Evaluation of CD4+ T Cells in HIV Patients Presenting with Malaria at the University of Ilorin Teaching Hospital Nigeria.

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CD4 count is an important immunological marker of disease progression in HIV seropositive patients. This study was carried out to determine the effect of malaria or fever of unknown origin on the population of CD4+ T lymphocytes of HIV seropositive patients attending the highly active antiretroviral therapy (HAART) clinic of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. 36 Subjects were selected for this study. Ongoing history of fever was used as a case definition for malaria and malaria was confirmed from microscopic examination of thick and thin film of blood sample obtained from the patients during presentation with fever. The CD4 count was evaluated during presentation of fever and post-fever using flow cytometry. There was significant decrease in CD4 count of the patients.

However, upon classifying the patients into 2 groups- those that returned to the clinic after a week and those that returned after a month; a significant increase in CD4 count was noticed in the group that returned after a week, while a significant decrease was noticed in the group that returned after a month (at p value of 95%). Further classification of the patients based on presence of malaria parasite, and body temperature resulted in varying effects on CD4 count post-fever (in the general group, 27 were positive for malaria parasites. Of these 27, there was an increase in CD4 count in 9 (33.3%). However in the group that returned after a week, all 6 (100%) that were positive for malaria parasites showed increase in CD4 count. Five (26.3%) of the 19 patients that had body temperature within the range of 35.5-37.4^{0}C showed an increase in CD4 count, while 7 (41.2%) the 17 patients that had body temperature of 37.5^{0}C and above showed an increase in CD4 count. The results led to the conclusion that while some components of the immune response to malaria could strengthen the immune system of HIV seropositive patients by increasing their CD4 count, other components will suppress their immunity by decreasing their CD4 count, accelerating the progression to AIDS.

Keywords: CD4 Count, HIV/AIDS, CD4+ T lymphocytes, Flow Cytometry, Malaria, Immune System

Human Immunodeficiency Virus (HIV) is the etiologic agent of the disease known as acquired immune deficiency syndrome (AIDS). It causes a progressive impairment of the body's cellular immune system by destroying CD4+ T cells leading to increased susceptibility to various infections. HIV/AIDS is a major

public health problem with socio-economic burden and a serious threat to development[1].

Nine out of 10 people living with HIV are in the developing world[2]. 60 to 70% of those are in Sub-Saharan Africa. But the disease is spreading in every region, with fierce epidemics threatening to tear through countries such as India, China, Russia and the islands of the Caribbean. As HIV spreads, it interacts with other infectious diseases, facilitated by the increase in numbers of immunosuppressed individuals and, because its own clinical course can be altered by other infections.

Malaria is by far the world's most important tropical parasitic disease, and kills more people than any other communicable disease except tuberculosis[3]. Malaria is a public health problem today in more than 90 countries, inhabited by a total of some 2400 million people - 40% of the world's population. Malaria is endemic in a total of 101 countries and territories, most of them in Africa.

In Africa, the HIV pandemic has been superimposed on the longstanding malaria infection which is endemic, where *P. falciparum* malaria is consistently one of the major causes of infant and child mortality. The high prevalence of both HIV and malaria infection in Africa means that even small interactions between the two could have substantial effects on populations[4]. In more recent years, one of the key issues focused on the relationship between malaria infection and HIV/AIDS.

The main question is whether malaria infection accelerates progression of HIV/AIDS or improves the immune level of the patient and is somehow protective. Many interesting studies have been conducted on the relationship between these two potentially fatal diseases, but a general consensus is yet to be reached on the effects, if any, of malaria parasitaemia on the underlying HIV infection in patients.

Studies conducted by[5] seemed to suggest that malarial infection leads to an increase in the HIV viral load, thereby potentially hastening the progression of HIV to full blown AIDS. The findings in that study indicated that this increased viral load was reversed by effective malaria treatment. Others have reported an increase in the CD4+ counts of HIV patients during the course of malarial infection, thereby improving the prognosis of the HIV infection[6]. The latter group has hinted at the possibility of repeatedly inducing malaria in HIV patients so as

to boost their immunity and prevent the progression to AIDS. This is referred to as malariotherapy, a treatment reminiscent of what was done for neurosyphilis in the 1920s by the Austrian doctor, Wagner Jauregg[6].

Plasmodium falciparum infection is endemic in most tropical countries and will definitely infect HIV patients living in this region at one time or the other during the course of their infection. Hence, it is important to determine the effect malaria parasites will have on the quality of life of HIV patients, especially since other intercurrent infections in HIV are being effectively controlled.

Methods

Study Area. The study was carried out at the University of Ilorin Teaching Hospital (UITH), a tertiary and referral hospital located in Ilorin, the Kwara state capital. It is a 486-bed hospital [7] involved in teaching, research and training of medical students and resident doctors. It is an affiliate of University of Ilorin through, the College of Health Sciences.

Subjects. Thirty six (36) HIV seropositive patients presenting with fever were recruited in this study following approval by the Ethics and research committee of the Hospital and due consent of the patients. Based on the sample size of this study, the subjects had to be grouped into two broad temperature categories, for proper statistical analysis. The temperature ranges were; 35.5^0C- 37.4^0C and 37.5^0C-39.8^0C.

Blood Collection. Aseptic procedures were adopted in the collection of the blood samples. 5ml of venous blood was obtained using sterile needle and syringe and dispensed into EDTA bottles[8].

CD4+ T cell Count. CD4 counts of the patients were evaluated during fever and post fever using a Partec Flow Cytometer. 20µl of whole blood was placed in a rohren tube, and 20µl of CD4+ T cell monoclonal antibodies was added. The tube was then incubated in the dark for 15 minutes at room temperature after which

800µl of buffer was added. The tube was then placed in the Partec flow cytometer. The value of CD4+ T cells per ml in the sample of blood was then obtained from a programmed computer connected to the instrument[8].

Preparation of Thin and Thick Blood Film. The preparation of both thick and thin blood films for confirmation of parasitaemia were carried out using the methods described by[9].

Microscopic Examination. A drop of immersion oil was placed on the stained thin film and another drop on thick film. They were examined systematically using x 100 objectives (oil immersion objective).Identification was done by the method described by[9].

Statistical Methods. The variation in CD4 count during and after episodes of malaria and fever are given as percentage proportion. Variations were categorized as increase and decrease. Differences between the categories were analyzed by T-test of equality in proportion.

In order to determine if any significant variation in CD4 count is due to the action of malaria parasite or fever, patients were examined for malaria parasite. The observations were analyzed for significance using T-Test of equality of proportion, a two-way analysis of variance (ANOVA) and Chi square.

Results. There was a decrease in the mean difference of the CD4 count of the patients. Of the total 36 samples evaluated, a decrease in the value of CD4+ T lymphocytes was observed in 24 (66.7%) samples, while an increase was noticed in the remaining 12 (33.3%) samples. However, when the subjects were divided into 2 groups based on the time of their return to the clinic (10 returned after a week while the remaining 26 returned after a month), the result varied. There was an increase in the CD4 count of the patients 1 week post fever presentation when compared with the count obtained at fever presentation. Of the 10 samples evaluated, an increase was noticed in 8 (80%), while a decrease in the value of

CD4+ T lymphocytes was observed in the 2 (20%) remaining samples. The CD4 counts of 26 patients were evaluated during fever, and 1 month post fever presentation. Those that returned after a month had a decrease in the mean values of the CD4 count. Of these 26 patients, 22 (84.6%) showed a decrease in CD4 count, while the remaining 4 (15.4%) showed an increase in CD4 count (Table 1).

There was also variation in CD4 count of the subjects in relation to presence of malaria parasite. Of the 36 patients involved in this study, 27 were positive for malaria parasites, while the remaining 9 were negative. Of the 27 samples that were positive for malaria parasite, there was an increase in CD4 count in 9 (33.3%), while there was a decrease in CD4 count in 18 (66.7%). Also, of the 9 samples that were negative for malaria parasite, 3 (33.3%) showed an increase in CD4 count, while 6 (66.7%) showed a decrease in CD4 count. Statistically, the difference in the blood malaria status, in patients that showed increase in CD4 count was significant (Table 2).

Table 1: Values of CD4+ T cells in HIV seropositive patients during fever and post fever presentation.

Health Status	Samples	Increase in CD 4 Count	Decrease in CD4 Count
General	36	12 (33.3%)	24 (66.7%)
1 Week post-Fever	10	8 (80%)	2 (20%)
1 month post- fever	26	22 (84.6%)	4 (15.6%)

Table 2: Influence of blood malaria status on variation in CD4 Counts in HIV patients after presentation with fever

Malaria Parasite	Increase in CD4 count (Mean Difference)	Decrease in CD4 count (Mean Difference)
Positive	9 (168.1)	18 (120.2)
Negative	3 (20.3)	6 (109.7)

When the variation in CD4 count in relation to malaria parasite in patients that reported back to the clinic after a week was checked, 6 of the 10 of them were positive for malaria parasite, while the remaining 4 were negative (Table 3). All 6 (100%) patients that were positive for malaria parasite showed an increase in CD4 count. Of the 4 that were negative for malaria parasite, 2 (50%) showed an increase in CD4 count, while the remaining 2 (50%) showed a decrease. Statistically, the difference in the blood malaria status, in patients that showed increase in CD4 counts was significant ($p< 0.05$).

Table 3: Influence of blood malaria status on variation in CD4 counts 1 week after presentation with fever.

Malaria Parasite	Increase in CD4 counts (Mean Difference)	Decrease in CD4 counts (Mean Difference)
Positive	6 (171)	0
Negative	2 (27)	2 (195.5)

Body temperature during fever did not have a significant influence on variation in CD4 count in the overall group (Table 4). It must be noted that for some of the subjects, their body temperature was below 37.5^{0}C, which is the body temperature indicative of fever. Nineteen patients had body temperature within the range of 35.5-37.4^{0}C, while 17 had body temperature of 37.5^{0}C and above. Of the 19 patients that had body temperature within the range of 35.5-37.4^{0}C, 5 (26.3%) showed an increase in CD4 count, while 14 (73.7%) showed a decrease. Of the 17 patients that had body temperature of 37.5^{0}C and above, 7 (41.2%) showed an increase in CD4 count while 10 (58.8%) showed a decrease. The difference was not statistically significant (p> 0.05).

Table 4: Influence of body temperature on variations in CD4 counts during fever, and after presentation with fever in HIV sero-positive patients.

Temperature (^{0}C)	Increase in CD4 count (Mean Difference)	Decrease in CD4 count (Mean Difference)
35.5-37.4	5 (109)	14 (126.4)
37.5-39.8^{0}C	7 (153.4)	10 (99.0)

Three patients had body temperature within the range of 35.5-37.4^{0}C, while 7 had body temperature of 37.5^{0}C and above. Of these total of 10 patients, 8 had increase in CD4+ T cells, while the remaining 2 had a decrease. Three out of the 8 that had an increase in CD count had body temperature within the range of 35.5-37.4^{0}C. The remaining 5 had body temperature of 37.5^{0}C and above. The difference was statistically significant (p<0.05) (Table 5). 16 patients had body temperature within the range of 35.5-37.4^{0}C, while 10 had body temperature of 37.5^{0}C and above. Of the 16 patients that had body temperature within the range of 35.5-37.4^{0}C, 2 showed an increase in CD4 count, while 14 showed a decrease. Of the 10 patients that had body temperature of 37.5^{0}C and above, 2 showed an increase in CD4 count while 8 showed a decrease. The mean differences was statistically significant (p< 0.05)(Table 6).

Table 5: Influence of body temperature on variations in CD4 counts during fever, and one week after presentation with fever in HIV sero-positive patients.

Temperature (^{0}C)	Increase in CD4 count (Mean Difference)	Decrease in CD4 count (Mean Difference)
35.5-37.4	3 (94.1)	0
37.5-39.8^{0}C	5 (160.2)	2 (195.5)

Table 6: Influence of body temperature on variations in CD4 counts during fever, and one month post fever presentation in HIV seropositive patients.

Temperature (^{0}C)	Increase in CD4 count (Mean Difference)	Decrease in CD4 count (Mean Difference)
35.5-37.4	2 (109)	14 (126.3)
37.5-39.8^{0}C	2 (136.5)	8 (82)

Discussion

This work has attempted to provide answers to some controversial issues of the consequence of malaria parasite infection in HIV seropositive individuals. It is not known whether malaria accelerates the progression of HIV to full-blown AIDS or ameliorates HIV infection by fever-inducing mechanism. To achieve this, the CD4+ T cell counts of the blood samples obtained from the 36 patients that participated in this study were evaluated during, and after episodes of malaria and fever of non-malarial origin. Ongoing history of fever was used as a clinical

definition of malaria and malaria was confirmed from microscopic examination of thin and thick film.

There were variations in the CD4 cell count, during and after fever, in all 36 patients, and the pattern of variation differed based on the different groups of the patients (Table 1). While some patients had increased CD4 counts post-fever, others showed a decrease. The variations in CD4 counts observed in this work are concomitant to similar studies that had been carried out previously confirming the fact that malaria does have an effect on the underlying infection in HIV seropositive patients. [6, 10, 11]all noted changes in the CD4 counts of HIV seropositive patients after episodes of malarial disease.

Generally, a decrease in CD4 count was observed in this study (Table 1). This seems to support the claims of[10, 12]. Hviid reported that malaria-specific T cell responses and even unrelated T cell responses are down regulated during the course of malaria infection[12]. The study carried out by[10] focused on the viral load of HIV patients that participated. They reported an increase in the viral load of the patients following episodes of malaria. Increase in viral load, if sufficient enough, will cause a decrease in CD4 count. This could also be responsible for the decrease in CD4 count noticed in thisstudy.

Some of the patients that participated in this study returned after a week while the rest returned after a month. A significant increase in CD4 count was noticed in the group that returned after a week. This is concomitant with[6], in which an increase in CD4 count in HIV patients infected with malaria parasites was reported. The presence of malaria parasite increases production of cytokines by the immune system in an effort to ward off the invading organism. The cytokines produced can also activate CD4+ T lymphocytes, strengthening the immune system[6]. This could account for the increase in CD4 count noticed in this group, and this observation seems to lend credence to Heimlich's argument that malaria can be used in boosting the immunity of HIV patients, a procedure termed "malariotherapy", improving their prognosis.

However, in the group that returned after a month, there was significant decrease in CD4 count post-fever, indicating possible acceleration of HIV infection towards AIDS. This result seems to confirm the observation reported by[5]. It was observed that an increase in HIV load could eventually lead to a

decrease in CD4 count. This could have been as a result of the action of pro - inflammatory cytokine such as TNF- . *P. falciparum* has been shown to stimulate HIV-1 replication through the production of cytokines such as TNF- and interleukin-6[13].

The results observed above (Table 1) suggest that the various components of the immune response mounted to challenge the presence of malaria parasites in the body will have varying effects on the T lymphocyte population of the patients that participated in this study. Cytokines have been reported to exert multiple actions that affect the activity of multiple cell types[14]. This is referred to as cytokine pleiotropy. TNF- and IL-10, which have been reported to be produced during malarial disease[15], have varying effects on different cell types, and at different times[16]. Differences in the quality of variation observed in these 2 groups suggest that the pleiotropic nature of some of the cytokines involved in the immune response of the body to malaria may have had varying effects on the population of CD4+ T cells.

Some patients that participated in this study had low level of parasitaemia as observed from the microscopic examination of their blood film (Table 4). TNF- has been reported to be involved in the cell mediated immune response against malaria, preventing parasitaemia by stimulating neutrophils and monocytes to phagocytosize parasites[17, 18]. This could account for the level of parasitaemia in patients in this group. There was significant decrease in CD4 count of these patients. [19] in a study into the effect of malaria on the viral load in HIV patients noticed a significantly higher HIV-proviral load in patients with malaria than those without, and this remained higher for at least 4 weeks after treatment. This increase in IHV load could lead to a decrease in CD4 count and may account for the decrease in CD4 count of patients in this group. This would suggest that malaria causes faster progression of HIV disease.

Another bone of contention in the overall debate as to the effect of malaria on HIV is whether any noticeable effect is due to the presence of malaria parasites or the fever induced during malarial disease. Comparison in CD4 count variation was made in subjects that were positive for malaria parasites and those that were negative (Tables 2 and 3). There was significant increase in CD4 count in those that had malaria parasites. [20] in a Study carried out in a children's hospital in

Kinshasa, Congo, followed 112 children with AIDS, 41 of whom also had malaria. They reported that none of the 41 children with malaria and AIDS died, all recovered from their malaria. However, of the remaining 71 children with AIDS without malaria, 25 (35%) died.

A similar result was observed in patients that returned after a week (Table 3). All of the patients in this group who were positive for malaria parasite showed an increase in CD4 counts. The continuous release of malarial pyrogenes that stimulate CD4+ T cells was lingering in those with malaria parasites such that there was still increase in CD4 count after a week as opposed to those who were malaria-parasite negative, who showed significant decrease in CD4 count within a week. The results observed in Tables 2 and 3 seem to suggest that malaria parasites play a key role in the stimulation of the immune system enough to produce increase in the population of CD4+ T lymphocytes which can be beneficial to HIV patients.

This study also attempted to investigate the effect of the quality of fever, as indicated by body temperature, on the CD4 cell count during malaria and fever of other origins. Based on the sample size examined in this study, the subjects had to be grouped into two broad temperature categories, for proper statistical analysis. The temperature ranges were; 35.5^0C- 37.4^0C and 37.5^0C-39.8^0C (Table 4). It must be noted that for some of the subjects, their body temperature was below 37.5^0C, which is the body temperature indicative of fever. It has been established that time affects the production of cytokines[21]. This is significant because the paroxysm associated with malaria is often experienced around evening time, while the temperature was taken in the morning (during clinic). This could account for the reason why some of the patients had body temperature less than that indicative of fever.

In the overall group, there was no significant variation in CD4 count of the subjects based on their body temperature during malarial disease (Table 4). This seems to suggest that the effect of malaria fever on the CD4 count of HIV positive patient does not depend on the quality of fever induced. If this is so, it reflects the results observed in some of the patients that underwent malariotherapy in the treatment of neurosyphilis. According to[6], some patients were cured even when the malaria did not produce high fevers.

However, when the effect of quality of fever on the CD4 count of the HIV patients that participated in this study were analyzed based on the time of their return to the clinic post fever, significant variations were noticed. In the group that returned after a week (Table 5), temperature had a significant effect as more of those that had body temperature equal to or greater than 37.5^0C had an increase in CD4 count, while for the group that returned after a month, a significantly higher number of them showed a decrease in CD4 count (Table 6). This seems to suggest that quality of fever may have a bearing on the effect of malaria fever on the CD4 count of HIV patients depending on the time interval between the peak of malarial disease and the remission of fever. This may be as a result of the diverse nature and effects exerted by the cytokines involved in the immune response to the presence of pathogens. The process of fever induction involves the activation of CD4+ T cells by pyrogens[22]. The activation of T-helper cells could have been strong enough to activate a high population of T cells. This activation will lead to an increase in CD4 count. The cytokines that normally induce fever were probably still activating CD4+ T cells in the group that returned after a week, as opposed to those that returned after a month, in which the effects of the fever-inducing cytokines have probably worn off. This could explain the significant increase in CD4 count observed in patients with body temperature indicative of fever in the patients that returned after a week, and significant decrease in those that returned after a month.

In conclusion, episodes of malaria fever in HIV patients elicit varying immune responses with attendant effect on the patients. It is therefore important to further investigate the various components of the immune system to malaria and the parts they play in the response to the presence of malaria parasites. Also, due to the fact that a significantly higher number of the patients eventually showed a decrease in CD4 count post-fever, it is imperative to continue the on-going efforts to protect HIV patients, as well as everybody from malaria attacks.

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Authors' contribution statements

All authors contributed extensively to the work presented in this paper.

O.O.A jointly conceived the study with T.O.A. T.O.A carried out the analysis while S.A.B and O.M.K gave technical support and advice. L.O.O assisted in the sampling of the patients. O.O.A, O.M.K, and T.O.A wrote and edited the manuscript. All authors discussed the results and implication and commented on the manuscript at all stages.

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