

Assessment of the immunological profile of HIV-positive patients co-infected with malaria parasite attending general hospital North-Bank, Makurdi, Nigeria

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Abstract

Malaria and HIV are the two most important infectious diseases and have comparable global disseminations. Given the eclectic geographical overlap on occurrence and the resulting co-infection; the interaction between the two diseases clearly has major public health implications, mostly in Sub-Saharan Africa. This study investigated the immunological alterations associated with HIV and Malaria co-infection in HIV positive individuals. The study was performed by sampling 600 adult HIV patients who routinely visit the General Hospital North-Bank Makurdi, Benue State, Nigeria. Blood samples were obtained for blood film microscopy identification of malaria parasites. Screening for immunological profiles was performed using the flow cytometer for the quantification of CD4+ cell count. Results: Of the 600 patients sampled, 221 (36.8%) had normal CD4+ count (≥ 500 cell/mm³), 320 (53.3%) had moderate CD4+ count (between 200 and 499 cell/mm³) while 59 (9.8%) had poor CD4+ (less than 200 cell/mm³). The mean CD4+ lymphocyte count of HIV-malaria co-infected patients was lower than HIV mono-infected patients. Malaria and HIV co-infection significantly reduced the CD4+ count of the subjects. In general, to achieve better management of all HIV patients in this setting, diagnosing malaria, prompt antiretroviral therapy, monitoring CD4 and some haematological indices on regular basis is important. In light of the epidemiological connection and global reputation of the two diseases, there is an urgent need for more research on a wider range in order to elucidate the impact of co-infection on host immune dynamics in HIV co-infected individuals.

Keywords: Human Immunodeficiency Virus (HIV); Malaria Parasite; Immunological Profile; Opportunistic; Assessed; Co-Infection; Cluster of Differentiation (CD4+).

1. Introduction

Human Immunodeficiency Virus (HIV) and malaria are among the two most important global health problems of developing countries, including Nigeria, which has been reported to cause more than 4 million deaths a year, with HIV infection increasing the risk and severity of malaria infection and burdens [1]. Furthermore, [1] reported that HIV facilitates the rate of malaria transmission which in turn causes strong CD4 cell activation and up-regulation of pro-inflammatory and cytokines production which create an ideal microenvironment for the spread of HIV among CD4 cells for rapid HIV-1 replication.

Malaria and HIV co-infections are increasingly reported worldwide and the interaction may lead to poorly understood effects on the disease outcome and clinical manifestations [2]. In an environment where malaria is common, the incidence of clinical malaria episodes is reported to be higher in patients with CD4 cell counts < 200 cells/ μ l than in those with CD4 cell counts > 500 cells/ μ l [3]. HIV and malaria both destroy important cells required for proper

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immunological and hematological functioning of the body [4]. Enhanced T-cell activation in HIV and malaria co-infected patients could worsen the immune response to both diseases [5].

It was reported by [6] that, "Opportunistic Infections in HIV-Infected Patients Differ Strongly in Frequencies and Spectra between Patients with Low CD4+ Cell Counts Examined Postmortem and Compensated Patients Examined Antemortem Irrespective of the HAART Era"

Malaria is the most important parasitic infection that attracts the greatest global attention due to its disproportionate distribution [7]. The World Health Organization affirms that malaria constitutes a global health threat with 228 million cases and 405,000 deaths in 2018 [8]. Malaria is caused by five species of Plasmodium namely; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Of the five Plasmodium species, *Plasmodium falciparum* accounted for most morbidities and consequently, highest mortality rates in Africa and other high-risk regions of the world [9, 10]. Therefore, infection with *P. falciparum* requires quick recognition, proper treatment and effective patient management [11].

Introduction of Human Immuno-Deficiency Virus (HIV) into host cells activates a complex network of protective responses originating from both the innate and adaptive immune systems [12]. These responses are either insufficient or too late to eliminate the virus. This enables life-long viral latency and chronic infection, which drives ongoing immune activation and progressive immunodeficiency, characterized by high cell turnover, apoptosis, and activation-induced death of immune cells [13].

There have been reports of increased cases of malaria and HIV in Nigeria by many researchers. This study is therefore designed to establish facts on the effects of Malaria-HIV Co-infection on the immune profile and advance possible solutions to the combat Co-infection. The results from this research will be of benefit to individuals so as to establish the relationship between Malaria/HIV and the immune system. The study would also give light to the level of trauma that the infected subjects undergo and the risk factors associated with such co-infection. This study provides useful information to policy makers and a clearer picture and understanding of the real position of these infections among the populace so that formulated policies can improve research work on the early detection and treatment of these diseases.

2. Materials and methods

2.1. Study population

The study population was 600 medically diagnosed HIV/AIDS patients who visit the study area for routine collection of Antiretroviral (ARV) Drugs and CD4 cell count.

2.2. Site

The study area was General Hospital, North-Bank, Makurdi, located behind North-Bank market, Makurdi, Benue state. The hospital is one of the HIV-designated centres, approved by Benue State Ministry of Health, for the screening of HIV/AIDS, checking of CD4 cell count and administration of antiretroviral (ARV) drugs.

2.3. Ethical Consideration

Ethical clearance was obtained from Benue state Ministry of Health ethical committee Makurdi. Informed consent was made available to all the study participants. All procedures were in accordance with the National Ethical Standards and results were treated with utmost confidentiality.

2.4. Protocol

In respect to the collection of demographic data, an easy – to – read and friendly questionnaire as well as participant's consent forms were provided.

2.5. Collection of Blood

The area where the blood was collected was cleaned with Methylated spirit swab to locate a prominent vein. A tunicate was tied round the patient's arm and fist closed. A vacutainer needle was then inserted in the prominent vein that is located and the tunicate was loosened and the fist opened. Five milliliters (5mls) of venous blood was collected and a cotton wool was placed on the vein to enable blood clotting. The blood collected was separated into test tubes, which was further used for Malaria screening, CD4 cell count, and full blood count respectively.

2.6. Determination of Malaria Parasites

A drop of blood was placed on a clean grease-free slide. A cover slip was used as a spreader to make thin and thick films. The slide was labeled with the patient's identification number or an alphabet and allowed to air dry and then stained with Giemsa solution. Eight (8) drops of the stain were added to the slide and allowed to stand for two (2mins) minutes. Twelve to fifteen (12-15) drops of buffered distilled water (pH 6.4) were added and allowed to stand for 4-8minutes. The slide was finally examined microscopically under $\times 10$ and $\times 40$ objective lenses.

2.7. Determination for Immunological profile

2.7.1. CD4 Cell count

The blood sample drawn in the vacutainer tube was placed on a mixer for 15minutes. Twenty micro-litres ($20\mu\text{l}$) of the CD4 absolute count reagent were pipetted into a separate sample tube and $20\mu\text{l}$ of the mixed blood was added to the tube containing the absolute count reagent and mixed, then incubated for 15minutes for dark reaction. Eight hundred micro liters ($800\mu\text{l}$) of no-lyse buffer were added to the blood in the tube and shaken well. The sample was analyzed using the flow cytometer (PARTEC CYFLOW COUNTER II, made in Germany). It contains a special diode laser for ultra violet, blue or red excitation. The device has a display screen that displays clearly the CD4 count with time and date. It has software which identifies the T-lymphocyte population and the capacity to calculate the absolute counts and display visually on screen.

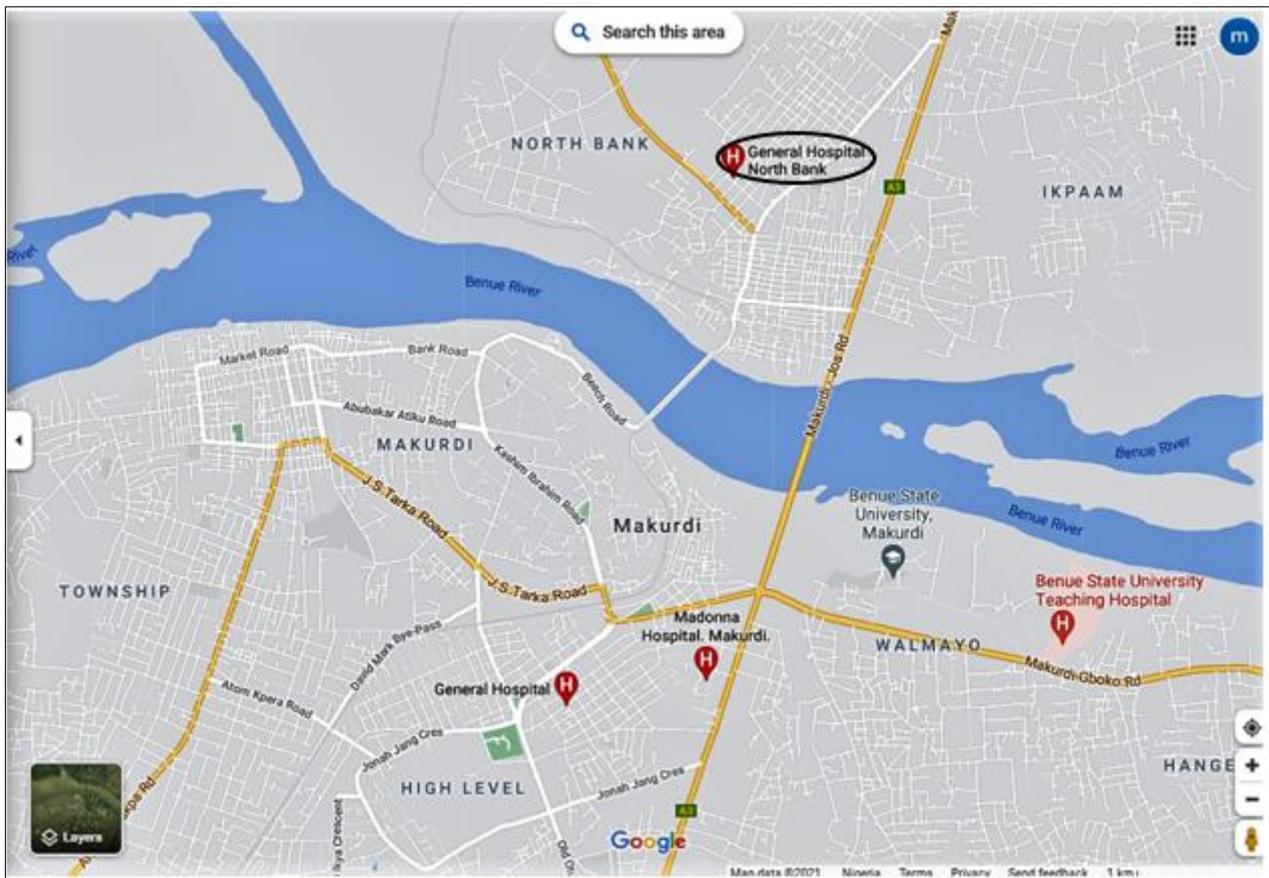


Figure 1 Map of Makurdi showing the study site (General Hospital North-Bank, Makurdi)

KEYS: Study site = ○
Hospitals = H

3. Result

The immunological profile of HIV positive patients co-infected by malaria parasite was investigated at the General Hospital North-Bank Makurdi. The results are expressed as follows;

The socio-demographic characteristics of the screened patients were shown in Table 1. In respect to Residence, the rural inhabitants had the highest frequency of 378 (63.0%). Based on age group, age 21-30 had the highest frequency of 227 (37.8%), while age 1-10 had the lowest frequency of 28 (4.7%). In respect to gender, the females had the highest frequency of 455 (75.8%). Based on educational qualification, the highest frequency was observed among the secondary with the value 345 (57.5%) while the lowest frequency was observed among the primary with the value 52 (8.7%). In respect to occupation, the farmers had the highest frequency of 312 (52.0%) while the applicants had the lowest frequency of 22 (8.7%). In respect to Marital status, the married had the highest frequency of 357 (59.5%).

The mean distribution of screened patients' CD4 count with respect to age is shown in Table 2. Age group 1-10 had the highest CD4 count of 995.25 ± 536.97 , while the least CD4 count was observed in age group 11-20 with the mean total value of 516.03 ± 316.47 . The highest attended number was age group 21-30 (227) 37.8% while the least attended number was age group 1-10 (28) 4.6%.

The sex distribution of participants' CD4 count is shown in Table 3. The females had the highest CD4 count with mean total value of 598.73 ± 347.78 , while the males had CD4 count with the mean total value of 524.06 ± 348.86 .

The distribution of CD4 count range of subjects screened with respect to age is recorded in Table 4. It was observed that 221 (36.8%) participants had good CD4 count greater than or equals $500\text{cell}/\text{mm}^3$. 320 (53.3%) participants had moderate CD4 between 200 and $499\text{cell}/\text{mm}^3$ and 59 (9.8%) participants had poor CD4 count less than $200\text{cell}/\text{mm}^3$.

The distribution of CD4 count range of subjects screened with respect to gender is shown in Table 5. It was observed among the females that 151 (33.2%) had CD4 count $\geq 500\text{cell}/\text{mm}^3$, 267 (58.7%) had CD4 count between 200- $499\text{cell}/\text{mm}^3$ and 37 (8.1%) had CD4 count less than $200\text{cell}/\text{mm}^3$. However, among the males, 61 (42.1%) had CD4 count $\geq 500\text{cell}/\text{mm}^3$, 65 (44.8%) had CD4 between 200- $499\text{cell}/\text{mm}^3$ and 19(13.1%) had CD4 count less than $200\text{cell}/\text{mm}^3$.

The distribution of CD4 count of subjects screened with respect to residence is recorded in Table 6. It was observed among the Urban residents that 78 (35.1%), had CD4 count $\geq 500\text{cell}/\text{mm}^3$, 127 (57.2%) had CD4 count between 200- $499\text{cell}/\text{mm}^3$ and 17 (7.7%) had CD4 count less than $200\text{cell}/\text{mm}^3$. However, among the Rural residents, 140 (37.0%) had CD4 count $\geq 500\text{cell}/\text{mm}^3$, 201 (53.2%) had CD4 between 200- $499\text{cell}/\text{mm}^3$ and 37 (9.8%) had CD4 count less than $200\text{cell}/\text{mm}^3$.

The distribution of participant's mean CD 4+ Count in respect to Marital Status is shown in Table 7. It was observed that the single had the highest mean CD4 count of 662.38 ± 376.66 , while the married had the lowest mean CD4 count of 525.07 ± 317.95 .

The Distribution of CD4 count range of HIV patients non-coinfected with malaria with respect to age is demonstrated in Table 8. It was observed that 221(36.8%) participants had good CD4 count greater than or equals $500\text{cell}/\text{mm}^3$, 320 (53.3%) participants had moderate CD4 between 200 and $499\text{cell}/\text{mm}^3$ and 59 (9.8%) participants had poor CD4 count less than $200\text{cell}/\text{mm}^3$.

The Distribution of CD4 count range of HIV patients non-coinfected with malaria with respect to gender is recorded in Table 9. It was observed among the females that 33(31.4%) had CD4 count $\geq 500\text{cell}/\text{mm}^3$, 1(1.0%) had CD4 count between 200- $499\text{cell}/\text{mm}^3$ and none had CD4 count less than $200\text{cell}/\text{mm}^3$. However, among the males, 69 (65.7%) had CD4 count $\geq 500\text{cell}/\text{mm}^3$, 2(1.9%) had CD4 between 200- $499\text{cell}/\text{mm}^3$ and none had CD4 count less than $200\text{cell}/\text{mm}^3$.

Table 1 Socio-demographic Characteristics of Co-infected Participants

Variables		Frequency (No.)	Percentage (%)
Residence	Rural	378	63.0
	Urban	222	37.0
Age Group (Years)	1 – 10	28	4.7
	11 – 20	37	6.2
	21 – 30	227	37.8
	31 – 40	192	32.0
	41 – 50	78	13.0
	Above 50	38	6.0
	TOTAL	600	100
Gender	Male	145	24.0
	Female	455	75.8
	TOTAL	600	100
Marital Status	Married	357	59.5
	Single	243	40.5
	TOTAL	600	100
Educational Qualification	Primary	52	8.7
	Secondary	345	57.5
	Tertiary	203	33.8
	TOTAL	600	100
Occupation	Applicant	22	3.7
	Civil servant	113	18.8
	Farmer	312	52.0
	Students	85	14.2
	Others	68	11.3
	TOTAL	600	100

Table 2 Mean distribution of screened Co-infected patient's CD 4+ Count with respect to age

Age	N. Obs	Mean	Std. Dev.
1-10	28	995.25	536.97
11-20	37	516.03	316.47
21-30	227	572.79	319.96
31-40	192	561.86	358.62
41-50	78	560.67	286.97
51 and above	38	520.29	255.74
Total	600		

Key: CD4 = Cluster of deferential 4, N. Obs = Number observed, Std. Dev. = Standard Deviation

Table 3 Sex Distribution of Co-infected participant's CD4+ Count

SEX	N. Obs	Mean	Std. Dev.
Female	455	598.73	347.78
Male	145	524.06	348.86
Total	600		

Key: CD4 = Cluster of deferential 4, N. Obs = Number observed, Std. Dev. = Standard Deviation

Table 4 Distribution of CD4 count range of Co-infected subjects screened with respect to age

Age group	CD4 count range		
	Greater or Equals 500 cell/mm cube(%)	Between 200 and 499 cell/mm cube (inclusive)(%)	Less than 200 cells/mm cube(%)
1-10	14(51.9)	11(39.3)	3(10.7)
11-20	22(59.5)	9(24.3)	6(16.2)
21-30	83(36.6)	118(52.0)	26(11.5)
31-40	65(33.9)	115(59.9)	12(6.3)
41-50	26(33.3)	45 (57.7)	7(9.0)
≥ 51	11(28.9)	22(57.9)	5(13.2)
Total	221(36.8)	320(53.3)	59(9.8)

Key: CD4 Count \geq 500 – Good health state; CD4 Count 200-499 – Moderate health state; CD4 Count range less than 200 – Poor health state

Table 5 Distribution of CD4 count range of co-infected subjects screened with respect to gender

Gender	CD4 count range			Total
	Greater Or Equals 500 cell/mm cube	Between 200 and 499 cell/mm cube (inclusive)	Less than 200 cells/mm cube	
Female	151(33.2)	267(58.7)	37(8.1)	455(100.0)
Male	61(42.1)	65(44.8)	19(13.1)	145(100.0)
Total	212(35.3)	332(55.3)	56(9.3)	600(100.0)

Table 6 Distribution of CD4 count of co-infected subjects screened with respect to residence

Residence	CD4 count range			Total(%)
	Greater or Equals 500 cell/mm cube(%)	Between 200 and 499 cell/mm cube (inclusive)(%)	Less than 200 cells/mm cube(%)	
Urban	78(35.1)	127(57.2)	17(7.7)	222(100.0)
Rural	140(37.0)	201(53.2)	37(9.8)	378(100.0)
Total	212(35.3)	332(55.3)	56(9.3)	600(100.0)

Table 7 Mean Distribution of co-infected participant's CD 4+ Count in respect to Marital Status

MARITAL STATUS		Mean \pm SD
	N. Obs	Mean
MARRIED	357	525.07 \pm 317.95
SINGLE	243	662.38 \pm 376.66
Total	600	

Key: N. Obs = Number observed, Std. Dev. = Standard Deviation

Table 8 Distribution of CD4 count range of HIV patients non-coinfected with malaria (control) with respect to age

Age group	CD4 count range		
	Greater or Equals 500 cell/mm cube (%)	Between 200 and 499 cell/mm cube (inclusive) (%)	Less than 200 cells/mm cube (%)
1-10	15(14.3)	0	0
11-20	10(9.5)	0	0
21-30	23(21.9)	0	0
31-40	41(39.0)	0	0
41-50	8(7.6)	1(1.0)	0
\geq 51	5(4.8)	2(1.9)	0
Total	102(97.1)	3(2.9)	0

Table 9 Distribution of CD4 count range of HIV patients non-coinfected with malaria (control) with respect to gender

Gender	CD4 count range		
	Greater Or Equals 500 cell/mm cube	Between 200 and 499 cell/mm cube (inclusive)	Less than 200 cells/mm cube
Female	33(31.4)	1(1.0)	0
Male	69(65.7)	2(1.9)	0
Total	102(97.1)	3(2.9)	0

4. Discussion

The immunological profile of HIV-positive patients co-infected with malaria parasite has been investigated among 600 medically diagnosed HIV patients attending General Hospital North-Bank, Makurdi, Benue State.

According to [14], the standard CD4 cell count range is as follows; ≥ 500 cell/mm³ (good health state), between 200-499 cell/mm³ (moderate health state) and less than 200 cell/mm³ (poor health state). From the results, females had the highest CD4 cell count with good health state compared to the males. This may be because the HIV present in semen is the most prevalent vector in transmission of this disease as reported by [15].

Among the 600 participants screened, on the basis of age group, 221 participants in this category had healthy CD4 counts. This may be due to the use of HAART (Highly Active Antiretroviral Therapy), as reported by, [16], who suggested that the use of HAART could reduce the immune suppression posed by HIV. However, it was observed that

the urban residents had higher CD4 count than the rural residents. This may be due to the rural residents being more susceptible to malaria and must have had repeated episodes of malaria infections due to the presence of various mosquito breeding sites compared to the urban areas. Thus, malaria leads to an increase in the viral load of HIV as reported by [17]

The result from this study therefore revealed that majority of the participants co-infected had CD4 counts lower than the normal reference range (≥ 500), implying that they had lowered immunity, which further leads to speedy progression of the HIV disease to AIDS and other infections which foster poor response to HIV treatments. This agrees with the report of [18] and that of [19], when CD4⁺ T cells decline to a critical level, cell-mediated immunity is lost and the body of the individual becomes susceptible to other opportunistic infections. It is also in consonance [20], who opined that the effect of Malaria-HIV Co-infection is greater in patients with advanced and suppressed immune function than those with strong immune system.

However, 105 HIV positive individuals who were not infected with malaria (control) were screened. It was observed that 97% of the participants had CD4 cell count ≥ 500 cell/mm³ (good health state). This may be due to the use of HAART which boosts the immune system thereby protecting them from other opportunistic infections that would've lowered their immune systems. This agrees with [14], who also suggested that the use of HAART could reduce the immune suppression posed by HIV.

5. Conclusion

This study revealed that, malaria infection in HIV positive individuals leads to further reduction in immunological indices required for patients to manage the disease and stay healthy. The immunological parameters were affected; the result showed a noticeable immune abnormality. It was evident from the result that Malaria and HIV interaction exponentially increases the adverse effects of one infection on the other, also has a negative impact on the prognosis, and complicates the prevention and treatment of both infections in patients infected. Their interactions promote the incidence of both infections. Thus, measures should be taken to prevent or treat as early as possible any co-infection in HIV patients to ensure an effective immune restoration in all HIV infected individuals undergoing therapy. Also, patients should ensure to take Antiretroviral Drugs which are immune boosters holistically to prevent immune system deterioration.

Recommendation

These findings highlighted the need for early confirmatory diagnosis of malaria in HIV infected individuals, as well as provision of malaria therapy when the diagnosis is established in order to prevent the consequences of the malaria parasites on the immune system.

Provision of amenities such as insecticidal treated mosquito bed-nets, insecticides and mosquito repellants should be made for individuals living in malaria endemic areas as well as antiretroviral drugs to help boost the immunity of infected persons.

Awareness should also be created on the risk of HIV/Malaria co-infection and the need for proper monitoring of CD4 cell count in HIV infected persons so as to enable early detection of changes in the immune profiles.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Benue State Health Management Board with an Issuance of an ethical clearance certificate from the ethics committee. All participants were informed of the details of the study before samples were collected.

Statement of informed consent

Informed consent was obtained from all individuals who participated in this study.

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