

A Clinical Study: Tumour Necrosis Factor Alpha as a Clinical Marker in Malaria in an Endemic Region, a Future Aid in Prognostication of Malaria.

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Abstract:

Background: Although the treatment and incidence of malaria have been well documented, the biochemical markers for its severity have not been well studied and limited data is available from this part of the world. Tumour Necrosis Factor alpha (TNF- α) level increase in Plasmodium vivax malaria (PVM) has been established in many previous studies

Objective: We aimed to investigate the TNF-alpha levels of Plasmodium falciparum malaria (PFM) and in Mixed Malaria (PFM+PVM) with an objective, if this could be used in early malaria diagnosis.

Methods: This study was a prospective non-randomized control study on 60 patients with proven malaria for the test sample and control of 20 volunteers. Tumour Necrosis factor Alpha levels were measured using commercially available ELISA human TNF- α kit.

Results: The TNF- α levels of the malaria patients (mean=19.67144.3333, \pm SD \pm 48.18 pcg/ml) were statistically higher in relation to those of the control group (4.63 \pm 9.05 pcg/ml; p <0.001)

Significant increase in TNF alpha in Mixed Malaria, PFM was seen. PVM also showed raise in titres of TNF- α levels compared to the normal subjects.

Interpretation: In our study, we found that the test group with malaria positive had significantly higher levels of TNF alpha.

Conclusion: The levels of tumour necrosis factor alpha co-related proportionally with disease severity in malaria and may be used as a predictor for prognosis of the disease.

Key Words: Mixed Malaria, plasmodium falciparum malaria, plasmodium vivax malaria, Tumour necrosis factor alpha

I. Introduction

The burden of malaria is vast in Mangalore; Dakshina Kannada a coastal district in southern India being endemic to malaria is at constant onslaught. Although the treatment and incidence of malaria has been well documented. The biochemical markers for its severity have not been well studied and limited data is available from this part of the world.

II. Background

The identification of these inflammation-related biomarkers in malaria may help to potentially utilize them as diagnostic tool to assess the severity of the disease and its management by identifying the patients at high risk for the disease

The secondary effects of these changes are the host response which include the immunological response to the parasite antigen and altered red cell surface membranes. There is stimulation of the reticuloendothelial system, changes in regional blood flow and vascular endothelium. Systemic complications of altered biochemistry, anaemia, tissue and organ hypoxia along with marked systemic inflammatory response characterized by release of cytokines such as tumour necrosis factor alpha and interleukins (IL). Pro- and anti-inflammatory cytokines are involved in the malarial pathogenesis and the outcome of malaria infection is determined by the balance between the pro- and anti-inflammatory cytokines.

The high TNF/IL-10 ratio observed in severe malaria suggests an imbalanced production of inflammatory cytokines contributed to anaemia¹. Studies have shown that in severe malaria there are elevated levels. Preceding the febrile paroxysms in vivax malariasharp increase in TNF- α levels has also been demonstrated³. Low spontaneous IL-10 production at admission resulting in higher TNF/IL-10 ratios than cerebral malarial cases than acute severe malaria has also been documented well various studies including in Ghana and Southern Zambia^{4,5}. The soluble TNF- α receptors are more reliable biomarker of parasite-induced inflammation

than short-lived TNF- α and other classical pro-inflammatory cytokines in malaria even in pregnant women⁶. The levels of TNF- α show a rapid decline following malarial treatment has also been well studied⁷.

III. Objective

In view of this we correlated that severity of plasmodium falciparum and plasmodium vivax infection can be estimated using TNF- α level in blood, additionally TNF- α can be helpful in predicting the outcome of Malaria benefitting our endemically affected region.

We aimed to investigate the TNF-alpha levels of Plasmodiumfalciparum malaria (PFM) and in Mixed Malaria (PFM+PVM) with an objective, if this could be used in early malaria diagnosis.

IV. Material And Methods

This study was a prospective non-randomized control study that was conducted at Yenepoya Medical College Hospital (YMCH) on patients with diagnosed malaria by thick blood smear or fluorescent test of venous blood selected by purposive sampling during 15 months (November 1st 2012 to 31st January 2014) which included 60 isolates (5 women, 55 men; mean age 33.3) of proven malaria for the test sample and control size of 20 healthy persons (20 men; mean age 27.75) who visited YMCH. TNF- α levels for the test and controls were measured. Ethical Clearance from the Ethics committee of Yenepoya University was obtained before the commencement of the study. Patients above the age 18 who were willing to participate after informed consent were included and patients with Chronic illness, Haematological conditions, Chronic Renal Failure, Malignancy, on-Hodgkin's Lymphoma, Asthma, Dermatitis Herpetiformis, Celiac Disease, Chronic Bronchitis, Severe Sepsis, Sarcoidosis, Leishmaniasis, Systemic Lupus Erythematosus and Meningococcal Infection were excluded from the study.

Malaria positive samples were further processed for TNF- α level using a commercially available standard ELISA human TNF- α kit for analysis. Samples were sent in batches and not done daily due to laboratory constraints. The data collected was tabulated and analysed.

V. Results And Observations

The younger age group was more affected with 19 cases of 60 belonging to the age group 21-30 and the mean age 33.3 (Table I). In our study, malarial cases predominated in the male sex with the male to female ratio being 4:1. On evaluation of the type of malaria, the Plasmodium vivax malaria constituted 75% and Plasmodium falciparum malaria constituted 25% of the cases. In our study, we found that the test group with malaria positive had significantly higher levels of TNF alpha (Fig I).The TNF alpha levels of the malaria patients (mean=19.67144.3333, \pm SD \pm 48.18 pcg/ml) were statistically higher in relation to those of the control group (4.63 \pm 9.05 pcg/ml; p<0.001) as seen in Fig II, Fig III.

VI. Conclusion

The levels of tumour necrosis factor alpha co-related proportionally with disease severity in malaria and may be used as a predictor for prognosis of the disease.

VII. Discussion And Interpretation

Studies in the past reported relationship of elevated TNF- α level in PVM Erken et al⁸ Also shown in 2008 by Sohail et al⁹ TNF- α can be used as a diagnostic marker in clinical management in PVM. Singh et al¹⁰ showed TNF level also can be a prognostic marker in clinical management.

In our study the male to female ratio in the test group was which correlates well with findings of studies by Chen¹¹, Wang¹², Arahman¹³, Reuben R¹⁴: in which similar findings were noted.

In our study we measured TNF- α level both in PVM (Mean =134.06 pcg/ml)and Mixed cases (Mean=175.153pcg/ml) which showed TNF level was increased in Mixed infection. It was different from previous studies in testing TNF level for PFM, PVM simultaneously. We had occurrence of only two species of Malaria.

The coastal region of Dakshina Kannada where this study was conducted is considered as an endemic zone for malaria, the study showed the levels tumour necrosis factor of co-related proportionally with disease severity in malaria and may be used as a predictor for prognosis of the disease and targeted therapy towards it can be thought off in modifying the occurs of the disease in future.

Certain limitations were noted in the study unequal distribution of test and control, age and sex standardization not done. The sample size was not adequate to the magnitude of the problem of malaria. The TNF- α value needs to be serially measured with treatment to monitor variation, which could not be done due to time and financial constraints of the study would have adversely affected completion of study.

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Competing Interests: Nil

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Remarks: Project was done as part of Postgraduate thesis work. Study was done after ethical clearance from the university ethics committee. TNF- α level were done in an outside lab.

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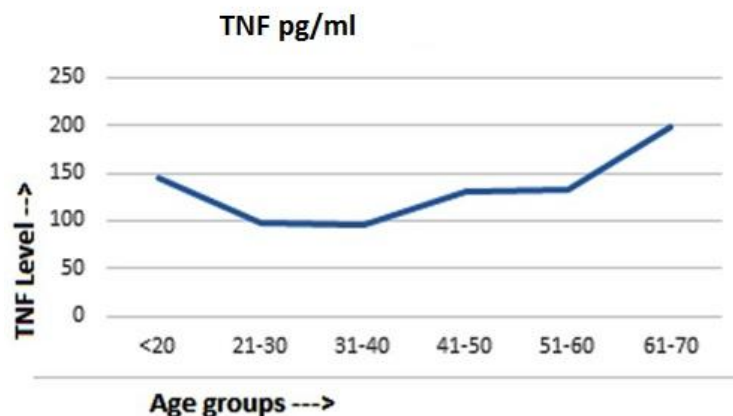


Fig I: Distribution curve for malaria for TNF- α level in picogram (pg) and age in years

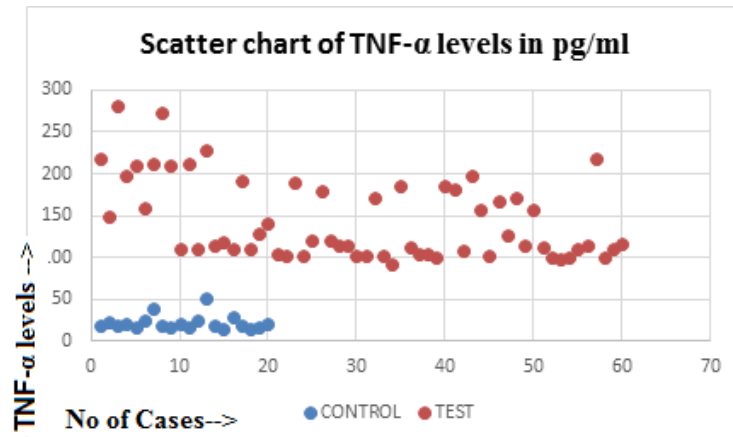


Fig II: Scatter Chart of TNF-α levels for control and test

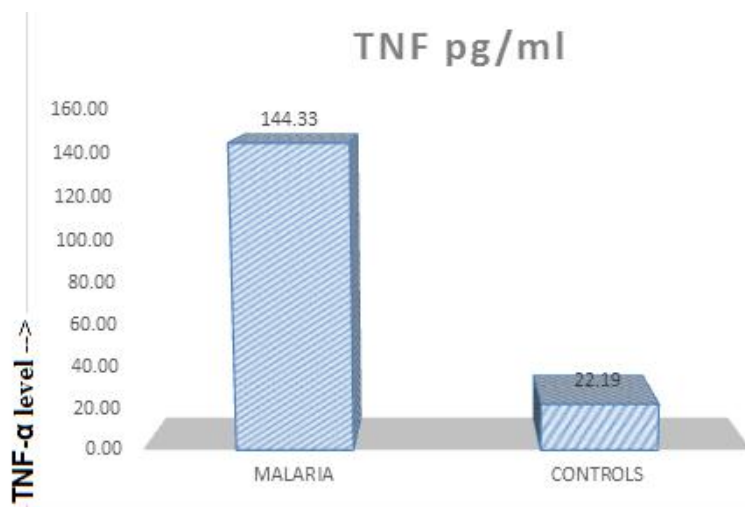


Fig III: Bar graph showing Average level of TNF-α for tests and control

	AGE GROUPS					
	<20	21-30	31-40	41-50	51-60	61-70
	Mean	Mean	Mean	Mean	Mean	Mean
TNFpg/ml	145.4417	97.8561	94.8636	130.9000	132.0143	197.7667

Table I: Mean TNF-α level in each age group in picograms/ml