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Studies on Some C-Reactive Protein, Complement 3, Complement 4 and Interleukin 6 in HIV-Malaria Co-Infected Subjects Attending General Hospital Awo-Omamma

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ABSTRACT: Malaria and HIV infection are two of the major public health issues in sub-Saharan Africa. High rates of morbidity and mortality are associated with both. In Awo-Omamma, Imo state, the study compared the levels of certain inflammatory mediators and immunoglobulins in HIV patients with co-infection with malaria and those who did not have malaria. 102 (34%) of the 300 volunteers that were enlisted for the study were men, and 198 (66%) were women.Of the patients in this study, one hundred (33.3%) had co-infections with malaria and HIV. The study's findings demonstrated a substantial variation in CD4 counts between the groups under investigation. (P=0.001, F=22.5). When compared to the average of HIV mono-infected individuals (488±100.5 cells/mm3) and control participants (909±102 cells/mm3), the mean CD4+ of HIV-malaria co-infected subjects (384.7±101.5 cells/mm3) was significantly lower. correspondingly
(P=0.0001, P=0.0001). When compared to the means of HIV mono-infected individuals (37.6±9.8 mg/dl) and control subjects (5.6±2.9 mg/dl), respectively, the mean ± SD value of C-reactive protein (CRP) in HIV-malaria co-infected participants (56±21.3 mg/dl) was substantially greater (P=0.0001, P=0.0001). HIV-malaria co-infected individuals had a statistically higher mean value of C3 (203.6±105 mg/dl) than HIV mono-infected individuals (152.3±90.5 mg/dl) and the control group (104.7±56.9 mg/dl), respectively (P=0.05, 0.04). In a similar vein, the mean IL-6 of individuals co-infected with HIV and malaria (54.4±19.6 mg/dl) was considerably greater than the averages of individuals infected with HIV alone (38.3±14.9 mg/dl) and control subjects (5.8±1.8 mg/dl) (P=0.0001, P<0.001). Additionally, it was noted that whereas CRP and IL-6 were higher in HIV mono-infected individuals than in control participants, there was no discernible difference between the two groups' C3 and C4. In summary, adult HIV/malaria co-infection heightens humoral and inflammatory responses, particularly for C-reactive protein (CRP), complement 3 (C3), and interleukin-6 (IL-6). Furthermore, ART demonstrated a drop in IL-6 and CRP levels.

KEY WORDS: C-Reactive Protein, Complement 3, Complement 4, Interleukin 6 , Hiv-Malaria Co-Infected, Awo-Omamma

INTRODUCTION

The lentivirus that causes HIV infection and acquired immunodeficiency syndrome (AIDS) is known as the human immunodeficiency virus (HIV) [1]. HIV infects key immune system components including dendritic cells, macrophages, and helper T cells (more especially, CD4+ T cells) in humans[2].Apoptosis of uninfected bystander cells [3], direct viral destruction of infected cells, and CD8 cytotoxic lymphocytes that identify infected cells are some of the processes by which HIV infection results in low levels of CD4+ T cells. Cell-mediated immunity is eliminated when CD4+ T cell counts fall below a certain threshold, making the body increasingly vulnerable to opportunistic infections [4]. Approximately 36.7 million people worldwide are HIV positive.
[5]. With a prevalence incidence of 3.1%, about 3.5 million adults in Nigeria between the ages of 15 and 49 are HIV positive [6]. Plasmodium parasites are the source of malaria. Female Anopheles mosquitoes carrying the parasites bite humans; these mosquitoes are known as "malaria vectors." Human malaria is caused by five different parasite species, the two most dangerous being P. falciparum and P. vivax. The malaria parasite P. falciparum is the most common in Africa. The majority of malaria-related deaths worldwide are caused by it. The predominant malaria parasite in the majority of non-sub-Saharan nations is P. vivax [7].
The burden of malaria remains disproportionately high in Sub-Saharan Africa compared to other regions of the world. 90% of malaria cases and 92% of malaria deaths in 2015 occurred in the region. Approximately 13 nations, mostly in sub-Saharan Africa, are responsible for 76% of malaria cases and 75% of deaths worldwide [8].
More than two million people die from HIV and malaria combined every year. A significant percentage of co-infections develop due to the significant geographic overlap between HIV/AIDS and malaria. HIV raises the risk of adult clinical malaria and malaria

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infection in regions where malaria transmission is persistent, particularly in individuals with severe immunosuppression. Adults living with HIV are more vulnerable to severe and complicated malaria, as well as death, in areas where malaria transmission is unstable [9].

Strong CD4+ cell activation and an increase in proinflammatory cytokines are linked to malaria infection, which creates an environment that is perfect for HIV 1 replication and virus transmission among CD4+ cells[10]

Due to their impact on inflammatory cells or the microvasculature, chemicals known as inflammatory mediators can partially or completely trigger inflammatory reactions [11]. Inflammatory mediator generation is a key mechanism shared by HIV and malaria infection. P. falciparum infections are frequently linked to the activation of multiple inflammatory pathways, which can lead to systemic and local inflammatory responses. These responses involve the production of a broad variety of cytokines from multiple cell types, including endothelial cells, leukocyte subsets, and various tissues cells like macrophages.The host may benefit from this inflammatory response that occurs during malaria infection because it may trigger anti-microbial responses in granulocytes, T cells, macrophages, and endothelial cells [12].

However, an overabundance of inflammatory response may be harmful to the host, leading to tissue damage and excessive systemic inflammation, which may increase the risk of morbidity and mortality in cases of falciparum malaria. According to research on rodents, the malaria parasites themselves release cytokines that impede the host's immune system [13]

.Despite the fact that severe immunodeficiency and the loss of CD4+ T cells are the immunological hallmarks of HIV infection. Additionally, it is characterized by a persistent inflammatory state, which through mechanisms such immunological fatigue will exacerbate immunodeficiency[14].Acute HIV infection is linked to a fast and high production of several cytokines (interferon-α, interferon-γ, tumor necrosis factor, inducible protein 10, IL-6, IL-10, IL-15)[15].Acute HIV infection also causes a significant rise in the frequency of activated T cells, with up to 50% of certain CD8+ T subsets being activated. A "steady-state" of T cell activation is reached following the resolution of acute infection, and this state is partially determined by the level of HIV replication and innate immune responses. [16]Nigeria, a nation in Sub-Saharan Africa, is confronted with the issue of co-infection between HIV and malaria. Research has indicated that interactions occur between HIV and malaria when they co-occur. Reports on the results of these interactions vary, nevertheless. This study will shed important light on the humoral and inflammatory immune responses in relation to co-infection between HIV and malaria. Policy makers can take this into account when creating efficient control and management policies.

MATERIALS AND METHODS

Study Area

This study was carried out at General hospital Awo-Omamma, Imo state. Awo-Omamma is a community in Oru east local government in Imo state, Nigeria. It covers about 89.2 square kilometres on the bank of Njaba River and lies in tropical rain forest, with hot and rainy seasons. Awo-Omamma is bounded in the North by Amiri, Imo State in Oru-East, and Mgbidi and Otulu both in Oru-West. In the East it shares boundaries with Okwudor in Njaba LGA. In the West Awo-Omamma is bounded by Akabo, Oguta LGA, Awa, Oguta LGA, Abiaziem and Ngbele communities in Oguta LGA and in the South by Eziama Obiato and Njaba River which runs through Oguta Lake and Orashi River on the other end into the sea. The Awo-Omamma General Hospital is a public hospital, located at Awo-Omamma 2. It is a 36-bed hospital that offers various health services including HIV/ AIDS Services.

Sample Size Sample size was calculated using the formula below determined. The lowest prevalence rate of preeclampsia is in southern Nigeria is 5 %

FORMULA: $n= Z^2p(q)/d2$

n = Minimum sample size

Z= Confidence interval = 1.96 (95% confidence interval).

P =Prevalence rate of HIV-malaria co-infection in Southern Nigeria = 5% (0.05)

q =1-P.

$n = 1.96^2$ X 0.05 (1-0.0.05)/0.05^2

n = 3.8146 X 0.0475/0.0025

n = 72.99

n = 73.

Study Population

Three hundred (300) subjects aged 18 to 67years were recruited for the study and categorized into three groups as follows; Patients with HIV-malaria coinfection, HIV seropositive patients without malaria and HIV seronegative subjects without malaria.

Selection Criteria

Inclusion Criteria.

- Subjects with HIV-malaria co-infection confirmed with standard methods

- HIV seropositive subjects without malaria confirmed with standard methods.
- HIV sero-negative subjects without malaria confirmed with standard methods.
- Subjects whom their informed consent were obtained

Exclusion Criteria

The following patients were excluded;

- Subjects below the age of 18years and above 67years were excluded.
- Subjects who were diagnosed with systemic diseases, tuberculosis and other functional diseases as well as subjects with chronic disease and complications were excluded.
- Pregnant women and children were excluded.

Ethical approval Advocacy and pre-survey contacts; A letter of introduction was obtained from the head of department medical laboratory science. It was submitted with the proposal to the ethical committee of general hospital Awo-omamma

- An ethical approval was issued by the ethical committee of general hospital Awo-Omamma for the collection of samples.

Informed Consent

Informed consent was obtained from the participants after a clear explanation of the study has been given to them. Along with questionnaires.

Specimen collection, preparation and storage

A standard venepuncture technique was employed. A sterile, plastic syringe of 10ml capacity was used for the collection of blood. Six millilitres (6mls) of blood was collected. Two and half millilitres (2.5mls) of blood was delivered into a bottle containing dipotassium salt of Ethylenediamine tetra-acetic acid (K_2-EDTA) at a concentration of 1.2mg/ml of blood. This was used for CD4 count by Cyflow method and malaria parasite detection by rapid test method. Five millilitres (5mls) of the blood was delivered into a plain specimen bottle. This was allowed to coagulate; serum was obtained by spinning at 1,500 RPM for five minutes. With the aid of pasture pipette, the serum was delivered into a fresh clean plain specimen bottle and stored at -20°C until required for the determination of inflammatory mediators using ELISA.

Laboratory Methods.

Determination of C-reactive protein (CRP), Complement 3 (C3), Complement 4 (C4), and Interleukin 6 (IL-6)) were by ELISA method

Statistical analysis

The data was analysed using Statistical Package for Social Science (SPSS) version 29. The significant of difference between groups was analysed using student T-test and within groups by analysis of variance (ANOVA). Games-Howell's post-hoc test was used to compare combination of groups. Results was regarded as significant at $P \leq 0.05$.

RESULTS

Table 1/Mean ± SD value of $CD4^+$and levels of inflammatory mediators (C-reactive protein (CRP), Complement 3 (C3), Complement 4 (C4) and Interleukin-6 (IL-6)) in HIV-malaria co-infected subjects, HIV mono infected, HIV seronegative subjects without malaria (Control).

PARAMETER	GROUP 1 (n =100)	GROUP 2 (n =100)	GROUP 3 (n =100)	F-value	P-value	1 vs 2 p-value	1 vs 3 p-value	2 vs 3 p-value
$CD4^+$(cells/mm^3)	384.7±101.5	488±100.5.	909±102	22.5	0.001*	0.0001*	0.0001*	0.0001*
CRP (mg/dl)	56±21.3	37.6±9.8	5.6±2.9	10.4	0.01*	0.0001*	0.0001*	0.0001*
C3(mg/dl)	203.6±105	152.3±90.5	104.7±56.9	8.40	0.002*	0.05*	0.04*	0.23
C4 (mg/dl)	58.1±31	49.8±21.3	44.3±18.6	5.09	0.004*	0.08	0.001*	0.09
IL-6(pg/ml	54.4±19.6	38.3±14.9	5.8±1.8	9.84	0.01*	0.001*	<0.001*	0.03*

KEY:

n = Sample size,

***** = Significant at $P \leq 0.05$,

Group 1 = HIV-malaria co-infected subjects.

Group 2 = HIV mono infected.

Group 3 = HIV seronegative subjects without malaria (Control).

P-value = P-value across all groups

1 vs 2 p-value = P-value comparison between HIV-malaria co-infected subjects and HIV mono infected

1 vs 3 p-value = P-value comparison between HIV-malaria co-infected subjects and HIV seronegative subjects without malaria (Control).

2 vs 3 p-value = P-value comparison between HIV mono infected and HIV seronegative subjects without malaria (Control).

The Mean ± SD value of $CD4^+$ count; 384.7±101.5 cells/mm^3 in HIV-malaria co-infected subjects, 488±100.5 cells/mm^3 in HIV mono infected and 909±102 cells/mm^3 in HIV seronegative subjects without malaria (Control), showed significant difference (F=22.5, P=0.001) among the groups. The mean $CD4^+$ of HIV-malaria co-infected subjects was significantly lower when compared with the means of HIV mono infected and control subjects respectively (P=0.0001, P=0.0001). Similarly, the mean value of CD4+ counts of HIV mono infected subjects was significantly lower when compared with the means of control subjects (P=0.0001).

The Mean ± SD value of C-reactive protein (CRP), in the three study groups are as follows; 56±21.3 mg/dl in HIV-malaria co-infected subjects, 37.6±9.8mg/dl in HIV mono infected subjects, 5.6±2.9 mg/dl in Control subjects. There was significant difference among the groups (F=10.4, P=0.01). Table 4.2. In the in-between group comparison, the mean CRP of HIV-malaria co-infected subjects was significantly higher when compared with the means of HIV mono infected and control subjects respectively (P=0.0001, P=0.0001). Also, the mean value of CRP of HIV mono infected subjects was significantly higher when compared with the means of control subjects (P=0.0001).

The Mean ± SD value of Complement 3 (C3) in the studied subjects are as follows; 203.6±105 mg/dl in HIV-malaria co-infected subjects, 152.3±90.5mg/dl in HIV mono infected, 104.7±56.9mg/dl in Control subjects. The compared means ± SD showed statistically significant difference among the groups (F=8.40, P=0.002). The in-between group comparison of C3 (mg/dl) levels showed that there was statistical higher mean value of C3 in HIV-malaria co-infected subjects when compared to HIV mono infected and control group respectively (P=0.05, 0.04). On the other hand, the mean ± SD of HIV mono infected showed no statistically significant difference when compared with Control subjects (P=0.23).

The Mean ± SD value of Complement 3 (C4) in the studied subjects were; 58.1±31mg/dl in HIV-malaria co-infected subjects, 49.8±21.3 mg/dl in HIV mono infected, 44.3±18.6mg/dl in Control subjects. The compared means ± SD showed statistically significant difference among the groups (F=5.09, P=0.004). Table 4.2. The in-between group comparison between HIV-malaria co-infected subjects and HIV mono infected subjects showed no statistically significant difference (P=0.08), However, the values in the HIV-malaria co-infection was significantly higher compared with values in control subjects (P=0.001). Conversely, the mean ± SD of HIV mono infected compared with the means of control subjects showed no statistically significant difference (P=0.09).

The Mean ± SD value of Interleukin-6 (IL-6) in the three study groups are as follows; 54.4±19.6 mg/dl in HIV-malaria co-infected subjects, 38.3±14.9mg/dl in HIV mono infected subjects, 5.8±1.8 mg/dl in Control subjects. There was significant difference among the groups (F=9.84, P=0.01). Table 4.2. In the in-between group comparison, the mean IL-6 of HIV-malaria co-infected subjects was significantly higher when compared with the means of HIV mono infected and control subjects respectively (P=0.0001, P<0.001). In the same vein, the mean value of IL-6 of HIV mono infected subjects was significantly higher when compared with the means of control subjects (P=0.03).

DISCUSSION

Malaria and HIV infection are two of the major public health issues in sub-Saharan Africa. High rates of morbidity and mortality are associated with both. Through their interactions with the host's immune system, HIV and malaria both result in complex immune cell activation and inflammatory responses. In Awo-Omamma, Imo state, a study was carried out to compare the levels of certain inflammatory mediators and immunoglobulins in HIV patients with co-infection with malaria and those without malaria. 102 (34%) of the 300 volunteers that were enlisted for the study were men, and 198 (66%) were women. One hundred (33.3%) of the study participants had co-infections with both malaria and HIV. This is consistent with the findings of [18], who found that the prevalence of HIV and malaria co-infection was 22.9% in Zaria, Kadunna state. The present analysis encompasses both HIV positive individuals on ART and those who are ART naïve, in contrast to the study by [19], which focused exclusively on HIV positive individuals on ART. However, 106 papers were included in a recent systematic review and meta-analysis by [20], which discovered that the average prevalence of malaria among people who are HIV positive was 27.3%. Co-infection with HIV and malaria has remained a global health burden for people. These two illnesses, which are thought to be the most critical health problems in developing countries like Nigeria, claim the lives of about 2 million people annually worldwide [20].

Significant fatality rates have been connected to opportunistic infections like malaria as well as the numerous other pathogenic ailments that HIV-positive people face [10].

The study's findings demonstrated a substantial variation in CD4 counts between the groups under investigation. (P=0.001, F=22.5). When compared to the means of HIV mono-infected and control patients, respectively, the mean CD4+ of HIV-malaria co-infected subjects was considerably lower (P=0.0001, P=0.0001).Similarly, there was a significant difference (P=0.0001) in the mean CD4+ count of HIV mono infected participants compared to the means of control subjects. According to a research by [13] on the prevalence and clinical characteristics of HIV and malaria in co-infected people in Osun State, Nigeria, patients with both HIV and malaria had a noticeably lower mean CD4+ cell count (P=0.0001).

Similarly, it has been observed that those who also have malaria and HIV had decreased CD4 counts, indicating a considerable reduction in CD4 count resulting from co-infection. In contrast, people who co-infect with HIV and malaria have greater CD4 levels. Nevertheless, untreated malaria infection appears to further lower the CD4 cell count, and HIV infection has been repeatedly linked to a decrease in the absolute numbers of CD4 count. HIV is a deadly infection that causes CD4 T cells in peripheral blood to be targeted and destroyed [21]. HIV-positive people who contract malaria experience an increase in their viral load, which further reduces their CD4+ T cell count.

[22]

HIV-malaria co-infected individuals had considerably greater concentrations of inflammatory mediators, such as C-reactive protein (CRP) and complement 3 (C3), than did HIV mono-infected and Control participants. Additionally, it was noted that whereas CRP and IL-6 were higher in HIV mono-infected individuals than in control participants, there was no discernible difference between the two groups' C3 and C4. Higher levels of the pro-inflammatory mediator C-reactive protein (CRP) have been linked to the advancement of HIV illness within the last ten years [23]. The liver cells produce C-reactive protein (CRP), one of the acute-phase proteins that is a well-known indicator of inflammation. The primary mediators of CRP synthesis are TNF-α, IFN-γ, and IL-6.

Previous research has also shown that CRP binds strongly to malaria-infected erythrocytes, activating the complement system [24]. One of the worst symptoms of malaria, anemia, is caused by hemolysis and erythrocyte clearance as a result of this complement activation [25].This could be the cause of the higher IL-6, CRP, and C3 levels among research participants who were also co-infected with malaria and HIV.

CONCLUSION

When HIV and malaria coexist, adults' inflammatory and humoral responses are heightened, particularly in relation to C-reactive protein (CRP) and complement 3 (C3). Furthermore, ART demonstrated a drop in CRP levels.

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