Original Article

# Parity May Determine Levels of Some Antioxidant Minerals in Pregnancy: An Experience From Rural South-Eastern Nigeria

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## ABSTRACT

**Background:** Maternal micronutrient and antioxidant status is critical to fetal growth and survival. Poor socioeconomic conditions in the rural areas portend undernutrition with inevitable micronutrients deficiencies. Presently, there are conflicting reports on the serum levels of some mineral antioxidants during pregnancy. **Aim:** To determine the serum concentrations of some antioxidant minerals - copper and manganese, in rural pregnant women of different parities. **Subjects and Methods:** This is a cross-sectional study, involving 195 rural pregnant women, in different trimesters and parities. They were recruited from antenatal clinics of some rural health centres in south-eastern Nigeria. The controls were 50 age-matched, nonpregnant, nulliparous, and apparently healthy women, who were not menstruating at the time of sample collection. **Results:** The results showed that copper was nonsignificantly lower ($P = 0.14$), while manganese was significantly higher ($P < 0.001$) in pregnant women than nonpregnant women. Over gestation, there was no significant changes ($P = 0.081$; $F = 2.268$; $r^2 = 0.028$) in copper concentrations as pregnancy progressed while manganese increased significantly ($P < 0.001$; $F = 18.370$; $r^2 = 0.195$), and remained relatively so, throughout gestation. However, both antioxidants increased progressively and significantly as parity increased ($P < 0.001$). **Conclusion:** Parity influences the levels of these antioxidant minerals in pregnancy and therefore should play significant role in the determination of the actual dose of supplements, if need be, of these antioxidants during pregnancy. Indeed assessment and supplementation of copper and manganese during pregnancy should be individualized to avoid deleterious fetomaternal consequences.

**KEY WORDS:** Copper, manganese, parity, pregnancy, rural area

## INTRODUCTION

Micronutrient status in pregnancy is critical to fetal growth and survival.[1] Deficiencies may be detrimental and can indeed adversely affect pregnancy outcome.[2-4] Pregnancy is a physiological process which may be complicated by unpleasant socioeconomic circumstance. In rural areas, it is complicated by unhealthy and filthy environments that encourage infections and infestations. This is further compounded by the burden of undernutrition, and high prevalence of malaria known to have more effects on primigravidae and secundigravidae than multigravidae.[5] The resultant effect is the generation of enormous free radicals and initiation of oxidative stress which have been implicated in uncountable perinatal and maternal illnesses.[6-8]

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Antioxidants include organic molecules (minerals and vitamins), enzymes, and dietary chemicals (phytochemicals) used in preventing the effects of oxidative stress. Defence against oxidative stress is largely dependent upon an orchestrated synergism between some of these endogenous and exogenous antioxidants.[9] Hence, it is widely believed that diet-driven antioxidants play a significant role in prevention of human diseases and that deficiencies may impair fetal and childhood development.[10] The major antioxidant minerals involved in reproduction include copper, manganese, selenium, and zinc. Several studies have presented inconsistent results concerning the status of copper during pregnancy. Indeed, there is no universally accepted pattern of plasma/serum copper concentrations over gestation. While some studies reported a significant increase as pregnancy progressed,[11-14] others reported no significant increase.[15,16] Indeed, some studies did not show any significant pattern,[17] while a study actually found a significantly reduced plasma copper level in

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pregnancy.[18] In nonsupplemented women, plasma manganese concentration is said to increase significantly as pregnancy progressed.[19,20] Though there is insufficient information on the need for manganese in pregnancy,[21] high exposure during pregnancy may have harmful effects on the developing fetus.[22] For instance, though umbilical cord manganese concentration was not found to be associated with birth weight,[23] the same study found a nonlinear association between maternal manganese and birth weight, suggesting that manganese may affect fetal growth. This needs to be properly investigated to avoid fetomaternal complications.

In addition to endogenous generation, mineral antioxidants are easily obtained exogenously from commonly eaten foods. For instance, copper is said to be readily available in many foods, with fruits, vegetables, and white potatoes providing more than other sources,[24] while manganese is found mainly in green leafy vegetables, spinach, walnuts, whole grains, liver, and kidney.[25,26] Though these elements are found in conventional foods, the prenatal dietary intake is often considered to be insufficient to meet increased requirements during pregnancy, particularly in developing countries;[4] hence, the need for supplementations. Moreover, earlier study[27] had reported that in these rural areas, some of the good sources of these antioxidants, especially green leafy vegetables, fruits, and potatoes are produced locally but are rarely consumed there. Instead, they are transported to the nearby urban areas for paltry financial returns, while the producers wallow in undernutrition. Before now, single drugs like vitamins C and B-complex, folic acid and fesolate (as iron table) were prescribed for pregnant women as routine antenatal drugs. These probably served for increased erythropoiesis and have not been able to meet ultimate nutritional needs of this category of patients in terms of antioxidation. Consequently, many obstetricians and other pregnancy caregivers now resorted to use of multivitamin capsules that contain both vitamins and some trace elements. Unfortunately, careful analysis of some of these multivitamin capsules have revealed gross inadequacies of over 60% of them in terms of the concentrations of antioxidant micronutrients in them, especially the mineral antioxidants, when compared with the recommended dietary reference intake for pregnant women in the developed countries.[28] Since general undernutrition, and specifically, micronutrient deficiency are said to be rampart in rural areas of developing countries,[27,29] the dietary requirements of pregnant women from these rural areas are expected to be higher than those from the urban areas. Thus, when matched with the constituents of the said multivitamin capsules, their inadequacies will be enormous. Hitherto, there is conflicting results on the actual need for supplementation of some of these antioxidants based on their serum/plasma concentrations during pregnancy. Factors causing these conflicts may include social, religious, and geographic factors, as well as endogenous factors like parity. It, therefore, becomes pertinent to investigate these factors to determine the actual need for supplementation of these antioxidants in pregnancy, especially in our rural areas, where prepregnancy care has not received the desired attention.[30] This work aims to determine the effects of parity on some antioxidant minerals – copper and manganese, in a rural setting.

## SUBJECTS AND METHODS

### Ethical clearance

Ethical clearance for this study was obtained from the University of Nigeria Hospital Ethics Committee, while additional consents were sought and obtained from the subjects.

### Study areas

The study areas were two rural communities of Ezzamgbo and Onueke in Ebonyi State of Nigeria. Inhabitants of these communities are mainly artisans and low level civil servants, as well as subsistent farmers. Public water supply is sparingly available; instead, they get their water supply primarily from commercial water vendors – tanker drivers, and sparingly available springs. They are mostly Christians and African traditional religionists. At the time of this work, each health centre was manned by a nurse/midwife and two or three health technicians, with occasional visits by qualified medical doctors – mainly on antenatal days. Commonly eaten foods in these areas include rice, yam, cassava, beans, and corn food as well as egusi, ogbono, orah, and vegetable soups.

### Subjects

Patients were 195 pregnant women who were attending the antenatal clinics of the health centers in the study areas for the first time during their current individual pregnancies. They were aged between 18 and 40 years and in different trimesters of pregnancy (51 in the first trimester, 66 in the second trimester, and 78 in the third trimester). Control subjects were 50 mobilized and counselled, age-matched, non-pregnant, nulliparous and apparently healthy women, who were not menstruating at the time of sample collection. Patients were first divided into three groups according to their gestational ages (0 – 13 weeks for first trimester; 14 – 28 weeks for second trimester; and 29 weeks and above for third trimester), and eventually into another three groups according to parity (0 – 3 life deliveries as group I; 4-6 life deliveries as group II, and 7-9 life deliveries as group III).

### Exclusion criteria

On first visit to the antenatal clinic, medical histories of the patients including the course of previous pregnancies were taken. Patients were also screened routinely in the laboratory to rule out new medical cases including malaria infections. Exclusion criteria included history of repeated cesarean sections, chronic alcohol consumption (including the locally tapped and constituted palm wine), and chronic disease conditions like diabetes, hypertension, and human immunodeficiency virus infection. Others include evidence of malaria infections and intake of routine antenatal drugs before attending antenatal clinics.

### Dietary index

The dietary indices of the study areas were calculated by using 24-h dietary recall. Each subject was asked to recall the foods she has taken in the past 24 h, both raw, locally prepared, or bought from the urban areas. The amount of nutrients taken by each subject within 24 h was then calculated based on the known compositions of commonly eaten foods in Nigeria[31] and other parts of the world.[32]

### Sample collection

Random blood samples were collected from both patients and controls between 9.00 am and 12.00 noon. A total of 6.0 mL of venous blood was collected from each subject, put into a chemically clean and dry test tube, and allowed to stand for 30 min at room temperature to clot and retract. This is then centrifuged for 10 min at 3000 rpm and serum obtained was stored frozen till analysis. The two trace elements were estimated using atomic absorption spectrophotometer-Buck Scientific Spectrophotometer, Model 205 (East Norwalk, Connecticut, USA).

### Statistical analysis

Values were expressed as mean (SDs). Differences between means were calculated using one-way analysis of variance and significance was taken at $P < 0.05$.

## RESULTS

Table 1 is the tabular presentation of mean (SD) of the antioxidant minerals in both pregnant women and control subjects. It shows that copper was nonsignificantly lower ($P = 0.14$), while manganese was significantly higher ($P < 0.001$) in pregnant women than in nonpregnant women. In trimesters, the mean copper levels were 1.65 ± 0.50 mg/dL in the first trimester, 1.34 ± 0.71 mg/dL in the second trimester, and 1.49 ± 1.06 mg/dL in the third trimester. These values showed non-significant differences between the trimesters ($P = 0.08$; $F = 2.268$; $r^2 = 0.028$)—Table 2. In the same Table 2, manganese concentrations were 0.27 ± 0.19mg/dL in the first trimester, 0.66 ± 0.58mg/dL in the second trimester, and 0.54 ± 0.47 mg/dL in the third trimester, showing statistically significant changes over trimester ($P < 0.001$; $F = 18.370$; $r^2 = 0.195$). When grouped according to parity, serum copper concentrations were 1.48 ± 0.73 mg/dL for those with 0 − 3 children, 1.52 ± 0.78 mg/dL for those with 4-6 children, and 1.88 ± 0.88 mg/dL for those with 7-9 children, showing progressive and significant increase ($P < 0.001$; $F = 11.83$; $r^2 = 0.138$) as parity increased [Figure 1]. Likewise, the serum concentrations of manganese over parity were 0.55 ± 0.41, 0.64 ± 0.66, and 0.69 ± 0.26 mg/dL, respectively, also showing significant changes ($P < 0.001$; $F = 7.490$; $r^2 = 0.087$) [Figure 2].

**Table 1: Comparison of serum copper and manganese between pregnant and nonpregnant women**

| Parameter (mg/dL) | Control (*n*=50) | Pregnancy (*n*=195) | P value | Remark |
|---|---|---|---|---|
| Copper | 1.68 (0.62) | 1.48 (0.84) | 0.141 | Nonsignificant |
| Manganese | 0.25 (0.09) | 0.47 (0.49) | <0.0001 | Significant |

## DISCUSSION

Serum copper concentration in our controls is consistent with some reference values earlier reported[33,34] but slightly higher than that obtained from another study in Spain.[13] The value decreased nonsignificantly in pregnancy. Over gestation, there were no significant changes between the trimesters. Though the figures from pregnant women in this study are consistent with that earlier reported in similar subjects;[14,17,35] those earlier studies, however, recorded a significant increase over gestation, which is not in agreement with the finding of this study. Instead, this study is consistent with decreased value reported by Ugwuja *et al.*,[18] from an urban area of the same state where the present work was done. This can be interpreted to mean that though the reference values may be the same in many countries and regions, metabolism during pregnancy is subject to other factors. The trace element, however, showed a progressive increase as parity increased, despite initial nonsignificant decrease. In fact, the value obtained in highly multiparous subjects (those with 7-9 life births) was significantly higher than the value from those with 0-3 life births. This is consistent with our earlier report which recorded relative increase in copper concentration

**Table 2: Serum copper and manganese in different trimesters of pregnancy**

| Parameters (mg/dL) | Controls (*n*=50) | 1st (*n*=51) | 2nd (*n*=66) | 3rd (*n*=78) | P | F | $r^2$ |
|---|---|---|---|---|---|---|---|
| Copper | 1.68 (0.62) | 1.65 (0.50) | 1.34 (0.71) | 1.49 (1.06) | 0.081 | 2.27 | 0.028 |
| Manganese | 0.25 (0.09) | 0.27 (0.19) | 0.66 (0.58) | 0.54 (0.47) | < 0.001 | 18.37 | 0.195 |

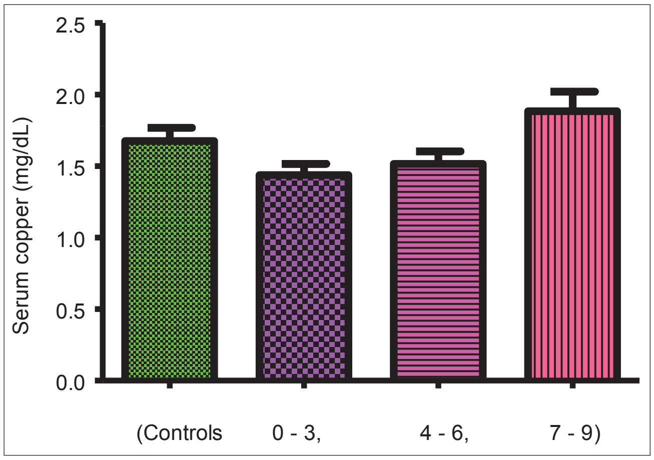


**Figure 1:** Mean (SD) of serum copper concentrations in different parity groups of pregnancy and controls

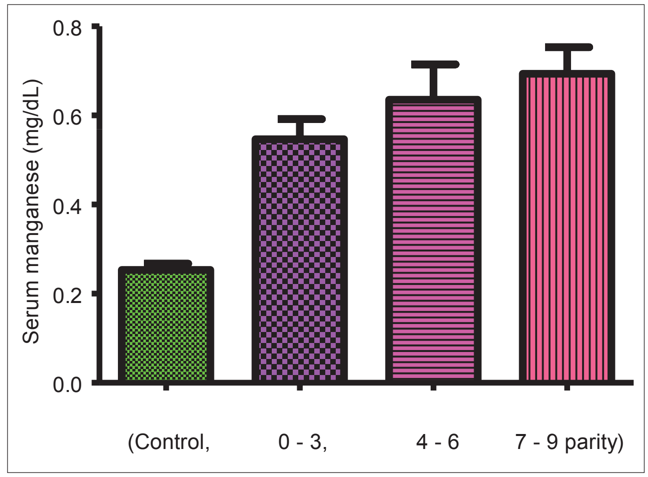


**Figure 2:** Mean (SD) of serum manganese concentrations in different parity groups of pregnancy and controls

in multiparous nonpregnant women over nulliparous nonpregnant women.[30] Since the significant increase recorded in this present study is not over gestation but parity, this study is of the opinion that copper concentration in the body over parity is cumulative and that those studies that recorded a significant increase in copper concentration in pregnancy might have used preponderance of multigravidae. Moreover, most of those studies did not specify whether the patients were taking their routine antenatal drugs. This clarification is necessary considering the fact that pregnant multigravidae from developing countries hardly register for antenatal care within the first and second trimesters of their pregnancies.[36] Instead, some will be taking the routine drugs prescribed during previous pregnancies till they are close to term, unless they develop complications before that time. This initial drug intake will, expectedly, contribute significantly to the concentration of the trace element under study, thus building high serum copper in some multigravidae. Though copper toxicity is said to be rare in the general population, its excess and deficiency have been reported to have profound and sometimes persistent effects on many foetal tissues and organs even in absence of clinical signs in the mother.[30] Therefore, care must be taken to avoid isolated toxicity, especially in multigravidae with infections or inflammatory stress that can induce acute phase action of interleukin-1.[11]

The mean serum concentration of manganese in the controls is less than the value earlier reported by Iyengar and Wolttlez[33] but higher than the value reported by West Midland Toxicology Laboratory.[34] This difference may not be unconnected with the differences in soil and food contents in these different areas. Since manganese is ubiquitous in foodstuff,[25] its concentration in the body will be dependent on dietary intake, which in turn is dependent on the soil content. When compared with the value from pregnant women, there was a significant increase of this element in pregnancy. This agrees with earlier studies on the same group of patients.[19,20,37] Manganese also showed a significant increase over gestation. This gradual increase will help to prevent inborn errors of metabolism and brain damage in neonates,[38,39] and ensure adequate utilization of other nutrients for normal growth.[26,40] However, more worrisome is the significant increase in the serum concentration of this element of each parity group over controls, to the tune of 2-3 times the control value. Manganese readily crosses the blood-brain barrier in developing foetuses, neonates, and even mature mammals, causing in-coordination.[41,42] Therefore, there is the need to monitor multigravidae to avoid isolated toxicity.

Micronutrient supplementation is essential to the optimal fetomaternal health and good pregnancy outcome, especially in poor socioeconomic environments. This study has shown that parity can affect the levels of some micronutrients especially in the rural areas of developing countries. This calls for more studies involving other micronutrients, including trace elements and vitamins, to establish the actual need or otherwise for supplementations of these micronutrients during pregnancy. Furthermore, these results and subsequent ones should certainly be taken into consideration when considering government policies for micronutrient replacement during pregnancy and lactation. Though, nonlongitudinal procedure in this study is a limitation, the results have given a good insight, while this procedure will be taken into consideration in subsequent studies. Also, 1 week dietary recall would have given better dietary index than 24-h recall and this will be addressed in further studies.

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