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Estimation of Ascorbic Acid Status in Normal Pregnancy

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Abstract: The study is to measure the serum concentration of ascorbic acid in pregnant women during all the trimesters. *Methods*: Total serum concentration was measured using the 2,4-Dinitrophenylhydrazine method, which involves the conversion of vitamin C to dehydroascorbic acid in the presence of copper ions and subsequent measurement of the resulting bishydrazone at 540nm. Results revealed that–total vitamin C concentration in all three trimesters values are significantly lower. In *Conclusion*: Serum vitamin C concentration were observed to be low in pregnant women in all the three trimesters when compared with non-pregnant women.

Key words: Ascorbic acid · Vitamin C · 2,4-Dinitrophenylhydrazine method

INTRODUCTION

Pregnancy is a memorable period in the life of a woman is subjected to a lot of nutritional, metabolic, hormonal and physiological demands and regulations. Nutritionally she requires enhanced inputs with respect to calories and macro and micro nutrients. As the metabolic activities are triggered the demand of oxygen is also increased and oxidation of reducing equivalents in electron transport chain (ETC) is enhanced. Various redox systems of electron transport chain and other redox systems are active. In this process, there is a like hood of developing reactive oxygen species as free radicals.

During pregnancy the demand for energy and oxygen requirement is high. This leads to intake and utilization of oxygen resulting in increased levels of oxidative stress and the acceleration in the production of reactive oxygen species. An imbalance between reactive oxygen species and anti-oxidants defence mechanisms of a cell leads to an excessive production of oxygen metabolites creating a condition known as oxidative stress.

The study aimed measure the serum concentration of total vitamin C as an antioxidant in the three trimesters of pregnancy considering the important role of this vitamin in the scavenging of reactive oxygen species and maintenance of healthy pregnancy, as vitamin C is considered as a strong antioxidant.

This work aimed to study the ascorbic acid status as one of the redox systems, playing a role as antioxidant in normal pregnancy.

MATERIALS AND METHODS

Total serum vitamin C (L-ascorbic acid) concentration was measured in 75 pregnant women with 25 number in each trimester. They were in the age group of 18-35 years. Age matched non-pregnant women were included as control group .The study had been conducted over a period of 9 months.

Institutional ethical committee clearance was obtained. Informed consent were obtained from all the participants.

Exclusion criteria included persons with diabetes mellitus, pregnancy toxemia, hypertension, asthma, renal disease and cancer.

The family and personal history data of Diabetes, Jaundice, hypertension and miscarriages were collected through a standard questionnaire.

Blood samples were collected. The heparinised blood samples were separated by centrifugation and plasma is used for the estimation of vitamin C. The same procedure of sample collection and estimation of vitamin C was adopted for control subjects.

Total serum vitamin C concentration was measured using the 2, 4-dinitrophenylhydrazine method [1].

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Assay for serum vitamin C. Serum L-ascorbic acid was assayed using the 2, 4-dinitrophenyhydrazine method which measures total vitamin C concentration by oxidizing all vitamin C to dehydroascorbic acid in the presence of copper ions and subsequent measurement of the dehydroascorbic acid-bis-hydrazone derivative at 540nm.

RESULTS

The mean serum concentrations of vitamin C in all the three trimesters were lower than the control. Average total vitamin C concentration in the first trimester was 20 % less than the control vitamin C concentration of 3.15±0.13 mg/dl, (p <0.05).

Total vitamin C concentration in the second trimester was 26 % less than the control concentration (p <0.05). The largest decrease was found in the third trimester, with a 75% drop relative to the control serum total vitamin C concentration (p <0.05) as shown in Table 1.

Table 1: Total serum vitamin C concentration in the three trimesters of pregnancy (mg/dl)

1.	First trimester	2.55 ± 0.82
2.	Second trimester	2.32± 0.40
3.	Third trimester	0.77±0.10
4.	Control	3.15±0.13

p<0.05 (comparisons with control)

DISCUSSION

As noted earlier, pregnancy is associated with increased requirement for dietary energy as a result of an elevated metabolic rate [2] due chiefly to increased oxygen consumption and subsequent utilization. The accelerated oxygen intake has been shown to lead to increased levels of oxidative stress via production of highly toxic reactive oxygen species [3]. If left unchecked, reactive oxygen species inflict a constant barrage of oxidative damage to DNA, proteins and lipids [4-6] .Evidence supporting this excessive reactive oxygen species production and accompanying oxidative stress during pregnancy include studies reporting low superoxide dismutase activity, low α-tocopherol and increased serum concentration of products of lipid peroxidation such as thiobarbituric acid reactive substances (TBARS) [7-9]. The progressive decline in serum vitamin C concentration observed in this study could indicate an increased utilization of the vitamin C by pregnant women to maintain normal reactive oxygen species homeostasis, as supported by reports of reduced levels of antioxidant nutrients in pregnancy. Since the lowest serum vitamin C concentration was found in the third trimester, it indicates that oxidative stress is highest during this trimester of pregnancy. This therefore underscores the need for vitamin C supplementations throughout the entire period of pregnancy since mean serum vitamin C concentration in all the three trimesters are significantly lower than the non-pregnant control values. It also corroborates the clinical evidence for the unequivocal benefits of antioxidant supplementation to protect against excessive reactive oxygen species during pregnancy [10]. Vitamin C supplementation is particularly important in pregnant women as its deficiency has been shown to affect placental structure and facilitates placental infection both of which results in increased risk of premature rupture of placental membranes and premature births [2, 11]. In addition the supplementation could help to prevent the development of such complications of pregnancy like gestational hypertension, intrauterine growth retardation and gestational diabetes, all known to be associated with high levels of oxidative stress, in addition to the numerous other benefits of this vitamin in human metabolism and health [12, 13].

CONCLUSION

Serum vitamin C concentration were observed to be low in pregnant women in all the three trimesters when compared with non-pregnant women. Therefore supplementation of vitamin C during pregnancy should be insisted inorder to avoid the complications associated with suboptimal vitamin C levels during pregnancy.

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