Original Article

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Serum FRAP Levels and Pre-eclampsia among Pregnant Women in a Rural Community of Northern India

Community Medicine Section

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ABSTRACT

Introduction: There is a balance between the oxidative and the anti-oxidative forces in human body. Some studies document decreased level of anti-oxidant in pre-eclampsia while other studies showed normal level of anti-oxidant in pre-eclampsia and the evidence is equivocal.

Aim: The aim of the present study was to assess enzymatic anti-oxidant activities in pre-eclamptic women and compare it with normotensive pregnant women with period of gestation between 28 to 36 weeks.

Materials and Methods: A community-based cross-sectional survey was conducted (from November 2012-December 2013) at the Ballabgarh Health and Demographic Surveillance System (HDSS) site which was managed by Centre for Community Medicine, All India Institute of Medical Sciences, New Delhi, India. All registered pregnant women with period of gestation between 28 weeks – 36 weeks were eligible for inclusion in the study. All eligible pregnant women were contacted through home visit. A total of 217 pregnant women were enrolled out of which 209 blood samples were collected from pregnant women. About three ml of blood from antecubital vein was drawn without use of tourniquet, under aseptic conditions. It was later analysed for the serum anti-oxidative measures {Malanoaldehyde, Vitamin C, Ferric Reducing Ability of Plasma (FRAP) levels}. Data were entered on Epi-Info version 3.5.4. Data management and analysis was carried out in Stata 11. The means were compared using t-test and p-value stated. Categorical data was analysed using chi-square test. Logistic regression was used and adjusted p-value was stated.

Results: A total of 217 pregnant women were eligible for the study and all were enrolled. Out of the 217 blood samples collected, eight samples accidently got destroyed. A total of 28 out of remaining 209 pregnant women (13.4%) had pre-eclampsia. Mean age (SD) was 22.4 (2.3) years, mean height (SD) was 156.6 (6.9) cm, mean weight (SD) was 65.1 (9.7) kg in pre-eclampsia group. In pre-eclampsia group mean serum levels of malanoaldehyde, vitamin C and FRAP was 4.2 (0.26) ng/dl, 0.83 (0.11) mg/dl, 678.0 (35.6) µmol/L. It was 4.5 (0.09) ng/dl,0.88 (0.03) mg/dl, 599.3 (13.3) µmol/L in normotensive pregnant women group respectively. The difference was statistically significant for FRAP levels only.

Conclusion: Serum anti-oxidant levels are not raised among women with pre-eclampsia.

Keywords: Anti-oxidant, Health and demographic surveillance system ballabgarh, Hypertension, Proteinuria

INTRODUCTION

In the human body, there are processes which lead to production of reactive oxidative species and the human body has anti-oxidative mechanism to counter the reactive oxidative species. Hence, there is a balance between the oxidative and the anti-oxidative forces [1]. Pregnancy is a stressful condition in which different metabolic functions are known to be deranged. Pre-eclampsia affects about 8-10% of pregnant women [2,3]. Pre-eclampsia is an important cause of morbidity, disability and death among mothers and babies [2-4]. The onset of a new episode of hypertension during pregnancy (with persistent systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mmHg) along with the occurrence of substantial proteinuria (>0.3 g/24 h/≥1+) is defined as pre-eclampsia. Literature suggests that increase in the reactive oxidative species cause damage to the endothelium of blood vessels leading to its dysfunction [5]. The endothelial dysfunction further leads onto proteinuria and hypertension i.e., pre-eclampsia. The pathogenesis of pre-eclampsia is only partially understood and it is said to be related to disturbances in placentation at the beginning of pregnancy, followed by generalized inflammation and progressive endothelial damage [3]. The Ferric Reducing Ability of Plasma (FRAP), is a novel method for assessing anti-oxidant power [6]. There are limited studies in India on anti-oxidant capacity of serum and its relation to pre-eclampsia in pregnant women. To the best of our knowledge, no community based study had been done. Some studies document decreased level of anti-oxidant in pre-eclampsia while other studies showed normal level of anti-oxidant in pre-eclampsia and the evidence is equivocal [7-13]. The aim of the present study was to assess enzymatic anti-oxidant activities in pre-eclamptic women and compare it with normotensive pregnant women with period of gestation between 28 to 36 weeks.

MATERIALS AND METHODS

A community-based cross-sectional survey was conducted (from November 2012-December 2013) at the Ballabgarh Health and Demographic Surveillance System (HDSS) site which was managed by Centre for Community Medicine, All India Institute of Medical Sciences, New Delhi. It was situated in Ballabgarh block of district Faridabad, Haryana. The Ballabgarh HDSS had two Primary Health Centres (PHCs), and one sub-district hospital (SDH) at Ballabgarh. A detailed description of Ballabgarh HDSS is available in one of our earlier publications [14]. All registered pregnant women with period of gestation between 28 weeks – 36 weeks were eligible for inclusion in the study. Pregnant women with known history of hypertension before 20 weeks of gestation were excluded. A total of 217 eligible pregnant women were identified from existing computerized Health Management Information System (HMIS). All the identified women who were eligible from the HDSS site were enrolled.

All eligible pregnant women were contacted through home visit. Three millilitre of blood from antecubital vein was drawn without use of tourniquet, under aseptic conditions. Blood was stored in previously labelled polypropylene tubes and allowed to clot spontaneously. Centrifugation at 6000 rpm for 5 minutes was done. Serum was stored at -20°C. It was later analysed for the anti-oxidative measures. The spectrophotometry machine used was Cary 100 UV-Vis, Agilent Technologies, Santa Clara, United States of America.

Serum Malanoaldehyde (MDA) levels were estimated using Thiobarbituric Acid (TBA). To the 250µl of sample (Serum), 250µl of saline (0.85%) and 500µl of acetic acid (20%, pH 3.5) were added. Then the solution was centrifuged and to the 500 µl of supernatant 1ml TBA and 500µl distilled water was added. Then it was kept in water bath at 95ºC for one hour. The absorbance was taken at 532nm after cooling [15]. Ascorbic acid levels were estimated colorimetrically using a DTCS reagent (dinitrophenyl hydrazine, thiourea and copper sulfate) [16]. Ascorbic acid concentration was expressed as mg/dL. The FRAP was measured using colorimetric assay. It measures the anti-oxidant capacity of the serum. It was expressed as microM/L [6]. The ferric reducing ability/anti-oxidant power (FRAP) assay [17] is recently developed, direct test of "total anti-oxidant power". Other tests of total anti-oxidant power are indirect methods of measuring anti-oxidative power. Measurement of inhibition assay requires specialized equipment and such tests can be time-consuming, technically demanding and may lack sensitivity [18]. In contrast to other tests of total anti-oxidant power, the FRAP assay is simple, fast, inexpensive and robust [19]. The FRAP assay uses anti-oxidants as reductants in a redox- linked colorimetric method. The tests were performed by one of the authors (SP) who had extensive experience in doing the test in Laboratory Medicine department.

Blood pressure was measured at first visit using a manual pneumatic sphygmomanometer (Industrial Electronic and Allied Products, Pune Maharashtra, India), with the pregnant women sitting comfortably, as recommended by the British Hypertension Society and the Nurses Hypertension Association. A clean-catch urine sample was tested by dipstick (Uristix, Bayer Healthcare, Siemens, Germany) at first visit for albuminuria [20]. Pre-eclampsia was defined as a systolic blood pressure higher than 140 mmHg and a diastolic blood pressure higher than 90 mmHg on two occasions at least 4 hours apart after 20 weeks of pregnancy in a woman with previously normal blood pressure, along with proteinuria (defined as N300 mg/24 h or ≥1+ (on a clean-catch dipstick in the absence of urinary infection) [21].

Ethical issues: All pregnant women were explained about the purpose of the visit in detail, and were provided with a participant information sheet in local language. Participants were informed regarding the consent process and then written informed consent was sought from the pregnant women. Ethical approval was obtained from the Ethics Committee of the All India Institute of Medical Sciences, New Delhi (Reference no. IESC/T-420/02/11/2012 dated November 8, 2012).

STATISTICAL ANALYSIS

Data entry and statistical analysis data were entered on Epi-Info version 3.5.4 (Distributed by Centers for Disease Control and prevention (CDC), Atlanta, USA). Data management and analysis was carried out in Stata 11 (StataCorp LP, Lakeway Drive, College Station, Texas, USA). Data was presented as number (%) as appropriate. The demographic characteristics were compared between the pre-eclampsia group and normotensive pregnant women group using student's t-test for independent samples. The difference in mean values of serum anti-oxidant level between the groups was compared using t-test followed by ANCOVA since weight of pregnant women was significantly different between the two groups. The p-value < 0.05 was considered statistically significant.

RESULTS

A total of 217 pregnant women were eligible for the study and all were enrolled. Out of the 217 blood samples collected, eight samples accidently got destroyed. Hence, data for those 8 pregnant women were excluded from analysis. A total of 28 out of remaining 209 pregnant women (13.4%) had pre-eclampsia. For the purpose of analysis pregnant women were divided into two groups i.e., pre-eclampsia group and normotensive pregnant women group. Mean age (SD) was 22.4 (2.3) years in pre-eclampsia group and was similar to the normotensive pregnant women group. About one-fourth pregnant women each had studied till high school and middle school in the pre-eclampsia group. And one-third of the pre-eclampsia group were primigravida which was also the case in normotensive pregnant women group. Mean height (SD) was 156.6 (6.9) cm. in pre-eclampsia group while it was 154.5 (6.2) cm. in normotensive pregnant women group but the difference was not statistically significant. Mean weight (SD) was 65.1 (9.7) kg in pre-eclampsia group while it was 56.5 (7.0) kg in normotensive pregnant women group and the difference was statistically significant ($p<0.01$) [Table/Fig-1].

The levels of serum oxidant MDA was compared in both the groups. In pre-eclampsia group mean (SE) was 4.2(0.26) ng/dl while it was 4.5(0.09) ng/dl in normotensive pregnant women group. The difference was statistically not significant. The levels of anti-oxidants vitamin C and FRAP were also analysed. Mean (SE) vitamin C level in serum was 0.83 (0.11) mg/dl in pre-eclampsia group and when compared with the other group no statistically significant difference was observed. The mean (SE) FRAP levels in serum adjusted for weight was 678.0 (35.6) µmol/L in pre-eclampsia group which was statistically higher (p-0.04) than the levels in normotensive group (599.3 (13.3) µmol/L) [Table/Fig-2].

DISCUSSION

This study provided information about the anti-oxidant level in pregnant women and its association with pre-eclampsia. There were no teen age pregnancies, which is an independent risk factor for pre-eclampsia. The mean age, height and education status in pre-eclampsia group and normotensive pregnant women group was similar. However, mean weight was higher in the pre-eclampsia group.

It is postulated that rise of oxidative stress cause damage to endothelial layer of blood vessels. Body tries to reduce the oxidative damage by raising the anti-oxidants. Pregnancy is a state of stress in which anti-oxidants try to counter the effect of oxidative stress. Pre-eclampsia is a state in which there is disruption of the endothelial layer of blood vessels. The body total anti-oxidant levels are measured by FRAP, which is a newer test. Very few studies have been conducted to measure FRAP. In our study the FRAP levels were significantly higher in the pre-eclampsia group as compared to the normotensive group. This was also documented by Kurlak et al., where they concluded that there was compensatory increase in anti-oxidant levels [22]. The FRAP-value could have been influenced by the serum uric acid. We had not measured serum uric acid. A study by Mistry et al., showed low levels of FRAP in first trimester pregnant women [23]. It is possible that in the first trimester of pregnancy the stress levels are low and therefore compensatory anti-oxidant mechanism are not required leading to lower levels of FRAP. As the period of gestation increases, the stress increases along with commensurate level of anti-oxidant as ascertained by the FRAP level. Traditional anti-oxidant vitamin C serum levels were not associated with pre-eclampsia. The newly developed test i.e., FRAP was found to have higher level among pre-eclampsia group. Theoretically, it ought to be better marker of total anti-oxidant capacity since it is a direct measurement as compared to other test which are indirect measurements. However, we need to be cautious in interpreting the results since more experience is needed in evaluating the performance of this test.

Variables		Pre-eclampsia group (n=28)	Normotensive Pregnant women group (n=181)	p-value t-test
Mean Age in years (SD)		22.4 (2.3)	22.7 (3.1)	0.58
Mean Height in cm (SD)		156.6 (6.9)	154.5 (6.2)	0.09
Mean Weight in kg (SD)		65.1 (9.7)	56.5 (7.0)	<0.01
Education status^	Professional	1 (3.6)	2 (1.1)	
	Post-graduate	3 (10.7)	19 (10.5)	
	Diploma	4 (14.3)	28 (15.5)	
	High school	7 (25.0)	38 (21.0)	
	Middle	7 (25.0)	41 (22.7)	
	Primary	4 (14.3)	20 (11.1)	
	Illiterate	2 (7.1)	33 (18.2)	
Gravida^	1	9 (32.1)	64 (35.4)	
	2	8 (28.6)	47 (26.0)	
	3	4 (14.3)	31 (17.1)	
	4	3 (10.7)	24 (13.3)	
	≥5	4 (14.3)	15 (8.2)	

[Table/Fig-1]: Distribution of pregnant women by independent variables.
^ Data presented as number (%)

Variables		Mean (SE)		Difference (CI)*	p-value*
		Pre-eclampsia group (n=28)	Normotensive Pregnant women group (n=181)		
MDA (ng/ml)	Unadjusted	4.3 (0.25)	4.4 (0.09)	-0.25 (-0.81, 0.31)	0.40
	Adjusted	4.2 (0.26)	4.5 (0.09)		
Vit C (mg/dl)	Unadjusted	0.83 (0.25)	0.88 (0.04)	0.06 (-0.28, 0.16)	0.60
	Adjusted	0.83 (0.11)	0.88 (0.03)		
FRAP (µmole/L)	Unadjusted	664.8 (34.6)	601.3 (13.1)	78.8 (2.7,154.8)	0.04
	Adjusted	678.0 (35.6)	599.3 (13.3)		

[Table/Fig-2]: Distribution of mean serum level of biomarkers (adjusted for weight) among pregnant women group
* Adjusted for weight (kg)

We did not find statistically significant difference in MDA levels between the two groups. Similar finding have also been reported by several other studies [9,13,22,24-28]. Some studies have documented an increase in the oxidative stress markers in pre-eclamptic women [28-33]. These were hospital based studies in which the pregnant women were patient and may have had other concomitant diseases with deranged oxidative stress status. We had recruited pregnant women from a rural community setting. We had not ascertained existence of any co-morbidity among them. However, these pregnant women were significantly less likely to have concomitant co-morbidity when compared with pregnant women who were recruited from the hospital settings.

We did not find any difference in vitamin C level between the pre-eclampsia group and normotensive group. No change in vitamin C level had been reported by others [8,25,34,35]. Vitamin C is a water soluble anti-oxidant which functions as first line of defence against oxidants. It is postulated that reduction in the level of vitamin C leads to leakage of oxidant from the endothelium leading to pre-eclampsia. Study conducted by Rao et al., was among pregnant women in the third trimester but in a hospital setting [34]. Their study reported 6.55±0.63 µmol/dl of vitamin C in normotensive group and 6.24±1.4 µmol/dl in pregnancy induced hypertensive group and the difference was not statistically significant. While study conducted by Sarwar et al., showed a decrease in vitamin C level in pre-eclampsia women [32]. They explained it as the failure of body anti-oxidant mechanism which led to pre-eclampsia. The measurement of vitamin C was taken after the disease had already developed in pregnant women. Hence, the causal role of vitamin C in development of pre-eclampsiawas not clear. Similarly other studies [10,12,34] also documented increase in vitamin C level in pre-eclampsia women.

Strength of study was that it was a rural community-based study. Therefore, extrapolation of results to other rural community in North India is possible. We have not come across any other community based study from India where estimation of serum anti-oxidant level for pregnant women and its association with pre-eclampsia had been done. All samples were tested in the laboratory which was housed in an apex teaching hospital.

LIMITATION

Limitation of study being, the association could have been underestimated in our study because we had visited the pregnant woman only once between 28-36 weeks. There was a possibility of pregnant woman developing oxidative stress after the visit. We made concurrent measurement of serum anti-oxidant level and incidence of pre-eclampsia at the time of visit. Therefore, findings are valid at the time of measurement. However, it is likely that some more pregnant women might have developed oxidative stress later on, which was not recorded.

CONCLUSION

In conclusion, the serum MDA and vitamin C level were similar in pre-eclampsia group and normotensive pregnant women group. The serum FRAP level was significantly higher in pre-eclampsia group as compared to the normotensive pregnant women group. FRAP levels have to be explored further for its utility in early diagnosis of pre-eclampsia and monitoring the progress of pre-eclampsia.

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