

Difference of Frap Levels Between Preeclamptic and Normotensive Women: A Cross-Sectional Analysis from a Tertiary Care Hospital, Pakistan

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ABSTRACT

Objective: To determine the association between FRAP levels and preeclampsia

Methodology: A case control study was conducted at Social Security Teaching Hospital Multan Road, Lahore between September 2019 March 2020. A total of 309 women were enrolled in the study using the non-probability convenience sampling technique. Women at 28 weeks or higher gestation, equal to or older than 18 years of age were included in the study. Those with a history of hypertensive issues before 20 weeks of gestation or other comorbidities were excluded from the study. For antioxidative analysis, the serum FRAP was measured using colorimetric assay. Patients sociodemographic, clinical, and reproductive history were recorded. For data analysis, SPSS version 24 was used and a p-value of 0.05 or less was considered as statistically significant.

Results: Preeclampsia was found in 40 (13%) pregnant women in our study. The mean age + SD for the group with preeclampsia was 21.56 + 4.2 years while the mean age + SD for the normotensive group was 22.12 +5.6 years. The mean + SD FRAP levels were significantly higher in women with preeclampsia than the normotensive control group (p=0.01); 684.2 + 21.2 µmole/L in the preeclampsia group and 603.1 + 15.6 µmole/L in the control group.

Conclusion: The FRAP levels were significantly higher in women with preeclampsia than the normotensive women indicating a correlation between high oxidative stress and risk of developing threatening pregnancy related issues.

Keywords: antioxidant, FRAP, gestational, oxidative stress, preeclampsia

INTRODUCTION

Pre-eclampsia is a widely known cause of maternal and fetal mortality, worldwide^{1,2}. Preeclampsia is defined as an onset of hypertension that is a blood pressure of 140/90 mmHg or higher with the occurrence of proteinuria of more than 3 mg per day³.

The mechanisms involved in induction of endothelial cell dysfunction are poorly understood. In the last decade or so, evidence showed that damage to the maternal endothelium plays a crucial role in the diverse clinical manifestations of preeclampsia. Literature suggests that increase in the placental and maternal reactive oxidative species causes damage to the vascular endothelium in preeclampsia^{4,5}. The uncontrolled formation of free radicals and highly reactive oxygen species results in cellular dysfunction & damage. Lipid peroxidation in this regard has received a great deal of attention as it results in the formation of lipid hydroperoxides – the highly reactive primary product of this process⁶. Many harmful endothelial changes in relevance to preeclampsia can be attributed to lipid peroxidation including: i) Structural injury to glomerular capillary endothelium and umbilical endothelium, ii) proteinuria, iii) Vasoconstriction and hypersensitivity to pressor agonists, iv) Impaired endothelial-dependent relaxation of isolated arteries, v) Hemolysis & increased red cell osmofragility, among other damaging functions⁴⁻⁷. The increased endothelial damage causes leakage of proteinuria and blood pressure irregularities. However, the pathogenesis of preeclampsia is still not entirely understood^{4,6}. It has been speculated that the condition is related to the abnormal placental development during early stages of pregnancy which subsequently leads to generalized inflammation and damage to endothelium⁷.

The Ferric Reducing Ability of Plasma (FRAP), is a new technique to measure the antioxidant power⁸. The FRAP is assessed to detect the ability of the body to fight off the oxidative stress and the subsequent damage^{9,10}. However, despite the evident link between FRAP and the severity of Preeclampsia, there are very limited studies on the subject from Pakistan. Hence, the aim of the study was to assess and evaluate the relationship between FRAP and preeclampsia by comparing the levels of

FRAP in women with preeclampsia with the levels of FRAP in normotensive women during pregnancy.

MATERIAL AND METHODS

A case control study was conducted at Social Security Teaching Hospital Multan Road, Lahore between September 2019 March 2020. A total of 309 women were enrolled using the non-probability convenience sampling technique. Pregnant women of 28 weeks gestation or greater and those equal to or older than 18 years of age were eligible to take part in study. Women with gestation period of 27 weeks or earlier, and those younger than 18 years and those with hypertension before 20 weeks of gestation or any comorbidity were excluded from the study.

For FRAP tests, 3 mL of blood from the cubital vein was extracted using the tourniquet and was sent to the laboratory for antioxidative analysis. Aseptic conditions were followed. Measurement of FRAP using colorimetric assay. The blood was centrifuged for at least fifteen minutes at 2200-2500 RPM within one hour of collection. The serum was stored at +4 °C. The test is used to measure the plasma antioxidant capacity. The ferric reducing antioxidant power (FRAP) assay is a novel test that measures the total antioxidative activity in the body. It is a quick, inexpensive, and a technically simple test to perform¹⁰.

All patients were screened for hypertensive irregularities upon their arrival for routine check-up at every visit 2.0 weeks apart. Blood pressure were monitored in all patients using the protocol given by the British Hypertension Society. [11] The patients with a systolic blood pressure of greater than 140 mmHg and a diastolic blood pressure of more than 90 mmHg at two occasions, four hours apart, in conjunction with proteinuria on urinalysis. For urinalysis, a clean catch urine sample was obtained. Proteinuria was considered positive when albumin/creatinine ratio of greater than 30 mg/mmol or 300 mg/g was reported. Data including patients' demographics, occupational, educational status, the reproductive history, blood pressure and serum FRAP levels were recorded on a predefined pro forma sheet.

Data analysis: The data was entered into excel and converted into .csv files. Statistical software SPSS version 24 was used to analyze the data. Continuous data was presented as mean and standard deviation while the categorical data was presented as frequency or percentages. Association between serum FRAP levels and preeclampsia was explored through independent t-test. A p-value of less than 0.05 was considered statistically significant.

Ethical considerations: Ethical clearance was obtained from the institutional review board prior to the study. All patient data were confidential and only the principal investigator had access to the data. The data was decoded and stripped off any patient identifiers like names, addresses, etc. Informed written consent was obtained from all patients before starting the study.

RESULTS

A total of 40 (13.0%) women in our study were pre-eclamptic while 269 (87%) were normotensive. The mean age + SD for the group with pre-eclampsia was 21.56 + 4.2 years while the mean age + SD for the normotensive group was 22.12 + 5.6 years.

The majority of the women were in their twenties; 20 (50%) in the preeclamptic group and 134 (49.8%) in the normotensive group. Over four-fifth women in our study were housewives. About 30 percent of the women in our study had an intermediate level education or higher. See table 1 for details. The mean + SD weight and height for the preeclamptic group was 69.2 + 10.5 kg and 151.5 + 12.5 cm respectively. In contrast the mean + SD weight and height for the control group was 64.2 + 8.5 kg and 154.2 + 10.4 cm respectively.

Table 1: Demographic and clinical profile of pregnant women in control group versus preeclampsia group n=309

Variable(s)	Pre-Eclamptic N (%)	Normotensive N (%)
Age group (in years)		
18 – 21	11 (27.30%)	72 (26.6%)
22 – 25	20 (50%)	134 (49.8%)
26 – 29 107	6 (15.37%)	39 (14.7%)
>=30 56	3 (8.05%)	24 (8.8%)
Occupational status		
Homemaker	38 (95.40%)	255 (94.7%)
Others	2 (4.60%)	14 (5.3%)
Educational status		
Intermediate and above	13 (32.61%)	86 (31.9%)
High school	9 (22.27%)	62 (23.0%)
Middle	7 (16.38%)	42 (15.7%)
Primary	5 (13.36%)	38 (14.1%)
Illiterate	6 (15.37%)	39 (14.7%)
Gravidarum		
1^	14 (35.20%)	97 (35.9%)
2^	12 (29.74%)	78 (29.0%)
3^	8 (19.83%)	55 (20.5%)
>=4	6 (15.23%)	39 (14.5%)
No. of abortions (n=)		
0	14 (35.63%)	98 (36.4%)
1	9 (22.70%)	59 (22.0%)
2	2 (4.74%)	15 (5.5%)
>=3	1 (1.72%)	3 (1.0%)

Table 2: shows the distribution of FRAP levels among the two groups i.e. for the preeclamptic group the mean + SD FRAP levels were significantly higher than the normotensive group (p=0.01).

Variable		Control group (n = 269)	Preeclampsia Group (n=40)	Confidence Interval (CI)	p-value
FRAP in (µmole/L)	Unadjusted	665.4 + 10.5	611.2 + 8.5	81.7 (3.7, 149.6)	0.01
	Adjusted	684.2 + 21.2	603.1 + 15.6		

DISCUSSION

In the present study, the association between pre-eclampsia and level of oxidative stress as determined by the FRAP test during pregnancy was explored. The Ferric Reducing Ability of Plasma

(FRAP), is a new technique for the evaluation of total antioxidant levels in the body¹². During pregnancy, preeclampsia can develop as a result of damaged endothelial layers of blood vessels due to the imbalance between oxidative stress and antioxidant activity¹³. Pre-eclampsia is a condition where a pregnant woman experiences high blood pressure and probable organ damage. It can lead to eclampsia if not promptly treated. Pre-eclampsia and eclampsia are greatly associated with maternal mortality and poor pregnancy outcomes¹⁴.

The present study showed that 40 (13.0%) women in our study were pre-eclamptic while 269 (87%) were normotensive. The mean age in pre-eclampsia group was 21.56 4.2 years while the mean age + SD for the normotensive group was 22.13 +5.6 years. The patient mean weight in with pre-eclampsia was greater than normotensive patients. But, the difference was significant statistically. Earlier studies have shown that obesity and younger age at the time of pregnancy were associated with a higher risk of developing pre-eclampsia or other pregnancy-related complications¹⁵⁻¹⁷. In a study by Aliyu et al, it was revealed that obese pregnant teenagers were four times likely to develop pre-eclampsia or eclampsia compared to the older nonobese mothers (adjusted odds ratio [95% confidence interval] = 3.79 [3.15–4.55])¹⁶.

In the present study, it was observed that the FRAP levels were greater significantly in the group with pre-eclampsia compared with the normotensive group. FRAP measures the antioxidant activity among mothers who are pregnant. Higher antioxidant activity indicates a more stressful condition which may be predictive of a more serious complication. During pregnancy, innumerable changes on macro and micro level are observed. One disruptive change is increased oxidative stress due to release of free radical charges¹⁸. It attacks the inner lining of the blood vessels, leading to hypertension and protein leakage, preeclampsia. To counterattack the oxidative stress, the body's inner mechanisms release the antioxidants hence, the increased FRAP levels¹⁹.

The findings of the present study are in line with a study from neighboring countries. In a study by Gupta et al, a comparison of antioxidative stress was done between pregnant women who were pre-eclamptic and those who were normotensive during the gestation period of 28 weeks to 36 weeks⁶. The study revealed that in pre-eclampsia group, both malonaldehyde and vitamin C levels were lower compared to normotensive pregnant female. However, the AFRAP levels were high significantly in female with pre-eclampsia compared to those with no blood pressure irregularities.

In another recent study from India, the authors evaluated the pre-eclamptic women for metabolic disturbances and oxidative stress during pregnancy²⁰. They reported that women with pre-eclampsia in conjunction with experiencing significantly higher antioxidative activity, more irregularities in their blood glucose level, had increased insulin resistance levels, and lipid metabolism was also disturbed compared to the control group (p<0.0001).

CONCLUSION

The FRAP levels were significantly higher in women with preeclampsia than the normotensive women indicating a correlation between high oxidative stress and risk of developing threatening pregnancy related issues.

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