Effect of Pregnancy on Serum Levels of Selected Antioxidant Vitamins and Minerals

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Abstract

Pregnancy places increased demand of adequate nutrients for the growing conceptus. Oxidative stress generated during normal pregnancy usually increases with decreased supply of antioxidant vitamins and minerals, resulting in adverse pregnancy outcome. In this study, the levels of antioxidant vitamins and minerals during pregnancy were evaluated. A total of 120 women of puberty age comprising 60 pregnant women attending antenatal care (ANC), Usmanu Danfodiyo University Teaching Hospital (UDUTH) and 60 apparently healthy non-pregnant women were recruited for this study. Serum levels of vitamin A, vitamin C and vitamin E were determined spectrophotometrically. The concentrations of zinc and copper were determined colorimetrically while BMI was evaluated using standard method. Results showed that serum levels of vitamin A, vitamin C and vitamin E in pregnant women (2.28±0.22, 44.67±3.09 and 17.94±1.5 respectively) were significantly lower (P<0.05) than their non-pregnant counterparts (5.33±0.36, 84.06±5.27 and 25.33±1.48 respectively). Our result further showed that the serum concentrations of zinc and copper in pregnant women (10.00±44 and 10.28±0.44 respectively) were significantly lower (p<0.05) (15.16±0.34 and 22.21±1.45 respectively) compared to the non-pregnant women. However, BMI were significantly higher (p<0.05) in pregnant women (28.76±0.08) compared to non-pregnant control (22.45±o.47). These findings suggest altered levels of vitamins and minerals during pregnancy. Therefore, supplement or food fortification will be a highly effective strategy to correct any adverse pregnancy outcome as a result of their deficiencies.

Keywords: Pregnant women, Oxidative stress, Antioxidant vitamins, Minerals

1.0 Introduction

Pregnancy is a stressful condition in which many physiological and metabolic functions are substantially altered. Consequently, remarkable and dramatic events occur during this period for sustaining mother and fostering the growth and maintenance of foetus [1]. It usually lasts around 40 weeks from the last menstrual period and normally ends in childbirth [2]. Reports showed that normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen which results in increased oxidative stress and antioxidant defences [3]. Adequate nutrition during pregnancy is important arising from increased energy and specific micronutrient requirements to ensure healthy growth of the foetus [4].

Antioxidants are substances that significantly delay or inhibit oxidation of a substrate, and protect cells of the body against free radicals. Antioxidants interact with and stabilize free radicals, thus preventing cellular damage [5]. Free radicals are atoms or molecules containing one or more unpaired electrons, making them very reactive [6]. Free radicals containing oxygen are called Reactive Oxygen Species (ROS). Free radicals are highly unstable because they have one or more unpaired electrons. They abstract one or two electrons or donate electrons, thereby damaging cells, proteins and DNA [7]. In healthy individuals, ROS remain in balanced state. However, when balance is disrupted towards an overabundance of ROS, oxidative stress occurs.

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Oxidative stress is a state of imbalance between generation of ROS and the level of antioxidant defence system [8]. Oxidative stress play important role during pregnancy, normal parturition and in initiation of preterm birth. Effects can be seen in pathophysiology of pre-eclampsia and birth defects [9]. The consequences of reduced antioxidant system in pregnancy include among others, reduced placental efficiency and calcification [9]. It could also be a cause of foetal malfunction, pregnancy complications such as pre-eclampsia, eclampsia as well as obstructive airway disease [10]. The aim of the study is to evaluate the level of serum antioxidant vitamins and minerals in pregnant women attending Usmanu Danfodiyo University Teaching Hospital.

2.0 Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents for this study are of analytical grade and purchased from Randox Company Ltd. They include distilled water, absolute ethanol, heptane, 12 M sulphuric acid, ferric chloride, xylene, 2,4-dinitro phenyl hydrazine, metaphosphoric acid thiourea, α-diprydyl, copper reagent and zinc reagent.

2.2 Study Population

A total of one hundred and twenty (120) subjects were recruited for this study. They consist of sixty (60) apparently healthy non-pregnant women as control and sixty (60) pregnant women attending antenatal care (ANC), Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria.

2.3 Ethical Approval

The approval of the study was sought and granted from the relevant Ethical Committee before the commencement of the study. The ethical clearance number of the study is UDUTH/HREC/2016/448.

2.4 Sampling Techniques

Arrangement was made with the clinicians where those that satisfied the study inclusion criteria were selected. The nature and reasons for the study was explained fully to the subjects using an appropriate language. Subjects consent was a priority and was obtained with their full history. Specimen collection was made and findings were documented in the proforma.

2.5 Anthropometric Measurements

The procedure of Bashar [11] was employed in the anthropometric measurements. Body Mass Index (BMI) was determined using the weight in kilogram (kg) divided by the square of the height in meters, measured using vernier calliper.

2.6 Analytical Methods

Serum level of vitamin A was estimated using the method of Bessey *et al*. [12]. Serum Vitamin C was determined spectrophotometrically according to Natelson [13], while Serum Vitamin E was estimated by the method of Hashim and Schuttringer [14]. Serum Zinc and Copper was estimated colorimetrically.

2.7 Statistical Analysis

The data obtained were analyzed using Statistical Package for social science (SPSS VERSION 20). Serum levels of antioxidant vitamins, Zinc and Copper obtained from the subjects were compared using the two tailed Student's t-test for matched samples. One way ANOVA was used for multiple comparisons. Correlations of anthropometric parameter with antioxidant vitamins, zinc and copper were carried out using Pearson's linear correlation analysis. The results generated were expressed as Mean ±Standard error of mean. A p-value at 5% ($p<0.05$) was considered as statistically significant.

3.0 Results

Socio-demographic characteristics of the study subjects are presented in Table 1 consisting of 60 pregnant women and 60 controls with the age range of 16-40 years. The distribution of pregnant women and controls by tribe shows that majority are Hausa/Fulani, 44(67.7%). The distribution of pregnant women based on educational status revealed that 28(43.1%) had tertiary education, 31(47.7%) had secondary, 5(7.7%) had primary education and 1(1.5%) had no formal education as compared with control where 48(73.8%) had tertiary education and 17(26.2%) had secondary education. With respect to occupation among the pregnant women, 13(20%) were civil servant, 13 (20%) engaged in business, 4(6.2%) were petty traders, 11(16.9%) were students, 23 (35.4%) were unemployed and only 1 (1.5%) was a farmer. For the non-pregnant controls, 10(15.4%) were civil servant, 11 (16.1%) were business women, 19 (29.2%) were students and 25(38.5%) were unemployed.

Table 2 shows anthropometric parameters among pregnant women and non pregnant women. The result indicated that BMI were significantly high ($p<0.05$) in pregnant women than in control. Table 3 presents the serum levels of antioxidant vitamins and some minerals in pregnant women and controls. The result showed a significant reduction ($p<0.05$) in serum levels of antioxidant vitamins and minerals in pregnant women when compared to control.

Table 1: Socio-demographic characteristics of pregnant women and non-pregnant controls

Variables	Pregnant women N=60	Percentage %	Controls N=60	Percentage %
Age				
16-25	25	41.66	28	46.66
26-40	35	58.33	32	53.33
Tribe				
Hausa/Fulani	41	68.33	40	66.66
Yoruba	6	10.00	12	20.00
Igbo	7	11.66	5	8.33
Others	6	10.00	3	5.00
Education				
Tertiary	25	41.66	45	75.00
Secondary	29	48.33	15	25.00
Primary	5	8.33	0	0
No formal	1	1.66	0	0
Occupation				
Civil servant	13	21.66	10	16.66
Business	10	16.66	9	15.00
Farmer	1	1.66	0	0
Petty trader	4	6.66	0	0
Student	11	18.33	19	31.66
Unemployed	21	35.00	22	36.66

Table 2: Anthropometric parameters (Mean±SEM) among pregnant women and non-pregnant controls

Parameters	N	Weight (kg)	Height (m^2)	BMI (kg/m^2)
Pregnant women	60	72.00±2.25	1.58 ± 0.007	28.76 ± 0.80
Controls	60	55.74 ± 1.11	1.81 ± 0.23	22.45 ± 0.47
p-value		<0.001	>0.05	<0.05

Table 3: Levels of antioxidant vitamins and some minerals (Mean ±SEM) in the serum of pregnant women and non-pregnant controls

Parameters	Vitamin A (μmol/l)	Vitamin C (μmol/l)	Vitamin E (μmol/l)	Copper (μmol/l)	Zinc (μmol/l)
Pregnant women	2.28±0.22	44.67±3.09	17.94±1.54	10.00±0.44	10.28±0.44
Controls	5.33±0.36	84.067±5.27	25.33±1.48	15.16±0.34	22.21±1.43
p-value	<0.001	<0.05	<0.001	<0.001	<0.05

The levels of antioxidant vitamins and some minerals in different stages of pregnancy are presented in Table 4. The data indicated a significant decrease in the serum levels of vitamin C, Copper and Zinc ($p<0.05$) as pregnancy progresses. However, the levels of vitamin A and Vitamin E significantly reduced ($p<0.05$) in first trimester and later increased by the second and third trimester.

Table 4: Levels of antioxidant vitamins and some minerals in the serum (Mean±SEM) of pregnant women at various stages of pregnancy

Parameters	Vitamin A (μmol/l)	Vitamin C (μmol/l)	Vitamin E (μmol/l)	Copper (μmol/l)	Zinc (μmol/l)
1st trimester	1.25±0.35	50.22±10.79	13.66±1.48	10.62±0.99	11.27±3.47
2nd trimester	2.95±0.34	45.77 ±4.92	17.52 ±2.82	9.94±0.66	10.77±1.25
3rd trimester	1.66±0.28	43.29±4.48	17.42±2.13	9.56±0.83	9.86±1.10
Controls	5 .33±0.36	84.06±5.27	25.33±1.48	15.16±0.34	22.21±1.43
Post Hoc Test					
Contol/ 1st	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
Control/2nd	P<0.05	P<0.05		P<0.05	P<0.05
Control/ 3rd	P<0.05	P<0.05		P<0.05	P<0.05

4.0 Discussion

Human pregnancy is associated with increased requirement for nutrients as a result of an elevated metabolic rate due to increased oxygen consumption and subsequent utilization [15]. The accelerated oxygen intake has been reported to cause oxidative stress via production of highly toxic reactive oxygen species [16]. Result of this study showed a significant reduction in serum antioxidant concentrations of vitamins A, C and E in pregnant women. This is in agreement with work of Patil *et al.* [17], where serum antioxidant levels of vitamins A, C and E were found to be significantly lowered in pregnancy. Other studies also reported decreased levels of alpha tocopherol and retinol in healthy pregnant women as compared to controls [18, 19]. The decreased level could be as a result of hemodilution in pregnancy, inadequate intake and increased oxidative stress among pregnant women [20]. The present study also revealed that vitamin C was significantly reduced in the pregnant women. This is in agreement with the work of Rao *et al.* [16], which showed a statistically significant decrease in levels of vitamin C in normal pregnancy. According to the study of Roes *et al.* [21] a reduction in the concentration of vitamin C was reported in uncomplicated pregnancy.

Regarding serum copper and zinc, the significantly reduced level of these minerals in pregnant women agrees with previous studies. Meram *et al.* [22] reported decreased plasma zinc concentration in pregnancy. Several other studies have also reported maternal plasma zinc reduction during pregnancy from the 24th to 33rd week of gestation [23-25]. Contrary to this study, a significantly higher serum level of copper has been reported in healthy pregnant Nigerian women than their non pregnant counterparts [26]. Similarly, increased in serum copper has been reported in Spanish and Turkish pregnant women [27]. The reduction in copper level may be due to iron supplement in pregnant women (with hemoglobin greater than 13.2 g/dL) which significantly reduced serum copper concentrations by the second and third trimesters [28].

From the present study, it was further observed that the anthropometric parameters in pregnancy were significantly higher than the controls. Similar finding was reported by Ukegbu *et al.* [29] in Abia, Nigeria. Contrary to this work, Okwu *et al*. [30] reported that pregnant women aged 24 years and below had significantly lower BMI in rural areas. The present study reported a reduction in serum vitamins A and E concentration in the first trimester and an increase in second and third trimester. This was also observed in previous studies [31, 32]. It has been well documented that severe copper deficiency can lead to reproduction failure and early embryonic death [33], whereas mild or moderate deficiency has little effect on either the number of live births or neonatal weight [34]. Alteration in zinc homeostasis may have devastating effects on pregnancy outcome, including prolonged labour, fatal growth restriction, or embryonic or fetal death [35]. Zinc deficiency has also been associated with preeclampsia [36].

Avitaminosis A in pregnancy has been shown to increase the risk of maternal mortality, night blindness [37], premature birth, intrauterine growth retardation, low birth weight and antepartum hemorrhage [38] due to abruption placentae. Vitamin A deficiency is also known to reduce leukocyte numbers, lymphoid tissue weights, complement, T-cells functions, tumor resistance, natural killer cell number, antigen-specific IgG and IgE [39]. Low serum concentration of vitamin E has been associated with abruption placentae in normal pregnancies [38]. Vitamin E is needed for fetal growth and recommends that intakes be increased by 25% in pregnancy [40]. However, avitaminosis E in pregnancy is rare except in malabsorption syndrome [40]. During pregnancy, serum vitamin C progressively decreases to about 50%, partly because of the extra uptake by the fetus and partly because of hemodilation [41]. Vitamin C requirement in pregnant women is 67% higher than that of non-pregnant, non-lactating women, to offset the losses from the mother's body pool [42].

In conclusion, this study suggested altered vitamins and minerals in pregnancy. Hence supplements or food fortification could be a highly effective strategy to correct any adverse pregnancy outcome as a result of their deficiencies. Future strategies focusing on providing nutritional guidance specifically in pregnancy will also be pivotal in helping to ensure optimal health of both mothers and fetus.

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