

MALARIA: HEMATOLOGICAL ASPECTS

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In spite of intensive worldwide efforts to reduce its transmission, malaria remains the most serious and widespread protozoal infection of humans. Over 40% of the world's population are at risk of contracting malaria, which is endemic in 91 countries, mostly developing.¹ Malaria has long featured prominently in the grey area between parasitology and hematology. In a classical European textbook of hematology published in the 1930s, malaria was defined as a "typical blood disease" characterized by fever, anemia and splenomegaly.² It is currently considered a typical example of a hemolytic anemia in more recent hematology textbooks, due to an acquired extra-corporeal cause. As parasites of the blood for the majority of their complex life cycle, they expectedly induce hematological alterations. The hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, and mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (DIC).^{3,4} There have also been reports of leucopenia and leucocytosis.³⁻⁵ Other hematological reactions to malaria that have been reported, include neutropenia, eosinophilia, neutrophilia and monocytosis.^{6,7} Some controversies appear to exist however. Many of the studies on the hematological abnormalities have been conducted in endemic countries, some only in children and some only in severe malaria patients.^{6,8-10} Relatively few studies have been done among non-immune or semi-immune travelers returning from endemic areas or patients returning from their endemic countries.^{3,11,12} The aim of this study was to investigate and find the hematological changes that may occur in acute malaria infection in non-immune and semi-immune patients returning from endemic areas.

Materials and Methods

The study design utilized in this review was a retrospective clinical case series study. All confirmed

cases of malaria who presented to King Fahd Hospital of the University (KFHU) at Al-Khobar in the Eastern Province of Saudi Arabia, between January 1988 and December 1999, were included in the study. A case was defined as a positive thin and/or thick blood film recorded in the Hematology Department laboratory records.

A specially designed form of three sections was used for the purpose of these studies. Section A (basic demographic data): included variables as age, sex, residence, education, occupation, and travel history to an endemic area. Section B (clinical data): inquired about fever pattern, presence of jaundice, organomegaly and lymphadenopathy. Section C (hematological data): recorded information about blood counts, complete blood counts (CBC), plasmodium species and the parasite level in some cases. CBC were performed using an automated Coulter counter STKS model and WBC differential was done on almost all the cases where CBC was done. All malaria-positive smears were reviewed by a hematologist for confirmation, identification of species, review of smear for platelet count, and in some cases, estimation of parasite level. Due to the different nationalities encountered, normal values (reference ranges) for the hematological findings were based on recommendations by Dacie and Lewis.¹³

The forms were completed by abstracting the relevant information from the medical records of the malaria cases diagnosed at KFHU during the specified period. Data was entered and analyzed using SPSS version 7.0.¹⁴ Statistical analyses included descriptive statistics, bivariate analysis i.e., *t*-test, chi-square and analysis of variance (ANOVA). Level of significance was set at <0.05.

Results

A total of 727 patients fulfilled the inclusion criteria for the diagnosis of malaria. The mean age was 25.43±14.34 years, with a wide range (2 months to 74 years). There was a male predominance, with a male to female ratio of 3.15:1. Saudi patients comprised 305 (42%) while the remaining 422 (58%) were non-Saudis. The non-Saudi patients were of different nationalities (Indian, Pakistani, Sudanese, Sri Lankan, Yemenis, and few cases who were Indonesian, Nigerian, Turkish, and Ghanaian). The majority of cases (75%) had a positive travel history to an endemic area. The most common type of malaria was *P. falciparum* (54.2%),

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followed by *P. vivax* (39%), then (mixed) infections in 17 (2.4%), while in 32 (4.4%) of the cases, the species could not be identified.

Hematological Results

A total of 430 patients (59.2%) were anemic at presentation, and the anemia was normochromic normocytic except in 129 cases (17.7%), where it was microcytic hypochromic. In these latter cases, 90 were found with iron deficiency anemia and 39 with thalassemia. More than half of the patients (55.6%) had thrombocytopenia, with high and normal mean platelet volume (MPV) values. WBC findings showed the vast majority 569 (78.3%) to be in the normal range. Differential leukocyte count showed neutrophil counts in the normal range in 494 (67.9%) cases, while 510 (70.2%) had a slight increase in band forms, mean band percentage 5.03 ± 5.78 . About half the patients (42.9%) had low lymphocyte counts (lymphopenia), while 227 (31.2%) had lymphocyte counts in the normal range, and atypical lymphocytes were found in 38.7%. Monocytes, eosinophils and basophils were in the normal range in the majority of the cases (66.6%, 57.2%, and 98.8% respectively, Table 1).

When hematological results were compared with age (children ≤ 14 years of age vs. adults), it was found that 81.8% of all children were anemic compared to only 48.8% of the adults (P value = <0.0001). Severe anemia (hemoglobin ≤ 7 g/dL) was occasionally, seen but mostly among infants and young children (40 cases, 5.5%). With regards to the WBC differential, there was no significant age differences, except for the lymphocyte count where it was higher (lymphocytosis) in children compared to adults (41.7% vs. 7.1%, P value = <0.0001), and the neutrophil percentage where it was low in 34% of children compared to only 6.4% in adults, and high in 6.4% of children compared to 10.4% in adults. These findings appear to be related more to age than malaria infection. When hematological values were compared with malaria species, anemia was seen more in *P. falciparum* cases, 257 (65.1%), compared to 126 (44.6%) in *P. vivax* cases (P value = 0.0002). Platelet counts were lower in most *P. vivax* cases (74.7%) compared to 59.9% of *P. falciparum* cases (P value = 0.0018). The neutrophil percentage was lower in *P. falciparum* (18.2%) compared to *P. vivax* (4.9%) (P value = 0.0018).

Discussion

Hematological abnormalities are considered a hallmark of malaria, and reported to be most pronounced in *P. falciparum* infection, probably as a result of the higher levels of parasitemia found in these patients.³ The findings presented in our study show that in acute malaria, there are several peripheral blood changes, including anemia which

FIGURE 1. Peripheral blood smear. Wrights stain showing an atypical lymphocyte with malaria ring forms.

FIGURE 2. Peripheral blood smear. Wrights stain showing a monocyte with malaria pigment and malaria ring forms.

FIGURE 3. Bone marrow aspiration from the patient with pancytopenia. Wrights stain showing increased phagocytic activity and malaria pigment.

TABLE 1. Summary of hematological values of malaria cases at KFHU (1988-1999).*

Hematological parameters	Low No. (%)	NR No. (%)	High No (%)	Reference range
Hemoglobin g/dL	430 (59.2)	286 (39.4)	11 (1.5)	15 \pm 2 g/dL
Total WBC $\times 10^9/L$	97 (13.3)	569 (78.3)	52 (7.2)	7 \pm 3 $\times 10^9/L$
Platelet count $\times 10^9/L$	404 (55.6)	200 (27.5)	12 (1.7)	150-400 $\times 10^9/L$
Mean platelet volume (MPV)	16 (2.2)	200 (27.5)	175 (24.1)	
Packed cell volume (PCV%)	520 (71.5)	187 (25.7)	5 (0.69)	45 \pm 5%

was a common presentation. Severe anemia (Hb ≤ 7 g/dL), however, was seen in a minority of patients (5.5%), especially infants and children. Anemia was normochromic and normocytic in the majority of cases, which is concordant with the reports of Facer and Beales.^{3,15} However, microcytic hypochromic anemia was seen in 129 cases (17.7%) mostly in children. In these latter cases, there was documented concurrent iron deficiency and/or a thalassemia. It is known that in heavily endemic malaria areas, it is almost inevitable that malarial infection will be associated with anemia, although malaria may not be the prime cause of it.^{3,15}

In previous studies, anemia was also found in patients who had imported malaria from endemic areas.^{11,12} The pathogenesis of anemia in malaria is extremely complex, multifactorial and incompletely understood. It is thought to result from a combination of hemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocently unparasitized red blood cells, depressed as well as ineffective erythropoiesis with dyserythropoietic changes, and anemia of chronic disease.^{3,4,16} Other factors contributing to anemia in malaria include decreased red blood cell deformability, splenic phagocytosis and/or pooling,⁹ so they have an increased rate of clearance from the circulation. Studies in experimental murine malaria have shown that the progressive defective erythropoietic activity of the bone marrow was the result of depletion of colony-forming unit-pluripotent stem cells, although the mechanism of this stem cell depletion is unknown.^{3,17} Tumour necrosis factor alpha (TNF- α) has been implicated, and may cause ineffective erythropoiesis.^{3,16} Other studies have also discussed the role of TNF- α in falciparum malaria.¹⁸ In a recent report, the investigators' observations

indicated that hemolysis is the prime cause of the anemia seen in acute falciparum malaria, but destruction of parasitized erythrocytes is not the only cause of the hemolytic process, and bone marrow suppression appears to have an insignificant role.¹⁹ It should be borne in mind also that red cell morphology in malaria patients may be influenced by their nutritional status i.e., patients could be iron deficient, folic acid or vitamin B₁₂ deficient or they may have a concurrent thalassemia, which aggravates the severity of the anemia, as was the case in some of the patients in this study.

P. falciparum malaria is one of the most common causes of anemia. The other plasmodia species rarely cause anemia because only selected red cell populations (reticulocytes in the case of *P. vivax* and *P. ovale* and older cells in *P. malariae*) are invaded. So the causes of anemia are through many multiple mechanisms, the most important being hemolysis as a result of direct invasion, where most of the erythrocytes hemoglobin is utilized. One should not forget the contribution of anemia of chronic disease, characterized by failure to utilize iron by the bone marrow related principally to increased levels of cytokines (TNF- α and interleukin 6). It has also been reported that anemia correlates with the severity of the infection.^{3,7,19}

In our study, the majority of patients (78.3%) had a normal total WBC count, unlike some studies which showed that leukopenia appears to be a common finding in both non-immune patients with falciparum malaria and semi-immune children living in malaria-endemic regions, where WBC may be as low as $1-2 \times 10^9/L$.³ The differential WBC showed a normal neutrophil count in the majority of cases, which differs from other studies, which reported either neutropenia or neutrophilia among malaria cases.⁶ Another finding, the presence of bands in the majority of

TABLE 2. Mean absolute blood counts of the malaria cases.

Absolute WBC counts	Low No. (%)	Mean absolute count (low)	NR No. (%)	Mean absolute count (normal range)	High No. (%)	Mean absolute count (high)	Reference range
Neutrophil	84 (11.6)	$1.4 \times 10^9/L$	494 (67.9)	$4.3 \times 10^9/L$	60 (8.3)	$8.4 \times 10^9/L$	$2-7 \times 10^9/L$ (40-80%)
Bands (STABS)**	–	–	200 (27.5)	$0.35 \times 10^9/L$	510 (70.2)	$0.75 \times 10^9/L$	$0-0.70 \times 10^9/L$ (0-3%)
Lymphocytes	312 (42.9)	$0.6 \times 10^9/L$	227 (31.2)	$1.73 \times 10^9/L$	99 (13.6)	$4.4 \times 10^9/L$	$1-3 \times 10^9/L$ (20-40%)
Monocytes	44 (6.1)	$0.19 \times 10^9/L$	484 (66.6)	$0.44 \times 10^9/L$	90 (12.4)	$1.1 \times 10^9/L$	$0.2-1 \times 10^9/L$ (2-10%)
Eosinophils	416 (57.2)	$0.02 \times 10^9/L$	298 (41)	$0.15 \times 10^9/L$	13 (1.8)	$0.44 \times 10^9/L$	$0.02-0.5 \times 10^9/L$ (1-6%)
Basophils	–	$0.05 \times 10^9/L$	718 (98.8)	$0.082 \times 10^9/L$	9 (1.2)	$0.12 \times 10^9/L$	$0.02-0.1 \times 10^9/L$ (<1-2%)
Atypical lymphocytes	–	–	–	–	281 (38.7)	–	–

Thrombocytopenia, which occurred in more than half of the patients, was a characteristic finding. Other studies of imported malaria have also reported that at least 50% of the malaria patients had thrombocytopenia, while only 28% of them had anemia.^{11,12} It appears from these different studies that thrombocytopenia may be more common than anemia in acute malaria infection. In the study of severe malaria cases in the Gizan area by Benzal et al.,²¹ thrombocytopenia was a common complication (50.4%). Thrombocytopenia is a classical feature of malaria, and a low platelet count is usually seen in about 85% of patients with uncomplicated malaria and all patients with severe falciparum malaria.³ It

is so characteristic of malaria that in some places, it is used as an indicator of malaria in patients presenting with PUO.^{3,22} It has also been observed that there is an inverse relationship between platelet count and parasite level.³

patients, even as little as 3%, was not stressed in previous reports. Two of our findings, however, coincided with previous studies, i.e., lymphopenia and the presence of reactive lymphocytes (Figure 1). In one study, the percentage of reactive lymphocytes in children was increased to a degree that mimicked a viral infection.²⁰ Although monocytosis has been reported to occur,^{6,7} the present study showed that the majority (66.6%) of malaria patients had a normal monocyte count. There was also no increase in eosinophils, which agrees with previous reports.³ However, eosinophilia has been reported to occur after initiation of antimalarial treatment.^{3,4}

Phagocytosis of malaria pigment by monocytes/macrophages, and less frequently by neutrophils, has been observed in peripheral blood cells and bone marrow of patients with malaria.^{3,6,8} Erythrophagocytosis of infected and uninfected red cells by monocytes/macrophages has also been observed.^{6,8} In some of the peripheral blood smears examined during this study, monocytes and rarely neutrophils (Figure 2), contained malaria pigment and in very rare cases, erythrophagocytosis by monocytes was also observed. Also, three patients needed bone marrow examination, two for pancytopenia with a positive malaria smear to exclude bone marrow etiology. The third patient was a case of pyrexia of unknown origin (PUO) with a negative peripheral blood smear, but with a high suspicion of malaria and history of recent travel to a malaria-endemic area. All bone marrows were essentially normal, however, showing increased phagocytic activity including phagocytosis of malaria pigment (Figure 3).

In our study, thrombocytopenia was more common in *P. vivax* than *P. falciparum*, which has also been observed in another study.¹² The mean platelet volume (MPV) is often raised confirming the presence of giant platelets on stained blood films. In our study, the MPV was found to be high in about 25% of the patients whenever reported. It has also been reported that thrombocytopenia occurs early in illness and resolves within a few days of treatment. The

precise mechanism behind thrombocytopenia, however, remains unclear. Decreased thrombopoiesis can be excluded because platelet-forming megakaryocytes in the marrow are usually normal or increased.³ Immune-mediated destruction of circulating platelets has been postulated, and it has been found that malaria patients have elevated levels of platelet-bound IgG.^{3,4,23} Some workers have suggested DIC as a major mechanism, but others have found no evidence of DIC in any of their patients, including those with severe thrombocytopenia.²³ Another proposed mechanism is that of platelets engulfing malaria parasites, and in the process becoming damaged and thus being removed from circulation.³ This has not been confirmed.

Hypersensitive platelets have been found in acute malaria infection as well as additional changes in platelet function. These include raised concentrations of platelet-specific proteins such as beta thromboglobulin (β TG), platelet factor 4 (PF4), and other changes such as enhanced production of thromboxane A₂ and prostacyclin, but spontaneous platelet aggregation did not occur in these studies.²³ It has also been postulated that these hypersensitive (hyperactive) platelets will enhance hemostatic responses, and maybe this is why bleeding episodes are rare in acute malaria infection, despite the thrombocytopenia.²³

There are conflicting reports as to the frequency of abnormalities of the coagulation mechanism in malaria. Prolonged PT, depletion of blood coagulation factors V, VII, VIII, IX and DIC have been reported.⁴ Only one patient in this study series was suspected of having DIC, i.e., prothrombin time, and the partial thromboplastin time were slightly prolonged, platelet count $48 \times 10^9/L$, and fibrinogen degradation products (FDPs) $>40 <80$ mg/mL (normal range <10 μ g/mL). Our patient recovered with treatment of malaria, however, and no further intervention was needed.

In conclusion, the hematological aspects of malaria infection constitute a very interesting area in various reports. Mainly anemia and thrombocytopenia are the classical changes. Changes in the white blood cell are less dramatic and there has been conflicting reports regarding these changes. It would be interesting to study further and compare the different reports discussing the hematological findings in both immune and semi-immune patients living in endemic areas, and those returning with imported malaria. It would also be beneficial to carry out prospective studies on other aspects, which have not been conclusive, e.g., coagulation parameters, bone marrow changes and the direct antiglobulin test (to help in understanding the anemia). In fact, results of direct antiglobulin tests and ⁵¹Cr red cell survival studies have shown some evidence for increased destruction of non-parasitized and parasitized erythrocytes by possible immune-mediated mechanisms. Laboratory and clinical studies, however, failed to establish the presence of antibodies on erythrocyte surface and

hemolysis in malaria infected patients. These aspects were studied by Facer³ and Abdalla.⁶

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