Utility Of Circulating Levels Of Tumour Necrosis Factor And Its Soluble Receptor 2 In Children With Plasmodium Falciparum Malaria

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Abstract: Tumour Necrosis Factor (TNF) constitutes a major signature pro-inflammatory cytokine in P.falciparum malaria. Low TNF levels are considered critical in controlling P. falciparum malaria parasitaemia; although high levels of TNF, that mediate a strong inflammatory response which if not well regulated, can lead to immunopathology and severe forms of malaria that may lead to fatalities. The biological activity of TNF can be regulated by soluble Tumour Necrosis Factor Receptors (sTNFRs), sTNFR1 and sTNFR2. Whereas sTNFR1 is constitutively expressed in most tissues sTNFR2 is tightly regulated during an inflammatory response. It was a case control study in age and sex-matched children under 5 years of age with P. falciparum malaria and healthy controls done at Bungoma County Hospital. A sample size of 48 children was derived based on a similar study, it was ultimately comprised of 23 P. falciparum malaria infected children and 24 non-malaria infected controls; they were selected by simple random sampling. Subjects were selected on the basis of parasitaemia, defined based on WHO criteria. The average age across all cohorts was 33 months. In cases on day 1: the average TNF levels were 295.7 pg/ml and sTNFR2 levels were 155.0 pg/ml and on day 3: TNF levels were 200 pg/ml while sTNFR2 levels were 52.1 pg/ml, compared to controls in which TNF levels were 68.2 pg/ml, while, sTNFR2 levels were 27 pg/ml. TNF and sTNFR2 levels were significantly elevated in this study which was indicative of TNF and sTNFR2 utility in distinguishing the various falciparum malaria disease states.

Index Terms: TNF, sTNFR2, Soluble, Utility, Plasmodium falciparum, Parasitaemia disease state.

1 INTRODUCTION

TNF is 26 kDa protein comprised of 233 amino acid residues. It is generated in precursor form and expressed as a cell surface type II polypeptide on activated macrophages and lymphocytes as well as other cell types. sTNFRs are generated by proteolytic cleavage from the cell surface into the extracellular milieu via different mechanisms, namely alternative messenger RNA splicing for sTNFR2 while sTNFR1 is cleaved via an inefficient enzymatic process utilizing a metalloproteinase[1,2]. On the other hand soluble TNF receptors act within complex receptor systems whose key roles are to attenuate or promote TNF signaling hence they are vital regulators of inflammation and immunity mediated through TNF action [3]. Malaria disease has been attributed to excessive TNF production [4,5]. However, there is a need for sufficient TNF levels to allow for immune activation to proceed normally during infections. sTNFRs have two key functions, namely to be antagonistic to TNF in times of excessive TNF production, while in the initiation of TNF response the two receptors work to stabilize TNF, increasing its bioactivity and lengthening the TNF half-life [6]. Whereas sTNFR1 is constitutively expressed in most tissues sTNFR2 is tightly regulated during an inflammatory response hence useful as a metric for comparison with circulating TNF levels in this study [7]. Since TNF levels are elevated in P.falciparum malaria infection combined with its key role in mediating P.falciparum malaria immunopathogenesis additional to directing the immunological intermediate cascade of P. falciparum malaria in concert with its downstream mediators, all the more tellingly suggest that measuring the circulating levels of TNF and its effective modulator sTNFR2 may be useful in children under 5 years of age with P.falciparum malaria.

2 MATERIALS AND METHODS

The sample population was drawn from patients attending the Bungoma County Hospital (BCH) in Bungoma town, of Bungoma County in Western Kenya. The County has a total population of 1,790,484 million with 313,000 being children under 5 years of age [8]. The study population comprised children aged between 12-59 months attending BCH from among whom the cases were malaria positive and the controls comprised non-malaria infected children recruited based on clinical assessment and a laboratory diagnosis. Cases and controls in this study were obtained based on a 1:1 or a 1:2 ratio for selecting and assigning participants as cases and controls. The age and sex matched study participants were assessed between day 1 pre-treatment and 3 post-treatment since in most cases P. falciparum malaria symptoms resolve in 3 days following treatment. A sample size of 48 children was deivered using the formula: Estimated sample size for two sample comparisons of means [9, 10]. It was ultimately comprised of 23 P. falciparum malaria infected children and 24 non-malaria infected controls; they were selected by simple random sampling. Data on the means and standard deviation of TNF was obtained from a study by Othoro et al, (1999) [11], which involved children with P.falciparum malaria in western Kenya and age and sex matched hospital controls and used to obtain a suitable sample. Subjects were selected on the basis of parasitaemia, defined based on WHO criteria. Blood and stool samples were collected on day 1(pre-treatment-maximum delay in treatment 2 hours) and day 3 (post-treatment) for assays to exclude other causes for elevation of TNF and sTNFR2. The study excluded children with other microscopically detectable parasitic infections The statistical analysis to determine the measures of dispersion and spread, within and between the parameters of interest were computed using the software Statistical Package for Social Sciences (SPSS) version 17 for Windows. TNF and sTNFR2 constituted the independent variables while age was the dependent variable. The Chi-square test was used for associations between categorical variables. Pearson correlation was used for testing the relationships between the study parameters and the t-test for determining the differences between means. A p
value of less than 0.05 was considered statistically significant. The study was granted formal ethical approval number FAN: IREC 1261 by the Institutional Research and Ethics Committee (IREC) of the Moi Teaching and Referral Hospital (MTRH) and Moi University School of Medicine (MUSOM). Parents or guardians gave informed consent through a duly signed informed consent form available in English and Swahili versions. Study participants’ identities were de-identified by use of codes tied to their actual identities that remained confidential during and after the study.

3 RESULTS
In total there were 24 controls and 23 cases matched for age and sex. One of the cases was lost to follow up on day 3 post-treatment registering incomplete data hence was excluded. In the cases the males were 56.5% while the females were 43.5%. The control cohort of our study struck gender parity in its constitution. Majority of the cases were in the 12-35 month age group that comprised 61% of all cases. The participants were stratified into 4 age groups.

The TNF levels were markedly elevated on day 1 and declining following treatment as seen on day 3. In our study the controls average circulating TNF levels were 68.2 ± 16.1 pg/ml, which when compared to the cases cohort on day 1 whose circulating TNF levels averaged 295.7 ± 37.6 pg/ml and eventually on day 3 the circulating TNF levels averaged 200 ± 38.2 pg/ml.

sTNFR2 levels were markedly raised considering that the average circulating sTNFR2 levels among the controls was 27.0 ± 4.3 pg/ml while the circulating sTNFR2 levels in the cases were 155.0 ± 20.8 pg/ml on day 1 while the circulating sTNFR2 levels on day 3 were 52.1 ± 8.9 pg/ml. sTNFR2 levels showed a greater decline in circulating levels on day 3 following treatment compared to TNF levels as seen in figure 3.

4 DISCUSSION
This study set out to assess the levels of TNF and sTNFR2 among P. falciparum malaria diagnosed children, the cases, and those diagnosed as non-malaria, the controls, in Bungoma County Hospital (BCH). Both the cases and controls were diagnosed to have no co-infections with other parasitaemias and without medium to high helminthiasis [12]. The mean age of our study participants for both cases and controls in BCH was 33 months representing the most susceptible group to paediatric in-hospital mortality due to malaria, a finding that was in concurrence with a study in Western Kenya [13]. The results indicated, that overall, the circulating TNF and sTNFR2 levels were significantly elevated in the cases compared to the controls. In this study, we determined that circulating TNF and sTNFR2 cytokines were present among cases and controls but these levels were distinctly and significantly elevated among the cases compared to the controls which also recorded some level of TNF and sTNFR2 levels, suggesting that circulating TNF and

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**Figure 1:** Bar Chart Showing Age and Sex Matched Study Cases and Controls Plotted by Age-Group.

**Figure 2:** A Box Plot Showing TNF Levels in the Controls, Cases Day 1 and Cases Day 3 Post-treatment During the Course of Disease across the Age-Groups.

**Figure 3:** A Box Plot Showing sTNFR2 Levels in the Controls, Cases Day 1 and Cases Day 3 Post-treatment during the Course of Disease across the Age-Groups.
sTNFR2 levels can be useful predictors of P. falciparum malaria disease in cases. [14]. In the cases, the elevation of the circulating TNF and sTNFR2 levels was attributed to P.falciparum malaria infection and their key roles were to control parasitaemia by facilitating parasite clearance as was noted among the cases on day1 and day3 of this study. Nonetheless, an overzealous response characterized by elevation of the circulating TNF and sTNFR2 levels caused the exacerbation of symptoms [14, 15]. The presence of circulating TNF and sTNFR2 levels in the non-malaria infected controls albeit significantly small in overall concentration can be attributed to the existence of relative imphycytosis in children as we noted some elevation of both cytokines among the controls in our study which has also been reported in other studies [5, 7, and 16]. The roles of this TNF and sTNFR2 levels in otherwise diagnosed non-parasitaemic children that formed our control study cohort remains unclear. We could not capture cerebral malaria cases throughout the time period allotted for data collection for this study, nonetheless, by random sampling of both acute and severe parasitaemia cases we captured valuable data that can be extrapolated to the various P. falciparum malaria disease classifications [17].

5 CONCLUSION

Overall the circulating levels of TNF and sTNFR2 were significantly elevated in cases on day 1 and day 3 post-treatment compared to controls. Since TNF levels are elevated in P.falciparum malaria infection combined with its key role in mediating P.falciparum malaria immunopathogenesis additional to directing the immunological intermediate cascade of P. falciparum malaria in concert with its downstream mediators, all the more tellingly suggest that measuring the circulating levels of TNF and its effective modulator sTNFR2 may be predictive of disease states in children under 5 years of age with P.falciparum malaria.

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REFERENCES


