Postnatal changes in maternal serum antioxidant vitamins
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Citation

Abstract
Objective: Aim of this study was to investigate the change in serum concentrations of antioxidant vitamins in postnatal mothers with respect to those in prenatal prenatal mothers.
Methods: The study comprised 39 healthy postnatal mothers, who delivered their babies at the Dhaka Medical College Hospital, Dhaka. Reversed phase HPLC and spectrophotometric methods were employed to determine the serum concentrations of vitamin E, A, and C. SPSS software package was used to analyze the data.
Results: The postnatal serum concentrations of vitamin E, C, and A were 15.30±5.04µmol/L, 18.41±10.76µmol/L and 0.87±0.35µmol/L respectively. Compared to the prenatal vitamins levels, a significant decrease in vitamin E concentration (15.30±5.04 vs 23.54±8.5µmol/L, p=0.000), and increase in vitamin C and A (18.41±10.76 vs 14.0±8.2µmol/L, p=0.000; 0.87±0.35 vs 0.85±0.24µmol/L, p=0.015) were obtained in the postnatal mothers. It was noted that the majority of postnatal mothers had vitamin E and A value in the middle and high range. It was in lower range for vitamin C.
Conclusion: Compared to the prenatal level, serum vitamin C and A levels were observed increase, while vitamin E level was lowered significantly (p<0.05) in postnatal mother. Report on postnatal antioxidant vitamins is scanty. However, this is by far the second report on the postnatal maternal serum antioxidant vitamins.

INTRODUCTION
The antioxidant vitamins act as the first line of defense against free radicals attack and lipid peroxidation. Vitamin E is by far the most abundant lipid soluble antioxidant in humans and belongs to the first line of antioxidant defence against lipid soluble peroxyl radicals (1, 2). Vitamin C is a quencher of free radicals as well as singlet oxygen. It also regenerates the vitamin E. Beta-carotene, by quenching singlet oxygen, also functions as an antioxidant. The antioxidant vitamins have been reported to play an important role in the regulation and eventual outcome of human pregnancy (3). It has been suggested that deficiency in antioxidant vitamins would be associated with the development of pregnancy complications like pre-eclampsia, eclampsia (4, 5). There has been a report on changes of antioxidant vitamins and malondialdehyde in postnatal maternal and neonatal serum as compared to those in the serum at delivery or in cord blood (6). We report here the serum concentrations of antioxidant vitamins (E, C, A) in postnatal mothers, which were compared changes in the maternal serum antioxidant vitamin concentrations.

MATERIALS AND METHODS
STUDY POPULATION
The study included 39 healthy postnatal mothers, who delivered their babies at the Dhaka Medical College Hospital, Dhaka. They were selected randomly irrespective of their socioeconomic status. Women with any pregnancy related or other long standing illnesses that could affect their nutritional status and/or women taking antioxidant vitamin supplements were excluded from the study. Prior informed consent was obtained from the participating mothers. Ethical permission was taken from Bangladesh Medical Research Council (BMRC), Dhaka. Serum antioxidant vitamin levels of postnatal mothers were compared to those of prenatal mothers. The prenatal data used were obtained from our earlier study of 35 healthy pregnant in their late trimester (7).

SERUM ANALYSIS
A 5ml venous blood sample was collected from the postnatal mothers on the second and third day of delivery in specimen tubes. Blood samples were spun at 3000rpm for 10min to serum, which was then aliquoted in eppendorf tubes and stored at −20°C for analysis of l-tocopherol and retinol.
Analysis of retinol and \( \beta \)-tocopherol. Reversed phase HPLC (LC-10AD, Shimadzu, HPLC 1991, Model-7125, Kyoto, Japan) was used for simultaneous determination of serum retinol and \( \beta \)-tocopherol concentrations as described by Islam et al (8). The retinol and \( \beta \)-tocopherol were isolated from the serum by liquid-liquid extraction using n-hexane (Merck, Germany), concentrated by evaporation under nitrogen stream and reconstituted with HPLC grade ethanol (Merck, Germany). The reconstituted sample (50µl) was injected into the chromatography on a C\(_{18}\) shim pack CLC-ODS (M) column of diameter 4.6mm (Shimadzu, Japan) with methanol:water (95:5) mobile phase. Retinol and \( \beta \)-tocopherol were detected spectrophotometrically at 291nm. The column was re-equilibrated with the mobile phase for 5min before injection of the next sample. To check the reproducibility, some samples were often injected consequently twice, and to verify the assay accuracy, standard analytes (Sigma Chemical Co, USA) were injected for every 15-20 test samples.

Analysis of ascorbic acid. To analyse serum ascorbic acid, extracted serum was immediately treated with 5% trichloroacetic acid (TCA) and centrifuged at 3000rpm for 10min. Clear supernatant thus obtained was stored at –20°C for analysis. The concentration of ascorbic acid in the serum was determined by spectrophotometric method using phenyl hydrazine indicator (Sigma Chemical Co, USA) as described by Islam et al (8). Absorbance was measured against a reagent blank at 520nm by a Spectrophotometer (UV-1201, UV-VIS, Shimadzu, Kyoto, Japan).

**STATISTICAL ANALYSIS**

SPSS software package (version 10.0, SPSS Inc. Chicago, USA) was used to analyse the data. Descriptive statistics were calculated for all variables. Values were expressed as percentage and mean±SD. Comparison of serum vitamin E, C and A concentrations between subjects and controls were performed by cross table variables and independent sample t-test.

**RESULTS**

Table 1 shows the postnatal maternal serum concentrations of vitamin E, C, and A, which were 15.30±5.04µmol/L, 18.41±10.76µmol/L and 0.87±0.35µmol/L respectively. The postnatal change in serum levels of vitamins E, C and A with respect to those in prenatal values are shown in table 2. Compared to the prenatal mothers, there was a significant decrease in serum vitamin E concentration (15.30±5.04 vs23.54±8.5µmol/L, p=0.000), and increase in vitamin C (18.41±10.76 vs 14.0±8.2µmol/L, p=0.000) and in vitamin A (0.87±0.35 vs 0.85±0.24µmol/L, p=0.015) in the postnatal mothers. It was noted that the majority of postnatal mothers had vitamin E and A value in the middle and top range, while the vitamin C was in lower range (table 1).

**DISCUSSION**

To our knowledge, this is by far the second report on the postnatal serum levels of antioxidant vitamins. Bolisetty et al (6) first reported the postnatal changes in maternal plasma antioxidant vitamins, where increase of plasma levels of vitamins E, A and \( \beta \)-carotene in postnatal mothers were documented. Our finding on the rise of postnatal serum vitamin C and A level is consistent with the report of Bolisetty et al (6), but decrease of vitamin E level is conflicting. It is not clear to us. Vitamin C, the most effective antioxidant in human plasma, provides major defense against the diseases caused by oxidative stress (3, 9). Therefore, the rise of postnatal serum antioxidant vitamins is suggested to be due to withdrawal of oxidative stress.
induced during pregnancy ($\alpha_{10}$). In conclusion, it was observed that compared to the prenatal vitamin level, serum vitamin C and A levels were observed increase, while vitamin E level was lowered significantly in postnatal mothers. This is by far the second report on the postnatal maternal serum antioxidant vitamins.

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