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Serum micronutrient levels and antioxidant defenses in Nigerians undergoing elective caesarean section

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ABSTRACT

Despite the role of Reactive Oxygen Species (ROS) in wound healing, its actions if not properly controlled by antioxidants, could be very deleterious. The interactions between micronutrient status and antioxidant defenses in wound healing in elective caesarean section (CS),has not been explored sufficiently. This is of particular interest in the developing countries where micronutrient malnutrition is common especially in women of reproductive age group. One hundred pregnant women booked for elective CS from the Obstetrics and Gynaecology department of the University College Hospital, Ibadan, Nigeria were recruited for this study. Blood samples were collected from each patient recruited for the study; before surgery(BS), day 1 post surgery(D1PS) and day 4 post surgery (D4PS) with PCV measurement.Furthermore, levels of Cu, Se, Zn, Mn, Vitamin C and E, Total antioxidant potential(TAP), total plasma peroxide(TPP), and oxidative stress index (OSI) were determined. Surgical wounds were assessed by a consultant Obstetrician and Gynaecologist on the fourth day post-surgery. Results were reported as mean±SEM for continuous variables while Analysis of Variance was used to assess difference between group means. Post hoc testing was performed for intergroup comparison using LSD. All results were considered statistically significant at $p \leq 0.05$. Though the levels of Mn and Se micronutrients measured decreased, they were not significant. There was a significant increase in TPP, and corresponding significant decreases in levels of Cu, Zn, vitamins C and E and TAP while decrease in D1PS levels of Mn, Se, OSI and PCV when compared to D4PS levels were not significant. Assessing micronutrients status in all elective CS patients before and after surgery may be considered an important part of surgical planning because of their possible involvement in wound healing.

Key words: Caesarean section. Wound healing, micronutrient, antioxidant, Oxidative stress.

INTRODUCTION

The rising global rate in Caesarean section (CS) deliveries presently is constituting a source of concern to obstetricians worldwide and Nigeria is not an exception [1,2]. Caesarean section is the delivery of a foetus, placenta, and membranes through an abdominal and uterine incision after viability. This major surgical procedure may be unplanned (emergency), if there are problems during labour or planned in advance, (elective), if there are signs that a vaginal birth is risky. However, reasons for elective CS vary, with key distinction being between hospital or doctor-centric reasons and mother-centric reasons [3].

Because caesarean section disturbs the integrity of the epidermis, dermis, connective tissue and the microcirculation resulting in a wound, one of the greatest expectations after CS is effective wound healing [4]. Wound healing is a complex process that proceeds through distinct yet step-wise and timed overlapping stages of hemostasis, inflammation, proliferation, and remodeling [5]. These stepwise stages result in increased cellular activity, causing intensified metabolic demand for nutrients and oxygen [5,6,7,8], leading to the production of free radicals which may be in the form of reactive oxygen species (ROS).

Although ROS may play a prominent role in oxidative bacterial killing and coregulate prevalent processes in wound healing [9], an increased level can transcend the beneficial effects and cause additional tissue damage leading to lipid peroxidation, which could be very deleterious when uncontrolled [5]. However, antioxidants intercept, modify or destroy the ROS thereby, maintaining homeostasis against the deleterious effects of these ROS [10]. Therefore, decreased levels of antioxidants or increased production of ROS or both can lead to oxidative stress [10].

Antioxidants include micronutrients like zinc(Zn), selenium (Se), copper (Cu) manganese (Mn) vitamin C and vitamin E among others. Zinc is also a cofactor for superoxide dismutase (SOD), an effective intracellular antioxidant and also, a cofactor for RNA and DNA polymerase and is, therefore, involved with DNA synthesis, protein synthesis, and cell proliferation and collagen synthesis which are all necessary processes for tissue regeneration and repair[11].

Copper is indispensable for ceruloplasmin; a broad spectrum oxidase whose main function is as an extracellular scavenger of superoxide and other oxygen radicals in Cu transport. Thus, through the action of Cu-ZnSOD and ceruloplasmin, Cu plays a pivotal role in antioxidant defence. Copper is also a cofactor for lysyl oxidase necessary for collagen and elastin cross-linking, which makes Cu indispensible for wound healing.

Manganese is another micronutrient that is a cofactor for superoxide dismutase in form of MnSOD, which is an intracellular antioxidant. Manganese also is a cofactor for prolidase, an enzyme that provides proline for the formation of collagen [12].

Selenium is an essential component of the antioxidant selenoproteins, including glutathione peroxidases [13]. These enzymes remove the products of attack by hydroperoxides and oxidized lipoproteins and so limit adverse effects on the endothelium. This action implicates Se as being protective against cell oxidation and lipid peroxidation[14].Thus, reduced selenium concentrations might adversely affect the functional activities of the selenoproteins, compromising protection against oxidative stress.

As a powerful antioxidant, vitamin C scavenges ROS in aqueous milieu and has been reported to have the capacity to decrease oxidative stress [15,16]. Vitamin C is also necessary for collagen synthesis, capillary wall intergrity, fibroblast and immunological function and therefore, necessary for healing [5]. Vitamin E, a fat soluble vitamin is the major powerful membrane bound antioxidant employed by the cell to protect against lipid peroxidation [17]. It is also established that vitamin E and vitamin C function together in a cyclic-type process to regenerate vitamin E [10,18].Measuring the above low molecular weight antioxidants in plasma in assessing in vivo antioxidant levels is essential. However, the number of different antioxidants in plasma or other biological samples makes it difficult to measure each antioxidant capacity. The possible interaction among different antioxidants in vivo could also make the measurement of any individual antioxidant less representative of the overall antioxidant status. Plasma total antioxidant potential (TAP), can therefore, be regarded as a better representative of the in vivo balance between ROS and antioxidants than the concentration of single, selected low molecular antioxidants [19].

Since these micronutrients have been reported to be involved in lowering ROS, thereby, combating oxidative stress and optimizing wound healing, abnormally low levels of these antioxidants may amplify metabolic derangements and cause complications in wound healing [20]. Studies exploring the interactions between micronutrient status and antioxidant defenses in wound healing in elective CS are scarce. This is of particular interest in the developing countries where micronutrient malnutrition is common especially in women of the reproductive age group [21].Therefore, the aim of this study was to determine the micronutrient and antioxidant status in women undergoing elective CS before and after surgery and their possible implications in wound healing.

MATERIALS AND METHODS

SUBJECTS

One hundred (100) pregnant women who were booked for elective CS from the department of Obstetrics and gynaecology department of University College Hospital, Ibadan, Nigeria were used for this study and each patient served as her own control. The women were in good general health and were not on any special diet or receiving any chronic drug therapy. The exclusion criteria were elective CS patients with multiple pregnancy, HIV or other viral infection, any form of malignant disease, recent exposure to chemotherapy or radiotherapy, gestational diabetes or previous history of diabetes and hypertension. Informed consent for participation was secured from each participant and an approval for the study was also obtained from the Ethical Committee of the hospital. Information on age, height, weight and parity was obtained. The pregnant women were made up of 50 primigravidae and 50 multigravidae.

SPECIMEN COLLECTION

Ten milliliters of venous, non-fasting (late morning; 11.00 am – 12.00 noon) blood was collected from each subject and dispensed into three specimen containers – 2.0mls were dispensed into EDTA containers free for packed cell volume (PCV) determination, 3.0mls into lithium heparin container to obtain plasma for while 5.0mls were dispensed into trace element-free and anticoagulant free tubes (Vacutainer, Becton Dickson, Rutherford, NJ) to obtain serum for biochemical assays. The blood samples were collected at 3 different times from the same patient; before surgery(BS) which served as baseline, day 1 post surgery(D1PS) and day 4 post surgery (D4PS) which served as follow up evaluation of parameters. Apart from PCV which was determined immediately, plasma and serum samples were kept frozen at -20^{0}C until time for analysis.

ANALYTICAL METHODS

Packed Cell Volume was determined by standard haematological procedures as described by Hughes-Jones et al., 2004 [22]. Serum levels of Cu, Se, Zn and Mn were determined using atomic absorption spectrophotometer(Buck 210VGP, USA) as described by Olaniyi and Ariola, 2007 [23]. Vitamin C and E were determined using HPLC (Buck BLC—20, USA) according to the method of Luo et al., 2008 [24].

Total Antioxidant Potential (TAP) and Total Plasma Peroxide(TPP),a marker of ROS, were based on colorimetric method of Erel, 2005 [25]. The ratio of TPP level to TAP was accepted as the Oxidative Stress Index (OSI) [19,25]. Surgical wounds were assessed qualitatively by a consultant Obstetric and Gynaecologist on fourth day post-surgery.

STATISTICAL ANALYSIS

All results were reported as mean±SEM. Difference between groups were assessed by ANOVA. And post hoc testing was performed for intergroup comparison using LSD. All results were considered statistically significant at $p<0.05$.

RESULTS

The mean ages, anthropometric indices and parity of women who participated in this study are shown in table 1. The mean age of participants was32.3 ± 3.66 years with mean BMI of 26.3±0. 433±65.0 gestational weeks and parity 3.0±0.5. Thirty percent of the cases had tertiary education, 60% secondary education and 10% primary education.

Table 2 shows the mean serum level of micronutrient of women undergoing Elective CS before surgery (BS), day 1 post surgery (D1PS) and day 4 post surgery (D4PS).
There was a significant decrease in serum copper levels (µg/L) in DIPS (65.27± 2.93) and D4PS (60.81 ± 3.97) compared to BS (68.23±2.92) ($p<0.05$).

A significant decrease was observed in serum zinc (µg/dl) in DIPS (65.21±9.34) and D4PS(61.22±10.67) compared to BS (68.53±10.80) ($p<0.05$).

Manganse (µg/dl), showed non significant decrease in D1PS (12.93±0.38) and D4PS (10.69 ± 0.30) compared to BS (13.47± 0.27) ($p>0.05$).

Selenium(µg/dl), showed non significant decrease in D1PS (38.67 ± 23.69) and D4PS (38.00 ± 24.24) compared to BS (39.53± 3.14) ($p>0.05$).

Vitamin C(µg/dl), showed a significant decrease in D1PS(0.54±0.17) and D4PS (0.52 ± 0.12) compared to BS (0.55± 0.15) ($p<0.05$).

Vitamin E (mg/dl), showed significant decrease in D1PS (0.80 ± 0.12) and D4PS (0.73±0.08), compared to BS (0.80±0.13) ($p<0.05$).

Packed cell volume (PCV)(%), showed non significant decrease in D1PS (33.54± 6.27) and D4PS (34.01 ± 3.35) compared to BS (34.75 ± 3.42).

Table 3 shows the mean level of oxidative stress indices of women undergoing Elective CS before surgery (BS), day 1 post surgery (D1PS) and day 4 post surgery (D4PS).

There was significant difference in the mean levels of Total plasma peroxide (TPP)(µmol/H_2O_2/l) in D1PS (11.12 ± 5.89) and D4PS (12.87± 5.17) compared to BS(10.28 ±1.80)($p<0.05$).

However, significant decrease was observed in the mean levels of Total antioxidant potential (TAP)(µmolTroloxequiv/L) in D1PS (938.07 ± 136.19) and D4PS (912.67±125.08), compared to BS(960.80± 144.71) ($p<0.05$).

But non significant increase was observed in the mean levels of oxidative stress index (OSI) in D1PS(1.30±0.69) and D4PS(1.61± 0.75) compared to BS (1.08± 0.39)($p>0.05$).

Table 1:Mean age, anthropometric indices and parity of women undergoing elective CS before surgery (BS)

Variable		Value
Age (yrs)		32.3 ±3.7
Weight (kg)		67.7 ± 1.3
Height (m)		1.60 ± 0.3
Body Mass Index (kg/m^2)		26.3 ± 0.4
Systolic Blood Pressure		113 ± 12.0
Diastolic Blood Pressure		74 ± 10.0
Parity		3.0 ± 0.5
Mid arm Circumference (cm)		25.4 ± 4.6
Gestational Age (weeks)		33 ± 65.0
Educational Level :	Primary	10 (10%)
	Secondary	60 (60%)
	Tertiary	30 (30%)

Table 2:Mean serum level of micronutrient of women undergoing elective CS before surgery (BS), day 1 post surgery (D1PS) and day 4 post surgery (D4PS)

Micronutrient	BS	D1PS	D4PS	p value
Copper (Ug/L)	68. 23±2.92	65.27±2.93[ab]	60.81±3.97[a]	0.03*
Zinc (Ug/dl)	68.53±10.80	65.21±9.34[ab]	61.22±10.65[a]	0.03*
Manganese (Ug/ml)	13.47±0.27	12.93±0.38	10.69±0.30	0.08
Selenium (µg/dl)	39.53±3.14	38.67±23.69	38.00±24.24	0.74
Vitamin C (Ug/dl)	0.55±0.15	0.54±0.17[ab]	0.52±0.12[a]	0.01*
Vitamin E(mg/dl)	0.80±0.13	0.80±0.12[ab]	0.73±0.08[a]	0.02*
PVC (%)	34.75±3.42	33.54±6.27	34.01±3.35	0.20

* *Significant at $P< 0.05$ (2-tailed)*
a *Value significantly different ($P< 0.05$) from BS value*
b *Value significantly different ($P<0.05$) from D4PS value*

Table 3: Mean level of oxidative stress indices of women undergoing Elective CS before surgery (BS), day 1 post surgery (D1PS) and day 4 post surgery (D4PS).

Parameter	BS	D1PS	D4PS	p value
Total Plasmaperoxide (µmol/H_2O_2/L)	10.28±1.80	11.12±5.89[ab]	12.87±5.17[a]	0.01*
Total Antioxidant Potential (µmolTroloxquiv/L)	960.80±144.71	938.07±136.19[ab]	912.67±125.08[a]	0.02*
Oxidative Stress Index	1.08±0.39	1.30±0.69	1.61±0.75	0.06

* *Significant at P< 0.05 (2-tailed)*
a Value significantly different (P< 0.05) from BS value
b Value significantly different (P<0.05) from D4PS value

DISCUSSION

Nutritional status is extremely important in wound healing. We evaluated the levels of micronutrients (Zn, Cu, Mn, Se, Vitamin C and Vitamin E) in women going for CS at 3 different times; before the surgery, 1 day after the surgery and 4 days after the surgery.

In this study, a significant decrease in the serum levels of Zn post- surgery compared with the BS level was observed. This decrease could have been as a result of increased utilization of this micronutrient during metabolism owing to the involvement of SOD in combating the free radicals generated during the hypermetabolic process of healing. Some studies have shown that zinc depletion is associated with increased oxidative stress in trauma patients and that supplementation of zinc attenuated indices of oxidative stress, improved immune function and/or attenuated cytokine production [2,26,27].

We also observed a significant decrease in serum Cu level D1PS compared to BS level. However, there was a significant increase on D4PS compared to the BS levels. The decrease could have been as a result of redistribution to wound site or increased utilization. The increase on the 4^{th} day could be attributed to the hepatic synthesis of ceruloplasmin mediated by cytokines. This finding is consistent with other reports[28,29].

The observed non- significant decrease in the level of manganese between D1PS and D4PS compared with BS level could be as a result of redistribution to the wound site or increased utilization.

We similarly observed a non- significant decrease in selenium concentration on D1PS compared to the BS value. However, there was a non-significant gradual rise on the 4^{th} day after surgery. This insignificant change could point to the fact that the role of antioxidant defense of Se, was not overwhelmed by oxidative stress. Again, Selenium is excreted in increased amounts in the urine after major injuries[30]. The D1PS non-significant decrease could have been as a result of increased urinary excretion of Se.

Again, we observed a significant decrease in plasma vitamin C level post- surgery compared to BS levels. This decline could be the result of redistribution in the body and consumption (increased demand) in a bid to combat oxidative stress. Other reports collaborate this finding [15,31].In our study, we observed a significant reduction in the level of vitamin E after surgery compared to the BS levels. This decrease may be as a result of increased utilization of vitamin E as an antioxidant [18]. This decrease in vitamin E could also be associated with decrease in vitamin C level in this study since the two micronutrients are synergistic.

The decrease in serum levels of these antioxidant micronutrients may actually be an adaptive, beneficial response, as some micronutrients at high doses act as pro-oxidants [32].

In our study, the significant decrease in post- surgery levels of TAP compared to controls reflects a reduced plasma antioxidant defense system. This observation could imply increase in utilization in the face of ROS.

Our data also showed a significant increase in post-surgery levels of total plasma peroxide(TPP), compared to baseline levels portraying increased ROS post- surgery and by implication, an increased oxidative bacterial killing and enhanced wound healing processes. This finding is in line with other reports [5,9] .

The actions of ROS to combat invading microorganisms and aid in cellular signaling require that the concentrations of these ROS be precisely controlled to avoid the cellular damage that occurs with excess oxidative stress [5].

Oxidative stress describes any challenge in which ROS predominate over antioxidants and CS patients have been reported to be at increased risk of oxidative stress owing to increased pro-oxidative state, due to ischemia-reperfusion processes[33]. In this study, we observed a non- significant increase in oxidative stress index post - surgery compared to baseline levels. This showed that the antioxidants were not overwhelmed by ROS; demonstrating proper management of the wound-inflammation-induced oxidative stress, which is a key component of cell protection during the healing process.

Adequate blood needs to be supplied to the site of wound for proper healing. Anaemia impairs wound healing through deficiency of nutrients and oxygen at the site of surgical wound [4,8]. The lack of significant change in the levels of PCV after surgery compared to baseline could indicate that the patients had minimal blood loss and were not anaemic after the surgery. All our study patients were discharged 4th day post-surgery without wound infection or dehiscence, as observed macroscopically by the consultant obstetric and gynaecologist in-charge.

The strength of this study is in the variety of antioxidant status measured pre-surgery, day one and four days after surgery. The impact of the nutritional intake on the nutritional status of the patients was not assessed in this study. Our report does not exonerate the use of subjective diet history and clinical judgment to assess micronutrients nutrition, but an alternate means of providing nutrients to elective CS patients who cannot eat any or enough food.

CONCLUSION

Assessing micronutrients status in all elective CS patients before and after surgery and increased consumption of fresh fruits, vegetables, dark and whole grain products, which are rich in antioxidant micronutrients, should be considered as an important part of surgical planning because of their possible involvement in wound healing. This will help the CS patients to cope better with this period of challenge.

Our study does not allow any conclusion whether impaired wound healing originates from micronutrient deficiency or not. This is because, decreased serum levels of these antioxidant micronutrients may not indicate actual deficiencies since the levels were within the reference range and all the wounds healed well. However, our data appear useful as early indices in assessing wound healing in elective CS.

Further studies would be comparing levels of micronutrients before and after surgery between CS women with wound infection and CS women without wound infection and demonstrating correlation (if any) between those parameters and the process of wound healing.

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