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Serum Vitamin E, C and A Status in Pre-Eclampsia and Eclampsia Patients, and Their Correlation with Blood Pressure: a Study in Dhaka, Bangladesh

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ABSTRACT

The aim of this study was to determine serum concentrations of vitamin E, C and A in pre-eclampsia and eclampsia patients, and to analyse their relationship with blood pressure. It was a cross-sectional case controlled study comprising forty-four pre-eclampsia, fifty eclampsia, and thirty-five normo-tensive pregnant women of singleton gestations in their third trimester from two hospitals in Dhaka, Bangladesh. HPLC and spectrophotometric methods were employed to determine the serum concentrations of vitamin E, A, and C. SPSS software package was used to analyse the data. Serum vitamin C was found to be significantly higher (F=6.266, p=0.003) in the pre-eclampsia group than in the pregnant control and eclampsia groups, while serum vitamin E and A in patients and control did not differ significantly. Vitamin C levels in the pre-eclampsia group were found to be influenced by their maternal age ($F_{(2,41)}$=3.197, p=0.05), and found to be positively related to the maternal age (r =0.250 and p=0.106). In the pre-eclampsia group, vitamin E showed a positive significant relationship with systolic pressure (beta co-efficient=0.303, P=0.052, R^2=0.101) and diastolic pressure (beta co-efficient=0.459, P=0.002, R^2=0.211). In the eclampsia group, vitamin C showed a negative significant relationship with systolic blood pressure (beta co-efficient=-0.502, P=0.000, R^2=0.302) but in the case of diastolic pressure, the relationship was reversed (beta co-efficient=0.443, P=0.001, R^2=0.237).

INTRODUCTION

Pre-eclampsia is one of the common complications in pregnancy (Erkkola, 1997). It is a leading cause of maternal and perinatal morbidity and mortality in the world (Staff *et al.,* 1999; Madazli *et al.,* 1999). The aetiology and pathogenesis of pre-eclampsia is still unknown, but several pathophysiological characteristics of pre-eclampsia such as altered vascular reactivity, loss of vascular integrity, activation of coagulation cascade and inadequate trophoblastic invasion of spiral arteries suggest that dysfunction of the vascular endothelium accompanies pre-eclampsia (Staff *et al.,* 1999; Madazli *et al.,* 1999; Hubel *et al.,* 1997; Gulmezoglu *et*

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al., 1997). It has been documented that pre-eclampsia is accompanied by oxidative stress that contributes to vascular dysfunction (Hubel *et al.,* 1997; Shaarawy *et al.,* 1998). The inadequate trophoblastic invasion results in a high resistance, low flow uteroplacental circulation that develop into placental ischaemia and hypoxia (Gulmezoglu *et al.,* 1997), which could trigger lipid peroxidation, inducing endothelial damage in pre-eclampsia (Hubel *et al.,* 1997; Gulmezoglu *et al.,* 1997). It has been postulated that the placental ischaemia initiates excessive free radical production and triggers multiple chain reactions, which ultimately results in widespread endothelial damage. Under hypoxic condition, the enzyme xanthine dehydrogenase is shifted to the oxidase generating free oxygen radicals (Gulmezoglu *et al.,* 1997) that attach unsaturated lipids and thiol containing proteins in cell membranes (Freeman & Crapo, 1982). This oxidative attack causes damage to enzymes, cell membranes, proteins and nuclear materials. The superoxide anion, an important free radical, can inactivate endothelium-derived-relaxing factor (Gryglewski, Palmer & Moncada, 1986) and inhibit prostacyclin synthesis (Wang *et al.,* 1991; Weiss, Turk & Needleman, 1979) resulting in the platelet aggregation and vasospasm, the two important characteristics of pre-eclampsia. Thus, oxidative stress arises from increased production of reactive oxygen species or deficiency in antioxidant nutrients.

It has been suggested that deficiency in antioxidant vitamins would be associated with the development of pre-eclampsia (Hubel *et al.,* 1997; Ehrenkrarnz, 1980). Antioxidant vitamin, with the ability to stabilise highly reactive free radicals, acts as the first line of defense against free radical attack and lipid peroxidation (Mayes, 1996). Vitamin E, vitamin C and β-carotene (pro-vitamin A) are known to be powerful antioxidants (Mayes, 1996; Czerinichow & Hercberg, 2001; Diplock, 1991). Vitamin E (α-tocopherol) is the major lipid-soluble antioxidant that protects cells against lipid peroxidation. Vitamin C is a quencher of free radicals as well as singlet oxygen. It also regenerates vitamin E. Beta-carotene, by quenching singlet oxygen, also functions as a potential antioxidant.

Antioxidant vitamins have been reported to have an important function in regulating blood pressure (Dakshinamuti & Dakshinamuti, 2001). Nitrous oxide (NO) is the most important endothelium dependent vasodilator and is highly susceptible to oxidative damage. The antioxidant vitamins C and E are able to inhibit formation of free radicals thereby inhibiting the oxidation of NO, and thus maintain the vasodilator status of blood vessels. β-carotene also carries out the same function. Vitamin C has been reported to have a direct acute effect on the inhibition of the constrictor response of resistance arteries to a variety of stimuli. It is also reported that there are synergisms between the actions of vitamin E and vitamin C, and between vitamin E and β-carotene (Islam *et al.,* 2001). However, there is little information regarding the functions of antioxidant vitamins in pre-eclampsia and eclampsia. We report here the serum concentrations of vitamin E, C and A in pre-eclampsia and eclampsia, and their relationship with blood pressure.

MATERIALS AND METHODS

Study population

It was a cross sectional case control study and was conducted prospectively in the Dhaka Medical College Hospital and Bangabandhu Sheikh Mujib Medical University, Dhaka. The study included forty-four pre-eclampsia, fifty eclamsia and thirty-five normotensive pregnant women of singleton gestation in their third trimester. The subjects were selected

under defined criteria. Pre-eclampsia patients were at 28 to 42 weeks of singleton gestation, one measurement of diastolic pressure of 110 mmHg or more, or two measurements of 90 mmHg or more on two consecutive occasions 6 hours or more apart, urinary protein 2+ or more (100mg/dl; dipstick reagent strip, Boehringer Mannheim, Germany). The patients who developed convulsions were considered to be suffering from eclampsia. The exclusion criteria included history of hypertension and proteinuria before conception or before 20 weeks of gestation, any associated medical disorders, a history of antioxidant vitamin therapy during the last one year, and smoking. Eclampsia patients with a history of convulsion before 48 hours of hospitalisation were also excluded from the study. As cohort control, age- and socio-economically matched healthy normotensives at 28 to 42 weeks of singleton gestation with no urinary protein were recruited by convenience. The controls were matched by group percent of age, education and income. Ethical clearance was taken from the Heads of the two hospitals involved.

Serum analysis

A venous blood sample (5ml) was collected from antecubital vein of each of the case and control subjects. The blood sample was kept undisturbed for at least 60 minutes and was then centrifuged at 3000rmp for 10 minutes. Serum thus extracted was stored at -20^{O}C for analysis of retinol and α-tocopherol. Reversed phase HPLC (LC-10AD, SHIMADZU, HPLC 1991, Model-7125, Kyoto, Japan) was used for simultaneous determination of serum retinol and a-tocopherol concentrations as described by Islam *et al.* (2001). The retinol and α-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 (50ml) 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 a-tocopherol were detected spectrophotometrically at 291nm. The column was re-equilibrated with the mobile phase for 5 minutes before injection of the next sample. In order to check the reproducibility of the method, some samples were injected consequently twice. To verify the assay accuracy, standard analytes (Sigma Chemical Co, USA) were injected for every 15-20 test samples.

For ascorbic acid analysis, the serum was treated immediately after extraction with 5% trichloroacetic acid (TCA) and then centrifuged at 3000rpm for 10 minutes. Clear supernatant thus obtained was stored at –20^{O}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.* (2001). 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 one way ANOVA. The ANOVA was also used to assess the influence of maternal age, gestational age, gravida, and proteinurea on the serum vitamin levels. Multiple linear regression analysis was performed to find a correlation between blood pressure and serum antioxidant vitamins.

RESULTS

Clinical parameters of the patients and normotensive controls are shown in Table 1. The mean maternal and gestational ages of the patients and control were found to be similar. They had different gravida distribution and had nearly equal proteinurea. As anticipated by definition, the systolic and diastolic pressures of pre-eclampsia and eclampsia patients were also significantly (P<0.005) higher than those of the pregnant control (Table 1).

Table 2 shows serum vitamin E, C and A status of the case subjects and normotensive control. It was observed that the majority of the cases and controls had serum vitamin E and A levels in the middle range (18-29 μmol/L, 0.76-1.25 μmol/L respectively), and in the lower range (1-25 μmol/L) for vitamin C. Serum vitamin E and A levels were not significantly different in pre-eclampsia, eclampsia and pregnant controls. Vitamin C concentration in pre-eclampsia was found to be significantly higher than that of eclampsia and pregnant control ($F_{(2,126)}$=6.266, p=0.003). Difference in the serum vitamin C levels between eclampsia and pregnant control was found to be insignificant.

One-way analysis of variance (ANOVA) showed that the maternal age, gestational age, gravida and proteinurea did not show any influence on serum vitamin E and A level. Vitamin C level in pre-eclampsia was found to be influenced by maternal age ($F_{(2,41)}$=3.197, p=0.05), (Table 3). The correlation between the age and vitamin C level was determined and it was found to be positively related with r=0.250 and p=0.106. In pregnant control, gestational age showed positive influence on vitamin C level ($F_{(1,33)}$=4.609, p=0.039) with r=0.350.

Multiple linear regression was employed to observe the effects of serum vitamin A, E and C contents on the blood pressure of pre-eclampsia and eclampsia patients, and pregnant control and the result is shown in Table 4. The results revealed that vitamin E had a positive significant relationship with systolic and diastolic blood pressure of pre-eclampsia patients. Although there was also a positive relationship in the case of eclampsia and control subjects, these were not statistically significant. Vitamin C showed

Table 1. Age and clinical characteristics of pre-eclampsia and eclampsia patients, and normotensive pregnant controls

Clinical characteristic		*Case subject*		
		Pre-eclampsia	*Eclampsia*	*Control*
Maternal age (year)		26.05±5.41	22.86±4.87	24.11±4.93
Gestational age (wk) at sampling		34.64±3.85	35.54±3.79	37.23±2.64
Gravida in no (%)	primi	18(41)	36(72)	12(34)
	multi	26(59)	14(28)	23(66)
Proteinurea	primi	2^+(26)	2^+(27)	0
	multi	3^+(18)	3^+(23)	
Systolic blood pressure (mmHg)		160.68±22.61	153.70±21.80	109.86±9.27
Diastolic blood pressure (mmHg)		109.16±15.21	106.60±13.83	73.43±8.81

Values are expressed in mean ± sd
Descriptive Statistics: frequencies, descriptives, crosstabs
Compare Means: Independent-samples t-test

Table 2. Serum concentrations of vitamin E, C and A in pre-eclampsia and eclampsia patients, and normotensive pregnant controls

Antioxidant vitamin (µmol/L)	*Pre-eclampsia*		*Eclampsia*		*Control*	
	n (%)	*mean±sd*	*n (%)*	*mean±sd*	*n (%)*	*mean±sd*
Vitamin E[1]						
06-17	5(11.4)	25.02±7.1	15(30.0)	22.17±8.5	6(17.1)	23.54±8.5
18-29	27(61.4)		28(56.0)		24(68.6)	
30-41	12(27.3)		7(14.0)		5(14.3)	
Vitamin C[2]						
01-25	25(56.8)	24.53±15.9	44(88.0)	16.95±15.0	30(85.7)	14.0±8.2
26-50	17(38.6)		4(8.0)		5(14.3)	
51-75	01(2.3)		01(2.0)			
76-100	01(2.3)		01(2.0)			
Vitamin A[3]						
0.35-0.75	21(47.7)	0.78±0.21	28(56.0)	0.78±0.32	10(28.6)	0.85±0.24
0.76-1.25	23(52.3)		18(36.0)		23(65.7)	
1.26-1.76			4(8.0)		2(5.7)	

sd: standard deviation
[1]F(2,126) = 1.452, p = 0.238
[2]F(2,126) = 6.266, p = 0.003
[3]F(2,126) = 0.877, p = 0.418
Descriptive Statistics: frequencies, descriptives, crosstabes.
Compare means: One-Way ANOVA (Descriptives, ANOVA)

Table 3. Effect of maternal characteristics on the serum vitamin E, C, A

Maternal characteristics	*Pre-eclampsia (F & P values)*			*Eclampsia (F & P values)*		
	E	*C*	*A*	*E*	*C*	*A*
Age	1.142	3.197	2.073	1.936	0.487	0.072
	0.329	0.05*	0.139	0.156	0.618	0.931
Gestational age	0.452	0.265	0.445	0.398	1.377	0.452
	0.639	0.769	0.644	0.674	0.262	0.639
Gravida	0.638	1.641	0.738	0.387	1.643	0.387
	0.534	0.206	0.484	0.681	0.204	0.681
Proteinurea	0.772	0.90	0.009	0.110	0.035	0.147
	0.924	0.766	0.924	0.896	0.966	0.864

*Significance: P < 0.05

Table 4. Multiple regression results of pre-eclampsia (PE), eclampsia (EC) and pregnant control

Variables	*Systolic*			*Diastolic*		
	PE	*Ec*	*Control*	*PE*	*Ec*	*Control*
R^2	0.101	0.302	0.084	0.211	0.237	0.137
F values	1.504 (0.228)	6.626 (0.001)	0.95 (0.428)	3.561 (0.022)	4.765 (0.006)	1.641 (0.200)
Beta co-efficient of						
Vitamin A	0.094 (0.538)	-0.216 (0.313)	-0.289 (0.111)	-0.008 (0.958)	0.010 (0.939)	-0.220 (0.208)
Vitamin E	0.303 (0.052)	0.147 (0.240)	0.013 (0.941)	0.459 (0.002)	0.175 (0.181)	0.004 (0.983)
Vitamin C	0.962 (0.681)	-0.502 (0.000)	0.122 (0.498)	-0.068 (0.632)	0.443 (0.001)	0.348 (0.052)

Note: Figures in parenthesis are P values

a negative significant relationship with systolic blood pressure of eclampsia patients but a reverse relationship in the case of diastolic pressure. In the pregnant control group, vitamin C had a significant positive relationship with diastolic blood pressure.

DISCUSSION

The aetiology of pre-eclampsia is elusive. Oxidative stress is thought to be associated with pre-eclampsia (Madazli *et al.,* 1999; Hubel *et al.,* 1997; Gulmezoglu *et al.,* 1997). Serum antioxidant activity has been reported to increase progressively throughout pregnancy (Gulmezoglu *et al.,* 1997; Wang *et al.,* 1991; Cranfield *et al.,* 1979). This study investigated serum vitamin levels in pre-eclampsia and eclampsia, and attempted to evaluate their correlation with the aetiology of this disease.

It was observed that serum vitamin E and A concentrations were not different among the patients and pregnant controls, which is consistent with the report of Valsecchi, Fausto & Grazioli (1995). While some reports documented an increase in serum vitamin levels in pre-eclampsia and eclampsia (Wang *et al.,* 1991; Schiff *et al.,* 1996), others have found a decrease (Hubel *et al.,* 1997; Shaarawy *et al.,* 1998). In this study, serum vitamin C concentration was found to be higher in pre-eclampsia than in eclampsia. The reason for this finding is not clear. It has been documented that vitamin C, as an effective antioxidant in human plasma, provides major defense against the diseases caused by oxidative stress (Dakshinamuti & Dakshinamuti, 2001; Frei, England & Ames, 1989). It is likely that severe oxidative stress in eclampsia utilises a higher amount of vitamin C to fight the oxidative stress leading to a depletion of the

vitamin. The higher vitamin C levels in pre-eclampsia may serve to prevent oxidation of NO (endothelium dependent vasodilator) to maintain the vasodilatation of blood vessels (Dakshinamuti & Dakshinamuti, 2001).

Serum vitamin E and A status were found to be independent of the maternal age and clinical parameters, which is consistent to some extent with the report of Hubel *et al.* (1997). The vitamin C levels in pre-eclampsia showed positive correlation with maternal age but the reason for this finding is not clear.

The results of multiple regression showed that the serum vitamin C had negative correlation with systolic, and positive correlation with diastolic pressure in eclampsia, which is in agreement to some extent with the report of Madazli et al (1999). In pre-eclampsia, vitamin E showed positive correlation with both diastolic and systolic blood pressure, which conflicts with its serum concentration. However, the coefficients are insignificant. Increasing blood pressure demands an increased amount of vitamin E to control the oxidative stress that is caused by oxidation of NO (Dakshinamuti & Dakshinamuti, 2001). In addition, the higher vitamin C in their sera might be involved in generating increased amounts of vitamin E that are needed in pre-eclampsia.

It is revealed that pre-eclampsia patients have higher serum vitamin C levels, but vitamin E and A levels remain unaltered in them. Vitamin E shows positive correlation with the blood pressure levels in pre-eclampsia. Vitamin C shows negative correlation with systolic pressure but positive correlation with the diastolic blood pressure in eclampsia.

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