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# HAEMATOLOGICAL ALTERATIONS IN HIV, MALARIA AND COINFECTED PATIENTS: 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 either malaria, HIV, or co-infection of both diseases. The study investigated haematological alterations in patients with HIV, malaria, and co-infection. HIV1/2 was screened using an immunochromatographic rapid test kit (DetermineTM HIV1/2), Total CD4 and CD3 cell counts were performed using the Partec flow cytometry, serum ascorbic acid was estimated by spectrophotometry, and the colorimeter method of Baker and Frank was used to determine Tocopherol (Vitamin E). The results showed that the mean ± SEM PCV, Hb, WBC, platelet, and neutrophil values of the HIV, malaria, and co-infected subjects were significantly lower ($P<0.05$) compared to the control groups. However, the mean ± SEM lymphocyte values of the HIV, malaria, and co-infected subjects were significantly higher ($P<0.05$) compared to the control subjects. The most significant reductions in PCV, haemoglobin, WBC, and platelets were observed in co-infected patients, indicating the additional burden of the two infections. Understanding these haematological profiles is crucial for improving clinical care, early interventions, and therapeutic management in patients with HIV, malaria, and co-infections.

**Keywords**: Malaria, Haemoglobin, Lymphocytes, Tocopherol, Patients

## INTRODUCTION

Haematologic changes are common in infectious diseases and can be valuable prognostic and diagnostic indicators. Among these, Human Immunodeficiency Virus (HIV) and malaria are significant global health concerns, especially in sub-Saharan Africa, where they often coexist (WHO, 2021). The impact of these infections on blood parameters, such as anemia, leukopenia, thrombocytopenia, and differential white blood cell counts, has been extensively researched, but few studies have compared HIV-infected, malaria-infected, and co-infected individuals (Olawumi and Olatunji, 2020).

HIV infection leads to progressive immunosuppression, resulting in changes in blood cell counts and increased susceptibility to opportunistic infections (Adetifa and Okomo, 2019). Anemia is common in HIV patients due to bone marrow suppression, chronic inflammation, and nutrient deficiencies (Adetifa and Okomo, 2019). Thrombocytopenia is also frequent in HIV-positive individuals, often caused by immune-mediated platelet destruction and reduced platelet production (Kagu et al., 2020).

Similarly, malaria caused by Plasmodium species, particularly Plasmodium falciparum, leads to significant hematological abnormalities, including hemolytic anemia, thrombocytopenia, and changes in leukocyte counts, primarily due to red blood cell invasion by the parasite and subsequent immune response (Muwonge *et al*., 2022). Severe malaria can also result in disseminated intravascular coagulation (DIC) and splenic sequestration, further complicating hematological status (Uneke, 2021).

The co-infection of HIV and malaria presents a unique challenge as both diseases contribute to overlapping hematological abnormalities. HIV's immunosuppressive effects may exacerbate malaria pathogenesis, leading to increased parasite loads and severe anemia in co-infected individuals (Van Geertruyden et al., 2020). Conversely, malaria infection can activate immune cells, potentially accelerating HIV progression by promoting viral replication and systemic inflammation (Ochola *et al*., 2019).

Despite the well-documented hematological changes in malaria and HIV, there is limited comparative research on their individual and combined effects. Understanding these differences is crucial for optimal disease management and treatment strategies. This study aims to investigate and compare hematological alterations in HIV, malaria, and co-infected patients to elucidate the interactions between these diseases and their clinical implications.

## MATERIALS AND METHODS

HIV Screening: HIV screening was conducted using the DetermineTM HIV1/2 immunochromatographic rapid test kit for the qualitative detection of HIV 1 and HIV 2 antibodies, as recommended by the World Health Organization (1993).

### Test Procedure

Fifty (50) µl of serum was applied to the sample pad, followed by a 15-minute waiting period before interpreting the results. A positive result was indicated by the presence of two red lines, while a negative result was indicated by one line on the conjugate pad.

### Malaria

The Giemsa staining method, a Romanowsky-type stain, was utilized to differentiate malaria chromatin dots from cytoplasm. Reagents included Solution 1 (absolute methyl alcohol and Giemsa stain), Solution 2 (Azure II-eosin, pine glycerol, and methyl alcohol), and Solution 3 (buffer solution with pH 7.0).

### CD4+/CD3+ Count

Total CD4 and CD3 cell counts were determined using the Partec flow cytometry system (Partec GmbH, Germany, 2012) following the manufacturer's instructions. Whole blood (20 µl) was mixed with a reagent containing PE-conjugated monoclonal antibody, incubated, and analyzed using a Partec device.

Antioxidants

### Ascorbic Acid (Vitamin C)

Serum ascorbic acid levels were estimated using the spectrophotometric method described by Ayekyaw (1978).

### LPHA Tocopherol (Vitamin E)

The estimation of serum alpha-tocopherol was done by colorimeter method of Baker and Frank (1968).

### Procedure

Clean test tubes were labeled to reflect the test, standard, and blank. Approximately 1.5 ml of serum, 1.5 ml of standard, and 1.5 ml of water (blank) were added to the respective tubes. In the test and blank tubes, 1.5 ml of xylene was added to all tubes, stoppered, mixed, and centrifuged. Then, 1 ml of the xylene layer was transferred into other stoppered tubes, taking care not to include any ethanol or residue. Next, 1 ml of dipyridyl reagent was added to each tube, stoppered, mixed, and 1.5 ml of the mixture was pipetted into colorimeter cuvettes. The extinction of the test and standard was read against the blank at 460 nm. Starting with the blank, 0.33 ml of ferric chloride was added, mixed, and after exactly 1.5 minutes, the extinction of the test and standard was read against the blank at 520 nm.

### Calculation

Serum tocopherol was calculated in mg/dl using the formula:

$$\frac{Extinction\ of\ unknown\ at\ 520\ nm - Extinction\ at\ 460\ nm \times 0.29}{Extinction\ of\ standard\ at\ 520\ nm}$$

### Haematological Analysis

All haematological analysis was analyzed with autoanalyzer (Abacus) were determined 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 presents a comparison of the mean ± SEM values of hematological parameters for all groups and the control subjects. The results indicate that the PCV, Hb, WBC, platelet, and neutrophil values of the HIV, malaria, and coinfected subjects were significantly lower ($P<0.05$) compared to the control group. However, the lymphocyte values of the HIV, malaria, and coinfected subjects were significantly higher ($P<0.05$) than those of the control subjects. Additionally, malaria-infected subjects exhibited higher levels of neutrophils and platelets compared to HIV-infected subjects. The mean ± SEM values of PCV, Hb, WBC, platelets, and neutrophils for the control subjects (39.38±0.484, 13.62±0.304, 5.03±0.227, 223.767±10.097, and 52.32±2.667) were reduced to (33.426±0.647, 58±0.313, 4.14±0.297, 185.67±7.214, 34.56±2.667) for HIV-infected subjects, (35.053±0.948, 12.56±0.252, 5.815±0.143, 198.416±8.124, 46.48±1.345) for malaria-infected subjects, and (30.551±0.614, 10.62±0.274, 3.9±0.164, 161.252±6.233, 26.76±1.240) for HIV and malaria coinfected subjects.

**Table 1: Haematological parameters in HIV, Malaria and Co-infection**

| Parameters | HIV and Malaria Coinfected Subjects | Malaria Infected Subjects | HIV infected Subjects | Control Subjects |
|---|---|---|---|---|
| PCV (%) | 30.551±0.614[a] | 35.053 ± 0.948[a] | 33.462 ±0.647[a] | 39.38± 0.48[a] |
| HB(G/DL) | 10.62 ± 0.274[a] | 12.56 ± 0.252[a] | 11.58 ± 0.313[a] | 13.62±0.304[b] |
| WBC x $10^9$/L | 3.9 ± 0.164[a] | 5.815 ± 0.143[b] | 4.14 ± 0.297[a] | 5.03 ± 0.227[b] |
| PLTx$10^9$/L | 161.252 ± 6.233[a] | 198.416 ± 8.124[a] | 185.67±7.214[a] | 233.767± 10.097[b] |
| NEU x $10^9$/L | 26.76±1.240[a] | 46.48±1.345[a] | 34.56±2.187[a] | 52.32±3.014[b] |
| LYM x $10^9$/L | 57.08±1.325[a] | 50.616±1.168[a] | 49.47± 2.166[a] | 36.39± 3.014[b] |

WBC – White blood cell count, NEU – Neutrophil, HB – Hemoglobin, LYPH – Lymphocyte, PCV - Packed cell volume, PLT – Platelet. Values not sharing the same superscript indicate a significant difference. Values sharing the same superscript indicate no significant difference. Values entered at ($P<0.05$

### Discussion

Haematological parameters are crucial indicators of disease severity in infectious diseases such as HIV and malaria. This study compared the haematological parameters of HIV-infected, malaria-infected, and co-infected individuals with those of healthy control subjects for reference. The results showed significant differences in packed cell volume (PCV), hemoglobin (HB), white blood cell (WBC) count, platelet count (PLT), neutrophils (NEU), and lymphocytes (LYM) among the groups.

PCV and HB values were significantly lower in all infected groups compared to control subjects. The co-infected group

had the largest decrease (PCV: 30.551 ± 0.614%, HB: 10.62 ± 0.274 g/dL), followed by HIV-infected patients (PCV: 33.462 ± 0.647%, HB: 11.58 ± 0.313 g/dL), and malaria-infected subjects (PCV: 35.053 ± 0.948%, HB: 12.56 ± 0.252 g/dL). This aligns with previous reports of Adetifa and Okomo (2019) indicating anemia as a common feature of both malaria and HIV infections. The lower values in co-infected individuals suggest a synergistic effect of the two infections in exacerbating hematological abnormalities, likely due to increased hemolysis, bone marrow suppression, and chronic inflammation (Olawumi and Olatunji, 2020).

WBC count was notably reduced in HIV-infected (4.14 ± 0.297 × $10^9$/L) and co-infected individuals (3.9 ± 0.164 × $10^9$/L) compared to malaria-infected (5.815 ± 0.143 × $10^9$/L) and control individuals (5.03 ± 0.227 × $10^9$/L). This reduction in HIV-infected and co-infected patients is consistent with HIV's immunosuppressive effects, leading to decreased immune cell counts (Van Geertruyden., 2020). In contrast, malaria infection alone appeared to result in elevated WBC levels, possibly due to an immune response to Plasmodium infection (Muwonge *et al*., 2022).

Neutrophil counts followed a similar pattern, with HIV-infected (34.56 ± 2.187 × $10^9$/L) and co-infected individuals (26.76 ± 1.240 × $10^9$/L) showing significantly lower levels than malaria-infected (46.48 ± 1.345 × $10^9$/L) and control individuals (52.32 ± 3.014 × $10^9$/L). Neutropenia is a common feature of HIV disease, attributed to direct viral effects on the bone marrow and increased peripheral destruction (Kagu *et al*., 2020). The malaria group exhibited high neutrophil counts, indicative of an acute immune response to parasitic invasion (Uneke, 2021).

Lymphocyte counts were significantly higher in co-infected (57.08 ± 1.325 × $10^9$/L), malaria-infected (50.616 ± 1.168 × $10^9$/L), and HIV-infected (49.47 ± 2.166 × $10^9$/L) patients compared to controls (36.39 ± 3.014 × $10^9$/L). The increase in HIV-infected patients is characteristic of CD4+ T-cell depletion, leading to compensatory lymphocytosis during the acute infection stage (Ochola *et al*., 2019). Immune stimulation and enhanced antigen-specific lymphocyte production may contribute to lymphocytosis in malaria (Muwonge *et al*., 2022).

Platelet counts were lowest in co-infected patients (161.252 ± 6.233 × $10^9$/L), followed by HIV (185.67 ± 7.214 × $10^9$/L) and malaria (198.416 ± 8.124 × $10^9$/L) patients, with control participants having the highest platelet counts (233.767 ± 10.097 × $10^9$/L). Thrombocytopenia is observed in both HIV and malaria infections due to bone marrow suppression, peripheral destruction, and splenic sequestration (Van Geertruyden*et al*., 2020). The more severe thrombocytopenia in co-infected individuals suggests an additive effect of the two infections, possibly due to immune-mediated destruction and endothelial injury (Olawumi and Olatunji, 2020).

## 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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