

## Original Research Article

# Levels of neutrophils, interleukin-10 and tumor necrosis factor-alpha amongst people living with coinfection of hiv and malaria in Benin City, Edo state.

### ABSTRACT

**Aim:** This study is aimed at investigating the levels of TNF- $\alpha$ , IL-10, and Neutrophils in study participants with coinfection of HIV and Malaria attending university of Benin teaching hospital.

**Study Design:** Cross-sectional study design was used for the study

**Place and Duration of Study:** The research project was carried out at the University of Benin Teaching Hospital (UBTH) in Benin City, Nigeria. The study lasted for a period of six months (From January, 2024 to June, 2024).

**Methodology:** The study was carried out at the University of Benin Teaching Hospital in Edo State, Nigeria. A total of 210 samples were collected and used in the study, which included adults with HIV infection's, with Malaria Infection and with the co-infection of HIV and Malaria who met the inclusion criteria and were reconfirmed throughout the study period. All samples were analyzed for full blood counts using Mindray BC-5000. Subsequently, the samples were processed for ELISA to evaluate the levels of TNF-alpha, andIL-10.

**Results:** Individuals with HIV-Malaria coinfection exhibited significantly elevated levels of Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) at 27.67 pg/ml compared to those with HIV (11.20 pg/ml) or malaria (13.23 pg/ml), with  $p < 0.001$  for both comparisons. Interleukin 10 (IL-10) levels were lower in coinfecting individuals (18.74 pg/ml) than in malaria only cases (25.86 pg/ml,  $p < 0.001$ ), while neutrophil percentages were significantly reduced in coinfecting individuals (51.71%) compared to those with HIV (59.23%,  $p < 0.001$ ) and malaria (57.74%,  $p = 0.001$ ). Correlations indicated that higher TNF- $\alpha$  levels were associated with lower neutrophil percentages and a negative correlation between IL-10 and TNF- $\alpha$  levels was observed.

**Conclusion:**This study revealed significant differences in the levels of TNF- $\alpha$ , IL-10, and neutrophils among individuals with HIV-Malaria coinfection compared to those with HIV or malaria alone. Notably, TNF- $\alpha$  levels were markedly elevated in the HIV-Malaria coinfection group compared to the other groups, while IL-10 levels were significantly higher in individuals with malaria alone. Neutrophil percentages were significantly lower in the HIV-Malaria coinfection group compared to the HIV-only group. Correlation analyses indicated a significant inverse relationship between TNF- $\alpha$  and neutrophils, while IL-10 was negatively correlated with TNF- $\alpha$ .

**Keywords:** HIV, Malaria, Co-infection, TNF-Alpha, IL-10

## 1.0 INTRODUCTION

Malaria alone affected almost 229 million people globally in 2019, with the majority (95%) of cases happening in 29 sub-Saharan African countries: Nigeria (27%), Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%), and Niger (3%) [1]. Malaria and HIV, two of the world's deadliest diseases, share a large portion of territory in Sub-Saharan Africa. As a result, malaria and HIV co-infection are prevalent in the region [2].

An estimated 3.3–3.7 million probable cases of malaria occur in healthcare facilities annually, despite notable progress in recent years to reduce the burden of this infection due to the implementation of several strategic programs for malaria control [3][4]. Thus, the disease is still widely distributed. The breadth of the antibody response to merozoite surface protein (MSP) antigens is limited by HIV infection, as previous studies have shown [5].

Important roles in immune control are played by pro-inflammatory cytokines like tumor necrosis factor (TNF- $\alpha$ ) and anti-inflammatory cytokines like interleukin 10 (IL-10). There are two types of TNF- $\alpha$ : transmembrane (mTNF- $\alpha$ ) and soluble (sTNF- $\alpha$ ), each having unique binding and functional characteristics [6]. Th1, Th2, Tr1, and regulatory T cells are among the T cell subsets that produce IL-10, which is essential for controlling immune responses and reducing inflammation [7]. The most common type of white blood cells, neutrophils, are critical for immune system modulation and pathogen removal [8].

The most common kind of white blood cells in the body are called neutrophils, also called polymorphonuclear cells [9]. [8]state that neutrophils can destroy pathogens by phagocytosis, the generation of Reactive oxygen species ( ROS), and other antimicrobial compounds, or the formation of extracellular traps (NETs) for neutrophils. They can also help to activate and regulate the immune response by secreting cytokines and chemokines and possibly presenting antigens [10].

In this study, we evaluated the levels of Neutrophils, interleukin-10, and tumor necrosis factor alpha in individuals who co-infect with HIV and malaria.

## **2.0 MATERIALS AND METHOD**

### **2.1 Study area**

The research project was carried out at the University of Benin Teaching Hospital (UBTH) in Benin City, Nigeria. UBTH, a premier tertiary healthcare institution in the country, provides an

ideal setting for extensive research due to its state-of-the-art facilities and diverse patient population.

## 2.2 Sample Size

Cochrane formula was used in calculating sample size.

Prevalence of malaria-HIV coinfection ( $p$ ) = 0.229 (22.9% expressed as a proportion) [11].

Margin of error ( $E$ ) = 0.0569

Z-score for a 95% confidence level ( $Z$ )  $\approx$  1.96

Using the formula:

$$n = \frac{Z^2 \times p \times (1-p)}{E^2}$$

Substituting the given values:

$$n = 1.96^2 \times 0.229 \times (1 - 0.229) / 0.0569^2$$

$$n = 3.8416 \times 0.176 / 0.00323$$

$$n = 0.6769 / 0.00323$$

$$n = 209.8$$

$$n \approx 210$$

Rounding up to the nearest whole number, a sample size of approximately 210 individuals was utilized.

## 2.3 Study Population

### 2.3.1 Inclusion criteria

Participants included in this research were 70 individuals diagnosed with both HIV and malaria, 70 individuals diagnosed with only malaria and 70 individuals diagnosed with only HIV confirmed through medical records or laboratory results at the University of Benin Teaching Hospital- were this information confirmed before study. To ensure consistency and reliability in the study population, only patients who met the clinical criteria for both infections as per established diagnostic guidelines were considered eligible for inclusion.

### **2.3.2 Exclusion criteria**

Individuals were excluded from the study if they met any of the following criteria:

1. Diagnosis of other types of infections or diseases that could interfere with immune responses or study outcomes.
2. Presence of severe co-morbidities or medical conditions that could significantly alter immune markers, such as cancer or autoimmune diseases.
3. Inability to provide informed consent or participate actively in the study due to cognitive impairment or other reasons.
4. Pregnancy, as hormonal changes during pregnancy can affect immune function and potentially confound study results.
5. Participation in any concurrent clinical trials or medical interventions that could influence the immune response or outcomes measured in this research.
6. HIV patients on ART

## **2.4 Equipments**

Haematology Analyzer (Mindray BC-5000), Elisa Machine

UNDER PEER REVIEW

## 2.5 Data Collection Methods

### 2.5.1 Sample Collection

Venipuncture was used to obtain blood samples, which were then separated into two tubes using standard procedures. These tubes held 5 mL of blood for the neutrophil count and a plain tube for the measurement of TNF- $\alpha$  and IL-10.

### 2.5.2 Determination of Tumor Necrosis Factor $\alpha$ (TNF $\alpha$ )

**Method:**ELISA[12].

**Principle:**Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) quantification assay uses sandwich ELISA technique: TNF- $\alpha$  from samples binds to microwells coated with a capture antibody specific to human TNF- $\alpha$ , which is followed by a biotin-conjugated detection antibody and streptavidin-HRP that binds to the detection antibody. Addition of a colorimetric substrate produces a color that is proportionate to TNF- $\alpha$  levels, measured at 450 nm.

**Procedure:**The process starts with prepping typical TNF- $\alpha$  dilutions and cleaning microwell strips. After washing, samples are added, and then the detecting antibody and streptavidin-HRP. An acid solution is used to monitor and halt the development of color. By comparing absorbance results against a standard curve made from known dilutions, TNF- $\alpha$  concentrations are obtained. This technique detects TNF- $\alpha$  in a variety of biological samples, such as serum and plasma, and is both sensitive and specific.

### 2.5.3 Determination of Interleukin-10 (IL-10).

**Method:**ELISA[13].

**Principle:**A microtiter plate coated with a monoclonal antibody specific to human IL-10 is used in the IL-10 test. Unbound components are removed by washing after the sample has been added. To identify bound IL-10, biotinylated antibody is added first, then streptavidin-HRP. Sulfuric acid stops the blue color that the TMB substrate produces in proportion to the levels of IL-10, turning the solution yellow. To calculate the amount of IL-10 present in the sample, the intensity of this yellow color is measured at 450 nm and compared to a standard curve.

**Procedure:**100  $\mu$ L of diluted standards and sample dilution buffer were added to designated wells for the IL-10 assay, and then 100  $\mu$ L of diluted samples were added. After two hours of

incubation at 37°C, the plate underwent three rounds of washing. After adding and incubating a working secondary antibody for an hour, another wash was performed. Subsequently, 100 µL of Streptavidin-HRP Conjugate was introduced and allowed to sit for half an hour. Following washing, blue-colored TMB Substrate was applied; this color changed to yellow when Stop Solution was introduced. At 450 nm, absorbance was measured.

#### **2.5.4 Determination of Neutrophil count**

**Method: Haematology Analyzer (Mindray BC-5000) [14].**

**Principle:**Both impedance-based and optical methods are used by the haematology analyzer to quantify neutrophils. Leukocytes are isolated from blood samples by diluting them with a lysing reagent. Impedance measurement measures changes in the cell as it passes through an electrical field, allowing for size and granularity differentiation. Simultaneously, optical analysis measures light scatter and fluorescence to obtain additional information on cell characteristics. This combined approach, processed through sophisticated algorithms, accurately determines the absolute neutrophil count (ANC) while correcting for potential interferences, ensuring precise neutrophil quantification in the sample.

**Procedure:**The sample was placed into the sample chamber of the haematology analyzer, and the researcher started the analysis. The analyzer then processed the data to determine the absolute neutrophil count, and the researcher checked the output on the analyzer's display and noted the results for additional analysis and reporting. The analyzer performed optical and impedance measurements to evaluate cell size and granularity during the analysis.

### 3. RESULTS

**Table 1** levels of Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) in individuals with HIV-Malaria coinfection compared to those with HIV or Malaria infection alone.

Parameter	HIV-Malaria coinfection n=70	HIV n=70	Malaria infection n=70	p-value
	<b>Mean <math>\pm</math>SD</b>			
TNF- $\alpha$ (pg/ml)	27.6667 $\pm$ 9.23976	11.1994 $\pm$ 2.17116	-	<0.001*
	27.6667 $\pm$ 9.23976	-	13.2317 $\pm$ 3.78970	<0.001*

\*p-value is significant at  $p < 0.05$

Table 1 presents the levels of Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) in individuals with HIV-Malaria coinfection compared to those with HIV or malaria infection alone. The table shows that the mean TNF- $\alpha$  level in individuals with HIV-Malaria coinfection was  $27.67 \pm 9.24$  pg/ml. In contrast, individuals with HIV alone had a mean TNF- $\alpha$  level of  $11.20 \pm 2.17$  pg/ml, and those with malaria infection alone had a mean TNF- $\alpha$  level of  $13.23 \pm 3.79$  pg/ml. Statistical analysis revealed a significant difference in TNF- $\alpha$  levels between the HIV-Malaria coinfection group and the HIV-only group ( $p < 0.001$ ), as well as between the HIV-Malaria coinfection group and the malaria-only group ( $p < 0.001$ ).

**Table 2** levels of Interleukin 10 (IL-10) in individuals with HIV-Malaria coinfection compared to those with HIV or Malaria infection alone.

Parameter	HIV-Malaria coinfection n=70	HIV n=70	Malaria infection n=70	p-value
	<b>Mean ± SD</b>			
IL-10 (pg/ml)	18.7350±5.15496	20.2414±8.89411	-	0.217
	18.7350±5.15496	-	25.8621±7.05089	<0.001*

\*p-value is significant at  $p < 0.05$

Table 2 displays the levels of Interleukin 10 (IL-10) in individuals with HIV-Malaria coinfection compared to those with HIV or malaria infection alone. The mean IL-10 level for individuals with HIV-Malaria coinfection was  $18.74 \pm 5.15$  pg/ml. For those with HIV alone, the mean IL-10 level was slightly higher at  $20.24 \pm 8.89$  pg/ml, but this difference was not statistically significant, as indicated by a p-value of 0.217. In contrast, individuals with malaria infection alone exhibited a significantly higher mean IL-10 level of  $25.86 \pm 7.05$  pg/ml compared to those with HIV-Malaria coinfection ( $p < 0.001$ ). The significant p-value highlights that the IL-10 levels in malaria-only cases are statistically different from those in HIV-Malaria coinfection cases, while no significant difference was observed between the HIV-only and HIV-Malaria coinfection groups.

**Table 3** levels of Neutrophils in individuals with HIV-Malaria coinfection compared to those with HIV or Malaria infection alone.

Parameter	HIV-Malaria coinfection n=70	HIV n=70	Malaria infection n=70	p-value
	<b>Mean ± SD</b>			
Neutrophil (%)	51.7089±9.440	59.2337±11.49514	-	<0.001*
	51.7089±9.440	-	57.7389±10.00316	0.001*

\*p-value is significant at p<0.05

Table 3 presents the levels of neutrophils in individuals with HIV-Malaria coinfection compared to those with HIV or malaria infection alone. The mean neutrophil percentage for individuals with HIV-Malaria coinfection was 51.71 ± 9.44%. This was significantly lower than the mean neutrophil percentage of 59.23 ± 11.50% observed in individuals with HIV alone (p < 0.001). Similarly, the mean neutrophil percentage in individuals with malaria infection alone was 57.74 ± 10.00%, which was also significantly higher than that in the HIV-Malaria coinfection group (p = 0.001). These results indicate a significant decrease in neutrophil levels in individuals with HIV-Malaria coinfection compared to those with either HIV or malaria infection alone.

**Table 4** Relationship between the levels of TNF- $\alpha$ , IL-10, and Neutrophils

		IL-10 (pg/ml)	TNF- $\alpha$ (pg/ml)	NEUTROP HILS (%)
IL-10 (pg/ml)	Pearson Correlation	1	-.138*	.099
	Sig. (2-tailed)		.045	.153
	N	210	210	210
TNF- $\alpha$ (pg/ml)	Pearson Correlation	-.138*	1	-.304**
	Sig. (2-tailed)	.045		.000
	N	210	210	210
NEUTROPHILS (%)	Pearson Correlation	.099	-.304**	1
	Sig. (2-tailed)	.153	.000	
	N	210	210	210

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\* . Correlation is significant at the 0.01 level (2-tailed).

Table 4 illustrates the relationships between the levels of TNF- $\alpha$ , IL-10, and neutrophils. The Pearson correlation analysis revealed a significant negative correlation between IL-10 and TNF- $\alpha$  ( $r = -0.138$ ,  $p = 0.045$ ), indicating that higher IL-10 levels were associated with lower TNF- $\alpha$  levels. However, the correlation between IL-10 and neutrophils was not significant ( $r = 0.099$ ,  $p = 0.153$ ). Conversely, a significant negative correlation was observed between TNF- $\alpha$  and neutrophils ( $r = -0.304$ ,  $p < 0.001$ ), suggesting that higher TNF- $\alpha$  levels were associated with lower neutrophil percentages. These findings highlight significant interrelationships among these biomarkers, with TNF- $\alpha$  showing notable associations with both IL-10 and neutrophil levels.

#### 4.0 DISCUSSION OF FINDINGS

The interplay between HIV and malaria presents a complex challenge to global health, particularly in regions where both infections are prevalent. Each disease exerts significant immunological and physiological impacts, and their co-occurrence could potentially exacerbate the severity of both conditions. Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), Interleukin-10 (IL-10), and neutrophil counts are critical biomarkers in understanding the immune response and inflammation associated with these infections[15][16][17]. TNF- $\alpha$  is a pro-inflammatory cytokine that plays a crucial role in the immune response but can also contribute to disease pathology when dysregulated. IL-10, an anti-inflammatory cytokine, often acts to counterbalance inflammation but may also influence disease outcomes through its immunosuppressive effects. Neutrophils, the most abundant white blood cells, are central to the body's acute inflammatory response and their levels can reflect ongoing inflammatory processes.

Table 1 shows that individuals with HIV-malaria coinfection had a mean TNF- $\alpha$  level of 27.67 pg/ml, which is significantly higher compared to those with HIV alone (11.20 pg/ml) and malaria infection alone (13.23 pg/ml). This elevated TNF- $\alpha$  level in coinfecting individuals aligns with several studies highlighting the enhanced inflammatory response in co-infected patients. For instance, previous research has demonstrated that co-infection with HIV and malaria leads to an increased production of pro-inflammatory cytokines, including TNF- $\alpha$ , compared to single infections [18][19][20].

The elevated TNF- $\alpha$  in HIV-malaria coinfection could be attributed to the synergistic effect of these infections on the immune system. Both HIV and malaria independently stimulate TNF- $\alpha$  production through different pathways. HIV infection triggers TNF- $\alpha$  production through the activation of macrophages and dendritic cells by the virus, while malaria induces TNF- $\alpha$  release primarily through the recognition of Plasmodium antigens by immune cells [21]. The

combination of these stimuli in coinfection may lead to an exacerbated inflammatory response, with TNF- $\alpha$  contributing to both increased immune activation and potential immunopathology.

As indicated in Table 2, the mean IL-10 level in individuals with HIV-malaria coinfection was 18.74 pg/ml, which was not significantly different from the IL-10 levels observed in individuals with HIV alone (20.24 pg/ml) but was significantly lower compared to those with malaria infection alone (25.86 pg/ml). This discrepancy in IL-10 levels is noteworthy as it contrasts with some studies which have shown elevated IL-10 levels in HIV-malaria coinfection [19].

The observed lower IL-10 levels in coinfecting individuals might be indicative of a dysregulated or exhausted anti-inflammatory response. In the context of co-infection, the immune system may be overwhelmed by the high inflammatory burden, leading to suboptimal IL-10 production. Molecularly, the interaction between HIV and malaria could disrupt the signaling pathways responsible for IL-10 production. For instance, HIV-induced changes in the function of regulatory T cells and monocytes may impair the ability to produce IL-10 adequately in response to malaria infection [22]. This imbalance could result in a less effective counter-regulatory mechanism against the elevated TNF- $\alpha$  levels observed in these patients.

Table 3 presents data showing that the mean neutrophil percentage in individuals with HIV-malaria coinfection was 51.71%, significantly lower than in individuals with HIV alone (59.23%) but not significantly different from those with malaria infection alone (57.74%). This finding is consistent with some studies suggesting that neutrophil counts can be altered in co-infections, with a trend towards reduced neutrophil levels in complex infections [23][24].

The lower neutrophil levels observed in HIV-malaria coinfection could be attributed to the immunosuppressive effects of HIV. HIV infection can lead to neutrophil apoptosis and impaired neutrophil function, which may be exacerbated when combined with malaria [25]. On the other

hand, malaria can also cause neutrophil sequestration in the spleen, leading to lower circulating neutrophil counts. The combined effects of HIV-induced neutropenia and malaria-induced sequestration might result in the observed neutrophil levels in coinfecting individuals.

Table 4 explores the correlations between TNF- $\alpha$ , IL-10, and neutrophil levels. A significant negative correlation was observed between TNF- $\alpha$  and IL-10 ( $r = -0.138$ ,  $p = 0.045$ ), suggesting that higher TNF- $\alpha$  levels are associated with lower IL-10 levels. Additionally, TNF- $\alpha$  levels were negatively correlated with neutrophil percentages ( $r = -0.304$ ,  $p < 0.001$ ), indicating that higher TNF- $\alpha$  levels are associated with lower neutrophil counts. Conversely, IL-10 and neutrophil levels showed a weak positive correlation ( $r = 0.099$ ,  $p = 0.153$ ), which was not statistically significant.

The significant inverse correlation between TNF- $\alpha$  and IL-10 supports the hypothesis of a disrupted balance between pro-inflammatory and anti-inflammatory responses in coinfecting individuals. Elevated TNF- $\alpha$  may suppress IL-10 production, leading to a less effective anti-inflammatory response. The negative correlation between TNF- $\alpha$  and neutrophils suggests that high TNF- $\alpha$  levels could be associated with neutrophil dysfunction or apoptosis. This correlation highlights the potential impact of TNF- $\alpha$  on neutrophil dynamics, further emphasizing the complex interplay between inflammation and immune cell function in HIV-malaria coinfection.

## **5.0 CONCLUSION**

This study revealed significant differences in the levels of TNF- $\alpha$ , IL-10, and neutrophils among individuals with HIV-Malaria coinfection compared to those with HIV or malaria alone. Notably, TNF- $\alpha$  levels were markedly elevated in the HIV-Malaria coinfection group compared to the other groups, while IL-10 levels were significantly higher in individuals with malaria alone. Neutrophil percentages were significantly lower in the HIV-Malaria coinfection group compared

to the HIV-only group. Correlation analyses indicated a significant inverse relationship between TNF- $\alpha$  and neutrophils, while IL-10 was negatively correlated with TNF- $\alpha$ .

## **6.0 RECOMMENDATION**

Based on the study's findings, it is recommended that clinicians consider the elevated levels of TNF- $\alpha$  and altered neutrophil counts in individuals with HIV-Malaria coinfection when devising treatment plans. Enhanced monitoring and targeted therapeutic interventions to address the inflammatory responses may improve patient outcomes. Further research is also recommended to explore the mechanisms underlying the observed correlations and their potential impact on disease progression and management.

## **CONSENT**

All authors unanimously declare that written informed consent was obtained from the participants for publication of this study finding.

## **ETHICAL APPROVAL**

Ethical approval was obtained from the ethics committee of the Edo State Ministry of Health.

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