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Predictive Role Of Oxidative Stress Biomarkers (Malondialdehyde, Glutathione, Catalase and Superoxide Dismutase) in Preeclamptic Pregnant Women In The Third Trimester Of Pregnancy

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Abstract.*The predictive role of oxidative stress in the pathophysiology of human pregnancy is of particular interest, as oxidative stress is increased in normotensive pregnant women through systemic inflammatory response, reactive oxygen species (ROS), and reactive nitrogen species (RNS). This study involved 40 consenting pregnant women recruited from St. Philomina Catholic Hospital, Edo State, Nigeria. Data obtained were analyzed using GraphPad Prism 9. Results were expressed as mean ± SEM, with a P-value of ≤ 0.05 considered statistically significant. Malondialdehyde (MDA) increased from 3.75 ± 0.42 U/L in normotensive women to 4.53 ± 0.59 U/L in preeclamptic women. However, this increase was not statistically significant (p<0.05; 0.2903). Catalase activity decreased from 114 ± 8.07 U/L in normotensive women to 84.02 ± 21.58 U/L in preeclamptic women, but this decrease was also not statistically significant (p<0.05; 0.1909). Superoxide dismutase (SOD) increased from 40.82 ± 1.23 U/L in normotensive women to 71.90 ± 5.61 U/L in preeclamptic women. This increase was statistically significant (p<0.05; <0.0001). Glutathione peroxidase (GSH) decreased from 66.74 ± 10.35 U/L in normotensive women to 50.63 ± 8.96 U/L in preeclamptic women, but this decrease was not statistically significant (p<0.05; 0.2466). These findings suggest the potential predictive roles of these biomarkers in identifying and monitoring preeclampsia.*

Keywords: *Catalase, Glutathione, Malondialdehyde, Preeclampsia, Superoxide dismutase,*

1. INTRODUCTION

Preeclampsia is a complex and multifactorial pregnancy complication characterized by high blood pressure and damage to vital organs such as the kidneys, liver, and brain. This condition affects approximately 2-8% of pregnancies worldwide, making it a leading cause of maternal and fetal morbidity and mortality. Preeclampsia can develop rapidly and poses a risk for serious complications such as eclampsia, HELLP syndrome (Hemolysis, Elevated Liver enzymes, and Low Platelets), and other organ dysfunctions. Therefore, early detection and prediction of preeclampsia are crucial to prevent severe complications and ensure timely intervention.

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One important aspect in the pathophysiology of preeclampsia is oxidative stress, which refers to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to counteract their harmful effects. Oxidative stress can lead to cellular damage, including damage to the vascular endothelium, which contributes to increased blood pressure and organ damage typical of preeclampsia. Oxidative stress biomarkers such as Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT), and Superoxide Dismutase (SOD) have been widely studied in relation to this condition.

Malondialdehyde (MDA) is a lipid peroxidation product used as an indicator of oxidative damage to cell membranes. Elevated levels of MDA indicate oxidative damage to body cells, which plays a role in the pathogenesis of preeclampsia. On the other hand, Glutathione (GSH) is one of the body's main antioxidants that helps combat oxidative stress by binding reactive oxygen species and converting them into less harmful forms. A decrease in GSH levels may indicate a deficiency in the body’s ability to combat oxidative damage, which can increase the risk of preeclampsia.

In addition to GSH, other antioxidant enzymes, such as Catalase (CAT) and Superoxide Dismutase (SOD), also help reduce the impact of free radicals in the body. CAT breaks down hydrogen peroxide into water and oxygen, while SOD converts harmful superoxide ions into oxygen and hydrogen peroxide, which can be more easily broken down by the body’s defense system. A decrease in the activity of these enzymes can exacerbate oxidative stress in pregnant women and potentially increase the risk of preeclampsia.

This study aims to determine the predictive role of oxidative stress biomarkers such as MDA, GSH, CAT, and SOD in preeclamptic pregnant women in the third trimester of pregnancy. Understanding the relationship between these biomarkers and preeclampsia will provide deeper insights into the mechanisms underlying the disease’s pathology. Furthermore, the use of oxidative stress biomarkers as predictors of preeclampsia offers a promising approach, as they are widely available and cost-effective. The findings of this study are expected to contribute to the development of personalized medicine approaches in the diagnosis and management of preeclampsia.

2. LITERATURE REVIEW

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and the antioxidant defense system. Excessive ROS can damage lipids, proteins, and DNA, disrupting cellular functions and contributing to various pathological conditions. Biomarkers of oxidative stress include malondialdehyde (MDA), 4-hydroxynonenal (4-HNE),

isoprostanes, and protein carbonyls, which indicate lipid peroxidation, protein oxidation, and DNA damage, respectively. Antioxidant biomarkers, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), reflect the body's capacity to counteract oxidative damage. These biomarkers are widely used to study oxidative stress in diseases, including preeclampsia (10). Preeclampsia is a hypertensive disorder of pregnancy characterized by new-onset hypertension and proteinuria after 20 weeks of gestation. It is a leading cause of maternal and fetal morbidity and mortality worldwide. The pathophysiology of preeclampsia involves poor placentation, endothelial dysfunction, and systemic inflammation, with oxidative stress playing a central role (11). During normal pregnancy, there is a physiological increase in ROS due to elevated metabolic demands and mitochondrial activity. However, in preeclampsia, this increase is exacerbated by reduced antioxidant defenses and increased placental oxidative stress. Impaired spiral artery remodeling leads to hypoxia-reoxygenation injury in the placenta, further contributing to oxidative stress (12).

Several studies have identified increased levels of oxidative stress biomarkers in preeclamptic women. Elevated MDA and 8-isoprostane levels have been reported, reflecting enhanced lipid peroxidation. Similarly, higher levels of protein carbonyls and 8-hydroxy-2'-deoxyguanosine (8-OHdG) indicate protein and DNA oxidative damage, respectively. Reduced antioxidant biomarkers such as GSH, SOD, and CAT have also been observed in preeclampsia, suggesting impaired antioxidant defenses (13). These alterations correlate with the severity of the disease, highlighting their potential as diagnostic and prognostic markers. The identification of oxidative stress biomarkers offers opportunities for early detection and targeted intervention in preeclampsia. Antioxidant therapies, such as vitamin C, vitamin E, and N-acetylcysteine, have been explored to mitigate oxidative stress, but their clinical efficacy remains inconclusive. Future research should focus on elucidating the molecular mechanisms linking oxidative stress to preeclampsia and developing novel biomarkers with higher sensitivity and specificity (14).

3. MATERIALS AND METHODS

Geographical Description of the Study Area

This research was carried out among Third Trimester Pregnant women in St. Philomina Catholic Hospital, Edo State , Nigeria. lies longitudinally at 04ºE and 43ºE and Latitude 05º44ºN and 07º34ºN. It geopolitical location is the South South and it has a population of 3.5 million people. Oredo land, Benin City, the State capital, is 100 km long. Edo State, South-South, Nigeria. Oredo is a Local Government Area of Edo State, Nigeria. Its headquarters are

in the town, Benin city. It has an area of 502 km² and a population of 500,000 at the 2006 census.

Majority of which are civil servants, traders, businessmen/women, transporter, farmers, teachers/lecturers and students by occupation. Oredo, since after its designation as headquarters and as the host of Oba of Benin Palace, the town has grown into an urban center.

Research Design

Forty (40) consenting pregnant subjects were recruited from St. Philomina Catholic Hospital, Edo State. These subjects consisted of twenty (20) normotensive pregnant women in their third trimester of pregnancy with blood pressure between 120/80mmHg to 130/90 mm/Hg without presence of proteinuria and twenty (20) preeclamptic women in their third trimester of pregnancy classified as having preeclampsia according to their blood pressure measured was above 130/90 mm/Hg with the presence of proteinuria taken two consecutive times at presentation at the antenatal clinic of the hospital

Sample Size

The Population of study was determined using the formula;

$N= Z^2pq/d^2$

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= previous survery prevalence of 2.23%

q= 1.0-p

d= acceptable error 5%.

Where N= sample size, Z=1.96, p=0.1% (0.01) and d=5% (0.05)

N= 39.8 subject.

Therefore, the sample for this study is 40 respondents who are normotensive and preeclamptic pregnant women from Oredo town, Benin City.

Ethical Approval and Informed Consent

Ethical clearance (REC Approval No:RECC/10/2023(07)) was obtained from the Research Ethics Committee of St. Philomina Catholic Hospital, Edo State.

Written informed consent was obtained from subjects prior to commencement of the study.

Blood Sampling

10 milliliters (10 ml) of venous blood was drawn from consenting participants and placed in a lithium heparin sample bottles. Blood samples was spun in a bucket centrifuge at 2500 RPM (rounds per minute) for 10 minutes after which plasma was collected and stored frozen in plain sample bottles and was analyzed for **Antioxidant activities (Malondialdehyde, Glutathione, Catalase and Superoxide dismutase) by spectrophotometric method**

Experimental Protocols

After the subjects were identified and recruited into the study, they were taken to the lab where their vital signs was taken, after which blood samples were collected by venipuncture and taken to the chemistry laboratory for analysis.

Study Area/Population

The study were conducted for three months at St. Philomina Catholic Hospital, Edo State, Nigeria.

Inclusion Criteria

Normotensive and Preeclamptic pregnant women in the third trimester of pregnancy, within the age range of 25 to 35years was used for this study. Pregnant women were recruited for this study and women who had given birth before and were pregnant for the second time.

Exclusion Criteria

Normotensive and Preeclamptic pregnant women who were on drugs and with a known history of hyperlipidemia, gestational Diabetes and other comorbidity.

Biochemical Examination

.**Antioxidant activities (Malondialdehyde, Glutathione, Catalase and Superoxide dismutase) by spectrophotometric method**

Determination of Catalase Activity

Microsomal catalase activity was determined according to the method of Sinha (1972).

Determination of catalase activity of samples

0.1ml of microsome was mixed with 4.9ml of distilled water to give 1 in 50 dilution of samples. The assay mixture contained 2ml of H_2O_2 solution (800 micromoles) and 2.5ml of phosphate buffer in test tubes (arranged in duplicates) (due to the one to be read at 0 sec and 60 sec). 0.5 ml of properly diluted microsome was rapidly added to the reaction mixture by a gentle swirling and the reaction proceeded at room temperature. 1ml portion of the reaction mixture was withdrawn and blown into 2ml dichromate/ acetic acid reagent at 60 second intervals (add to the 2ml, then wait for 60sec add to the other . and read both). The H_2O_2 contents of the withdrawn solutions were determined by the method described above.

Assessment of lipid Peroxidation

Lipid peroxidation in post mitochondrial fraction was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method as described by Varshey and Kale (1990).

Procedure

0.4 ml of the post mitochondrial fraction was mixed with 1.6ml of Phosphate buffer and 0.5 ml of 30 % TCA was added, 0.5 ml of 75 % TBA was added and placed in a water bath for 45 minutes at 80˚C, cooled in ice and centrifuged at room temperature for 10minutes at 3,000 rpm. The absorbance of the clear supernatant was measured against refe blank (distilled water) at 532 nm using spectrophotometer.

Determination of Superoxide Dismutase (Sod) Activity

Superoxide dismutase was estimated according to the methodology proposed by Kono (1978).

Procedure

In the test cuvette, the reaction mixture containing 1.3 ml sodium carbonate buffer, 500 µl NBT and 100 µl Triton X-100 was taken. The reaction was initiated by the addition of 100 µl hydroxylamine hydrochloride. After 2 minutes, 70 µl of the enzyme extract was added. The percentage inhibition in the rate of NBT reduction was recorded as an increase in absorbance at 540 nm.

Determination of Reduced Glutathione (GSH) Level

The method of Beutler *et al*. (1963) was adopted in estimating the level of reduced glutathione (GSH) in some organs of rats treated with hesperidin and 4 vinylcyclohexenediepoxide.

Assay Protocol

0.4 ml of sample was added to 0.4 ml of precipitating solution which was vortexed and centrifuged at 4000 rpm for 5 minutes. Thereafter, 0.5 ml of the supernatant was added to 1.5 ml of Ellman's reagent. The absorbance of the reaction mixture was read at 412 nm against a reagent blank.

Data Analysis

Data obtained from this study were analysed using Graph Pad Prism 9. Results generated were expressed as mean ± SEM and a P-value of ≤ 0.05 were considered satistically significant. The significance of difference among the groups were used to assess the repeated-measures analysis of variance (ANOVA). Independent students' t-test were used to compare normotensive and preelclamptic pregnant women groups.

4. RESULTS AND DISCUSSION

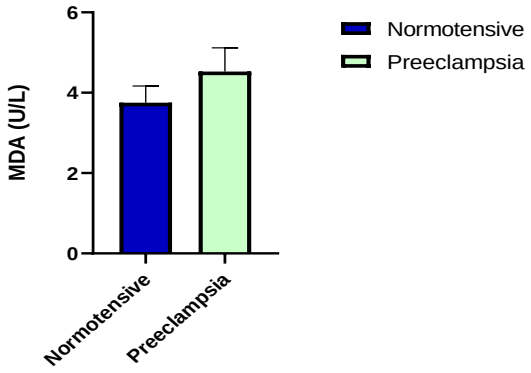

Figure 1: Mean ± SEM of Malondialdehyde (MDA) level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference.

Figure 1 shows the levels of Malondialdehyde (MDA) in Normotensive and pre-eclamptic women in their third trimester of pregnancy. Malondialdehyde (MDA) increased from 3.75 ± 0.42U/L in Normotensive women to 4.53 ± 0.59U/L in pre-eclamptic women. However, this increase was not statistically significantly ($p<0.05$; 0.2903).

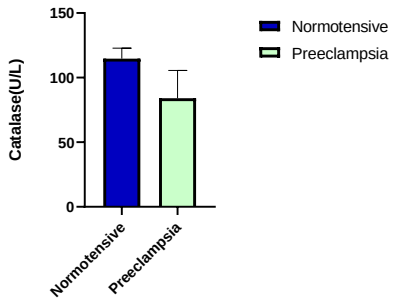

Figure 2: Mean ± SEM of Catalase level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference.

Figure 2 shows the levels of Catalase activities in Normotensive and pre-eclamptic women in their third trimester of pregnancy. Catalase activities decreased from 114 ± 8.07U/L in Normotensive women to 84.02 ± 21.58U/L in pre-eclamptic women. However, this decrease was not statistically significantly ($p<0.05$; 0.1909).

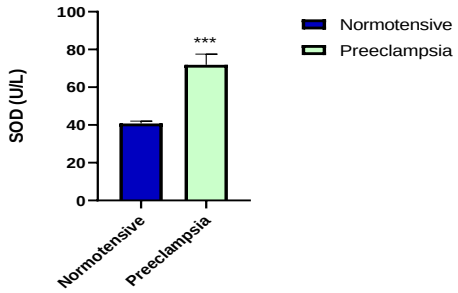

Figure 3: Mean ± SEM of Superoxide Dismutase (SOD) level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference. * represents p<0.001**

Figure 3 shows the levels of Superoxide Dismutase (SOD) in Normotensive and pre-eclamptic women in their third trimester of pregnancy. Superoxide Dismutase (SOD) increased from 40.82 ± 1.23U/L in Normotensive women to 71.90 ± 5.61U/L in pre-eclamptic women. This increase was found to be statistically significant ($p<0.05$; <0.0001).

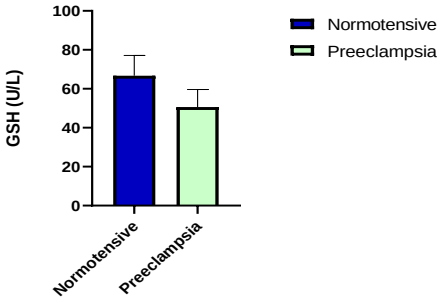

Figure 4: Mean ± SEM of Glutathione Peroxidase (GSH) level in normotensive (n=20) and preeclampsia (n=20). The t-test was carried out to access any significant difference.

Figure 4 shows the levels of Glutathione Peroxidase (GSH) in Normotensive and pre-eclamptic women in their third trimester of pregnancy. Glutathione Peroxidase (GSH) decreased from 66.74 ± 10.35U/L in Normotensive women to 50.63 ± 8.96U/L in pre-eclamptic women. However, this decrease was not statistically significantly ($p<0.05$; 0.2466).

5. DISCUSSION

SOD is an antioxidant enzyme that plays a crucial role in protecting cells from oxidative stress by catalyzing the conversion of superoxide radicals into hydrogen peroxide and oxygen [3] . Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, has been implicated in the pathogenesis of preeclampsia [8].Figure 1, the significant increase in superoxide dismutase (SOD) level observed in pre-eclamptic women compared to normotensive women suggests a potential predictive role of SOD in preeclampsia which correlate with the research of [4]. Elevated SOD levels have been reported in women with preeclampsia compared to normotensive pregnant women, suggesting a role for SOD as a biomarker for oxidative stress in preeclampsia. The significant increase in SOD levels in pre-eclamptic women may reflect a compensatory response to increased oxidative stress associated with the disorder. In contrast, the non-significant changes in malondialdehyde (MDA), glutathione (GSH), and catalase levels between normotensive and pre-eclamptic women suggest limited predictive roles of these antioxidants in preeclampsia. MDA is a marker of lipid peroxidation, while GSH and catalase are important antioxidants that neutralize ROS and protect against oxidative damage [2]. Although oxidative stress has been implicated in the pathogenesis of preeclampsia [9], the inconsistent findings regarding MDA, GSH, and catalase levels in this study suggest that these antioxidants may not be reliable biomarkers for predicting preeclampsia. However, the significant increase in SOD levels in pre-eclamptic pregnant women highlights the importance of oxidative stress in the pathophysiology of preeclampsia and suggests that SOD may have potential utility as a biomarker for oxidative stress and disease severity in preeclampsia.

CONCLUSION

The present study showed that there was statistically significant increase in SOD was observed in preeclamptic women compared to normotensive pregnant women, indicating various underlying pathophysiological processes such as oxidative stress abnormal cascades activation. This finding suggest the potential predictive roles of this biomarker in identifying and monitoring preeclampsia. However, levels of malondialdehyde (MDA), glutathione (GSH)

and Catalase and did not show significant differences between preeclamptic and normotensive women, suggesting that these biomarkers may not be reliable predictors of preeclampsia based on the current study's findings.

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