Original Article

Impact of Malnutrition on Serum Levels of Pro-inflammatory Cytokines among *Plasmodium falciparum* Malaria Subjects in Nigeria

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Abstract

Background: Understanding the impact of malnutrition on innate immune response in *Plasmodium falciparum* (*Pf*)-infected subjects is critical for malaria control. **Aims and Objectives:** This study aims to investigate the nutritional status and innate immune response of *Pf*-infected subjects in Lagos, Nigeria. **Materials and Methods:** A total of 1183 patients with a history of fever or axillary temperature ≥37°C were screened microscopically for *Pf* at Ijede General Hospital, Lagos, Nigeria. Malnutrition was determined according to the U.S National Center for Health Statistics (NCHS) as stunting, wasting, or underweight when the *Z*-score is <−2 in the participants aged <20 years. Serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and IL-12 were determined by capture ELISA while hematological parameters were measured using an automated hematology system. **Results:** A total of 384 volunteers were positive for *Pf*, of which 114 were <20 years with a median age of 10 years. Overall malaria prevalence was 20.89%. The malnutrition rate was 89.5%; 24 (21.05%) were stunted, 30 (26.32%) were underweight, and 48 (42.11%) were wasted. Pro-inflammatory cytokine responses were not affected by the type of malaria. TNF-α was higher in participants <5 years ($P = 0.001$) and in malnourished patients ($P < 0.05$). **Conclusion:** Together, it could be deduced that nutritional status influences *Plasmodium falciparum* malaria outcomes and progression pattern.

Keywords: Cytokines, IL-12, TNF- α malnutrition, necrosis, plasmodium

Résumé

Contexte: Comprendre l'impact de la malnutrition sur la réponse immunitaire innée chez les sujets infectés par *Plasmodium falciparum* (Pf) est essentiel pour la lutte contre le paludisme. **Buts et objectifs:** Cette étude vise à étudier l'état nutritionnel et la réponse immunitaire innée des sujets infectés par Pf à Lagos, au Nigeria. **Matériels et méthodes:** Un total de 1183 patients ayant des antécédents de fièvre ou une température axillaire ≥37°C ont fait l'objet d'un dépistage microscopique de Pf à l'hôpital général Ijede, Lagos, Nigeria. La malnutrition a été déterminée selon le National Center for Health Statistics (NCHS) des États-Unis comme un retard de croissance, une émaciation ou une insuffisance pondérale lorsque le score Z est <-2 chez les participants âgés de moins de 20 ans. Les taux sériques de facteur de nécrose tumorale alpha (TNF-α), d'interleukine-1β (IL-1β) et d'IL-12 ont été déterminés par capture ELISA, tandis que les paramètres hématologiques ont été mesurés à l'aide d'un système d'hématologie automatisé. **Résultats:** Au total, 384 volontaires étaient

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Access this article online

Quick Response Code:

Website: www.annalsafrmed.org

DOI: 10.4103/aam.aam_209_23

How to cite this article: Ajibaye O, Adaramoye VO, Olalere S, Ibekpobaoku AN, Olasope MA, Eniafe GO, *et al*. Impact of malnutrition on serum levels of pro-inflammatory cytokines among *plasmodium falciparum* malaria subjects in Nigeria. Ann Afr Med 0;0:0.

Submitted: 18-Dec-2023 **Revised:** 24-Jan-2024
Accepted: 02-Feb-2024 **Published:** 08-Jun-2024

positifs pour le Pf, dont 114 étaient âgés de moins de 20 ans avec un âge médian de 10 ans. La prévalence globale du paludisme était de 20,89 %. Le taux de malnutrition était de 89,5 % ; 24 (21,05 %) souffraient d'un retard de croissance, 30 (26,32 %) d'une insuffisance pondérale et 48 (42,11 %) d'émaciation. Les réponses des cytokines pro-inflammatoires n'ont pas été affectées par le type de paludisme. Le TNF-α était plus élevé chez les participants de moins de 5 ans (P = 0,001) et chez les patients souffrant de malnutrition (P < 0,05). **Conclusion:** On peut en déduire que l'état nutritionnel peut influencer les résultats et le schéma de progression du paludisme.

Mots-clés: Cytokines, IL-12, TNF- α, malnutrition, necrosis, plasmodium

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Introduction

Malaria is still a leading global health concern. With 3.4 billion people at risk and 198 million clinical cases, sub-Saharan African countries have the highest burden.[1] Plasmodium species associated with human infections are *Plasmodium falciparum* (*Pf*), *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale,* and *Plasmodium knowlesi*.[2] *Pf* is the most virulent species of *Plasmodium* and is responsible for the adverse consequences of malaria.

Pf malaria and malnutrition are major causes of child morbidity and mortality with common consequences ranging from cognitive impairment to decreased school performance,[3-6] yet their precise interaction remains underreported. Moreover, undernutrition is highly prevalent in developing countries and is considered to be the underlying cause of more than 50% of all childhood deaths in the world.[7,8] In sub-Saharan Africa, 38% of under-five children suffer from chronic malnutrition or stunting (height-for-age [HAZ] *z*-score below − 2 of the international growth reference), and acute malnutrition or wasting (weight-for-height [WHZ] *z*-score below − 2) affects 9% of preschool children.[7]

Malnutrition is estimated to cause about half of the world's 12 million annual deaths in children <5 years of age as well as substantial proportions of infectious disease morbidity.[9,10] Although many studies have shown that *Pf* infection can result in acute weight loss, improvements in growth and other anthropometric indices have been described in children protected from malaria.[11-13] Nutritional status hence is considered to be one of the major determinants of host resistance to infection.[14] In sub-Saharan Africa, the impact of chronic malnutrition or stunting (HAZ *z*-score below − 2 of an international growth reference) and acute malnutrition or wasting (WHZ *z*-score below − 2) is debilitating, especially in children.[14] In Nigeria, stunting or chronic malnutrition has been reported in about 10 million children resulting in increased susceptibility to infections, more commonly malaria.[15] This is because malaria is one of the most common parasitic infections occurring in comorbidity.[16]

Malnutrition is the primary cause of immunodeficiency worldwide; however, there have been conflicting reports on the influence of malnutrition on the immune response of malaria patients. Malnutrition could influence the development of the protective immune response acquired during infection and regulate antimalarial immunity. Cytokines play both protective and pathological roles in malaria; however, an early and effective inflammatory response is crucial in the control of parasitemia and resolution of malaria infection through the mechanism of tumor necrosis factor-alpha (TNF-α).[17] In response to infection, the immune system first executes innate and then subsequently acquired host defense functions of high diversity. Both processes involve the activation and propagation of immune cells and synthesis of an array of molecules requiring DNA replication, RNA expression, and protein synthesis and secretion and therefore consume additional anabolic energy.

Pro-inflammatory cytokines, which are mediators of inflammation further, increase the catabolic response acclaiming much necessity for protein–energy and nutritional status of infected hosts. Interleukin-1beta (IL-1β), TNF-α, and IL-12 are pro-inflammatory cytokines crucial in malaria immunity.[7] However, a cohort study reported the downregulation of malaria-specific immunity in contrast to the report from a study in Papua New Guinea, which indicated that malnutrition protected children from *Pf*[18] while Müller *et al*.[19] and Müller *et al*.[20] reported no association.

There is a need for better understanding of the impact of malnutrition on innate immune response in *Pf* malaria-infected individuals though it is widely recognized that malnutrition compromises the immune function, resulting in a higher risk of infection. However, there is paucity of data on the relationship between malaria, malnutrition, and antimalarial immunity in Nigeria. It is therefore pertinent to investigate their inter-relationship.

Methodology

Study site and enrollment

In this cross-sectional study, participants were recruited at Ijede General Hospital (IJGH), Ikorodu Local Government Area of Lagos State, Nigeria [Figure 1]. Ijede is a low socio-economic settlement with ecological features favoring malaria. IJGH is one of the centers characterized in 2011 as sentinel sites for malaria research in Nigeria; on average, there are 400 outpatients per week. Patients were recruited at the peak of malaria transmission period (i.e. May–July) in 2021. Patients presenting at the IJGH with symptoms suggestive of malaria (axillary temperature ≥37.5°C or history of fever in the last 48 h) and with *Pf* mono-infection who consented were enrolled in the study.

Parasitemia assessment

Finger-pricked blood samples were collected from each participant. Parasite densities were estimated in thick

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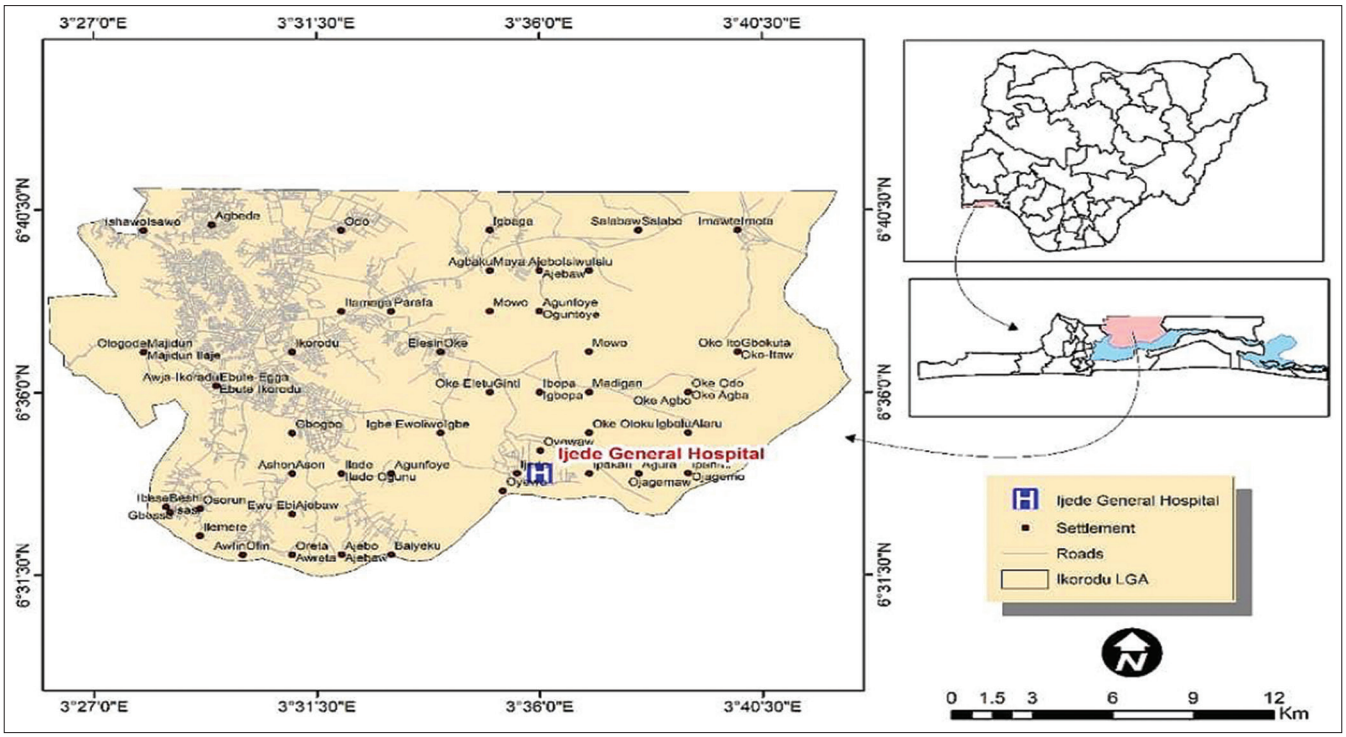


Figure 1: Map showing the location of the study site (Ijede General Hospital), Ikorodu Local Government Area of Lagos State

blood films, assuming an average white blood cell count of 8000/µl. All slides were Giemsa stained and read by two certified microscopists, and ties were broken by a third expert microscopist. Venous blood samples of positive patients and 40 uninfected controls were collected into EDTA bottles for ELISA and hematological assays.

Anthropometric measurements

Anthropometric data were collected in accordance with internationally recommended procedures.[4,5,9,21] Weight measurements were taken using a scale (Téfal, Paris, France) precise to the nearest 100 g. 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). Body mass index (BMI) (kg/m^2) was calculated and used as an index of nutritional status for the participants; those within the range of 18.5 − 24.9 were grouped as normal, while those less than or above this range were categorized malnourished or overweight, respectively.

Evaluation of cytokine response

Levels of TNF-α, IL-12, and IL-1β were assessed in sera of included participants using antigen capture ELISA.

Statistical analyses

The nutritional indicators HAZ, weight-for-age (WAZ), and WHZ were computed in *z*-scores of the WHO/National Center for Health Statistics (NCHS) reference using Epi Info software V.6 for participants below 20 years after BMI computation. Stunting, underweight, and wasting were defined for values below − 2 for HAZ, WAZ, and WHZ, respectively. SPSS V.20

Table 1: Nutritional status and cytokine levels of participants

Parameters	Uncomplicated malaria		
	Infected, *n* (%)	Uninfected, *n* (%)	*P*
Age (years), mean±SEM (years)	13±12	15.5±2.7	
<5	37 (32.8)	14 (35.00)	
>5	77 (67.2)	26 (65.00)	
Mean parasitemia/µL (years)			
<5	136,267±1449	-	0.04
≥5	32,912±1256	-	
Gender			
Female	55 (48.44)	20 (50.00)	
Male	59 (51.56)	20 (50.00)	
Mean parasitemia/µL			
Female	37,203±4751	-	0.43
Male	44,239±2985	-	
Nutritional status (participants <20 years)			
Normal	12 (10.56)	14 (35.00)	
Stunted (HAZ)	24 (21.05)	2 (5.00)	
Wasted (WHZ)	48 (42.11)	14 (35.00)	
Underweight (WAZ)	30 (26.32)	10 (25.00)	
Cytokines (ng/L)			
TNF-α	362.5±40.00	97.5±10.80	0.00016
IL-12	83.44±2.14	57.25±2.80	0.003
IL-1β	23.14±2.30	10.47±0.30	0.02

HAZ=Height-for-age, WHZ=Weight-for-height, WAZ=Weight-for-age, SEM=Standard error of mean, IL-12=Interleukin-12

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IBM, New York, USA was used for analysis of variance, multivariate regression, and correlation analysis. Differences were considered significant at $P < 0.05$.

Ethics

Approval was obtained from the NIMR Institutional Ethics Review Board for clearance to carry out the research.

Results

A total of 384 participants were included in this cross-sectional study meeting the inclusion criteria from the 1838 screened. Malaria prevalence was 20.89%. Of these, 252 (65.6%) were malnourished, 96 (25%) normal, and 36 (9.4%) overweight. In the control group (uninfected participants), 50% were malnourished [Table 1]. A total of 114 infected participants were <20 years. Using the WHO/NCHS reference, 48 (42.11%) of participants <20 years were wasted, 24 (21.05%) were stunted, and 30 (26.32%) of them were underweight [Table 1]. There was no significant difference in infection intensity by parasitemia between the male and female participants ($P = 0.43$); however, malaria parasite density (MPD) was significantly higher ($P < 0.05$) children <5 years than in older subjects (<20 years) [Table 1].

Table 2: Effect of malaria parasite density on cytokine response of participants

Cytokines (μ/L)	Malaria parasite density groups (parasites/μL), mean±SD				
	≤1000	≤100,000	≤600,000	*P*	*n*
IL-12	90±0.6	83.5±3.1	82.7±2.1	0.85	114
IL-1β	50±1.2	21.34±2.4	23.81±2.3	0.08	114
TNF-α	250±21.1	365±53.3	368.2±67.5	0.88	114

SD=Standard deviation, TNF-α=Tumor necrosis factor-alpha, IL-Iβ=Interleukin-1 beta, IL-12=Interleukin-12

Table 3: Cytokine levels with respect to nutritional status of participants

Nutritional status	Mean±SEM (test/CTRL)		
	TNF-α (μ/L)	IL-1β (μ/L)	IL-12 (μ/L)
Stunted			
Infected[a,c]	446.70±52.59	28.88±2.97	86.76±2.97
Uninfected	111.00±18.51	10.15±0.43	50.50±6.43
P	0.001*	0.12	0.06
Wasted			
Infected[b]	445.75±52.12	26.14±3.22	88.77±15.25
Uninfected	100.05±38.22	9.44±0.88	51.02±10.10
P	0.03*	0.04*	0.05
Normal			
Infected[a,b]	356.25+79.3	26.85±6.29	81.25±2.95
Uninfected	91.25±11.94	11.19±0.42	63.75±5.49
P	0.002*	0.3	0.82
Underweight			
Infected[c]	351.0±115.47	23.33±5.83	80±5.77
Uninfected	188±10.44	11.23±1.33	38±7.89
P	0.008*	0.05	0.05

t-test, ANOVA: *Significant at $P<0.05$ (stunted vs. wasted and normal), [a,b,c]Tukey's. SEM=Standard error of mean, TNF-α=Tumor necrosis factor-alpha, IL-Iβ=Interleukin-1 beta, IL-12=Interleukin-12, CTRL=Uninfected controls

Correlation of tumor necrosis factor-alpha, interleukin-12, and interleukin-1beta levels with intensity of *Plasmodium falciparum* infection

It has been shown that Th1 responses directed to wide ranges of *Pf* antigens were associated with *Pf* infection.[22-24] In the present study, levels of IL-12 decreased with increasing intensity of infection (from ≤1000 to ≤600,000 pRBCs/μL of blood) while TNF-α increased with increasing intensity of infection although not significantly ($P > 0.05$) [Table 2].

TNF-α levels were significantly higher in malnourished patients ($P < 0.0001$) whereas there was no significant difference in the levels of IL-12 and IL-1β in all the nutritional status and the control ($P < 0.05$) [Table 3]. From Table 2, with increasing parasitemia, IL-1β response reduced significantly but not so with IL-12. Moreover, all the pro-inflammatory cytokines measured were significantly higher in infected than uninfected participants irrespective of the nutritional status ($P < 0.05$) [Table 3].

Hematological parameters

The frequency of anemia was 9.5% in the malnourished group and 0% in the normal and control groups [Table 4]. Approximately 26% of the malnourished infected participants had platelet counts <150 × 10^9/L (altered platelet count) as

Table 4: Anemia and altered platelet counts in study participants

Parameter frequency	Malnutrition (HAZ, WHZ, and WAZ) (%)	Normal, *n* (%)	Control, *n* (%)
Anemia (Hb <7 g/dL)	24 (9.5)	0	0
Fever (auxiliary temperature ≥37.5°C)	80 (31.7)	15 (15.6)	0
Altered platelet count <150×10^9/L	65 (25.8)	40 (41.6)	20 (50)

HAZ=Height-for-age, WHZ=Weight-for-height, WAZ=Weight-for-age, Hb=Hemoglobin

Table 5: Cytokine levels with respect to malaria infection status (i.e., asymptomatic or symptomatic)

Cytokines	Malaria infection status (mean±SEM)		
	Asymptomatic	Symptomatic	*P*
TNF-α	395.11±17.31	417.74±24.74	0.15
IL-1β	67.88±29.26	25.77±1.69	0.08
IL-12	87.52±1.57	88.39±2.22	0.22

Values are mean levels of pro-inflammatory cytokines±SEM (*t*-test: $P>0.05$). SEM=Standard error of mean, TNF-α=Tumor necrosis factor-alpha, IL-Iβ=Interleukin-1 beta, IL-12=Interleukin-12

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compared to 5 (12.5%) uninfected controls having similar platelet counts. Only the malnourished infected participants 80 (31.7%) had fever (temperature ≥ 37.5°C) in the study.

Pearson's correlation showed that IL-12 was negatively correlated ($r = -0.327$, $P = 0.03$) with platelet counts in the test participants with a multiple regression coefficient of − 2.48 and $P = 0.0018$ [Table 5].

Discussion

This cross-sectional study conducted at the onset of the rainy season in 2021 set out to investigate whether nutritional status affects the cellular immune response of malaria patients in Lagos, Nigeria. The findings showed that the malaria burden is relatively high in rural areas of Nigeria similar to what was reported by Afolabi *et al*.[25] This may not be unconnected with the noncompliance of people to the National Malaria Control Program guidelines for malaria control in Nigeria. The geometric mean parasite density was significantly higher in males than females due to higher immunity in females and body exposure by males consistent with Akanbi *et al*.[26] and Mandel and Whit.[27]

TNF-α was higher in <5 in response to slowly reducing MPD due to low immunity and increased TNF-α response at this age. Malnourished patients presented high levels of TNF-α, a potent protective immune component, of which (low) leptin-induced high levels have been reported in malnourished individuals[28] and associated with cachexia seen in malnourished malaria subjects. However, no association was found between malnutrition and IL-1β although IL-12 levels have been reported to substantiate levels of TNF-α in severe malaria.[29-31]

Infection intensity was found to be nonuniformly related to the pro-cytokines studied signifying that after induction, recruitment of these immune components is independent of parasite factors.

With negative socio-economic indices, the poverty level is high in Nigeria negatively affecting the standard of living, nutritional status, and susceptibility to infections resulting in a high rate of wasting in children, especially of rural dwellers as found in this study.

Altered platelet (platelet count <150 × 10^9/L) and anemia (Hb <7 g/dL) were predominant in infected subjects, resulting from cytokine inhibition of erythropoiesis. Analysis of the hematological indices showed that IL-12 was negatively correlated with mean cell hemoglobin, granulocyte, and platelet in the test participants as IL-12 plays a significant role in the induction of Th1 immunity while IL-1β has an early protective role in malaria.[32] Platelets play a major role as first-line inflammatory mediators. This is evident from their capability to recruit early inflammatory cells such as neutrophils, granulocytes, and monocytes and even to exhibit direct antimicrobial activity, hence they might have delayed the immune functions of pro-inflammatory cytokines in malaria-infected subjects. However, cytotoxic properties of blood platelets are induced by cytokines including interferon-gamma and TNF-α.[33-36] Moreover, numerous studies have also demonstrated that nutritional status could modulate the immune response directed to malaria antigens[7,37-39] and therefore to vaccine candidates in Nigeria.[40-44]

The main findings from this study indicated that malnutrition may modulate Th1 response in *Pf* malaria, especially affecting the TNF-α production. However, IL-12 levels were also raised and have been linked to significantly higher levels of TNF-α. Susceptibility to malaria and clinical episodes is therefore associated with elevated TNF-α, of which high values of IL-12 are a major contributor. This should also be taken into consideration in designing vaccine trials in Nigeria as malnutrition influences patients' immunity to malaria and disease progression possibly via TNF-α.

Conclusion

Nutritional status is a key determinant of outcomes during infection with *Plasmodium falciparum*.

Limitation of study

The sample space was limited to one study site as funding was limited.

Acknowledgment

We acknowledge all the members of the staff of Ijede General Hospital for their cooperation during sample collection. Dr Faneye Dayo, at the Department of Microbiology, Nigerian Institute of Medical Research for the ELISA equipment support.

Financial support and sponsorship

The study was self-sponsored.

Conflicts of interest

There are no conflicts of interest.

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