Original Research Article

Assessment of Inflammatory Markers Across Varying Malaria Parasite Densities in Patients at Rivers State University Teaching Hospital, Port Harcourt

ABSTRACT

Aim: to assess the levels of some inflammatory markers in malaria parasite-infected subjects attending Rivers State University Teaching Hospital, Port Harcourt, Nigeria.
Study design: Cross-sectional study
Place and Duration of Study: Rivers State University Teaching Hospital and Pamel Laboratories & Diagnostics Limited, both in Port Harcourt, between July 2023 and July 2024.
Methodology: A total of 150 individuals were initially screened for various health conditions, resulting in 89 subjects deemed suitable for the study. This group comprised 43 males and 46 females, with 29 participants serving as controls and 60 participants as malaria-positive subjects. Blood specimens were obtained from each subject, and serum samples were extracted after centrifugation. These serum samples were used to assay IL-6, TNF-α using the ELISA method. Additionally, subjects were categorized based on parasitaemia severity and malaria treatment history. Data was analyzed using GraphPad Prism version 9.02. Descriptive statistics involving the use of Mean and Standard Deviation. Inferential Statistics involving the use of one-way ANOVA (PostHoc: Tukey's multiple comparison test), students statistical t-test, and Pearson's correlation. Statistical significance was set at $p < 0.05$.
Results: The study found no significant differences in IL-6 (p=0.8878), TNF-α (0.0961), levels between control and malaria-positive subjects. However,based on malaria severity, there were no significant differences in TNF-α (p=0.1993)levels among mild, moderate, and high parasitaemia cases. However, IL-6 (p=0.0002) levels were elevated in high parasitaemia cases. Malaria treatment normalized all markers within 1-8 months, except IL-6 (p=0.0423), which remained significantly elevated in subjects with a history of malaria within 9-12 months. These results suggest that malaria parasitaemia does not significantly alter inflammatory markers such as IL-6 and TNF-α. The severity of parasitaemia influences IL-6, while malaria treatment typically returns most markers to baseline within 8 months, except for IL-6.
Conclusion: Further research studies need to explore the mechanisms behind persistent IL-6 elevation post-malaria treatment and the potential long-term effects of other inflammatory markers in malaria patients.

Keywords: Malaria Parasitaemia Severity,Inflammatory Markers, Rivers State University Teaching Hospital, Port Harcourt.

1. INTRODUCTION

Malaria stands as one of the most significant health challenges worldwide, particularly affecting regions with tropical and subtropical climates. The burden of malaria is particularly

pronounced in sub-Saharan Africa, where the disease poses a substantial threat to public health and socioeconomic development [1]. In 2019 alone, there were an estimated 229 million cases of malaria globally, with approximately 409,000 deaths attributed to the disease [1]. Most of these deaths occurred among children under the age of five in sub-Saharan Africa, highlighting the disproportionate impact of malaria on vulnerable populations [1].

Malaria remains a significant public health concern in Nigeria, particularly in regions like Port Harcourt, where the prevalence of 77.5% was recorded[2]. While efforts to control malaria have primarily focused on vector control and antimalarial treatment, there is a growing recognition of the role played by inflammatory responses in the pathogenesis of the disease [3]. Therefore, this study will address this critical gap in knowledge and contribute to the overall understanding of malaria pathophysiology and management. A better understanding of the role of inflammation in malaria pathogenesis could lead to the development of novel therapeutic strategies aimed at reducing the severity of malaria and preventing complications.Malaria triggers an inflammatory cascade orchestrated by cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), produced by the immune system in response to infection [4]. While these cytokines play a crucial role in combating the parasites, excessive inflammation can lead to tissue damage and organ dysfunction, exacerbating the clinical manifestations of malaria like severe anemia, cerebral malaria [5]. Understanding how inflammation is regulated during malaria infection is crucial for developing new therapies [6].

In spite of the considerable research on malaria, it is pertinent to evaluate the relationship between malaria parasitaemia and inflammatory markers in specific geographic contexts, such as the population attending Rivers State University Teaching Hospital in Port Harcourt, Nigeria. Therefore.The aim of this study was to assess the effect of varying degrees of malaria parasitaemia and how they affect the levels of some inflammatory markers in subjects attend the Rivers State University Teaching Hospital (RSUTH), Port-Harcourt, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Rivers State University Teaching Hospital (RSUTH), Port-Harcourt, Rivers State, Nigeria. The Rivers State University Teaching Hospital (RSUTH) was formerly known as Braithwaite Memorial Specialist Hospital (BMSH) a government owned hospital, named after Eldred Curwen Braithwaite, a British doctor. It is in old GRA, PH and was transformed into a state-of-the-art Teaching Hospital in 2018 for the training of health care professionals. The facility has 375 bed capacity with about 20 departments.

2.2 Study Design

This research employed a cross-sectional study design.

2.3 Study Population

This study recruited a total of 150 participants aged between 18 and 77 years using who indicated interest in the study. Among them, 62 were males and 88 were females. However, a preliminary screening for various infections including HIV, HBsAg, HCV, TB, Syphilis, and *Salmonella spp* identified 32 individuals who tested positive for HIV, HBsAg, HCV, TB, Syphilis or *Salmonella spp* were subsequently removed from the study. Hence, the final sample consisted of 118 individuals, of which were 43 males and 46 females who tested positive for malaria. Additionally, 29 participants served as controls, while 60 were classified as test subjects, having tested positive for malaria.

A well-structured questionnaire was used to obtain relevant information about each subject, such as the demographic information, malaria symptoms, as well as food and drug intake.

2.4 Inclusion Criteria

In this study, the inclusion criteria are as follows: Individuals between the ages of 18 and 77 years, who tested positive to malaria, who tested negative (NMPS) to malaria, (control subjects) and those who were willing to provide informed consent to participate in the study.

2.5 Exclusion Criteria

Individuals who tested positive to HIV, HBsAg, HCV, TB, Syphilis, and/or Salmonella spp were excluded from the study.

2.6 Sample Collection and Processing

Eight millilitres (8mL) of venous blood were collected from each subject using sterile hypodermic syringes and needles and were dispensed into plain bottles. The collected blood specimens were transported from the point of collection to the laboratory in a specimen transportation box. The blood specimens were spun using a centrifuge at 3,500 rpm for 5 minutes, followed by the separation of the serum, which was used to assay for tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6). Prior to this, the serum samples were stored in the refrigerator at -4^{o}C until time for analysis.

2.7 Sample Analysis

2.7.1 Determination of Serum Tumour Necrosis Factor Alpha (TNF-α)

2.7.1.1 Method (Bioassay Technology Laboratory) Enzyme-linked Immunosorbent Assay (ELISA)

2.7.1.2 Principle

The microplate provided in the kit is precoated with antibodies specific to Human TNF-α. When TNF-α present in the sample is added, it binds to the antibodies immobilized on the wells of the plate. Subsequently, biotinylated Human TNF-α Antibody is introduced, which also binds to TNF-α present in the sample. Following this, Streptavidin-HRP is added, which binds to the Biotinylated TNF-α antibody. After an incubation period, any unbound Streptavidin-HRP is removed by washing the plate. Substrate solution is then added, and colour development occurs proportionally to the amount of Human TNF-α present in the sample. The reaction is halted by the addition of an acidic stop solution, and the absorbance is measured at 450 nm.

2.7.2 Determination of Serum Interleukin 6 (IL-6)

2.7.2.1 Method (Elabscience Biotechnology Inc.) Enzyme-linked Immunosorbent Assay (ELISA)

2.7.2.2 Principle

The testing methodology utilized in this kit employs a Sandwich enzyme immunoassay approach. The microtiter plate included in the kit has been precoated with an antibody that specifically targets Human IL6. Additionally, separate wells pre-coated with Human IL6 standard plates using protein-related techniques are provided. Standard/Sample Diluent Buffer or samples are then added to the appropriate wells of the microtiter plate, followed by the addition of a HRP-conjugated antibody specific to Human IL6. Upon adding the TMB substrate solution, only those wells containing Human IL6 and HRP-conjugated antibody will undergo a colour change. The enzyme-substrate reaction is halted by adding a sulfuric acid

solution, and the resulting colour change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Human IL6 in the samples is subsequently determined by comparing the optical density (OD) of the samples to the standard curve.

2.8 Statistical Analysis

Data was analyzed using GraphPad Prism version 9.02. Descriptive statistics involving the use of Mean and Standard Deviation. Inferential Statistics involving the use of one-way ANOVA with Post Hoc, students statistical t-test, and Pearson's correlation. Statistical significance was set at $p < 0.05$.

3. RESULTS

Table 1: Inflammatory Markers in test and control subjects

Parameters	Control (n=29)	MP Positive (n=89)	P value	Remark
IL-6 (pg/mL)	0.80±0.20	0.84±0.14	0.8878	NS
TNF-α (ng/L)	140.6±43.35	127.5±30.27	0.0961	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha, NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 2: Inflammatory Markers in Subjects with Varying Degree of Malaria Parasitaemia

Parameters	Mild Parasitaemia (+) (n=46)	Moderate Parasitaemia (2+) (n=29)	High Parasitaemia (3+) (n=14)	F value	P value	Remark
IL-6 (pg/mL)	$0.53±0.18^{a}$	$0.70±0.14^{a}$	$2.24±1.30^{b}$	10.28	0.0002	S
TNF-α (ng/L)	124.1±23.16	135.5±37.24	144.0±31.26	1.659	0.1993	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at p<0.05. Post Hoc: Values within same row with different superscript differ significantly at p<0.05.

Table 3: Influence of Previous Malaria Parasitaemia on Inflammatory Markers Based on Last-Treatment Regimen (1-4 Months) between Control and Malaria Parasitaemia Subjects

Parameters	Control (1-4 months) (n=24)	Malaria Parasitaemia (1-4 months) (n=43)	P value	Remark
IL-6 (pg/mL)	0.88±0.24	0.75±0.15	0.5983	NS
TNF-α (ng/L)	141.8±83.62	134.6±53.76	0.6708	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant. Statistically Significant at p<0.05.

Table 4: Influence of Previous Malaria Parasitaemia on Inflammatory Markers Based on Last-Treatment Regimen (5-8 Months) between Control and Malaria Parasitaemia Subjects

Parameters	Control (5-8 months) (n=2)	Malaria Parasitaemia (5- 8months)	P value	Remark

		(n=8)		
Ileu-6 (pg/mL)	0.62±0.12	0.56±0.30	0.8131	NS
TNF-α (ng/L)	125.0±12.73	123.9±17.59	0.9356	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha,. NS=Not Significant. Statistically Significant at p<0.05.

Table 5: Influence of Previous Malaria Parasitaemia on Inflammatory Markers Based on Last-Treatment Regimen (9-12 Months) between Control and Malaria Parasitaemia Subjects

Parameters	Control (9-12 months) (n=3)	Malaria Parasitaemia (9- 12 months) (n=9)	P value	Remark
IL-6 (pg/mL)	0.35±0.05	0.61±0.19	0.0423	S
TNF-α (ng/L)	508.7±351.3	145.6±53.28	0.0770	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 6: Influence of Previous Malaria Parasitaemia on Inflammatory Markers Based on Last-Treatment Regimen

Parameters	Malaria Parasitaemia (1- 4 months) (n=43)	Malaria Parasitaemia (5- 8 months) (n=2)	Malaria Parasitaemia (9- 12 months) (n=9)	P value	Remark
IL-6 (pg/mL)	0.74±0.14	0.56±0.11	0.61±0.19	0.7933	NS
TNF-α (ng/L)	134.6±53.76	123.9±17.59	145.6±53.28	0.6793	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant.Statistically Significant at p<0.05.

Table 7: Influence of Age (18-37 Years) of Malaria Parasitaemia Subjects on Inflammatory Markers between Control and Malaria Parasitaemia Subjects

Parameters	Control (18- 37 years) (n=12)	Malaria Parasitaemia (18- 37 years) (n=28)	P value	Remark
IL-6 (pg/mL)	1.05±0.48	0.77±0.21	0.5416	NS
TNF-α (ng/L)	167.6±98.48	133.0±36.40	0.1102	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 8: Influence of Age (38-57 Years) of Malaria Parasitaemia Subjects on Inflammatory Markers between Control and Malaria Parasitaemia Subjects

Parameters	Control (38- 57 years) (n=13)	Malaria Parasitaemia (38- 57 years) (n=22)	P value	Remark
IL-6 (pg/mL)	0.54±0.18	0.56±0.19	0.7375	NS
TNF-α (ng/L)	210.3±83.64	121.3±7.283	0.1758	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha.NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 9: Influence of Age (58-77 Years) of Malaria Parasitaemia Subjects on Inflammatory Markers between Control and Malaria Parasitaemia Subjects

Parameters	Control (58 – 77 years) (n=4)	Malaria Parasitaemia (58 – 77 years) (n=10)	P value	Remark
IL-6 (pg/mL)	0.90±0.57	0.61±0.16	0.1480	NS
TNF-α (ng/L)	158.3±81.47	119.4±17.98	0.1580	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 10: Influence of Age of Malaria Parasitaemia Subjects on InflammatoryMarkers Across Varying Age Groups

Parameters	Malaria Parasitaemia (18- 37 years) (n=28)	Malaria Parasitaemia (38- 57 years) (n=22)	Malaria Parasitaemia (58 – 77 years) (n=10)	F value	P value	Remark
IL-6 (pg/mL)	0.77±0.21	0.56±0.19	0.61±0.16	0.4870	0.6170	NS
TNF-α (ng/L)	133.0±36.40	121.3±7.283	119.4±17.98	1.0330	0.3624	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 11: Influence of Sex (Female) of Malaria Parasitaemia Subjects on Inflammatory Markers

Parameters	Female Control (n=19)	Female Malaria Parasitaemia (n=46)	P value	Remark
IL-6 (pg/mL)	0.95±0.31	0.70±0.11	0.1690	NS
TNF-α (ng/L)	135.2±45.75	130.7±30.49	0.6924	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha,. NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 12: Influence of Sex (Male) of Malaria Parasitaemia Subjects on InflammatoryMarkers

Parameters	Male Control (n=10)	Male Malaria Parasitaemia (n=43)	P value	Remark
IL-6 (pg/mL)	0.5510±0.1935	0.7967±0.1863	0.4777	NS
TNF-α (ng/L)	152.9±49.70	141.2±68.08	0.6165	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at p<0.05.

Table 13: Influence of Sex of Malaria Parasitaemia Subjects on Inflammatory Markers

Parameters	Female Malaria Parasitaemia	Male Malaria Parasitaemia (n=43)	P value	Remark

	(n=27)			
Ileu-6 (pg/mL)	0.70±0.11	0.7967±0.1863	0.3134	NS
TNF-α (ng/L)	130.7±30.49	141.2±68.08	0.4649	NS

Key: MP=Malaria Parasitaemia, IL-6=Interleukin-6, TNF-α=Tumour Necrotic Factor-alpha. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$.

4. DISCUSSION

The aim of this study was to assess the levels of some inflammatory markers in malaria parasite-infected subjects attending RSUTH, Port Harcourt, Nigeria. A total of 150 subjectsconsented to participate in the study, with 41.3% males and 58.7% females indicating interest. This gender balance is important for ensuring representative samples in research studies. The preliminary screenings for various health conditions highlighted some important findings. A notable portion of the participants tested positive for HIV (16.7%) and HBsAg (18.0%), thus highlighting the importance of addressing these prevalent health issues. However, the relatively low prevalence of HCV (0.7%), absence of TB, and *Treponema pallidum* infections are encouraging from a public health perspective. On the other hand, the detection of *Salmonella* spp. in 5.3% of participants raises concerns about potential food or waterborne infections within the community.

Recordings of the body temperature showed that most of the sampled subjects had normal temperatures, indicating overall good health among the cohort. This finding suggests that systemic infections may not be widespread among the study population. After the screening process, 89 participants were deemed suitable for the study, reflecting a rigorous selection process. The age distribution of the recruited participants varied across different intervals, with a significant proportion falling within the 18 - 37 years age range. Regarding malaria status, a substantial proportion of recruited participants tested positive for malaria (40.0%), indicating the endemic nature of malaria in the study area. Further categorization based on the degree of malaria parasitaemia revealed varying levels of infection severity, with a significant portion classified as having moderate to severe parasitaemia. Finally, data on the duration since the last malaria treatment regimen provided information into the participants' recent exposure to malaria treatment.

The mean IL-6 levels between control and test (malaria-positive) subjects showed no significant difference, indicating comparable inflammatory responses regardless of malaria parasitaemia status. This report disagrees with that of Wilairatana et al. [7], demonstrating elevated IL-6 levels in individuals with malaria parasitaemia. This disagreement could be due to variations in the locality for both studies. Similarly, the mean TNF-α levels did not significantly differ between the two groups, suggesting consistent immune responses irrespective of malaria parasitaemia. These results disagree with report from studies by Budiningsih et al. [8] and Mukthayakka et al. [9], demonstrating elevated TNF-α levels in individuals with malaria parasitaemia. This disagreement could be due to variations in the locality for both studies.

Notably, the mean levels of IL-6, an important pro-inflammatory cytokine, show a substantial increase as the severity of parasitaemia rises. Specifically, subjects with high parasitaemia exhibit significantly elevated IL-6 levels compared to those with mild and moderate parasitaemia. This finding suggests a potential correlation between the degree of parasitaemia and the inflammatory response mediated by IL-6. This report agrees with that of Wilairatana et al. [7] who noted significantly elevated levels of IL-6 in patients with severe malaria compared with those patients with non-severe malaria, thus that indicating IL-6 as a candidate marker for severe malaria. In contrast, the mean TNF-α levels across subjects

with varying degrees of parasitaemia show no significant differences, indicating that TNF-α may not be strongly influenced by the severity of malaria parasitaemia. This report disagrees with that of Kinra and Dutta [10] who noted elevated TNF-α levels in severe cases of malaria compared to the control.

Similarly, the influence of previous malaria parasitaemia on Inflammatory markers, specifically examining the last-treatment regimen within the last 5-8 months, among both control subjects and those with malaria parasitaemia was assessed. It revealed that there were no significant differences observed in the mean levels of IL-6 and TNF-α between control subjects and those with malaria parasitaemia. Interpreting these results, it appears that within the 5–8-month period post-treatment, there was no significant impact of previous malaria parasitaemia on the levels of these Inflammatory markers. This suggests that any potential alterations in immune response parameters may have normalized by this stage, with no residual effects observed.

Additionally, the impact of previous malaria parasitaemia on Inflammatory, specifically examining the last-treatment regimen within the last 9-12 months, among both control subjects and those with malaria parasitaemia was assessed. The data reveals that individuals with a history of malaria parasitaemia within the last 9-12 months exhibited significantly higher mean levels of IL-6 compared to control subjects. This elevation in IL-6 levels suggests a potential ongoing inflammatory response in individuals with recent malaria exposure, even after several months post-treatment. However, no significant differences were observed in the levels of TNF-α was observed.

The influence of previous malaria parasitaemia on Inflammatory based on the last-treatment regimen, comparing malaria parasitaemia subjects within different timeframes: 1-4 months, 5-8 months, and 9-12 months post-treatment showed that, for IL-6 levels, no significant difference was observed among malaria parasitaemia subjects within the different timeframes. Similarly, TNF-α, across the 1-4 months, 5-8 months, and 9-12 months intervals, with no statistically significant difference noted. These findings suggest that the Inflammatory responses following malaria infection may stabilize over time, irrespective of the duration since the last treatment regimen.

The study on the impact of age (18-37 years) on Inflammatory markers among malaria parasitaemia subjects compared to controls revealed that for both inflammatory markers, IL-6 and TNF-α, there were no statistically significant differences observed between control subjects and malaria parasitaemia subjects within the specified age group. Specifically, for IL-6 levels, no significant difference was noted between control and malaria parasitaemia subjects aged between 18 and 37 years. These findings suggest that in individuals aged 18-37 years, malaria parasitaemia may not exert a significant effect on Inflammatory markers compared to control subjects.

The study on the impact of age (58-77 years) on Inflammatory markers in malaria parasitaemia subjects compared to controls revealed that across various markers including IL-6, TNF-α, there were no statistically significant differences observed between control subjects and malaria parasitaemia subjects within the specified age group. Specifically, for IL-6 levels, no significant difference was noted between control and malaria parasitaemia subjects aged between 58 and 77 years.

The study on the influence of sex on Inflammatory markers among malaria parasitaemia subjects, comparing female and male subjects with malaria parasitaemia revealed no significant differences in the levels of various markers (IL-6, TNF-α) between female and male malaria parasitaemia subjects. These findings suggest that sex does not significantly

influence the Inflammatory markers measured in this study among malaria parasitaemia subjects. The lack of significant differences between female and male malaria parasitaemia subjects implies that sex may not be a major determinant of these specific markers in malaria parasitaemia subjects.

5. CONCLUSION

The study found no significant differences in IL-6 and TNF-α levels between control and malaria-positive subjects, indicating comparable inflammatory responses regardless of malaria parasitaemia status. Severity of parasitaemia influenced IL-6 levels, with higher levels in subjects with severe parasitaemia, suggesting a correlation between parasitaemia degree and inflammatory response. TNF-α levels did not vary significantly with parasitaemia severity. IL-6 levels were higher in those with a history of malaria within 9-12 months, suggesting inflammation.Additionally, age and sex did not significantly influence inflammatory markers.

CONSENT

All authors declare that informed consent was obtained from the patient for publication of this original research article and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Rivers State Teaching Hospital Health Research Ethics Committee, Port Harcourt and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Disclaimer (Artificial intelligence)

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1. NO AI TOOL WAS USED FOR THE ENTIRE WORK
2.
3.

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