Original Article

Tumor Necrosis Factor (TNF) and Parasite Density in Determining Disease Severity in Falciparum Malaria

Disease Severity in Falciparum Malaria

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ABSTRACT

Objective: This study was designed to investigate the TNF and parasite density in determining disease severity in falciparum malaria by using Giemsa stained slides. Our major aim was to explore the association of TNF levels with anemia, disease severity and mortality ratio.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Teaching Hospital of Peshawar Institute of Medical Sciences between February 2019 to November 2019.

Materials and Methods: Total 300 patients were analyzed and 70 infected patients were recruited. For comparison total of 60 healthy individuals were selected. A structured questionnaire was produced for the age, gender and socioeconomic status, educational status, and clinical manifestations of the participants. From each participant, two thin and thick blood with 4% Giemsa stained slides were prepared and observed under the microscope for Giemsa-stained peripheral blood smears and confirmation of Plasmodium presence.

Results: Correlation analysis was performed to understand the relationship of parasite burden with anemia, we observed a negative correlation between HB vs parasitemia, decreased Hb level VS TNF, parasitemia vs red blood count, and parasitemia vs IL-6 level. On the other hand, a positive correlation was formed between HB level vs RBC levels and TNF vs parasitemia.

Conclusion: TNF and IL-6 serves as an early marker of prognosis as TNF was high in patients with even moderate levels of parasitemia and so these patients must be managed indoor and high vigilant care to be given with close monitoring to prevent deterioration.

Key Words: Plasmodium falciparum malaria, Cytokines, parasitemia, Giemsa stained slides

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INTRODUCTION

Malaria is still one of the important global issues in the 21st century. Around three billion people are at risk of malaria every year. In 2015, almost 212 million cases were reported along with 429000 deaths[1]. Pakistan is a region that reports a higher number of deaths with Malarial infection. Plasmodium vivax and plasmodium falciparum are the two most prevalent species of Malaria that cause high morbidity and mortality ratio in Pakistan[2]. In 2013, a WHO report explored that 12% population of Pakistan was affected by plasmodium falciparum[3].

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Annually fifty thousand malarial cases are reported along with the same ratio of death in Pakistan[4]. On the other hand, our neighboring countries Afghanistan and Iran reported a 37% prevalence every year[5]. Northwest areas of Pakistan including Khyber Pakhtunkhwa and especially those areas which share the borders are on the hit list of Malaria[6,7]. Malaria is a seasonal disease that turns into a huge epidemic in areas of Khyber Pakhtunkhwa, Sindh and. Balochistan province. In Khyber Pakhtunkhwa, three districts Banu, Dera Ismael Khan, and Laki Maarwat reported a high prevalence of Malarial infection[8]. The survey reported that August to December is the peak seasons for transmission of plasmodium falciparum in Pakistan[9]. A study conducted on tribal areas of Pakistan reported 13.8% positive cases of plasmodium falciparum using the polymerase chain method[10]. A study conducted in 2020 reported a high prevalence of plasmodium falciparum in Bannu, Hangu, and Thall districts. The prevalence ranges from 16% to 25% in the age group > 14 years[11].
This study was designed to investigate the TNF and parasite density in determining disease severity in falciparum malaria by using Giemsa stained slides. Our major aim was to explore the association of TNF levels with anemia, disease severity and mortality ratio.

MATERIALS AND METHODS

This study protocol was approved by the Ethical committee of Peshawar institute of Medical sciences. The study was conducted between February 2019 to November 2019. This cross-sectional study was conducted in the teaching hospital of Peshawar institute of medical sciences. This hospital is located at Phase V Hayatabad which provides free medical assistance to poor individuals. Malaria-infected patients who were presented to the wards were included in this study. For comparison, we asked healthy individuals who were attending blood banks for donations for voluntary participation. All the adult participants were selected without any biases of ethnicity and socioeconomic status. The objectives of the research were well explained to the participants and written consents were asked before any procedure. The sample size was estimated by raw calculator with a prevalence rate of 28% as described in the previous study of Kwenti et al[17]. Total 300 patients were analyzed and 70 infected patients were recruited. For comparison total of 60 healthy individuals were selected. A structured questionnaire was produced for the age, gender and socioeconomic status, educational status, and clinical manifestations of the participants.

Senior physicians treated patients and identified the severe cases of Malarial infection as described by the WHO guideline until discharge[18]. All the patients >18 years positive for plasmodium parasites with a temperature ≥of 37.5°C were recruited for this research. Patients less than 18 years, pregnant women were excluded. All those patients who were tested positive for dengue virus, typhoid, hepatitis B, and C were also excluded from this research. We performed Malaria diagnosis with rapid diagnostic kits (RDT) and microscopic examination of Giemsa-stained peripheral blood smears. Lactate dehydrogenase of PV in human blood and histidine-rich protein II antigen of Pf were examined by using Ag Pf/Pv rapid diagnostic test kit.

From each participant, two thin and thick blood with 4% Giemsa stained slides were prepared and observed under the microscope for Giemsa-stained peripheral blood smears and confirmation of Plasmodium presence. Parasite densities were measured as parasites/µl of blood (number of parasites counted/number of white blood cells (WBCs) counted × total number of WBCs per µl of blood) or (number of parasites counted/number of RBCs counted × total number of RBCs per µl of blood). For parasitemia, we used the formula as (number of parasites per µl of blood/number of RBCs per µl of blood) × 100[19].

Participants were divided into three major categories; healthy participants, mild cases of malaria, and severe cases of malaria. In the category of healthy individuals those who were tested negative for malarial infection by blood smear and RDT kits.We recruited patients under the SM category with HB level less than 5 g/dl, serum level ≥3 mg/dl, serum bilirubin ≥3 mg/dl, plasma bicarbonate <15 mmol/l), spontaneous bleeding, hypoglycemia (plasma glucose <40 mg/dl), hyperparasitemia (≥10% parasitemia.The intensity of anemia was divided into four categories; non-anemic (Hb ≥11 g/dL), mild anemia (between 8 and 10 g/dL), and severe (5-7 g/dL)[20].

For statistical analysis, we used SPSS 23.0 software. The comparison was performed between cytokine levels and the intensity of anemia. Pearson correlation was used for showing a correlation between continuous variables whereas one-way non parametric Kruskal-Wallis test was performed for multiple comparison. P-value was set as 0.05 for statistical significance.

RESULTS

A total of 70 patients was found to be infected with plasmodium falciparum Malaria type. We observed 32.4 years as the mean age group. During observations, we found that the male population was more prone to malarial infection. Total 45 (64.2%) males were infected with malaria. Among the 70 infected patients, a total of 8 (11.4%) patients were suffered from severe malaria and need hospital admission for treatment (Table 1).

We observed levels of HB, RBC, and plasma levels of cytokines among anemic and nonanemic malarial patients in table 2. We observed a significant increase in the mean percentage of parasitemia ($P \leq 0.0001$). We observed that HB and RBC levels of anemic patients significantly decrease throughout the infection. Whereas the cytokines levels especially TNF and IL-6 increased in the anemic group. However, we did not find any statistical significance between age, gender, and inflammatory cytokines.

Table No. 1: Demographic characteristics of participants

Variables	Plasmodium falciparum n= 70	Healthy Control n= 60
Mean Age group	32.4 (18-65)	29.7 (18-58)
> 45	8 (11.4%)	7 (11.6%)
36-45	24 (34.2%)	24 (40%)
26-35	28 (40%)	24 (40%)
18-25	10 (14.2%)	15 (25%)
Gender		
Female	25 (35.7%)	20 (33.3%)
Male	45 (64.2%)	40 (66.6%)
Mild Malaria	62 (88.5%)	0
Severe Malaria	8 (11.4%)	0

Table No.2: Hematological analysis and cytokines profile of Anemic versus non Anemic patients

Variables	Healthy control - n= 60	Plasmodium falciparum (n= 70)	
		Anemic (n=53)	Non-anemic (n=17)
Hemoglobin (g/dl)	14.1±0.8	7.2±2.3	13.1±1.5
IL-10 (pg/ml	134.1±103.7	549.05±460.5	632.83±414.6
Red blood cells ($\times 10^3/\mu l$)	4.99±0.7	3.71±1.0	4.99±0.7
IL-6 (pg/ml)	87.48±54.3	269.19±188.5	211.11±163.2
Hematocrit	40.88±4.1	27.20±9.6	41.82±6.2
TNF-α (pg/ml)	66.73±29.3	328.87±217.7	212.16±150.0
Mean cell hemoglobin (pg)	26.83±2.9	25.66±2.9	25.95±5.1
Parasitemia	-	0.58±0.7	0.14±0.2
Mean corpuscular volume (fL)	86.59±6.1	82.61±13.7	84.69±9.4
Mean corpuscular hemoglobin concentration (g/dl)	30.76±1.8	29.93±3.6	30.93±3.3

Table No.3: Correlation between Hematological findings and inflammatory cytokine levels

Parameters	Correlation coefficient (r)	p- value
HB VS parasitemia	−0.3426	$P \leq 0.0001$
Parasitemia Vs RBC	−0.2158	0.007
HB VS RBC	0.3854	$P \leq 0.0001$
TNF levesl Vs parasitemia	0.3669	$P \leq 0.0001$
Low HB level VS TNF	−0.4412	$P \leq 0.0001$
Parasitemia Vs IL-6	−0.2892	$P \leq 0.0001$

Table No.4: Inflammatory cytokine levels at different intensity of anemia

Variables	Severe Anemia	Moderate Anemia	Mild Anemia	No Anemia
No. of patients(%)	15 (21.4%)	18 (25.7%)	20 (28.5%)	17 (24.2%)
IL-10 (pg/ml)	155.4 (101.4-183.1)	385.2 (186.4-727)	487.7 (274.2-1047)	585.7 (328-934)
Hb (g/dl)	4.2 (3.5-4.6)	7.2 (6.7-7.7)	10.2 (9.4-10.6)	13 (12.2-13.7)
IL-6 (pg/ml)	445.4 (306.7-516.6)	262.2 (147.6-576.9)	324.1 (198.4-427.1)	181.7 (128.7-324.1)
TNF-α (pg/ml)	678.7 (561.5-882.2)	356.4 (132-591.5)	195.3 (146.4-310.9)	86 (45.9-233)

In table 3, correlation analysis was performed to understand the relationship of parasite burden with anemia, we observed a negative correlation between HB vs parasitemia, decreased Hb level VS TNF, parasitemia vs red blood count, and parasitemia vs IL-6 level. On the other hand, a positive correlation was formed between HB level vs RBC levels and TNF vs parasitemia.

In table 4, patients were classified according to an anemic category, we observed a significant increase in TNF levels, IL-6 levels whereas a decrease was found in the IL-10 levels of all groups. In table 5, observed seven severe anemic patients with severe malarial complications including two cases of jaundice, acute renal function, metabolic acidosis, and hepatic dysfunction. However, one case of hypoglycemia, hyperparasitemia was reported during studies.

Table No.5: Frequency of severe amemia with severe Malarial Complications.

Severe Malarial Complications	Pf (n=7)
Hepatic dysfunction	2 (28.6%)
Acute renal failure	2 (28.6%)
Hyperparasitemia	1 (14.3%)
Jaundice	2 (28.6%)
Hypoglycemia	1 (14.3%)
Metabolic acidosis	2 (28.6%)

DISCUSSION

All around the world, anemia is the major public health problem during Malaria. This not only increases the morbidity duration but also enhances the mortality frequency in many regions[22]. Generally, Plasmodium falciparum malarial cases have more associated with anemia as compared to the other types. But studies by Rahimi et al[23] and Alexandra et al[12] observed found a high association of anemia with plasmodium vivax infection despite the low parasite burden during the PV infection. For this present study, our major goal was to evaluate the effect of PF Malaria on hemoglobin and red blood cells and specify the association of cytokines level with complications of malaria in patients from Peshawar and surrounding areas. We found that Pf malaria is more prevalent in the male gender as compared to females. The affected male patients were mostly laborers who worked in construction buildings which are known to provide ideal breeding and spread of infection[24]. In our study, we observed a significant decrease in the number of red blood cells and hemoglobin levels of the patients whereas the results show a significant increase in parasitemia. On the other hand, we failed to find any significant correlation between increasing parasitemia and decreasing Hb and

RBC levels. Total 8.7% of severe cases were diagnosed during the study period.

Cytokines levels are a huge mediator in the development and severity of Malaria[12]. We further explored the influence of cytokines especially IL-6, IL-10 TNF on the degree of anemia. These cytokines were compared at varying degrees of anemia Usually, TNF-α is released by the macrophages in response to Malaria[13]. Previous studies found a huge contribution of TNF-α to the destruction of RBC levels and suppression of bone marrow when combined with other cytokines[14]. Evidence from a vitro study demonstrates that TNF-α suppressed the proliferation of erythroid progenitor cells in human marrow cultures[15]. We also found a significant positive correlation between parasite density and TNF-α level. These results are in correspondence with the previous study of Lamb et al[25]. On the other hand, a negative correlation was observed between HB and TNF-α levels which depicts that TNF-α level has a huge contribution in anemic severity during malaria[26]. IL-6 also plays a great role in the pathophysiology of severe malarial anemia. TNF-α levels are responsible for the production of IL-6 levels to react together to reduce the parasite burden[27]. In our study, we observed a reciprocal association between IL-6 and parasitemia. During severe malarial anemia, patients reported increased levels of IL-6. This increased level has a strong association with high receptor density and low transferrin synthesis. These results both the Malawian study[28] in which they found a significant correlation between increased IL-6 levels and iron deficiency among children.

CONCLUSION

TNF and IL-6 serves as an early marker of prognosis as TNF was high in patients with even moderate levels of parasitemia and so these patients must be managed indoor and high vigilant care to be given with close monitoring to prevent deterioration.

Author's Contribution:

Concept & Design of Study:	Shaista Alam
Drafting:	Aysha Sarwar
Data Analysis:	Saman Hussain
Revisiting Critically:	Shaista Alam, Aysha Sarwar
Final Approval of version:	Shaista Alam

Conflict of Interest: The study has no conflict of interest to declare by any author.

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