



# **Original article**

# Status of some selected antioxidants in pregnant Nigerian women

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## ABSTRACT

It has been a known fact that normal pregnancy is associated with high metabolic demand accompany by elevated tissue oxygen requirements and increased oxidative stress. The question is, does this suggest that during pregnancy the antioxidant defense mechanism is defenseless? This study therefore investigated the status of some selected antioxidants in pregnant Nigerian women. The study comprised of 200 apparently healthy Nigerian women between the ages of 20 and 40, among which 180 were pregnant (test group) and 20 were non pregnant (control group). Test group was divided into three subgroups which comprised of 60 each representing the three trimesters of pregnancy. Whole blood was collected from each subject and the selected antioxidants (Catalase, Vitamin C and Vitamin E) levels were analyzed using standard laboratory method. Results showed overall reduction in antioxidants concentration which is not significant in Catalase but significant in Vitamin C and E. Also, this reduction was observed to be more with advancing age, gestation period, increasing parity and amongst civil servants. Conclusively, pregnancy appears to impinge on the balance between the formation of oxidants and the antioxidant defense mechanism. It is therefore recommended that during pregnancy, women should improve on their antioxidants intake.

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#### 1. Introduction

Pregnancy is regarded as a condition which is usually accompanied by oxidative stress (Chen and Scholl, 2005; Arikan et al., 2001; Idonije et al., 2011). This is reported to be due to the physiological changes that occur during pregnancy coupled with the increased basal oxygen intake and consumption as well as elevated metabolic demand, finally leading to the overproduction of reactive oxygen species and decreased activity of antioxidant enzymes (Casanueva and Viteri, 2003; Walsh and Wang, 1993). Under normal physiological conditions there is a balance between the formation of free radicals and the antioxidant defense system (Fehér, 1985, Kehrer 1993). Furthermore, according to Agarwal et al., (2005) scavenging molecules known as antioxidants convert reactive oxygen species (ROS) to water (H<sub>2</sub>O) to prevent overproduction of ROS which are normally produced. However, previous study on pregnant Nigerian women shows pregnancy to be accompany by oxidative stress, which is worsen as age advances, in inactive women and multi-parous individual (Idonije et al., 2011). On this regard, it becomes reasonable to solicit if the reported oxidative stress noted during pregnancy implies that during pregnancy? This present study was therefore designed to determine the levels of some selected antioxidants in pregnant Nigeria women.

#### 2. Materials and methods

#### 2.1. Subjects

A total of two hundred (200) subjects between the ages of 20 and 40 years formed the study population. Group 1; the control comprises of twenty (20) apparently healthy non pregnant volunteers of Nigerian origin. Group 2; the test group; involved 180 apparently healthy pregnant Nigerian women, sub- divided into three subgroups; each made of sixty (60) subjects representing 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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, 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 and other morbid conditions.

## 2.2. Sample collection and analysis

5ml 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 5000rpm for 5 minutes and the plasma removed and stored at 4  $^{\circ}$ C pending assay of the selected antioxidants.

Catalase activity was determined spectrophotometrically by the method of Aebi (1984). Ascorbic acid (vitamin C) levels were estimated as described by Beulter (1988) and  $\alpha$ -Tocopherol (vitamin E) was measured by high-performance liquid chromatography (HPLC) as per the modified method of Omu et al., (1999).

## 2.3. 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.

# 3. Results

Table 1 showed the antioxidant levels of pregnant Nigeria women in relation to socio-demographic characteristics. Results obtained from the present study showed that the concentration of virtually all the antioxidants studied reduced with increasing age and parity during pregnancy. No significant change was seen with catalase while significant changes were observed with Vit C and E. With respect to occupation, civil servants showed the lowest level of antioxidants (catalase, Vit C and Vit E) and were observed to be significant with vit C and E compared to other occupations and this is same in respect to parity.

Table 2 showed the mean values of antioxidants in pregnant women of Nigeria origin at different gestation compared with the controls. Mean serum levels of virtually all the antioxidants studied were reduced in pregnant women compared with the control. This observed reduction was gestational dependent, that is, as gestation period changes from 1<sup>st</sup>, 2<sup>nd</sup> to 3<sup>rd</sup>, antioxidants levels reduces. Catalase levels were not significantly reduced compared with the control. Vit C and Vit E showed significant reduction compared to the control. In term of gestation, significant reductions were seen in the 3<sup>rd</sup> trimester of pregnancy in Vit C and E compared to levels in 1<sup>st</sup> trimester. However, 2<sup>nd</sup> trimester value was not significantly different in Vit C compared to 1st and 3<sup>rd</sup> trimester levels. The results obtained for antioxidants vitamins showed that the levels of vit E was mostly effected in pregnant women compared to the changes observed with Vit C and catalase.

#### Table 1

Socio-demographic characteristics in relation to antioxidant level of pregnant Nigerian women.

Socio-demographic Characteristics	Ν	Antioxidants			
		Catalase (ug/ml)	Vit C (mg/dl)	Vit E (mg/dl)	
Age: 22 – 25	18	0.48 ± 0.05	$1.76 \pm 0.23^{a}$	$2.22 \pm 0.53^{\circ}$	
26 – 29	53	0.48 ± 0.03	$1.75 \pm 0.19^{a}$	$2.17 \pm 0.54^{a}$	
30 – 33	82	$0.46 \pm 0.04$	$1.69 \pm 0.19^{a}$	$1.83 \pm 0.26^{a}$	
34 – 37	27	0.47 ± 0.03	$1.67 \pm 0.17^{a}$	$2.03 \pm 0.52^{a}$	
Occupation: FHW	28	$0.47 \pm 0.04$	1.77 ± 0.22	$2.24 \pm 0.56^{a}$	
Traders	80	0.47 ± 0.03	1.73 ± 0.19	1.99 ± 0.45	
Civil servants	56	$0.46 \pm 0.04$	$1.65 \pm 0.19^{b}$	$1.84 \pm 0.26^{b}$	
Farmers	16	0.47 ± 0.03	1.75 ± 0.13	2.19 ± 0.62	
Parity: 1 <sup>st</sup> 2 <sup>nd</sup> 3 <sup>rd</sup>	14	0.48 ± 0.05	$1.81 \pm 0.20^{\circ}$	$2.34 \pm 0.55^{\circ}$	
$\geq 4^{\text{th}}$	49	0.47 ± 0.03	$1.77 \pm 0.20^{\circ}$	$2.09 \pm 0.51^{\circ}$	
	71	0.47 ± 0.03	$1.68 \pm 0.19^{\circ}$	$1.90 \pm 0.38^{\circ}$	
	46	$0.46 \pm 0.04$	$1.67 \pm 0.18^{\circ}$	$1.96 \pm 0.43^{\circ}$	

Values are mean ± standard deviation; Vit = Vitamin; FHW = full house wife; a=significant reduction with advancing age; b=significant reduction when compared with other occupation; c=significant reduction with increase in parity.

#### Table 2

Comparism of antioxidant level among non-pregnant and pregnant Nigerian women and the trimester of pregnancy.

Parameter	Control (n = 20)	Pregnancy (n = total 180)	Test group			
			1 <sup>st</sup> trimester (n = 60)	2 <sup>nd</sup> trimester (n = 60)	3 <sup>rd</sup> trimester (n = 60)	
Catalase	$0.48 \pm 0.08$	$0.47 \pm 0.04$	$0.48 \pm 0.04$	0.47 ± 0.03	0.46 ± 0.04	
Vit. C	$1.93 \pm 0.37^{a}$	$1.71 \pm 0.19^{b}$	$1.77 \pm 0.20^{b}$	$1.71 \pm 0.18^{bc}$	$1.66 \pm 1.18^{\circ}$	
Vit. E	$2.38 \pm 0.44^{a}$	$2.00 \pm 0.46^{b}$	$2.17 \pm 0.53^{a}$	$1.97 \pm 0.41^{bc}$	$1.86 \pm 0.38^{\circ}$	

Values are mean  $\pm$  standard deviation; vit = vitamin; Values in a row having different super scripts are significantly different (p < 0.05).

#### 4. Discussion

Antioxidants may be group into two, known as enzymatic and no-enzymatic (Van Langendonckt et al., 2002; Pierce et al., 2004). Enzymatic antioxidants (also known as natural antioxidants) are composed of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. They are known to causes reduction of hydrogen peroxide to water and alcohol, neutralize excessive ROS and prevent it from damaging the cellular structure (Agarwal et al., 2005). Non-enzymatic antioxidants (synthetic antioxidants or dietary supplements) are vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, taurine, hypotaurine, glutathione, beta carotene, and carotene (Van Langendonckt et al., 2002; Szczepanska et al., 2003; Agarwal et al., 2003; Pierce et al., 2004). The present study showed reduction in both enzymatic and no-enzymatic antioxidant during normal pregnancy among Nigerian women. Several other studies have reported similar cases of antioxidants reduction in normal pregnant among Nigeria women (Uchenna and Fidelis, 2005) and in other parts of the globe (Jonathan et al., 1998). Interestingly, our previous study on status of oxidative stress in pregnant Nigerian women clearly showed circulating levels of oxidative stress marker (thiobarbituric acid- reactive substances) to be significantly elevated in women with normal pregnancy and levels increased as the gestation period advances (Idonije et al., 2011). This may provide an explanation for the exhibited reduction in antioxidants concentrations in maternal circulation in the present study. This decrease in antioxidant concentration would no doubt propagate lipid peroxidation leading to the biological cascade of exceeding oxidative stress reported in our previous study (Idonije et al., 2011). Although reports showed that plasma free radical trapping and antioxidant potential are able to counteract oxidative stress in normal pregnancy through enzymatic induction and activity (catalase, SOD, glutathione peroxidase, transferase and reductase, glucose 6-phosphate dehydrogenase) as well as through nonenzymatic free radical protectors and scavengers (vitamins C and E, uric acid, protein thiols) (Yoshioka et al., 1990; Wang et al., 1991; Wang and Walsh, 1996; Watson et al., 1997; Poranen et al., 1998; Myatt and Miodovnik, 1999; Kharb, 2000). Pregnancy is a state where this adaptation and equilibrium are easily disrupted as evidenced by the propensity toward the development of gestational hypertension and insulin resistance that in some extreme cases can lead to gestational diabetes (Casanueva and Viteri, 2003). Thus, reconfirming pregnancy to alter physiological and metabolic functions and consequently, remarkable and dramatic events occur during this period in the woman's life. Although it has been shown that women with naturally higher levels of antioxidant enzymes are less likely to miscarry (Baxter et al., 2001), the impact of peri-conceptional antioxidant supplementation on early pregnancy failure rates in the general population remains to be investigated.

In addition, the present study showed maternal antioxidant concentration to be affected during pregnancy by socio demographic characteristics such as age, parity and occupation. The effect was observed to be poorer with advancing age, increasing parity and amongst civil servants. This was supported by our previous study from which we concluded that pregnancy is associated with oxidative stress which is horrifying as age and gestation advances, in multi-parity individuals and amongst physically inactivity women (Idonije et al., 2011). It is our finding also that as pregnancy advances, antioxidants become poorer. Report showed that micronutrients deficiencies such as vitamin A, zinc, iron, calcium etc are more common in pregnant women in developing countries (Stephenson et al., 2000). These deficiencies may contribute to an increased risk of parasite infection such as malaria (Mahomed, 2000) and also the reported status of oxidative stress. Considering the detrimental effects of ROS reported in several studies, one would expect a negative correlation between levels of oxidative stress and total antioxidant capacity in pregnancy; a finding of this study. Vitamin C has been shown to take part in bone formation, folic acid metabolism, formation and maturation of red blood cells and immune response mechanisms (Garba et al., 2003). Deficiency of vitamin C may affect immune system as well as causing anaemia which are involved in resistance to malaria. This is supported by a clinical trial in women at risk of pre-eclampsia which has shown that antioxidant vitamin C and E supplementation during the second trimester of pregnancy improves biochemical incidences of oxidative stress and decreases the occurrence of the clinical disease in high-risk women (Chappell et al., 1999, 2002b).

Conclusively, we recommend dietary supplementation of antioxidants during pregnancy considering the observed findings of this study. This however is in accordance with several findings reported in literature. However, the significant importance of caution in therapeutic antioxidants vitamins supplementation cannot be overemphasized considering dosage toxicity during pregnancy. Evidence accumulates that the quality of nutrient supply to pregnant women affects maternal health and well-being, pregnancy outcome, the rate of complications and fetal growth

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