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JOURNAL OF ADVANCED PHARMACEUTICAL SCIENCE AND TECHNOLOGY
ISSN NO: 2328-0182

Review Article

DOI: 10.14302/issn.2328-0182.japst-20-3347

Invivo Impact of Malaria and HIV Co-Infection on CD4 Cell Count of Infected Patients of Niger Delta Extraction

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Abstract

The study evaluated the impact of co-infection of malaria parasitaemia, and HIV positive indices on the CD4 cell count of 120 HIV infected subjects, who were already diagnosed and visiting Braithwaite Memorial Specialist Hospital Port Harcourt for routine Medical check-up. Also, a control group of 40 HIV negative were included as part of the study control group. The subjects were between the age ranges of ≤10–79 years respectively. A double check laboratory assay was conducted to detect the presence of antibody to HIV as confirmed using immunocomb 11 and Determine for HIV status. A thick Blood film stained with field stain (A and B) was used to detect the presence of malaria parasite in the subject's blood. Furthermore, CD4 cell count was assayed using Partec cyflow counter (Partec, Germany). Excel and Graphpad statistical software were used for analysis of the data generated. The result among the HIV positive subjects and control subjects revealed that the highest positive for malaria infection was observed among ≤10 years age group as 2 (100%) and 11 (84.61%) respectively. In the HIV positive subjects, the distribution of malaria infection among sex revealed a high rate in male 42(77.78%) than in female 44 (66.67%). Similarly, the control recorded a high rate of malaria infection in male 11 (57.89%) than in female 7 (33.33%). However, 86 (71.67%) had malaria and HIV co-infection while 34 (65%) had only HIV mono infection. The positive HIV subjects who had CD4 cells count below 200 cells/mm^3 were 15%, above 200-499cells/mm^3 were 58.3% while 500 cells/mm^3 and above had normal CD4 cells counts for 26%. Nonetheless, for the control subjects, no CD4 cells count of below 200cells/ mm was observed, 2.5% fell within the moderate category while 75% had normal CD4 cells count. Statistical analysis using ANOVA and t-test showed that there is significant difference between CD4 of seropositive and seronegative subjects infected with or without malaria ($p=0.00$). In addition, a t-test further demonstrated Comparison of Mean CD4 Cell Count among HIV and Malaria Infected and Non-Infected Subjects. MP/HIV Co-Infection and Mono Infection with No Infection showed strong mean difference ($p=0.00$) in the various CD4 counts while HIV Mono-Infection and others only had a non significant ($p=0.44$) mean difference between HIV Mono-Infection and No HIV or Malaria Infection. A robust and effective malaria and HIV control management programme should be strongly underpinned; so as to improve the quality of life of patients and HIV patients should be encouraged to live a healthy life style, through the provision of antiretroviral drugs and regular health education engagement, even as the provision of antimalarial treated net would be helpful to the subjects.

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Keywords: Impact, Malaria, HIV, Co-Infection, CD4 Cell Count, Infected Patients, Partec cyflow counter, Niger Delta

Received: Apr 28, 2020 **Accepted:** May 03, 2020 **Published:** May 08, 2020

Editor: Fatma Mohammed Mady, Department of Pharmaceutics, Minia University, Egypt.

Introduction

Malaria and HIV infection are two important commonest Public Health infections probably prominent in sub-Saharan Africa, even as these calls for more concerted effort of multi-dimensional approach, if we must root them out completely or reduce their clinical and socio –economic impact significantly in developing countries, especially in our remote communities that are strongly believed to be harbouring more vulnerable subjects when probably compared with the urban cities across the globe. Nevertheless, it is estimated that 29.4 million Africans are infected with HIV [1]. Therefore, any clinical interaction between the two infections will be of major public health importance for researchers to study. The HIV/AIDS pandemic in regions where *Plasmodium falciparum* is endemic has generated concern about potential interactions between the two infections, especially in sub-Saharan Africa with huge endemic land scale coverage [2]. Nonetheless, there is always an increase in HIV replication progression when subjects are infected with *malaria parasite, especially* in blood monomamanial cells, also when exposed to malaria antigens during an invitro set up [3] and furthermore, in transgenic mice-infected with *P. chabaudi* [4]. These significant increases occurs especially when an individual has a parasites density more than 2000 parasites per micro-litre of blood, and base line CD4 count of 300 cells/mm^3 [5]. However, HIV-1 RNA concentrations and CD4 cells counts are moderately but inconsistently associated with *parasitaemia outcome*. Nonetheless, a high parasite density with fever is associated with HIV-1 seropositivity. Thus, low CD4 cell count infection with HIV causes progression of cellular immune suppression, and any impairment in immune response resulting to *malaria* might be associated with failure to prevent infection, or to suppress *parasitaemia* [6].

However, a laboratory based studies have found that some components of human immune response to *P. falciparum* are modified by HIV-1, but that others are unaffected. It has also been shown to increase the potential reservoir for HIV in the placenta by increasing the number of *CCR5+* positive macrophages [3]. However, a study from Malawi showed that HIV-1 plasma viral loads are significantly higher in patients with *malaria* than in those without, and these levels remain higher for at least four weeks after treatment [7]. On the other hand, another studies in the same country revealed that increased HIV virus concentration was reversible within eight to nine weeks in individuals who had been treated for malaria and their viral load had reached almost the baseline level [5]. In all, the spread of HIV and malaria infection is very vital and should be critically managed and diagnose in good time to reduce the untold clinical consequences that may be linked to the above scenario if left undiagnosed and poorly managed in good time.

Nonetheless, HIV is not spread through casual contact or by mosquitoes or other insect vectors like malaria [8], although, malaria and HIV also share some common similarities. One common feature between malaria and AIDS is that, there is no effective vaccine for immunization against these two diseases, which have continued to threaten the existence of man in Africa [9]. Just like HIV, malaria parasite can be transmitted through transfusion and communal use of infected syringes by drug addict [10]. Transmission route mechanism remains of very massive interest to epidemiologist and other vital Public Health stake holders in the field of social and preventive medicine, so as to activate better and fast control and preventive strategies. However, studies in men and non-pregnant women shows that the underlying epidemiological perspectives, and the overall intensity of the malaria

transmission remains very critical factor for determining the consequences of co-infection in an area of stable and unstable malaria endemicity. Transmission is usually intense and continuous, although seasonal variations may occur in some cases. Young children and pregnant women are at the greatest risk of morbidity and mortality from malaria as reported by Whitworth *et al.,* [11] in their studies.

Nevertheless, mono infection of malaria and HIV is of great burden and concern especially in the Sub Sahara Africa, but more critical morbidities and mortalities would be associated in co-infection of malaria and HIV outcome in a subject. Unarguably, this is a serious Public Health problem that calls for regular monitoring and evaluation in developing counties like Nigeria where there is scarcity of data in this direction. Thus, the study would attempt to evaluate the infection rate and impact of malaria and HIV co-infection on the CD4 cell count of the infected population. Also, the study would established the age group and sex mostly infected amongst the study population, and it is strongly believed that data generated would prompt enhance much needed advocacy, that would improve the management and effective diagnosis of the infection in our hinterlands, where access to functional health care infrastructures remains visibly lacking in large scale and the few available ones are in a state of decay with no attempt of rebuilding them in the nearest future for the good of all.

Research Location

The quantitative study was performed in Rivers State, Nigeria. HIV infected patients accessing care at Braithwaite Memorial Specialist Hospital were recruited into the study. Rivers State is one of the wealthiest states by gross domestic product and foreign exchange revenue from the oil industry, crude oil is its main natural resources used for export and Rivers State remains the headquarter of all Niger Delta States of Nigeria. The area has an average temperature between 25°C – 28°C in the city. It has a coordinates of about 4.57°N°E. The areas occupy is 186Km^2, land of 1700km^2, water 16km^2 and metro of 462km^2. It has a population in urban 2,667,435 with varying ethnicities.

Ethical Approval

The ethical approval of the study was sought and the approval was granted by the ethical committee board of Rivers State Hospital Management Board and the study was conducted in line with the consideration and recommendation of the board, even as the consent and confidentialities of the subject's personal data status were highly secured and maintained throughout the study period and beyond.

Research Design/ Inclusion/Exclusion Criteria

A non-proportionate stratified random sampling method was used to form the study groups into two strata of HIV positive and control. A sample size of 160 using the descriptive formula by Cochran; excluding subjects who did not give consents, those outside the hospital care and those with known cases of active Tuberculosis and Hepatitis however, both secondary and primary data sources were used.

Experimental

5ml of whole blood sample were aseptically collected into EDTA blood container using a sterile needle and syringe, about 2ml part of whole blood was used for the preparation of thick blood film for malaria parasite examination while the remaining 3 ml were centrifuged at 1500 rpm for 5 minutes to separate the blood cells from plasma for HIV screening analysis respectively

The hospital based descriptive study utilized ImmunoComb 11 and BiSpot test kit, which is an indirect solid phase enzyme immunoassay. The solid phase is a card with 12 projections of teeth. Each tooth is sensitized at three spots for HIV and the microscopic method of malaria estimation was explored, using field stains (A & B) for thick film. However, the Partec cyflow was used for CD4 enumeration as described Cheesbrough, [12].

All laboratory protocols followed the manufacturer's instructions strictly. Study ethics guided the research and confidentiality was held very high as both informed/written consent was obtained. Appropriate unit heads gave approval before carrying out the study like the hospital and laboratory heads. Secondary data sources were referenced respectively.

Statistical Analysis

Statistical analysis was done using Graphpad software for frequency and percentages. ANOVA and

t-test compared mean differences and decision taking at 95% confidence level. The data obtained were presented in tables for clarity purposes respectively

Results

A total of 160 subjects (120 HIV infected subjects and 40 control subjects) were screened for HIV, malaria and CD4 counts was estimated for all subjects. For the HIV infected subjects, the highest prevalence of malaria infection was observed among ≤ 10 years age group (100%), while for the control subjects, a high prevalence of malaria infection was also observed among ≤ 10 years age group (Table 1).

The distribution of malaria infection among sex group revealed a high percentage of positive in male (77.78%) than in female 66.67% for the HIV positive subjects, while for the control subjects, a high rate of malaria infection was also found in male (57.89%) than in female (33.33%). See Table 2.

General distribution of malaria among HIV infected subjects showed that, 71.67% had malaria and HIV co-infection while 65% had only HIV infection (Table 3).

The study further compared mean distribution of CD4 Cell Count. The overall ANOVA Distribution of CD4 Cell Count among HIV/Malaria Infected and Non Infected Subjects; F=59.975, df=159, p=0.00. By implication, a significant mean difference exist in the CD4 Cell Count. See Table 4.

In addition, a t-test further demonstrated Comparison of Mean CD4 Cell Count among HIV and Malaria Infected and Non Infected Subjects. MP/HIV Co-Infection and Mono Infection with No Infection showed strong mean difference (p=0.00) in the various CD4 counts while HIV Mono-Infection and Others only had a non-significant (p=0.44) mean difference between HIV Mono-Infection and No HIV or Malaria Infection (Table 5).

Discussion

The distribution of malaria was higher in HIV infected patients (71.67%) than in HIV negative individuals (37.5%). This is supported by that of Patnalk *et al.* [6], who reported that there is high rate of malaria in HIV positive individuals than in negative individuals. The overall positive rate of malaria infection among HIV seropositive patients are higher (77.78%) in male than in female (66.67%) respectively. This finding disagrees with the studies of Warren *et al.* [9]. Furthermore, study also observed that females are at higher risk of malaria infection when compared to their male counterparts [9]. The high rate of malaria infection in male could be attributed to their social behaviour such as dressing code which in most cases involves short sleeves and singlet thereby exposing parts of their bodies to mosquito attack particularly in the tropics, been an endemic region. Male often stay out late during mosquitoes biting hours drinking in clubs and in an open bars that is probably not recently fumigated to keep mosquitoes and other dangerous insects away

On the other hand however, the peak of infectivity was observed among children within the age group of ≤ 10 years. This strongly agrees with Joklik *et al.* [10], who observed and reported in the United States of America that children suffer most from malaria infection. The susceptibility of children to malaria infection could be attributed to their low immunity, which in this context might be due to HIV infection, which has degenerative effects on the immune system. It can also be attributed to careless attitude of parents which may be the direct consequence of poverty or inadequate knowledge about the mode of transmission of malaria parasite [10]. Whitworth *et al.* [11] have similar view that children and pregnant women are more vulnerable. The health implication of malaria attack to children has been linked to anaemia, fatigue and loss of weight and more importantly death in severe cases [13, 14]

The mean CD4 count of HIV patients infected with malaria is significantly lower than HIV patients without malaria infection. This finding was in conformity with the work of Hoffman *et al.*, [7], which in a study in Malawi showed that there is decrease in CD4 count of HIV infected patients with malaria infection than in those without malaria infection. This may due to HIV-1 plasma viral load higher in HIV patients with malaria infection in HIV patients without malaria infection. Reason could also be that HIV infection with *P. falciparum* stimulates HIV-1 replication through the production of cytokines (interleukin 6- and tumor necrosis Factor-alpha) by activated lymphocytes, as reported in higher parasitaemia.

Table 1. Percentage Distribution of Malaria among different Age Group of Study Subjects.

HIV Infected (Test) Group					Control Group			
Age Group (Years)	Number Tested	Number Positive	Number Negative	Percent Positive	Number Tested	Number Positive	Number Negative	Percent Positive
≤10	2	2	0	100	13	11	2	84.61
11-20	3	2	1	66.67	1	0	1	0.00
21-30	53	36	17	67.92	10	3	7	30.00
31-40	34	23	11	66.65	8	2	6	25.00
41-50	15	13	2	56.67	3	1	2	33.33
>50	13	10	3	76.92	5	1	4	20.00
Total	120	86	34	71.67	40	18	22	45

Table 2. Showing the malaria distribution among male and female HIV positive and control subjects.

HIV Infected (Test) Group					Control Group			
Sex	Number Tested	Number Positive	Number Negative	Percent Positive	Number Tested	Number Positive	Number Negative	Percent Positive
Male	54	42	12	77.78	19	11	8	57.89
Female	66	44	22	66.67	21	7	14	33.33

Table 3. Malaria Distribution among HIV Positive Patients.

	No Positive HIV	Percent Positive
Malaria/HIV Co- infection	86	71.67
HIV without malaria infection	34	65%
Total	**120**	

Table 4. ANOVA Distribution of CD4 Cell Count among HIV and Malaria Infected And Non Infected Subjects.

Subjects	No. of patients	Mean ±SD (cells/mm^3)	F-value	Df	p-value
MP/HIV Co-Infection HIV	86	368.8±196.9			
HIV Mono-Infection	34	766.2±161.9	59.975	159	0.00
MP Mono-Infection	15	876.7±148.4			
No HIV or Malaria Infection	25	725.6±242.2			

Table 5. Comparison of Mean (t-test) CD4 Cell Count among HIV and Malaria Infected and Non Infected Subjects

Subjects	Number	Mean +SD (cells/mm^3)	t-test	df	p-value
MP/HIV Co-Infection and Mono Infection with No Infection					
MP/HIV Co-Infection	86	368.8±196.9	10.4472	118	0.00
HIV Mono-Infection	34	766.2±161.9			
MP/HIV Co-Infection HIV	86	368.8±196.9	9.5138	99	0.00
MP Mono-Infection	15	876.7±148.4			
MP/HIV Co-Infection HIV	86	368.8±196.9	7.5595	109	0.00
No HIV or Malaria Infection	25	725.6±242.2			
HIV Mono-Infection and Others					
HIV Mono-Infection	34	766.2±161.9	2.2563	47	0.03
MP Mono-Infection	15	876.7±148.4			
HIV Mono-Infection	34	766.2±161.9	0.7717	57	0.44
No HIV or Malaria Infection	25	725.6±242.2			
MP Mono-Infection	15	876.7±148.4	2.1770	38	0.04
No HIV or Malaria Infection	25	725.6±242.2			

P<0.05=Significant; p>0.05=Not Significant.

Surprisingly, HIV-related immune-suppression may increase the rate of malaria infection and clinical malaria disease, but does not increase the rate of severe or complicated malaria. The odds of parasitaemia and risk of *malaria* fever increase with decreasing CD4 count and increasing viral load. These findings suggest that HIV infection may interfere with parasite control, and cause the loss of antitoxic immunity, which protect subjects from parasitaemia or clinical disease [15]. In regions of unstable *malaria*, transmission is intermittent, less predictable, and epidemic outbreak may occur. The disease burdens are similar in all age groups because pre-existing anti- malarial immunity is limited. As a result of this, *malaria fever* rates are direct function of parasite transmission rates. Thus, HIV co-infection has its impact on disease presentation, with an increased risk of complication and severe malaria leading to death in most case.

Nevertheless, in this study, decrease CD4 cells count was found in some subjects without HIV or malaria infections. This agrees with Joe *et al*. [16] in one of his study, which observed that apart of HIV and malaria, other factors such as tuberculosis, viral infection, bacterial infection, stress and other factors which are yet to be identified could be present on the subjects without malaria or HIV infection which could be the cause of decrease in their CD4 count hence the cause of death in such a subject may be due to complications from the underlining pathological circumstances.

Conclusion

The risk of co-infection with malaria is high among HIV patient living in malaria endemic areas. Infection of HIV with malaria has been associated with low CD4 cell count. CD4 cells depletion could weaken the immune response paving to pathogen including opportunistic infections to be established. Also parasitaemia in the generality of patient could not be completely resolved after therapy leaving the patients with a possibility of recrudescence this is because Co-infection of HIV and malaria enhances the impact of each disease. HIV and malaria causes' maternal anemia, increase in the burden of maternal and infant mortality and increase risk of mother to child transmission of HIV. Finally, the study has shown the prevalence of malaria infection among HIV patients, which is about one third of the total population studied.

Recommendation

HIV infected patients should be encouraged to routinely check and treat malaria to avoid overburden of co-infection. CD4 monitoring is paramount. All protective measures against malaria should be practiced to help prevent and manage cases if noticed. The age group of 0-10years should be cared for since they are still dependents especially those infected with HIV/MP. Besides, Malaria control programme should be incorporated in HIV control scheme instituted in health institutions, as the need for enacting and implementing health policies aiming at subsidizing, and distributing effective anti-malaria drugs can never be over emphasized, otherwise the Roll Back malaria vision would only remain a mirage and it will die in a round table meeting discussion with a cap of coffee.

Acknowledgement

We wish to immensely thank all the subjects who willingly supported us to be enrolled in the study, we are also eternally grateful to laboratory technical staff who supported us through their technical setting up of all the procedures of wet laboratory protocols. Finally, we are also grateful to Prof. S.D Abbey, Prof G.N Wokem and Dr Azuonwu Goodluck for their moral support and continuous prayers.

Conflict of Interest

None reported among authors till date

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