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HAEMATOLOGICAL ALTERATIONS IN HIV, MALARIA, AND CO-INFECTED PATIENTS ATTENDING THE HIV/AIDS CLINIC OF FEDERAL MEDICAL CENTER OWERRI IMO STATE, NIGERIA: A COMPARATIVE STUDY

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ABSTRACT

Blood parameters are typically altered in Human Immunodeficiency Virus (HIV) and malaria infections, and the extent of these alterations may vary due to several factors. These parameters can be used to determine the degree of damage resulting from the infections. The aim of this study was to assess changes in hematological parameters in individuals suffering from malaria, HIV, or co-infection of both diseases. With the aid of purposive sampling, a total of 165 adults attending the HIV/AIDS clinic at the Abia State University Teaching Hospital were recruited for the study after providing consent. They were categorized into Group I (30 HIV-positive individuals), Group II (53 malaria-positive individuals), Group III (52 individuals co-infected with malaria and HIV), and Group IV (30 individuals with neither HIV nor malaria). HIV and malaria parasite infections were screened using standard procedures. Hematological indices were examined using standard procedures. The PCV, Hb, WBC, platelets, and neutrophils were reportedly highest in HIV patients but lowest in co-infected patients. However, a contrary observation was made on the lymphocytes. The hematological indices reported for the control group were significantly ($p<0.05$) higher than those reported for each of the groups I-III. Thus, it can be deduced from this study that malaria and HIV co-infection have deleterious health consequences on sufferers. Therefore, government policies aimed at ameliorating the severity and hardships experienced by HIV patients should ensure that the chances of co-infection are significantly reduced.

Keywords: Malaria, Haemoglobin, Lymphocytes, Tocopherol, Patients

INTRODUCTION

The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). It belongs to the Retroviridae family and is classified under the Lentivirus genus. This virus primarily targets CD4+ T-helper lymphocytes, resulting in significant immune system suppression and a progressive decline in these cells. Such immune suppression compromises the body's defenses and leads to various clinical symptoms, which can be effectively analyzed through specific hematological indices (Meissner *et al*., 2022). According to the WHO HIV Factsheet, by the end of 2022, there were 39 million individuals living with HIV, with the majority, 25.6 million, residing in Sub-Saharan Africa (WHO, 2020).

Malaria is a severe illness caused by *Plasmodium* parasites, transmitted to humans through the bites of infected female Anopheles mosquitoes. The infection begins when a person is bitten by one of these mosquitoes, leading to the injection of sporozoites into the bloodstream. These sporozoites migrate to the liver, where they multiply asexually over a period of 7 to 10 days. Once they transform into merozoites, they exit the liver cells in vesicles and travel through the heart to the lung capillaries. The vesicles break down, releasing the merozoites into the bloodstream, where they invade and replicate within red blood cells (erythrocytes). As these cells rupture, the parasites spread to additional erythrocytes. Clinical symptoms, such as fever, coincide with the destruction of infected erythrocytes and the subsequent release of cellular debris, including malarial pigment (hemozoin) and glycosylphosphatidylinositol, which is believed to act as a 'malaria toxin' (Schofield *et al*. 1993; Clark and Cowden, 2003). Malaria poses a significant public health challenge in developing regions, particularly in the subtropics and tropics. Sub-Saharan Africa accounts for over 90% of global malaria cases and fatalities Symptoms associated with malaria include severe anemia, fever, thrombocytopenia, chills, headaches, vomiting, muscle pain, loss of appetite, rigor, diarrhea, abdominal pain, cough, seizures, respiratory distress, hypoglycemia, metabolic acidosis, hyperlactatemia, and coma linked to increased intracranial pressure (cerebral

malaria), as well as retinopathy, all of which may have hematological implications (Langhorne *et al*. 2008).

Due to the endemic nature of the malaria parasite in Sub-Saharan Africa, where HIV infection is also prevalent, it is reasonable to anticipate a range of health complications resulting from the interaction of these two diseases. These complications can be better understood by analyzing specific hematological indices in affected individuals and has formed the basis for conceptualizing this study.

MATERIALS AND METHODS

Study Location

A cross-sectional study was conducted at the Federal Medical Centre (FMC) in Owerri, Imo State, located in South-East Nigeria. According to the 2006 census, Imo State has an estimated population of approximately 3.9 million, with Owerri being the largest city. The population consists of 62,990 males and 64,223 females, predominantly from the Igbo ethnic group, as well as several other tribes. The city is situated between latitudes 29 and 30°E. FMC Owerri is a significant public health institution in Imo State, offering services for HIV and malaria.

Study Size

A purposive sampling method to select patients from the clinic based on the inclusion and exclusion criteria. Patients were recruited consecutively until the sample size is reached.The study involved 165 adults of both sexes, including 30 HIV-infected patients, 53 malaria-infected patients, 52 HIV and malaria co-infected patients, and 30 individuals with neither HIV nor malaria. The sample size was determined using a sample size calculator with a confidence level of 95%, a margin of error of 5%, and a population proportion of 50%. The population size was 273.

Inclusion and exclusion Criteria

Person considered for inclusion in this study were at least 18 years of age and were attending the HIV/AIDS clinic at FMC Owerri. They were either positive with malaria or HIV or were co-infected with malaria and HIV. Pregnant women as well as those were suffering from illnesses that may affect haematological parameters sickle cell disease, haemophilia, excluded in the study Patients on antimalarial or antiretroviral therapy during the study period (if the therapy has the potential to influence haematological parameters were also not included in the study.

Collection of Blood Samples

Blood samples (approximately 5 mL) drawn from each patient were divided into three aliquots for hematological analysis, HIV screening, and malaria parasitemia quantification. The aliquot designated for HIV screening was collected in microcentrifuge tubes and left to settle at room temperature. The samples were centrifuged at 1000×g for 10 minutes using a refrigerated centrifuge. The resulting serum was carefully transferred into new centrifuge tubes prior to screening.

HIV Screening

HIV screening was conducted using the DetermineTM HIV1/2 immunochromatographic rapid test kit, which detects antibodies for HIV 1 and HIV 2, following the World Health Organization's guidelines. The process included applying 50 µL of serum to the sample pad and waiting for 15 minutes before interpreting the results. A positive outcome was confirmed by the presence of two red lines, while a negative outcome was shown by a single line on the conjugate pad.

Malaria Microscopic

To conduct the microscopic examination of samples, a thin blood smear was prepared according to the standard protocol established by Pembele *et al*. (2014). Blood films were created by placing a drop of blood on one end of a microscope slide and using a spreader slide to evenly distribute the blood along the length of the slide. The aim was to achieve a monolayer area where the cells are adequately spaced for accurate counting and differentiation. Once prepared, the slide was allowed to air dry, and the blood was fixed by briefly immersing the slide in methanol. Following fixation, the film was stained with Giemsa stain for 15 minutes. After staining, the slide was quickly rinsed with distilled water and left to dry. Two drops of immersion oil were then applied to the film, which was examined under a microscope using a 100x objective lens to identify the characteristic features of Plasmodium, as described by Obimakinde *et al*. (2018).

Haematological Analysis

Packed cell volume (PCV), white blood cells (WBC), platelets, neutrophils, and lymphocytes were analyzed using an automatic haematological analyzer (Coulter STKS, Beckman) (Yang *et al*., 2019).

Statistical Analysis

Data were entered into Microsoft Excel 2010 (Microsoft Corporation Inc., USA) and transferred to the software package SPSS for Windows version 20.0 for analysis. Differences between the group means were compared using the Student's t-test and standard error of the mean for analysis of variance (ANOVA). Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 summarizes the haematological indices of individuals with HIV and malaria infections. The levels of PCV, WBC, platelets, lymphocyte and neutrophils were significantly lower ($p<0.05$) in patients co-infected with malaria and HIV compared to those with HIV alone, who also had significantly lower levels ($p<0.05$) than individuals with malaria only. Furthermore, the values for PCV, WBC, platelets, neutrophils and lymphocyte in malaria patients, and HIV patients were significantly lower ($p<0.05$) in the co-infected group compared to the control group.

Table 1: Haematological indices in HIV- and malaria-infected individuals

Parameter	HIV & Malaria patients	Malaria patients	HIV patients	Control
PCV%	30.55±0.61^{a}	35.05±0.94^{c}	33.42±064^{b}	39.38±0.48^{d}
Hb (g/dL)	10.62±0.27^{a}	12.56±0.25^{bc}	11.58±0.31^{b}	13.62±0.30^{d}
WBC (cell/µL)	3.90±0.16^{a}	5.81±0.14^{c}	4.14±0.29^{b}	5.03±0.22^{c}
Platelets (platelets/µL)	161.25±6.23^{a}	198.41±8.12^{c}	185.67±7.21^{b}	233.76±10.09^{d}
Neutrophil (Neutrophil/µL)	26.76±1.24a	46.48±1.34c	34.56±2.18b	52.32±3.01d
Lymphocytes (lymphocyte/µL)	36.39±3.01^{a}	50.61±1.16^{c}	49.47±2.16^{b}	57.08±1.32^{d}

Results are expressed as mean ± standard deviation of three determination. Values with the different superscript in a column are significantly ($p>0.05$) different.

Discussion

Hematological parameters are essential monitoring tools for assessing treatment and prognosis in HIV and malaria. Changes in these parameters can be influenced by various disease states, particularly endemic diseases like malaria and HIV/AIDS, which can impact human health in multiple ways. Hematological alterations are common complications associated with malaria and play a significant role in its pathogenesis. These changes primarily involve essential cell types, including red blood cells (RBCs), leukocytes, and platelets (Bakhubaira, 2013). The most significant leukocyte changes observed in malaria infections are related to neutrophil and lymphocyte counts. The decrease in lymphocyte counts in this study may suggest a redistribution of these cells, potentially due to sequestration in the spleen (Erhart *et al*., 2004). In contrast, the reduction in neutrophil counts associated with malaria infection may result from increased production or release of neutrophils from the bone marrow or reduced clearance from peripheral circulation (Maina *et al*., 2010). These findings are consistent with the results of Kotepui *et al*. (2014), who reported decreased neutrophil and lymphocyte levels in malaria patients. Additionally, the observed declines in packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBC), and platelets in malaria patients support the findings of Kotepui *et al*. (2014), who noted lower PCV, Hb, WBC, platelet counts in individuals with *falciparum* malaria.

A variety of blood-related disorders, such as cytopenia (including anemia, leukopenia, neutropenia, and thrombocytopenia), as well as disruptions in the plasma coagulation pathway, are mainly associated with human immunodeficiency virus (HIV) infection in individuals (Fan *et al.*, 2020; Kibret *et al*., 2019). Platelet destruction and inadequate platelet production are observed in HIV patients. Numerous studies suggest significant sequestration and destruction of platelets in the spleen in cases of HIV-related thrombocytopenia (Noy and Gulick, 2014). Antiretroviral therapy has been shown to reduce the prevalence of anemia, lymphopenia, thrombocytopenia, and other abnormal hematological parameters. The decrease in PCV, Hb, platelets, lymphocyte and WBC levels observed in HIV patients is consistent with the results reported by Ngwu and Eneh (2022), which showed that these parameters improved in HIV patients receiving antiretroviral therapy compared to those not undergoing such treatment. This study emphasizes the positive impact of antiretroviral therapy on these conditions. The decreased lymphocyte count in HIV patients could be attributed to the destructive impact of the virus on CD4+ lymphocytes. This finding is consistent with the results of a study conducted by Soriano *et al*. (1998), which showed that the duration of HIV infection and the level of plasma viremia independently influenced the CD4+ lymphocyte count in people with HIV infection. The significantly decreased PCV, Hb, WBC, platelet, neutrophil, and lymphocyte levels observed in malaria and HIV co-infected individuals could be attributed to the combined effects of both diseases. This finding is consistent with the study by Sanyaolu *et al*. (2013), which showed that patients co-infected with malaria and HIV were more likely to experience anemia.

CONCLUSION

The results of this research emphasize the significant hematological changes in patients with HIV, malaria, and co-infections. The most notable decreases in PCV, hemoglobin, WBC, and platelets were observed in co-infected patients, highlighting the cumulative impact of both infections. Understanding the hematological profiles is crucial for improving clinical care, implementing early interventions, and managing treatment in patients with HIV, malaria, and co-infections. Further research is needed to explore the molecular mechanisms underlying these hematological changes and to determine the potential benefits of targeted medication therapy.

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