A Comparative Study of the Status of Oxidative Stress in Pregnant Nigerian Women

1O.B. Idonije, 2O. Festus, 3O. Okhiai and 1U. Akpamu

1Department of Chemical Pathology, 2Medical Laboratory Science, 3Nursing, 4Physiology, Ambrose Alli University Ekpoma, Edo State, Nigeria

ABSTRACT

Normal pregnancy has been found to be associated with a high metabolic demand and elevated requirements for tissue oxygen which results in increased oxidative stress and antioxidant defenses. Hence this study evaluates in pregnancy the state of oxidative stress express by product of lipid peroxidation via the level of ThioBarbituric Acid-Reactive substances (TBARS). It involves 180 apparently healthy pregnant Nigerian women age between 20-40 years of different gestation period parity and socio status. In addition 20 apparently healthy tested non pregnant women formed the control group. The test groups were women attending ante-natal clinic at the University of Benin Teaching Hospital (UBTH) Benin City Edo state, Nigeria. Data from standard laboratory procedure were then subjected to statistical analysis using the paired sample t test of SPSS software version 17. Result shows increase in plasma TBARS as age advances and as the number of pregnancy increases. TBARS was lowest in women indulge in physical activity as in farm works (7.6±3.24 µM) and highest among the physical inactivity as in full house-wife (16.28±6.69 µM). A significant rise in TBARS in pregnant women (13.09±2.34 µM) was observed compared to the non pregnant women (the control 4.41±0.50 µM). There was a steady increase in TBARS as the gestation period advances and this was significantly different (p<0.05) from the control. Conclusively, normal pregnancy is associated with oxidative stress. We recommend therefore that there is a need for moderate physical activity during pregnancy and antioxidant supplementation which should increase as age, gestation and number of pregnancy (parity) advances.

Key words: Pregnancy, oxidative stress, peroxidation, parity, antioxidant

INTRODUCTION

Pregnancy has been known to be associated with alteration in physiological and metabolic functions of the woman’s life. Consequently remarkable and dramatic events occur during this period for sustaining mother and fostering the growth and maintenance of fetus (Qanungo and Mukherjea, 2000). Report shows normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen which results in increased oxidative stress and antioxidant defenses (Knapona et al., 1999). Oxidative stress is the presence of Reactive Oxygen Species (ROS) in excess of the buffering capacity of available antioxidants (Palan et al., 2001). While according to Sies (1991) and Page (1993) it is a disturbance in the prooxidant-antioxidant balance in favour of the former, leading to potential damage. The role of ROS in various diseases of the female reproductive tract has been investigated and can affect
a variety of physiological functions in the reproductive tract as excessive levels can result in precipitous pathologies affecting female reproduction (Agarwal et al., 2005).

Although the generation of free radicals is a normal physiological process but increased production of free radicals can act on lipids to cause lipid peroxidation (Tiwari et al., 2010). It has been suggested that free radicals are likely promoters of maternal vascular malfunction, as reactive oxygen species particularly superoxide anions evoke endothelial cell activation (Palan et al., 2001). These ROS have been implicated in atherosclerosis cancers pre-eclampsia and many other human diseases (Zhang et al., 2001). Although lipid peroxidation is an oxidative process which occurs at low levels in all cells and tissues excess production is known to be detrimental to health. Under normal conditions a variety of antioxidant mechanisms serve to control this peroxidative process (Sies, 1991). The question now is does pregnancy affect this mechanisms that check peroxidation processes? This present study was to evaluate the state of oxidative stress express by product of lipid peroxidation via the level of plasma thiobarbituric acid-reactive substances (TBARS) in pregnant Nigerian women.

MATERIALS AND METHODS

Subjects: A total of two hundred subjects between the ages of 20 and 40 formed the study population. Group 1; the control comprises of 20 healthy non pregnant volunteers of Nigerian origin. Group 2 the test involve 180 apparently healthy pregnant Nigerian women sub-divided into three groups X, Y and Z, each made of 60 subjects distributed into and 3rd trimester of pregnancy, respectively. The test subjects were selected among those attending ante natal clinic at the University of Benin Teaching Hospital (UBTH) Benin City Edo state Nigeria. The study was conducted in compliance with the Declaration on the Right of the Patient (WMA, 2000) after approval by the Ethical Committee of University of Benin City Edo state Nigeria. Also an informed consent was obtained from all subjects enrolled for the study.

Inclusion criteria include healthy non pregnant and pregnant women of Nigerian origin and are consumers of normal mixed food.

Exclusion criteria include pregnant women with gestational diabetes mellitus anemia hypertension obesity smoking alcoholism and HIV.

Sample collection and analysis: Five milliliter of venous blood was collected from the antecubital vein under aseptic precaution from each subject into EDTA anticoagulant bottles. The blood was then centrifugated at 2500 g for 5 min and the plasma removed and stored at 4°C pending assay of product of lipid peroxidation.

Oxidative stress was analyzed by measurement of the level of plasma ThioBarbituric acid-Reactive Substances (TBARS) by the method described by Buege and Aust (1978).

Statistical analysis: The data was analysed using SPSS software package version 17. The paired sample t-test was used to test the level of significance and p<0.05 was considered significant. Results were then presented in suitable Tables.

RESULTS

Table 1 shows the relationship of socio-demographic characteristic with Oxidative stress by measurement of the level of plasma ThioBarbituric Acid-Reactive Substances (TBARS). The test
Table 1: Socio demographic characteristics in relation to oxidative stress (TBARS) status of pregnant Nigerian women

<table>
<thead>
<tr>
<th>Socio demographic</th>
<th>Control group</th>
<th>Test group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBARS (UM)</td>
<td>TBARS (UM)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-25</td>
<td>5.4±0.17*</td>
<td>5.3±1.10*</td>
</tr>
<tr>
<td>26-29</td>
<td>4.4±0.11*</td>
<td>6.3±2.47*</td>
</tr>
<tr>
<td>30-35</td>
<td>4.4±0.15*</td>
<td>11.2±2.51*</td>
</tr>
<tr>
<td>34-37</td>
<td>4.4±0.22*</td>
<td>13.9±1.83*</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHW</td>
<td>5.4±0.10*</td>
<td>14.4±1.64*</td>
</tr>
<tr>
<td>Traders</td>
<td>4.4±0.11*</td>
<td>8.7±2.79*</td>
</tr>
<tr>
<td>Civil servants</td>
<td>4.4±0.16*</td>
<td>9.5±4.12*</td>
</tr>
<tr>
<td>Farmers</td>
<td>4.2±0.10*</td>
<td>6.0±1.96*</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0</td>
<td>8.2±2.68*</td>
</tr>
<tr>
<td>2nd</td>
<td>0</td>
<td>8.5±3.94*</td>
</tr>
<tr>
<td>3rd</td>
<td>0</td>
<td>9.4±4.23*</td>
</tr>
<tr>
<td>≥4th</td>
<td>0</td>
<td>11.3±2.49*</td>
</tr>
</tbody>
</table>

Values are Mean±SD. TBARS: Thiobarbituric acid-reactive substances. FHW: Full house wife. Values in a row and in each column having different superscripts are significantly different (p<0.05).

Table 2: Oxidative stress (TBARS) status of pregnant Nigerian women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 20)</th>
<th>Pregnancy (n = total 180)</th>
<th>1st trimester (n = 60)</th>
<th>2nd trimester (n = 60)</th>
<th>3rd trimester (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (µM)</td>
<td>4.4±0.10*</td>
<td>9.6±3.80*</td>
<td>5.2±0.99</td>
<td>9.8±1.93</td>
<td>13.7±1.68</td>
</tr>
</tbody>
</table>

Values are Mean±SD. TBARS: Thiobarbituric acid-reactive substances. Values in a row having different superscripts are significantly different (p<0.05).

Group comprised 180 apparently healthy pregnant women and the control group 20 apparently healthy non-pregnant women both of Nigerian origin with age ranging from 22 to 37 years. Result shows plasma thiobarbituric acid-reactive substances to increase as age advances and follow a similar pattern in both the non-pregnant and pregnant women. However, the values of the pregnant women were significantly higher (p<0.05) than those of the non-pregnant women. Also TBARS values were found to be influence by the type of occupation these women are involve in. It was lower in women involve in physical activities (as in farm work 4.2±0.15 for control and 6.07±1.95 for test) compared to other occupation while those classified as full house wife presented the highest value and were statistically significant (p<0.05). Furthermore, TBARS was observed to increases with significant differences (p<0.05) as the number of pregnancy (parity) increases.

Table 2 is a comparative table of oxidative stress status in non-pregnant and pregnant women and the gestation period of the pregnancy. Comparatively with the non-pregnant women (4.4±0.16 µM) result presented significantly higher TBARS in pregnant women (9.6±3.80 µM). Also TBARS increases as the gestation period advances and was statistically significant (p<0.05).

DISCUSSION

Oxygen toxicity is an inherent challenge to aerobic life and reactive oxygen species can modulate cellular functions and oxidative stress can impair the intracellular milieu resulting in
diseased cells or endangered cell survival (Agarwal et al., 2005). The results of the present study clearly showed that circulating levels of oxidative stress marker (thiobarbituric acid-reactive substances) is significantly elevated in women with normal pregnancy and levels were marked as the gestation period increases. This finding is in agreement with the findings of Toescu et al. (2002), Upadhyaya et al. (2005) and Patil et al. (2007) who reported Markers of lipid peroxidation (MDA) to be increased during the progression of normal pregnancy and Little and Gladen (1999) reporting lipid peroxidation to be enhanced in the second trimester and tapers off later in gestation and decrease after delivery. Although there is a report of placental production of lipid peroxides to decreases as normal gestation advances most likely because of an increase in the activity of superoxide dismutase and catalase (Watson et al., 1997). Interestingly increased lipid peroxidation, as evidenced by increased levels of the biomarker malondialdehyde has been noticed in term labor (Mocatta et al., 2004). In a case controlled study the serum levels of hydroperoxides were higher in patients in labour compared to the controls who were not in labour (Fainaru et al., 2002). The role of oxidative stress in initiation of labor is not known. Pregnancy is a physiological state accompanied by a high metabolic demand and elevated requirements for tissue oxygen (Spatling et al., 1992) and causes an increase of reactive oxygen species production (Goto et al., 1993) similar to findings in this present study. Moreover the placenta is a major source of oxidative stress because of its enrichment with PUFA (Gitto et al., 2002). Falkay et al. (1977) suggested that the increase in the lipid peroxide levels was due to the increased prostaglandin synthesis in the placenta. Placental oxidative stress has been suggested to play a role in the pathogenesis of pre-eclampsia (Mutlu-Turkoglu et al., 1998; Takagi et al., 2004) and fetal growth retardation (Takagi et al., 2004; Karowicz-Bilinska et al., 2002; Scholl and Stein, 2001). Monitoring of the oxidative stress in pregnant women is important to enable an understanding of the relationship between oxidative stress and pregnancy outcome (Kim et al., 2005).

The present study also showed oxidative stress marker (thiobarbituric acid-reactive substances) to increase with age. This relationship of oxidative stress with age is significantly elevated in women with normal pregnancy. Oxidative stress has been reported to influences the entire reproductive span of women’s life and even thereafter (i.e. menopause) (Agarwal et al., 2005). It has been suggested that the age-related decline in fertility is modulated by oxidative stress (De Bruin et al., 2002) which agreed with the increased oxidative stress seen in older women in this study. There is some understanding of how reactive oxygen species affect a variety of physiologic functions such as oocyte maturation ovarian steroidogenesis, ovulation, implantation, formation of blastocyst, luteolysis and luteal maintenance in pregnancy (Suzuki et al., 1999; Jozwik et al., 1999; Ishikawa, 1995; Vega et al., 1998; Sugino et al., 2000). It has been reported that reactive oxygen species may damage the oocytes (Tarin, 1996). Also there is an age related decline in the number and quality of follicles in females. This age related decline in oocyte quality also results in increased incidence of congenital anomalies in children (Agarwal et al., 2005). The ageing of the oocytes affects many biochemical pathways which have a deleterious effect on pre- and post implantation development of the embryo (Tarin et al., 2000). The pre- and postovulatory ageing of the oocytes have also been associated with congenital anomalies behavioral alterations and learning disabilities in later life and constitutional diseases such as diabetes mellitus and schizophrenia (Agarwal et al., 2005). Oxidative stress occurs at menopause because of loss of
estrogens which have antioxidant effect on low-density lipoproteins. Estrogens confer cardioprotection by lowering protein oxidation and antioxidant properties (Artesaga et al., 1998). Diminished antioxidant defense is associated with osteoporosis in post-menopause. Modulation of the estrogen receptors α and β has been reported to be effected in vitro by oxidative stress (Tamir et al., 2002).

Furthermore the present study reveal physical inactivity (sedentary life style) as in full house wife to increase the production of oxidative stress marker and those involved in physical activities as in farm work to present the lowest values of oxidative stress in both pregnant and non-pregnant women. To this regards and supporting this finding is the study of Laufs et al. (2005) who reported inactivity to increases vascular NADPH oxidase expression activity and enhances vascular ROS production which contributes to endothelial dysfunction and atherosclerosis during sedentary as opposed to physically active lifestyle. Interestingly recent study in Palliative worker shown occupational stress to increases oxidative stress levels probably as a response to increased generation of reactive oxygen species and that working during the evening and night shifts increases oxidative levels and burnout levels (Casado et al., 2011). Oxidative stress has also been shown to have a major role in the causality of some disorders that have higher prevalence in shift workers such as cardiovascular disorders (Tenkanen et al., 1997). This may be suggestive of the high oxidative stress value in civil servants and traders reported by this study. Furthermore the results of the study of Sharifian et al. (2005) showed that shift work can act as an oxidative stressor and as age and Body Mass Index (BMI) rise the antioxidant system becomes more disabled against oxidative stress.

Circulating markers of oxidative stress has been shown to increase during pregnancy (Little and Gladen, 1999; Morri et al., 1998; Toescu et al., 2002) similar to findings in this present study and the question as to whether this returns to normal after parturition and whether this might contribute to the increased risk for cardiovascular disease observed in older multiparous women (Dhawan et al., 2004, 2005; Grunblatt, 1998; Koski-Rahikkala et al., 2006; Lawlor et al., 2003; Ness et al., 1993, 1994) had not previously been addressed. The present study indeed demonstrates that there is increased oxidative stress during pregnancy and that the number of pregnancy also influences oxidative stress. It was observed that there is an increased marker of oxidative stress as the number of pregnancy increases as oxidative stress marker was lowest in first pregnancy and becomes higher in consecutive pregnancies. In accordance to this finding is the study of Tawfik et al. (2008) which suggest multiparity to induce endothelial dysfunction through decreased NO bioavailability and increased reactive oxygen species formation. In rats it has been reported that repeated pregnancies are associated with degradation of vascular elastic tissue (Wexler, 1970) and an increase in the incidence of spontaneous arteriosclerosis of the aorta and of the mesenteric and renal vascular beds (Wexler, 1964, 1981). Epidemiological studies have shown an increase in cardiovascular morbidity and mortality among postmenopausal women with four or more children (Grundy and Tomassini, 2005; Koski-Rahikkala et al., 2006). In addition, Lawlor et al. (2003) found multiparous women to have a higher risk for coronary heart diseases which was associated with an adverse lipid profile and diabetes. Pregnancy is associated with an increase in low-density lipoprotein (LDL) total cholesterol and triglycerides levels (Heliovaara and Aromaa, 1981; Humphries et al., 2001). Some of these
changes in the lipid profile persist into postpartum long after reproductive activity has ceased (Humphries et al., 2001). Hyperlipidemia especially high LDL is responsible for the formation of oxidizable particles. Increased oxidative stress and atherosclerosis leading to endothelium dysfunction and decreased vascular distensibility (Giannattasio et al., 2001; Martin et al., 1999; Ramirez et al., 2001; Toescu et al., 2002; Vapaatalo and Mervaala, 2001).

CONCLUSION

Conclusively this study has shown pregnancy to be associated with oxidative stress which is worse as age advances in inactive women and multi-parous individual. It is therefore suggested that pregnant women be indulged in physical activities and their diet be supplemented with antioxidants.

REFERENCES


