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Full length Research Article

Influence of pregnancy and lactation on vitamin D serum levels and antioxidant status in randomized women in Zaria

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Summary: Background: Pregnancy and lactation are normal physiological conditions that tend to influence numerous biological processes. The aim of this study was to identify the impact of pregnancy and lactation on serum vitamin D level and antioxidant status in some women in Zaria, Nigeria. Methods: A cross sectional descriptive study conducted at Ahmadu Bello University Teaching Hospital, Zaria for a period of three (3) months. Blood samples were collected, serum catalase, superoxide dismutase, lipid peroxidation and vitamin D, were determined using standard methods. Data were presented as mean ± SD, analysis was performed using one-way ANOVA and Pearson's correlation analysis. Values were considered significant at $p \leq 0.05$. Results: There was a significant difference ($p < 0.01$) serum malondialdehyde level, superoxide dismutase activity and glutathione peroxidase activity during the various trimesters of pregnancy and lactating group. However, the levels of these markers were highest in the lactating group. Furthermore, serum level of vitamin D and catalase activity was highest in the 2nd trimester and lowest in the control and lactating group respectively. Conclusion: Pregnancy and lactation altered serum level of Vitamin D, CAT, SOD, MDA and GPx suggesting a variation in oxidative stress at different trimester of pregnancy and lactation.

Keywords*: Pregnancy, lactation, oxidative stress, vitamin D*

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INTRODUCTION

There are certain physiologic conditions that alter the antioxidant status of an individual. Physiological phases such as pregnancy and lactation usually alters the level of antioxidants in women. Reports show that normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen which results in increased oxidative stress and antioxidant defences (Idonije *et al*., 2011; Ogbodo *et al*., 2014). Vitamin D has been reported to be involved in the regulation of physiologic factors that controls calcium signaling and reactive oxygen species (Berridge, 2016). The deficiency of Vitamin D causes an increase in oxidative stress (Wimalawansa, 2019). Augmented levels of oxidative stress may occur because of the increased cellular uptake and utilisation of oxygen, associated with pregnancy (Idonijie *et al*, 2011). It has been observed that pregnant women are more susceptible to oxidative damage than non-pregnant women, as evidenced by decreased antioxidants in non-pregnant women (Patil *et al*., 2007; Ugwa *et al*., 2014). Oxidative stress is the presence of reactive oxygen species (ROS) in excess of the buffering capacity of available antioxidants (Pizzino *et al*., 2017). Although the generation of ROS is a normal physiological process, their increased production in the body causes lipid peroxidation (Tiwari *et al*., 2010). The ROS scavenging mechanisms include enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH-Rx) and catalase, which limit the cellular concentration of ROS and prevent excessive oxidative damage (Scott, 1994). Furthermore, evaluating the activity of serum biomarkers of oxidative stress may provide useful information on the effects of pregnancy on antioxidant status which will further help understand the role of oxidative stress during pregnancy and lactation as such studies are lacking in the available literature. This study is therefore aimed at assessing the influence of pregnancy and lactation on serum Vitamin D level and some biomarkers of oxidative stress (malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase) in some pregnant and lactating women in Zaria.

MATERIALS AND METHODS

Demography of Study Area: Zaria is a heterogeneous city in Kaduna State. It is inhabited by about 1,018,827 people4. Kaduna is located in the North-west geopolitical zone of Nigeria, Kaduna State is one of the 36 states in Nigeria with its capital in Kaduna city. Zaria occupies a portion of the high plains of Northern Nigeria, 652.6 meters above sea level and some 950 kilometers from the coast at11o031N, 7o42E.

Study Design and Study Population: This study is a cross-sectional descriptive study that was conducted between within a period of three (3) months. A total of one hundred and seventy nine (179) women attending antenatal, family planning and immunisation clinics at the Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria, Maternal and Child Health Centre, ABUTH, Ban Zazzau, Zaria and Comprehensive Health Centre, ABUTH, Sabon Gari, Zaria were recruited for the study. A structured questionnaire about pregnancy history was administered. Blood samples were collected by a competent Laboratory Technologist at the Departments of Obstetrics and Gynaecology, ABUTH, Shika and measurements (body weight and height) were also recorded from the subjects. Body mass Index (BMI) was calculated from data collected from the anthropometric indices.

Experimental Grouping: The women were grouped as follows: (n = 179)
Group 1: Non-pregnant women: Control group (n = 15)
Group 2: Pregnant women in the first trimester (n = 40)
Group 3: Pregnant women in the second trimester (n = 41)
Group 4: Pregnant women in the third trimester (n = 53)
Group 5: Lactating women (n = 30)

Inclusion and exclusion Criteria: Healthy consenting women attending antenatal, family planning and immunization clinics at Ahmadu Bello University Teaching Hospital (ABUTH) Shika; Women and Child Centre, ABUTH, Ban Zazzau; Women Centre, ABUTH Sabon Gari, Antenatal Clinic, Sickbay, Ahmadu Bello University. Pregnant women with any disorder that affects metabolism of calcium or bone, history of endocrine, renal or liver illnesses, hypertension of pregnancy, gestational diabetes, thyroid disorders, or treatment with anti-tubercular or antiepileptic drugs in the previous 3 months were excluded from the study.

Ethical Approval Statement: Approval was obtained from the Scientific and Ethical Committee on Human Research, ABUTH, Shika, Zaria (Reference number: ABUTHZ/HREC/ K30/2014) and verbal consent was obtained from all subjects.

Collection and processing of samples: Exactly 10 ml of blood sample was collected by vein puncture, transferred to clean plain serum bottles immediately and allowed to stand at room temperature (270C) for two hours and centrifuged at 3000 g for 5 minutes to obtain the serum. The separated serum was stored at -20°C in a deep freezer at the department of Chemical pathology until needed for assay.

Determination of serum antioxidants: Serum level of vitamin D, catalase activity, superoxide dismutase activity glutathione peroxidase activity and malondialdehyde were measured using methods described by Aebi (1974), Fridovich (1989), and Ellman (1959) respectively.

Data Analysis: Data was collected, curated, cleaned up and was presented as mean ± SD. Analysis of data was done using One Way ANOVA followed by Holm-Sidak post hoc test and level of significance was tested at $P<0.05$. Pearson's correlation analysis was done to determine the inter-relationship between the variables.

RESULTS

Figures 1, 2 and 3 show the pattern of changes in concentration of vitamin D, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in the serum of subjects.

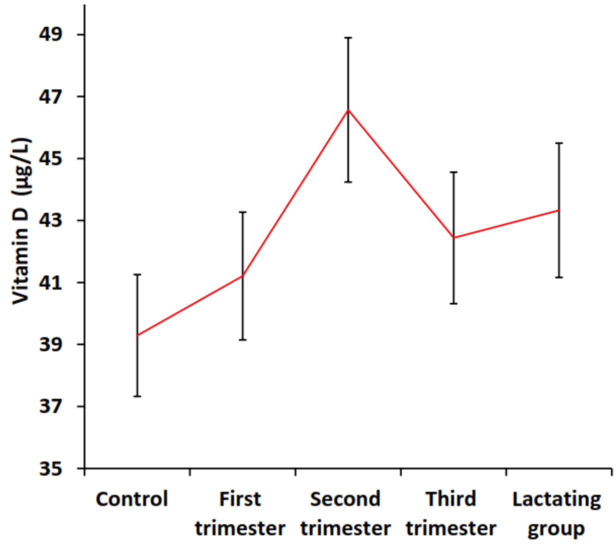

Figure 1:
Changes in the serum concentration of vitamin D in control, Pregnant and lactating women in Zaria, Nigeria

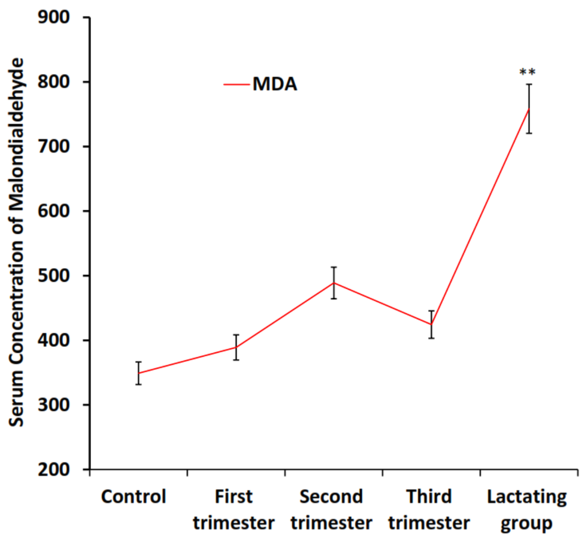

Figure 2:
Pattern of change in serum concentration of malondialdehyde in control, pregnant and lactating women in Zaria, Nigeria

The Vitamin D concentration was highest in the 2nd trimester group (46.50 ± 20.90) and lowest in the control group (39.29±4.51), MDA activity was highest in the lactating group (349.03±102.25 nmol/mg protein) and this difference was statistically significant ($p < 0.01$). There was a statistically significant ($P < 0.001$) increase observed in the activity of SOD between the different groups. This difference was observed in the lactating group, where it was higher, 14.87±1.61 U/ml. The GPx activity was also highest in the lactating group 47.43±26.00 ug/ml; and lowest in the control (11.71±1.83 ug/ml), and this difference was statistically significant ($p < 0.05$). The activity of serum CAT was highest (17.56±8.81 u/mg) in the 2nd trimester and lowest in the lactating group (3.14±0.78 u/mg), However, the difference was not statistically significant ($p > 0.05$).

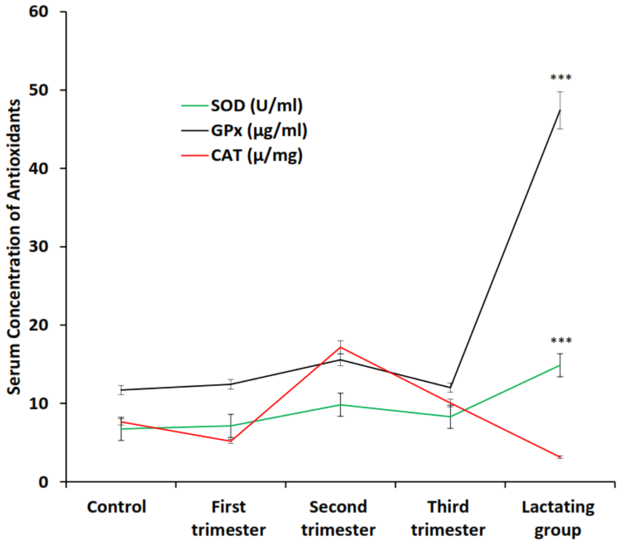

Figure 3:
Pattern of change in serum concentration of superoxide dismutase, glutathione peroxidase and catalase in control, pregnant and lactating women in Zaria, Nigeria.

DISCUSSION

Pregnant women are at an increased risk of elevated inflammation and oxidative stress especially during the third trimester. These conditions might result from micronutrient deficiency, increased maternal adipose tissue, and production of hormones by the placenta (Asemi *et al.*, 2016).

It can be inferred that pregnant women with increased serum levels of inflammatory biomarkers are at elevated risk of adverse pregnancy outcome, premature delivery, and disturbances of calcium homeostasis. To reduce maternal and foetal complications resulting from unfavourable metabolic profile, various strategies have been proposed such as the consumption of antioxidants, calcium and vitamin D supplementation, which help to reduce systemic inflammation and oxidative stress (Asemi *et al.*, 2016).

The result from this study showed that serum malondialdehyde concentration was highest in the 2nd trimester of pregnancy and lowest in the lactating group. Higher MDA levels in the 2nd trimester than non-pregnant control has been reported in pregnancy due to increased generation of oxygen radicals following increased oxygen demand in pregnancy and the reduction in the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Ugwa *et al.*, 2014)

In a study by Fedirko *et al.*, 2010, it was observed that antioxidant enzymes in humans function in combination with low-weight antioxidant compounds. Vitamin D activates the expression of antioxidant enzymes, which may not function properly in the antioxidant-depleted environment. Therefore, it is possible that vitamin D effects on the oxidative DNA are modified by the presence or absence of various pro-oxidant or antioxidant exposure. Thus, the use of Vitamin D3 supplements may decrease oxidative DNA damage in mucosa cells.

This study is in agreement with this report in that there was an observed correlation between Vitamin D with SOD and GSH which are antioxidant enzymes. Similarly, in an earlier study by Ekici *et al*, (2009), increased GSH activity was seen with Vitamin D3 supplementation in both cortex and corpus striatum in rats. Zhang *et al*., 2014 demonstrated that vitamin D supplementation in pregnant women resulted in increased GSH levels and a significant difference in plasma MDA levels. Asemi *et al*. (2014) had previously presented data in support of this, revealing that vitamin D supplementation significantly increased GSH levels just as There was strong negative correlation between vitamin D and calcium as well as vitamin D and phosphate (Avidime *et al*. 2022).

In the light of the findings in this study, it seems reasonable to speculate that the synthesis of CAT may reduce oxidative stress in the uterine environment especially during the second trimester. In addition to the suggestion that CAT might come from different sources in the serum. The pregnant human myometrium might be very efficient in the elimination of MDA (Biberoglu *et al*., 2016). Thus, the higher concentration of MDA, SOD, GSH and also CAT, in the serum samples during the 2nd trimester and indeed significantly in the lactating group (SOD), may reflect the overflow of these markers from the uterus to the circulation. It could also be that there is higher placental oxygen in the 2nd trimester than in the 1st trimester. This may possibly explain why MDA and CAT levels were observed to be low during the first trimester. A similar relationship has been previously demonstrated between the placenta and umbilical cord blood in pregnant women (Wang *et al.*, 1996).

In accordance with the ischemia-reperfusion phenomenon, re-oxygenation would facilitate the transfer of oxidation markers from the myometrium to the maternal circulation, not only to play a local protective role, but also as a means of responding to systemic oxidative stress (Biberoglu *et al*., 2016). An alternative explanation could be that, independent from the systemic antioxidant process, a well-functioning myometrial system might be active enough to rescue the pregnant myometrium from reactive oxygen species, as reflected by the values obtained in the 2nd trimester when compared with the control and the 1st trimester values obtained in this study. Additionally, the reactive oxygen species may promote local myometrial increase in oxidative stress, which are rapidly detoxified, thereby elevating oxidative markers in the circulation only slightly and for a short time, which also explains the high

MDA and antioxidant levels in the circulation by the 2nd trimester observed in this study (Biberoglu *et al*., 2016).

It may well be that the increased levels of antioxidants enzymes in serum samples of pregnant women observed in this study is an indication of the effort to compensate for the elevated MDA concentration in serum, especially during the 2nd trimester. Another finding of this study is that serum CAT activity was reduced though not statistically significant during lactation. This may be attributed to the fact that the subjects were drawn from a population of women lactating at different stages (i. e. colostrum, transitional and mature milk). Another way to put the contradictory findings into perspective is the methodological problems in measuring free radicals and absolute levels of in vivo oxidative stress. In the present study, we measured four basic oxidative stress and antioxidant activity markers. Given that not all markers change in the same direction, measuring more markers makes the interpretation of the results more problematic. Moreover, to what extent the tissue-specific oxidative damage is reflected systemically and is easily measured in the serum sample is not too clear (Biberoglu *et al*., 2016).
In conclusion, we believe that increased generation of oxygen free radicals (oxidative stress), defense mechanisms and calcium homeostasis are closely associated. Yet, the oxidative stress in circulation must be interpreted with great caution. The increased or decreased oxidative stress marker levels in the serum samples may represent reactive changes in the context of an endothelial or inflammatory status. Thus, larger multicenter studies may be required to clarify the discrepancies seen.

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