



Oxidative Stress Markers in Malaria Patients: Assessing the Impact of Parasitaemia Severity at Rivers State University Teaching Hospital, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EI and OUA designed the study. Author EI performed the statistical analysis. Author OJ managed the analyses and the literature searches of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study aimed to assess the levels of some oxidative stress markers in malaria parasite-infected subjects attending Rivers State University Teaching Hospital, Port Harcourt, Nigeria.

Background: Malaria can be fatal, the severity of malaria infection is closely linked to parasite density, the number of malaria parasites per microliter of blood. Higher parasite densities are associated with increased risk of severe malaria complications, such as cerebral malaria, anemia, and death.

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Methodology: This Cross-sectional study was carried out at Rivers State University Teaching Hospital and Pamel Laboratories & Diagnostics Limited, both in Port Harcourt, between July 2023 and July 2024. 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 SOD, TAS, TOS, and OSI 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 SOD ($p=0.4877$), TAS ($p=0.4877$), and TOS ($p=0.4877$) levels between control and malaria-positive subjects, but OSI was higher ($p=0.0430$) in malaria-positive subjects. Based on malaria severity, there were no significant differences in SOD ($p=0.7853$), TAS ($p=0.7853$), and TOS ($p=0.7853$) levels among mild, moderate, and high parasitaemia cases. However, OSI ($p=0.0490$) levels were elevated in high parasitaemia cases. Malaria treatment normalized all markers within 1-8 months. These results suggest that malaria parasitaemia is associated with increased oxidative stress. The severity of parasitaemia influences OSI levels, while malaria treatment typically returns most markers to baseline within 8 months.

Conclusion: Further research should explore the potential long-term effects of oxidative stress in malaria patients.

Keywords: Malaria parasitaemia severity; oxidative stress; markers; Rivers State University Teaching Hospital; Port Harcourt.

1. INTRODUCTION

Malaria, a mosquito-borne infectious disease caused by *Plasmodium* parasites, remains a significant public health concern globally, with an estimated 241 million cases and 627,000 deaths in 2021, primarily occurring in sub-Saharan Africa [1,2]. In Nigeria, malaria is the leading cause of morbidity and mortality, accounting for approximately 25% of all hospital admissions and 11% of all under-five deaths [3]. The causative agents of malaria, Plasmodium parasites, are transmitted through the bites of infected female Anopheles mosquitoes [4]. Upon infection, the parasite invades red blood cells, disrupting their function, undergo complex life cycles within both mosquitoes and human hosts, leading to a range of clinical manifestations [5]. Symptoms include fever, chills, fatigue, and muscle aches [6]. In severe cases, malaria can be fatal, the severity of malaria infection is closely linked to parasite density, the number of malaria parasites per microliter of blood [1]. Higher parasite densities are associated with increased risk of severe malaria complications, such as cerebral malaria, anemia, and death [7].

The immune response to malaria infection often leads to oxidative stress, characterized by an

imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses [8]. Antioxidant defenses neutralize these free radicals and prevent them from causing damage [9]. However, malaria infection disrupts this balance. The Plasmodium parasite itself contributes to an increase in free radicals, leading to a condition called oxidative stress [10]. This heightened oxidative state can induce cellular damage, exacerbating the pathology of malaria and contributing to disease severity [11].

Despite the considerable research on malaria, there is a need for a comprehensive understanding of the relationship between malaria parasitaemia, oxidative stress, markers in specific geographic contexts, such as the population attending Rivers State University Teaching Hospital in Port Harcourt, Nigeria.

Therefore, this study seeks to address this gap by systematically assessing and correlating oxidative stress markers in malaria-infected subjects attending Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Nigeria. This research will provide valuable insights into the pathophysiology of malaria and potentially contribute to the development of

novel diagnostic and therapeutic strategies for both local and global malaria control strategies.

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 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, 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 milliliters (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 superoxidase dismutase (SOD), total oxidant status (TOS), and total antioxidant status (TAS). Prior to this, the serum samples were stored in the refrigerator at -4°C until time for analysis.

2.7 Sample Analysis

2.7.1 Determination of serum superoxidase dismutase (SOD)

2.7.1.1 Method (elabscience biotechnology inc.) enzyme-linked immunosorbent assay (ELISA)

Principle: This ELISA kit operates on the Competitive-ELISA principle. The microplate included in this kit is pre-coated with Human SOD1. During the assay, Human SOD1 in the samples or standard competes with a fixed amount of Human SOD1 on the solid phase for binding sites on the Biotinylated Detection Antibody specific to Human SOD1. Any excess conjugate and unbound sample or standard are washed away, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubated. Subsequently, a tetramethylbenzidine (TMB) substrate solution is added to each well. The enzyme-substrate reaction is stopped by adding a stop solution, and the colour change is measured spectrophotometrically at 450 ± 2 nm. The concentration of Human SOD1 in the samples is determined by comparing the optical density (OD) of the samples to a standard curve.

2.7.2 Determination of Serum Total Oxidant Status (TOS)

2.7.2.1 Method (elabscience biotechnology Inc.) enzyme-linked immunosorbent assay (ELISA)

Principle: In acidic conditions, oxidizing agents in the sample can transform Fe²⁺ into Fe³⁺, which then forms a highly stable blue-purple complex with xylenol orange. At a pH between 2 and 3, this complex has a maximum absorption wavelength around 590 nm. The depth of the colour correlates with the concentration of oxidizing substances in the sample over a certain range and period, enabling the indirect calculation of the sample's total oxidative state.

2.7.3 Determination of Serum Total Antioxidant Status (TAS)

2.7.3.1 Method (fortress diagnostics) enzyme-linked immunosorbent assay (ELISA)

Principle: ABTS (1,2'-Azino-di-(3-ethylbenzthiazoline sulfonate) is reacted with peroxidase (metmyoglobin) and hydrogen peroxide (H₂O₂) to generate the ABTS⁺ radical

cation. This radical cation exhibits a stable blue-green colour, which is detectable within the wavelength range of 600-660 nm. The presence of antioxidants in the sample inhibits the development of this colour, and the extent of this inhibition correlates with the concentration of antioxidants in the sample.

2.7.4 Calculation of Oxidative Stress Index (OSI)

To calculate OSI, the following formula was used:

$$OSI = (TOS, \mu\text{mol/L}) / [TAS, (\text{mmol TroloxEquiv/L})] \times 100 \quad [12]$$

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 AND DISCUSSION

Table 1. Oxidative Stress Markers in test and control subjects

Parameters	Control (n=29)	MP Positive (n=89)	P value	Remark
SOD (pg/mL)	1827±456.3	1895±422.1	0.4877	NS
TAS(10 ³)(mmol/L)	12.97±3.24	13.46±2.99	0.4877	NS
TOS (10 ⁵) (μmol/L)	18.53±4.62	19.22±4.28	0.4877	NS
OSI	0.035±0.016	0.079±0.046	0.0430	S

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at p<0.05

Table 2. Oxidative Stress Markers in Subjects with Varying Degree of Malaria Parasitaemia (+, 2+, 3+)

Parameters	Mild Parasitaemia (+) (n=46)	Moderate Parasitaemia (2+) (n=29)	High Parasitaemia (3+) (n=14)	F value	P value	Remark
SOD (pg/mL)	1962±402.4	1904±330.8	1862±168.0	0.2428	0.7853	NS
TAS (10 ³) (mmol/L)	13.93±2.86	13.52±2.34	13.22±1.19	0.2428	0.7853	NS
TOS (10 ⁵) (μmol/L)	19.91±4.08	19.31±3.35	18.88±1.70	0.2428	0.7853	NS
OSI	0.014±0.009 ^a	0.0228±0.0072 ^a	0.086±0.003 ^b	1.9558	0.0490	S

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. 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 Oxidative 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
SOD (pg/mL)	1817±514.6	1910±342.1	0.3820	NS
TAS (10 ³) (mmol/L)	12.89±3.65	13.56±2.43	0.3820	NS
TOS (10 ⁵) (µmol/L)	18.43±5.22	19.37±3.47	0.3820	NS
OSI	0.045±0.032	0.024±0.007	0.1019	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant. Statistically Significant at p<0.05

Table 4. Influence of Previous Malaria Parasitaemia on Oxidative 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) (n=8)	P value	Remark
SOD (pg/mL)	1724±30.41	2113±524.8	0.3452	NS
TAS (10 ³) (mmol/L)	12.237±2.16	15.01±3.73	0.3452	NS
TOS (10 ⁵) (µmol/L)	17.48±3.08	21.43±5.32	0.3452	NS
OSI	0.039±0.029	0.055±0.017	0.7221	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant. Statistically Significant at p<0.05

Table 5. Influence of Previous Malaria Parasitaemia Oxidative 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
SOD (pg/mL)	1646±436.7	1795±445.4	0.6236	NS
TAS (10 ³)(mmol/L)	11.68±3.10	12.75±3.16	0.6236	NS
TOS (10 ⁵) (µmol/L)	16.69±4.43	18.21±4.52	0.6236	NS
OSI	0.054±0.043	0.035±0.015	0.5940	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at p<0.05

Table 6. Influence of Previous Malaria Parasitaemia on Oxidative 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
SOD (pg/mL)	1910±342.1	2113±524.8	1795±445.4	0.2347	NS
TAS (10 ³) (mmol/L)	13.56±2.43	15.01±3.73	12.75±3.16	0.2347	NS
TOS (10 ⁵) (µmol/L)	19.69±3.47	21.43±5.32	18.21±4.52	0.2347	NS
OSI	0.024±0.007	0.055±0.017	0.035±0.015	0.6827	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at p<0.05

Table 7. Influence of Age (18-37 Years) of Malaria Parasitaemia Subjects on Oxidative Stress Markers between Control and Malaria Parasitaemia Subjects

Parameters	Control (18- 37 years) (n=12)	Malaria Parasitaemia (18- 37 years) (n=28)	P value	Remark
SOD (pg/mL)	1987±573.3	1966±371.0	0.8913	NS
TAS (10 ³) (mmol/L)	14.11±4.07	13.96±2.63	0.8913	NS
TOS (10 ⁵) (µmol/L)	20.15±5.81	19.94±3.76	0.8913	NS
OSI	0.012±0.002	0.014±0.004	0.8017	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

Table 8. Influence of Age (38-57 Years) of Malaria Parasitaemia Subjects on Oxidative Stress Markers between Control and Malaria Parasitaemia Subjects

Parameters	Control (38- 57 years) (n=13)	Malaria Parasitaemia (38- 57 years) (n=22)	P value	Remark
SOD (pg/mL)	1731±336.4	1891±477.2	0.2947	NS
TAS (10 ³)(mmol/L)	12.28±2.39	13.429±3.388	0.2947	NS
TOS (10 ⁵) (µmol/L)	17.55±3.41	19.18±4.84	0.2947	NS
OSI	0.007±0.005	0.01±0.004	0.0930	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

Table 9. Influence of Age (58-77 Years) of Malaria Parasitaemia Subjects on Oxidative Stress Markers between Control and Malaria Parasitaemia Subjects

Parameters	Control (58 – 77 years) (n=4)	Malaria Parasitaemia (58 – 77 years) (n=10)	P value	Remark
SOD (pg/mL)	1660±324.4	1857±156.8	0.1413	NS
TAS (10 ³)(mmol/L)	11.78±2.30	13.182±1.11	0.1413	NS
TOS (10 ⁵)(µmol/L)	18.32±3.290	18.83±1.59	0.1413	NS
OSI	0.011±0.003	0.015±0.004	0.5497	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

Table 10. Influence of Age of Malaria Parasitaemia Subjects on Oxidative Stress Markers 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
SOD (pg/mL)	1966±371.0	1891±477.2	1857±156.8	0.3794	0.6860	NS
TAS (10 ³) (mmol/L)	13.96±2.63	13.429±3.388	13.182±1.11	0.3794	0.6860	NS
TOS (10 ⁵) (µmol/L)	19.94±3.76	19.18±4.84	18.83±1.59	0.3794	0.6861	NS
OSI	0.014±0.004	0.01±0.004	0.015±0.004	0.4560	0.6360	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

Table 11. Influence of Sex (Female) of Malaria Parasitaemia Subjects on Oxidative Stress Markers

Parameters	Female Control (n=19)	Female Malaria Parasitaemia (n=46)	P value	Remark
SOD (pg/mL)	1851±523.6	1923±421.5	0.6139	NS
TAS (10 ³) (mmol/L)	13.142±3.718	13.65±2.99	0.6139	NS
TOS (10 ⁵) (µmol/L)	18.77±5.31	19.50±4.28	0.6139	NS
OSI	0.015±0.042	0.019±0.009	0.2780	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

Table 12. Influence of Sex (Male) of Malaria Parasitaemia Subjects on Oxidative Stress Markers

Parameters	Male Control (n=10)	Male Malaria Parasitaemia (n=43)	P value	Remark
SOD (pg/mL)	1782±309.8	1948±323.0	0.1582	NS
TAS (10 ³) (mmol/L)	12.65±2.19	13.83±2.29	0.1582	NS
TOS (10 ⁵) (µmol/L)	18.07±3.14	19.75±3.27	0.1582	NS
OSI	0.022±0.011	0.018±0.005	0.7565	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

Table 13. Influence of Sex of Malaria Parasitaemia Subjects on Oxidative Stress Markers

Parameters	Female Malaria Parasitaemia (n=27)	Male Malaria Parasitaemia (n=43)	P value	Remark
SOD (pg/mL)	1923±421.5	1948±323.0	0.7947	NS
TAS (10 ³) (mmol/L)	13.65±2.99	13.83±2.29	0.7947	NS
TOS (10 ⁵) (µmol/L)	19.50±4.28	19.75±3.27	0.7947	NS
OSI	0.019±0.009	0.018±0.005	0.9396	NS

Key: MP=Malaria Parasitaemia, SOD= Superoxide dismutase, TAS=Total Antioxidant of System, TOS=Total oxidative of System, OSI=Oxidative stress index. NS=Not Significant, S=Significant. Statistically Significant at $p<0.05$

This study was aimed at assessing oxidative stress markers in malaria parasite-infected subjects attending RSUTH, Port Harcourt, Nigeria. Initially, the study attracted a total of 150 individuals willing to participate, demonstrating a diverse gender distribution, with 41.3% males and 58.7% females showing 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.

Body temperature assessments revealed that most participants 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. However, the lack of information regarding the occupation and level of education of the participants limits our understanding of the socioeconomic and educational backgrounds of the study populations. 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 [13].

Regarding Oxidative Stress Markers, the mean SOD level did not significantly differ between control and test subjects. This report differs from that of Kotepui et al. [14], who found that malaria patients exhibited reduced levels of SOD in their blood in comparison to individuals without malaria infection. Similarly, the mean TAS and TOS levels did not significantly differ between control and test subjects, indicating similar antioxidant and oxidant levels in both groups. These results disagree with that of Ebrahim et al. [15] who reported significantly increased level of TOS in the serum of malaria patients compared to healthy control subjects. However, it is noteworthy that the mean OSI level was significantly higher in test subjects compared to controls, suggesting increased oxidative stress in individuals with malaria parasitaemia. This report agrees with that of Ebrahim et al. [15] who reported significantly increased level of OSI in the serum of malaria patients compared to healthy control subjects.

Similarly, no significant differences are observed in the mean levels of SOD, TAS, and TOS among subjects with mild, moderate, and high parasitaemia, suggesting that these Oxidative Stress Markers may not be directly associated with the severity of parasitaemia. This report contradicts that of Babalola et al. [16] who reported elevated levels of SOD, TAS, and TOS in patients with severe malaria infection compared to those with mild infection. However, the Oxidative Stress Index (OSI) demonstrates a notable trend, with subjects having high parasitaemia exhibiting significantly higher OSI levels compared to those with mild and moderate parasitaemia. This implies a potential role of oxidative stress in the pathogenesis of severe malaria, as reflected by the OSI. The influence of previous malaria parasitaemia on oxidative stress markers, specifically focusing on the last-treatment regimen within the last 1-4 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 SOD, TAS, TOS, and OSI between control subjects and those with malaria parasitaemia. Interpreting these findings, it appears that within the 1–4-month timeframe post-treatment, there is no significant impact of previous malaria parasitaemia on the levels of these Oxidative Stress Markers. This suggests that the immune response and oxidative stress parameters may have returned to baseline levels following successful treatment, with no lingering effects observed during this period.

Similarly, the influence of previous malaria parasitaemia on Oxidative Stress 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 SOD, TAS, TOS, and OSI between control subjects and those with malaria parasitaemia. Interpreting these results, it appears that within the 5–8-month period post-treatment, there is no significant impact of previous malaria parasitaemia on the levels of these oxidative stress markers. This suggests that any potential alterations in immune response and oxidative stress parameters may have normalized by this stage, with no residual effects observed.

Additionally, the impact of previous malaria parasitaemia on oxidative Stress Markers, specifically examining the last-treatment regimen within the last 9-12 months, among both control subjects and those with malaria parasitaemia was assessed. However, no significant differences were observed in the levels of SOD, TAS, TOS, or OSI between control subjects and those with malaria parasitaemia within this timeframe.

The influence of previous malaria parasitaemia on Oxidative Stress Markers 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 was assessed. Similarly, SOD, TAS, TOS, and OSI levels remained consistent across the 1-4 months, 5-8 months, and 9-12 months intervals, with no statistically significant difference noted. These findings suggest that the oxidative 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 Oxidative Stress Markers among malaria parasitaemia subjects compared to controls revealed that across various markers including SOD, TAS, TOS, and OSI, there were no statistically significant differences observed between control subjects and malaria parasitaemia subjects within the specified age group. Similarly, SOD, TAS, TOS, and OSI levels exhibited no significant differences between the two groups within this age range. These findings suggest that in individuals aged 18-37 years, malaria parasitaemia may not exert a significant effect on Oxidative Stress Markers compared to control subjects.

The study on the influence of age (38-57 years) on Oxidative Stress Markers among malaria parasitaemia subjects compared to controls demonstrated that across various markers including SOD, TAS, TOS, and OSI, there were no statistically significant differences observed between control subjects and malaria parasitaemia subjects within the specified age group. Similarly, SOD, TAS, TOS, and OSI levels exhibited no significant differences between the two groups within this age range. These findings suggest that in individuals aged 38-57 years, malaria parasitaemia may not exert a significant effect on Oxidative Stress Markers compared to control subjects.

The study on the impact of age (58-77 years) on Oxidative Stress Markers in malaria parasitaemia subjects compared to controls revealed that across various markers including SOD, TAS, TOS, and OSI, there were no statistically significant differences observed between control subjects and malaria parasitaemia subjects within the specified age group. SOD, TAS, TOS, and OSI levels exhibited no significant differences between the two groups within this age range. These findings imply that in individuals aged 58-77 years, malaria parasitaemia may not induce significant alterations in Oxidative Stress Markers compared to control subjects. The study of the influence of sex (female) on Oxidative Stress Markers in malaria parasitaemia subjects, comparing these markers between female control and female malaria parasitaemia subjects revealed no significant

differences in the levels of various markers (SOD, TAS, TOS, and OSI) between female control and female malaria parasitaemia subjects. These findings suggest that sex (female) does not significantly influence the Oxidative Stress Markers measured in this study among malaria parasitaemia subjects. This report differs from that of Babalola et al. [16], indicating sex-related variations in oxidative stress markers in malaria patients.

The study on the influence of sex on Oxidative Stress Markers among malaria parasitaemia subjects, comparing female and male subjects with malaria parasitaemia revealed no significant differences in the levels of various markers (SOD, TAS, TOS, and OSI) between female and male malaria parasitaemia subjects. These findings suggest that sex does not significantly influence the Oxidative Stress 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.

4. CONCLUSION

Oxidative stress markers (SOD, TAS, and TOS) did not differ significantly between the groups, though the OSI was higher in malaria-positive subjects, suggesting increased oxidative stress. The study found no significant differences in oxidative stress markers among different parasitaemia severities, except for higher OSI levels in severe cases. Additionally, age and sex did not significantly influence oxidative stress markers, indicating that malaria parasitaemia does not affect these markers differently across age groups or between sexes. Overall, while malaria parasitaemia is associated with increased oxidative stress.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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