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Effects of parity on antioxidant status in pregnant women in a Nigerian population

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ABSTRACT: Controversial reports on the effects of multiparity on pregnancy outcome have been documented in literature. While some authors have strongly supported the popular belief that multiparity is associated with complicated pregnancy outcome, others have claimed that there is no association. Moreover, little or no information has been documented about the effect of multiparity on antioxidant status of pregnant women in Nigeria. This is the purpose of this study.

The study involved a total of one hundred and fourteen (114) subjects which were randomly selected, this comprises of thirty seven(37) non pregnant women as control subjects and seventy seven (77) pregnant women as test subjects, with different parity status and all within the ages 15-50 years. All the subjects had normal blood pressure (not more than 125/80mmHg).The test subjects were all in the third trimester with no underlying disease and not on any chronic drug therapy. They were grouped into five groups according to their parity status. Plasma estimations of their vitamin C, vitamin E, albumin, uric acid, selenium, copper, and zinc were determined; and the control group results compared with the test group results.

The results showed that the albumin concentration of the test group was significantly lower than those of the control group (**P**<0.001), and amongst the test sub –groups the albumin was significantly reduced in the multipara (**P**<0.001).

The reverse was observed for plasma uric acid, where there was an increase in the test group than the control (**P**<0.001) and the increase was more in the multipara in the test group.

For the vitamins ; Plasma vitamin E concentration was significantly decreased in the test group than in the control group and the decrease was more as parity increased.

However, vitamin C in all the six groups (control group and all the test subgroups) was not significantly different from one another (**P**<0.149).

For the minerals (trace elements); copper showed no statistically significant difference (P<0.143) among the groups. Selenium concentration was observed to be significantly decreased (P<0.001) in the test group than the control and the decrease was more in the multipara. Lastly, zinc was obviously less in test group than control group and the reduction was more as parity increased (P<0.001).

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These results indicated that pregnancy significantly reduced the serum levels of some of the antioxidants such as albumin, vitamin E, selenium and zinc. The reduction in each of these analytes was more pronounced in multiparity than in low parity or primip and this is an indication that multiparity causes more oxidative stress. While vitamin C and copper levels were not significantly affected by pregnancy or multipartiy, uric acid level increased in pregnancy and this increase was more in multipara than in low parity or primp. The increase in uric acid however may be due to effect of pregnancy or multiparity on the kidney. It is suggested from the findings of this study that food supplements rich in antioxidant substances (vitamin E, albumin, zinc, and selenium) will be beneficial in pregnancy, especially in the multiparous, this may reduce oxidative stress and hence reduce complications of pregnancy as well as reduce maternal mortality, hence improve safe motherhood.

Key words: Parity, Antioxidant Status, Pregnant Women, Nigerian Population.

Introduction

An antioxidant is a substance whose presence in relatively low concentration significantly inhibits the rate of oxidation of the major targets of oxidative activity (Halliwell and Gutterridge, 1994), and oxidative activity is a result of free radicals. A free radical is any molecular species capable of independent existence that contains an unpaired electron in an atomic orbit (Young and Woodside, 2001). Free radicals are highly reactive molecules generated during normal metabolism or from certain drugs (like paracetamol), and examples include the superoxide (O^{O}_{2}), hydroxyl (OH^{O}), nitric oxide (NO^{O}), lipid peroxyl (ROO^{O}) ,and hydroperoxy (ROH^{O}).

A lot of biochemical activities occur in the human body and are aimed at generating high energy molecules for our daily life activities, such activities require oxygen consumption and they lead to an increase in the production of reactive oxygen species **(ROS).**

Pregnancy is a physiological state accompanied by a high-energy demand and increased oxygen requirement (Spatling et al,1992). The tendency in pregnancy therefore is enhanced production of ROS. For instance, certain peculiar processes occur in pregnancy; the human placenta produces steroid hormones in increased concentrations and this has been partly implicated with the formation of free radicals capable of mediating tissue damage in the mother and the fetus (Murray, 1990). Also, the placenta produces lipid peroxides that are secreted mainly to the maternal side of the placenta (Walsh and Wang, 1993a). In pregnancy the consumption of oxygen increases by 20%, causing increased respiratory rate (Sembulingam and Sembulingam, 2000) and this will favour free radicals generation. In late pregnancy basal metabolic rate increases due to increased secretion of hormones (thyroxine, cortisol, sex hormones), this will increase free radical generation. These processes lead to increased oxidative stress and consequently reduced maternal anti-oxidant levels and activities.

High parity is often associated with obstetric complication, which often affects the mother or the fetus or the pregnancy outcome generally. A woman's age and parity have been said to affect her chances of dying during labour (Bai et al, 2002). The deleterious effect of oxidative stress on living tissues is probably one of the factors involved in the complications associated with multiparity. There are some important body antioxidants, examples include vitamin E, vitamin C, uric acid, albumin and certain transition metals (selenium, copper and zinc as examples).

For the purpose of this study the concentrations of some antioxidants will be determined and used as the basis of assessing the concentrations of free radicals generated in the subjects (both control and text groups). This study looked at the plasma levels of some antioxidants (albumin, uric acid, vitamin C, vitamin E, selenium, copper and zinc) in non-pregnant women (control subjects) and pregnant women (test subjects). The pregnant women will be of various parity levels, involving nulliparous or primigravide (no previous child), low parity (1 or 2 previous children), high parity (3 or 4 previous children) and grand multiparity (5 or more previous children), at their third trimester.

The aims of this study are therefore to:

(i) determine the concentrations of some antioxidants (albumin, uric acid, vitamin C, vitamin E, selenium, zinc and copper) in disease free pregnant women (test subjects) and disease free non-pregnant women (control subjects) and compare the results of the control with that of test subjects and see if pregnancy has any effect on antioxidant level in women of childbearing age.
(ii) compare the effect of parity status on antioxidants in pregnant women.

Materials and Methods

A total of one hundred and fourteen (114) subjects were randomly selected for the study, comprising of seventy-seven (77) pregnant women at different parity levels and thirty-seven (37) non-pregnant women. The seventy-seven (77) pregnant women were at their third trimester and served as the test subjects and consisted of 24 primigravidae, 20 para 1, 14 para 2, 11 para 3 and 8 para 4 and above.

They were selected from some antenatal clinics in Ile-ife, both the public and private health facilities (Obafemi Awolowo University teaching hospital, a health centre, Osun state General Hospital, and some private health facilities in the town). The exclusion criteria included blood pressure greater than 125/80mmHg, presence of glycosuria or proteinuria and chronic drug therapy. The control subjects (37 non pregnant women) were disease free women selected from the healthy population within the town. Both the test group and control group were age matched, and all were within age bracket 15-50 years. While 110 subjects fall within age bracket 20-44years, only 3 fall within 15-19years and 1 within 45-50years (see table 2). They were also matched in terms of anthropometric parameters.

For every participating subject the demographic characteristics, obstetric history, anthropometric parameters and blood pressure were taken and urinalysis conducted to ascertain suitability for the study. About 10mls of venous blood was collected from each subject, non-fasting sample, between 10.00am–12.00pm into a plain bottle to obtain the plasma. The sera were then kept frozen at –20C until the time for analysis. The various analyses were carried out and the concentrations of the antioxidants were determined using the methods as indicated in the table below.

Assays

Vitamin C was determined by the method of Witson and Guillan (1969), Vitamin E by the method of Hausen and Warwick method (1996). Plasma Albumin was also estimated by the method of Rodkey (1965) and uric acid by the method of Fossati, Prencipe and Berti (1980). The minerals selenium and copper were estimated by flame atomic absorption spectrophotometric method while zinc was estimated by the flame photometric method of Davies et. al. (1968). The results obtained were then subjected to statistical analysis.

Statistical Analysis

The statistical tools used were SPSS Computer package 11.1, Analysis of Variance (ANOVA), post HOC test (multiple comparisons) and t-test

Results

Tables 1 and 2 show the results of the study. Table 2 shows the age distribution of the controls and the pregnant women in the different groups. While only 3 of the subjects fall within age 15-19 years (1 control and 2 primip), majority of the subjects fall within 20 – 44 years and only 1 subject is in the age range 45 – 50 years. This justifies the age matching.

Table 1: Age distribution of the controls (non-pregnant) and test group (pregnant women) in the different age groups

Age (Years)	Groupings					
	Control n = 37	**Primip n = 24**	**Para 1 n = 20**	**Para 2 n = 4**	**Para 3 n = 11**	**Para4 n=08**
15 – 19	1	2	0	0	0	0
20 - 24	14	2	0	0	0	0
25 – 29	10	13	13	4	0	1
30 – 34	4	7	4	6	4	2
35 – 39	4	0	1	2	3	3
40 – 44	4	0	1	2	4	2
45 – 50	0	0	1	0	0	0
Total	37	24	20	14	11	8

Note: N = Number of Subjects, Primip= Primiparous, Para = Parity.

The concentrations of the various analytes (antioxidants) studied in these subjects are as presented in Table 2.

Table 2: Concentrations of Analytes (X± SD) in control and pregnant subjects

Subjects	**Albumin (X± SD) (g/L)**	**Uric Acid (X± SD) (mmol/L)**	**Vit. C (X± SD) (mg/100ml)**	**Vit. E (X±SD) (ugl/ml)**	**Selenium (X±SD) (umol/L)**	**Copper (X± SD) (umol/L)**	**Zinc (X± SD) (umol/L)**
Control (n=37)	39±4.6	0.15±0.08	0.33±0.09	11±2.4	2.0±0.050	18.0±9.7	27.7±5.8
Primip (n=24)	32±4.0	0.22±0.1	0.35±0.07	11±1.9	1.2±0.16	22±10.3	23.5±5.8
Para 1 (n=20)	32±2.1	0.21±0.11	0.38±0.08	8±1.7	1.0±0.08	25.5±9.7	23.8±2.9
Para 2 (n=14)	31±2.1	0.25±0.11	0.32±0.06	9±1.9	1.1±0.01	27±11.8	19.1±5.1
Para 3 (n=11)	31.0±2.7	0.26±0.09	0.4±0.01	6±0.84	1.6±0.39	28±8.4	17.4±5.7
Para 4+ (n=8)	29±2.5	0.31±0.07	0.37±0.9	6±1.0	1.0±0.09	29±9.5	14.3±1.3

The figures represent the means ± standard deviation (SD)
n = number of subjects; Primip = Primiparous; Para = Parity

The study revealed that the plasma albumin for the control group was 39±4.6g/L and 32±4.0g/L for the primip group. This showed that there was a reduction in the plasma albumin of the pregnant women compared with the control and the reduction was statistically significant ($P<0.05$). This difference is also obvious comparing the para 1 with the multipara, with the latter having less, which is also statistically significant ($P<0.05$). Unlike albumin, the plasma uric acid increased consistently as parity increases and there was an increase in the values of the pregnant women compared with the control, with values being 0.22±0.1 and 0.15±0.08 respectively. These differences were statistically significant.

For vitamin C, there was no notably consistent pattern of change when the control group was compared with the test group, and no statistically significant difference. On the other hand, vitamin E was significantly reduced in the pregnant subjects when compared with the controls, especially when the multipara is compared with either the primip or the control group (table 3). These differences were statistically significant ($P<0.05$).

As for the transition metals, while selenium and zinc showed obvious reductions in the pregnant women than the control group, and the primip women have more than the multigravid, there was no notable change in the copper level of the control and test subjects .Significant differences occured in plasma levels of selenium and zinc when control and test subjects were compared.

Discussion

Pregnancy is associated with an increase in oxidative stress as a result of certain factors; these include increase in metabolic rate, energy consumption**,** oxygen intake and increased hormones synthesis. Also, in pregnancy more Estriols are produced by the fetal Adrenal gland and the placenta, leading to increased formation of free radicals. Invariably, a reduction in antioxidants levels may be anticipated in pregnancy. This study has shown some significant differences between the test group and the control group.

The observed reduction in albumin could be attributed to two factors, one of which is the fact that albumin moves to the fetus in the pregnant woman compared to the non pregnant control. Secondly, albumin is an important antioxidant because of its possession of cysteine residue, which confers on it the ability to neutralize peroxy radicals as it helps to transport free fatty acids (Stocker and Frei, 1991). These explanations mean that Albumin has probably been used.

The increase in uric acid in the test group compared with the control is simply due to reduced GFR in the pregnant women. This reduction in GFR is worse with increased multiparity as seen in this study. It has been documented that as age advances serum urate level rises (Anetor et al, 2003), also a pregnancy may have its untoward effect on the kidneys and hence, multiparity may be associated with higher plasma urate as seen in this study though an ealier study reported a contrary opinion (Anetor et al, 2003).

Vitamin C did not show any statistically significant difference, this observation may mean that multiparity has no effect on the role of vitamin C as an antioxidant . However, Vitamin C had been reported to reduce in patients with pre-eclampsia when compared with pregnant women without eclampsia as controls (Sharma, et al, 2006).

Vitamin E on the other hand showed obvious reduction in the pregnant women compared with controls, with a significant difference ($p<0.001$). This suggests that vitamin E has an active role to play in pregnancy, especially to prevent against perioxidation of polyunsaturated fatty acids. The observation that Vitamin E is reduced in pregnancy agrees with previous finding (Moris et al, 1998), and multiparity as observed in this study has further worsen the position of vitamin E in pregnancy. The role of Vitamin E can not be overemphasized because lipid peroxidation plays a role in some pregnancy related pathological conditions. For instance, current theory suggests that in pre-eclampsia there is an increase in the lipid peroxidation products and this leads to decrease in serum antioxidants except uric acid, contributing to the pathogenesis of pre-eclampsia. (Kashinakunti, et al, 2010). The serum antioxidants are excessively utilized to counteract the cellular changes mediated by free radicals. Another study investigated the role of oxidative stress in the pathophysiology of malaria in pregnancy using lipid peroxidation product - Malondialdehyde (MDA) level in plasma, the activity of erythrocyte antioxidant defence enzymes (Superoxide dismutase and catalase), as well as the ability to resist oxidative stress by the Ferric Reducing Ability of Plasma (FRAP) assay. It was reported that an imbalance existed between the oxidants and the antioxidants (Tiyoung et al, 2009).

The finding that selenium was reduced with significant difference ($p <0.001$) in pregnant women when compared with the control and that this reduction is more in the multipara than the primip is not strange. The reduction in selenium in multiparity compared with the nulliparous or primip is in literature (Osman et al, 2000), and

reduction of selenium in pregnant women compared with non-pregnant women has also been documented (Anetor et al, 2003).

Copper was found to show a steady increase as parity increased, however there is no significant difference. This increase in Copper concentration during pregnancy is in literature (Buamah, et al, 1984) and it is thought to be due to increased Copper retention in pregnancy due to decreased billiary excretion of copper induced by hormonal changes during pregnancy.

For Zinc, it was found to have a significantly different reduction ($p < 0.001$) in the test groups than the control and the observation was more marked in the multipara. Similar observation was documented by Osman and his colleagues (Osman, et al, 2000).

Animal studies had indicated that oxidative stress is a pathologic factor in tissue damage. For instance, in the brain elevated Malondialdehyde level (marked lipid peroxidation) has been proposed to be one of the major mechanisms of secondary damage in traumatic brain injury (Weighand et al, 1999). Also, Malondialdehyde level in the liver may be used to investigate the oxidative damage of protein and lipoproteins which is a possible mechanism for liver injury (Kojic et al, 1998).

It is a known fact that pregnancy is not a disease, however, the higher generation of free radicals in pregnancy than the non pregnant state predisposes the pregnant woman to disease condition, and multiparity worsens the scenario. It had been documented that multiparity induced vascular endothelial dysfunction by facilitating the formation of reactive oxygen species(Huda E. Tawfik, et al,2008). The importance of antioxidants in pregnancy had prompted some workers to attempt using amniotic fluid antioxidants as Biomarkers of complication of pregnancy, like pregnancy induced hypertension and gestational diabetes, but their use is limited as amniotic fluid volume varies between patients(Mirjana Bosavac, et al, 2011).

Finally, evidence that free radicals are influenced by diet exits, for instance feeding animals with saturated fats resulted in increased generation of free radicals (Odutuga and Amballi, 2007), an important pathologic factor in most diseases.

This study has shown that multiparity caused reduced antioxidant status due to increased oxidative stress. From above, an additional factor such as preeclampsia or malaria in a multiparous woman will worsen the effect of oxidative sress and hence a greater risk to the woman. Likewise the ingestion of saturated fats (Odutuga and Amballi, 2007) by a multiparous woman is likely to pose a greater risk to the woman. We therefore suggest that supplements rich in antioxidants, especially vitaminE should be encouraged and saturated fats should be avoided in pregnancy, especially in multiparous women. This will play a role in reducing maternal morbidity and mortality and in ensuring safe motherhood.

References

Anetor J.I, Adelaja O, Adekunle A.O. (2003) Serum Micronutrient level, nucleic acid metabolism and antioxidant defences in pregnant Nigerians: implications for foetal and maternal health. Afri. J Med. Sci. 32:257 -263.

Bai J, Wang F.W, Bauman A, Mohsin M (2002:) Parity and Pregnancy Outcomes. Am. J. Obstetric and Gyneacology 186 (2): 274 – 8.

Barry Halliwell (2009). The Wanderings of a free radical (Review article). Free Radical Biology and Medicine, 46: 531 – 542.

Buamah P.H, Rusell M, Wilford – Ward A, Taylor P., Robberts D.F (1984): Serum Copper concentration significantly less in abnormal pregnancies. Clin. Chem. 30: 1667 – 1670.

Halliwell Barry and Gutteridge J.M.C (1994): Antioxidants in nutrition, health and disease, Pg. 26, Oxford University Press Oxford.

Huda E. Tawfik, Jonathan Cena, Richard Schulz & Suzan Kaufman (2008) Role of Oxidative stress in multiparty – Induceed endothelial dysfunction. American Journal of Physiology Heart Circulatory physiology. AJP – Heart (2008) Vol. 295 No. 4. H1736 – H1742.

Kashinakunti S.V., Sunitha H, Gurupappa K., Shankarprasad D.S., Suryaprakash G., and Ingin J.B. (2010). Lipid peroxidation and Antioxidant status in preeclampsia. Al Ameen J. Med. Sci. 3 (1): 38 - 41.

Kojic G., Vlahonics P., and Ravlonre O (1998). The possible importance of the binding site for the oxidative modification of liver nucleosidase. Arch. Physiol. Biochem; 106 : 91 – 99.

Mirjana Bogavac, Neda Lakic, Natasasimin, Aleksandra Nikolic, Jan Sudji, Biljana Bozin (2011) Biomarkers of Oxidative stress in amniotic fluid and complications in pregnancy. Journal of Maternal – Fetal and Neonatalmedicine. Doi: 10.3109/14767058.

Moris J. .M, Gopaul N.K, Endresen M.J, Knight M, Linton E.A, Dhir S, Anggard E.E, Redman C.W (1999): Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclempsia. Br. J. Obstet. Gynaecol 106 (7): 751.

Murray R.K. (1990). Biochemistry and disease. Happers Biochemistry, Murray R.K, Granner D. K, Mayes P.A and Rodwell. V.W(eds). (Appleton and Lange, Connecticut). PP 852 - 863.

Odutuga A.A. & Amballi A.A (2007). Lipodystrophic and Lipo-peroxidative effects of Hydrogenated coconut oil diet in Rats. Pakistan Journal of Nutrition 6(6): pp 593 – 596.

Osman K, Akesson A, Berlund M, Bremme K, Schutz A, Ask K, Vahter M (2000). Toxic and essential elements in placentas of Sweddish women. Chm. Biochem; 33(2): 131 – 8.

Sembulingam K and prema Sembulingam (2000) Essentials of Medical Physiology. 2nd Edt. Publisher Jaypee Brothers, New Delhi.

Sharma, J.B; Sharma A; Bahadur, A., Vimala, N; Satyam, A $ Mittal S(2006). Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. International Journal of Gynaecology and Obsterics 94: 23 – 27.

Spattling L., Fallen Stein F, Huch R and Rooth G. (1992). The variability of cardiopulmonary adaptation to pregnancy at rest and during exercise. Bitish Journal of Obst. And Gynae 99: 1 – 40.

Stocker R & Frei. B (1991). Endogenous antioxidant defences in human blood plasma. London: Academic press, pg 212 – 213.

Tiyong Ifoue S.H., Teugwa mofor C., Gouado I., Teto G., Asonganyi T. and Amvam Zollo P.H. (2009). Evaluation of oxidative stress and antioxidant status of pregnant women suffering from malaria in Cameroon. Indian Journal of Clinical Biochemistry, 24 (3) 288 – 293.

Walsh S.W and Wang Y (1993). Deficient glutathione peroxidase activity in preeclampsia is associated with increased placental production of thromboxane and lipid peroxides. Am. J. of Obst. And Gynae; 169: 1456 – 1461.

Weighand , M.A., Laipple. A. and Plascke, K. (1999). Concentration changes of malondialdehyde across the cerebral vascular bed and shedding of L-selectin during carolide endarterectomy. Stroke, 30: 306 – 311.

Young I.S. and Woodside J.V. (2001). Antioxidants in health and disease. J. Clin. Pathol; 54: 176 – 186.