Antioxidant Vitamin Status of Healthy Man, Woman and Pregnant Mother : a Baseline Study

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Abstract

A cohort of one hundred and fifteen subjects, comprising fifty man, thirty non-pregnant woman and thirty five pregnant mother, have been investigated for their serum α-tocopherol, ascorbic acid and retinol levels. HPLC was employed to analyse the serum α-tocopherol and retinol, and spectrophotometric method was used for estimation of serum ascorbic acid level. Mean serum α-tocopherol levels for men, women and pregnant mother were found to be 14.9 ± 3.2, 13.1 ± 5.96 and 22.5 ± 8.07 μmol/L respectively, while their serum ascorbic acid were found to be 31.6 ± 11.4, 30.7 ± 20.4 and 14.0 ± 8.5 μmol/L respectively. Their serum retinol levels were found 1.38 ± 0.33, 0.99 ± 0.20 and 0.85 ± 0.25 μmol/L respectively. Serum α-tocopherol for man (14.9 ± 3.2 μmol/L) was found significantly ($P < 0.001$) lower than that for pregnant mother (22.5±8.07 μmol/L), but the level compared well with that for non-pregnant women (13.1 ± 5.96 μmol/L). Serum ascorbic acid for man (31.6 ± 11.4 μmol/L) and non-pregnant woman (30.7 ± 20.4 μmol/L) were also found to be equivalent, but were significantly ($P < 0.001$) higher than that for pregnant mother (14.0 ± 8.5 μmol/L). Serum retinol level for man (1.38 ± 0.33 μmol/L) was found to be significantly higher than that for non-pregnant (0.99 ± 0.20 μmol/L, $P < 0.0001$) and pregnant mother (0.85 ± 0.25 μmol/L, $P<0.001$). Pregnant mother were found to have higher level of serum α-tocopherol, but lower levels of serum ascorbic acid and retinol as compared to the non-pregnant woman. The later two serum vitamins were observed to be higher for man as compared to those for pregnant mother. Amongst pregnant mother, 22.9%, 45.7% and 20.0% mothers were found to have respectively low serum levels of α-tocopherol, ascorbic acid and retinol.

Key Words : Antioxidant Vitamins, Men, Non-pregnant Women and Pregnant Mothers.

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Introduction

An antioxidant has the ability to stabilize highly reactive, potentially harmful free radicals, that are reactive oxygen intermediates (ROI) or reactive oxygen species (ROS). The destructive chain reaction initiated by free radicals can be broken or stopped by antioxidants. They act as first line defense against lipid peroxidation[1,2]. Antioxidant vitamin controls free radical production. Alpha-tocopherol, ascorbic acid and β-carotene (pro-vitamin A) as well as retinol are known to be powerful antioxidant nutrients[3-5]. Vitamin E (α-tocopherol), the major lipid-soluble antioxidant, is recognized to protect cells against lipid peroxidation. Vitamin C is a quencher of free radicals as well as singlet oxygen ant it can also regenerate the vitamin E. Beta-carotene, by quenching singlet oxygen, functions as an antioxidant. However, there is only a substantial report on the antioxidant function of vitamin A (retinol) itself[5].

The vitamin E, C and A are potent immunoenhancers[6-8] and play an important role in the maintenance of immunity[9-10]. As the antioxidant vitamins have important role in immune functions, they would be classified as immunonutrients. The antioxidant vitamin via their antioxidant potentiality acts as anti-aging agent. An insufficient intake of these vitamins produces immunonutritional deficiency resulting in oxidative stress to immunosuppression, and thus may increase the risk of some killer diseases including AIDS, cancers, cardio-vascular diseases[3,7,11-13]. Alcohol drinking and cigarette smoking also induce antioxidant deficiency and oxidative stress[14-17]. Dietary or therapeutic supplementation of antioxidant vitamins as nutraceuticuticals has been reported to protect cells or tissues against oxidative stress and improve immunity. As there is, so far, no data on vitamin E, C, and A status for the people of Bangladesh, this study has, therefore, attempted to focus this information for a sub-group of the population.

Subjects and Methods

Study population

The study included one hundred and fifteen healthy people comprising fifty men and sixty-five women. Amongst women thirty-five were pregnant mother and thirty were non-pregnant mother. It was conducted during July 1998 to October 1999. Pregnant mother, who were admitted into the Dhaka Medical College Hospital (DMCH) and Bangabandhu Sheikh Mujib Medical University (BSMMU) were recruited randomly and the healthy men were selected

preferably from patient attendants and community. The study population represented the general group of people having good health. None of the study subjects was smoker or alcoholic or was in antioxidant vitamin medication.

Serum analysis

A 5ml venous blood sample was collected from antecubital vein of each of the subjects. Blood sample was kept undisturbed for at least 60min and was then centrifuged at 3000rmp for 10min. Serum thus extracted was stored at -20^{0}C for analysis of retinol and α-tocopherol. Reversed phase HPLC (LC-10AD, SHIMADZU, HPLC 1991, Model-7125, Japan) was used for simultaneous determination of retinol and α-tocopherol in the sera as described by Bieri et al[18]. The analytes retinol and α-tocopherol were isolated from the serum by liquid-liquid extraction using n-hexane, concentrated by evaporation under nitrogen stream and reconstituted with HPLC grade ethanol. The reconstituted sample (50μl) was injected into HPLC instrument on a C_{18} shim pack CLC-ODS (M) column of diameter 4.6mm (Shimadzu, LC Column, 4.6x250mm, No. 1256168, Japan) with methanol-water (95:5) mobile phase. The column was re-equilibrated with the mobile phase for 5min before the next injection. Standard analytes were injected for every 25 test samples. Retinol and α-tocopherol were detected spectrophotometrically at 291nm. Standards (retinol, α-tocopherol, retinyl acetate and α-tocopheryl acetate) were purchased from Sigma Chemical Co, USA and solvents (HPLC grade) were from Merck (Darmstadt, Germany).

For ascorbic acid analysis, serum immediately after extraction was treated with 5% trichloroacetic acid (TCA) and then centrifuged at 3000rpm for 10min. Clear supernatant thus obtained was stored at -20^{o}C until analysis. The concentration of ascorbic acid in the serum was determined by spectrophotometric method using phenylhydrazine indicator as described by Lowry et al[19]. Absorbance was measured against a reagent blank at 520nm by a Spectrophotometer (UV-1201, UV-VIS, Shimadzu, Japan). Chemicals used in the analysis of serum ascorbic acid were purchased from Merck (Darmstadt, Germany) and Sigma Chemicals Co, USA.

Statistical analysis

SPSS software package (version 9.0) was used to analyze the data. Descriptive statistics were done for all variables. Values were expressed as percentage, mean and standard deviation. Comparison of serum vitamin E,

C and A concentrations between men and women, and non-pregnant women and pregnant mothers were performed by cross table variables and independent sample t-tests.

Results

Table 1 depicts the serum antioxidant vitamin levels for men, women and pregnant mothers. Serum α-tocopherol levels were found to be 14.9 ± 3.2, 13.1 ± 5.96 and 22.5 ± 8.07μmol/L for men, women and pregnant mothers respectively, while their serum ascorbic acid levels were found to be 31.6 ± 11.4, 30.7 ± 20.4 and 14.0 ± 8.5 μmol/L respectively. Serum retinol levels for men, women and pregnant mother were found to be 1.38 ± 0.33, 0.99 ± 0.2 and 0.85 ± 0.25 μmol/L respectively.

Table 1. Serum α-tocopherol, ascorbic acid and retinol levels of men, women and pregnant mothers

Serum antioxidant vitamin (μmol/L)	Healthy men (n = 50)		Non-pregnant women (n = 30)		Pregnant mother (n = 35)	
	%(n)	Mean ± SD	%(n)	Mean ± SD	%(n)	Mean ± SD
α-tocopherol						
<17.8	84.0(42)	14.9 ±3.20	80.0(24)	13.1±5.96	22.9(8)	22.5±8.07
17.8-26.7	16.0(8)		13.3(4)		51.4(18)	
> 26.7	0		6.7(2)		25.7(9)	
Ascorbic acid						
< 11.4	0	31.6±11.4	13.4(4)	30.7±20.4	45.7(16)	14.0±8.5
11.4-34.1	62.0(31)		53.3(16)		54.3(19)	
> 34.1	38.0(19)		33.3(10)		0	
Retinol						
< 0.70	0	1.38±0.33	3.3(1)	0.99±0.21	20.0(7)	0.85±0.25
0.70-1.75	84.0(42)		96.7(29)		80.0(28)	
>1.75	16.0(8)		0		0	

Human normal serum levels of α-tocopherol, ascorbic acid and retinol are 17.8-26.7μmol/L[20], 11.4-34.1μmol/L[21] and 0.70-1.75μmol/L[22] respectively.

Serum α-tocopherol for man (14.9±3.2 μmol/L) and non-pregnant women (13.1±5.96 μmol/L) were found equivalent but both the levels were significantly (P<0.001) lower than that for pregnant mother (22.5±8.07 μmol/L) (table 2, 3). Serum ascorbic acid for man (31.6±11.4 μmol/L) also compared well with that for non-pregnant woman (30.7±20.4 μmol/L), but it was found significantly (P<0.001) higher than that for the pregnant mother (14.0±8.5 μmol/L) (table 2, 3). For man, serum retinol level (1.38±0.33 μmol/L) was found to be significantly higher than that for non-pregnant woman (0.99 ± 0.20 μmol/L, P<0.0001) and pregnant mother (0.85±0.25 μmol/L, P<0.001) (table 2, 3). Pregnant mother were found to have higher levels of serum α-tocopherol, but lower levels of serum ascorbic acid and

retinol as compared to the non-pregnant woman. Serum ascorbic acid and retinol levels were observed to be lower for pregnant mother as compared to those for man. Amongst pregnant mother, 22.9% were found to have low serum α-tocopherol, 45.7% were found to have low ascorbic acid, while 20.0% had low serum retinol.

Table 2. Serum α-tocopherol, ascorbic acid and retinol levels of men and non-pregnant women

Serum antioxidant vitamin (μmol/L)	Healthy men (n=50)		Non-pregnant women (n=30)		P value*
	%(n)	Mean±SD	%(n)	Mean±SD	
α- tocopherol					
<17.8	84.0(42)	14.9±3.20	80.0(24)	13.1±5.96	t=1.73
17.8-26.7	16.0(8)		13.3(4)		P=0.08
>26.7	0		6.7(2)		
Ascorbic acid					
<11.4	0	31.6±11.4	13.4(4)	30.7±20.4	t=0.23
11.4-34.1	62.0(31)		53.3(16)		P=0.82
>34.1	38.0(19)		33.3(10)		
Retinol					
<0.70	0	1.38±0.33	3.3(1)	0.99±0.21	t=5.65
0.70-1.75	84.0(42)		96.7(29)		P=0.00
>1.75	16.0(8)		0		

*significant (P<0.05).

Table 3. Serum α-tocopherol, ascorbic acid and retinol levels of non-pregnant women and pregnant mothers

Serum antioxidant vitamin (μmol/L)	Non-pregnant women (n=30)		Pregnant mother (n=35)		P value*
	%(n)	Mean±SD	%(n)	Mean±SD	
α- tocopherol					
<17.8	80.0(24)	13.1±5.96	22.9(8)	22.5±8.07	t=5.28
17.8-26.7	13.3(4)		51.4(18)		P=0.00
>26.7	6.7(2)		25.7(9)		
Ascorbic acid					
<11.4	13.4(4)	30.7±20.4	45.7(16)	14.0±8.5	t=4.46
11.4-34.1	53.3(16)		54.3(19)		P=0.00
>34.1	33.3(10)		0		
Retinol					
<0.70	3.3(1)	0.99±0.21	20.0(7)	0.85±0.25	t=2.52
0.70-1.75	96.7(29)		80.0(28)		P=0.01
>1.75	0		0		

*significant (P<0.05).

Discussion

This study addressed the baseline data for antioxidant vitamins for a sub-section of the people of Bangladesh. The study showed that there had significant variations in the serum α-tocopherol, ascorbic acid and retinol levels between men, women and non-pregnant mothers. Serum α-tocopherol level for pregnant mother was found to be significantly high as compared to that for men and non-pregnant women. Although, the requirement of α-tocopherol for adult men and non-pregnant women is somewhat same, but pregnant mother need higher content of α-tocopherol than the non-pregnant female[9]. This increased need of α-tocopherol, which is produced by physiologic system, is required to stabilize increased free radicals that are generated, possibly by placenta in pregnancy[23]. However, our finding is consistent with the reports of other investigators[24-25].

Pregnant mothers were found to have reduced ascorbic acid content. It is possibly because of increased utilization of vitamin C in pregnancy by the mothers, fetus and placenta for collagen synthesis and other vital functions in the body [26]. In addition to that, vitamin C is used to regenerate vitamin E to meet its increased demand in pregnancy. Therefore, it is well practice that vitamin C supplementation is usually given during pregnancy. Compared to men, retinol level was found predominantly low in women, particularly its level was found significantly low in pregnant mother. The reason for it may be because of its increased demand in pregnancy by the mothers and fetus[27].

In conclusion of this study, it is to be mentioned that the general healthy people of Bangladesh have predominantly reduced level of vitamin E as compared to the international reference value[20]. It would be because of their less intake of vitamin E containing foods. Their vitamin C[21] and vitamin A[22] values are found to be within the international reference value.

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