

Haematological Changes in Falciparum Malaria and Tumor Necrosis Factor

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Abstract

Tumor necrosis factor and various haematological parameters were studied in 90 patients suffering from falciparum malaria. They were divided into three groups on the basis of haemoglobin level. The difference in haemoglobin level between group-I (Hb<7 gm/dl) and group-2 (Hb 7-10 gm/dl), as well as group-I and group-3 was statistically significant. The geometric mean TNF α concentrations in group-I (193.9 pg/ml) and group-2 (132.2 pg/ml) were higher as compared to group-3; however, the difference was statistically non-significant. The TNF concentration in group-2 correlated negatively with haemoglobin level ($r=-.43$, $p=.05$). As a whole, 21% patients had leukocytosis, 3% leukopenia, 46% increased ESR and 26% elevated levels of fibrin degradation products. The platelet count was done only in 4 patients with bleeding problems. Twenty-five healthy subjects were included in the study as controls. The difference between TNF and haemoglobin level in group-I and controls was statistically significant ($p<.05$, $p<.001$ each). The role of tumor necrosis factor in the production of these changes is discussed (JPMA 46:198, 1996).

Introduction

Patients suffering from malaria present with a number of haematological changes of which anaemia is a major complication especially in those infected with *Plasmodium falciparum*¹. Other changes include eosinopenia, neutropenia, reactive lymphocytosis, thrombocytopenia and neutrophil leukocytosis. *Falciparum* malaria also causes accelerated turnover of coagulation cascade. In severe disease, there is increased fibrinogen consumption, but in most cases plasma concentrations are either normal or elevated².

The pathogenesis of anaemia remains obscure. It results either from defective production or excessive destruction of red blood cells or a combination of both. In tropics, repeated attacks of malaria eventually lead to profound anaemia, especially, if there is associated chronic blood loss –from hookworm infection, malnutrition, pre-pregnancy and persisting relapsing or recrudescing parasitaemia.³ Oxford and Nairobi workers have studied tumor necrosis factor alpha (TNF α) in Kenyan children with severe, mild and without malaria. They confirmed, that TNF α levels correlate with parasite density and with the severity of malaria. What part this cytokine plays in the pathophysiology of malaria is not yet established, but the possible role of anti TNF α antibodies in cerebral malaria is under investigation. It was reported in 1993 that monoclonal antibodies against TNF α reduce fever in children with cerebral malaria⁴.

The aim of this study was to study the haematological changes in patients with *Falciparum* malaria, correlate them with tumor necrosis factor and compare them with other studies.

Patients and Methods

Sixty-nine Pakistani adults (52 males and 17 females) and 21 children (16 males and 5 females), admitted in District Headquarter Hospital, D.I. Khan between August, 1994 and February, 1995, with

acute symptoms of malaria due to *Plasmodium falciparum* were studied. Diagnosis of malaria was made on blood film. Parasite count was done by counting the number of asexual *Plasmodium falciparum* parasites against 200 leukocytes in a thick film. Twenty five healthy subjects (20 males and 5 females) were used as controls. Patients were divided into three groups on the basis of haemoglobin level, white blood cell count and FDP level. Group I patients had haemoglobin of less than or equal to 7 gm/dl (n=9). group 2 between 7-10 gm/dl (n=16) and group 3 over 10.0 gm/dl (n=65). Blood samples were taken within 24 hours of admission, before the initiation of antimalarial treatment. Laboratory investigations like total leukocyte count, haemoglobin level, red blood cells count, erythrocyte sedimentation rate (ESR) were done by conventional method and fibrinogen degradation products (FDP) by latex agglutination method. Platelet count was done only in patients with bleeding problems due to *Plasmodium falciparum* malaria.

For the determination of tumor necrosis factor alpha (TNF α) venous blood samples were taken, sera separated and stored at -20°C until cytokine assay. Serum concentrations of TNF α were measured by the quantitative immunoenzymometric, 'sandwich' technique. Microtitre plates were coated with monoclonal antibodies specific to TNF α . Standards with known amounts of TNF α and samples were pipetted into the wells and any TNF α present was bound by the immobilized antibody. After washing away any unbound sample protein and enzyme linked polyclonal antibody specific for TNF α was added to the wells and allowed to bind the TNF α which was bound during the first incubation.

Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added as described by Kwiatkowski⁵ using an enzyme linked immunosorbent assay.

Results

Of 90 cases, 69 were adults (52 males and 17 females) and 21 children (16 males and 5 females). Their ages ranged from 1 month to 70 years.

Haemoglobin level in patients with *falciparum* malaria

In group-I there were 9 patients (10%). Their mean haemoglobin level was 5.9 ± 0.5 gm/dl and geometric mean of TNF α concentration 193.9 pg/ml. In group-2 there were 16 patients (19%) with mean haemoglobin of 9.0 ± 0.93 g/dl and a geometric mean TNF concentration of 132.2 pg/ml. In group-3, there were 65 (71%) patients with mean haemoglobin level of 12.5 ± 1.09 gm/dl and geometric mean TNF level of 108.94 pg/ml. The relationship between haemoglobin level and tumor necrosis factor in different groups of *falciparum* malaria is shown in Figure 1.

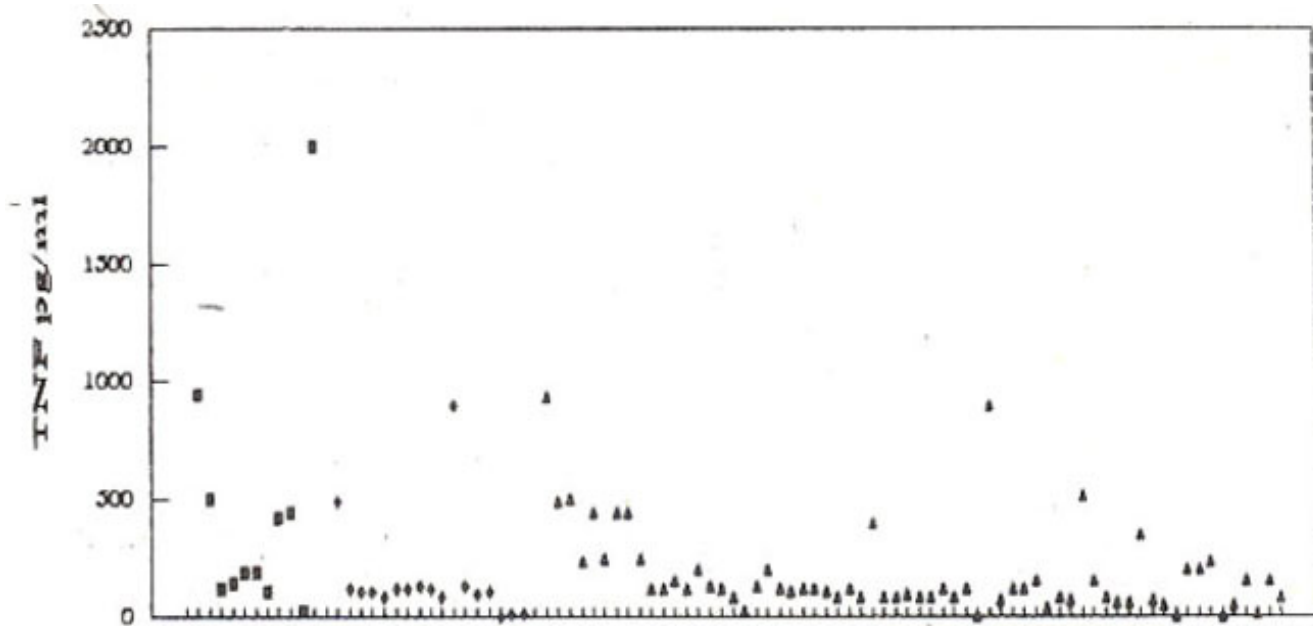


Figure 1. TNF and HB level in patients with Falciparum malaria. 1=Hb<7 g/dl, 2=Hb 7-10 g/dl, 3=Hb>10 g/dl.

White blood cell count in patients with falciparum malaria with cell count in Group 1 (3 cases) was $<4.0 \times 10^3/\mu\text{l}$ in Group 2 (68 cases) $4-11 \times 10^3/\mu\text{l}$ and in Group 3 (11 cases) it was $>11 \times 10^3/\mu\text{l}$ (n=11).

Sixty-eight patients had normal leukocyte count, 3 had leukopenia and 19 (21%) had leukocytosis. The relationship between WBC count and tumor necrosis factor is shown in Figure 2.

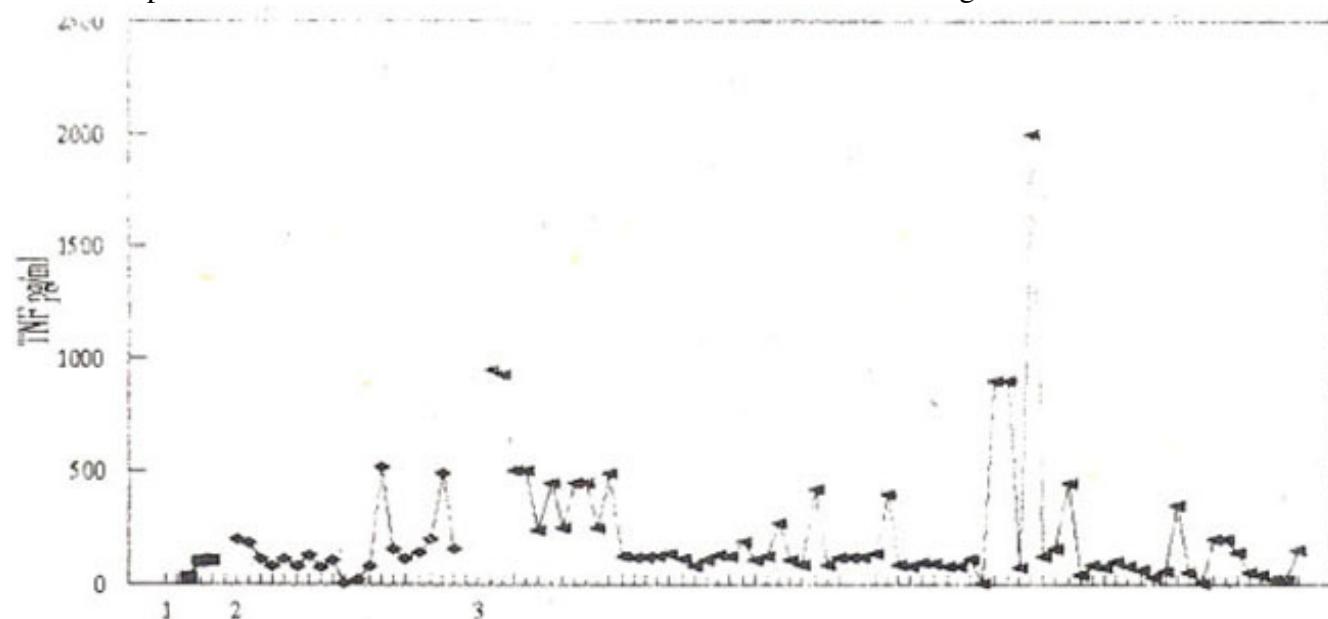


Figure 2. TNF and WBC count in patients with Falciparum malaria. 1=WBC $<4,000/\text{cmm}$, 2=WBC $4-11,000/\text{cmm}$, 3=WBC $>11,000/\text{cmm}$.

Fibrinogen degradation products (FDP level) in patients with falciparum malaria.

FDP level was less than 10 ug/dl in 74 cases belonging to group 1; between 10-40 ug/dl in 11 cases of group 2 and more than 40 jig/dl in 5 cases of group 3.

Seventy-four patients had normal FDP level (reference range 0-10 ug/dl, 11 patients between 10-40 ug/dl and 5 had levels above 40 ug/dl. The relationship between FDP level and tumor necrosis factor is shown in Figure 3.

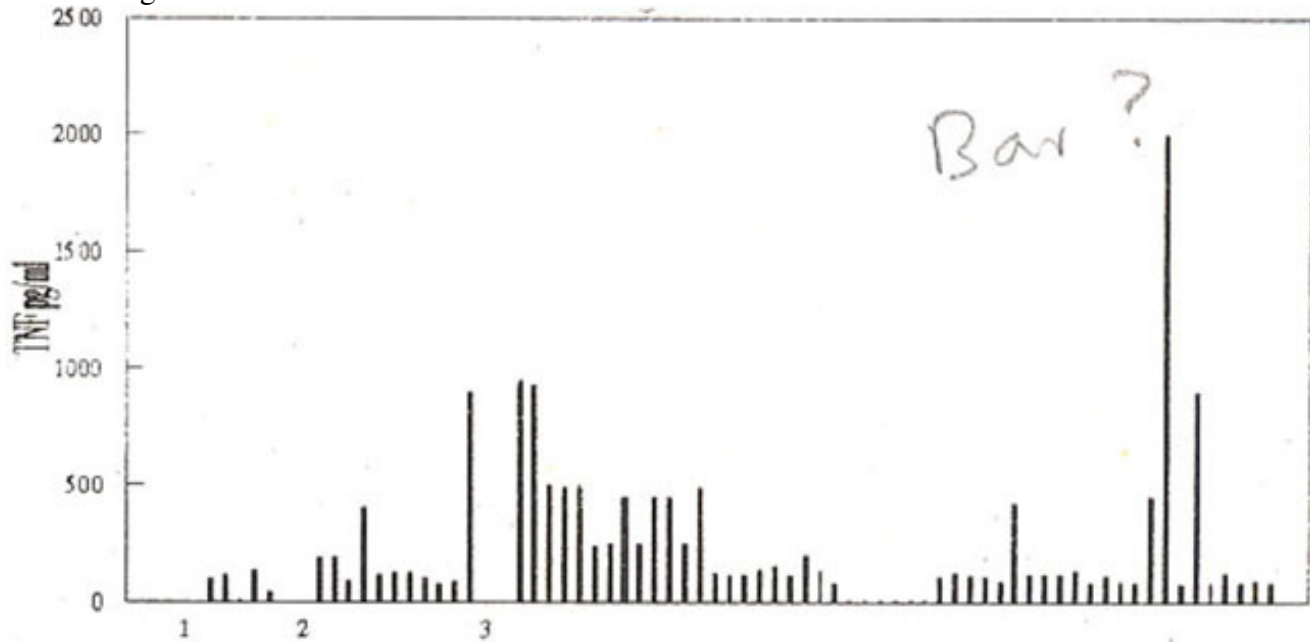


Figure 3. TNF and FDP level in patients with Falciparum malaria. 1=FDP >40μg/dl, 2=FDP 10-40μg/dl, 3=FDP <10μg/dl.

Platelet count in patients with falciparum malaria

Platelet count was done in 4 patients with bleeding problems, one had normal platelet count (150-400x10⁹/i), while in 3 it was reduced (80-98x10⁹/l). In 25 controls, the mean haemoglobin level was 14.5±1.5. The geometric mean TNF level was 97.3 pg/ml (reference range 5-150 pg/ml) as shown in the Table.

Table. Tumour necrosis factor and haemoglobin levels in different groups of Falciparum malaria patients and control subjects.

S.No.	Hb g/dl	TNF pg/ml
Group 1	5.9±.49	193.9
Group 2	9.0±.93	132.2
Group 3	12.5±1.09	108.9
Controls	14.5±1.5	97.3

In 3 patients with icukopenia TNF levels were with in reference range (5-150 pg/dl). Of 68 patients with normal white blood cell count, 25 (37%) had elevated TNF levels. Similarly in 19 patients with leukocytosis. 7 had increased TNF level. In 5 patients with markedly elevated FDP levels, TNF levels were normal. In 11 patients with moderately elevated FDP levels. 4 had elevated TNF levels. In 74

patients with normal FDP levels, 20 had increased TNF concentration.

Discussion

This study shows that falciparum malaria is associated with a number of haematological changes like anaemia, leukocytosis, leukopenia, thrombocytopenia, high levels of fibrinogen degradation products and elevated erythrocyte sedimentation rate. It is further shown that the anaemia associated with falciparum malaria is related to the levels of tumor necrosis factor alpha (TNF α).

Anaemia is an inevitable consequence of erythrocyte parasitization by the plasmodium falciparum as all infected cells are destroyed at schizogony. The survival of non-parasitized erythrocytes was found to be reduced for several weeks after clearance of parasitaemia in patients with falciparum malaria³.

Many workers have described the correlation between high TNF level, cerebral malaria and hypoglycaemia, however, very few have reported the correlation between high TNF level and severe anaemia. Shafer et al found significant elevated TNF levels in children with severe anaemia in Kinshasa (Zaire)⁶. Gruss et al⁷ demonstrated that elevated TNF α and interleukin 6 (IL-6) levels are markers for complicated falciparum malaria. They also found higher incidence of severe anaemia in their patients as compared to our study. Eighteen percent of their patients had haemoglobin below 7.0 gm/dl.

There is now considerable evidence linking TNF with various aspects of malarial pathology¹⁰. The geometric mean TNF levels were much higher (193.9 pg/ml) in patients with severe anaemia (Hb 5.9 $\pm$ 0.49) than in patients (108.94 pg/ml) with mild anaemia (Hb 12.5 $\pm$ 1.09). There was negative correlation between TNF levels and severe anaemia ($r=-0.4$, $p=0.05$). This observation shows that TNF may be involved in the production of anaemia in patients with falciparum malaria. Elshoura⁸ reported several ultrastructural changes in non-parasitized erythroblasts and erythrocytes in the blood of 28 Saudi patients who were anaemic and suffering from acute falciparum malaria. Ultrastructural changes of these cells were suggested to be due to dyserythropoiesis and ineffective erythropoiesis which was attributed to an imbalance in metabolism as they were being overproduced in response to infection. Numerous haemoglobin like particles are liberated through erythrocyte plasma membrane, indicating severe haemolysis which is one of the major factors producing anaemia during malarial infection.

Shofield and Hackett⁹ identified a dominant glycolipid toxin of plasmodium falciparum. They showed that chemically it is glycosylphosphatidylinositol (GPI) and it induces tumor necrosis factor and interleukin 1 production by macrophages. Thus GPI of plasmodium is a potent glycolipid toxin that may be responsible for novel pathogenic process, exerting pleiotropic effects on a variety of host cells⁹. The lower frequency of severe and moderate anaemia in our falciparum malaria patients may be due to the genetic diversity of the malarial parasite. The clinical immunity in our population may also play a relative protective role, as high infection rate with low prevalence of disease has already been demonstrated in rural Punjabi community in Pakistan¹⁰.

Leukocytosis as well as leukopenia has been reported in falciparum malaria. Abdalla and co-workers showed that 29% of their patients with falciparum malaria and severe anaemia had leukocytosis¹¹. Fourteen percent of our patients suffering from severe falciparum malaria had leukocytosis, while 3% with mild malaria had leukopenia. Mild leukopenia has been described in uncomplicated malaria, but neutrophil leukocytosis is an important abnormality in patients with severe falciparum malaria and is associated with a bad prognosis³. None of our patients even with marked leukocytosis died from severe falciparum malaria. Tumor necrosis factor may be responsible for leukocytosis which may be associated with a complicating Gram negative rod or other bacteraemia. However, 4 patients in our series with leukocytosis had TNF levels above 150 pg/ml³.

Serum fibrin degradation products are elevated in falciparum malaria. Tanabe and co-worker found that

57% of falciparum malaria patients had elevated FDP levels. Twenty-nine percent of these patients had evidence of disseminated intravascular coagulation (DIC)¹². Twenty-six percent patients in our series had elevated FDP levels. Eleven patients in the present study had cerebral malaria, but none of these had elevated FDP levels as reported by other workers¹³.

Thrombocytopenia is common in falciparum and vivax malarias. Its degree has some prognostic significance. Platelet survival is reduced to 2-4 days in severe falciparum malaria. Increased numbers of large abnormal looking megakaryocytes have been found in the marrow and the circulating platelets may also be enlarged suggesting dyspoietic thrombopoiesis. Our study shows that although FDP levels are elevated in falciparum malaria, they have no pathological significance. The same observations have been made by Pukntayakamee and co-workers after studying coagulation profile in falciparum malaria¹⁴.

Our study shows that TNF α levels are elevated in patients with falciparum malaria. Our data provide further confirmation of the association of haematological changes with falciparum malaria and that tumor necrosis factor may be directly or indirectly responsible for the production of anaemia and other pathological changes. The degree of pathology produced, can be the consequence of genetic variation in the host propensity to produce tumor necrosis factor. The decreased frequency of haematological abnormalities in our patients may be due to the presence of clinical immunity in this community.

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