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THE IMPACT OF HIV AND MALARIA CO-INFECTION ON CARDIAC BIOMARKERS AND CD4$^+$ T CELL COUNTS IN ADULT HIV SEROPOSITIVES IN NAUTH NNEWI, SOUTH EASTERN, NIGERIA.

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ABSTRACT

Aim: To determine the impact of HIV and malaria co-infections on Cardiac biomarkers and CD4$^+$ T cell counts in adult HIV seropositives in NAUTH Nnewi, South Eastern, Nigeria. **Methods**: A total of 400 subjects aged between 17 and 58 (38 ± 9) years were recruited by convenient sampling technique from patients that attended HIV Clinic, NAUTH, Nnewi for this study. The study design was a case study. Ethical approval was obtained from the Ethics Review Committee from NAUTH, Nnewi. The subjects were grouped based on WHO criteria for staging HIV infection and the presence or absence of malaria as follows: (1) Symptomatic HIV on Antiretroviral therapy (ART) (n = 100) of these, 50 were symptomatic HIV on ART with malaria co-infection. (2) Symptomatic HIV not on ART (n = 100) of these, 50 were symptomatic HIV not on ART with malaria co-infection. (3) Asymptomatic HIV subjects (n = 100) of these, 50 were asymptomatic HIV with malaria co-infection. (4) HIV seronegative control subjects (n = 100) of these, 50 were malaria positive. Enzyme linked Immuno sorbent Assay (ELISA) was used for Myoglobin and Troponin I. Spectrophotometric method was used for enzyme cardiac markers- total creatine kinase (T-CK), CK-MB, lactate

dehydrogenase (LDH) and aspartate transaminase (AST) and Cyflow SL-Green for CD4 counts. Student t test was used for data analysis. **Results**: The results showed that the mean serum CK-MB level was significantly higher in symptomatic HIV on ART with malaria infection compared to symptomatic HIV on ART without malaria infection at P< 0.05. But the mean blood CD4 count was significantly lower in symptomatic HIV on ART with malaria infection compared to symptomatic HIV on ART without malaria infection at P< 0.05. The mean serum Myoglobin level was significantly higher in symptomatic HIV not on ART with malaria infection compared to symptomatic HIV not on ART without malaria infection at P< 0.05. Similarly, the mean serum Troponin I was significantly higher in asymptomatic HIV with malaria parasitaemia compared to asymptomatic HIV without malaria parasitaemia at P< 0.05. Also, the mean serum Myoglobin, Troponin I, T-CK, CK-MB, LDH and AST were significantly higher in Control subjects with malaria parasitaemia compared to Control subjects without malaria parasitaemia at P< 0.05 respectively. **Conclusion:** Malaria endemicity poses a threat to the improved cardiac function as observed with increased serum levels of CK-MB, myoglobin, and Troponin I in HIV malaria co-infection with or without ART and with the reduction of CD4 count in HIV malaria co-infection with ART. These markers T-CK, CK-MB, LDH, AST, myoglobin, and Troponin I were raised in apparent control groups with malaria parasitaemia. This calls for concern in the management of patients with HIV malaria co-infection as well as in controls with malaria infection.

KEYWORDS: HIV, malaria, Cardiac Biomarkers, $CD4^+$ T cell.

INTRODUCTION

The Human immunodeficiency virus (HIV) was a major global health issue despite various interventional measures and improved management scheme in recent time. The HIV targets the immune system, infecting the immune cells and progressively weakens the defense system of the body against infection and renders the individual more susceptible to opportunistic infections (OPs), normally cleared by the immune system of a healthy individual. The CD4 + T cells are majorly affected by HIV.[1,2]

In 2015, about 36.7 million people worldwide were infected with HIV, 1.8 m of it were children. About 30% of these HIV populations were ignorant of their HIV status. In 2016, 1 m people died of AIDS-related illness.[3,4] The HIV pandemic is most severe in Sub-Saharan Africa. Over 60% of HIV populations reside within the region. East and South Africa has the

largest population of HIV individuals globally (19.6 m), followed by Nigeria (3.2 m), and Philippines (2.1m).[1,2,3,4]

In 2015, there were 2.1 m new HIV infections but in 2016, there was a decline to 1.8 m new HIV infections. New HIV infections among children globally were halved, from 300,000 to 160,000 in 2000 to 2010. Young women especially have been reported to be at risk, with 59% of new infections among young people with age limit of 15 – 24 years.[4]

Reports have it that more than half of all HIV populations (53%) now have access to life-saving treatment. 7.5 m were on Antiretroviral therapy (ART) in 2010. The number has increased to 19.5 m people on ART in 2016. It is estimated that the world will meet its global target of 30 m people on treatment by 2020[5]. US$19 billion were funded on treatment of people on HIV/AID in low-and middle income countries by United Nations General Assembly and they have mapped out US$19 billion for the response to epidemic in 2020. Although, most countries, provide strong source of domestic investment on ART.[4] However, despite the progress made across the 69 countries which witnessed a decline in new infections, UNAIDS warned that the progress in combating viral transmission is still not very fast to meet global targets.[4]

Malaria and HIV are the most common infections in sub-Saharan Africa. Both diseases kill millions of people annually and both diseases are scourges of developing Nations in Africa, South Asia, India, and South America. The sub-Saharan Africa populace was reported to have the highest prevalence rate of HIV infection globally. Malaria is caused by plasmodium species. The *plasmodium falciparum* is the most virulent of the species.[6] Malaria was observed to be endemic in Nigeria and it is the leading cause of morbidity and mortality in the country.[7,8]

Reports have it that individuals infected with HIV have increased risk of developing cardiovascular diseases.[9,10] The HIV infection has also been implicated in cardiovascular disorders especially in advanced stage of the infection.[11] Some enzymes have been implicated in HIV infection such as aspartate transferase alkaline transferase, alanine phosphatase.[12] Both infections interact bi-directionally and synergistically with each other. Both affect CD4 counts.[13]

ART should be given to everyone with HIV at any CD4 cell count.[14] Antiretroviral drugs are used as chemotherapeutic interventions of HIV/AIDS infection, many a times on long term basis. The drugs may present with side effects, most which are not uniquely associated with a particular drug and sometimes may be difficult to identify the cause.[10,15] HIV itself is capable of producing many of the symptoms that may also occur as drug side effects. Hence it is needful to investigate the effect of HIV, malaria and Antiretroviral therapy on some biomarkers of cardiac status in HIV infection.

MATERIALS AND METHODS

Subjects

The study was conducted in Nnamdi Azikiwe University (NAUTH), Nnewi in Anambra state, South East Nigeria. Based on 3.1% prevalence rate of HIV in Nigeria[16] and using the formular of Naing *et al*[17] for sample size calculation, a total of 400 adult subjects were recruited by random sampling technique from subjects that attended HIV Clinic and ART unit of NAUTH, Nnewi, South Eastern Nigeria. All the participants participated voluntarily, with a written informed consent. Ethical approval was sort from the Ethics committee of NAUTH, Nnewi. The subjects were screened for both HIV and malaria infection routinely and the subjects were classified, using WHO 2006 HIV staging as guide[18] into: (1) Symptomatic HIV on Antiretroviral therapy (ART) (n = 100) of these, 50 were symptomatic HIV on ART with malaria co-infection. (2) Symptomatic HIV not on ART (n = 100) of these, 50 were symptomatic HIV not on ART with malaria co-infection. (3) Asymptomatic HIV subjects (n = 100) of these, 50 were asymptomatic HIV with malaria co-infection. (4) HIV seronegative control subjects (n = 100) of these, 50 were malaria positive. Questionnaires were used to obtain background information of participants such as age, sex, time of HIV infection, time of intake of ART, any or absence of disease.

Sample collection

6 ml of fasting blood samples were collected from all the participants in this study. 2ml of blood samples were collected into EDTA sample tubes for HIV and malaria screening and detections, and CD4+ T cell count. The remaining 4 ml of blood sample were collected into plain tube and allowed to clot, centrifuged, the serum separated and analyzed for serum Myoglobin, Troponin I, total-CK, CK-MB, LDH and AST.

Quality control measures

Quality control sera were ran along test in each batch of analysis these were compared with the reference values of the control sera. Standard deviation and coefficient of variation were calculated on them.

Methods of assay

Enzyme linked Immunosorbent Assay (ELISA) as described by Tiez[19] was used for Myoglobin and Troponin I. Spectrophotometric method as described by Tiez[20] was used for enzyme cardiac markers- total creatine kinase (T-CK), CK-MB, lactate dehydrogenase (LDH) and aspartate transaminase (AST) and Cyflow SL-Green for CD4 counts. HIV was determined by the methods of Abbott determine TM HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay (Abbott Japan Co.Ltd.Tokyo, Japan) 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 (CHEMBIO Diagnostic system, Inc, New York, USA). Determination of $CD4^+$T cells counts was by CyFlows SL-Green method. Detection of Plasmodium falciparum parasite was by thick and thin films as described by W.H.O[21] and by screening method using antigen rapid test device as described by the manufacturer-Access Bio, Incorporated, New Jersey, USA. The result of the analysis was statistically analyzed. Students't-test was used to compare means. The analysis was performed with the use of Statistical *Package for Social Sciences* (SPSS) statistical software package, version 16.0. $P < 0.05$ is considered statistically significant.

RESULTS

Pair wise comparisons showed that serum activity of CK-MB was significantly higher in symptomatic HIV infected subjects on ART with malaria parasitaemia compared to symptomatic HIV infected subjects on ART without malaria parasitaemia at $p<0.05$. But, the CD4 count was significantly lower in symptomatic HIV infected subjects on ART with malaria infection compared to symptomatic HIV infected subjects on ART without malaria infection at $p<0.05$. However, the values of Myoglobin, Troponin, the serum activities of CK-T, LDH and AST were the same in symptomatic HIV infected subjects on ART with malaria infection compared to symptomatic HIV infected subjects on ART without malaria infection at $p>0.05$ respectively.

Between group comparisons showed that the serum levels of CK-T, CK-MB, LDH, AST and blood CD4 count were the same in symptomatic HIV infected subjects without ART with

malaria infection compared to symptomatic HIV infected subjects without ART without malaria infection at p>0.05 respectively. But the serum level of myoglobin was significantly higher in symptomatic HIV infected subjects without ART with malaria infection compared to those without malaria infection at p>0.05.

Between group comparisons showed that the serum level of troponin I was significantly higher in asymptomatic HIV infected subjects with malaria parasitaemia compared to asymptomatic HIV infected subjects without malaria parasitaemiaat p<0.05. However, the serum levels of CK-T, CK-MB, LDH, AST, myoglobin and blood CD4 count were the same in asymptomatic HIV infected subjects with malaria parasitaemia compared to asymptomatic HIV infected subjects without malaria parasitaemia (p>0.05 respectively).

Pair wise comparisons showed that the serum levels of Myoglobin, Troponin I, CK-T, CK-MB and LDH were significantly higher in control subjects with malaria infection compared to control subjects without malaria infection at p<0.05 respectively. But, the values of AST and CD4 were the same in control subjects with malaria infection compared to control subjects without malaria infection at p>0.05 respectively. Also, pair wise comparisons showed that the serum levels of LDH was significantly lower in control subjects with malaria infection compared to control subjects without malaria infection (p<0.05). Also, significant differences were observed in levels of Myoglobin, Troponin, CK-T and CK-MB in control subjects with malaria infection compared to control subjects without malaria infection (p<0.05, in each case) (see table 1).

Table 1: Comparison of Mean ± SD serum levels of Cardiac markers and CD4 in subjects studied with and without malaria parasitaemia.

GROUPS	Myoglobin (NG/ML)	Troponin (Ng/Ml)	T-CK (IU/L)	Ck-Mb (IUL)	Ldh (IU/L)	Ast (IU/L)	Cd4 (/µL)
Symptomatic HIV on ART mp^+ (n=50)	43.60 ± 41.30	1.46 ± 0.15	97.10 ± 7.52	8.37 ± 1.83	189.46 ± 24.00	23.60 ± 5.66	535.58± 368.91
Symptomatic HIV on ART mp- (n=50)	37.90 ± 38.82	1.31 ± 0.11	94.60± 7.87	6.78 ± 2.11	185.70 ± 23.93	22.61 ± 5.27	706.58± 400.11
p-value	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	<0.05
Symptomatic HIV without ART mp^+ (n=50)	76.72 ± 36.82	1.72 ± 0.22	128.9 ± 18.40	16.03 ± 4.04	197.50± 21.63	35.28 ± 5.81	390.06± 130.75
Symptomatic HIV without	44.08 ± 16.85	1.47 ± 0.22	116.02± 14.20	11.88 ± 3.64	176.94± 24.58	27.99± 10.64	359.50± 110.90

ART mp^+ (n=50)							
p-value	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Asymptomatic HIV mp^+ (n=50)	39.87 ± 13.14	0.77 ± 0.14	106.50 ± 14.54	5.48 ± 1.57	157.78± 25.49	22.97 ± 8.78	416.18± 130.47
Asymptomatic HIV mp- (n=50)	42.99 ± 14.27	0.54 ± 0.04	98.06 ± 17.85	4.28 ± 1.50	156.46± 25.25	17.49 ± 7.65	457.92± 126.94
Control subjects mp^+ (n=50)	22.77 ± 11.33	0.35 ± 0.15	68.18 ± 24.54	3.10 ± 1.51	163.36± 0.93	14.03 ± 5.34	935.02± 151.15
Control subjects mp- (n=50)	22.77 ± 11.33	0.26 ± 0.15	53.50 ± 30.13	1.92 ± 1.27	147.04± 23.11	10.52 ± 4.99	946.26± 147.83
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05

DISCUSSION

This study showed that the serum CK-MB was significantly higher in symptomatic HIV subjects on ART with malaria parasitaemia than in those without malaria parasitaemia. The elevated level may be due to the effect of HIV-malaria co-infections. But blood CD4 count was significantly lower in higher in symptomatic HIV subjects on ART with malaria parasitaemia than in those without malaria parasitaemia. The prevalence of malaria as co-infection in HIV-infected individuals in sub-Saharan Africa has been established. Cohen *et al*[11], observed an increased prevalence of severe malaria in HIV-infected adults in South Africa. On the other hand, Onyenekwe *et al*,[22] observed also an increased prevalence of severe malaria in HIV-infected malaria subjects in endemic area of Southern Nigeria. Ezeugwunne *et al*[23] observed that HIV and malaria co-infections were capable of lowering the values of CD4 counts in HIV individuals.

In this study, the serum level of Myoglobin was significantly higher in symptomatic HIV subjects not on ART with malaria parasitaemia than in those without malaria parasitaemia. Ezeugwunne *et a* [24] observed higher level of Myoglobin in symptomatic HIV participants not on antiretroviral therapy compared with asymptomatic HIV participants.

The study revealed increased Troponin I level in asymptomatic HIV subjects with malaria parasitaemia than in those without malaria parasitaemia. *Plasmodium falciparum* has been found to stimulate HIV replication through the production of cytokines (Interleukin -6 (IL-6) and tumor necrosis factor (TNF-alpha)) by activated lymphocytes.[25] This may suggest that malaria may speed up the progression of HIV disease.[26] Previous study showed that Troponin I levels were significantly higher in symptomatic HIV participants not on ART compared with HIV asymptomatic HIV participants.[24]

The serum Myoglobin, Troponin I, total creatine kinase, creatine kinase-MB, Lactate dehydrogenase and Aspartate transaminase levels were significantly higher in the control group with malaria parasitaemia. Malaria is caused by plasmodium species. The *plasmodium falciparum* is the most virulent of the species. It causes up to 25 % death when great amount of infected red blood cells are destroyed in a single burst.[6] Malaria was observed to be endemic in Nigeria and it is the leading cause of morbidity and mortality in the country.[7,8]

The serum Myoglobin, Troponin I, total creatine kinase, creatine kinase-MB, Lactate dehydrogenase and Aspartate transaminase levels are cardiac markers biomarkers that are measured to evaluate heart function. They are reduced in blood during heart damage. [27] So their evaluation in apparent healthy individual with malaria parasitaemia may imply that malaria infection can have undesirable effect on the heart.

CONCLUSION

Malaria endemicity poses a threat to the improved cardiac function as observed with increased serum levels of CK-MB, myoglobin, and Troponin I in HIV malaria co-infection with or without ART and with the reduction of CD4 count in HIV malaria co-infection with ART. This calls for concern in the management of patients with HIV malaria co-infection as well as in controls with malaria infection as these cardiac biomarkers were also raised in malaria infection.

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Conflict of interest

There is no conflict of interest whatever with anyone or group of persons. The studied was sponsored by Tertiary Education trust Fund (TETFUND), Nigeria.

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