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**Original Research Article**

# Parity-dependent changes in serum levels of some antioxidant micronutrients in rural areas of Eastern Nigeria

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## Abstract

**Objective:** Wide range antioxidant micronutrients deficiencies during pregnancy have been reported in rural areas. This study was undertaken to determine if parity influences the serum levels of antioxidant micronutrients during subsequent pregnancy.
**Method:** This is a cross-sectional study where pregnant women with varying number of previous pregnancies and attending routine antenatal visits for the first time in the current pregnancies were recruited for the study. The controls were age-matched, non-pregnant and non-menstruation women from the same environment and with similar dietary indices. Antioxidant vitamins (A, C, E) were determined by spectrophotometric methods while the trace elements (Cu, Mn, Se, Zn) were estimated using atomic absorption spectroscopy.
**Results:** All vitamin antioxidants in different parity groups decreased significantly when compared with values obtained from control group. However, while vitamin A level had no correlation with parity, vitamin C level showed negative correlation and vitamin E level showed positive correlation with parity. On the other hand, all the antioxidant minerals showed positive correlations with parity. However, while copper level at only the first parity group was significantly lower than that of control, selenium and zinc levels at all the parity groups were significantly lower and manganese levels at all parity groups were significantly higher than that of control group.
**Conclusion:** The results showed that parity can cause variations in the serum levels of these antioxidant micronutrients. We are of the opinion that there is absolute need for pre- and early pregnancy individualized assessment of antioxidant micronutrients status to determine the actual need for supplementation.
**Keywords:** Parity; Antioxidant micronutrients; Rural areas.

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## 1. Introduction

Good nutritional status during pregnancy, determined by nutritional intake and dietary planning, is one of the predictors of optimal pregnancy outcome.[1] The food a pregnant woman eats is the source of nutrient for her body and the fetus, and as the pregnancy progresses the requirement increases.[2] Thus, it is believed that the micronutrient status of a pregnant woman is an important determinant of fetal growth and survival.[3] Unfortunately, multiple micronutrient deficiencies during pregnancies are common in developing countries.[4] Generally, during gestation, there are a lot of physiological changes occasioned by increased demands and change in plasma volume. These changes range from decrease in micronutrients, especially the antioxidants [5,6] to decrease in macronutrients concentrations.[1] These micronutrient

deficiencies during pregnancy, which may not be subsequently corrected, in addition to significant increase in oxidative stress markers like glutathione peroxidase (GPx), superoxude dismutase (SOD), malonyldialdehyde (MDA), volatile organic compounds (VOCs) and protein oxidation products are indications of oxidative stress or pregnancy-induced hypertension (PIH) – preeclampsia[7-10]. These resultant effects are probably the major causes of preterm delivery and other untoward pregnancy outcomes. Actually, oxidative stress influences the entire reproductive lifespan of a woman and even menopausal period.[11]

Some Studies have shown that in normal pregnancy, the earliest stages of development take place in a low oxygen environment – tissue hypoxia. [12] This tissue hypoxia has been found to promote release of reactive oxygen species (ROS) that are potentially damaging to the cardiovascular system.[13] Fortunately, physiological hypoxia of early gestational sac is beneficial because it protects developing fetus against deleterious and teratogenic effects of ROS.[12] This is quite different from what sets in as gestational age increases, producing free radicals implicated in pre-eclamptic toxaemia (PET), other abnormalities in pregnancy and even failed reproductive performances like infertility, miscarriage and diabetes-related congenital malformations. [14-16] These changes, brought about by oxidative processes, are fought and corrected by antioxidants as gestation progresses and even immediately after parturition. However, while some of these micronutrients may tend to return fully to pre-pregnancy values some months after delivery through physiological compensatory mechanisms, some may not. The later may tend to decrease with subsequent pregnancies, exposing these pregnant women to dangers of insufficiency as parity increases. This is most probable in the rural areas where proper nutrition and adequate micronutrient supplementation during pregnancy are hardly met.

On the other hand, it is possible that some micronutrients which have been found to increase during pregnancy, like copper and manganese [17-19] can reach toxic level as parity increases. From a decade ago, micronutrients like trace elements and vitamins are being given more attention in the nutrition of pregnant women in poorer regions of developing countries due to their antioxidant and immune boosting properties. [20] Unfortunately, there is dearth of information on the effects of parity on these micronutrients to serve as a guide on the need and extent of supplementation during pregnancy in these areas. For this, we studied the effect of parity on some of these antioxidant micronutrients in order to establish the necessity and extent or otherwise of micronutrient supplementation during pregnancy in our rural areas. This will help obstetricians and other pregnancy care-givers to give optimum and individualized antenatal care with expectant healthy babies, given that the principal cause of micronutrients deficiencies in infants is the preceding deficiencies of these micronutrients in their mothers during gestation.[20]

## 2. Materials and Methods

### 2.1 Ethical clearance

The ethical clearance for this study was obtained from Health Research Ethics Committee of University of Nigeria Teaching Hospital, Enugu. Additional informed consents were obtained from the subjects who also willingly filled the questionnaires after counseling.

### 2.2 Study areas

The study was undertaken in two rural communities - one community each from two different states in the south eastern part of Nigeria with similar environmental, religious and socio-economic characteristics. The inhabitants are mainly subsistent farmers, artisans and low level civil servants with cassava, yam, rice and corn as their staple foods. They are mainly Christians and African traditional religionists.

### 2.3 Subjects

Our patients were pregnant women (aged between 18 and 40 years) attending the antenatal clinics of the Health Centers in the study areas for the first time during their current respective pregnancies. A total of 315 pregnant women were initially enrolled for the study but only 195 passed the exclusion criteria and were subsequently enlisted. Controls were 50 age-matched, non-pregnant, unmarried and apparently healthy women, who were not menstruating at the time of sample collection. All the subjects were divided into 4 groups – controls and 3 parity groups. The 3 parity groups were made up of 84 who have had 0 – 3 pregnancies (0-3 parity group), 69 who have had 4 – 6 pregnancies (4-6 parity group) and 42 who have had 7 – 9 pregnancies (7-9 parity group) before the present pregnancy. Ultra-sound reports of all the patients showed that they were singleton.

### 2.4 Exclusion criteria

After initial enrollment, those who were already taking routine antenatal drugs were excluded from the study. Other exclusion criteria were as earlier reported. [21]

### 2.5 Dietary index

A 24-hour dietary recall and estimated food records were used to calculate the dietary indices of the areas under study as earlier reported.[21] Prior to the take-off of the study, twenty (20) non-pregnant women were randomly selected from each study area and interviewed on their food intake. Each woman was asked to recall and explain the type of food consumed within the previous 24 hours – including raw, locally prepared and industrially processed foods. From this, the amount of nutrients intake by each respondent within the 24 hours were calculated based on the known compositions of commonly eaten foods

in Nigeria.[22] This was used to estimate the dietary indices of these areas and it showed that the areas have similar dietary indices. When the study commenced, a section of questionnaire given to each subject after counseling was a 7-day retrospective dietary record. The answers to this section were also used to calculate dietary indices and the answer was similar to the one obtained before the commencement of the study, thus confirming the close relationship among the study areas in terms of nutrient intake.

**2.6 Sample collection**:

8.0ml of venous blood was collected from each subject into a chemically clean and dry glass test tube and transported in a sample pack wrapped with black polythene to protect the analytes from direct sunlight. After 30 minutes at room temperature, the sample was centrifuged for 10 minutes at 3000 rpm. The serum obtained was divided into two and stored frozen at -20°C until needed for analysis. Vitamins were estimated within 48 hours of collection while the trace elements were estimated within two weeks of collection.

**2.7 Laboratory analysis**:

Antioxidant vitamins (vitamins A, C, E) were estimated spectrophotometrically while the trace elements were estimated using atomic absorption spectroscopy (AAS) as earlier reported.[21] Vitamin A was estimated by method recommended by International Vitamin A Consultative Group (IVACG) in 1982 as modified by Ene-Obong *et al*,[23] vitamin C was estimated by 2,4-dinitrophenylhydrazine method as modified by Ene-Obong *et al* [23] while vitamin E was estimated using method of Pearson.[24] Trace elements – Copper (Cu), Manganese (Mn), Selenium (Se) and Zinc (Zn), were estimated using atomic absorption spectroscopy (Buck Scientific Spectrophotometer Model 205, East Norwalk, Connecticut, USA).

**2.8 Statistical analysis**

Generated data were analyzed using Graph Pad Prism version 5.03. Descriptive values were analyzed and differences between means were calculated. The level of statistical significance was set at $p < 0.05$. Analysis of variance (ANOVA) was used to study the variations of the means at different parity groups.

## 3. Results

Figure 1 is the mean (±SEM) serum vitamin A concentrations in different parity groups and controls. The values obtained were 46.77 ± 1.54µg/dL for controls, 14.85 ± 0.62µg/dL for 0 – 3 parity group, 16.32 ± 0.78µg/dL for 4 – 6 parity group and 17.16 ± 1.18µg/dL for 7 – 9 parity group. All the values from different parity groups were significantly lower ($p<0.05$) than the value from control group, though there was no significant difference between the values obtained from one group and another ($p>0.05$).

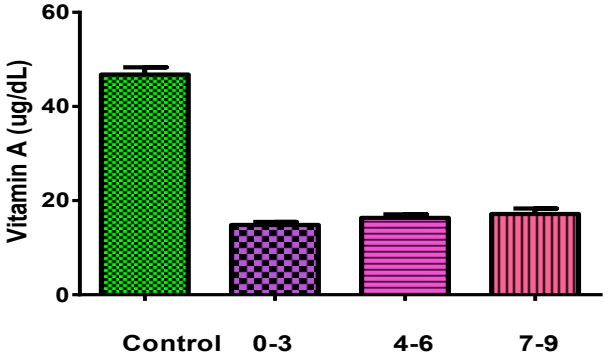


**Figure 1: serum vitamin A concentrations in different parity groups and controls**

Figure 2 is the mean (±SEM) serum vitamin C concentrations in different parity groups and controls. The values obtained were 75.65 ± 2.10mg/dL for control, 42.95 ± 2.46 mg/dL for 0 – 3 parity group, 30.15 ± 1.07 mg/dL for 4 – 6 parity group and 34.11 ± 1.53 mg/dL for 7 – 9 parity groups. The levels in all the parity groups were significantly lower than that from control group and decreased as parity increased.

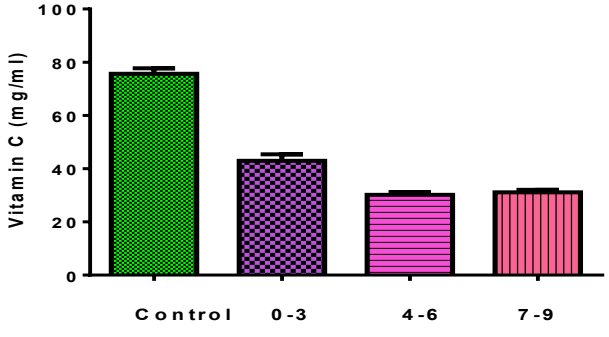


**Figure 2: Serum vitamin C concentrations in different parity groups and controls**

Figure 3 is the serum vitamin E concentrations obtained for different parity groups and control – 0 – 3 (1.82 ± 0.22 mg/dL), 4 – 6 (1.50 ± 0.15 mg/dL) and 7 – 9 (2.99 ± 0.29 mg/dL). They were all significantly decreased ($p<0.001$) when compared with the value obtained from the controls (4.91 ± 0.19 mg/dL).

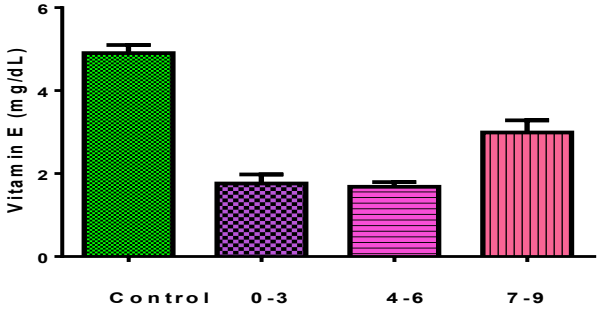


**Figure 3: Serum vitamin E concentrations obtained for different parity groups and control**

Figure 4 presents the mean (±SEM) serum copper concentrations in different parity groups and controls. This shows that women in 0 – 3 parity group had a mean serum copper concentration of 1.44 ± 0.08mg/dL, those in 4 – 6 group had 1.52 ± 0.09mg/dL, while those in 7 – 9 group had 1.88 ± 0.14mg/dL, indicating that only the first parity group (0 – 3) had significantly lower value (p=0.001) than that from controls (1.68 ±0.09mg/dL) and that copper increases as parity increases.

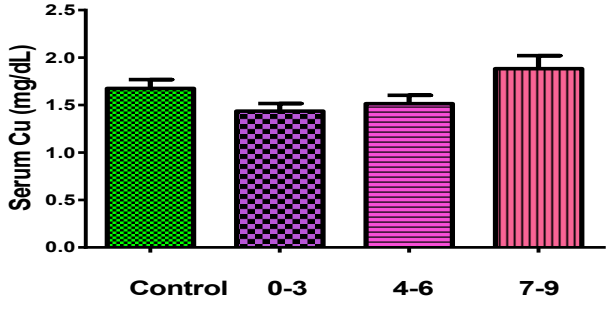


**Figure 4: Serum copper concentrations in different parity groups and controls**

Figure 5 shows the mean (±SEM) serum manganese concentrations in different parity groups and controls with 0.55 ± 0.04mg/dL for those in 0 – 3 parity group; 0.65 ± 0.08mg/dL for those in 4 – 6 group and 0.69 ± 0.06mg/dL for those in 7 – 9 group while control was 0.25 ± 0.01mg/dL. These values from parity groups were significant higher (p<0.05) than the value from control group and still increased as parity increased.

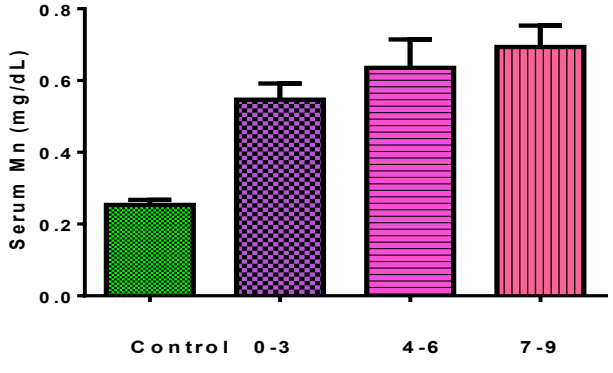


**Figure 5: Serum manganese concentrations in different parity groups and controls**

Figure 6 is the mean (±SEM) serum selenium concentrations in different parity groups and controls with 89.65 ± 2.28µg/dL for those in 0 – 3 group, 84.90 ± 2.09µg/dL for those in 4 – 6 group and 94.32 ± 2.38µg/dL for those in 7 – 9 group while 108.9 ± 2.09µg/dL was obtained from the controls. From these values, the difference between the serum selenium concentration in each parity group and the control was significant (p≤0.003), while there was no significant difference between one group and another (p>0.05).

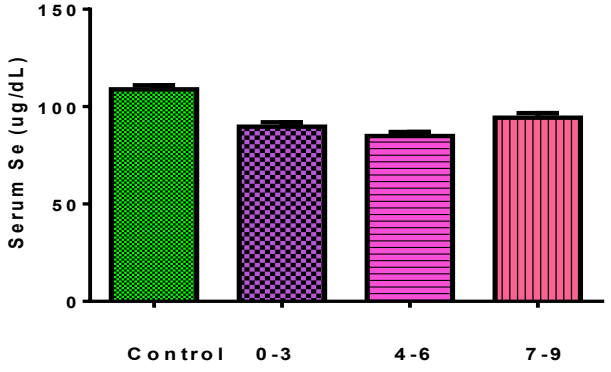


**Figure 6: Serum selenium concentrations in different parity groups and controls**

Figure 7 is the mean (±SEM) serum zinc concentrations in different parity groups and controls with 3.07 ± 0.13mg/dL, 2.92 ± 0.12mg/dL, and 3.95 ± 0.26mg/dL, from those in 0 – 3, 4 – 6 and 7 – 9 parity groups respectively while 4.46 ± 0.29mg/dL was obtained from the controls. All the parity groups showed significantly decreased values when compared with that of control subjects (p<0.001 each).

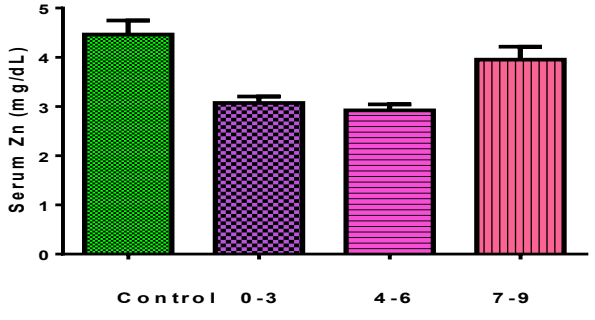


**Figure 7: Serum zinc concentrations in different parity groups and controls**

Table 1 is the analysis of variance of the different means of the antioxidant micronutrients in different parity groups. The results show that vitamins C and E, copper, selenium and zinc vary significantly as parity increased while vitamin A and manganese did not show statistically significant variation as parity increased.

**Table 1**: **One-way analysis of variance of the mean antioxidant micronutrients concentrations in different parity groups**

| Parameter | 0-3 | 4-6 | 7-9 | R2 | P-value |
|---|---|---|---|---|---|
| Vitamin A | 14.85(0.62) | 16.32(0.78) | 17.16(1.18) | 0.021 | 0.129 |
| Vitamin C | 42.75(2.46) | 30.15(1.07) | 34.11(1.53) | 0.112 | <0.001** |
| Vitamin E | 1.76(0.22) | 1.68(0.11) | 2.99(0.29) | 0.088 | <0.001** |
| Copper | 1.44(0.08) | 1.52(0.09) | 1.88(0.14) | 0.049 | 0.008** |
| Manganese | 0.55(0.04) | 0.64(0.08) | 0.69(0.06) | 0.012 | 0.300 |
| Selenium | 89.67(2.27) | 84.90(2.09) | 94.33(2.38) | 0.035 | 0.034* |
| Zinc | 3.07(0.13) | 2.92(0.12) | 3.74(0.24) | 0.061 | 0.002** |

**(*) = weak correlation** **(**) = strong correlation**

## 4. Discussion

Increased nutrients' demand during pregnancy is one of the main causes of nutrients' deficiencies in pregnancy, some of which continue to remain low even months after delivery. This study shows that there was significant decrease of serum vitamin A in all the parity groups when compared with the controls (Figure 1).

Though the concentration tend to increase as parity increased, there was no significant change ($p>0.05$ each) between one parity group and another and no significant correlation between parity and its serum concentration. This suggests that the number of previous births does not influence the serum level of the vitamin during subsequent pregnancy. Thus, even if there is full recovery from initial pregnancy-induced deficiency, ensuring adequate vitamin A level before subsequent pregnancy, its deficiency will still occur subsequently.

Therefore, the attention given to multigravid pregnant women in terms of supplementation should be the same for primigravidae and secundigravidae. On the other hand, though serum vitamin C concentration also decreased significantly in all parity groups, the decrease was also from one parity group to another ($p\leq0.038$) with strong negative correlation with parity.

The implication of the lower values of these vitamins and the negative correlation of vitamin C level with parity is possible continuous reduction in immune status and increase in susceptibility to infection and oxidative stress on subsequent pregnancies as a result of inadequate recovery from the previous deficiencies. This is at variance with earlier submission that primigravidae and secundigravidae usually have lower immune status than multigravidae.[25]

This therefore, calls for proper evaluation of mothers at onset of any pregnancy, especially multigravid mothers, to determine the need and appropriate dose of supplementation for the individual. Likewise, there was significant decrease of serum vitamin E concentration in all the parity groups when compared with the control ($p<0.001$). But unlike others, there was positive correlation between the later and parity, indicating steady improvement as parity increases.

There have been disagreements over the trend of vitamin E in pregnancy; while some studies reported significant increase during pregnancy, [26] others reported significant decrease as pregnancy progressed. [21]

This disparity may be due to the patients' population used in each study. For instance, the earlier researchers might have used preponderance of multigravidae in their study as this present study may suggest. If that is the case, it is possible that some multigravidae may have vitamin E adequacy or even be prone to vitamin E toxicity in subsequent pregnancies. Fortunately, since many families in the rural areas are no more interested in raising many children due to economic conditions, fear of vitamin E toxicity should not be entertained.

Only first parity group showed significant decrease of serum copper concentrations when compared to value obtained from the control group. Most importantly, not only that the antioxidant showed strong positive correlation with parity but the last parity group has significantly increased value over that of control group. There is dearth of information on the effect of parity on serum copper concentration. Moreover, there have been conflicting results on effect of gestational age on serum copper level; while some studies reported decrease over gestation,[21,27] others reported increase as pregnancy progressed [18,28].

However, these differing studies were from different environments; those who recorded decrease over gestation used subjects from rural areas while those who recorded increase used subjects from urban areas. Therefore, the difference between the values from different areas could be as a result of the differences in both the copper content of the soil/food and the social status of the subjects from those areas. Alternatively, those who recorded increase over gestation might have used more multigravidae in their studies. If that is the case, copper supplementation during pregnancy must be based on individual assessment to avoid copper deficiency, prompting poor immune function and weakness[29] or toxicity that may precipitate heart problems, liver damage, kidney failure, coma and even death.[30,31]

Furthermore, the present study shows that there were significant increases in the serum concentrations of manganese as parity increased. Even the level in each of the parity group was significantly higher than that of controls ($p\leq0.005$), and the difference between each group and the subsequent one was also significant.

This result is in agreement with previous studies [19,21] which have reported significant increase in manganese concentration over gestational age. Though manganese toxicity is said to be rare in humans, especially if consumed orally,[32] the possibility of isolated toxicity in multigravidae cannot be ruled out; hence the need for pre-pregnancy, early pregnancy and individualized assessment as earlier advocated,[33] especially for all multigravidae.

This study recorded significant decrease in serum concentrations of selenium and zinc in all parity groups when compared with that of control group. However, while selenium had negative correlation with parity, zinc showed positive correlation with parity. Selenium and zinc are known to play important role in reproduction, modulation of antioxidant status and immune functions of the body, [20,34,35] among other functions; hence the need to maintain their optimum concentrations during pregnancy. Serum levels of both minerals are known to decrease with gestational age [18,21,24,36,37,38,39] and low level of

selenium during pregnancy has been implicated in miscarriages and preeclampsia.[34] A study of King *et al* [40] has also indicated that zinc level increases immediately after delivery, though it is possible that the recovery may not be complete or remains highly sensitive to influence by subsequent pregnancies as the present study suggests.

Therefore, the present study supports the need for selenium and zinc supplementations during pregnancies, though it is advised that the supplementation should be individualized after assessment.

## 5. Conclusion

With the exception of copper and manganese, all antioxidant micronutrients studied were significantly decreased in pregnancy. However, they showed varying response as parity increased; thus while some have negative correlation with parity, others have positive correlation. Even those that have positive correlation still have lower serum concentrations at the last parity group when compared with the control group, signifying that they need to be carefully and individually evaluated before supplementation.

For copper and manganese, care must be taken to avoid isolated toxicity, especially in multigravidae. The results obtained from the present study therefore support our earlier deposition that there is need for pre- and early pregnancy assessment of antioxidant micronutrients status to ascertain the actual need for supplementation. [33]

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