Pro- and anti-inflammatory response profile modulates Plasmodium falciparum malaria outcomes among subjects from Baiyeku, Ikorodu, Lagos, Nigeria.

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Abstract

**Background** Available evidence indicates that the various stages of the malaria parasite life cycle elicit specific immune responses of which the relative levels of pro-inflammatory cytokines are key to disease progression, killing the parasite and mediating disease outcomes. This study will inform immunological interventions against malaria and thus malaria vaccine developments programs/efforts.

**Methods** A total of four hundred and sixty-two participants were screened in a community survey for *Plasmodium falciparum* (*P. falciparum*) malaria in Baiyeku, Lagos, Nigeria. *P. falciparum* parasitaemia was determined by Microscopy using thick and thin blood films stained by Giemsa method using World Health Organization parasitology laboratory protocol whilst the serum levels of IL-10, IFNγ and TNFα were determined by Enzyme linked immunosorbent assay [ELISA].

Data analysis was done by One-way Analysis of Variance (ANOVA), Chi square (*X²*) and Student’s T-test in statistical package for the social sciences (SPSS) version 24 was used to test statistical significance between the symptomatic groups and asymptomatic in relation to age, gender and BMI of the participants.

**Results** A total of 70 (15.2 %) participants were microscopically positive for *P. falciparum* of which 70% were female, 30% were males while children aged 1-17 years were 65.7%. The geometric mean parasite density (GMPD) was significantly (*p*=0.001) higher among females than males. The GMPD of participants < 5 years was also significantly (*p*=0.001) higher than other age groups. About 46.8% of the participants were underweight (BMI < 18.5) also had the highest parasite intensity. The TNFα, IFNγ and IL-10 levels were significantly (*p* < 0.05) higher in the infected than the uninfected participants. IFN-γ values were significantly (*p*=0.014) elevated among the symptomatic than the asymptomatic participants while there was no significant difference (*P*>0.053) in the levels of TNF-α and IL-10 (*P*>0.093) between the symptomatic and asymptomatic participants. Notably, the IL-10 levels were the most elevated amongst the participants with the highest parasite density.

**Conclusion** The prevalence of *P. falciparum* obtained in this study area which is endemic for malaria is 15.2% suggesting a significant reduction of the disease over time. The awareness of the disease which is now more than before seems to contribute to the lowering of prevalence of the disease in the community. There was a positive relationship between TNF-alpha levels and body temperature. However, compared with the anti-inflammatory cytokine (IL-10) in this study, the levels of the pro-inflammatory cytokines (IFN-γ and TNF-α) were lower due to the negative action of the anti-inflammatory cytokines. IL-10 value increased as parasitemia increased (*p*=0.073). These findings suggest that higher levels of anti-inflammatory cytokines, especially IL-10 levels may contribute to pathogenesis of uncomplicated malaria.

**Background**
Available evidence indicates that the various stages of the malaria parasite life cycle elicit immune responses [1, 2]. The pro-inflammatory cytokines tend to play an important role in preventing malaria and killing the parasites. Furthermore, the relative levels of pro- and anti-inflammatory cytokines are essential mediators of malaria anemia production and outcomes [3]. Early production of pro-inflammatory T-helper 1 (Th1) cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-12 and interferon (IFN)-gamma may limit progression to severe complications from uncomplicated malaria. They actually limit the growth of parasites and stimulate monocyte phagocytosis to improve clearance of parasitized erythrocytes while IL-6 is a major mediator of the acute phase response [3, 4]. Often involved in the immune response to *Plasmodium* are other pro-inflammatory cytokines such as IL-17 and IL-22, developed by other cell subtypes including Th17 cells [5]. An increase in peripheral blood IL-17-producing CD4+ T cells has been reported during *P. vivax* infection, along with the production of the pro-inflammatory cytokines IFN-γ, IL-10 and the transforming growth factor (TGF)-beta [5]. During murine infection IL-22 activation defends against liver damage [6]. However, if these pro-inflammatory responses during the acute infection are not properly regulated, severe malaria complications may arise [7, 8]. Hence, the need for anti-inflammatory responses to regulate the development of pro-inflammatory cytokines and subsequent cytopathic consequences. Regulatory cytokines such as IL-10 play a major role in infection with *Plasmodium*, neutralizing excessive development of inflammatory Th1 cytokines [9, 10]. Anti-inflammatory Th2 cytokines including IL-4 and IL-13 control the humoral immune response, leading to the clearance of parasites and inhibiting the development of Th1 cytokines [11, 12].

Given the significance of the development of pro-and anti-inflammatory cytokines in the human immune response to infection with *P. falciparum* malaria, this is not well established in Nigerians.

**Methods**

**Description of Study Area**

Baiyeku is a rural settlement with favorable ecological features for all year round transmission of malaria. It is an ancient town in the Ikorodu Local Government Area located along the Lagos Lagoon on Latitude 6° 37’ 0”N and Longitude 3° 36’ 30”. Baiyeku shares boundary with Ogun State and is inhabited by about 800, 000 people [From 2006 census]. Baiyeku has a variety of ethnic groups. The predominant languages spoken are Yoruba and English. The majority of the people are subsistence farmers, petty traders or fishermen. The population consists of Muslims, Christians and traditionalists [Fig. 1].

**Study Design And Sample Collection**

This study was a cross-sectional prospective study involving community survey of *Plasmodium falciparum* malaria in the study area of Baiyeku town in Ikorudu Local Government Area of Lagos State, Nigeria [Fig. 1]. The transmission of malaria in this area typically occurs all year round. Sampling was conducted at the peak of transmission (April to August, 2017).

The sample size for the study was derived using an anticipated population proportion of 50%, confidence level of 95% and absolute precision of 5 percent point. The sample size was calculated using the
following formula [13]:

\[ n = \frac{Z^2 \times P (1 - P)}{d^2} \]

Where \( z \) is the critical value of the standard normal distribution at 5% level (1.96), \( n \) is the minimum sample size, \( P \) is the anticipated population proportion and \( d \) is the absolute precision at 5 percentage point (0.05).

This gave an approximate sample size of 384 participants. A total of 462 were enrolled in the study.

**Ethical Approval**

Ethical approval of the study was obtained from Nigerian Institute of Medical Research, Institutional Review Board (NIMR-IRB).

Before the commencement of the study, the investigators visited the site and explained the study to the chief of the community who is the traditional ruler known as Baale. The Baale gave his consent and permitted the town announcer to announce our mission to the community. The Baale provided us with the venue (canopy, chairs, and tables). Interested persons in the community that came out for the survey were interviewed and recruited as participants in the study. Informed consent was obtained from the study participants. Socio-demographic data consisting of age, gender, height and weight as well as body temperature were obtained before venous blood samples were collected by standard phlebotomy for malaria parasites testing.

**Anthropometric Measurements**

Anthropometric data were obtained in accordance with internationally recommended procedures [14]. Weight measurements were taken using a scale (Tefal, Paris, France) precise to the nearest 100g. Recumbent length measurements were taken for children under 2 years of age, while standing height was measured for older participants using a stadiometer (SECA, Hamburg, Germany). BMI (Kg/m²) was calculated for the participants, those within the range 18.5–24.9 were grouped as normal, those below this range were categorized as underweight, those having BMI range of 25.0 – 29.9 were grouped as overweight while those who had BMI above 30 were categorized as obese. The body temperatures of the volunteers were obtained using non-contact infrared thermometer (Proactive Medical, NY, USA) at 5 cm away from the participant’s forehead. Participants with body temperatures below 37.5°C were asymptomatic participants whereas body temperatures 37.5°C or above were categorized symptomatic.

**3.2.2 Sampling and Analyses**
3ml of venous blood was obtained from each participant using standard phlebotomy practice and aseptic techniques for malaria diagnosis and immunoassay for (TNF-α), Interferon Gamma (IFN-γ) and Interleukin-10 (IL-10) by ELISA method. Thick and thin blood films for malaria diagnosis were made for each participant and examined under the light microscope for *P. falciparum* following the World Health Organization [14] procedure. Serum for ELISA assay was obtained by dispensing the blood into plain tube, allowed to clot and the serum separated [2]. Malaria parasite density MPD (Parasite per μl of blood) was calculated using the formula: \[ \text{Parasites per } \mu l = \frac{\text{number of parasites} \times 8000}{\text{number of leucocytes}}. \]

Levels of TNF-α, IFN-γ and IL-10 were assessed in sera of the study participants using antigen capture ELISA (Enzyme Linked Immunosorbent Assay) kits based on antigen-antibody reaction employing the manufacturer’s protocol. The concentrations were extrapolated from the standard curve obtained using values for the standards [15].

**Statistical Analysis of Data**

One-way Analysis of Variance (ANOVA) was used to analyze the group means. Chi square (χ²) statistics was used to test statistical significance between the symptomatic groups and asymptomatic in relation to age, gender and BMI of the participants. Where appropriate Chi-square (χ²) test was used to compare data sets generated. P value <0.05 was considered statistically significant. Geometric Mean Parasite Density (GMPD) for parasite density was determined using SPSS statistical software version 21. The geometric mean parasite density (GMPD) was estimated for the positive participants.

**Results**

**Demographic Profile of Participants**

A total of 462 participants, consisting of 136 (29.4%) males and 326 (70.6%) females, were screened for *Plasmodium falciparum* malaria in this study. Seventy of these were microscopically positive for *Plasmodium falciparum*, corresponding to a prevalence of 15.2% (Table 2). Seventy percent were females and 30% males while 65.7% were participants below 17 years of age and 34.3% were aged 18 years and above. Overall, the median age of the participants was 21 years.

**Table 1: Demographic profile of study population.**
Profile | Total
--- | ---
Number of participants tested for *P. falciparum* malaria | 462
Number of participants microscopically positive for *P. falciparum* malaria | 70
Prevalence of malaria (%) | 15.15%
Gender of participants (positives) | Male 21  
Female 49  
(Negatives) Male 441  
Female 413

Tests Age Groups

| Positive | <5 years | 10 | 5-9 years | 26 | 10-17 years | 10 | 18-60 years | 24 |

**Malaria parasite Density**

Geometric Mean Parasite density (GMPD) was significantly (p=0.001) higher among female participants than the males (Table 3) and among participants < 5 years (p=0.001) than other age groups (Table 2).

**Table 2: Malaria Parasite Density of Infected Participants**
| GROUP        | % (Frequency) | Geometric mean parasite density (GMPD) Parasite/uL | P-value |
|-------------|--------------|-----------------------------------------------|--------|
| Male        | 30 (21)      | 1148.459974 ± 3920.9515077                  | 0.001  |
| Female      | 70 (49)      | 2032.656172 ± 7711.7352504                  |        |
| <5years     | 14.3 (10)    | 3074.974498 ± 8889.8975496                  | 0.001  |
| 5-9years    | 37.1 (26)    | 1862.417209 ± 16966.2337278                 |        |
| 10-17years  | 14.3 (10)    | 1249.296051 ± 384.2184604                   |        |
| 18-60years  | 34.3 (24)    | 1259.127428 ± 471.4340281                   |        |

**BMI Status of Participants**

A total of 46.8% of the participants were underweight (Table 3). However, 27.3% had normal BMI (BMI=18.5 – 29.5) and 13.4% were obese.

**Table 3: BMI Status of Participants**
| STATUS         | Frequency | %    |
|----------------|-----------|------|
| BMI (kg/m²)    |           |      |
| Underweight    | 216       | 46.8%|
| (BMI <18.5)    |           |      |
| Normal         | 126       | 27.3%|
| (BMI 18.5-24.9)|           |      |
| Overweight     | 58        | (12.6%)|
| (BMI 25.0 – 29.9)|         |      |
| Obese          | 62        | (13.4%)|
| (BMI >= 30)    |           |      |
| Total          | 462       | (100%)|

Parasite Intensity in Different BMI Status.

Among the participants included in this study, the category with parasitemia ≥1,000 but 100,000 had more parasitemia than other BMI groups. The intensity of *Plasmodium falciparum* parasitemia was highest among the participants underweight BMI (Table 4).

**Table 4:** *Plasmodium falciparum* parasitemia & Intensity among different BMI Status.
Evaluation of Cytokine response of Tested Participants

Serum levels of TNF-Alpha, IFN gamma and IL-10 were determined for all the participants in this study. The levels of IFN gamma was highest among the cytokines tested in the participants (Table 5). The serum levels of all the cytokines (TNFα, IFNγ and IL-10) were significantly (p 0.05) higher in the infected than the uninfected participants (Table 6). The IL-10 levels were the most elevated amongst the participants with the highest parasite densities relative to TNF-α and IFN-γ, although not statistically significant. Table 7.

Serum levels of TNF alpha increased with increasing parasitemia (Table 8).

Table 5: Cytokine Values of Test Participants

| Cytokine       | Mean ± S.E.M |
|----------------|--------------|
| TNF-α (pg/ml)  | 1.98 ± 0.015 |
| IFN-γ (ng/ml)  | 2.46 ± 0.051 |
| IL-10 (pg/ml)  | 2.21 ± 0.054 |

n = 462

Table 6: Mean Cytokine Levels in Infected and Uninfected Participants

| GROUP | TNF-α (pg/ml) | IFN-γ (ng/ml) | IL-10 (pg/ml) |
|-------|---------------|---------------|---------------|
|       |               |               |               |
INFECTED (70)  
0.06 ± 0.028  
0.74 ± 0.072  
1.31 ± 0.103

UNINFECTED (392)  
0.01 ±0.006  
0.16 ± 0.022  
0.84 ± 0.036

P-value  
0.022  
0.046  
0.006

Values are mean ± S.E.M

Table 7: Malaria Parasite Density in Relation to Cytokine Levels of Participants.

| CYTOKINE | Malaria Parasite Density Groups (parasite/µl) | P-value |
|----------|-----------------------------------------------|---------|
|          | 1,000 (n=30) | ≥10,000 (n=38) | ≥100,000 (n= 2) |
| TNF-α (pg/ml) | 1.5758 ± 0.6849 | 1.3760 ± 0.858 | 1.7668 ± 0.4412 | 0.033 |
| IFN-γ (ng/ml) | 1.2500 ± 0.9029 | 1.8500 ± 0.0764 | 1.333 ± 0.2108 | 0.084 |
| IL-10 (pg/ml) | 1.5714 ± 0.1373 | 1.5833 ± 0.1028 | 2.000 ± 0.5774 | 0.073 |

Values are Mean ± S.E.M

Cytokine levels in Asymptomatic and Symptomatic Participants

IFN-γ values were significantly (P=0.014) higher among the symptomatic participants than the asymptomatic, while there was no significant difference (P>0.05) in the levels of TNF-α and IL-10 between the symptomatic and asymptomatic participants (Table 8).

Table 8: Cytokine levels in Asymptomatic and Symptomatic Participants

| CYTOKINE | Asymptomatic | Symptomatic | P-value |
|----------|--------------|-------------|---------|
| TNF-α (pg/ml) | 1.1515 ± 0.0445 | 1.5000 ± 0.2887 | 0.053 |
| IFN-γ (ng/ml) | 1.0833 ± 0.0563 | 1.2500 ± 0.0693 | 0.014 |
| IL-10 (pg/ml) | 1.2500 ± 0.0903 | 1.1429 ± 0.0673 | 0.093 |

Values are Mean ± S.E.M
Cytokine levels in the asymptomatic and symptomatic infected participants compared with uninfected (controls).

Serum level of TNF-α, IFN-γ and IL-10 were significantly (P< 0.05) higher amongst the test participants (asymptomatic and symptomatic groups) than the uninfected participants (figure 2a, 2b and 2c).

Discussion

In many parts of Africa, the burden of *Plasmodium falciparum* malaria is gradually decreasing. It is however characterized by the spatial and temporal viability presenting with new and evolving challenges for malaria control programmes. Nigeria currently contributes 25% of the global malaria burden, with the highest malaria prevalence in sub-Saharan Africa [16]. Protective immune responses in malaria are mediated by both T-cell and humoral responses, which are important in malaria control strategies. Tumor necrosis factor- alpha (TNFα) and Interferon gamma (IFNg) are T helper 1 (Th1) anti-parasitic pro-inflammatory cytokines with established protective roles in malaria, while interleukin-10 is a T helper 2 (Th2) cytokine with demonstrated immune-regulatory anti-inflammatory roles protective in malaria [17].

This study describes prevalence of *Plasmodium falciparum* malaria and its relationship with pro- and anti-inflammatory innate immune responses (TNFα, IFNg and interleukin-10 (IL-10)) among asymptomatic and uncomplicated malaria subjects in Baiyeku community of Ikorodu, Lagos State, Nigeria.

A prevalence of 15.2% was observed among participants in all age groups and gender in tandem with the results obtained by Aina [18] which reported prevalence of 14.7% in Ibeshe coastal community, Ikorodu, Lagos state. However, this result contrasts with that of Olukosi [19] who reported 5.7% malaria prevalence in Ijede in Lagos State during the dry season and that of Sam-Wobo [20] in Abeokuta, Ogun Sate with a prevalence of 71.1%. This difference can be attributed to the seasonal variability in the design of the studies. Olukosi’s work, although community-based, was conducted during the dry season when the vectoral population is naturally to be low [21]. The study conducted by Sam-Wobo was notably hospital-based, of which subjects were symptomatic. Some authors have reported strong correlation between high malaria prevalence and low levels of education; for instance a population that is well informed about the use of long lasting Insecticidal Nets (LLIN), indoor residual spraying (IRS) and intermittent preventive treatment of malaria in pregnancy (IPTp) could help reduce the prevalence of the disease [20,22]. South-West Nigerian communities have the highest malaria prevalence and transmission because rainfall is all year round with appropriate climatic conditions for vector breeding [23, 24].

Participants in the age group less than five years (<5 years) showed higher geometric mean parasite density (GMPD) compared to those above 5 years. The higher GMPD peaks observed in children are the result of less developed immune system that could not effectively clear parasites as observed in adults. Parasite density reduced with increasing age group which could be as result of developed immunity and premonition against malaria.
In Uganda, a negative but significant correlation \( (r = -0.09271; p < 0.0214) \) was found [25] between parasite density and age, suggesting that mature individuals’ clear parasites more effectively than children. This antimalarial immunity modulates infection outcomes and is more pronounced in children over 15 years of age and adults who have been previously exposed [25].

Body mass index (BMI) is a measure of nutritional status of which malaria have a negative effect on the nutritional status of children less than 5 years [26]. However, there has been only a few studies addressing the relationship between malnutrition and malaria in the face of relevant pro and anti-inflammatory cytokines. This study revealed higher infection intensities (GMPD) among participants with low BMI (underweight) than those having normal BMI which constituted 27.3%. This inverse relationship between malaria parasitaemia and BMI corroborates the assumption that malaria thrives among the poor while nutrition plays an important role in the development of immunity against malaria [27].

The serum levels of selected innate immune responses, TNF-\( \alpha \), IFN-\( \gamma \) and IL-10, were assessed amongst participants in this study. Serum levels of IFN-\( \gamma \) were significantly \( (P=0.014) \) higher in the symptomatic than the asymptomatic participants. This infection-induced increase could be directly linked to the effectiveness of IFN-\( \gamma \) as a pro-inflammatory Th-1 cytokine. This result is consistent with the study by Perara [28] in which both IL-10 and IFN-\( \gamma \) levels were reported elevated in \textit{P. falciparum}-infected patients.

In this study, serum levels of TNF-\( \alpha \) was significantly \( (P= 0.03) \) high among participants within the highest parasitemia group. This is an agreement with the knowledge that TNF Alpha, and other pro-inflammatory cytokines protect the host against asexual blood stages of malaria parasite [29, 30]. T cell studies have shown that mounting a rapid TNF-\( \alpha \) and IL-2 response may protect against severe disease and reinfection [31]. TNF-\( \alpha \) is a pro-inflammatory cytokine which mediates inflammation that is crucial in malaria immunity [32].

Conversely, however, low levels of TNF-\( \alpha \) were observed in this study, relative to IL-10. This could be explained by the negative action of IL-10 on pro-inflammatory responses. IL-10 completely abolishes TNF-\( \alpha \) production in response to malarial antigens. IL-10 is a critical anti-inflammatory cytokine [33, 34]. There was an increased IL-10 serum levels in infected patients in this study compared to non-infected patients, IL-10 levels increased as parasitemia increased. These results are consistent with other studies in which the severity of malaria and increased parasitemia have been associated with increased IL-10 levels [3]. Perara [29] found that a high circulating TNF-alpha levels and an inadequate IL-10 response in severe malaria (SM) patients carrying TNF2 allele could have contributed to the development of the severe \textit{falciparum} malarial disease. Findings by Perara [28] had suggested that IL-10 down-regulates the pro-inflammatory response to \textit{P. falciparum}.

There was no statistically significant difference in the serum levels of TNF-\( \alpha \) among symptomatic and asymptomatic participants although Perara [28] reported a strong positive correlation between TNF-\( \alpha \) levels and body temperature as shown in their study. TNF-\( \alpha \) is a critical mediator of malarial fever [30].
These findings suggest that higher levels of anti-inflammatory cytokines, especially IL-10 levels may contribute to pathogenesis of uncomplicated malaria by inhibiting the production of IFN-γ and TNF-α.

Declarations

Ethical Approval and Consent to participate

Ethical approval (Ref no: IRB/17/021) was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research (NIMR), Lagos to conduct the study. Due advocacy and necessary permission was obtained from the Head of the community as well and as written informed consent from study participants.

Consent for publication

Not applicable

Availability of supporting data

Data is not restricted

Competing interests

The authors declare that they have no competing interests

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Authors’ contributions

DO: Designed the study, collected the data, contributed to the analysis and wrote the manuscript.

AO: Contributed to data collection, analysis and manuscript writing.

PAM: Contributed to the manuscript and data analysis.

IN: Contributed to the manuscript and data analysis.

DAD: Supervised the entire project and contributed to the manuscript and data analysis.

All authors read and approved the final manuscript.

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Figures
Figure 1

Map showing Baiyeku in Ikorodu, Lagos State, Nigeria. (Source: Remote Sensing & GIS Lab; Department of Geography, University of Lagos, 2019).

Figure 2

Cytokine Levels in Infected and Uninfected Participants
Figure 3

Serum levels of TNF, IFN and IL-10 among the study participants compared to uninfected participants. [2a: TNF-α (pg/ml), 2b: IFN-γ (ng/ml), 2c: IL-10 (pg/ml)].