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Evaluation of Cytokines Response in *Plasmodium falciparum* Infection among Adolescents in Port Harcourt, Nigeria

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Abstract
This study evaluated the immunomodulatory pattern of cytokine in adolescent infected with plasmodium falciparum, using observational longitudinal design. The study was carried out in Nigeria National Petroleum Corporation Medical services Akpajo, Port Harcourt Rivers State, with the aim of evaluating the changes in cytokine level among adolescent with Plasmodium falciparum. A total of 91 adolescents participated in this study, comprising of 61 (67.0%) subjects with mild to moderate malaria infection and 30 (33.0%) non-infected as control. There were 49 male and 42 female adolescents in the study population. Cytokine level such as IL-6, IL- 8, IL-10 and TNF were assayed by using Elab science reagent ELISA method, while malaria parasite diagnosis was done using RDT, quantitative buffy coat for malaria parasite and thick blood film, infected subject were treated with antimalaria drug. There were statistically significant increase in the mean value of IL-6, TNF and IL-8 in test group compared to control group at baseline. Comparison of male and female showed statistically significant increase in TNF, and IL-8 for male compared to female. After treatment there were significant decrease in TNF, IL-6 and IL-8 $P < 0.05$. Gender effect showed statistically significant increase in TNF and IL-8 in male compared to female. Others showed no significance. This study reveals several changes in cytokine such as tumor necrosis, interleukin-6, interleukin-8 which were all increased in malaria infection and could exacerbate disease condition.
Keywords: *Cytokines, Malaria, Adolescent, Port Harcourt.*

Introduction

Plasmodium falciparum is a major public health challenge in the tropical region, which causes the infectious disease called malaria. It is associated with 300 -500 million cases per annum, leading to the death of about one million annually the death [1][2]. *Plasmodium falciparum* malaria is the major cause of death in Africa affecting mostly children, adolescent and women in poor communities[3][4]. Malaria causes significant burden on population economy[5].

Malaria is caused by a protozoa parasite, human malaria account for different species which includes *P. falciparum, P.ovale, P vivax, P. Malariae. P. Knowles*i. It is transmitted through the bite of female Anopheles mosquito which is

the causative agent. Human becomes infected when mosquito takes a blood meal resulting in the release of sporozoite with further replication [4].

The degree of severity, differential response and underlying pathophysiological process by which severe *P.falciparum* malaria progress to cerebral malaria are not well understood. Evidence suggesting that pathogenesis of cerebral may be caused by vascular occlusion and inflammation [6].The concept of vascular occlusion to cerebral malaria is based on the ability of erythrocytes infected with mature *P.falciparum* sequestered into the microvasculature binding with *P. falciparum* erythrocyte membrane 1 (PFEMP1) present on erythrocyte endothelial cells which could leads to circulatory disturbance and inflammation[7][8][9][10].

Plasmodium falciparum causes abnormalities such as severe malaria anaemia and cerebral malaria is found in non-immune individual, children and pregnant women having high mortality and morbidity rate. However, adolescents are more protected from *Plasmodium falciparum* malaria due to past episode or exposure to malaria during childhood stage which enable them to have some level of acquired immunity. In the host immune system, malaria symptoms produce complex interaction between *Plasmodium* and immune systems[11]. Severity of *P.falciparum* in children especially those living in endemic region has increased density of parasitaemia and severe malaria anaemia but less common in cerebral malaria[12][13].

Immune responses against parasite have more direct effect of parasite on the immune system, the interaction of *Plasmodium falciparum* with immune system brings about the production of cytokines. Malaria result in the interaction between *Plasmodium* and immune responses, the presence of parasite in the blood triggers the immune system, mediated by the secretion of cytokine molecules by immune cell [14].The immune system is capable of affecting both innate and adaptive immunity involving the pathogenesis of malaria caused by infection leading to the appearance of symptom of varying severity [15][16].However, parasitemia is brought under control by regulatory cytokine such as interleukin-10 and transforming growth factor that causes further reduction in the severity of disease [17].

Cytokines like tumor necrosis factor (TNF), interleukin-12 and interleukin-18 stimulate immune response to produce macrophage which destroy parasite by phagocytes, other cytokine such as interleukin-10, interlukin-4 and tumor growth factor (TGF) both cushen the effect of immune system by immune regulation [18].The role of cytokine as well as the stability of pro and anti-inflammatory cytokines is of utmost importance in immune modulation and pathogenesis of severe falciparum malaria, in adolescent alteration could lead to disease condition[19]. Pro inflammatory cytokines, act in specific cytokines inhibition that regulate the immune system responses, while anti-inflammatory cytokines promote healing and reduces inflammation.

Immune response due to pro-inflammatory cytokine result in classical symptoms of malaria fever [20]. Increase in the level of cytokine such as interleukin-6, interlukin-10, interleukin -12 and tumor necrosis factor (TNF) are related to severity of *Plasmodium falciparum* infection [21].In adolescent, natural immunity is developed, characterized by decrease of severity of the disease incidents found in higher level of parasiteamia[22]. These acquire immunity protects and keeps malaria asymptomatic or uncomplicated in adolescent due to type1 and type 2 producing T cells in pathogenesis of malaria infection [23].

In this study, cytokines such as interleukin-6, interleukin-8, interleukin-10, and tumor necrosis factor (TNF) were assessed in other to detect the changes in cytokines responses in *Plasmodium falciparum* infection amongst adolescents in Port Harcourt.

Materials and Methods

Study Design

Observational longitudinal design was used in this study.

Study Area

The study was carried out at (NNPC) Akpajo, Port Harcourt, Rivers State with geographical coordinate of 4.819^0 N, 7.0900^0 E. Port Harcourt is the state capital of Rivers state situated in Niger delta of southern part of Nigeria. It lies along the bonny rivers of 41 miles (66 kilometers upstream from the Gulf of Guinea.[24].

Study Population

The study was limited to adolescent male and female subjects aged 11-17years, infected with *Plasmodium falciparum* malaria in Port Harcourt as study group and non-plasmodium falciparum infected adolescent in Port Harcourt as control groups.

Inclusion and Exclusion Criteria

Male and female adolescent age of 11-17years who tested positive for *Plasmodium falciparum* served as the study group while those who tested negatives who had not been exposed to malaria was used as control, also those who were over the age of 17years and those suffering from other disease conditions were excluded from the study.

Sample Size Calculation

G-power version 2.0.10 was used to calculate the sample size; with parameters such as error of probability at 0.05, power (1-β error) at 0.95 (95%), and effect size of 0.5. This yielded sample size of sixty-one (61) adolescent infected with *Plasmodium falciparum* and thirty (30) apparently healthy adolescents without *Plasmodium falciparum* giving a sum total of ninety-one (91) subjects

Sample Collection

5 milliliters of blood were collected from test and control subjects, 3milliliters was dispensed into ethyl diamine tetra acetic acid (EDTA) anticoagulant for the analysis of full blood count and malaria parasite, the remaining 2 milliliters was dispensed into the plain container and was used for cytokine profile. The control subjects were apparently healthy adolescent who had not been exposed to malaria for the past one month.

Methods of Analysis

Determination of *Plasmodium falciparum* Infection

Three methods of malaria parasite identification were employed: Rapid diagnostic test (RDT) as decribed by (WHO, 2010; Akdis *et al.*, 2016), Quantitative buffy coat (QBC) and Microscopy (Thick blood film**)** as described by[25].

Determination of Interleukin-6

This was done using Enzyme linked-immunosorbent assay (ELISA) commercially available Elisa kits specific for research purpose from (Elabscience Biotechnology Inc). Catalog NO: E-EL-H0102, Lot NO: AK0017SEP13031, expiry date on 2018/12 was used according to manufacturer direction and specification. (Elabscience.com).

Determination of Interleukin-8

Sandwich Enzyme linked-immunosorbent assay (ELISA) commercially available Elisa kits specific for research purpose from (Elabscience Biotechnology Inc). Catalog NO: E-EL-H0110, Lot NO: AK0017SEP13035, expiry date on 2018/12 was used according to manufacturer direction and specification. (Elabscience.com).

Determination of Interleukin-10

Sandwich Enzyme linked-immunosorbent assay (ELISA) commercially available Elisa kits specific for research purpose from (Elabscience Biotechnology Inc). Catalog NO: E-EL-H0111, Lot NO: AK0017SEP13033, expiry date on 2018/12 was used according to manufacturer direction and specification. (Elabscience.com).

Determination of Tumor Necrosis factor alpha (TNF alpha)

This was done using Enzyme linked-immunosorbent assay (ELISA) commercially available Elisa kits specific for research purpose from (Elabscience Biotechnology Inc). Catalog NO: E-EL-H0109, Lot NO: AK0017SEP13034, expiry date on 2018/12 was used according to manufacturer direction and specification. (Elabscience.com).

Statistical Analysis

The statistical analysis was performed using the graph pad prism, Microsoft window 7 and Microsoft excel. Results obtained were presented in Charts, Graphs and Tables. Normality of data was tested by Kolmogorov-smirnov test. Data description was presented as means ± standard deviation (SD). (P-value) of <0.05 was considered significant and 95% confidence intervals (CI) were calculated.

Results

Demographic Details of Study Population

Total of ninety-one (91) adolescent participated in this study, comprises of sixty-one (61) subjects with mild to moderate *Plasmodium falciparum* infection and thirty (30) control subjects apparently healthy adolescent who had not been exposed to *Plasmodium infection* for one month. There are forty-nine (49) males and forty-two (42) females recruited in this study population within the age range of thirteen (13) to seventeen (17) years with mean age of 13.72. The adolescents were residing in Port Harcourt. Table 1.1 shows the demographic details of the study population.

Table 1.1 Demographics Details of the Study Population

Items	Status
No. of subjects	91
No. of test group	61
No. of Control	30
Males	49
Females	42
Age range of subjects	
Years	13 – 17
Mean age	13 – 72

Table 1.2 shows cytokine profile of study population at baseline there were statistically significant increase in the mean values of tumor necrosis factor (TNF), interleukin -6, interleukin -8 and interleukin-10 (P>0.05) of test group compared to control group at baseline, portraying the active involvement of macrophages the main site of cytokine production.

Table 1.2 Cytokines Profile of Study Population at Baseline

	TNF pg/ml	IL6 pg/ml	IL8pg/ml	IL10pg/ml
Control N=30	27.8 ± 39.41	32.51 ± 41.94	24.5 ± 55.81	4.31 ± 6.82
test N= 61	41.47 ± 68.15	63.8 ± 11.1	61.01 ± 16.7	5.81 ± 1.87
p-value	0.230	0.028	0.064	0.340

NS – Non-Significant P > 0.05, S - Significant P<0,05

Table 1.3 shows comparison of cytokine profile of male and female at baseline. There was statistically significant increase in mean values of tumor necrosis factor (TNF) and interleukin -8 in male (P < 0.02, P < 0.03) respectively. Whereas the female shown statistically significance increase in the mean value of interleukin -6 and interleukin -10 (P > 0.05) respectively.

Table 1.3 Comparison of Mean ± SD of Cytokines Profile of Male and Female at Baseline

	TNFpg/ml	IL6pg/ml	IL8pg/ml	IL10pg/ml
Female Test n= 27	21.54 ± 8.78	88.14 ± 16.7	13.86 ± 6.41	7.70 ± 4.7
Male N=34	57.3 ± 8.83	44.46 ± 35	98.45 ± 21.8	4.36 ± 2.7
p-values	0.025	0.177	0.031	0.132

NS – Non -Significant P > 0.05, S - Significant P <0.05.

Table 1.4 display comparison of cytokine response of malaria infected subject after treatment, there was statistically significant decrease in mean values of tumor necrosis factor (TNF), interleukin-6 (IL-6), interleukin-8 (IL-8) before treatment compared to after treatment. P < 0.05. However, there was statistically significant increase in the mean value of interleukin-10 (IL-10) after treatment P >0.05.

Table 1.4 Comparison of Mean ± SD of Cytokines Response of Subjects with Malaria Following Treatment

	TNF pg/ml	IL6pg/ml	IL8pg/ml	IL10pg/ml
Before treatment N= 61	41.47 ± 68.15	63.8 ± 11.1	61.01 ± 16.7	5.81 ± 1.87
After treatment n= 61	28.96 ± 8.93	22.89 ± 6.22	44.39 8.98	6.13 ± 3.6
p-values	0.042	0.005	0.025	0.838

NS - Non-Significant P > 0.05, S - Significant P<0,05.

Table 1.5 shows the effect of gender on cytokine level on malaria infected subject after treatment, there was statistically significantly increase in the mean values of tumor necrosis factor and interleukin -8 with P < 0.05 respectively. Whereas there was no statistically significant difference in interleukin-6 and interleukin -10 P>0.05 in the female compared to the male after treatment.

Table 1.5 Effect of Gender on Cytokine levels of Subjects After Treatment

	TNF pg/ml	IL-6 pg/ml	IL-8 pg/ml	IL-10 pg/ml
After treatment Female n = 27	15.37 ± 5.88	22.94 ± 16.97	10 ± 3.4	6.75 ± 3.11
After treatment Male N=34	41.29 ± 23.8	22.85 ± 7.2	75.55 ± 12.86	5.58 ± 3.1
p-values	0.002	0.987	0.0001	0.532

NS - Non-Significant P > 0.05, S - Significant P <0,05.

Discussion

In this study, there was significant increase in interleukin-6 in malaria infected subject with P < 0.05. There were also increase in the mean value for tumor necrosis factor, interleukin-8 in malaria and the mean value of interleukin-10 in infected subject compared to the control group, previous report specify that increase level of tumor necrosis factor (TNF), in malaria parasitized subjects is associated to marrow suppression, this has also been demonstrated in report that malaria infection is associated with circulating level of cytokine such as interleukin-6, tumor necrosis factor and interleukin-10.

Comparing the cytokine profile of subjects at baseline, the result reveal there was significant increase in tumor necrosis factor and interleukin -8 among infected male than female which reported increase in tumor necrosis factor and interleukin-10, from this study interleukin-10 was significantly low in malaria infected subject. This however commensurate with the strong positive correlation or association existing between tumor necrosis factor and interleukin-8 portraying increase in the parameter of tumor necrosis factor leading to increase in interleukin-8 vice versa. Other causes of increase in tumor necrosis factor in male could be due to male having high level of stress hormone especially cortisol and adrenaline, it may also be attributed to timely reporting to clinic, the female report early for better management of disease condition compared to male. However, increase in tumor necrosis factor, interleukin-6 and interleukin-8 production contributes to the pathogenesis of malaria consequently failure to down regulate inflammatory response leads to immune pathology.

The increase in tumor necrotic factor may be contributory to immune protection against malaria by limiting parasite progression, further clearance may be required to know the reason for low interleukin-10, which could lead to immune response. However, it has been observed in this study that anaemia is associated with reduction in interleukin-10. Investigating the pattern of changes in cytokine level, correlation matrix was used to determine the relationship between haematological and cytokine parameters their immunological using spearman's rank of correlation co-efficient with P< 0.05 being statistically significant. Various combination of variables was analyzed to identify the pattern of changes in cytokine profile demonstrated in pre and post treatment of malaria infected subject.

It was observed that interleukin-6, interleukin-8 and tumor necrosis factor were all significantly reduced after treatment, these significant

reductions demonstrate the activities of T helper cells which initiates immune response according to[26]. Cytokine profile and haematological relationship also shown that a strong positive correlation between mid-cell and interleukin-8, haematocrit and interleukin-8 respectively, this demonstrated an ongoing parasitaemic infection due to immune system response either in up regulation or down regulation.

Furthermore, it was observed that interleukin-10 is negatively correlated with white blood cell reason being that interleukin-10 inhibit the activities of other cytokines especially interleukin-6 and interleukin-8 and tumor necrosis factor as agree by the work done by [27].

Interleukin-10 was predominated in early anti – inflammatory response in *Plasmodium falciparum* infected subjects but drastically reduced following malaria treatment, its reduction correlates with recovery due to the relapse in clinical manifestation of malaria and disappearance of signs and symptoms, this agrees with previous work done by [27].However, anti-inflammatory cytokines have also been implicated in immune response against *Plasmodium falciparum*, interleukin-10 function as immunoregulator during malaria infection counteracting the effects of other cytokines produced by T-helper (Th)1 this report is in accordance with the findings of [26]. Presently evidence suggested that regulatory T cells are the main anti-inflammatory cells that are fatal in limiting inflammatory response whereas the pro-inflammatory play an important role in protection and clearance of parasite during malaria infection, the mutual effect of pro and anti-inflammatory acts as mediator in the final outcome of malaria as early or timely production pro-inflammatory cytokine such as tumor necrosis factor help in the reduction or limiting progression of disease from uncomplicated to complicated.

Conclusion

Cytokine response to *Plasmodium falciparum* provides indebt understanding in the pathophysiology and defense against malaria infection in adolescent the effect of cytokine released by the host immune cell are responsible for the symptoms of the disease. Studies has shown that cytokine are required in the protection against malaria. Cytokines are incriminated in different stages of *Plasmodium falciparum* infection, their mechanism of action could be beneficial or harmful, hence pro-inflammatory and anti- inflammatory cytokines are associated with disease severity thus the balance between should be regulated and maintained.

This study has evaluated the role of cytokine level such as tumor necrosis factor, interleukin-6, interleukin-8 and interleukin-10 in malaria among adolescent, which effect was seen in anaemia, lymphopenia, thrombocytopenia. Upon infection with malaria parasitise showed significant increase in cytokine profile except interleukin-10 which showed a very mild increase. However, after treatment the value for these cytokine levels were drastically reduced, very low reduction of interleukin -10 has a fatal outcome in malaria infection.

This study reveals several changes in cytokines such as tumor necrosis, interleukin-6, interleukin-8 which were all increased in malaria infection and could exacerbate disease condition.

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