical level, the cell – mediated immunity is lost, and the body become progressively more susceptible to opportunistic infections like Malaria, tuberculosis, hepatitis B and so on (Afolabi and Bakare 2022). HBV and HCV infection has been suggested to negatively impact $CD4^+$ cell count restoration and cirrhosis is associated with depressed CD4 cell counts, independent of HIV, HBV or HCV infection (Mc Govoven, 2007). Also the detrimental effect of HBV and HCV on HIV infection includes a significant reduction of CD4 cells and total CD4 present (Jajadi *et al.*, 2010).

Therefore, this study was conceptualized to ascertain current prevalence of *P.falciparum* and other diseases among HIV infected patients, and in view of the morbidity and mortality associated with HIV infection, Hepatitis B and C virus affects the immune response to *Plasmodium* determining more frequent occurrence of clinically severe malaria. The study will aid information and policy making in the provision of adequate control measures for these infections.

## 2.0. MATERIALS AND METHODS

### 2.1 *Study Area*

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria, (Latitude 4º 53' 58' and Longitude 6º 55'43E) in Obio-Akpor Local Government Area. This hospital is one of the main treatment facilities for HIV infected and malaria in Rivers State, Nigeria, Southern Nigeria. It is bounded by the states of Imo and Anambra on the North, Akwa-Ibom and Abia on the East and Bayelsa and Delta on the west. It is characterized by constant rainfall during the wet season with resultant mangrove swamp vegetation.

### 2.2 *Study Design*

A hospital-base cross-sectional study design was adopted for the present study, which seeks to determine HBV, HCV and *plasmodium falciparium* infections among HIV–infected individuals attending University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria.

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### 2.2.1. Ethics Statement

Administrative approval for this study was obtained from the ethical committee of University of Port Harcourt, Rivers State, Nigeria. The University of Port Harcourt Research Ethics committee received the work for ethical issues and approved the standards for research involving human beings. Before samples were taken and processed, everyone who participated gave informed consent.

### 2.2.2. Sampled Population

A total of three Hundred and Seventy Eighty (378) patients from the University Teaching Hospital, Port Harcourt (UPTH), Rivers State, Nigeria, willingly gave informed consent and volunteered to examine their blood samples. These include 189 patients from each of the anti-retroviral clinic who had been tested and confirmed positive of the HIV infection and within the patients that tested negative malaria parasite and the groups used as the control group.

### 2.2.3. Sample Collection

Intra-venous blood was obtained from all enrolled study patient by trained laboratory scientists working in the selected healthcare facility. These samples (store in ethylene diamine tetra acetate bottles) were used to prepare thick and thin smear for microscopic investigation and as well as determining hemalogical parameters of each study participants.

### 2.3 *Serological Analysis*

A known quantity (5ml) of blood was collected using the sample droppers. 5µl drawn blood was load into the sample Port 1(s) then a drop of 5µl of buffer solution was added into the buffer pool 2 (B) on the test device. Blood samples were taken and examined at the virus and Genomic Research Unit, Department of Microbiology, University of Port Harcourt, for ABs Ag, HCV and *Plasmodium falciparum* antigen using appropriate test kits. Laboratory testing was carried out according to the manufacturer's instructions and all tests were run using quality controls according to standard operating procedures. (Egbom, 2022).

### 2.4 *Data Analysis*

McNmemar Chi-square was used for paired and Pearson independence chi-square comparisons using statistical package for social science (SPSS) version 17, 2017. The data will be presented in frequencies percentage and means while a P-value less than 0.05 considered significant. Also the level of Association is used to ascertain between HBV, PCV and CD4+ Cell count at P<0.01.

## 3.0. RESULTS

A total number of three hundred and seventy eight (378) respondents enrolled in the study, with 28(10.2%) infected and 244(89.7%) not infected in men at P-value of 0.012, while the female had 23(21.7%) infected and 83(78.3%) not infected at P-value of 0.012 and accounting to total number of 51(13.5%) and 327 (86.5%) in the study area, as shown in Table 1.

**Table 1: Overall Malaria Prevalence according to Sex**

| Sex | No Examined | Infected (%) | Not Infected (%) | P-value |
|---|---|---|---|---|
| Male | 272 | 28(10.2%) | 244(89.7%) | 0.012 |
| Female | 106 | 23(21.7%) | 83(78.3%) | |
| **Total** | **378** | **51(13.5%)** | **327(86.5%)** | |

Table 2: represents overall malaria, HBV, HCV and HIV-co-infection, according to sex, of the 378 participants, men had 9(3.3%) infected with malaria, HBV and HIV co-infection, 263(96.7%) not infected, 1(0.4%) infected while 271(99.9%) not infected at P-value of 0.518. while the female had 106 examined participants, 6(5.7%) infected, 100(94.3%) not infected for malaria, HCV & HVC had Nil (zero) and not infected also had (zero) at P-value of 0.518. therefore total infected for both male and female is 14(3.7%), while infected had 1(0.26) and not infected 27(7.1%) respectively.

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**Table 2: Overall Malaria HBV, HCV and HIV – Co-Infection According to Sex**

| Sex | No Examined | Infected for Mal, HBV & HIV Co-infection | Not Infected for Mal, HBV & HIV Co-infection | Infected for Mal, HCV & HIV Co-infection | Not Infected for Mal, HCV & HIV Co-Infection | P-value |
|---|---|---|---|---|---|---|
| Male | 272 | 9(3.3%) | 263(96.7%) | 1(0.4%) | 271(99.6%) | 0.518 |
| Female | 106 | 6(5.7%) | 100(94.3%) | - | - | |
| **Total** | **378** | **14(3.7%)** | **363(96.8%)** | **1(0.26%)** | **27(7.1%)** | |

Also Table 3 shows overall malaria prevalence according to intensity in Relation to Co-infections, in which parasite intensity were classified into low (%), medium (%) High (%) and very High (%). Out of the 51 patients examined, malaria alone had 35 which is broken down into 91.4 % (3-10) low, 8.6 %(10-19) medium, and 0.0%(19-30) high. Also malaria and HBV had 15, (60%) low, (26.7%) medium and (13.3%) high, while malaria and HCV had 1, zero (low), 100% (medium) and high had zero percentage.

**Table 3: Overall Malaria Prevalence According to Intensity in Relation to co-infections**

| Intensity of Malaria Parasite (%) | | | | |
|---|---|---|---|---|
| Low (%) Medium (%) High (%) Very High (%) | | | | |
| Parasite Load per Microscopic Field | | | | |
| **Study Group** | **No Examined** | **(3-10) Low** | **(10-19) Medium** | **(19-30) High P-value** |
| Malaria alone | 35 | 32(91.4%) | 3(8.6%) | - |
| Malaria + HBV | 15 | 9(60%) | 4(26.7%) | 2(13.3%) 0.508 |
| Malaria + HCV | 1 | - | 1(100%) | - |
| **Total** | **51** | | | |

Also, the CD4 count level according to sex as shown in Table 4, shows that total of 378 participants were examined 272 of male had 801-1000µl of malaria alone, 405-800µl had malaria and HCV while 930-1120µl shows no malaria, HBV and HCV, while that of the female, out of 106 examined, 800-1110µl, malaria alone, 400-800µl malaria & HBV, malaria & HCV had nil while 920-1120µl had no malaria, HBV & HCV and their P-values are 0.020, 0.004 NA and 0.405 respectively.

**Table 4: CD4 Count Level According to Sex**

| | | CD4 Count Level | | | |
|---|---|---|---|---|---|
| **Sex** | **No. Examined** | **Malaria alone** | **Malaria + HBV** | **Malaria + HCV** | **No Malaria HBV & HCV** |
| Male | 272 | 801-1000µl | 405-800µl | 400µl | 930-1120µl |
| Female | 106 | 800-1110µl | 400-800µl | - | 920-1120µl |
| **P-value** | | 0.020 | 0.504 | NA | 0.405 |
| **Total** | **378** | | | | |

## 4. DISCUSSION

HIV and malaria have similar risk factors, and HIV/HBV/HCV co-infection speeds up the development of AIDS, which results in millions of deaths annually throughout the world (Boraschi, 2008). The study shows the current prevalence of *Plasmodium falciparum* , *Hapatitis* B and C virus among HIV infected parents in Port Harcourt, Rivers State, Nigeria, with some developing co-infections. As far as we know, no literature recorded in Port Harcourt or the Sourth-South region that considered these four illnesses simultaneously and inconsistent domestically and internationally (Shrestha, 2022). The overall HIV/HCV prevalence rate recorded was 13.5% infected parents and 86.5% non infected parents. It has similar findings with a 4.0% HIV/HCV co-infection rate

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obtained in Yenagoa, Bayelsa State (Okonko *et al*, 2023), 4.4% in Port Harcourt, Rivers State (Baeka *et al*, 2021) and 4.7% from a South Eastern State (Nhaknyi *et al*, 2020), all in Nigeria. Moreover, Cookey *et al*, (2021) recorded a zero percent HIV/HCV co-infection prevalence among highly infected HIV patients in Rivers State. However our result contrasts with 14.6% recorded in a rural community in Northern Nigeria (Adesegun *et al*, 2020).

Low infection rates of Hepatitis C 1(100%) between (10-19) with a significant different of P<0.508 and HIV/AIDS (13.5%) might be as a result of increase in the public enlightenment in the area. The people living in endemic area like Port Harcourt metropolis are aware of the various preventive measures for the two diseases. Low prevalence of hepatitis C recorded in the study areas is supported by Denue *et al*, (2013) at Medical words of University of Maiduguri Teaching Hospital, Nigeria where a low prevalence of 12.3% for hepatitis B virus was reported among HIV positive patients. The distribution of malaria among the groups shows that prevalence of the disease is higher among the male patients as compare to that of female patients. This shows that the lower groups are more predisposed to malaria infections than the older age groups. This might be because the low groups are still building up immunity against malaria parasites compared to the other groups where their immunity has been fully developed to combat the malaria parasites.

The prevalence of HBV/HIV co-infection was found to be higher among male patients 271(99.6%) than females 100(94.3%) in this study P=0.518, with non-significant difference. This finding corroborates reports from Jos, North central Nigeria (Irisena *et al*, 2004) and India (Slud *et al*, 2001). The observation may have been accounted for by the fact that men are more likely to have multiple sex partners and also practice unprotected sex in polygamous setting. We also observed a significantly higher prevalence of HCV antibody among HIV infected patients as compared to HIV negative blood donors. 14(3.7%) vs 27(7.1%) respectively, P<0.5. The difference is statistically significant. The reason for the difference may be due to share modes of transmission of both viruses in the study patients, it is essential to note the increased prevalence of HBV and HCV dual infection in this study compared to prior studies (Ogwu-Richard, 2015). Further, these studies have shown that triple HIV/HBV/HCV infections or dual HIV/HBV/HCV infections are the most prevalence. However, the incidence of these infections depends on risk categories, the type of exposure involved and geographic locations (Zhou, 2011, Zhang, 2002 and Bao and Liu, 2009).

*Plasmodium* spp and HCV are known to infect liver cells, hence given that the hepatitis C and malaria epidemics overlap in some parts of the world, it is conceivable that they might infect and reproduce in the same cell. Moreover, it is conceivable that these two illnesses could co-infect, in which case one pathogen could influence the other's severity to rise or fall and vice versa. Furthermore, despite substantial research on HCV attachment and entrance due to the virus's rising prevalence worldwide, more research needs to be done on *plasmodium* entry into host hepatocytes. Investigating the co-infection of the three diseases was imperative since it would aid in creating novel treatments and testing. In Port Harcourt, Rivers State, Nigeria, the study found 7.1% triple infections with HIV, HCV and *Plasmodium falciparum.*

To our knowledge, the data regarding association between $CD4^+$ T cells count and HIV-HBV-HCV co-infection in Port Harcourt is scarce. Our study showed that HIV patients with $CD4^+$ T cells count less than 930 cells/$mm^3$ were at 0.020 time, P-value have the higher risk of triple co-infections as compared to patients with $CD4^+$ T Cells count of more than 930 cells/$mm^3$. The study by Bhattaral *et al.* (2022), showed that HIV patients with $CD4^+$ T cells more than 200 cells/$mm^3$ were 8.1% and less likely to have HIV-HCV Co-infection (Bhattaral *et al*, 2022). The depleting CD4+ T cells count is a marker of immune dysfunction and HIV Progression (Wang *et al*, 2015 and George *et al*, 2015) and indicators of acquiring multiple opportunistic infections and co-infections.

## 5. CONCLUSION

The HBV and/ or HCV co-infection among HIV patients is a significant health threat in Port Harcourt. The physicians involved in care of HIV/AIDS should be vigilant to screen for these infections frequently. The policy makers should integrate the Hepatitis B vaccination into the HIV prevention program. Similarly, the rapid initiation of ART in those diagnosed with HIV can help to maintain CD4+ T Cells count at the optimum level and this can help to bring down the HIV-HBV-HCV co-infection in them. Besides, patients with HIV need proper counselling and awareness about their disease so that the patients understand the risks of blood borne diseases and transmission due to unhygienic needles in drug use and engagement in unsafe sexual intercourse. It is necessary to do additional research on clinical patterns and risk variables to comprehend how HIV/HCV/HBV and malaria co-infection emerged in the study area for better management practice.

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## CONFLICT OF INTEREST

Authors declared the article with no conflict of interest

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