Assessment of Serum Amyloid Protein A, Tumor Necrosis Factor Alpha and some Liver Enzymes in HIV seropositive with Malaria co-infection in NAUTH Nnewi, Nigeria

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Abstract-
Objective: Co-infection with malaria is a common problem in HIV-infected patients living in endemic areas, particularly in sub-Saharan Africa. Co-infection of HIV with malaria increases mortality and is associated with strong CD4+ cell activation and up-regulation of pro-inflammatory cytokines, providing an ideal micro-environment for the spread of the virus among CD4+ cells and thus for rapid HIV-1 replication. This is a cross-sectional case controlled study aimed to evaluate the levels of serum amyloid protein A (SAA), tumor necrosis factor alpha (TNF- α) and some liver enzymes in HIV infected individuals attending the Antiretroviral Therapy Unit (ART) in Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria.
Methodology: A total of 130 participants aged between 18 and 65 years were randomly recruited and the participants were grouped into Group A: HIV co-infection with Malaria (n=32), Group B: HIV positive subjects without Malaria (n=33), Group C: HIV negative subjects Malaria (n=32) and Group D: apparently healthy individuals (n=33) as the control. 6 milliliters (6ml) of blood sample was collected from each of the participants for determination of SAA and TNF- α using the ELISA method. Alanine transaminase and aspartate aminotransferase (ALT, AST) were done using colorimetric method. A standard questionnaire was used for the collection of their demographic data. Clinical Data including CD4 counts were obtained from the patient's data in the register.
Results: The results showed significantly higher serum SAA, TNF- α, AST and ALT levels in HIV/malaria co-infected individuals than in those with single infections and in control subjects (p≤.05 respectively). Similarly, BMI, SBP and DBP were significantly higher in HIV/mal co-infected individuals when compared with HIV seropositive, malaria positive individuals and control participants (p≤.05 respectively).
Conclusion: The findings revealed significant variations in the levels of SAA, TNF- alpha and liver enzymes in co-infected individuals indicating the presence of active inflammatory response and immune activation, shedding light on disease severity and liver disease progression due to a potential synergistic effect of co-existing HIV and malaria infections on liver health. The significantly higher BMI, SBP and DBP values shows evidence of overweight and possible hypertension which suggests risk of developing cardiovascular disease and may be exposed to heart failure if neglected

Keyword: **Inflammatory marker, HIV, Malaria, co-infection, liver enzymes, HAART, Nigeria**

INTRODUCTION

Co-infection with malaria is a common problem in HIV-infected patients living in endemic areas, particularly in sub-Saharan Africa, Nigeria inclusive [1]. HIV infection weakens the immune system, making people more susceptible to infections, including malaria. Co-infection with HIV and malaria may exacerbate inflammation and liver damage, which can have serious consequences for patients' health and wellbeing [2].

However, despite the introduction of highly antiretroviral therapy to ameliorating the adverse complications of HIV infection and to increase the life expectancy of the affected individuals worldwide, several research has documented some degrees of organ damage following ART administration [3]. Some of the organ mostly affected include the liver, heart, kidneys and others [2]. ART hepatotoxicity and heart failure have been documented as the foremost occurring adverse toxicity of ART which may be hazardous and deadly [4]

Serum amyloid A (SAA) and liver function tests (LFT) are two commonly used laboratory tests that can provide important information about inflammation and liver function [5]. SAA is an acute-phase protein that is produced in response to inflammation and can be used as a marker of systemic inflammation. LFTs are a group of blood tests that can evaluate liver function, including tests for liver enzymes, bilirubin, and albumin. SAA is predominantly synthesized by hepatocytes and hematoma cells in response

to pro and anti-inflammatory cytokines particularly with cooperation of IL-1, L-6 and TNF-alpha [6], [7]. Furthermore, it has been shown that TNF alpha in synergy with SAA and other chemokines can increase the leukocyte migration to the site of inflammatory reactions [8]. HIV-malaria co-infection is known to stimulate various inflammatory pathways with some certain cytokines release into the circulation which can destroy the host immune cells with subsequent disease progression and eventual death [9]. This can easily occur due to immunosuppression which is exacerbated by malaria infection [10] especially in endemic places of higher transmission [11].
Previous studies have suggested that HIV-infected patients with malaria may be at increased risk of liver damage and inflammation, but the underlying mechanisms of this association are not fully understood [12], hence the present study design.

MATERIALS AND METHODS

Study site
This study was conducted on HIV infected patients with Malaria at Nnamdi Azikiwe University Teaching Hospital (NAUTH)

Study design
This is a cross-sectional design to evaluate the levels of serum amyloid A and some liver enzymes in HIV-infected patients with and without malaria attending the institute of human virology (IHVN) clinic.
Inclusion and exclusion criteria
HIV positive individuals who were confirmed positive for malaria infection and HIV positive individuals without malaria infection were included in the study. HIV negative individuals with confirmed malaria infection were also included. Apparently healthy individuals who were with neither HIV nor malaria infection were included and regarded as control group. The participants were both male and females aged between 18-65 years old. Pregnant and /or breastfeeding mothers were excluded from the study. Participants with history of liver disease and renal disease were excluded. Participants who have a history of chronic inflammatory diseases such as rheumatoid arthritis, lupus, or inflammatory bowel disease were also excluded from the study

Ethical approval and Informed consent
The ethical approval for this research was sought and obtained from the board of ethics committee of Nnamdi Azikiwe University teaching hospital (NAUTH), Nnewi, Nigeria. Written informed consent was obtained from each of the participants prior to the study.

Sample collection
Venous blood samples (5ml) was collected aseptically by venipuncture from each subject via the antecubital vein using a plastic syringe. The whole blood sample was dispensed into a plain tube, allowed to clot and centrifuged at 5000 rpm for minutes using table top centrifuge to obtain the serum for the evaluation. Sample was stored at -20 degree centigrade (-20°c) before analysis.

Methods

Screening for HIV Infection
The participants were screened for HIV infection using Immunoassay and immunochromatographic method. Antibodies to HIV-1 and HIV-2 in human plasma was determined using Abbott determine TM HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay [13] and HIV-1 and 2 STAT-PAK Assay kit, which is an immunochromatographic test for the quantitative detection of antibodies to HIV-1 and HIV-2 in Human plasma [14].

Screening for Malaria Infection
The participants were screened for malaria using Abbot Malaria Rapid Test (RTDs) kit as described by Abbot Laboratories and giemsa staining technique antigens [15]. The test qualitatively detects plasmodium antigen in human whole blood samples. This test applies lateral flow immuno-chromatography which is a tool in the diagnosis of malaria.

Determination of Serum Amyloid A
The serum amyloid A protein level was determined through enzyme-linked immunosorbent assay (ELISA) using commercially available kits, as was described by Cheng et al.[16] while, Human TNF-α Immunoassay was determined bas was described by Hedeyati et al. [17].

Determination of Liver Function Test (LFT)
The liver function tests (LFTs) AST and ALT were performed using commercially available kits by Siemens Dimension Vista 1500 Intelligent Lab System and Siemens Advia 1800 Chemistry System [18], [19].

Data collection
Socio-demographic data was collected from the participants using a questionnaire while, biochemical data was obtained from the results of the biochemical parameters assayed

Statistical Analysis

Statistical package for social sciences (SPSS) version 25 was used for the statistical analysis. The data generated was analyzed using Analysis of variance (ANOVA) to compare more than two independent variables with and student's t-test for two independent

variables. Pearson correlation was used to correlate different parameters. Values were considered statistically significant if p value ≤ .05

RESULTS

Comparison of the mean values of age and anthropometric parameters among HIV serpositive, co-infection, malaria positive and control groups.

The Systolic blood pressure and diastolic blood pressure were significantly higher in HIV seropositive (119.60±10.20, 79.60±12.74), malaria positive (117.05±11.09, 79.47±7.80) individuals and HIV/mal co-infected individuals (147.08±14.89, 86.67±12.39) when compared with control participants (115.24±9.28, 74.76±10.78) (p≤.05 respectively). Similarly, SBP and DBP were significantly higher in HIV/malaria co- infected individuals when compared with HIV infected individuals and malaria positive individuals (p≤.05 respectively). The mean BMI was significantly higher in HIV seropositive individuals (25.98±4.27) and HIV/mal co-infected individuals (26.02±4.34) when compared with the apparently normal individuals (24.31±3.51) (p≤.05 respectively). Similar observation was also made between HIV seropositive (25.98±4.27) and malaria positive individuals (24.07±4.10) (p≤.05).

Table 1. Comparison of the mean values of age and anthropometric parameters among HIV seropositive, HIV/mal co-infection, malaria positive and control participants.

Group	N	Age (years)	SBP (mmHg)	DBP (mmHg)	BMI (kg/m^2)
HIV Positive (A)	32	37.84±12.15	119.60±10.20	79.60±12.74	25.98±4.27
Malaria Positive (B)	33	37.16±12.36	117.05±11.09	79.47±7.80	24.07±4.10
HIV/mal Co-infection (C)	32	36.38±15.51	147.08±14.89	86.67±12.39	26.02±4.34
Control (D)	33	36.05±18.95	115.24±9.28	74.76±10.78	24.31±3.51
F-value		2.598	4.095	3.931	3.418
P-value		0.723	0.009	0.029	0.014
AvsB		0.936	0.290	0.852	0.021
AvsC		0.680	0.002	0.007	0.819
AvsD		0.472	0.020	0.034	0.011
BvsC		0.789	0.007	0.009	0.000
BvsD		0.753	0.321	0.366	0.480
CvsD		0.848	0.000	0.011	0.000

*Significance is set at p≤.05. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. BMI: Body mass index.

Comparison of mean ALT, AST, SAA and TNF –α levels among all the study groups

The results showed that the mean serum ALT, AST, SAA and TNF- α levels were significantly different in all the test groups when compared with control group (p≤.05 respectively). The mean serum ALT, AST and TNF- α were significantly higher in HIV seropositive individuals (14.74±1.09, 17.24±0.56, 593.14±221.17) when compared with malaria positive participants (12.64±0.08, 11.98±0.48, 419.02±146.09) (p≤.05 respectively). Similar observation were made in the whole parameters between HIV seropositive individuals and HIV/mal co-infected individuals (p≤.05 respectively). However, the mean serum ALT, AST, SAA and TNF- α were significantly lower in HIV seropositive (14.74±1.09, 17.24±0.56, 3.76±0.49, 593.14±221.17) and malaria positive (12.64±0.08, 11.98±0.48, 2.01±1.21, 479.02±146.09) individuals when compared with HIV/malaria co-infected individual (27.21±1.18, 45.88±0.85, 5.78±0.35, 876.26±285.72) (p≤.05 respectively).

Table 2. Comparison of mean serum ALT, AST, SAA, and TNF- α levels among all the study groups

Group	N	ALT (IU/L)	AST (IU/L)	SAA (ug/ml)	TN F- α (pg/ml)
HIV seropositive (A)	32	14.74±1.09	17.24±0.56	3.76±0.49	593.14±221.17
Malaria positive (B)	33	12.64±0.08	11.98±0.48	2.01±1.21	479.02±146.09
HIV/mal co-infection (C)	32	27.21±1.18	45.88±0.85	5.78±0.35	876.26±285.72
Control (D)	33	10.52±0.87	10.67±0.48	1.74±1.01	321.10±84.05
F-value		9.667	7.680	5.750	61.213
P-value		0.001	0.000	0.000	0.000
AvsB		0.031	0.011	0.079	0.000
AvsC		0.008	0.000	0.014	0.001
AvsD		0.005	0.025	0.023	0.000
BvsC		0.000	0.000	0.007	0.000
BvsD		0.301	0.797	0.820	0.029

CvsD		0.000	0.000	0.000	0.000

*Significance is set at p≤.05. ALT- alanine transaminase, AST- aspartate aminotransferase, SAA- serum amyloid protein A, TNF –α – tumor necrosis factor alpha

Comparison of mean serum ALT, AST, amyloid A, and TNF- α levels by gender in all HIV seropositive participants with/without malaria co-infection.

The results showed that there were no significant differences (p>.05) in the serum ALT and AST levels between male (23.00±1.0, 35.88±0.35) and female (23.31±1.25, 36.88±0.50) HIV with/without malaria co-infection. However, the mean level of SAA in female HIV seropositive individuals (5.80±0.39) was significantly higher when compared with their male counterparts (3.75±0.34) (p≤.05). Contrastingly, the mean TNF-α in female HIV seropositive participants with /without malaria (792.62+312.53) was significantly lower when compared with their corresponding males (616.94 ±234.10) (p≤.05).

Table 3. Comparison of mean serum ALT, AST, amyloid A, and TNF- α levels by gender in HIV seropositive participants with/without malaria co-infection.

Gender	N	ALT (IU/L)	AST (IU/L)	SAA (ug/ml)	TN F- α (pg/ml)
Male	14	23.00±1.07	35.88±0.35	3.75±0.34	616.94+ 234.10
Female	18	23.31±1.25	36.88±0.50	5.80±0.39	792.62+312.53
P-value		0.552	1.090	4.751	64.926
F-value		0.664	0.841	0.000	0.000

*Significance is set at p≤.05.

Comparison of mean serum ALT, AST, amyloid A, and TNF- α levels by gender in in malaria infected individuals.

The results showed that there were no significant differences (p>.05) in the serum alanine transaminase, aspartate transaminase levels between male and female individuals with malaria infection. However, the mean levels of SAA and TNF-α in female HIV seropositive individuals (2.80±0.39, 481.18+130.07) were significantly higher when compared with their male counterparts (2.05±0.34, 374.87+113.76) (p≤.05 respectively).

Table 4. Comparison of mean serum ALT, AST, amyloid A, and TNF- α levels by gender in malaria infected individuals.

Gender	N	ALT (IU/L)	AST (IU/L)	Amyloid A (ug/ml)	TN F- α (pg/ml)
Male	15	12.00±1.87	13.88±0.35	2.05±0.34	374.87+113.76
Female	18	13.31±1.05	14.88±0.50	2.80±0.39	481.18+130.07
P-value		0.552	2.090	3.751	41.535
F-value		0.364	0.110	0.036	0.024

Comparison of mean serum ALT, AST, Amyloid A and TNF- α in all HIV seropositive individuals with /without malaria infection based on CD4 T-cell count ≤500 and >500

The results shows that the mean values of ALT, AST, SAA and TNF- α in all HIV seropositive individuals with/without malaria co-infection with CD4 count ≤500 (27.45±1.14, 46.73±0.87, 5.76±0.84, 678.6.14) were significantly higher when compared with those with CD4 count > 500 (11.38±1.38, 23.49±0.84, 3.79±0.45, 452.21+173.42) (p≤.05 respectively).

Table 5. Comparison of mean serum ALT, AST and SAA in all HIV seropositive individuals based on CD4 T-cell count ≤500 and > 500 with/without malaria infection

*Significance is set at p≤.05

DISCUSSION

HIV infection poses as a critical global public health challenge, with its impact reverberating worldwide [20]. The profound toll of HIV and AIDS has been felt universally, and Nigeria in particular houses over nine percent of the global population living with HIV [21]. Antiretroviral therapy (ART) has introduced a sense of optimism and has notably elevated life expectancy among HIV-positive individuals, although not without challenges. Beyond the detrimental effects instigated by the human immunodeficiency

virus itself, ART has been linked to renal toxicity, emerging as a pressing health concern. Coincidentally, malaria and HIV, two significant infectious diseases, exhibit widespread prevalence across intersecting geographical regions [21].
Within the context of this study, the subjects' mean age range signifying a predominantly youthful demographic within the active workforce age bracket of 18 to 65 years. This demographic distribution holds considerable implications for both the workforce and overall economic productivity of the nation. These findings are in accordance with existing research that consistently underscores the higher vulnerability of young individuals to HIV and AIDS. UNAIDS has highlighted the elevated HIV infection rates among young people aged 15 to 49 years, potentially leading to substantial economic repercussions [22].
Regarding blood pressure (BP) measurements, the mean SBP and DBP demonstrated significant elevation in HIV/mal co-infected individuals when compared with control participants. The findings in this study is in line with other previous reports [23]-[26], although our study focused on HIV with malaria co-infection while the authors reported on HIV individuals on HAART alone. Study has shown that malaria alone is capable of deregulating the blood pressure and causing hypertension which can subsequently result to heart disease [27], [28].
Some authors however, reported lower prevalence of hypertension in HIV seropositive individuals on HAART [29] which they attributed reduced CD4 T- cell count in the HAART naive individuals. Other studies documented no significant shifts in SBP and DBP among HIV infected individuals irrespective of their HAART status when compared to controls [30], [31]. The reasons for variations in blood pressure of these individuals could result from the age and gender differences, ethnicity, BMI and other environmental factors [32], [33]. The use of HAART and HAART duration on the other hand, have been shown to contribute significantly to the increased incidences of hypertension in HIV infected individuals [34], [35]. The effect of long period of ART administration may be dependent on the age and increased weight gain due to HAART as well as endothelial cell dysfunction [36], [37].
In relation to body mass index (BMI), the present study also reported significant increase in the HIV/mal co-infected Individuals when compared to the control participants. The increase in BMI in the present study may have contributed to the elevated blood pressure level observed in this patients. Many other studies have attributed incidence of overweight and obesity to increased risk of developing hypertension [38], [39]. This suggests that HIV infected patients on HAART with higher BMI values are at risk of developing cardiovascular disease and may be exposed to heart failure if neglected.

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels were significantly higher in HIV/mal co-infected group when compared with the control group. These important finding sheds light on the interplay between co-infection with HIV and malaria and liver function. Previous reports have documented increased levels of ALT and AST levels in malaria infection as well as HIV infected individuals especially in cases of hepatocellular injuries [40]- [42]. Although the levels in our study are still within the normal clinical values, the combine increases with the SAA levels as observed in this study can subsequently exposed these individuals to adverse liver complications if not properly managed. The elevated AST and ALT levels in the study participants may be attributed to several factors. Firstly, both HIV and malaria infections can trigger an inflammatory response in the body. The co-occurrence of these infections might lead to an intensified immune response that impacts the liver. Immune activation and inflammation can contribute to liver damage and variable increases in these liver enzymes [43]. Additionally, the use of antiretroviral therapy and antimalarial drugs might contribute to drug interactions that affect liver function [43] Furthermore, pathogenic mechanisms associated with both HIV and malaria infections can directly or indirectly affect liver function. HIV can directly infect liver cells, leading to inflammation and cell damage [44]. Similarly, malaria parasites can invade liver cells during their life cycle, causing liver inflammation and dysfunction [45]. Metabolic changes induced by infections can also impact liver function. The alteration of metabolic pathways due to infections might influence AST and ALT production and release into the bloodstream [46].
Surprisingly, HIV seropositive individuals with CD4 count of less than 500 cells/μL, also have significantly increased AST and ALT showing some association of immunosuppression and disease progression with increased liver enzymes. Antiretroviral medications have been implicated in increased liver enzymes in HIV-infected patients [47], [48]. The definitive process by which HIV and malaria infections exacerbates liver damage have not been fully understood. However, some authors have attributed the cause to mitochondrial damage and programmed cell death [44], [49].
Serum Amyloid A was significantly higher in HIV/malaria co-infected participants when compared with HIV seropositive individuals and control participants. This suggests a synergistic inflammatory response when both infections co-exist. This could be attributed to the cumulative effect of immune activation triggered by both pathogens [50], [51]. Increased levels of SAA and C-reactive protein in malaria infection has been shown to be important prognostic markers in malaria severity as well as inflammatory reaction in HIV infection [52]. The elevated SAA level in the co-infected individuals in this study may therefore reflect the body's attempt to mount a heightened immune response to combat both HIV and malaria infection especially in this case where the affected individuals are on ART. This response could involve the activation of pro-inflammatory pathways, recruitment of immune cells, and release of cytokines. The intricate interplay between these infections might lead to the modulation of immune signaling networks, potentially impacting disease progression and outcomes [53]. Furthermore, SAA being an acute phase protein that is produce in the liver represent a very sensitive biomarker for inflammatory reactions in liver diseases including, presence of hepatitis B, autoimmune liver diseases and drug-induced liver injury. However, our study excluded individuals with clinically diagnosed hepatitis B and other liver problems. Increased SAA level can also exacerbate inflammatory messengers that can enhance atherothrombotic events and this can stimulate immune dysfunction and enhance cardiovascular disease risk [54], [55]. This is evidenced by the increased risk of hypertension observed in the present study. Furthermore, SAA was lower in HIV/mal co-infected individual with CD4 T cell in those above 500 u/l than in those less/equals 500 u/I irrespective of malaria infection. This shows the positive effects of ART in reducing the active inflammatory reaction hence, improving the immune response

However, Reports has shown that HIV infected individuals exhibit a low-grade inflammatory state which can disrupt the body's metabolic process despite ART administration [56], [57].

Furthermore, this study observed significantly higher TNF-α in all the test participants when compared with control individuals. Same observation was made in HIV/malaria co-infected individuals when compared with those with single infections. The increased level of TNF alpha has been reported previously in pregnant women with HIV and malaria co infection [58], [59]. HIV seropositive individuals have been documented to aggravate pro-inflammatory cytokines release including TNF-α, SAA, interleukins and other intracellular signaling molecules [60]. Our study observed decreased level of TNF alpha in the single infected individuals showing the degree of combined effect of HIV and malaria infection on disease severity and progression irrespective of ART administration on these individuals. The value was significantly raised when compared between individuals with CD4 T-cell count less/equals 500 than in those above 500 u/lit indicating evidence of immunosuppression and active inflammatory response which might have been worsened by malaria co-infection irrespective of ART. Meanwhile, reports has documented increased production of inflammatory mediators during malaria infection which increasingly, correlates with severe malaria as well as disease progression [61]. Additionally, persistent elevation in TNf- alpha has been recorded in HIV infected individual irrespective of consistent administration of ART [62]. This shows that the synergistic effect of combined HIV and malaria infection could be more sever and detrimental to the health of the affected individuals. This may progressively lead to irreversible tissue injury if not properly managed.

In Conclusion, the findings revealed significant variations in the levels of SAA, TNF- alpha and liver enzymes in co-infected individuals indicating the presence of active inflammatory response and immune activation, shedding light on disease severity and liver disease progression due to a potential synergistic effect of co-existing HIV and malaria infections. These findings emphasize the need for vigilant monitoring of liver function in individuals with co-infections, especially those undergoing antiretroviral and antimalarial treatments. Concerted efforts should be geared towards identifying more protein markers for rapid diagnosis, monitoring and treatment of increasing inflammatory conditions that masks with HIV and malaria co-infection especially in endemic regions. Longitudinal studies are also recommend to make clearer the adverse effect for increased liver enzymes in co-infection

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