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ASSESSMENT OF THE LEVELS OF SERUM MALONDIALDEHYDE (MDA), GLUTATHIONE PEROXIDASE, AND CATALASE IN PLASMODIUM FALCIPARIUM INFECTED INDIVIDUALS

BY

Otaye, O.M[1], Christian Ugege[2],Osazuwa T.I[3], John C.B[4], Ohiwerei W.O[5], Ogedegbe, I.S[6] , Eidangbe, A.P[7]

[1] Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University Ekpoma, Edo State, Nigeria

[2]Department of Research and Training, Ohilux Global Research Diagnostic and training center, Ekpoma Edo State

[3]Department of Medical Laboratory Science, Faculty of Health Science Edo State University, Uzairue, Edo State, Nigeria

[4]Edo Specialist Teaching Hospital, Benin City, Edo State, Nigeria.

[5]Hospital St-Bartholomew Hospital London

[6]Hospital of Watford United Kingdom

[7]Narrow Way Clinic, Benin City, Edo state

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Abstract

Malaria is a disease triggered by microscopic single-celled organisms known as protozoan parasites from the Plasmodium genus, which invade and annihilate red blood cells. The objective of the research was to evaluate the concentrations of Malondialdehyde (MDA), Glutathione peroxidase, and catalase in individuals infected with Plasmodium falciparum within the Ekpoma region and its surrounding areas. In this forward-looking investigation, a comprehensive set of 150 blood samples was gathered. Among these, 100 samples were obtained from patients afflicted with malaria, with 40 exhibiting a parasiteamia degree of one (+), 40 displaying a degree of two (++), and 20 demonstrating a degree of three (+++). Additionally, 50 control subjects participated in the study, consisting of 24 males and 26 females. The findings of the study disclosed the distribution of age and gender within the study population. A total of 150 samples were enrolled in this research, including fifty (50) control samples, with an equal split of twenty-five (25) males and twenty-five (25) females. Additionally, the study included fifty (50) male patients testing positive for malaria and an equal number of fifty (50) female patients also diagnosed with malaria. In this investigation, a significant ($p \leq 0.05$) decline was observed in the experimental groups when compared to the control group, highlighting significant findings. The present study identified an elevation in Malondialdehyde (MDA) activity among malaria-positive patients, suggesting an augmented generation of reactive oxygen species. However, this observation did not attain statistical significance. No significant ($p \geq 0.05$) decrease in catalase activity was noted when compared to the control group. In conclusion, the current study demonstrated that malaria infection led to the occurrence of oxidative stress to a certain degree, as evidenced by the presence of non-significant findings.

INTRODUCTION

Stress is a natural physiological reaction to situations that evoke a sense of threat or danger to the body. The stress response is the body's mechanism of safeguarding itself, whereby the nervous system reacts by releasing a surge of stress hormones, such as adrenaline and cortisol. Malaria poses a significant public health challenge in the majority of tropical countries. Malaria stands as a leading cause of both mortality and morbidity, impacting an estimated 300 to 500 million individuals annually across 100 developing nations. Alarmingly, the most vulnerable group affected by this disease consists primarily of children (UNICEF, 2000). In Nigeria, approximately 96 million individuals are at risk of malaria exposure, with around 64 million people becoming infected each year. Tragically, the general population

*Corresponding Author: **Otaye, O.M**

witnesses nearly 300,000 reported deaths annually due to this disease (Alaribe, 2006). Nonetheless, the existing data suggests that malaria holds the highest frequency among reasons for outpatient visits. It occupies a position within the top five leading causes of death across all age groups (FMOH, 1990). Malaria also imposes significant social burdens in terms of school absenteeism and decreased economic productivity. As a result, an individual afflicted with malaria typically experiences an average loss of ten working days. Malaria inflicts a financial toll on African nations, accounting for over one percent of their gross domestic product and approximately ten percent of their health expenditure (UNICEF, 2000).

Malaria is the result of infection by protozoan parasites from the plasmodium genus, which invades and annihilates red blood cells. Malaria in humans is caused by four distinct plasmodium species: Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, and Plasmodium vivax. Among these, Plasmodium falciparum poses the greatest peril as it represents the most hazardous form of malaria. It poses a life-threatening risk, particularly in populations lacking protection or immunity, leading to significant rates of illness and mortality. Previous research has provided evidence of the presence of oxidative stress during the acute phase of malaria infection, which includes a reduction in antioxidant levels (Das *et al.*, 1993) Elevated levels of plasma lipid per oxidation and modifications in the fluidity of erythrocyte membrane have been observed as a result of the malaria infection (Sibmooh*et al.*, 2000). The occurrence of oxidative stress arises from the immune response of the host, functioning as an acute phase reaction, as well as the metabolic activities of the parasite within the red blood cells. While oxidative stress seems to be a prevalent occurrence during acute malaria infection, it can potentially lead to distinct outcomes in the progression of the disease. The severity of malaria is closely associated with oxidative stress, which can arise from various sources, including intracellular parasitized red blood cells (Atamma and Ginsburg, 1993) Furthermore, oxidative stress in malaria can also originate from extra-erythrocytic sources, which can be attributed to factors like hemolysis and the host's immune response. (Das *et al.*, 1993).

Malondialdehyde (MDA), Glutathione peroxidase, and catalase are organic compounds that are produced as a result of the presence of reactive oxygen species (ROS). Hence, these compounds are examined as in vivo biomarkers of oxidative stress. Malaria infection has been linked to the occurrence of lipid peroxidation, which coincides with a decrease in the antioxidant capacity of the affected individuals, particularly in cases of Plasmodium falciparum infection. Numerous studies have confirmed that malaria infection is accompanied by heightened production of reactive oxygen species, indicating the conducive conditions for oxidative stress within the body (Akanbi*et al.*, 2009). The malaria parasite demonstrates sensitivity to oxidative stress, and the intensity of oxidative stress is influenced by the severity of the malaria infection (Farombi*et al.*, 2003). While oxidative stress has the capability to eliminate malaria parasites, it can also render host tissues, such as red blood cells, more susceptible to oxidative damage. As a consequence, this vulnerability can lead to anemia in individuals infected with malaria (Egwunyenga*et al.*, 2004). A previous investigation has validated that the levels of MDA can potentially serve as an indicator of the severity of a disease progression (Das *et al.*, 1993). In the case of malaria infection caused by Plasmodium falciparum and Plasmodium vivax, oxidative stress occurs as a result of an excessive accumulation of reactive oxygen species. This leads to an elevation in oxygen free radicals and a disruption of the regular defense mechanisms, ultimately resulting in increased levels of antioxidants in the bloodstream.

The microscopic analysis of stained blood films is widely regarded as the benchmark for routine diagnosis of malaria. Accurate assessment of parasite density is crucial for determining appropriate thresholds of parasitemia in order to effectively manage the condition. Moreover, in cases of Plasmodium falciparum infections, parasite counts assume significant importance as these infections are consistently regarded as potentially perilous. Plasmodium falciparum has the ability to infect red blood cells at any stage, which can result in the development of high levels of parasitemia. Due to the rapid multiplication of this parasite, the parasite count can escalate up to 20 times within a 48-hour span if left untreated. In the clinical setting, the level of parasitaemia is useful as one of the criteria in defining “severe P. falciparum malaria” and to monitor the effect of anti-malarial therapy (Dubey*et al.*, 1999). In a clinical context, the degree of parasitemia serves as a valuable criterion for defining "severe P. falciparum malaria" and for monitoring the efficacy of anti-malarial treatment. The objective of this study is to assess the degree of stress caused by infection with the malaria parasite.

MATERIALS AND METHODS

GEOGRAPHICAL DESCRIPTION OF THE STUDY AREA

This investigation was conducted in the town of Ekpoma, located in the state of Edo, Nigeria. Edo state is positioned longitudinally between 04^{o}E and 43^{o}E, and latitudinally between 05^{0}44^{o}N and 07^{0}34^{0}N. It is located within the South-South geopolitical region and is inhabited by around 3.1 million people (World Gazzetter, 2007). Esan land is adjacent to the southern region of Benin City, the southeastern region of Agbor, the northern and eastern regions of Etsako, and the western region of River Niger. The distance from Ewu to the capital city of the State, Benin City, spans approximately 100 kilometers. The specific region is situated within the latitudes of 6^{O} 43' and 6^{0} 45' north of the Equator, as well as the longitudes of 6^{0} 5' and 6^{0} 8' east of the Greenwich Meridian (Aziegbe, 2006). The inhabitants reside in various regions including Uromi, Ewohimi, Ewatto, Igueben, Irrua, Ubiaja, Ogwa, Ebele, Ekpoma, Ohordua, and Ewu within the central part of Edo State, located in the South-South region of Nigeria. Esan West constitutes a Local Government Area within the state of Edo, situated in Nigeria. Its headquarters are in the town of Ekpoma. Spanning across an expanse of

*Corresponding Author: **Otaye, O.M**

502 square kilometers, Esan West is home to a population of 125,842 individuals according to the 2006 census. The populace comprises predominantly of civil servants, traders, entrepreneurs, transporters, farmers, educators, and students, each pursuing their respective occupations. Following its establishment as the administrative center and as the home of a state-owned university (Ambrose Alli University), Ekpoma has experienced notable urbanization, evolving into a bustling urban hub (Aziegbe, 2006).

SAMPLE SIZE

The determination of the required sample size for this study followed specific guidelines, taking into consideration the upper limit to achieve a 95% confidence level, with an anticipated prevalence of approximately 5% (excluding retrospective studies). The precise formula was employed to arrive at the appropriate sample size:

The selection of the sample size for this study was determined based on the specified upper limit criteria, ensuring a 95% confidence level, considering the expected prevalence derived from a pilot study of approximately 55%. The application of the precise prevalence formula was employed to arrive at the final sample size.

$$N = \frac{z^2pq}{D^2}$$ (Araoye, 2004)

Where N= the desired sample size (when population is greater than 10,000)
z= is a constant given as 1.96 (or more simply at 2.0) which corresponds to the 95% confidence level.
P= expected prevalence
q= 1.0-p
d= acceptable error 5%.

$$N = \frac{(1.96)^2 \text{ X } 0.07 \text{ X } (1 - 0.07)}{(0.05)^2}$$

$$N = \frac{(1.96)^2 \text{ X } 0.07 \text{ X } 0.93}{(0.05)^2}$$

N = 100.034

To make up for the sampling error or dropouts, a minimum of 150 samples was collected and used for the research.

RESEARCH DESIGN

In a forward-looking investigation, a collection of 150 blood samples was obtained, consisting of 100 patients infected with malaria. Among the infected individuals, there were 40 cases with a parasiteamia degree of one (+), 40 cases with a degree of two (++), and 20 cases with a degree of three (+++); a group of 50 control subjects was included, consisting of 24 males and 26 females who voluntarily participated. A total of 100 malaria subjects, including 59 males and 41 females, ranging in age from 16 to 55 years, were randomly chosen from different central hospitals located in the Esan land region. All participants provided their informed consent prior to the study.

INCLUSION CRITERIA AND EXCLUSION CRITERIA

This study exclusively enrolled individuals between the ages of 16 and 55 who were diagnosed with stable malaria, while those who tested negative were included as the control group. Individuals with a history of smoking, chronic alcoholism, diabetes mellitus, rheumatoid arthritis, sickle cell anemia, pregnancy, or cancer were excluded from participation in this study.

ETHICAL CONSIDERATION

Ethical considerations were addressed and obtained from the ethics review committee of the health management board, Ambrose Alli University Ekpoma, as well as the review committee of ISTH, granting permission for the study. Consent for sample collection was obtained by informing the subjects about the research and requesting both verbal and documented consent. Those who willingly provided their consent were included in the study.

SAMPLE COLLECTION AND LABORATORY DIAGNOSIS METHODS

Collection of blood samples

A baseline blood sample of approximately 6ml was obtained from each participant on their initial hospital visit. From the collected 6ml blood sample, 3ml was placed into plain bottles for further analysis. The serum was extracted from the blood sample to conduct assays for malondialdehyde (MDA) using the Thiobarbituric Acid Assay method, as well as for glutathione peroxidase and catalase. Malaria parasite screening was conducted using a commercially prepared rapid test kit. Additionally, both thick and thin blood films were prepared for the gold standard microscopic diagnosis of malaria parasite infection. Malaria parasite detection, parasite count, and identification of the malaria parasite species were also performed.

Parasitological examination

The existence and concentration of P. falciparium in every blood sample were assessed using Giemsa-stained thin and thick blood films (Cheesbrough, 2005). A slide was considered negative when no parasites were observed after examining approximately 100 high-power fields (at 100x magnification) for a duration of around 30 minutes. The number of parasites in positive smears was tallied to assess the magnitude of the infection. Positive smears were categorized into three groups:

- Low parasitemia was defined as a parasite density of fewer than 1000 asexual forms per milliliter of blood
- Moderate parasitemia was characterized by a parasite density ranging from 1000 to 10,000 asexual forms per milliliter of blood.
- High parasitaemia characterized by a high density of >10,000 asexual forms per milliliter of blood.

ASSESSMENT OF LIPID PEROXIDATION

The measurement of lipid peroxidation in the post-mitochondrial fraction was conducted using the thiobarbituric

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acid reactive substances (TBARS) method, following the protocol outlined by Varshey and Kale (1990), and the absorbance was measured using a spectrophotometer. For a comprehensive description of the methodology, please refer to Appendix B.

DETERMINATION OF CATALASE ACTIVITY

The microsomal catalase activity was assessed following the procedure outlined by Sinha (1972). This procedure relies on the conversion of dichromate in acetic acid to chromic acetate upon heating in the presence of H_2O_2, leading to the formation of an unstable intermediate known as perchromic acid. The chromic acetate so produced is measured colorimetrically at 570-610nm. For detailed method see Appendix B.

DETERMINATION OF REDUCED GLUTATHIONE (GSH) LEVEL

The approach described by Beutler et al. (1963) was utilized to measure the concentration of reduced glutathione (GSH) in various organs of rats subjected to treatment with hesperidin and 4 vinylcyclohexenediepoxide.

This technique relies on the formation of a relatively persistent yellow-colored substance when 5,5′-dithiobis-2-nitrobenzoic acid (DTNB; also known as Ellman's reagent) is introduced to sulfhydryl compounds, with glutathione being the predominant sulfhydryl compound present in tissues. The resultant chromophoric product exhibits its highest absorption at a wavelength of 412 nm. For a comprehensive description of the procedure, please refer to Appendix B.**STATISTICAL**

ANALYSIS

The statistical analysis was performed using SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows. A significance level of P<0.05 was considered statistically significant.

RESULTS

AGE AND SEX DISTRIBUTION OF THE STUDY POPULATION

Table 4.1 presents the distribution of age and gender among the participants included in the study. A total of 150 samples were enrolled, including 50 control samples. Among the control samples, there were 25 males and 25 females. It also included fifty male patients with malaria and fifty female patients with malaria. In this current study, the highest occurrence of Malaria patients was observed among the age groups of 20-29 and 30-39, with 22 (44%) in male subjects and 16 (32%) in female subjects. However, it is noteworthy that the lowest occurrence was observed among the age groups of 0-9 and 10-19 in both male and female subjects.

TABLE 4.1: AGE AND SEX DISTRIBUTION OF THE STUDY POPULATION

		Malaria patients	
	Control	**Male**	**Female**
Age (Years)	**N= 50(n/N)**	**N=50;(n/N)**	**N=50:(n/N)**
0-9	2(4)	1(2)	3(6)
10-19	7(14)	6(12)	2(4)
20-29	11(22)	22(44)	14(28)
30-39	16(32)	9(18)	16(32)
40-49	9(18)	8(16)	7(14)
>50	5(10)	4(8)	8(16)

4.2: Comparative study of Antioxidant enzymes and MDA in malaria and nonmalaria subjects

Table 4.2 presents a comparative analysis of antioxidant enzymes and MDA levels between malaria and non-malaria subjects. The average MDA value for the control group was 3.92 ± 2.19, while the test group showed a mean of 4.98 ± 3.46. Regarding GSH levels, the control group had an average of 58.50 ± 24.51 with a standard deviation, while the test group showed a mean of 38.50 ± 15.61. As for catalase, the control group had a mean of 7.33 ± 7.77, whereas the test group exhibited a mean of 6.81 ± 7.44.

Table 4.2: Comparative study of Antioxidant enzymes and MDA in malaria and nonmalaria subjects

	Control Mean±SD	**Test mean±SD**	**t-value**	**p-value**
MDA (um/L	3.92 ± 2.19	4.98 ± 3.46	-1.152	0.258
GSH (U/gmHb%)	58.50 ± 24.51	38.50 ± 15.61	3.077	0.004*
CATALASE (umol/sec)	7.33 ± 7.77	6.81 ± 7.44	.214	0.832

*: Statistically significant at p<0.05

4.3: Comparative study of Antioxidant enzymes and MDA in male and female malaria subjects

In Table 4.3, a comparative analysis of antioxidant enzymes and MDA levels among male and female malaria subjects is presented. The mean and standard deviation (SD) of MDA values were 3.11 ± 2.14 for males and 4.98 ± 3.46 for females. Regarding GSH levels, the mean and standard deviation (SD) for males were 59.10 ± 22.1, while for females it was 38.50 ± 15.61. For catalase, the mean and SD were 7.33 ± 7.77 for males and 6.12 ± 7.54 for females.

Table 4.3: Comparative study of Antioxidant enzymes and MDA in male and female malaria subjects

	Male Mean±SD	**female mean±SD**	**t-value**	**p-value**
MDA (um/L)	3.11 ± 2.14	4.98 ± 3.46	-1.152	0.58
GSH (U/gmHb%)	59.10 ± 22.1	38.50 ± 15.61	2.16	0.09
CATALASE (umol/sec)	7.50 ± 6.70	6.12 ± 7.54	0.112	0.56

*: Statistically significant at p<0.05

DISCUSSION

The malaria parasite demonstrates susceptibility to oxidative stress, and the degree of oxidative stress is affected by the

*Corresponding Author: **Otaye, O.M**

severity of the malaria infection, as indicated by plasma parameters. These findings align with the results obtained in the current investigation (Sibmooh*et al*., 2004). While oxidative stress can eliminate malaria parasites, it can also increase the susceptibility of host tissues, including red blood cells, to oxidative harm. As a consequence, this can lead to the development of anemia in individuals infected with malaria (Egwunyenga*et al*., 2004).

The elevated Malondialdehyde (MDA) levels observed in this study among malaria-positive patients suggest an augmented generation of reactive oxygen species. However, it should be noted that these findings did not reach statistical significance. Prior investigations have validated the association between Malondialdehyde (MDA) levels and the intensity of a disease condition, as also observed in the present study (Das *et al*., 1993). In cases of Malaria caused by Plasmodium falciparum and Plasmodium vivax, the oxidative stress arises from an excessive accumulation of reactive oxygen species, resulting in elevated levels of oxygen free radicals and compromised normal defense mechanisms. This, in turn, leads to an upregulation of antioxidant blood serum levels (Hunt and Stocker, 1990). Nevertheless, this discovery contradicts the results of numerous prior investigations that have demonstrated elevated levels of MDA in individuals infected with malaria parasites (Hunt and Stocker, 1990; Egwunyenga*et al*., 2004; Akanbi*et al*., 2009). Notably, studies have indicated that the malaria parasite has the ability to produce substantial amounts of reactive oxygen species (ROS) both independently and through its interaction with the phagocytic cell system (Kremsner*et al*., 2000). The oxidative stress arises from the breakdown of red blood cells, leading to an imbalance between the production of reactive oxygen species and the protective antioxidant defense system (Bonnefont-Rousselot*et al*., 2000; Mendis*et al*., 2001). The development of an infectious agent leads to an excessive production of free radical species and impairment of the normal defense mechanisms, resulting in a reduction in antioxidant levels and a compromised elimination of reactive substances (Thurnhann*et al*., 1988). One of the outcomes of oxidative stress is the occurrence of anemia in individuals with malaria (Kremsner*et al*., 2000; Clark and Hunt, 1983).

Moreover, individuals with uncomplicated acute P. falciparum or P. vivax malaria have been documented to exhibit reduced catalase activity compared to individuals without the infection (Pabon, *et al*., 2003). This finding aligns with the current study, where a comparable but statistically insignificant decrease was observed in comparison to the control group. The decreased catalase activity can result in the buildup of hydrogen peroxide (H_2O_2), which can readily react with iron in ferriprotoporphyrin IX (FP) and generate hydroxyl radicals, thereby contributing to tissue damage (Seixas*et al*., 2009)

Cells have developed numerous defensive mechanisms to mitigate the harmful impact of the reactive radicals. These defense mechanisms involve the presence of antioxidants such as glutathione (GSH) and enzymes like catalase (CAT), glutathione-S-transferase (GST), and superoxide dismutase (SOD). They facilitate the breakdown of superoxide anions (O2-) and hydrogen peroxide (H2O2) into harmless water (H2O), utilizing GSH in the process (Rinola*et al*.. 2008). GSH, in its reduced form, assumes a crucial role in safeguarding cells against the detrimental effects of oxidative stress and harmful substances.

The diminished utilization of GSH results in elevated levels of cellular GSH and overall intracellular-SH groups, thereby exposing red blood cells to the potential dangers of oxidative stress. The rise in sulfhydryl (–SH) groups in red blood cells under oxidative stress conditions is attributed to the decline in the activity of erythrocyte GST (glutathione-S-transferase). The reduced activity of GST (glutathione-S-transferase) may result in an extended binding with sulfhydryl (–SH) groups of harmful substances, potentially causing their buildup within the red blood cells (Bernabucci*et al*., 2000). In this research, a notable reduction was observed in the experimental groups compared to the control group, which aligns with the findings of a previous study conducted by Amit et al. (2017) investigating the antioxidant status in individuals with malaria.

Human red blood cells infected with Plasmodium experience heightened oxidative stress caused by the malaria parasite (Eaton *et al*., 1976; Golenser*et al*., 1991). The malaria parasite has the ability to produce Reactive Oxygen Species (ROS) inside red blood cells, and the ROS generated through immune activation can cause additional harm to uninfected red blood cells (Rath*et al*., 1991) This observation aligns with our study findings, indicating an elevated level of oxidative stress in the examined population.

CONCLUSION

In conclusion, the current study demonstrated that malaria infection resulted in the occurrence of oxidative stress to a certain degree, as evidenced by the observed lack of statistical significance.

RECOMMENDATION

Therefore, we recommend considering the inclusion of antioxidants in the treatment regimen for malaria patients, either through dietary supplementation or in combination with anti-malaria medications, as part of their management plan. Furthermore, the significance of conducting further research in this regard cannot be overstated.

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*Corresponding Author: **Otaye, O.M**