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Effect of Plasmodium Infections on CD4 Cells, Neutrophil and Lymphocytes

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Abstract

Knowledge of malaria-immunity is essential for understanding the pathology, treatment & vaccine production. So, changes in CD4 in malaria patients were studied at a teaching hospital in Enugu, Nigeria. 45 patients on doctor's provisional diagnosis of malaria were examined for *Plasmodium* infections and the degree of parasitaemia (0/+/ ++/ & +++). Positive/(+) and negative/(0) samples were thereafter counted for CD4 using Flow Cytometry. CD4 cell count of *Plasmodium*-negative specimens were within reference ranges (RR): (464-1308); mean value: 835. Fifteen (+) parasitaemia showed lower ranges of CD4 count (502-1282); mean: 678; with immune falls in one (427). 9/12 (++) parasitaemia showed crash in CD4 counts, range: 301- 415; mean: 399. Seven (+++) parasitaemia showed crashes in CD4 counts, range: 160-357; mean: 225. As parasitaemia rises, neutrophils, Total WBC and lymphocyte significantly rise in numeric. Malaria causes immunosuppression; unlike prevailing reports, *should* accelerate complications in immunodeficients-patients, Haemolytic-anaemia results only from chronic cases. CD4 counts should be a paradigm in malaria investigation.

Keywords: CD4 cells, *Plasmodium* spp, flow cytometry, malaria, immunity

1. Introduction

Malaria syndrome, or relatively, "Whiteman's grave of West Africa," has been one of man's toughest combat against diseases. There have been three main measures against it, which were measures directed against: 1) the parasite in man; 2) the vector and; 3) prevention of mosquitoes-man contact.

Among the most interesting were measures directed against the vector-mosquitoes; because it was believed that vector control is the primary tool to control vector-borne diseases (Wilson, et al., 2020); indeed, that was primarily the approach that United States of America used to eradicate malaria from North America to-date. The method against the vectors can target either the immature stages (by the use of predator species and chemical or biological larvicides or by the modification of the habitat), or the adult vectors (by the use of nets, topical repellents, and insecticides). (Anand, et al., 2021). Furthermore, novel vector control methods are under development such as the genetic manipulation of mosquitos (Hammond & Galizi, 2017), bacterial infection of vectors (e.g., Wolbachia) (Flores & O'Neill, 2018) and eave tubes with insecticide-laden electrostatic netting

(Knols et al., 2016). However, the development of novel control tools for measures directed against the parasites in man is still needed and is supreme, and it is not going to reside only in therapeutics; because the age long adage that prevention is better than cure is sacrosanct in disease management and control. That is where issue of an immunity factor cannot be overemphasized.

According to Wikipedia (1), the immune system is a network of biological systems that protects an organism from diseases; it detects and responds to a wide variety of pathogens, from viruses to parasitic worms, as well as cancer cells, and in fact, any objects at all, such as wood splinters, that distinguish them from the organism's own healthy tissue. Further, according to Medical News Today (2023) the immune system is the body's tool for preventing or limiting infection; its complex network of cells, organs, proteins, and tissues enable it to defend the body from bacteria, viruses, parasites, and more. Likewise, it is known that cell-mediated immunity or cellular immunity, the bedrock of this investigation, is an immune response that does not involve antibodies, rather, cell-mediated immunity is the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen (Wikipedia.com, 2023). According to Science Direct (2023), T cells are critical for immunity to malaria, not only because they function as helper cells for an antibody response, but also because they serve as effector cells; such cellular immunity is directly implicated in protection from sporozoites and plays an important role in protection from blood-stage parasites.

Yet, issue of non-immunity in malaria infection and disease has been a very long problem of morbidity, with very long-standing attempts to resolve with vaccine production. It is currently a very good news that a new effective malaria vaccine has been on clinical trial. But it will not be good to relax yet on that achievement because of its numerous past disappointments of later development of resistance to therapeutics.

Another routes to approach this are to understand the roles of immune cells during active malaria disease, which will as well be very essential in understanding the immune responses to the pathology. It could also aid efforts towards immunotherapy parri passu ventures on vaccinations against the syndrome. This is because, in the pathology of disease dynamism, the ability of a pathogen to establish infection, and later be able to cause diseases is an interplay of two major factors: the pathogen's virulence factor (on one hand) and the host's resistance measure (on the other hand). Disease only ensues if the former overwhelms the later, colonize, disseminate and damage tissues. Among virulence factors of pathogens, are their ability to impair the host's defence mechanisms, then invade, colonizes and disseminates. So it is essential to look at the population of the cells of the immune system in the course of the disease malady in order to verify their roles.

Besides, some reports indicated that malaria does not seem to affect the progression of HIV infection (Greensberg, *et al*., 1919; Kalyesubula, *et al.,* 1997). This was a surprise. The expectation was that addition of another debilitating and immunosuppressive disease (such as malaria), especially one that should aggravate the already state of immune depression (Amadi, 2008), to an already grave burden of HIV infection and consequent immunodepression, should create graver consequences.

Apparently, among P. *falciparum*'s virulent factors, could be its ability to also cause immunosuppression in the host, as can be discerned from Amadi (2008). This fact also needs exploration. There could be many ways of investigating this assertion; i.e., CD4 cell count in malaria pathology.

It was on these premises of this need for further explorations that the effects of Plasmodium infections on CD4 cells were initiated.

2. Aim and Objectives

Aim: The aim of this work is to determine the effect of Plasmodium spp infection on CD4 cells.

Specific Objectives: The Specific Objectives are:

1) To select patients on medical doctors' provisional diagnosis of malaria.
2) Collect their blood samples.
3) And analyze these blood samples for:
    i. CD4 cells counts, using Partec Flow Cytometry.

3. Materials and Methods

3.1 Collection of Blood Samples for Analysis

The patient's sleeve was raised above the left elbow, and a tourniquet tied to the upper arm. With the patient's fist clenched, the area where the needle will be inserted was swabbed with 70% methylated spirit soaked in a cotton wool, then the cover of the hypodermic needle was sterilely removed without touching the bare needle and its tip, then the now sterilely opened needle, in a slanting position, was gently inserted into one of the most prominently displayed veins in the arm. Then the syringe was gently drawn up to suck in the blood. After about10ml of blood sucked, the tourniquet was loosened, and the hypodermic needle gently withdrawn from the

patient's arm. The methylated spirit soaked in a cotton wool was also used to cover the point of insertion of the needle for few minutes to control bleeding. The blood sample were then evenly share into two different respective bottles for the CD4 cells counts and the full blood counts.

3.2 CD4 Cells Count

3.2.1 Principle of Flow Cytometry

The basic principle of flow cytometry is the passage of cells in a single file in front of a laser so they can be detected, counted and sorted. Cell components (such as CD_4^+ TEST cells) are labelled and then excited by the laser to emit light at varying wavelengths.

3.2.2 Procedure

The CD4 cells count was done with Partec Flow Cytometer Code No. CY-S-3022.

The instrument main power was switched on at the back of the instrument and then the green button was pushed on the left of the instrument.

3.2.3 Cleaning

Sample tube was first plugged with cleaning solution and inserted into the sample port, then the bottom of the flow cytometer was pressed to start the measurement; after the measurement had stopped automatically, another sample tube containing a decontamination solution was plugged into sample port. When the cleaning procedure had stopped automatically the process was repeated with 1.6ml of sheath fluid in order to remove residual cleaning solution.

3.2.4 Quality Control (Count Check Beads Green)

A sample tube with well mixed 850µl count check beads green was plugged into the sample port and the start button pressed to begin measurement. When the measurement and cleaning procedure have stopped automatically, the result is indicated at the result area on the screen. This was compared with the lot specific number and check if it was within the allowed 10% range.

3.2.5 Sample Preparation for Absolute CD4 Count (Wet)

20 µL of antibody m Ab PE was pippeted into Partec tube. 20 µL of whole blood was then added into the tube containing the antibody. This was mixed gently and incubated at 15 minutes in a dark field. Then 800µL of no lyse buffer solution was added and analyzed with the C.Y flow counter. For the analysis, the script for CD4 measurement was loaded. Then sample tube with the prepared blood sample was inserted into the machine. Before measurement, the gain value and gating for proper CD4 T-cell measurement is selected. Then the measurement started. After the measurement, the machine cleans automatically. The CD4 Count result is then displayed on the screen, read, printed out and recorded.

4. Results

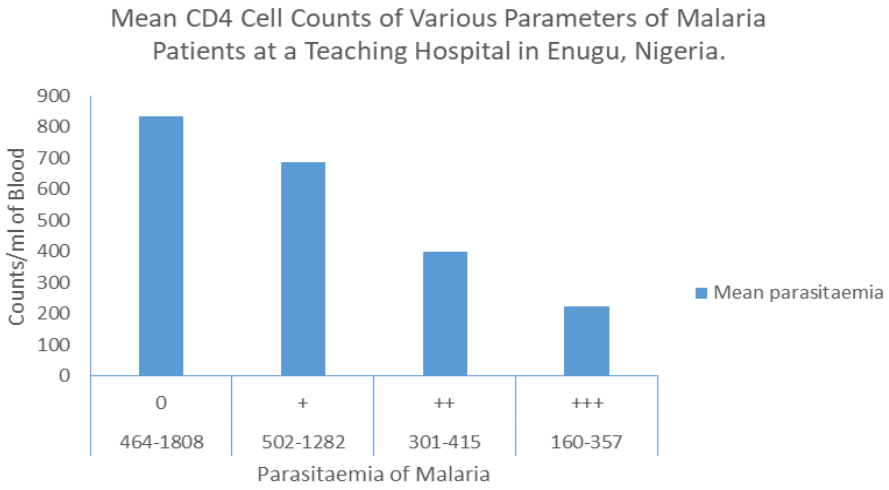

Figure 1. Histogram of the Mean CD4 cells counts at the various levels (0, +, ++, and +++) of *Plasmodium* parasitaemia in the patients at the teaching hospital in Enugu, Nigeria

Key: 0 = Mean zero *Plasmodium* parasitaemia; + = Mean plus one *Plasmodium* parasitaemia; ++ = Mean plus two *Plasmodium* parasitaemia; +++ = Mean plus three *Plasmodium* parasitaemia

Figure 1 showed that the *Plasmodium*-negative (0) specimens were within reference ranges for CD4 cell count (464-1308); mean value: 835. Fifteen (+) parasitaemia showed lower ranges of CD4 count (502-1282); mean: 678; with immune falls in one (427). Nine from twelve (++) parasitaemia showed crash in CD4 cells counts, range: 301-415; mean: 399. Seven (+++) parasitaemia showed crashes in CD4 cell counts, range: 160-357; mean: 225.

Table 1. Reference Ranges and the Mean Numeric Counts of the white blood cells, total (TWBC), Neutrophils and, Lymphocytes at various levels of *Plasmodium* parasitaemia (0, +, ++, and +++) in the blood samples of the patients at the teaching hospital in Enugu, Nigeria

Parameters ($x10^9$)	**TWBC ($x10^9$)**	**N**	**L**
Mean (0)	5.65	2.24	2.94
Mean (+)	6.10	2.52	3.09
Mean (++)	7.37	3.91	3.52
Mean (+++)	5.59	3.8	1.42
Ref. Ranges	***4 - 10***	***2.0 - 7.0***	***0.8 - 4.0***

Keys: N = Neutrophils; L = Lymphocytes; TWBC = White blood cells; 0 – +++ = Various plasmodium parasitaemia

As shown in Table 1, with increase in *Plasmodium* parasitaemia, there was significant steady rise in the numbers of Total White blood Cells, but which numerically remained within the reference ranges. There was also steady significant rise in the numbers of neutrophils with increase in *Plasmodium* parasitaemia, but which also remained within the reference ranges. Similarly, there was steady increase in lymphocytes number/numerics with corresponding increase in *Plasmodium* parasitaemia (Table 1).

5. Discussion

The result of this work showed that *Plasmodium* spp causes immunosuppression in patients (Figure 1). Thus one of its virulent factors is the ability to destroy the immune regulator of the host, thereby impairs the host's immune defense system, as seen from the steady decline in CD4 percentage and numeric counts (Figure 1) and decrease in lymphocyte percent, with resultant steady rise in plasmodia parasitaemia, as demonstrated by the results in the flow cytometry analysis. (Figure 1).

According to National Institute of Health.gov (2017), CD4+ T-, B-, and NK cells play an important role in the immune response induced by malaria parasite during the erythrocyte stage since immunity depends on memory B-cell production and lifespan, following infection. This explained that the recorded numeric rise in lymphocytes with rise in the parasitaemia, against depletion in the CD4+ cells, were from NK cells and B-cells.

Therefore, it confirms that plasmodia infection target only the CD4 cells; such that one of its apparent virulent factors is to destroy the immune regulator, hence suppress immunity of the host, because. lymphocyte decrease in percent (apparent shortage is from the CD4), but with steady increase in the numeric value. This thus confirmed (Immunopaedia.org, 2023) that: hosts can mount a level of protective immunity, which can occur following initial infection and render the host shielded against subsequent disease; further supported by the steady rise in neutrophils' percent and numeric values throughout the study. This is also in line with Stevenson and Riley (2004) description of innate immunity to malaria and some of the presumed mechanisms of adaptive immunity to malaria.

It doesn't appear that any of our patients has either the genetic trait of resistance to malaria or the natural acquired immunity because they were all from medical doctor's provisional diagnosis of active malaria, not expected if they were immune or are with asymptomatic infection. Asymptomatic infection is the predominant presentation seen in patients with malaria immunity and is believed to confer some form of immunity through premunition (Cohen, et al, 1961). (It is important to state categorically that this immunity is non-sterilizing and can be lost if there is no exposure to infection for 3–5 years) On the other hand, naturally acquired immunity to malaria is achieved with ongoing exposure to infections and subsequent acquisition of anti-malarial antibodies. Antibodies against merozoite antigens and VSA are thought to play key roles in conferring immunity against malaria (Web 1. How long does malaria immunity last?) Genetic immunity to malaria is inborne acquisition of certain genetic traits that alter the shape of red blood cells (RBCs), which include sickle cell trait, hemoglobin AC, thalassemia and hereditary ovalocytosis gene; the shape changes interfere with the ability of *Plasmodium* to invade these RBCs and offer some protection against malaria infection. (Genetic immunity to malaria, 2023) Sickle cell trait (genotype HbAS) confers a high degree of resistance to severe and complicated malaria yet the

precise mechanism remains unknown to-date. Sickle hemoglobin (HbS) and hemoglobin C (HbC) are both caused by point mutations in the beta globin gene, and both offer substantial malaria protection. Corollary, individuals with blood group "A" have been found to be highly susceptible to falciparum malaria whereas blood group "O" is said to confer protection against complicated cases and creates low parasitaemia. Another first line of defense against malaria is exerted by glucose-6-phosphate dehydrogenase deficiency gene.

Lastly, consequently, if immunotherapy is to be approached in malariology, it should be geared towards therapeutics that will improve CD4 count unfailingly, aid neutrophilia, improve appropriate lymphocytosis, encourage inflammatory response and not to forget the role of antibody as had been recorded in literature. That way, even if there is resistance to a drug, an efficiently assisted immune counter-reaction will provide an immunotherapy.

6. Conclusions

In conclusion:

1) *Plasmodium* infection causes immunosuppression in patients.
2) Thus, one of the virulent factors of *Plasmodium* spp is the ability to impair host's immune defense, as seen from the steady decline in CD4 count.
3) CD4 cell count should be a paradigm in the investigation of malaria diseases; it will corroborate the degree of parasitaemia, and guide the physician's therapeutics and/or radical cure.
4) Malaria infection in the immunodeficients patients *should* accelerate the complications, including death, unlike prevailing reports.
5) It is high time immunotherapy should also be properly studied and considered in malaria therapy.

As speculated by Feachem et al. (2019), Malaria eradication within a generation: is ambitious, achievable, and necessary.

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