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Evaluation of Some Antioxidant Parameters (Vitamin E & C), MDA and Hemoglobin Levels, in Women of Reproductive Age, Based on Mode of Child Delivery, in Owerri, Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Childbirth indicates the positive outcome of pregnancy in humans, in which mode of child delivery, which is either by vaginal delivery (VD) or cesarean-section delivery (CS) is considered critical in maintaining maternal health. Antioxidants delay or inhibit cellular damage, mainly through their free radical-scavenging property. Changes in vitamin E and vitamin C antioxidants, lipid peroxidation (MDA) and hemoglobin (Hb) can lead to complications, forming reactive oxygen species (ROS) and cellular imbalance, bringing about oxidative stress (OS). These parameters remove the ROS efficiently and protect the body from the adverse effects of oxidative stress. The purpose of our study was to evaluate the effect of mode of child delivery on maternal VE, VC, MDA and Hb status, in women of reproductive age in Owerri, Imo state, Nigeria. It involved 200 pregnant women, within the reproductive ages of 20 to 39 years, with 100 subjects being those who gave birth through VD and 100 being those who gave birth through CS. Data were analyzed using SPSS version 23, with results expressed as mean ± standard deviation and presented in tables. Independent t-tests and ANOVA were used for comparisons, with p≤0.05 considered significant. The results from this study showed that, women who gave birth through VD had significantly lower VE ($p<0.001$) and VC (p<0.001) levels compared to those who had CS. Similarly, the levels of MDA were significantly (p<0.001) reduced in women who gave birth through VD compared to those who had CS. The antepartum Hb of VD women was significantly higher than postpartum levels, while the antepartum Hb of CS women was significantly lower compared to the postpartum levels. The results indicate that women who gave birth through VD had significantly reduced VE, VC, MDA and Hb levels, after childbirth, compared to births of CS delivery. It is recommended that antioxidant parameters (Vitamin E & C), MDA and hemoglobin (Hb) levels, be assessed in women during childbirth in order to help in the management of their postpartum conditions.

Keywords: Vaginal delivery (VD); cesarean section (CS); malondialdehyde (MDA); reactive oxygen species (ROS); vitamin E (VE); vitamin C (VC); hemoglobin (Hb); enzyme-linked immunosorbent assay (ELISA); oxidative Stress (OS).

1. INTRODUCTION

Mode of delivery otherwise delivery mechanism is a spontaneous process [1], which advances in medical technology in maternity care have drastically reduced maternal and infant morbidity and mortality. Mode of delivery such as vaginal delivery (VD) and cesarean section (CS) delivery is associated with maternal oxidative stress [2]. Fear and pain of normal vaginal delivery (VD) is the first or second reason for cesarean section (CS) [3,4].

Antioxidants are often used to protect cells and tissues and reverse oxidative damage caused by oxidative stress, as oxidative stress is a common denominator in the pathogenesis of many chronic diseases [5]. After birth delivery periods otherwise called perinatal or postpartum or parturition periods are associated with complications due to oxidative stress to the mother and baby, which is characterized as an up regulation in the production of oxidative free radicals and a concomitant decrease in the availability of antioxidant species, thereby creating a state of health imbalance [6]. Several studies have reported increased oxidative stress during

parturition (that is labor and delivery) for both mother and neonate, implying an increased production of free radicals that must be controlled by their antioxidant system [7].

A study on elective CS and its comparison with a control group (Vaginal delivery) indicated that the content of degradation products from lipids (i.e. lipid peroxidation or malondialdehyde) was greater in women undergoing CS than that in vaginal delivery [8].

Child birth has been associated with oxidative stress injury. Following the adverse effects of deficiency and excess of antioxidants (like vitamin E & C) on health of both, the mother and the baby, resulting to complications of pregnancy, including physical and mental conditions that affect the health of the pregnant, their baby and both [9]. It is now being recommended that women of child-bearing age and their healthcare providers should work together to assess and address the antioxidant status, more of the non- enzymatic vitamin E & C, during and after pregnancy. Nutritional deficiencies occur in pregnancy and after child delivery, and an impact is felt more in poor socioeconomic settings prevalent in Sub-Saharan Africa, including Nigeria [10].

Each of these modes of delivery has its own effects both on the baby and mothers. During parturition (or childbirth), this stress increases more profoundly [11], this is due to increasing energy demand and metabolic activity by the contraction of skeletal muscle during any type of delivery exercise, combined with a rise in using oxygen, is known to result in increased levels of ROS. Oxidative stress increases several folds during labor because of repeated uterine contractions leading to Ischemia followed by reperfusion, resulting in increased production of reactive oxygen species (ROS) [12]. Reported study explains that the severity of this oxidative stress in mothers after delivery may be related to stress of different modes of delivery [13].

Moreover, it was pointed out that the increase of oxidative stress in the delivery period was influenced by exertion of skeletal muscles and uterine muscles over a long period of time [14]. Bearing in mind the stress of labor as the fetus navigates the birth canal, an assumption can be made that free radicals may be generated more in women and babies delivered through vaginal delivery than those delivery by cesarean section (CS) delivery [13]. The event that may lead to cesarean section (CS) may however be associated with generation of more free radicals and an increased consumption of antioxidants. The status of these antioxidants and oxidants (index of free radical due to plasma MDA) will give an idea of the maternal oxidative stress.

The body is provided with non-enzymatic antioxidant vitamins E and C which can remove the ROS efficiently and protect the body from the adverse effects of oxidative stress [15]. In some reported researches, there is an imbalance between the antioxidant defense and oxidative stress in complications of pregnancy and during labor [16]. Though blood loss in the immediate postpartum period is expected for all women, an excessive amount (i.e. postpartum hemorrhage (PPH)) can have serious consequences and is the leading cause of maternal morbidity and mortality globally [17].

Postpartum blood loss and changes in postdelivery hemoglobin levels are presumed to be highly correlated, however, several studies report poor correlation between the two [18], which may suggest that the exact amount of blood loss below this amount may play a small role in

impacting a woman's physiological well-being after delivery [19], as postpartum anemia can be a consequence of a clinically important loss of blood during delivery, that has negative consequences for maternal and child health in the postpartum period [20].

The knowledge of the antioxidant/oxidative status after each mode of child delivery (Vaginal and Cesarean delivery) could be of help to better understand the physiological mechanisms involved in the postpartum maternal period, the non-enzymatic antioxidant (vitamin E & C)/oxidative status, lipid peroxidative (MDA) status and hemoglobin, along with the general physiological conditions associated immediately after childbirth/delivery, which will better indicate possible therapeutic targets [7]. However, despite the importance of these afore mentioned aspects, the knowledge gained on this issue is still very limited in certain aspects. There are also scarce studies aimed at assessing the relationship between the maternal antioxidant/oxidative status and the effect of each mode of child delivery in healthy conditions [21]. Hence, this led to the aim of this study, the evaluation of some antioxidant parameters (vitamin E & C), MDA and hemoglobin (Hb) levels in women of reproductive age based on their mode of child delivery, in Owerri, Imo state, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Location

This study was carried out in Owerri Municipal Area of Imo state, South Eastern Nigeria. Imo state with 27 local government areas divided into three senatorial zones (Owerri, Orlu and Okigwe Zones) is located at Latitude $4^{\circ}45'N$ and $7^{\circ}25'E$ and Longitude 6° 50'E and 7° 25'E with an estimated population of 4, 978,758 [22] in an area of 5,100km²(Imo Government, 2010). The state is one of the states in the South -Eastern geopolitical zone. In the heart of the East, the state is bordered by Abia State, Enugu State, Anambra State and River State to the South [23].

2.2 Subject Characterization and Selection

A total of 200 pregnant subjects, sample size, of this cross-sectional research work was used, of which 100 were pregnant subjects that delivered through vaginal mode of delivery (VD) and the other 100 were pregnant subjects that delivered through cesarean- section delivery (CS). All mothers were in their 38 weeks gestational period and were recruited by simple randomization. The sample size was determined using G^xpower software (version 3.1.9.2) at power of 95%.

The study/target population are women at point of child delivery in Federal Medical Center Hospital, Owerri, Imo State. They are between the reproductive ages of $20 - 39$ years. This study involved pregnant subjects that were of full-term gestational weeks (37 – 38 weeks), who were in normal progressive labor for vaginal delivery (VD), and those scheduled for Cesarean Section (CS) within the reproductive age of twenty to thirty-nine years (20 – 39 years) and who gave their informed consent. The inclusion criteria were full-term pregnant women (37–38 weeks) in normal labor for vaginal delivery or scheduled for Caesarean section, aged 20–39 years, with informed consent, while the exclusion criteria included those outside the gestational age range, under 20 or over 39 years, lacking consent, or with complications such as hypertension, gestational diabetes, anemia, sepsis, or abnormal fetal presentation.

2.3 Sample Collection and Analysis

Ten milliliters (10 ml) of blood samples were collected aseptically from each subject by proper venipuncture technique in the antecubital vein using sterile syringe and needle before and after delivery for each mode of child delivery. This blood collection was done by thoroughly washing of hands, wearing hand gloves, using aseptic technique and observing standard precautions throughout the procedure, explaining the procedure to the subject, applying tourniquet above the antecubital fossa sites which is most often the easiest to assess, disinfecting the area/collection site with diluted alcohol, having syringe with needle securely attached. The blood samples were put into K₃EDTA and plain bottles, 4ml and 6ml of blood respectively for hematological and biochemical analysis. Samples were centrifuged for 5 minutes at 3000 RPM for biochemical analysis. The plasma samples were separated into plain tubes. The results were read using Mindray hematology autoanalyzer for hemoglobin (Hb) (BC 2800) and enzyme-linked immunosorbent assay (ELISA) microplate reader for biochemical parameters (Vitamin E, Vitamin C and MDA) (BMG LABTECH).

The samples were collected at point of delivery (Labor phase) and after delivery (post-partum phase). The pregnant subjects were attending ante-natal clinic at Federal Medical Centre Hospital in Owerri, Imo State, Nigeria. The biodata and medical history of the subjects were obtained from their medical records. These included duration of pregnancy and age of the subjects.

2.4 Statistical Analysis

Analysis of data from this study was done using Statistical Package for Social Sciences (SPSS) version 23. All values were expressed as mean ± standard deviation and presented in tables. Comparison of means of parameters was done using independent t-test (one tail) and ANOVA, with p≤0.05 being considered statistically significant.

3. RESULTS

3.1 Demographic Characteristics of the Study Subjects

The study involved 200 pregnant subjects; women who had reached 38 weeks (mean gestational weeks) of pregnancy and are in labor at the point of delivery. One Hundred (100) of the pregnant women delivered by vaginal mode of delivery (VD) while 100 delivered through Cesarean Section (CS). The mean age of subjects was 27 ± 5 years for vaginal mode of delivery and 29 \pm 5 years for Cesarean Section mode of delivery. Also, the mean weight of the subjects that had a vaginal mode of delivery was 84.14 \pm 10.77kg and cesarean section mode of delivery, 84.32 ± 11.63 kg.

3.2 Comparison of Maternal Mean Values of the Parameters for Vitamin E (VE), Vitamin C (VC) (Non-enzymatic) Antioxidants and MDA in the Labor (Antepartum) Period and after Delivery (Postpartum) Period of Vaginal Mode of Child Delivery

The mean maternal values for non-enzymatic antioxidants in the antepartum period were 26.81 $± 1.48$ nmol/ml for VE and 76.19 $± 1.36$ ng/ml for VC relatively to the postpartum periods which showed significant decrease of 23.21 ± 1.80 nmol/ml for VE and 64.48 ± 2.28 ng/ml for VC respectively ($p < 0.001$). The mean values of 6.30 ± 0.50 nmol/ml for MDA in the antepartum period showed significant difference on the decrease in the postpartum values (after delivery) period with MDA 5.69 \pm 0.38 nmol/L $(p<0.001)$.

3.3 Comparison of Mean Maternal Values for Non-enzymatic Antioxidants (VE & VC) and MDA in the Labor (Antepartum) Period and after Delivery (Postpartum) Period of Cesarean Section Mode of Child Delivery

The mean maternal values for non-enzymatic antioxidants in the antepartum periods were 30.10 \pm 1.19 nmol/ml for VE, 80.88 \pm 0.68 ng/ml for VC showed significant increase in postpartum CS periods of 34.31 ± 1.56 nmol/ml for VE and 90.36 ± 2.34 ng/ml for VC respectively (p<0.001). The mean values in antepartum (labor) periods of 6.72 ± 0.21 nmol/L for MDA showed significant difference on the increase with MDA values of 8.78 ± 0.50 nmol/L in the CS postpartum periods $(p<0.001)$.

3.4 Comparison of Mean ± SD of Nonenzymatic Antioxidants (VE & VC) and MDA in Postpartum (after Delivery) Periods of VD and CS Modes of Child Delivery

The non-enzymatic antioxidant levels for vaginal delivery were 23.21 ± 1.80 nmol/ml for VE and 64.48 ± 2.28 ng/ml for VC, while Cesarean nonenzymatic antioxidant levels had relatively significantly higher VE of 34.31 ± 1.56 nmol/ml

Table 2. Comparison of mean maternal levels for non-enzymatic antioxidant (VE & VC) and MDA in the labor (Antepartum) period and after delivery (Postpartum) period of vaginal modes of child delivery

**VE= Vitamin E; VC= Vitamin C; MDA= Malondialdehyde*

Table 3. Comparison of mean maternal levels for non-enzymatic antioxidants (VE & VC), MDA in the antepartum and postpartum periods of cesarean section mode of child delivery

**VE= Vitamin E; VC= Vitamin C; MDA= Malondialdehyde*

Table 4. Comparison of Mean ± SD of non-enzymatic antioxidants (VE & VC) and MDA in postpartum (after Delivery) periods of VD and CS modes of child delivery

**VE= Vitamin E; VC= Vitamin C; MDA= Malondialdehyde*

and 90.36 ± 2.34 ng/ml for VC (p<0.001). The results of MDA in postpartum periods of VD, 5.69 ± 0.38 nmol/L for MDA showed a higher significant difference of 8.78 \pm 0.50 nmol/L for MDA in the postpartum period of CS mode of delivery (p<0.001).

3.5 Comparison of Mean Maternal Hemoglobin (Hb) Values of the Antepartum and Postpartum Period in Vaginal and Cesarean Section Modes of Child Delivery

The mean value of 10.43 ± 0.66 g/dl for Hb in the antepartum period showed significant difference on the decrease in the postpartum (after delivery) period, 9.56 ± 0.68 g/dl (p<0.001) for Vaginal mode of Child Delivery. The mean values in antepartum (labor) periods of 9.96 ± 1.67 g/dl for Hb showed significant difference on the increase with values of 12.03 \pm 0.71 g/dl for Hb in the Cesarean Section postpartum periods (p<0.001).

3.6 Percentage Age Distribution of Subjects that had Vaginal and Cesarean Section Mode of Delivery

The 27 \pm 5 years age distribution of women for vaginal births were 48% as compared to 52% for 29 \pm 5 years age distribution of women with Cesarean Section mode of delivery (for Fig. 1).

Table 5. Comparison of mean maternal levels for hemoglobin (Hb) in the labor (Antepartum) period and after delivery (Postpartum) period of VD and CS modes of child delivery

3.7 Percentage Weight Distribution of Subjects that had Vaginal and Cesarean Section Mode of Delivery

The 83.14 \pm 10.77 kg weight distributions of women for vaginal births were 49.6% as compared to 50.4% for 84.32 ± 11.63 kg weight distribution of women with Cesarean Section mode of delivery (for Fig. 2).

3.8 Graphical Representation of Changes in the Hemoglobin Levels of the Antepartum and Postpartum Period in Vaginal and Cesarean Section Mode of Child Delivery

The hemoglobin levels of the vaginal postpartum period decreased than the antepartum hemoglobin level. Also, there was a relative increase in the hemoglobin postpartum level of the Cesarean Section mode of delivery to the antepartum level. Consequently, the hemoglobin level of the postpartum CS delivery increased significantly more than that of the postpartum vaginal mode of delivery (p<0.001) (for Fig. 3).

4. DISCUSSION

The results of this study indicated that the ages of mothers with cesarean section mode of delivery were significantly higher than that of the ages of mothers with vaginal mode of delivery. Advanced maternal age with delayed pregnancy at childbirth was suggested as contributing to the increase in cesarean delivery with pre-pregnancy morbidity and associated risk factors. This delayed pregnancy leading to CS delivery could be due to social, educational and demographic changes, resulting in the women postponing their pregnancies until later in their fertile life, at higher ages [24]. Research studies have consistently found an association between the CS and increased weight in relation to high body mass index (BMI) compared with a similar group of younger pregnant women [25], as increased weight has effect on the physiological changes in the pregnant population in relation to the body mass index or weight [26]. The mode of child delivery with cesarean section showed a significant increase in the vitamin E (Nonenzymatic antioxidant) in the postpartum periods as compared to the vitamin E levels of vaginal

Fig. 2. Percentage weight distribution of subjects that had vaginal and cesarean section mode of delivery

Fig. 3. Changes in the hemoglobin levels of the antepartum and postpartum period in vaginal and cesarean section mode of delivery **Significantly different (p≤0.05)*

mode of child delivery. This result explains an increase in extracellular antioxidant level of pregnancy outcome, as vitamin E, concentrations increased for mothers who delivered via CS compared to vaginal deliveries [27]. Vitamin E is associated with clinical outcomes, owning to their ability to modulate inflammation associated with CS. Vitamin E plays a protective role for both the mother and infant during and after pregnancy because of its role as a chain-breaking antioxidant and as the primary lipid peroxyl radical [28] scavenger in the human body. This protection is necessary since there is unbalanced oxidant stress [29] and Vitamin E has powerful anti-oxidant property with the potential to impact health outcomes.

There was significant decrease in the vitamin C levels of the postpartum maternal vaginal

delivery as compared to that of the postpartum cesarean section mode of child delivery, where vitamin C concentrations in the maternal plasma of vaginal mode of delivery were significantly lower. The lower levels of vitamin C after vaginal birth of child delivery could be because, during labor at term, uterine contractile activity may generate reactive oxygen species (ROS) through the process of repetitive ischemia and reperfusion as water soluble vitamin C scavenges ROS in the aqueous phase [30] and recycles lipid-soluble vitamin E to combat ROSinduced tissue damage. The oxidative effects of ROS are controlled by exogenous circulating antioxidants such as vitamin C and E [31]. The increased concentrations of vitamin C may be because, vitamin C reduces the levels of stress hormones and increases the levels of antioxidants and antibody that fights against germs and viruses in both stressed and unstressed conditions [32]. Vitamin C is helpful in preventing some of the DNA damages caused by free radicals, which may contribute to the aging process and the development of diseases [33]. Vitamin E and C, have beneficial effects on the antioxidative/oxidative balance in the postpartum periods of cesarean section delivery, by increasing the antioxidative potential thereby reducing lipid and protein peroxidation [34].

Malondialdehyde level (MDA) of the cesarean mode of child delivery was significantly higher than the MDA levels in vaginal delivery of the postpartum periods, as MDA reflects the degree of lipid peroxidation. This is also, as a result of intrauterine growth retardation and weakness causing vessel constriction, thereby generating oxidative stress as abdominal operations are carried out. The MDA concentration in CS delivery increased, due to rise to almost three times volume of amniotic fluid than normal vaginal delivery where there is deliberate removal of amniotic fluid before birth [35]. This also suggests that although MDA levels may predict the level of oxidative stress before and during birth, it may not determine birth outcomes, immediately after birth [36]. MDA is a measurement for oxidative capacity of free radicals, [37] as free radical attack on membrane lipids, produces Malondialdehyde (MDA) as one of the intermediate products of these dangerous reactions [38]. Free radical generation brings up the effect of lipid peroxidation, thereby increasing the MDA. This poses the eliciting of total antioxidant status/capacity to counteract the effect of these free radicals [38,39]. The plasma MDA has been shown to be an index of free radical (oxidant) injury on membrane lipids, as the Cesarean section (CS) involves a surgical opening of the uterus, given a surgical injury on the membrane lipids, which brings oxidative stress and in turn raises the MDA level during cesarean section mode of child delivery. This oxidative stress generates Reactive Oxygen Species (ROS) which can change membranes, proteins and nucleic acids resulting in lipid peroxidation or protein and nucleic acid modification, were MDA is a product of such ROS – induced damage and reflects the degree of lipid peroxidation [40].

The result from this study indicated significant decreases in the maternal postpartum hemoglobin level of vaginal mode of child delivery in relation to the antepartum periods. This is in agreement with the work of Kavle et al*.,*

[41], probably because of blood loss during vaginal delivery, which ranges from 197ml to 505ml and blood loss during intra and postpartum periods which changes hematologic conditions of women and this blood loss occurs in some (5-6%) of women having vaginal delivery [42], leading to iron deficiency anemia [43]. The decrease in hemoglobin level coincides with the effect of vaginal mode of child delivery on the degree of hemoglobin levels and hematocrit measurements as compared to the hemoglobin levels in CS mode of delivery [44]. This effect of decrease in Hb, shows the physiologic patterns of Hemoglobin (Hb) and hematocrit (HCT) following vaginal mode of child delivery in patients with or without postpartum hemorrhage [45]. The data from this study, also showed that the maternal hemoglobin levels in the postpartum periods of CS mode of child delivery were significantly higher than the hemoglobin level in the antepartum period. This may be due to supporting blood transfusion after the CS (post operative period) as the percentage of intraoperative erythrocyte and platelet infusion increased the blood volume and also supplemented coagulation factors along with cryoprecipitate which would decrease the cases of bleeding or severe hemorrhage, thereby limiting some postpartum complications and with some other recuperative administrations based on guidelines recommended treatment in postpartum periods, having it that there will be make-up of moderate to severe anemia with intravenous iron supplements such as iron sucrose [46]. Also, Cesarean section could also be identified as one of the commonest indications for blood transfusion in obstetric practice, because of the risk of major intraoperative blood loss [47].

5. CONCLUSION

This study aimed to evaluate the non-enzymatic antioxidants (vitamin E and C), Malondialdehyde (MDA) and hemoglobin (Hb) parameters with different modes of child delivery on reproductive mothers in Owerri, Imo State. This result indicated that the levels of the non-enzymatic antioxidant (VE & VC) and MDA parameters were significantly reduced in the postpartum period compared to antepartum state of the subjects that gave birth through vaginal delivery compared to the significantly higher levels of the cesarean section postpartum periods. The results of the hemoglobin assay showed that, for subjects who had vaginal delivery, the antepartum hemoglobin levels were significantly higher compared to the postpartum levels, while for cesarean section subjects, the antepartum hemoglobin levels were significantly lower than the postpartum hemoglobin levels. The maternal demographics of age were higher in the cesarean section modes of child delivery, comparable to the vaginal delivery, indicating maturity before childbirth. Also, the weights of the mothers could be a determinant to the mode of delivery, judging from the body weight. These showed effects of modes of child delivery on the determined parameters.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

Ethical approval was obtained from Health Research Ethics Committee, Federal Medical Centre, Owerri Imo State, with the approval number FMC/OW/HREC/192.

CONSENT

Informed consent was obtained from all individual participants included in the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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