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Evaluation of the level of thiol-disulphide homeostasis in patients with mild and severe preeclampsia

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1. Introduction

Although the formation of free radicals is a part of human metabolism, increased concentrations of free oxygen radicals play a significant role in the pathogenesis of many diseases, as a result of their potential to harm vital biologic systems [1,2].

Pre-eclampsia is a significant complication of pregnancy characterized by proteinuria and hypertension, which can increase both maternal and fetal morbidity and mortality [3]. Although the etiology of pre-eclampsia is not yet fully understood, there are studies in the literature showing that oxidative stress plays a role in its pathophysiology [4–7]. The effect of maternal circulation and the endothelial damage caused by the oxidative stress radicals formed in the placenta that is the most significant reason for the pathophysiological changes noted in pre-eclampsia has been shown in previous studies [8–11].

Living organisms have developed complex antioxidant systems in order to reduce the effects of free radicals and eliminate the damage they create. Thiols are a class of organic compounds, also known as mercaptans, which include the sulfhydryl (—SH) group that has a critical role in preventing the formation of any oxidative stress situation in cells [12].

Thiol (—SH) groups may be converted into reversible disulphide (S—S) bond structures by being oxidized by oxidant molecules in the environment [13]. The disulphide bond structures thus formed can be reducted into thiol (—SH) groups again, and thus a thiol-disulphide homeostasis is maintained. This thiol-disulphide homeostasis, which is a recently defined oxidative stress indicator, is of vital importance [14] (Fig. 1).

The contribution of the dynamic thiol-disulphide homeostasis to antioxidant protection, detoxification [15], apoptosis [16], the regulation of enzymatic activity and cellular signal mechanisms [17], and also the pathogenesis of various diseases such as diabetes [18], cancer [19], chronic renal disease, liver disorders [20] and cardiovascular diseases [21] have also been shown.

Currently, there is no method that simultaneously measures the dynamic plasma thiol-disulphide balance by colorimetry [22]. Whereas the double-sided thiol-disulphide balance can only be measured unilaterally since 1979 [23], it can be fully assessed with a new colorimetric method recently developed by Erel & Neşelioğlu that is easy, reliable, sensitive, cheap, fast, highly accurate and repeatable, and which can be operated both manually and fully automatically [17].

In the light of all these studies mentioned, oxidative stress clearly plays a pathophysiological role in pre-eclampsia. In this study we examined whether serum thiol-disulphide homeostasis can be used as an indicator in foreseeing the severity of pre-eclampsia, assuming that disturbed thiol-disulphide homeostasis can be present in severe and mild pre-eclamptic patients when compared to the control group. In this study, we aimed to compare the plasma thiol-disulphide levels in normal healthy, mild and severe pre-eclamptic pregnant women and assess the severity of the disease and the relationship between the homeostatic parameters.

2. Materials and methods

This study was planned as a prospective case controlled study. The informed voluntary consent of all patients was taken. The study protocol was performed according to the principles of the Declaration of Helsinki and was approved by the institutional ethics committee of our University. (Project ID number: 77).

2.1. Study population

The patients included in this study were all within the age range of above 18 years and below 40 years, and with a single live fetus in gestation at week 24 and above. The patients applied to the Sakarya University, Training and Research Hospital, Department of Obstetrics and Gynecology between May 2015 and January 2016.

The blood pressure (BP) of the patients was measured in the sitting position following a resting period of 10 min or more. After resting for 10 min, the patients with high blood pressure were taken for resting and their blood pressure was measured again 6 h later. Those whose systolic blood pressure was $\geqslant$140 mmHg and diastolic blood pressure was $\geqslant$90 mmHg were defined as hypertensive. In the spot urine analysis performed, the patients

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Fig. 1. Thiol disulphide homeostasis.

among whom 1+ and more protein was determined at least twice, were diagnosed with pre-eclampsia and were classified as such in the study [3].

These patients were further assessed as having severe pre-eclampsia where at least one of the following criteria defined by the American Society of Obstetrics and Gynecology was found [3].

- Blood pressure ⩾160/110 mmHg (two measurements made at intervals of at least 6 h).
- Thrombocyte count <100,000/microliter.
- Impaired liver functions shown with abnormally high blood concentrations of liver enzymes, seriously persisting Epigastric or right upper quadrant pain.
- Progressive renal failure (that serum creatinine is >1.1 mg/dl or the serum creatinine level doubles in the absence of renal disease).
- Pulmonary edema.
- New onset cerebral or visual disturbances.

Whereas pre-eclamptic patients that are not included in the severe pre-eclampsia group are regarded as having mild pre-eclampsia, normotensive healthy patients without any systemic disease were included in this study as the control group.

Those pregnant women possessing pre-eclampsia properties and who had tonic-clonic convulsion at least once were regarded as having eclampsia. The case was excluded from this study if another reason for convulsions was determined. Those who have a history of pre-eclampsia in their previous pregnancies, a history of chronic disease and drug use affecting renal and liver functions, chronic hypertension, gestational diabetes mellitus, Type I or Type II diabetes mellitus, connective tissue disease, chronic renal and liver disease, hyper/hypothyroidism, hematologic disease, a baby with chromosomal or congenital fetal anomaly, multiple pregnancies, and who are found to have infection in the spot urinalysis were not included in this study.

2.2. Sample collection

All the patients were hospitalized and monitored in the clinic of our Perinatology Department until delivery. Gestational age was confirmed in all the pregnant women by a routine ultrasonographic examination performed during the first trimester of gestation. The full blood count, blood biochemistry and full urinalysis results that are routinely requested during hospitalization were recorded.

All biochemical analyses (aspartate aminotransferase [AST], alanine aminotransferase [ALT], albumin, total protein, and creatinine) were performed in the biochemical laboratory of Sakarya University, Faculty of Medicine with the use of a fully automatic analyzer (Architect C160000; Abbott Laboratories, Lake Bluff, IL, USA). All dipstick urine analyses were also performed in the same laboratory with the use of an H800 FUS200 Automated Urine Analyzer (DIRUI, serial number: 1300200FUS0117). Test results were determined; U/L (AST, ALT), g/dL (albumin, total protein), mg/dL (creatinine).

Venous blood was drawn from all patients without using anticoagulants for evaluating thiol-disulfide homeostasis after 12 h of fasting, when they were not in the active stage of delivery. All the samples were immediately centrifuged at 1500 rpm for 10 min to separate the plasma. Then, all the plasmas were stored at −80 °C until the analysis. At the end of this study, blood samples were transferred to Yıldırım Beyazıt University, Biochemistry Laboratory in a styrofoam (foam) box containing dry ice or ice batteries that prevent the melting of the serum samples.

2.3. Serum thiol/disulphide homeostasis

The blood thiol-disulphide homeostasis from the samples collected was examined using the spectrophotometric measurement method that was recently developed by Erel & Neselioglu [17]. Measurements were made using a Cobasc 501 instrument (Roche Diagnostics, Mannheim, Germany). Serum thiol/disulphide homeostasis values were presented as mmol/L. The principle reagent of the new assay is sodium borohydride ($NaBH_4$), which is used to reduce the disulphide bonds to form the free functional thiol groups. The sum of existing thiol groups and reduced thiol groups gives the total thiol [(S—S) + (—SH)]. The unused reductant sodium borohydride was consumed and completely removed with formaldehyde. Hence, the extra reduction of 5,5′-dithiobis-(2-nitro benzoic acid) (DTNB) is prevented. The dynamic disulphide amount is a value which can be calculated automatically as a half of the difference between the values of total thiols and the native thiols. After the native thiols (—SH) and total thiols had been determined, the disulphide (—SS) amounts, disulphide/total thiol percent ratios (—SS/—SH + —SS), disulphide/native thiol percent ratios (—SS/—SH), and native thiol/total thiol percent ratios (—SH/—SH + —SS) were calculated. Measurements were made by using an automated clinical chemistry analyzer (Cobas c501, Roche), and the results were presented as μmol/L.

2.4. Statistical analyses

NCSS (Number Cruncher Statistical System) 2007 (NCSS, LLC Kaysville, Utah, USA) software was used for statistical analyses. While evaluating the study data, the conformity of descriptive statistical methods (mean, standard deviation, median, frequency, and percentage), as well as the variables with normal distribution, was assessed with the Kolmogorov-Smirnov test. The One-Way ANOVA test was used in the intergroup comparison of the parameters showing normal distribution, and the Tukey's HDS test was used in *post hoc* evaluations. The Kruskal-Wallis test was used in the intergroup comparisons of those variables not showing normal distribution, and the Mann-Whitney *U* test was used in their *post hoc* comparisons. The Yates Continuity Correction, Fisher's Exact test and the Fisher-Freeman-Halton test were used in the comparison of qualitative data. Diagnostic screening tests (sensitivity, specificity, PKD, NKD) and ROC curve analysis were used in the cut-off determination for variables. The results were found to be within the confidence interval of 95% and the significance level of $p < 0.05$.

3. Results

A total of 99 pregnant women were enrolled in the study: 32 severe pre-eclampsia, 30 mild pre-eclampsia patients and 37 healthy pregnant controls. The baseline characteristics of the severe, mild pre-eclampsia patients and controls are given in Table 1. There was no statistically significant difference among age, gravida and parity between the groups ($p > 0.001$). Statistically significant differences among systolic, diastolic, and mean arterial BP, serum