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Evaluation of Plasma Levels of Interferon-Gamma, Interleukin-6, and Transforming Growth Factor-Beta in Under-Five Children with Malaria Parasitaemia

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Abstract

BACKGROUND: The interaction between the pro- and anti-inflammatory cytokines is known to play key roles in the immune response to infectious diseases. The pathogenesis of malaria parasitemia, including its progression to symptomatic manifestation, also seems to be strongly related to this interplay.

AIM: The study evaluated the plasma levels of interferon-gamma (IFN-γ) and interleukin-6 (IL)-6, which are pro-inflammatory cytokines, and transforming growth factor-beta (TGF-β), which is an anti-inflammatory cytokine; in the 6–60 months age-group children, when infected with *Plasmodium falciparum*, to show their relevance in the development of immunity against malaria.

METHODS: The study was a cross-sectional study involving children with uncomplicated malaria parasitemia. In the study, malaria parasitemia was confirmed by microscopy, using the Giemsa stain. The enzyme-linked immunosorbent assay (ELISA) method was used to evaluate the plasma levels of IFN-γ, IL-6, and TGF-β in the under-five children infected with *P. falciparum*, and their counterparts who were not infected with the parasite.

RESULTS: The median plasma IFN-γ, IL-6, and TGF-β levels in participants with malaria parasitemia were 225.15 pg/mL, 123.31 pg/mL, and 2091.02 pg/mL, respectively. The difference in the plasma levels of TGF-β in the infected and uninfected participants was statistically significant with a $p < 0.001$.

CONCLUSION: The findings in this work showed that malaria parasitemia in under-five children is associated with significant depression in the plasma level of TGF-β when compared to their uninfected counterparts.

Introduction

Malaria is a protozoal infection caused by certain species of *Plasmodium* (Ghosh and Stumhofer, 2021). Globally, in 2021, there was an estimated incidence of about 247 million malaria cases in 84 malaria-endemic countries, with the greater number coming from the WHO African Region. Four countries in the sub-Saharan Africa, with Nigeria inclusive, accounted for 48% of malaria cases globally [1]. In 2021, malaria death in Africa was estimated at 593,000; with children younger than 5 years contributing 76% of the mortality. Thus, the disease is ranked by the WHO as the largest single component of disease burden in Africa [1].

The six species of Plasmodium known to cause malaria in humans are: *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium ovale curtis*, *Plasmodium ovale wallikeri*, *Plasmodium malariae*, and *Plasmodium knowlesi*, and are transmitted by the bite of infected female *Anopheles* mosquitoes which are widely distributed throughout Africa, Asia, and Latin America [2]. However, *P. falciparum* which, incidentally, is also the main cause of severe malaria, is also the most prevalent of the parasites on the African continent and responsible for most malaria deaths globally [3].

In the sub-Saharan African countries, where the rate of transmission of malaria is quite high, children are particularly vulnerable to severe complications of the infection, with its accompanying high morbidity

and mortality [4]. However, outside these areas of high endemicity of the infection, where the populations do not acquire significant immunity, the risk of the disease is spread across all age groups and depends on the level of exposure to bites from infected female *Anopheles* mosquitoes [5], [6].

Various studies have shown that mediators of inflammation are important in explaining the pathogenesis of many infectious diseases, with malaria inclusive [7], [8], [9]. Elevated plasma levels of the pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), are often associated with severe forms of malaria, suggesting that these mediators play a significant role in the pathogenesis of the disease [8]. However, TNF-α may contribute to the protective immune response to malaria by its induction of fever, which is detrimental to the malaria parasite development, and helpful in stimulating effector cells [9]. Adequate production of anti-inflammatory cytokines such as interleukin (IL)-10, is however, required to regulate TNF-α production and prevent pathologic consequences; thus showing that IL-10 can also play a protective role in children with severe malaria [10], [11].

Hence, the clinical course of severe malaria is influenced by the relative imbalance between the pro- and anti-inflammatory cytokines levels [12]. In the quest for a possible potent vaccine against malaria parasitemia, to reduce or eliminate the morbidity and mortality resulting from *P. falciparum* infection, a more critical review of the interplay between these cytokines in malaria endemic zones could go a long way in actualizing this goal.

This study was designed to evaluate the plasma levels of two pro-inflammatory cytokines-interferon-gamma (IFN-γ) and IL-6 and one anti-inflammatory cytokine-transforming growth factor-beta (TGF-β), in under-five Nigerian children infected with *P. falciparum*; in attempt to show any relationship between the plasma levels of the cytokines and age and gender differences in them. It is hoped that the findings could aid in designing a potent malaria vaccine.

Materials and Method

Study area

The study was carried out at the children out-patient clinic (ChOPC) of Federal Teaching Hospital Abakaliki (FETHA) with Children on Clinic visitation and with pupils of Redeemer's International School Abakaliki (RISA). Both facilities are located in Abakaliki Local Government Area of Ebonyi State, South-Eastern Nigeria. Ebonyi State shares a border with Benue State to the north, Enugu State to the west, Imo and Abia States to the south, and Cross River State to the east.

FETHA, being a tertiary health facility, provides specialized tertiary health care services, with ChOPC having between 100 and 160 children in attendance, on a daily basis.

Study participants

Children aged between 6 and 60 months, who presented at the ChOPC of the hospital, and apparently healthy-looking pupils from RISA, were enrolled, with consent from their caregivers, after detailed information on what the study was all about.

Study design

The study was a cross-sectional descriptive study, carried out between August and September 2018.

Inclusion criteria

All children aged between 6 months and 60 months with febrile illness and any healthy-looking child within the age bracket (as controls).

Exclusion criteria

Severely malnourished child/Grade III malnutrition, using Gomez's Classification i.e. 60% or less of Weight for Age [13]; Child with confirmed immunosuppressive disease, such as HIV/AIDS, Malignancy, Measles, Tuberculosis, cough of more than 2 weeks and Child with burns.

Ethical consideration

Ethical Approval for the research was obtained from the Research Ethics Committee of the Federal Teaching Hospital, Abakiliki (FETHA). Only the children whose parent/guardian gave their consent were enrolled in the study. Confidentiality was maintained throughout the study. Caregivers were privy to the results of the investigations carried out on their wards if they wished. As a form of incentive, children who tested positive for malaria infection were given free-malaria-treatment with Artemisinin-based Combined Therapy, following the dose prescription by the resident doctor at the Clinic, whereas, those that tested negative were given haematinic syrup.

Sample collection

The Nurses at the ChOPC of FETHA assisted in taking the biophysical profiles of the children, during the registration. A total of 2 ml of blood sample was collected from each enrollee in the survey, throigh venepuncture, and was promptly transferred into the sample bottle which contains ethylene diamine tetraacetic acid. Each

batch of samples collected was taken to the Research laboratory of FETHA for processing, within 20 min of their collection from the participants. The sample collection spanned from August 6th to 22nd, 2018.

Laboratory procedures

In the laboratory, for each specimen, blood films (thick and thin films) for malaria parasite microscopy were quickly made on the glass slides, for malaria parasite identification. The remaining sample is centrifuged for 15 min, and the supernatant (plasma) is separated into a plain specimen bottle, and then frozen at –20°C. The frozen plasma specimens were transported, under cold chain maintenance, to Ohize Medical Center, Benin City, Edo State in Nigeria, where the cytokine analysis took place, using the commercial standard enzyme-linked immunosorbent assay (ELISA) kits.

The Malaria Parasite Microscopy

Making a thin blood film (for intracellular parasite identification)

A drop of blood sample (using a capillary tube) was placed on each properly identified dry glass slide. The blood drop was immediately spread using a smooth-edged slide-spreader and allowed to air-dry. Afterward, the slide was placed horizontally on a staining rack and a drop of methanol was applied. This was allowed for 2 min to fix the thin blood film.

Making a thick blood film (for specie identification)

Twenty-five microlitres of blood sample (using an automatic pipette) was applied on properly identified clean slide. The blood sample was gently spread to make the thick smear, which evenly covered an area of about 15 × 15 mm on each slide. With the slide in a horizontal position, it was allowed to thoroughly air-dry.

Staining of the slides

The slides (both the thick and thin blood films) were faced upwards, supported on two rods in a staining trough, flooded with 10% Giemsa stain solution, and allowed for 10 min. Afterward, clean water was used to flush the stain from the slides. The back of the slides were wiped clean and placed in a draining rack to air-dry, before viewing under the microscope at ×100 (oil immersion).

Statistical analyses

The data collected were analyzed using Statistical Package for Social Sciences (SPSS) version 23. The study population was partitioned into 5 age groups: 6–11 months, 12–23 months, 24–35 months, 36–47 months, and 48–59 months. It was also partitioned according to sex. The malaria infected and the uninfected (control) populations were also, separately, partitioned according to age and sex. The median plasma levels of INF-γ, IL-6, and TGF-β for the infected and uninfected groups were determined, after which a comparison of the plasma levels of the cytokines in both groups was done using Mann–Whitney U test. Analyses for age-related differences in plasma levels of INF-γ, IL-6, and TGF-β in the population with malaria parasitemia were done using the Chi-square test of independence. Mann–Whitney U test was also used to determine the differences in the plasma levels of INF-γ, IL-6, and TGF-β, in relation to the sex, among the population infected with malaria. Analyses for correlations between plasma levels of INF-γ, IL-6, and TGF-β and age in the infected population were done using the Pearson's Correlation Test. Positive Pearson's correlation coefficients, (+ r-values) were taken as positive correlation, and negative Pearson's correlation coefficients, (- r-values) were taken as negative correlation. A probability value (p-value) of < 0.05 was considered to be statistically significant.

Results

Sample characteristics

A total of 89 children, aged between 6 months and 59 months were enrolled in the survey. Analyses for age distribution showed 10 were within 6–11 months age group, 18 were within 12–23 months, 27 were between 36 and 47 months, and 17 were between 48 and 59 months (Table 1). For the sex distribution, 42 were female, while 47 were male; with female to male ratio of 1:1.1 (Figure 1).

Table 1: Age distribution of all the participants, showing the infected and uninfected population

Age (months)	Participants (n)	Infected, n (%)	Uninfected, n (%)
>6–11	10	5 (10.0)	5 (12.8)
12–23	18	8 (16.0)	10 (25.6)
24–35	27	16 (32.0)	11 (28.2)
36–47	17	7 (14.0)	10 (25.6)
48–59	17	14 (28.0)	3 (7.7)
Total	89	50 (100.0)	39 (100.0)

Malaria parasitaemia

The presence of the malaria parasite was noticed in 50 out of the 89 samples collected from the study population, as shown in Table 1. The 39 children without malaria parasitemia were used as a control group in the study. The age distributions of the infected and uninfected groups are both illustrated in Figure 1. The age group of 24–35 months had the highest number of children (16 children) with malaria parasitemia, whereas,

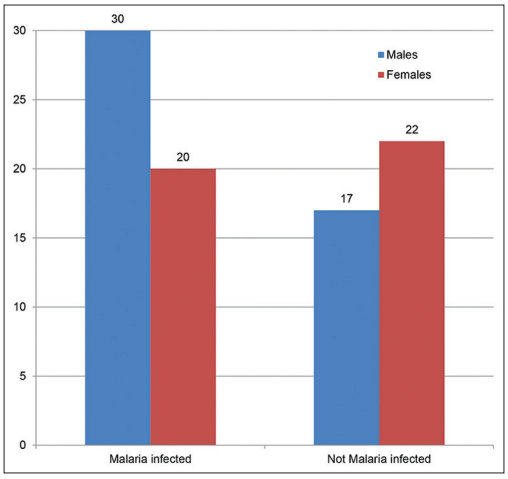

Figure 1: 3-D cones showing sex distribution of the participants

children aged between 6 and 11 months had the least (5 children). For the uninfected population (control group), the age group of 24–35 months, also had the highest number of children (11 children), whereas, children aged between 48 and 59 months had the least (3 children).

The sex distribution of the infected and uninfected population

The males appeared to be more infected with the malaria parasites than the female population; with a total of 30 out of the 50 infected participants being males. The proportion of the affected males was 30 out of the 47 male participants (i.e., 63.8% of the male participants), unlike the females in which out of the 39 participants, 20 had malaria parasitemia, (i.e. 51.2% of the female participants). These are illustrated in Figure 1.

Description of the cytokines levels

For the population with malaria parasitemia, the median levels for INF-γ, IL-6, and TGF-β were 225.15 (110.84–549.15) pg/mL, 123.36 (53.55–168.25) pg/mL, 2091.02 (1182.76–3685.17) pg/mL. For the population not infected with malaria, the mean levels for INF-γ, IL-6, and TGF-β, were 178.86 (107.61–272.70) pg/mL, 65.78 (37.60–180.71) pg/mL, and 4813.74 (2013.51–6414.23) pg/mL. These are shown in Table 2.

Table 2: The cytokines levels in the infected and uninfected population

Cytokines	Infected group (n = 50), median	Uninfected group (n = 39), median
IFN-γ (pg/mL)	225.15 (110.84–549.15)	178.86 (107.61–272.70)
IL-6 (pg/mL)	123.36 (53.55–168.25)	65.78 (37.60–180.71)
TGF-β (pg/mL)	2091.02 (1182.76–3685.17)	4813.74 (2013.51–6414.23)

IFN-γ: Interferon-gamma, IL-6: Interleukin-6, TGF-β: Transforming growth factor-beta.

Comparison of the plasma levels of the cytokines in those with malaria parasitaemia and those without malaria parasitaemia

Mann–Whitney U test was used to compare the medians of the levels of INF-γ, IL-6, and TGF-β in children with malaria parasitemia and those without malaria parasitemia. The difference in the mean plasma levels of TGF-β was significant with a $p < 0.001$, whereas IFN-γ and IL-6 levels were not, with the $p = 0.081$ and 0.179, respectively, as shown in Table 3.

Table 3: Mann–Whitney u-test comparing the levels of cytokines in the infected and uninfected groups

Cytokines	Mann–Whitney u	Wilcoxon w	z	p
IFN-γ	765.000	1545.000	−1.737	0.082
IL-6	812.500	1592.500	−1.344	0.179
TGF-β	516.500	1791.500	−3.791	0.000*

*p < 0.001. IFN-γ: Interferon-gamma, IL-6: Interleukin-6, TGF-β: Transforming growth factor-beta.

Discussion

Evaluation of the IFN-γ

From this study, it was observed that the plasma level of this pro-inflammatory cytokine was higher in the population with malaria parasitemia than in the uninfected participants. Meanwhile, the difference between the plasma levels of the cytokine in the infected group and the uninfected (control) group was not statistically significant. A study carried out among Ghanaian children, aged below 15 years, however, showed a significant increase in the level of IFN-γ in children with malaria parasitemia, when compared with the uninfected control group [12]. This significant difference could be as a result of the incorporation of children with submicroscopic infections, which they detected with the aid of polymerase chain reaction (PCR) technique [12].

The age-related difference in plasma levels of IFN-γ in participants with malaria parasitemia was not significant and this was in keeping with the study in Ghana, by Frimpong *et al.*, among under-15 children with asymptomatic malaria; where they observed no significant increase in serum level of IFN-γ with increase in age [12].

The median plasma level of IFN-γ in the males with the parasitemia appeared lower than those of their female counterparts. However, the difference in the plasma levels of the cytokine, when compared was not significant.

Evaluation of the IL-6

The plasma level of plasma IL-6 in the population with malaria parasitemia appeared higher than that of those without the parasite. However, the difference between the plasma levels of the cytokine in the infected and the control group was not significant. This was in contrast to a work carried out in Gabon, among children (aged between 6 and 168 months) infected with malaria, where malaria parasitemia was found to be associated with a significant increase in the level IL-6 [14]. A similar study carried out in Ghana among under-15 children, also showed a significant increase in the level of IL-6 when compared with the uninfected control

group [12]. However, this significant difference could still be a result of the incorporation of children with submicroscopic malaria infections, which they detected with the aid of PCR technique [12].

The age-related differences in the plasma level of IL-6 in infected children were not significant. This was not in keeping with the studies by Farrington *et al.*, which showed that younger children, infected with malaria, exhibited much higher levels of IL-6 compared to older ones [15].

The difference in the plasma level of IL-6 in those with malaria parasitemia, with respect to sex, was not statistically significant.

TGF-β

In this study, the plasma level TGF-β in the infected population was found to be lower than that in the uninfected/control population. The difference in the plasma levels was very significant. This was in keeping with findings in Ugandan children, where serum TGF-β level was significantly diminished in uncomplicated malaria cases when compared with the healthy control group, and even further decreased in cerebral malaria cases [16]. Furthermore, similar to this was the finding from the meta-analysis carried out by Kotepui *et al.*, comparing the levels of TGF-β in patients with uncomplicated malaria and healthy control group, which showed that the patients with uncomplicated malaria had lower TGF-β levels than the uninfected/control group [17]. This shows that elevation of this anti-inflammatory cytokine may be associated with the conferment of some sort of protection against *P. falciparium* malaria, whereas its decrease may be associated with increased susceptibility to the parasitemia, due to reduction in the immune-modulatory activities of the TGF-β, and consequent exaggeration of the activities of the pro-inflammatory cytokines. Thus, showing that with proper modulation of the activities of the pro-inflammatory cytokines by immune-modulatory cytokines, like the TGF−β, early clearance of parasites, at the pre-patent/pre-symptomatic stages of malaria parasitemia, that is as early as the end of the hepatic phase (exoerythrocytic phase) or early blood phase is obtainable [18]. This can also explain why the apparently healthy children, who were also living in the same malaria endemic area, but with relatively higher plasma TGF-β, did show any malaria parasitemia, possibly due to the clearance of the parasites at the exoerythrocytic phase or merozoites formation in the red cells.

The age-related difference in the plasma level of TGF-β in infected children was not significant, and the difference in the plasma level of TGF-β in those with malaria parasitemia, with respect to sex, was also not statistically significant.

Summary

In this study, children of age 24–35 months accounted for the highest number of children with malaria parasitemia, whereas, children aged between 6 and 11 months had the least. The study also showed a higher incidence of malaria parasitemia in males than the females. The plasma level of the pro-inflammatory cytokines, under study, (INF-γ and IL-6) in the group with malaria parasitemia appeared higher than that in the level of IFN-γ in the control group, though the difference was not statistically significant. However, the level of the anti-inflammatory cytokine (TGF-β), was significantly higher in the uninfected group than in the infected group. There was no age or sex-related differences in the levels of the cytokines in the children with malaria parasitemia.

Conclusion

The findings in this work showed that, not withstanding the malaria endemicity in Nigeria, under-five Nigerian children infected with *P. falciparum* exhibit statistically significant depression in the plasma level of TGF-β, and some elevations (though, not statistically significant) in the levels IFN-γ and IL-6, when compared to the uninfected population. These early cellular cytokines responses to the parasitemia, may be important correlates of immunity and risk of symptomatic malaria episodes.

Limitations

- Not determining the parasite density.
- Not determining the submicroscopic infections using PCR.

Recommendations

From the results obtained the following recommendations are made:

1. These findings can be of help in the development of an effective malaria vaccine.
2. Further research of this sort determined the inflammatory cytokines levels in other common inflammatory diseases, among these age groups (6–60 months) and relating the levels to those with complicated and those with uncomplicated malaria.
3. A similar but more robust research involving young adults, who are seemingly not prone to severe forms of *P. falciparum* malaria.
4. An animal study that involves the incorporation of an inducer of TGF-β, as an adjuvant in the development of malaria vaccine.

References

1. World Health Organization. World Malaria Report 2022. Geneva: World Health Organization; 2022. Available from: https://www.who.int/teams/global-malaria-program [Last accessed on 2023 Jan 25].
2. Lalremruata A, Jeyaraj S, Engleitner T, Joanny F, Lang A, Belard S, *et al*. Species and genotype diversity of *Plasmodium* in malaria patients from Gabon analysed by next generation sequencing. Malar J. 2017;16(1):398. https://doi.org/10.1186/s12936-017-2044-0
   PMid:28974215
3. World Health Organization. Guidelines for Malaria. Geneva: World Health Organization; 2022. Available from: https://apps.who.int/iris/handle/10665/351995 [Last accessed on 2023 Jan 25].
4. Awosolu OB, Yahaya ZS, Haziqah MT, Simon-Oke IA, Fakunle C. A cross-sectional study of the prevalence, density, and risk factors associated with malaria transmission in urban communities of Ibadan, Southwestern Nigeria. Heliyon. 2021;7(1):e05975. https://doi.org/10.1016/j.heliyon.2021.e05975
   PMid:33521357
5. White M, Watson J. Age, exposure and immunity. Elife. 2018;7:e40150. https://doi.org/10.7554/elife.40150
   PMid:30129437
6. Sato S. *Plasmodium*-a brief introduction to the parasites causing human malaria and their basic biology. J Physiol Anthropol. 2021;40(1):1. https://doi.org/10.1186/s40101-020-00251-9
   PMid:33413683
7. Sebina I, Fogg LG, James KR, Soon MS, Akter J, Thomas BS, *et al*. IL-6 promotes $CD4^+$ T-cell and B-cell activation during *Plasmodium* infection. Parasite Immunol. 2017;39(10):e12455. https://doi.org/10.1111/pim.12455
   PMid:28748530
8. Kurup SP, Butler NS, Harty JT. T cell-mediated immunity to malaria. Nat Rev Immunol. 2019;19(7):457-71. https://doi.org/10.1038/s41577-019-0158-z
   PMid:30940932
9. Mohamedahmed KA, Abakar AD, Ahmed MO, Muktar MM, Nour BY. The role of TNF-α level as predictive diagnostic biomarker among children with severe falciparum malaria in endemic area in Sudan. Int J Acad Health Med Res. 2019;3(7):1-6.
10. Ortega-Pajares A, Rogerson SJ. The rough guide to monocytes in malaria infection. Front Immunol. 2018;9:2888. https://doi.org/10.3389/fimmu.2018.02888
    PMid:30581439
11. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. Int J Mol Sci. 2019;20(23):6008. https://doi.org/10.3390/ijms20236008
    PMid:31795299
12. Frimpong A, Amponsah J, Adjokatseh AS, Agyemang D, Bentum-Ennin L, Ofori EA, *et al*. Asymptomatic malaria infection is maintained by a balanced pro-and anti-inflammatory response. Front Microbiol. 2020;11:559255. https://doi.org/10.3389/fmicb.2020.559255
    PMid:33281757
13. Ann A. Nutrition, food security and health. In: Kliegman R, editor. Nelson Textbook of Pediatrics. 20th ed. Philadelphia, PA: Elsevier Inc.; 2015. p. 295-306.
14. Oyegue-Liabagui SL, Bouopda-Tuedom AG, Kouna CL, Maghendji-Nzondo S, Nzoughe H, Tchitoula-Makaya N, *et al*. Pro-and anti-inflammatory cytokines in children with malaria in Franceville, Gabon. Am J Clin Exp Immunol. 2017;6(2):9-20. https://doi.org/10.1051/parasite/2016032
    PMid:28337387
15. Farrington L, Vance H, Rek J, Prahl M, Jagannathan P, Katureebe A, *et al*. Both inflammatory and regulatory cytokine responses to malaria are blunted with increasing age in highly exposed children. Malar J. 2017;16(1):499. https://doi.org/10.1186/s12936-017-2148-6
    PMid:29284469
16. Hanisch BR, Bangirana P, Opoka RO, Park GS, John CC. Thrombocytopenia may mediate disease severity in *Plasmodium falciparum* malaria through reduced transforming growth factor beta-1 regulation of proinflammatory and anti-inflammatory cytokines. Pediatr Infect Dis J. 2015;34(7):783-8. https://doi.org/10.1097/INF.0000000000000729
    PMid:25886788
17. Kotepui KU, Kwankae w P, Masangkay FR, Mahittikorn A, Kotepui M. Transforming growth factor-β concerning malarial infection and severity: A systematic review and meta-analysis. Trop Med Infect Dis. 2022;7(10):299. https://doi.org/10.3390/tropicalmed7100299
    PMid:36288040
18. Kurup SP, Obeng-Adjei N, Anthony SM, Traore B, Doumbo OK, Crompton PD, *et al*. Regulatory T cells impede acute and long-term immunity to blood-stage malaria through CTLA-4. Nat Med. 2017;23(10):1220-5. https://doi.org/10.1038/nm.4395
    PMid:28892065