

Selenium and Glutathione Levels in Pregnant Women With and Without Preeclampsia: A Cross-sectional Comparative Study in Ibadan, Nigeria

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Abstract

Background

Preeclampsia is a major cause of maternal and perinatal morbidity and mortality worldwide, particularly in low- and middle-income countries. Oxidative stress plays a significant role in its pathogenesis. Selenium and glutathione are important antioxidants that mitigate oxidative stress, but previous studies have reported conflicting findings regarding their levels in preeclamptic women.

Objective

This study aimed to determine and compare serum selenium and glutathione levels among women with preeclampsia, normotensive pregnant women, and normotensive non-pregnant women in Ibadan, Nigeria.

Methods

This comparative cross-sectional study was conducted at the University College Hospital and Adeoyo Maternity Teaching Hospital, Ibadan, Nigeria, between 2019 and 2020. Participants included women with antepartum preeclampsia, normotensive pregnant women, and normotensive non-pregnant fertile women. Blood samples were collected for selenium assay using atomic absorption spectrophotometry and glutathione determination using Owen's method modified by Owen and Butterfield. Data were analyzed using SPSS version 23. Descriptive statistics and analysis of variance (ANOVA) were used, with statistical significance set at $p < 0.05$.

Results

The mean serum selenium levels among preeclamptic women, normotensive pregnant women, and normotensive non-pregnant women were 70.93 ± 22.56 µg/dl, 54.56 ± 12.42 µg/dl, and 57.40 ± 11.51 µg/dl, respectively ($p < 0.001$). Mean glutathione levels were 2.592 ± 0.263 µmol/L, 2.395 ± 0.324 µmol/L, and 2.742 ± 0.139 µmol/L, respectively ($p < 0.001$). Selenium levels increased with gestational age among women with preeclampsia, although this trend did not reach statistical significance.

Conclusion

Contrary to previous reports suggesting antioxidant depletion in preeclampsia, this study found significantly higher selenium and glutathione levels in preeclamptic women. These findings may suggest a transient adaptive antioxidant response to oxidative stress in preeclampsia. Further longitudinal studies are needed to clarify these findings.

Background

Preeclampsia is a disorder of pregnancy characterized by hypertension and significant proteinuria, discovered for the first time in the second half of pregnancy[1]–[5].

Being a progressive disease, when preeclampsia is not diagnosed or treated promptly, it could lead to eclampsia, in which a pre-eclamptic woman with no previous neurologic problem presents with tonic-clonic convulsion[3], [4].

Hypertensive disorder of pregnancy complicates about 2-8% of all pregnancies, 5-7% of which will be preeclampsia; though up to 17% prevalence has been reported in an African study[6]–[8]. It is one of the leading causes of maternal morbidity and mortality. There is no universally accepted biomarker to predict, prevent, or prognosticate for preeclampsia. However, preeclampsia, being an oxidative-stress-associated disease, some researchers have reported a decline in the levels of many oxidative stress markers, including nitric oxide, superoxide dismutase, glutathione peroxidase activities, and catalase, among others[9]–[12]. Therefore, this study aims to measure selenium and glutathione levels in women with preeclampsia and compare them with those in normotensive pregnant and non-pregnant women. The choice of Glutathione (GSH) was because it is required by the enzyme Glutathione peroxidase to convert hydrogen peroxide to water, thus reducing oxidative stress, while Selenium is an important component of many metalloenzymes that mop up oxidative stress at the cellular level[13], [14]. If the pattern of change in Selenium or Glutathione concentrations could be determined, it could be a promising marker or substance for the management of preeclampsia.

Organic compounds containing selenium are called organo-selenium and have greater bioavailability and less toxicity compared with inorganic selenium. The body does not synthesize selenium, but it is obtained from diets in the form of inorganic and organic compounds. Selenium is obtained from diets and absorbed from the distal part of the small intestine. Selenium is mostly stored in the thyroid gland and skeletal muscles[11], [15], [16]. The liver synthesizes most of the selenoproteins and regulates the excretion of selenium metabolites. Selenium performs many roles in the body, such as being a constituent of selenoproteins, activating anti-carcinogenic factors, preventing diseases of the cardiovascular system, and exhibiting anti-proliferative and anti-inflammatory activities. It has also been noted to stimulate the immune system and act as an antagonist to heavy metals such as arsenic, cadmium, lead, and mercury. Increased nutritional demand in pregnancy makes selenium levels lower in pregnancy than in a non-pregnant state. Glutathione levels are also lower in pregnant than in non-pregnant states[10], [13]–[15], [17], [18].

Glutathione (GSH) is made up of three peptides, which are glycine, cysteine, and glutamic acid, with glutamic acid linked to cysteine via a γ -carboxyl group. Though it could also be obtained from the diet, Glutathione is synthesized in the liver following ingestion of the appropriate amino acids and sulphur-containing foods. GSH is water-soluble and serves as an antioxidant and regenerator of vitamin E and carotenoids, as well as an intracellular enzyme[19].

Preeclampsia is characterized by oxidative stress, leading to the depletion of agents such as Glutathione and Selenium that combat this unfavourable state. It is, therefore, necessary to assess the level of these two substances in women with preeclampsia and compare it with that of normotensive pregnant and non-pregnant women.

Previous studies evaluating selenium and glutathione levels in preeclampsia have reported inconsistent findings. While several studies have demonstrated reduced levels in preeclamptic women, others suggest the possibility of compensatory increases in antioxidant activity in response to oxidative stress.

This study aimed to determine serum selenium and glutathione levels among women with preeclampsia and compare them with those of normotensive pregnant and non-pregnant women in Ibadan, Nigeria.

Methods

This comparative cross-sectional study was conducted in Ibadan Oyo state in 2019-2020 at the University College Hospital and Adeoyo Maternity Teaching Hospital, both in Ibadan and are major referral centres. The participants included in the study were consenting women meeting the inclusion criteria which were women diagnosed with pre-eclampsia, who presented to either of the two study centres and normotensive women with previous pregnancy experience but not pregnant presently and had come to the family planning clinic at the study centres to uptake a family planning method. A non-probability purposive sampling technique was used to select the participants. The inclusion criteria included: consenting pregnant women, women who were diagnosed with pre-eclampsia, and presented to either of the two study centres and women with previous pregnancy history, but not pregnant at the time of this study, and had come to the family planning clinic at the study centres to uptake a family planning method. The excluded women included females who were less than 18 years old, women with multiple pregnancies; pregnant women whose babies have foetal anomalies, inability or refusal to give informed consent, and non-pregnant women who were hypertensive. The diagnosis of preeclampsia was made based on blood pressure of at least 140/90mmHg measured on two occasions at least 4 hours apart, or a single measurement of at least 160/110mmHg with significant proteinuria of at least 300mg in a 24-hour urinary sample or dipstick proteinuria of at least 2+. An appropriate formula was used to calculate the sample size, and 80 participants were determined to be in each arm of the study, with a total of 240 participants. The three groups were women with preeclampsia, normotensive pregnant women, and normotensive non-pregnant women.

Sample collection, transport, storage, and analysis

A proforma was used to obtain the relevant information about the patient. Ten millilitres of venous blood were collected from each of the 240 consenting patients. Five millilitres were dispensed into a Lithium Heparin container for the estimation of glutathione level, and the remaining 5ml was dispensed in a plain bottle, allowed to stand for about 30 minutes, and the supernatant (serum) was dispensed into another container for the analysis of selenium level. The samples were labelled appropriately for easy identification. The heparinized blood samples were centrifuged in the laboratory at 1000-2000rpm for 10

minutes, and the supernatant (plasma) was collected in another heparinized bottle. All samples were then stored at -20 °C until the time of analysis.

Selenium was assayed in the blood samples collected using the Atomic Absorption Spectrophotometry technique[20]. Selenium absorbance was read at a wavelength of 196nm.

Total glutathione determination was according to Owen's method (Owen and Belcher 1965) as modified by Owen and Butterfield (2010)[21], [22]. The absorbance was read at a wavelength of 412nm. The laboratory analysis was conducted at the main laboratory of the Department of Chemical Pathology of the University College Hospital, Ibadan, Oyo State, Nigeria. Ethical approval was obtained from the joint ethical board of the University of Ibadan and the University College Hospital (UI/UCH) Ibadan (UI/UCH committee assigned number: UI/EC/18/0375). The tenets of ethics were adhered to throughout the study.

Data obtained was input into Statistical Products and Services Solution (SPSS) Version 23. Categorical variables were summarized with tables, frequency, and proportions, while numerical variables were summarized with mean and standard deviation. ANOVA was used for the test of significance of quantitative variables. Significance was considered at p-value < 0.5.

RESULTS

Table 1 shows the socio-demographic characteristics of the participants: age, educational status, religion, tribe, residential area, and occupation. Three categories of women were studied: pregnant women with preeclampsia, normotensive pregnant women, and normotensive non-pregnant women. In all three groups of women, the majority were in their third to fourth decade of life, most had a tertiary level of education, with 60% among the preeclamptic, 74% among the normotensive pregnant, and 45.7% among the non-pregnant women. Most women in each category were Christians and of the Yoruba tribe. Most resided in semi-urban areas, and the majority of the women were employed. The women had comparable socio-demographic characteristics.

Table 2 shows the obstetric history of the studied women. The majority of the women in each group were multigravida, and this accounted for 48.8%, 58.4%, and 46.3%, respectively, among the preeclamptic, normotensive, and non-pregnant women. An almost equal proportion of the preeclamptic and normotensive pregnant women had one child alive at the time of this study, representing 25% and 28.6%, respectively. The 7.6% among the non-pregnant women with no child alive represented the women who had been pregnant at one point in time, with pregnancy not resulting in a livebirth, but were not desirous of pregnancy at the time of this study. Women with high parity were not many among the studied population. These women across the three groups likewise had comparable obstetric histories.

Table 3 shows the mean selenium and total glutathione levels among the studied women. The selenium level was measured in micrograms per decilitre (µg/dl) and the total glutathione level in micromoles per litre (µmol/L). The range of values of selenium obtained from the 76 preeclamptic women was 22.09

-117.10 µg/dl with a mean value of 70.93 ± 22.56 µg/dl. The mean serum selenium levels among the normotensive pregnant women and normotensive non-pregnant women were 54.56 ± 12.42 µg/dl and 57.40 ± 11.51 µg/dl, respectively. The differences among these three means were statistically significant with a p-value <0.001.

The glutathione level in the preeclamptic women was 2.592 ± 0.263 µmol/L, while those of normotensive pregnant and non-pregnant women were respectively 2.395 ± 0.324 µmol/L and 2.742 ± 0.139 µmol/L. The difference among the three groups was statistically significant with a p-value < 0.001.

Table 4 shows the trend of selenium and glutathione levels among preeclamptic and normotensive pregnant women studied. When classified according to the gestational ages at which the diagnosis of preeclampsia was made into pre-viable (gestational age of <28 weeks as generally used in Nigeria), preterm (28- 36 weeks gestational age), term (from 37 -41 weeks), and prolonged (≥ 42 weeks) pregnancies; it was observed that the serum selenium level increased with increasing gestational age among the women with preeclampsia. Among the women with pre-viable pregnancy at the time of diagnosis, the mean serum selenium level was 56.02 ± 6.96 µg/dl and this value increased progressively with advancing gestational age at diagnosis of preeclampsia with a finding of a mean selenium level of 66.61 ± 23.83 among those with preterm pregnancy, 77.16 ± 19.85 among those with preeclampsia at term, and ultimately the largest value of 93.80 ± 11.54 among those with prolonged pregnancy. However, there is no statistically significant difference in these mean values as shown by a p-value of 0.065. Among the normotensive pregnant women, serum selenium was also lowest in pre-viable pregnancy, with a level of 45.03 ± 15.80 , although the mean value of 53.33 ± 11.81 in term pregnancy was smaller than 56.00 ± 12.30 found in the preterm pregnant women. This difference, however, is not statistically significant with a p-value of 0.02. Total glutathione level was within the same range among the categories of preeclamptic women, with levels of 2.577 ± 0.38 among the women with pre-viable pregnancy at diagnosis, 2.595 ± 0.29 among those with preterm pregnancy, 2.595 ± 0.23 and those with term pregnancy, and 2.490 ± 0.07 among those with prolonged pregnancy. These differences are not statistically significant, nonetheless ($P = 0.96$). A similar pattern was also noticed in the normotensive pregnant women. Though the total glutathione level among the preterm women is slightly lower than in the pre-viable and term groups (No prolonged pregnancy), this difference is not statistically significant ($p = 0.42$).

Discussion

This study measured serum selenium and total glutathione levels in women with antepartum preeclampsia, normotensive pregnancy, and normotensive non-pregnant women. These women had comparable sociodemographic and obstetric characteristics. Selenium is an important component of antioxidant enzymes in the body, especially the seleno-enzymes such as glutathione peroxidase, and they mitigate oxidative stress in the body. Glutathione is an essential substrate for the activities of this glutathione peroxidase in the body. The levels of glutathione and selenium in the body are directly proportional to the activities of the antioxidant enzyme glutathione peroxidase. Women with

preeclampsia were found to have a significantly higher level of serum selenium than normotensive pregnant and non-pregnant women. This was contrary to the report of most other researchers who had reported selenium levels in preeclamptic women in comparison with normotensive pregnant women. Rayman and co-workers had reported lower levels of selenium in preeclamptic than in normotensive UK women in a randomized controlled trial[18], In Imo State, Nigeria, Ikaraoha et al found a significantly lower selenium level in preeclamptic than in normotensive pregnant and non-pregnant women[23] so also was Eze et al[12], Mistry et al[24], and many others[25], [26]. However, in a meta-analysis by Taravati and Tohidi, where 2953 preeclamptic cases were compared with 3261 controls, the authors concluded with a hypothesis that in a condition of established oxidative stress, there is the possibility of an induced protective adaptive response that leads to an increase in some antioxidants[14]. In light of this hypothesis, the preeclamptic patients might probably have an adaptive elevation in the selenium levels to enhance the antioxidant effects of seleno-enzymes, at the time the blood sample was being collected, though this response may be for a short time. To further buttress this assumption, it is known that next to the thyroid gland, the bulk of body selenium is stored in skeletal muscles[27]–[29], It is, therefore, possible that during this adaptive period to the oxidative stress associated with preeclampsia, selenium might have been released from the muscles into the blood circulation, thus accounting for this rise in selenium levels in the preeclamptic than the normotensive pregnant and non-pregnant women. The finding of a higher selenium level in non-pregnant women than in normotensive pregnant women is consistent with the reports of other researchers within and outside Nigeria. Diet, being the main source of selenium for the body, a diet high in selenium or selenium-containing supplements could also have contributed to the high selenium level in the preeclamptic group.

Furthermore, among the women with preeclampsia (Table 4), serum selenium levels increased with increasing gestational age, but this did not reflect any statistical significance (p : 0.07). This could be a result of the small sample sizes of 3 and 2, respectively, in the pre-viable and prolonged pregnancy groups. Among the normotensive pregnant women, too, there was no significant difference in selenium levels from one gestational age to another (p = 0.2).

Glutathione is an essential substrate for seleno-enzymes, especially glutathione peroxidase. In the face of oxidative stress, there may be increased consumption of the substrate, and this may lead to low levels in preeclamptic women. Gutman and Parilla reported a lower level of glutathione in preeclamptic clients[30]. This study found a higher glutathione level in preeclamptic women than in normotensive pregnant women. This is in sharp contrast to the findings of Uzun in Turkey, among the 29 preeclamptic and 32 healthy pregnant women studied, where the mean glutathione level was more than twice as high in the normal as in the preeclamptic women[11]. The small sample size of 29 preeclamptic women may be a limitation of the Turkish study because, in the United States of America, Mackenzie et al compared the glutathione peroxidase levels in preeclamptic and normotensive women, but found no significant difference between them[10]. This again brings to mind the submission of Taravati and Tohidi in a meta-analysis that some antioxidant compounds, including glutathione, may increase as an adaptive response in the presence of oxidative stress[14]. It was possible that as the selenium level in the preeclamptic experienced a presumed adaptive increase, glutathione also had the same adaptive change. Among the

preeclamptic women as well as among the normotensive pregnant women, there was no significant change in glutathione levels from one gestational age to another, and this is consistent with the report of Mackenzie and co-workers in the USA, among patients of the same diagnosis.

CONCLUSION

This study did not have enough power to disapprove the prevailing knowledge that oxidative stress in preeclampsia leads to low levels of selenium and other antioxidant products, though this study reported higher levels in this study, however; it has raised the possibility of an adaptive response in the presence of oxidative stress as seen in preeclamptic women which may cause a momentary rise in the level of oxidative stress mediators like selenium and glutathione. Further work therefore, needs to be done to confirm this hypothesis

LIMITATION

This was a cross-sectional analytical study; the samples were only collected once, and this cannot fully explain the changes happening in the participants. A longitudinal study in which the participants would be followed up, and samples collected at different points of the pregnancy, could have given a better understanding of the progressive changes in the antioxidant status of the pregnant participants

Abbreviations

Abbreviation	Full Meaning
ACOG	American College of Obstetricians and Gynecologists
ANOVA	Analysis of Variance
GSH	Glutathione
kg	Kilogram
L	Litre
mg	Milligram
ml (mL)	Millilitre
mmHg	Millimetres of Mercury
nm	Nanometre
NICE	National Institute for Health and Care Excellence
p-value	Probability Value
rpm	Revolutions Per Minute
SD	Standard Deviation
Se	Selenium
SPSS	Statistical Product and Service Solutions (commonly Statistical Package for the Social Sciences)
UCH	University College Hospital
UI	University of Ibadan
UI/EC	University of Ibadan Ethics Committee
UI/UCH	University of Ibadan / University College Hospital
UK	United Kingdom
USA	United States of America
µg/dl	Micrograms per Decilitre
µmol/L	Micromoles per Litre
°C	Degrees Celsius

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the University of Ibadan/University College Hospital Joint Ethical Review Committee (UI/EC/18/0375). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ASA conceived the study, collected data, analyzed data, and drafted the manuscript. OAA and OO supervised the study, contributed to the study design, interpretation of findings, and manuscript revision. All authors approved the final manuscript.

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Tables

Tables 1 to 4 are available in the Supplementary Files section.

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