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Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) Levels Among Women with Spontaneous Abortion in Sokoto Metropolis, North Western Nigeria.

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Abstract

Spontaneous abortion or miscarriage is a global public health problem and the commonest complication of pregnancy that occurs in 10% to 15% of clinically recognizable pregnancies. A case control study was undertaken to evaluate the role of oxidative stress among women with miscarriage. Forty-five (45) women with spontaneous abortion as case and women without any history of miscarriage as control were enrolled in to study. Biomarkers of oxidative stress (malondialdehyde and total antioxidant capacity) were estimated using chemical method. The result showed that the mean and standard deviation of mean of malondialdehyde concentrations of the cases and controls were 2.2 ± 0.3 (nmol/mL) and 1.6 ± 0.2 (nmol/mL) respectively. The concentration of MDA was higher in the cases compared to the controls but not significant ($p=0.2057$). The mean and standard deviation of concentration of the antioxidant capacity in the cases and controls group were 1586 ± 185 and 2412 ± 252 ($\mu\text{mol/L}$) respectively ($p= 0.0098$). The outcome of this study provides further proven evidence of the effect of oxidative stress on Spontaneous abortion.

Keywords: Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC), Spontaneous abortion, Oxidative stress, Sokoto.

Introduction

Spontaneous abortion or miscarriage refers to pregnancy loss at 12 weeks (early miscarriage) or from 12 to 24 weeks (late miscarriage) of gestation in the absence of elective medical or

surgical measures to terminate the pregnancy (Scroggins *et al.*, 2000) It occurs in 10%-15% of clinically recognized pregnancies. As many as 80% of miscarriages occur before 12 weeks of gestation, with miscarriage rates decreasing sharply after the first trimester. (Chen and Creinin, 2007). There are many causes of miscarriage including chromosomal abnormalities, uterine abnormalities, infection, endocrinological abnormalities, and autoimmune diseases (Michels and Tiu, 2007). It is one of the most common yet under-studied adverse pregnancy outcomes. The health impact of miscarriages includes physical pain and bleeding, septicaemia and sometimes later development of cardiovascular disease. It is also associated with increased financial burden on the family searching for cure (Lok *et al.*, 2010). Feelings of loss, grief, depression and even threat to matrimonial life especially in this part of the globe are some of the psychology and mental health challenges that those affected can suffer (Engelhard *et al.*, 2001).

Oxidative stress (OS) Oxidative stress is defined as the unbalancing between production of free radicals, molecules characterized by high reactivity due to one or more unpaired electrons in the external orbital, and antioxidant defenses in the biological systems (Ashok *et al.*, 2012). This ratio can be altered by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), or a decrease in antioxidant defense mechanisms. A certain amount of ROS is needed for the progression of normal cell functions, provided that upon oxidation, every molecule returns to its reduced

state (Al-Gubory *et al.*, 2010). Excessive ROS production, however, may overpower the body's natural antioxidant defense system, creating an environment unsuitable for normal female physiological reactions. This, in turn, can lead to a number of reproductive diseases including endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility (Al-Gubory *et al.*, 2010). It can also cause complications during pregnancy, such as spontaneous abortion and recurrent pregnancy loss (Al-Gubory *et al.*, 2010). However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens (Segal, 2005). Overwhelming placental OS has been proposed as a causative factor of spontaneous abortion. Placenta in normal pregnancies experiences an oxidative burst between 10 and 12 weeks of gestation. This OS returns to baseline upon the surge of antioxidant activity, as placental cells gradually acclimate to the newly oxidative surroundings (Jauniaux *et al.*, 2000). In cases of miscarriage, the onset of maternal intraplacental circulation occurs prematurely and sporadically between 8 and 9 weeks of pregnancy in comparison to normal continuous pregnancies (Hempstock *et al.*, 2003; Jauniaux *et al.*, 2003). Markers of apoptosis have been reported in the villi, suggesting oxidative damage to the trophoblast with subsequent termination of the pregnancy (Burton and Jauniaux, 2010). Antioxidant enzymes are unable to counter increases in ROS at this point, since their expression and activity increases with gestational age (Jauniaux *et al.*, 2000). When OS develops too early in pregnancy it can impair placental development and/or enhance syncytiotrophoblastic degeneration, culminating in pregnancy loss (Gupta *et al.*, 2007). Since spontaneous abortion is one of the commonest complications of pregnancy outcomes yet under studied, this study was intended to evaluate the involvement of oxidative stress in the etiopathogenesis of miscarriage through quantification of MDA and TAC as biomarkers of OS.

Material and Methods

The research was a hospital-based study and included 45 clinically confirmed cases of miscarriages attending Specialist Hospital

Sokoto and Maryam Abacha Maternity and Children Hospital, Sokoto. Sokoto is the capital city of Sokoto State. Women with miscarriages served as the case subjects and women without any history of miscarriage served as control subjects

Exclusion criteria for the study subjects

Women with miscarriages who refuse to offer a verbal informed consent to voluntarily participate in this study, subjects with chronic diseases (Cancer, diabetes, hypertension, atherosclerosis, leukaemia, malaria, patients with Tuberculosis and Human Immunodeficiency Virus infection) were excluded from participation in this study.

Control Subjects

Women attending going to the post-natal clinic in Specialist Hospital Sokoto and Maryam Abacha Maternity and Children Hospital, Sokoto that have at least given birth were enrolled as controls. Subject with history of ectopic pregnancy, preterm birth, with chronic diseases (cancer, diabetes and hypertension), HIV and tuberculosis were excluded.

Sampling Technique

Purposive sampling technique was used in recruiting the subjects until the desired sample sized was attained.

Ethical Approval

Ethical approval was sought and obtained from the Sokoto State Ministry of Health (SMH/1580/V. IV). Informed consent was obtained from all the subjects in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

Blood Sample Collection and Processing

Five (5) ml of blood samples was collected from subjects using standard venipuncture phlebotomy. The blood was carefully and gently dispensed into sterile plain sample container. The tubes were labeled appropriately with participant's identification number. Sera from these blood samples were separated by allowing the blood to clot at room temperature then by centrifugation at 2500rpm for 10 minutes.

Thereafter transferred into serum aliquot containers and stored at -20°C until analysis.

Estimation of Serum Malondialdehyde concentration

Serum concentration of malondialdehyde will be estimated by the chemical method of Shah and Walkers (1989) using chemical reagents obtained from Nums Diagnostics Nigeria.

Determination of Total Antioxidant Capacity (TAC): TAC was estimated using Ferric Reducing Antioxidant Power (FRAP) reagent as described by Benzie and Strain (1999).

Principle: At low pH, reduction of 2, 4, 6-tripyridyl-s-triazine (TPTZ)-ferric complex to ferrous form (which has an intense blue colour). The colour change can be monitored by measuring the change in absorbance at 593 nm. The reaction is non-specific, any half reaction that has a lower redox potential under reaction conditions than that of ferric-ferrous half reaction will drive the ferrous ion formation. The change in absorbance is therefore directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

Statistical analysis

Results generated from study were analyzed

using Statistical Package for Social Sciences (SPSS) version 20, California Inc. USA. Data was reported as mean \pm SD (for continuous variables). Numbers and percentages (for categorical variables). Continuous variables were compared between case and control subjects using Student's t test, while categorical variables were compared using chi-square test that was corrected for continuity. One-way analysis of variance was done for continuous variable of the mean and standard deviation of mean of the MDA and TAC at 95% confidence interval (95% CI) between study groups.

Results

The mean and standard deviation of mean of malondialdehyde concentrations of the cases and controls are 2.2 ± 0.3 (nmol/mL) and 1.6 ± 0.2 (nmol/mL) respectively. The concentration of MDA was higher in the cases compared to the controls but not significant (p-value of the student t-test was 0.2057) as shown in figure.1.

The mean and standard deviation of mean concentration of the antioxidant capacity in the cases and controls group are 1586 ± 185 and 2412 ± 252 ($\mu\text{mol/L}$) respectively. The results shows a significant difference between the study groups ($p < 0.05$) as shown in figure 2

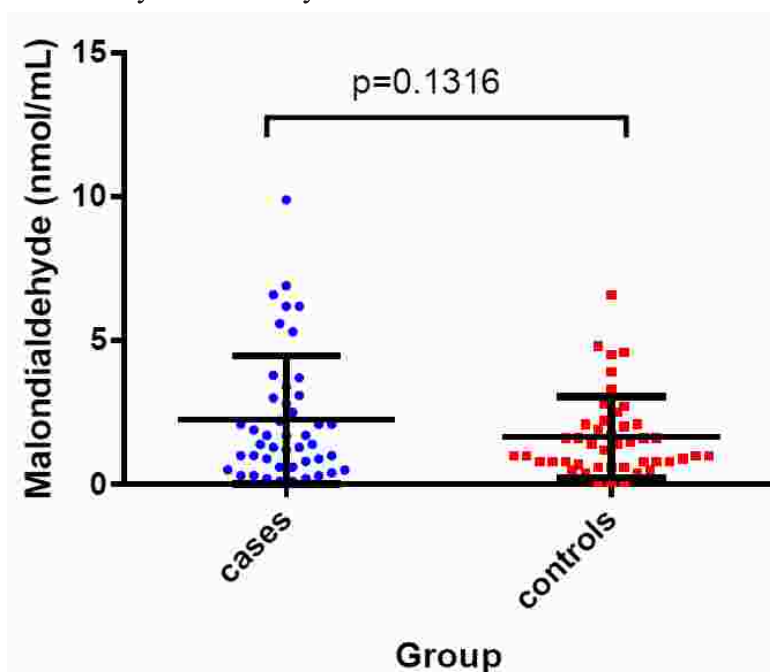


Figure 1: Malondialdehyde (MDA) values distribution among group of women with miscarriage and control group (without miscarriage). A scatter plot showing MDA values among the study population and controls.

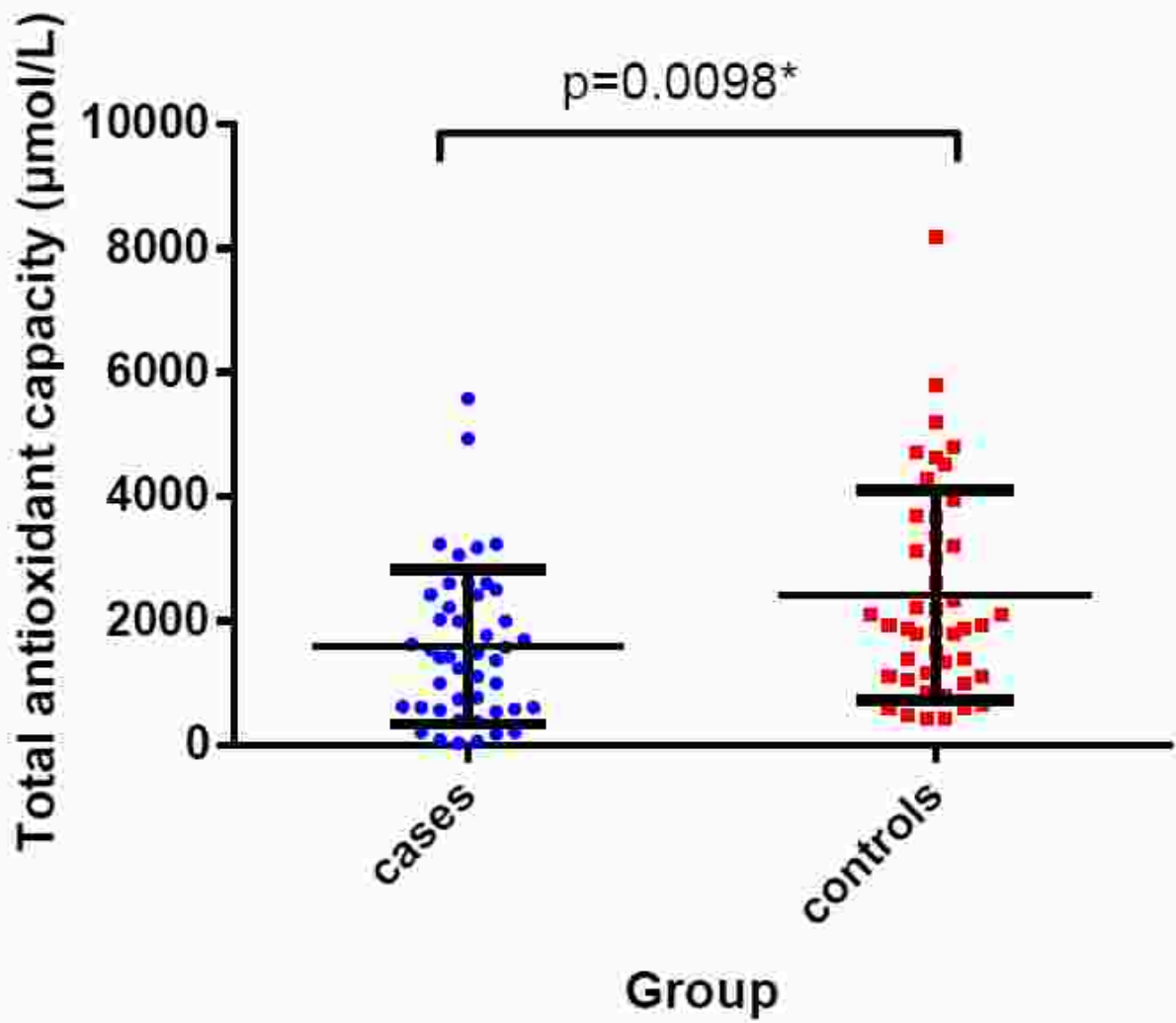


Figure 2: Total antioxidant capacity distribution among group of women with miscarriage and control group (without miscarriage). A scatter plot showing TAC values among the study population and controls.

Discussion

Overwhelming placental OS has been proposed as a causative factor of spontaneous abortion. Our results of MDA was higher in the miscarriage group (case) compared to their control counterpart but is not statistically significant ($p > 0.05$), however, many studies have shown a statistically higher levels of MDA in the patients with miscarriages compared to controls (Ramandeep *et al.*, 2017; Sumith *et al.*, 2015; Ahmed *et al.*, 2012; Hempstock *et al.*, 2003; Arikani *et al.*, 2001; Sugino *et al.*, 2000). Markers of apoptosis have been reported in the villi, suggesting oxidative damage to the trophoblast with subsequent termination of the pregnancy (Burton and Jauniaux, 2010). Antioxidant enzymes are unable to counter increases in ROS at this point, since their

expression and activity increases with gestational age (Jauniaux *et al.*, 2000). When OS develops too early in pregnancy it can impair placental development and/or enhance syncytiotrophoblastic degeneration, culminating in pregnancy loss (Gupta *et al.*, 2007). A negative correlation with lipid hydroperoxide was also observed in these patients, indicating their high susceptibility to lipid peroxidation (Toy *et al.*, 2009).

Persistence of endoplasmic OS can increase decidual cell apoptosis and resulting in early pregnancy loss (Liu *et al.*, 2011). Apoptosis of placental tissues may result from OS induced inflammatory processes triggered by a variety of factors including infections. Several etiologies may underlie improper initiation of maternal

blood flow to the intervillous space; yet it may be through this mechanism by which both spontaneous and recurrent pregnancy loss occur (Al-Kunani *et al.*, 2001). Many mechanisms on how free radical can induce termination of pregnancy have been proposed. MDA is a byproduct of lipid peroxidation, therefore an elevation in the MDA levels usually reflect an excessive production of lipid peroxides and or impaired antioxidant defense mechanism. This lipid peroxides are produced mainly in the placenta due to membrane disruption induced by reactive oxygen species. The peroxides inhibit prostaglandin I₂ synthase enzyme activity, the altered prostaglandin status might provoke vasospasm with platelet aggregation and exacerbation of placental ischemia, increased cell damage, this may lead to abnormal placentation and increased lipid peroxidation (Agarwal *et al.*, 2006). The results of TAC show a significant reduction in the level in the miscarriage group compared to control group. This results from excessive production of ROS that overpower the body's antioxidant defense mechanism.

Yigenoglu *et al.* (2011) established that the values of total oxidant level and oxidative stress index are higher, while total antioxidant capacity is lower in the miscarriage patient group (Yigenoglu *et al.*, 2011). Some studies suggest that low antioxidant level increases the risk of spontaneous abortion (Zhu *et al.*, 2014; Vural *et al.*, 2000; Barrington *et al.*, 1996). Earlier documented studies (Enkins *et al.*, 2000; Simsek *et al.*, 1998) report a reduction in Superoxide dismutase which is one of the antioxidants defenses mechanisms that scavenge free radicals. Apart from the normal pregnancy that generate reactive oxygen species, many factors that cause miscarriage are also associated with induction of free radical generation such as persistent urogenital infections, the body's defense as ways to kill microbes stimulate the polymorphonuclear cells to generate free radical and attacks to pathogen in so doing it can also attacks the trophoblast of the developing embryo leading to spontaneous expulsion of the product of conception.

Conclusion

The results of this study provide further evidence on the effect of increased oxidative stress on the

incidence of early spontaneous abortion in the first trimester of pregnancy. High serum MDA levels and low TAC during pregnancy were two risk factors for spontaneous abortion. The present findings support the hypothesis that oxidative stress plays a key role in the etiopathogenesis of spontaneous abortion. Further studies are required for assessing the preventive role of antioxidant therapy in this complication.

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