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Haematological studies in malaria affected patients in North Chennai, Tamil Nadu

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ABSTRACT

Malaria is a potentially fatal blood disease caused by a parasite that is transmitted to human and animal hosts by the Anopheles mosquito. The human parasite, Plasmodium falciparum, is dangerous not only because it digests the red blood cell's haemoglobin, but also become it changes the adhesive properties of the cell it inhibits. This change in turn causes the cell to stick to the walls of blood vessels. It becomes especially dangerous when the infected blood cells stick to the capillaries in the brain obstructing blood flow, condition called cerebral malaria. A study was undertaken to compare certain serological parameters viz. Hb content, total blood cell count, WBC differential count, platelet count, E.S.R, P.C.V, M.C.V and M.C.H.C between normal persons and malaria affected patients coming for check-up in Primary Health Centres in North Chennai to find out any specific variations.

Key words: Malaria, Anemia, Parasitemia, Leukopenia

INTRODUCTION

Malaria has been known since time immemorial, but it was centuries before the true causes were understood. Previously, it was thought that "miasma" (bad air or gas from swampa-"mal air ia") caused the disease. Surprisingly in view of this, some ancient treatments were remarkably effective. An infusion of qinghao (*Artemesia annua*) has been used for at least the last 2000 years in China, its active ingredient (artemisinin) having only recently been scientifically identified. The antifebrile properties of the bitter bark of (*Cinchona ledgeriana*) were known in Peru before the 15th century. Quinine, the active ingredient of this potion was first isolated in 1820 by the pharmacists.

The vector efficiency of a mosquito depends on a number of factors, some related to the species of Anopheles and of the malaria parasite, others to environmental conditions. One commonly refers to the main or principal vector and to the secondary vector or vectors.

The percentage of Anopheles caught in nature showing either sporozoites in the glands or oocysts in the stomach is known as the anopheles infection rate or rate of natural infection (but not all sporozoite or oocyst infections are of human origin). The percentage of experimentally infected anopheles showing either sporozoites in the glands of oocysts in the stomach is the rate of experimental infection.

A crude acetone/water (50/50) extract of neem leaves (IRAB) was evaluated by [1] for activity against the asexual (trophozoites/schizonts) and the sexual (gametocytes) forms of the malarial parasite, *Plasmodium falciparum, in*

vitro. This extract, if found safe, may provide materials for development of new antimalarial drugs that may be useful both in treatment of malaria as well as the control of its transmission through gametocytes.

Epidemiological data point to an increased risk of HIV-1 mother-to-child transmission in pregnant women with malaria, by unknown mechanisms. The surface binding of a recombinant *Plasmodium falciparum* adhesion to chondroitin sulphate, A proteoglycans increases HIV-1 replication in the human placental cell line BeWo, probably by a *P. falciparum* adhesion-induced long-terminal repeat-driven TNF-alpha stimulation. This suggested that placental malaria could increase the risk of HIV-1 transmission in utero [2].

The blood concentration profiles of most antimalarial drugs vary considerably between patients. The interpretation of antimalarial drug trials evaluating efficacy and effectiveness would be improved considerably if the exposure of the infecting parasite population to the antimalarial drug treatment could be measured. Artemisinin combination treatments are now recommended as first-line drugs for the treatment of falciparum malaria [3].

Malaria is a significant health problem causing morbidity and mortality worldwide. Vaccine development has been an imperative for decades. However, the intricacy of the parasite's lifecycle coupled with the lack of evidence for robust infection-induced immunity has made vaccine development exceptionally difficult. [4] reviewed some of the key advances in the field and discuss potential ways forward for a whole-organism vaccine. The early cloning of malaria antigens has fuelled rapid development of subunit vaccines. [5] described reproductive health issues among pregnant women in a rural area of Kenya with a high coverage of insecticide treated nets (ITNs) and high prevalence of HIV (15%). In blood estimates, 36% and 53% of women had malaria paraitemia and anaemia respectively.

[6] reported a case of *Plasmodium vivax* infection manifested as severe thrombocytopenia, bilateral hydronephrosis and hypotension in a returning traveler from a malaria-endemic area in Venezuela. While most of the efforts to prevent malaria in travelers focus on the life-threatening consequences of *Plasmodium falciparum* malaria, nonimmune travelers who encounter infection with *P. vivax* may also develop serious complications. This case highlights the importance of preventing malaria cases among nonimmune or semi-immune individuals traveling to *P. vivax*-endemic areas.

In spite of arid conditions prevailing in desert part of Rajasthan, malaria is a major public health problem. A longitudinal study on social determinants of malaria has been undertaken in different villages of Ramgarh PHC of Jaisalmer district, Rajasthan [7]. The study aimed to know treatment seeking behavior of malaria patients in the desert communities which is significantly different than the non-desert part of India.

Malaria and malnutrition coexist within the poorest regions of the world. In the regions of Columbia where malaria is endemic, malnutrition is also a public health problem. The prevalence of malnutrition among malarial patients was higher in males and in children [8].

Since the outbreak of malaria was in Thiruvotriyur, North Chennai, the present study was undertaken.

MATERIALS AND METHODS

Seropositive malaria cases (25-40 years of age) coming for a check-up in Primary Health Centres in and around North Chennai were taken for study. 20 patients (10 males and 10 females) were analyzed for the clinical laboratory profile. Blood samples were collected after following aseptic procedures and the laboratory investigations carried out included the haemoglonin level, total erythrocyte count, total and differential leucocyte counts, erythrocyte sedimentation rate (E.S.R), platelet count, packed cell (P.C.V), mean corpuscular volume (M.C.V), mean corpuscular haemoglobin (M.C.H) and mean corpuscular haemoglobin concentration (M.C.H.C) [9]. The control values were taken into account after the patients become normal. The results obtained were subjected to statistical analysis.

RESULTS AND DISCUSSION

The vector for malarial parasite is female *Anopheles stephensi* (Fig: 1); the gametes of malarial parasites observed are *Plasmodium vivax* (Fig: 2); the gametes of *Plasmodium falciparum* (Fig: 3); ring stage of *Plasmodium falciparum* (Fig; 4) and the transmission cycle of malaria (Fig: 5).

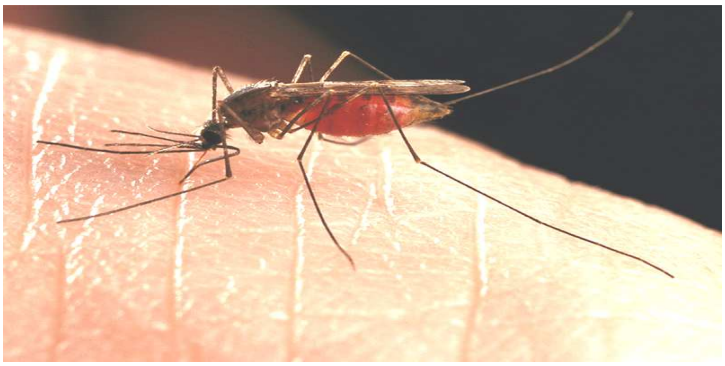

Fig: 1 *Anopheles stephenci* (female)

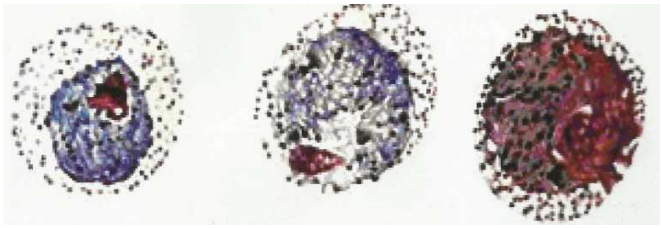

Fig: 2 Gametes of *Plasmodium vivax*

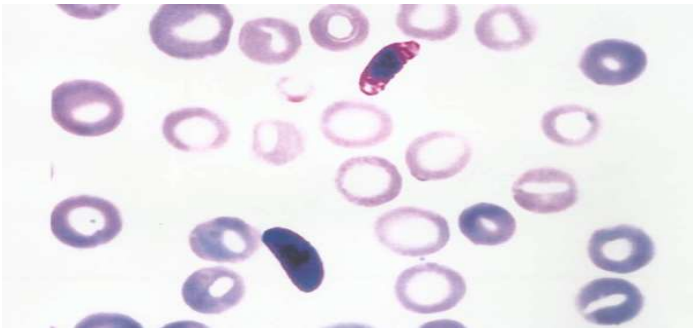

Fig: 3 Gametes of Plasmodium falciparum

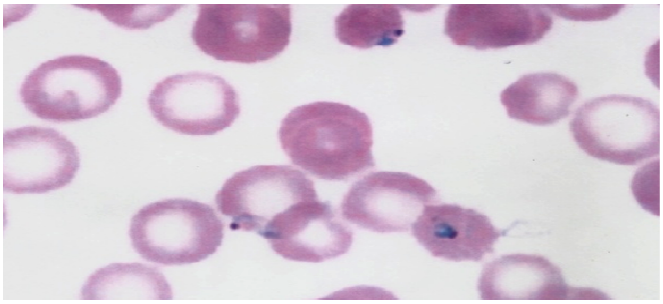

Fig: 4 Ring stage of Plasmodium falciparum

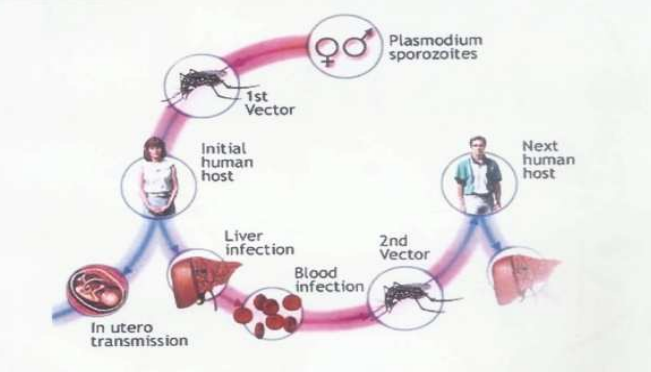

Fig: 5 Transmission cycle of malaria

The haematological parameters viz; haemoglonin level, total erythrocyte count, total and differential leucocyte counts, erythrocyte sedimentation rate (E.S.R), platelet count, packed cell (P.C.V), mean corpuscular volume (M.C.V), mean corpuscular haemoglobin (M.C.H) and mean corpuscular haemoglobin concentration (M.C.H.C) were studied in 20 patients; 10 males (Table: 1) and 10 females (Table: 2) affected by malaria.

Table: 1 Haematological parameters in malaria affected male patients

EP	Hb mg/ml	RBC-TC mi/cu.mm	WBC-TC /cu.mm	WBC-DC-N %	WBC-DC-L %	WBC-DC-E %	WBC-DC-B %	WBC-DC-M %	PC L/cu.mm	ESR mm/hr	PVC %	MCV cu.mic	MCH pg	MCHC %
C	15.25	5.3	7500	53	40	4	1	2	2.75	10	46	86	29	34
T1	15	4.84	8100	57	38	5	0	0	0.76	46	38.8	89	28	33.8
T2	12.7	4.41	7800	56	39	5	0	0	1.61	44	33.8	87	28.9	32.6
T3	12	4	7650	55	36	9	0	0	1.03	39	36	90	29	33.3
T4	12.6	4.37	8500	66	27	6	0	1	0.43	42	38	87	28.5	33.2
T5	13.6	4.82	14880	73	21	5	0	1	1.8	48	42.8	88.8	28.2	31.8
T6	12.3	4.1	10700	56	38	6	0	0	2.19	44	37	90	27	33
T7	12.4	4.5	10600	57	38	5	0	0	1.52	44	37.9	90.2	28	33.3
T8	13.5	4.52	14580	72	22	5	0	1	1.85	41	42.6	87.2	28.5	31.9
T9	13.2	3.91	9980	73	20	6	0	1	1.92	38	43	88	24	30
T10	13.2	4.15	13670	73	21	5	0	1	2.01	42	41.8	89	28	28.5
SD	0.8669	0.3220	2811.87	8.28	8.45	1.25	0.00	0.52	0.58	3.04	3.21	4.53	2.04	3.10
SE	0.2743	0.1019	889.83	2.62	2.67	0.39	0.00	0.16	0.18	0.96	1.01	1.43	0.64	0.98

EP – Experimental parameters; Hb – Haemoglobin; RBC-TC – Red Blood Corpuscles-Total Count; WBC-TC – White Blood Corpuscles-Total Count; WBC-DC – White Blood Corpuscles-Differential Count; N – Neutrophil; L – Lymphocyte; E – Eosinophil; B – Basophil; M – Monocyte; PC – Platelet Count; ESR – Erythrocyte Sedimentation Rate; PCV – Packed Cell Volume; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration; SD – Standard Deviation; SE – Standard Error

Malaria is a potentially fatal blood disease caused by a parasite that is transmitted to human and animal hosts by the Anopheles mosquito. The human parasite, *Plasmodium falciparum*, is dangerous not only because it digests the red blood cell's haemoglobin, but also become it changes the adhesive properties of the cell it inhibits. This change in turn causes the cell to stick to the walls of blood vessels. It becomes especially dangerous when the infected blood cells stick to the capillaries in the brain obstructing blood flow, condition called cerebral malaria. Scientists using the x-ray microscope are hoping to learn more about how the parasite infects and disrupts the blood cells and the blood vessels of an infected host. The life cycle of the malaria parasite in a human or animal begins when an infected mosquito transmits malaria sporozoites to a new host. The sporozoites travel to the liver, where they invade hepatocytes (liver cells) and multiply thousands of times over the following two weeks before rupturing out of the liver into the blood stream. During the first 48 hours after infecting a red blood cell, a parasite goes through several phases of development. The first phase is the ring stage, in which the parasite begins to metabolize haemoglobin. The next phase is the trophozoite stage, during which the parasite metabolizes most of the heamoglobin, gets larger, and prepares to reproduce more parasites. Finally, the parasite divides asexually to form a multinucleated schizont. At the end of the cycle, the red blood cell burst open and the parasites are dispersed to infect more red blood cells.

Table: 2 Haematological parameters in malaria affected female patients

EP	Hb mg/ml	RBC-TC mi/cu.mm	WBC-TC /cu.mm	WBC-DC-N %	WBC-DC-L %	WBC-DC-E %	WBC-DC-B %	WBC-DC-M %	PC L/cu.mm	ESR mm/hr	PVC %	MCV cu.mic	MCH pg	MCHC %
C	13.75	4.8	7500	53	40	4	1	2	3.4	12.5	42	86	29	34
T1	5.7	2.66	13400	75	20	5	0	0	0.12	16	18.4	89	21.5	31.2
T2	4.6	1.52	11000	57	38	5	0	0	0.12	16	12.2	87	28	33.1
T3	9.3	3.86	9400	58	36	5	0	1	1.91	37	34.1	88	24.1	27.2
T4	11	3.6	11900	77	17	6	0	0	1.25	37	33	90.1	28.5	33.3
T5	12	4.32	11850	54	39	6	0	1	1.6	42	38.4	91	27.5	33.9
T6	12.3	4.28	12560	56	37	6	0	1	1.48	42	35.7	90.6	29	33.2
T7	12.5	3.8	8900	54	38	7	0	1	1.4	35	38.9	90.7	28.5	33
T8	10.9	3.9	8090	76	18	5	0	1	1.12	39	34.2	87.9	23.6	29
T9	9.5	3.85	9010	55	38	6	0	1	0.89	37	32	87	23.3	30.2
T10	11.7	3.2	10180	73	21	5	0	1	1.1	36	35	87	23	31.3
SD	2.7609	0.8504	1777.02	10.23	9.87	0.69	0.00	0.48	0.49	7.94	8.76	6.82	3.64	3.01
SE	0.8737	0.2691	562.34	3.23	3.07	0.22	0.00	0.15	0.15	2.51	2.77	2.16	1.15	0.95

EP – Experimental parameters; Hb – Haemoglobin; RBC-TC – Red Blood Corpuscles-Total Count; WBC-TC – White Blood Corpuscles-Total Count; WBC-DC – White Blood Corpuscles-Differential Count; N – Neutrophil; L – Lymphocyte; E – Eosinophil; B – Basophil; M – Monocyte; PC – Platelet Count; ESR – Erythrocyte Sedimentation Rate; PCV – Packed Cell Volume; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration; SD – Standard Deviation; SE – Standard Error

The interval between the date of the infection and the time when the malaria parasites are detectable in the peripheral blood is known as the pre-patent period. This should be distinguished from the incubation period which is related to the first appearance of clinical symptoms of the disease.

The merozoites relased from the tissue schizone invade the enythrocytes. This process has now been observed and analyzed with the help of the electron microscope. Merozoites contain at their apex certain organelles (rhoptries and micronemes) which, after having come into contact with the erythrocyte, cause an invagination of its surface; that the resulting dimple deepens gradually, so the merozoite slips into the erythrocyte. During this process merozoite loses two of its three membranes and, on entering the erythrocyte, assumes a rounded form. Antibodies present in the blood of partially immune patients may cause mature merozoites to cluster and this prevents invasion of fresh erythrocytes. The merozoite enters the cell by five stages: initial recognition and attachment, formation of a junction, creation of a vacuole membrance continuous with the red cell membrane, entry into the vacuole through the moving junction and sealing of the erythrocyte after entry.

The youngest stages in the red blood cell are small, more or less rounded bodies, some of which contain a vacuole which displaces the cytoplasm to the periphery, while the nucleus is situated at the pole. In option section the cytoplasm has an annual appearance and the young parasites are known as Ring forms. As these grow in size they become more irregular in shape. All these early stages of the parasite are termed trophozoites. In the course of their development they absorb the haemoglobin of the red blood cell, leaving as the product of digestion a pigment called haemozoin, a combination of haematin with protein. The enzyme involved is probable cysteine proteinase. This iron containing pigment is seen in the body of the parasite in the form of dark granules which are more obvious in the later stages of development.

The early studies confirmed that the erythrocytic parasites are intracellular, within vacuoles formed by the erythrocytic internal membrane; they feed by pinching off small amounts of the cytoplasm of the hose, through a mouth-like structure (cytostome). The ring appearance of young trophozoites is due to distribution of the cytoplasm and the nucleus in the form of a concave disk. As it grown the trophozoite becomes globular and is filled with ribosomes, while the pigment from the ingested haemoglobin accumulates in the form of granules. The trophozoites grow through the proliferation of endoplasmic retirulum; the vacuole's membrane increases in area and small particles of it's accumulate on the inner membrane of the infected erythrocyte, producing the characteristic stippling seen in stained films. In *P. falciparum* the vacuole membrance forms longer loops touching the cytoplasm of the host cell and this may explain the aspect of 'Maurer's dots' seen in stained blood films. The effect of chloroquine is to disrupt the action of the food vacuole and to a lesser extent, the mitochondria and other organelles. Much research has been carried out in the case of calcium channel blockers (such as verapamil and its analogue desipramine) which appear to inhibit the resistant strains of *P. falciparum* from preventing this disruption and thus, potentially, revert chloroquine to its potency.

After a period of growth the trophozoite undergoes an asexual diving process of erythrocytic schizogony. The nucleus of the parasite divides 3-5 times into a variable number of small nuclei. This is soon followed by the division of cytoplasm forming a schizont. Mature schizonts are fully developed forms in which, as a result of the segmentation of the nucleus and the cytoplasm a number of small rounded forms (merozoites) are produced. When the process of schizogony is completed the red blood cell bursts and the merozoites are released into the blood stream. The merozoites then invade fresh erythrocytes in which another generation of parasites is produced by the same process. This erythrocytic cycle of schizogony is repeated over and over again in the course of infection, leading to a progressive increase of parasitaemia under the process is slowed down by the immune response of the host.

The development of parasites in the red blood cell brings about certain changes of it; among these abnormal appearances the most important are enlargement, decolorization and certain forms of stippling, now though to be associated with the transport of malaria protein through the membranes to the surface of the erythrocyte. These charges are characteristic for the particular species of Plasmodium involved.

The length of the erythocytic phase is known as schizogonic periodicity. It differs according to the species of the parasite, being 48 hours in vivax, ovale and falciparum malaria and 72 in quartan. In the early stage of infection there may be groups ('broods') of parasites developing at different times so that the febrile symptoms show no characteristic periodicity. Later, the schizogonic periodicity is better synchronized and the febrile paroxysms assume a more definite 3 or 4 day pattern.

While merozoites originating from pre-erythrocytic schizogony may also give rise to sexually differentiated forms (gametocytes), it is usually only after several rounds of blood schizogony that these forms are produced in greater numbers. After invading fresh erythrocytes these sexual forms grow, but the nucleus remains undivided. The mature gametocytes have different *P. falciparum* they are usually crescent-shaped when mature, while in other species they are round. In all species of Plasmodia the female (macrogametocyte) has a deeply stained cytoplasm and a small compact nucleus while the male (macrogametocyte) stains pale blue or pink and has a larger, diffuse nucleus. Both contain numerous pigment granules.

In synchronous infection of some species of Plasmodia gametocytes mature at night and it has been suggested that this represents an adaptation of the parasite to the nocturnal feeding habits of Anopheles mosquitoes.

Anaemia is an inevitable consequence of erythrocyte parasitization as all infected cells are destroyed at schizogony. The survival of non-parasatized erythrocytes was found to be reduced for several weeks after clearance of parasitaemia in patients with falciparum and vivax malarias. In Gambian children, initial studies showed a correlation between severe anaemia and a positive direct antiglobulin test, implying immune haemolysis. Later studies failed to confirm this finding. In Thailand adults with falciparum malaria, there was no increase in IgG coating of erythrocytes and no correlation between the number of IgG molecules/cell and the severity of anaemia. However, IgG-coated cells appeared to be cleared more rapidly by the spleen for a period of several weeks after the acute infection. Iron sequestration, erythrophagocytosis and dyserythropoiesis were found in the acute phase of falciparum malaria. Maturation defects were present in the marrow for at least 3 week after clearance of parasitaemia. Survival of radio-isotope-labelled compatible donor erythrocytes was significantly shorter than that of the patient's own (autologous) erythrocytes which were presumably survivors or the enhanced splenic clearance of ageing or subtly altered cells. Patients with splenomegaly showed markedly accelerated clearance of labeled heat-damaged erythrocytes and a lower mean haematocrit than those without splenomegaly. In many parts of the tropics, repeated attacks of malaria eventually lead to profound anaemia, especially if there is a background of chronic blood loss from hookwork infection, mal-nutrition, pregnancy and persisting, relapsing or recrudescent parasitaemia.

Thrombocytopenia is common with falciparum and vivax malarias. Its degree, if not its presence, has some prognostic significance. Platelet survival is reduced to 2-4 days in sever falciparum malaria. Platelet associated IgG and IgG-coating of platelets has not been a consistent finding. Increased numbers of large abnormal-looking megakarocytes have been found in the marrow and the circulating platelets may also be enlarged suggesting dyspoietic thrombopoiesis. Enhanced splenic uptake or sequestration may contribute to thrombocytopenia and in patients with disseminated intravascular coagulation (DIC), platelets may be removed from the circulation at sites of fibrin deposition. Surprisingly, platelets are rarely found in cerebral blood vessels in patients dying with cerebral malaria.

Mild leucopenia has been described in uncomplicated malarias, but a neutrophil leucocytosis is an important abnormality in patients with severe falciparum malaria and is associated with a bad prognosis. Tumour necrosis factor may be responsible for this leucocytosis which may be associated with a complicating Gram-negative rod or other bacteraemia.

Haematological changes in malaria infected blood stored in blood bank refrigerator have been studied [10]. Platelet counts and serum potassium levels in malaria infected blood were significantly different from that of non-infected blood; while the PCV was significantly different. This study confirms with the results of the present investigation. Parasitemia and hematological alterations in malaria, a study from the highly affected zones; the infected patients tended to have significantly lower platelets, haemoglobin, and red blood cell counts [11]. These results also correlated with the present study. Reduction in the counts of lymphocytes and reduced packed cell volume were observed in the studies made by [12]. Children infected with Plasmodium falciparum malaria exhibited important changes in some haematological parameters with low platelet count and haemoglobin concentration being the two most important predictors of malaria infection in children in Western Kenya [13]. Some biochemical and haematological studies on the methanolic extract of *Anthocleista grandiflora* stem bark were carried out with the aim of ascertaining the significance of interaction in the treatment of malaria infected mice induced with *Plasmodium berghei*. The infected extract treated animals had significant difference in PCV, Hb, WBC and platelet count [14]. Studies were undertaken by [15] on the clinical and hematological pattern in patients with *Plasmodium vivax* infection and anemia and thrombocytopenia were more frequently observed.

The present study showed an increase in the total count of white blood corpuscles and this might be due the geographical area and the nutritional factors by which the infected patients develop their immunity towards the infection with high production of white blood cells. To conclude, the present study indicates alterations in blood parameters and therefore recommends proper monitoring during treatment in order to reverse them to normal levels.

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