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The Role of TNF-α Levels as Predictive Diagnostic Biomarker Among Children with Severe Falciparum Malaria in Endemic Area in Sudan

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Abstract: *Tumor necrosis factor alpha (TNF-α) level is a central proinflammatory cytokine. Their production associated with severe falciparum malaria. The purpose of this study was to compare TNF-α levels between severe falciparum malaria, uncomplicated malaria and control. Furthermore to evaluate the association between TNF-α levels and malaria parasitemia and malaria parasite count. A case control hospital based study was included 300 Sudanese children (100 severe falciparum malaria (with mean age 8.63 ± 3.40 years; 61% male; 39% female), 100 uncomplicated falciparum malaria (with mean age 8.83 ± 4.20 years; 45% male; 55% female) and 100 normal healthy children controls (with mean age 10.08 ± 3.58 years; 50% male; 50% female). The malaria parasitemia was estimated using thick blood film, while parasite count was counted using thin blood film. TNF-α levels measured using Human TNF-α ELISA MAX™ Deluxe Sets - BioLegend, Inc. The data were analyzed using SPSS software (V 20.0) and Statdisk software (V 13.0). The average of TNF-α levels were markedly elevated in serum of severe and uncomplicated falciparum malaria (200.98 ± 92.77 and 112.42 ± 35.52 pg/ml respectively) versus normal healthy children controls (38.81 ± 22.23 pg/ml), giving statistically highly significant difference (P value = 0.000). Furthermore, the levels of TNF-α in malaria parasitemia had statistically highly significant differences and significant positive correlation (P value = 0.000 for both). On the other hand, the levels of TNF-α had significant positive correlation within parasite count (P value = 0.000). This study showed that TNF-α levels had association with falciparum malaria severity, and level of parasitemia. The results obtained in this study will help clinicians to diagnose and to improve the management of severe malaria cases.*

Keywords— TNF-α levels, severe falciparum malaria, level of parasitemia, ELISA, Sudanese children.

1. INTRODUCTION:

Falciparum malaria accounts for up to 80% of malaria cases globally (1) and about 87.6% of malaria cases in Sudan (2). Malaria caused by *P. falciparum* is considered to be the most virulent and pathogenic, and linked well with high morbidity and mortality in humans (3). The disease is especially important among groups of high risk including children under 5 years, pregnant women and non-immune adults in endemic areas (4).
In fact, about 285,000 children died before their fifth birthdays in 2016 in Africa According to the World Health Organization (1); therefore malaria remains the largest cause of children death in Africa (5).
The tumor necrosis factor alpha (TNF-α) is 26 kd transmembrane proinflammatory cytokines involved in multiple inflammatory and immune responses and plays an important role in the pathogenesis of many infectious diseases including falciparum malaria (6, 7). During *P. falciparum* malarial infection, the TNF-α has been described as both protective and pathogenic. At low levels, TNF-α kills the parasite by macrophage activation and subsequent release of cytokines, whereas high TNF-α level has been associated with severe manifestations like acute respiratory distress and cerebral malaria (8). Severe malaria susceptibility have been associated with high TNF-α plasma levels and TNF-α gene polymorphisms (9-16). So TNF-α is thought to be a critical factor in malaria pathogenesis (15).
The inflammation-related biomarkers as TNF-α levels in malaria identification may help as diagnostic tool to assess the disease severity and its management by identifying the patients at high risk for the disease.

2. METHODOLOGY:

This case control study is part of a wider study project comprising role of TNF-α levels and TNF-α 238 alleles polymorphisms in malaria severity, malaria anemia and malaria thrombocytopenia conducted at Wad Medani Pediatric Hospital in collaboration with Faculty of Medical Laboratory Sciences, Gezira University on 300 Sudanese children. Samples were

collected from 100 subjects previously diagnosed as severe falciparum malaria by blood film and WHO criteria (17); 100 subjects previously diagnosed as uncomplicated falciparum malaria by blood film or ICT and 100 normal healthy controls according to inclusion and exclusion criteria.

All study procedures were approved by the Ethics Committees of both Ministry of Health – Gezira State and Faculty of Medical Laboratory Sciences – Gezira University – Wad Medani – Sudan. Informed consent was written from each participant parents.

2 ml venous blood sample was collected by clean venipuncture in plain container for all patients. Thin and thick films were prepared immediately. Serum was obtained immediately after blood collection by blood centrifugation at 1200 rpm for 10 min (18).

The parasitemia was estimated on thick blood film using plus system (17). The parasitized red cells counting (%) was determined from thin film by calculating number of parasitized red cells and divided by 20 (19). ELISA was further processed for TNF-α level using Human TNF-α ELISA MAX™ Deluxe Sets (BioLegend, Inc).

Data were presented as means with their standard deviations. The SPSS (V 16.0) and Stat disk (V 13.0) were used for data analysis. T test, correlation test and One Way ANOVA were used to compare the results, at 95% confidence interval, P value < 0.05 was considered as significant.

3. Results:

The study was conducted on 100 severe falciparum malaria (with mean age 8.63 ± 3.40 years; 61% male; 49% female), 100 uncomplicated falciparum malaria (with mean age 8.83 ± 4.20 years; 45% male; 55% female) and 100 normal healthy children controls (with mean age 10.08 ± 3.58; 50% male; 50% female) from Gezira state, Sudan (Table 1).

The average of TNF-α levels in severe malaria, uncomplicated malaria and healthy control were (200.98 ± 92.77, 112.42 ± 35.52 pg/ml and 38.81 ± 22.23 pg/ml respectively) giving statistically highly significant difference (*P value* = 0.000) (Table 2; Figure 1).

The hyperparasitemia represented 93 % in severe malaria, 32 % in uncomplicated malaria and 61.5 % for all falciparum malaria. Otherwise the average of parasite count for severe and uncomplicated malaria were (0.88 ± 0.42 and 0.39 ± 0.30 respectively).

Furthermore, the average of TNF-α levels in malaria parasitemia (+, ++, +++, ++++) were (127.56 ± 63.86, 114.56 ± 46.82, 153.76 ± 87.53 and 188.64 ± 88.34 pg/ml respectively) giving highly significant differences between them (*P value* = 0.000) and strong significant positive correlation (r +0.306; *P value* = 0.000) (Table 3; Figure 2).

The levels of TNF-α had strong significant positive correlation within parasite count (r 0.254; *P value* = 0.004; critical r ±0.148) (Figure 3).

Table 1: Demographic characteristics of study participants:

Factors	Severe malaria (SM) – N= 100	Uncomplicated malaria (UM) – N = 100	Falciparum malaria All – N = 200
Age (years)			
Mean ± SD	8.63 ± 3.40	8.83 ± 4.20	8.73 ± 3.81
Age group (years)			
Less than 5 years	19 (19 %)	24 (24 %)	43 (21.5 %)
6 – 10 years	47 (47 %)	41 (41 %)	88 (44 %)
11 – 15 years	33 (33 %)	29 (29 %)	62 (31 %)
More than 15 years	1 (1 %)	6 (6 %)	7 (3.5 %)
Gender			
Male	61 (61 %)	45 (45 %)	106 (53 %)
Female	39 (39 %)	55 (55 %)	94 (47 %)
Parasitemia			
+	4 (4 %)	43 (43 %)	47 (23.5 %)
++	3 (3 %)	25 (25 %)	28 (14 %)
+++	21 (21 %)	14 (14 %)	33 (16.5 %)
++++	72 (72 %)	18 (18 %)	90 (45 %)
Parasite count (%) (Mean ± SD)	0.88 ± 0.42	0.39 ± 0.30	0.64 ± 0.44

Table 2: Comparison of TNF-α levels between uncomplicated (UM), severe falciparum malaria (SM) and control:

Parameters	SM	UM	Controls	P value *
TNF-α pg/ml Mean ± SD	200.98 ± 92.77	112.42 ± 35.52	38.81 ± 22.23	**0.000**

* P value > 0.05

Figure 1: Comparison of TNF-α levels between uncomplicated (UM), severe falciparum malaria (SM) and control

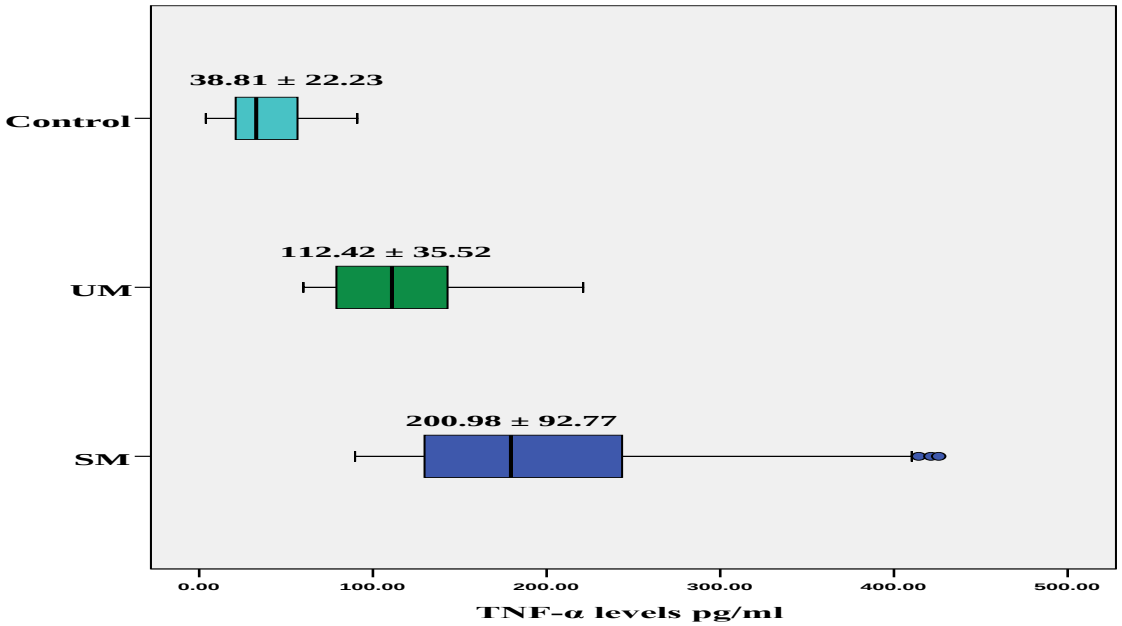
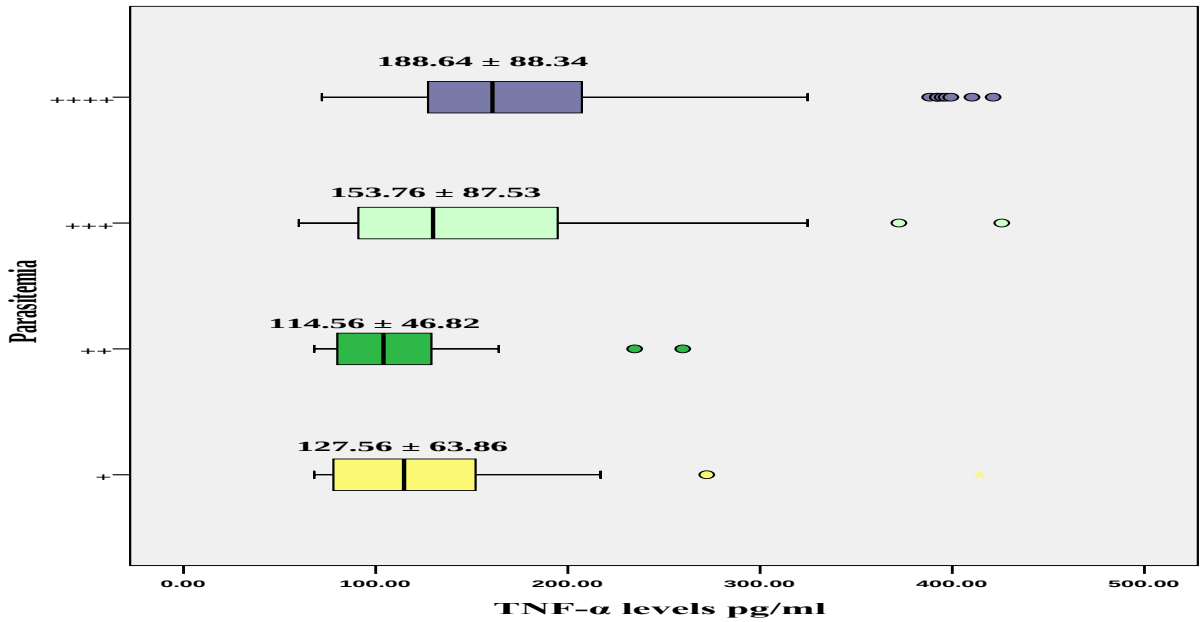


Table 3: Comparison of TNF-α levels between falciparum malaria parasitemia

Parameters	+	++	+++	++++	P value *
TNF-α pg/ml Mean ± SD	127.56 ± 63.86	114.54 ± 46.82	153.76 ± 87.53	188.64 ± 88.34	**0.000**

* P value > 0.05

Figure 2: Comparison of TNF-α levels between falciparum malaria parasitemia



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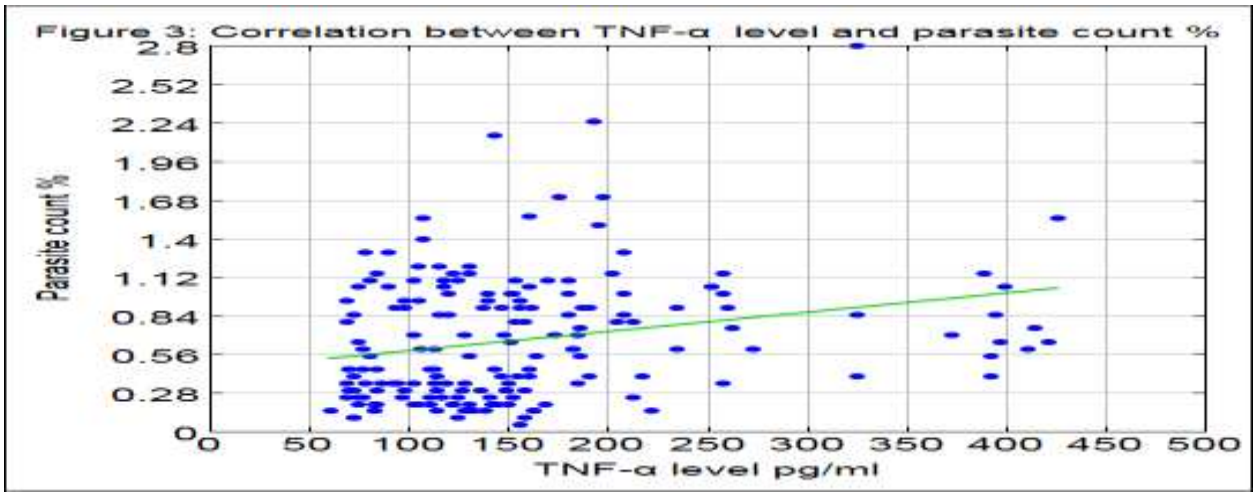

4. Discussion:

Falciparum malaria is still a major health problem in Sudan accounts for 87.6%. Poor sanitation and absence of majors protective is significantly leading to increased prevalence of the disease. Children suffer more malaria episodes and are more prone to severe malaria compared to adults.

Tumor necrosis factor (TNF-α) level is a central proinflammatory cytokine. their high level and it is association with severe falciparum malaria and is an equivocal.

The current research aimed to focus on TNF-α levels association with malaria severity, level of parasitemia.

The present study conducted on 200 Sudanese children with falciparum malaria and 100 healthy control. Similar studies were reported from different countries like Nigeria (20), Ethiopia (21), and Ghana (22).

The average of TNF-α levels were markedly elevated in serum of falciparum malaria (156.70 ± 64.15 pg/ml) as compare to normal healthy children controls (38.81 ± 22.23 pg/ml), giving statistically highly significant difference between them (*P value* = 0.000) (Table 2; Figure 1). Harischandra et al. reported the TNF-α levels of the malaria patients (196.71 ± 48.18 pg/ml) were statistically higher in relation to those of the control group (46.30 ± 9.05 pg/ml) ($P < 0.001$) (23). The high level TNF-α could be related to overproduction of TNF-α are associated with more rapid resolution of fever and parasite clearance but predisposes to severe disease (24).

Interestingly, the TNF-α levels of severe falciparum malaria (200.98 ± 92.77 pg/ml) were higher in relation to those of uncomplicated falciparum malaria (112.42 ± 35.52 pg/ml) (*P value* = 0.000). These findings are inconsistence with the previous reports on higher TNF-α levels in severe falciparum malaria patients compared to the uncomplicated falciparum malaria patients, from studies conducted from studies conducted in other malaria endemic regions (9, 10, 25-32) respectively. Severe malarial infection is associated with rupture of parasitized red blood cells releasing malaria pigment and other soluble antigens and toxins that stimulate production of TNF-α in human monocytes and may also stimulate intense T helper type 1-like response locally, in tissues of vital organs which would upregulated the expression of endothelial receptors. This in turn leads to increased parasite adherence and subsequent microvascular obstruction, decreasing oxygen delivery, and/or possibly the release of Nitric oxide from the endothelium which may ultimately contribute to the pathogenesis.

In the present study, the average of TNF-α levels in malaria parasitemia by plus system (+, ++, +++, ++++) were (127.56 ± 63.86, 114.56 ± 46.82, 153.76 ± 87.53 and 188.64 ± 88.34 pg/ml respectively) giving highly significant differences between them (*P value* = 0.000), and significant strong positive correlation (r +0.306; *P value* = 0.000). Furthermore, the levels of TNF-α had significant positive correlation within parasite count (r +0.254; *P value* = 0.004) similar to studies done in Uganda, Gabon and Zambia (26, 33, 34) respectively. In contrast Perera et al. reported no correlation was found with TNF-α levels and parasitemia (27). Possibly reason because TNF-α overproduction are associated with falciparum parasite clearance and elimination.

From the children with severe malaria; 99 % of them had TNF-α levels more than 100 pg/ml. While, 94 (87%) of children with hyperparasitemia had TNF-α levels more than 100 pg/ml. Song et al. reported the elevated expression of TNF-α was found in severe malaria and in hyperparasitemia, a primary clinical feature of severe disease (30).

5. Conclusion:

Our findings suggest the secretion of TNF-α is essential for elimination and clearance of *P. falciparum* parasite but overproduction associated severity of disease.

In conclusion the study showed the TNF-α levels had association with falciparum malaria severity, parasitemia and parasite count and can discriminate between severe and uncomplicated falciparum malaria, so may be used as a predictor and prognostic factors of the disease.

6. Competing interests:

The authors declare that they have no competing interests.

7. Acknowledgements:

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