Investigate the Relationship Between Serum Levels of Homocysteine, Oxidative Stresses and Lipid Profiles in Women with Pre-Eclampsia.

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ABSTRACT
Pre-eclampsia is a syndrome which is characterized by the increase in blood pressure to above 140/90, oedema and proteinuria, and is the second leading cause of maternal death in developing countries, including Iran. A total of 5% of pregnant women are affected worldwide. Oxidative stress is one of the key factors involved in the development of pre-eclampsia. The present study aims to investigate the effect of the concentration of homocysteine, oxidative stress and lipid profiles on pre-eclampsia. This case-control study was conducted on healthy pregnant women visiting Taleghani and Al Zahra hospitals in Tabriz, Iran, as well as on women with pre-eclampsia admitted to these two hospitals. Fifty-eight samples were selected as controls and 58 subjects were selected as cases. The blood samples were prepared with their contents. Parameters measured included lipid profiles (total cholesterol, triglyceride, LDL-c and HDL-c), MDA as a marker of lipid peroxidation, homocysteine concentration in blood, glutathione peroxidase (GPX) enzymes, superoxide dismutase (SOD) and the total antioxidant status (Canakci et al.). In addition, a questionnaire was completed to collect information on age, gestational stage, height, weight, weight before pregnancy and folic acid (B9) intake. The results obtained in this study are based on the inhibition of confounding variables and indicate significant differences in homocysteine status, total antioxidant status (Canakci et al.) and TG in both groups. However, there was no significant difference in the concentration of GPX-SOD enzymes, OSI index, SOD and MDA.

INTRODUCTION
Pre-eclampsia is a syndrome which is characterized by the increase in blood pressure above 140/90, oedema and proteinuria, and is one of the leading causes of maternal death and of complications in pregnancy that can occur in the second half of pregnancy [26] Approximately 20% of pregnant women in developing countries and 5% of pregnant women worldwide are affected [59], and it is the second leading cause of maternal death in developing countries, including Iran [42,38,19,13,45]. Oxidative stress is one of the most important factors in pre-eclampsia; this is a special type of chemical stress caused by an imbalance between production of free radicals and their consumption by organisms during vital mechanisms. It is associated with risks because it does not cause any symptoms and so diagnosis with conventional means is difficult [41,22].

All chemical reactions in the body use respiratory oxygen, but chemical reactions use these to produce free radicals that in excessive amounts lead to toxicity and oxidative damage, targeting vulnerable organs such as the...
polyunsaturated fatty acids’ fragile membrane, the thiols in proteins and DNA nucleic acids [60]. Therefore, the determination of free radicals is an indicator of oxidative stress that is in direct proportion to the organism’s condition, as oxidative stress plays an important role in homocysteine metabolism [49,24,25]. Conditions of oxidative stress include lipid peroxidation, which is one of the autocatalytic mechanisms leading to oxidative damage to the cell membranes [5,40]. Oxidative stress is involved in aging and in chronic diseases such as diabetes and artherosclerosis; diets rich in antioxidants such as vitamin C and carotenoids are associated with reduced risk of these diseases [31,36].

ROS (reactive oxygen species) in biological systems are constantly produced by normal metabolism in the body and are necessary for keeping biological balance through various functions. However, excessive production of these ROS causes damage to the molecules necessary for the structure and function of the cell. Production of ROS such as anion superoxide (-•O2), hydroxyl radical (HO•), alkyl peroxide Radical (•ROO) and alcoxy radicals (• RO) can be enzymatic and non-enzymatic. Mitochondria are the most important cell organelles for the production of radicals, especially •-O2 and H2O2 in mammals [35,27,61].

Lipid peroxidation products are used as biomarkers for oxidative stress measurements in biological systems. They include thiobarbituric acid or isoprostanes, among other compounds, which have a short carbonyl chain and can be used as a biomarker because reactions between free radicals and polyunsaturated fatty acids are caused. Among the toxic products of lipid peroxidation, malondialdehyde MDA, 4-hydroxy nonenal (4-HNE) and 2-alkenal can be noted as oxidative stress biomarkers [15,23,34].

Homocysteine (2-amino-4-mercapto-butric acid), is a sulphur-containing amino acid without special codon and is created by an essential amino acid methionine demethylation from the diet. Homocysteine has sulphhydryl group, which makes it more susceptible to other thiol oxidation and disulphide formation [32,48,9,29]. Homocysteine by superoxide anion (O2•-) resulting from the oxidation, leads to lipid oxidation in low-density lipoprotein and LDL [11,55,2]. Also, oxidized homocysteine produces thiolactone, which causes acetylation of extracellular proteins such as apoB and LDL lipoprotein. Nitrogen guanidine-L-arginine atoms’ oxidation effects are caused by nitric oxide synthase NO enzyme, which has vasodilatory effects including on the mode of inhibition of oxidative homocysteine in which L-arginine converts into L-citrulline. During homocysteine oxidation with nitric oxide, the nitric oxide’s stronger oxygen radical is deactivated and single oxygen (O) and nitrite proxy (ONO-) are produced [54,20,28]. The human body has antioxidant barriers against oxidative stress that either prevent the formation of free radicals, or inhibit the formation of the auto-oxidation chain. There are also antioxidant enzymes such as superoxide dismutase (SOD) or glutathione peroxidase (GPX) that cause hydroperoxides and hydro-lipid peroxide to revive the water and the alcohol [53,37]. On the other hand, the measurement of the total antioxidant capacity of plasma TAS will vary depending on a person’s diet. The total capacity of water-soluble antioxidants such as vitamin C, uric acid, thiol groups of proteins and lipid-soluble antioxidants, such as tocopherols and carotenoids, can be obtained [46,1,52].

The present study aims to examine the relationship between serum levels of homocysteine, oxidative stress and lipid profiles in women affected by pre-eclampsia.

MATERIALS AND METHODS

Sample Selection:

This case-control study was conducted based on conscious informed consent of Medical Ethics Committee on 116 pregnant women admitted to teaching hospitals in Tabriz (Taleghani and Al Zahra hospitals) in 2014 and was based on objective selection criteria for participation in the study: not smoking materials drugs, lack of diseases such as diabetes, heart disease and digestive disease, no history of stroke, asthma or thyroid disorders, and not taking medication. The sample included 58 patients with pre-eclampsia; the pre-eclampsia criteria were blood pressure higher than or equal to 140/90 and proteinuria higher than 300 mg per 24 hours’ urine. Other questions were asked, such as age, the gestational stage, parity, height, weight before pregnancy, weight at hospitalization and folic acid intake. After getting permission from Tabriz University of Medical Sciences and introduction letters to the hospitals, the admitted women selected were informed of the study and a participation satisfaction and consent questionnaire was completed by them. To assess the condition of the samples, tests for superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), total antioxidant status [10], low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides total cholesterol (TC), homocysteine (hcy) and oxidative stress index were performed. Fifty-eight pregnant women in the gestational stage of the second half of pregnancy who were referred to the hospital for routine tests were selected as a control group to identify confounding or interfering parameters.

Blood Samples:

Full blood samples were taken over three months from two hospitals affiliated with Tabriz University of Medical Sciences. Samples and centrifuged serums between this time period and the measuring time were frozen at a temperature of -20°.
Homocysteine Measurement:

The ELISA method was used to measure homocysteine levels. In this method, the separation of free decreased homocysteine to three thiols and then converted it to SAH. After removal of anti-SAH antibody, monoclonal antibody labelled with peroxidase was added, and peroxidase activity of the antioxidant enzymes glutathione peroxidase and superoxide associated with homocysteine concentration was measured using a spectrophotometer absorbed in the samples.

Measuring SOD and GPX:

To measure glutathione peroxidase (GPX), the UV method was used. The method is based on that of Valentine and Paglia. For GPX enzymatic measurements, a method introduced which consists of 4 mmol/L glutathione; glutathione reductase, NADPH 34 mmol / L phosphate buffer to a 5 0/0 mmol/L; 4.3 mmol/L of EDTA; and hydroproxy, 0.81 mmol /L. In addition, a vial of reagent with an appropriate volume of buffer containing 6.5 ml for Kit (RS 504) and 10 ml for Kit (RS 505) and 30 ml for Kit (RS 506) are mixed for 48 hours at 2-8 °C or 8 hours at 15-25 °C were stored, and 50/0 ml of all blood heparin was diluted with 1 ml of reagent and incubated for 5 min. Then, 1 ml of the diluted reagent is incubated for 5 min and 1 ml of Drabkin’s reagent is added in the presence of glutathione oxidized glutathione reductase, which quickly causes a revival mode. Next, the decreased rate in the absorption intensity at 340 nm wavelength is measured, which indicates GPX activity. To measure SOD, after washing the total blood four times with 0.9 % NaCl in a 3 ml wash. Each time after washing, it was centrifuged and mixed with cold water for 15 minutes at 40 °C and then mixed with 0.01 mmol/L phosphate buffered saline. The reagents used include xanthine 0.05 mmol Int, to a value of 0.025 mmol/L, a buffer containing CAPS at an amount of 40 mmol/L and EDTA to the amount of 0.94 mmol/L, and xanthine oxidase at 80 μmol based on standard dilution, which reacted with INT producing red formazan. Superoxide dismutase activity is measured by the degree of inhibition of this reaction (Enquobahrie et al., 2004)) an auto-analyser device was used for the measurement of these two enzymes.

Lipid Profile Measurement:

Lipid profiles were measured enzymatically by auto-analyser device.

After preparing the reagent, 10 microlitres of serum was mixed with 1 mL reagent and put in a water bath at 37 °C for 15 minutes. After examining the optical density, we read it at a wavelength of 520 nm against the blank reagent and using mathematical formulas, we calculated their levels. To calculate HDL value, 500 microlitres of serum was centrifuged, then 20 microlitres of the stuffing clear supernatant was removed and mixed with 1 ml prepared solution. To prepare standard samples, 20 microlitres of standard calibration solution was mixed with 1 ml ready-mixed. Both are put in a water bath at 37 °C for 15 minutes. Then, the optical absorption and standard are calculated at a wavelength of 520 nm against the blank reagent and HDL levels; then LDL values are calculated by the William Firedewald formula.

\[
LDL = TC - (HDL - \frac{TG}{5})
\]

Malondialdehyde Measurement:

First, 500 microlitres is solved in 3 ml of 1 % solution of phosphoric acid and aftervortexing, 1 ml thioharbituric acid (0.67 %) was added to the test tube and after a complete vortex placed in a boiling water bath for 45 minutes. Then the test tubes were cooled down under cold water and 3 ml normal butanol was added and vortexed for one to two minutes and centrifuged for 10 minutes with 3000 rpm. After removing the supernatant, it is measured at an absorbance of 532 nm wavelength, versus normal butanol as a blank, using a spectrophotometer. The results are transferred to a standard curve, and then the samples’ serum MDA concentration is determined.

Total Antioxidant Status (Canakci et al.) Measurement:

Repaginated serum is used to measure TAS, as well as a reagent containing 80 mmol/L with ferrophosphatechromogen, 6.1 ABTS– mmol/lit with a value of 610 micromoles per litre – sub strove 250 μmol lit, H2O2, which is standard. This method is incubated by 2,2-Azino-di-[ 3 –ethylbenthiazolinesulphonate] with peroxidase and H2O2 to produce the radical cation ABTS R +, which is stable in blue-green. The colour intensity is measured at 600 nm and the sample antioxidant may suppress the production of the colour in order

\[ A_{600} = B - C \]
to fit the antioxidants’ concentrations. The auto-analyser was also used to measure TAS. OSI index calculation

Statistical analysis:

Statistical analysis was performed with SPSS version 18.0 software. Statistically comparisons were between
the control group and cases. Differences between mean values were carried out using one-way analysis of
variance. Data are represented Mean ± SEM. The differences were considered significant when *P < 0.05.

Results:

The following table shows details of the two groups’ average concentration parameters as measured by the
method above.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean in control</th>
<th>Mean in case</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCY (ng/l)</td>
<td>7.458±3.667</td>
<td>11.12±4.511</td>
<td>0.005</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>2.97±1.40</td>
<td>2.76±1.51</td>
<td>0.393</td>
</tr>
<tr>
<td>GPX (Unit /gr Hb)</td>
<td>63.77±16.10</td>
<td>74.84±17.67</td>
<td>0.52</td>
</tr>
<tr>
<td>SOD (Unit /gr Hb)</td>
<td>1.33±376</td>
<td>1.35±361</td>
<td>0.795</td>
</tr>
<tr>
<td>OSI (mmol/l)</td>
<td>2.83±1.49</td>
<td>2.16±1.31</td>
<td>0.082</td>
</tr>
<tr>
<td>MDA/GPX</td>
<td>0.51±0.3</td>
<td>0.36±0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>MDA/SOD</td>
<td>0.002±0.001</td>
<td>0.002±0.005</td>
<td>0.859</td>
</tr>
<tr>
<td>TAS (mmol/l)</td>
<td>1.07±0.236</td>
<td>1.43±0.367</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

HCY: homocysteine, MDA: malondialdehyde, GPX: glutathione Peroxidase, SOD: super oxide dismutase,
TAS: total antioxidant status. OSI: oxidative stress index

All the data was compared with control groups through ANOVA tests. Note. Data are represented Mean±
SEM. The differences were considered significant when *P < 0.05.

As can be seen, there are significant differences only in the homocysteine and total oxidant capacity
variables among the groups of women with and without pre-eclampsia, while there are no significant differences
in terms of other variables.

As shown in the figure 1, there are significant differences in serum homocysteine concentration among the
groups of women with and without pre-eclampsia, so that women with pre-eclampsia (11.12±4.511) show
higher homocysteine levels than women without pre-eclampsia (7.458±3.667) (P<0.05).

Fig. 1: Mean of homocysteine concentrations between the groups of women with and without pre-eclampsia.
The differences were considered significant when *P < 0.05. Homocysteine concentration (ng/l).
Fig. 2: Mean total antioxidant capacity (Canakci et al.) in the two groups (controls and cases)

The differences were considered significant when *P < 0.05. TAS: total antioxidant capacity (mmol/l)

As also shown in this diagram, there is another significant difference between the two groups, which is that the women with pre-eclampsia (1.43+0.376) show greater total antioxidant capacity than women without pre-eclampsia (1.07+0.236) (P <0.002). Here the age of pregnancy and folic acid intake were considered confounding factors that were inhibited, while other variables did not show any confounding effects.

Table 2: Mean lipid profile concentrations measured, including total cholesterol, triglycerides, LDL-C and HDL-C in controls and cases.

<table>
<thead>
<tr>
<th>Me in control(mg/dl)</th>
<th>Me in case(mg/dl)</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL 55.22+8.230</td>
<td>55.30+11.462</td>
<td>0.848</td>
</tr>
<tr>
<td>LDL 167.861+51.06</td>
<td>186.20+74.933</td>
<td>0.333</td>
</tr>
<tr>
<td>TG 224.44+75.133</td>
<td>320.45+114.185</td>
<td>0.000</td>
</tr>
<tr>
<td>TC 52.882+250.37</td>
<td>55.924+262.96</td>
<td>0.354</td>
</tr>
</tbody>
</table>

HDL: High density Lipoprotein, LDL: Low density lipoprotein TG: Triglycerides TC: Total cholesterol all the data was compared with control groups through ANOVA tests. Note. Data are represented Mean± SEM. The differences were considered significant when *P < 0.05.

As Table 2 shows, there is a significant difference in triglycerides variables in the parameters related to the lipid profiles of the groups of women with pre-eclampsia and without pre-eclampsia (P <0.05), so that the women with pre-eclampsia (320.45+114.18) show higher triglyceride levels than women experiencing a normal pregnancy (224.44+75.13) (P <0.05).

Fig. 3: The mean triglyceride concentration in both groups of controls and cases

The differences were considered significant when *P < 0.05.

Discussion:

Pre-eclampsia or gestational hypertension is a multifactorial disorder that increases the sulphur-containing amino acid homocysteine. The aetiology is of general interest, since homocysteine is a marker of vascular disease [51]. Some foods contain antioxidants, including vitamins C-E and folic acid, which reduce
homocysteine and pre-eclampsia [43,50,14]. Some researchers have studied the effect of reducing the amount of antioxidant enzymes in the oxidative stress conditions involved in pre-eclampsia [7], and believe that in times of oxidative stress, lipid per oxidation and pre-eclampsia increase; that is, under the effects of genes related to the expression of renin, angiotensin and aldosterone [56]. This leads to an increase in malondialdehyde as a biomarker of lipid per oxidation in women with pre-eclampsia compared to women with normal pregnancies [10]. Based on the results of recent research, there is a significant increase in homocysteine levels in women with pre-eclampsia; however, there were no significant differences between the two groups. It seems that special care given to women with pre-eclampsia admitted to the hospital could control oxidative stress and reduce lipid peroxidation, since the level of malondialdehyde production is affected by total and systemic oxidative conditions [4]. Moreover, the malondialdehyde levels in hospitalized women with pre-eclampsia are reduced by magnesium infusion because it reduces lipid peroxidation [57]. There was no significant difference regarding glutathione peroxidase antioxidant enzyme or superoxide dismutase between the two groups; this is probably due to hospital care and medication. The results of some studies on glutathione peroxidase show decreased enzyme in women with pre-eclampsia and suggest that glutathione peroxidase activity inhibition causes increased production of lipid peroxide and thrombaxane, as well as increasing the ratio of thrombaxane to prostacyclin, while the presence of glutathione peroxidase limits prostaglandin synthetase activity and reduces the peroxide anions [33,58]. However, the increased homocysteine level reduces the amount of antioxidant enzymes, including key elements in maintaining cellular homeostasis and reducing oxidative damage [30,3]. In general, antioxidant enzymes such as superoxide dismutase and glutathione peroxidase are used to eliminate free radicals from lipid peroxidation in pre-eclampsia. Oxidative stress reduces antioxidant enzymes [17]. In the present study, the results for the TAS show a significant difference between women admitted to hospital and pregnant women without pre-eclampsia, which can be attributed to the type and amount of antioxidants in the food served in the hospital, which enhances the TAS levels in women with the condition. Also, there was no significant difference regarding the amount of OSI (MDA/TAS), MDA/GPX or MDA/SOD found in some studies of TAS reduction in women with pre-eclampsia compared with normal pregnant women [21]. The use of proper antioxidants to enhance total antioxidant capacity and reduce vascular disease is recommended [8]. Tryptophan-fortified cereals are factors that enhance TAS [39]. Studies of the effects of tea and mint, and other herbal extracts, on TAS in women with pre-eclampsia show they reduce oxidative stress and increase TAS in both mother and foetus [47]. Results show significant differences regarding the lipid profile in the triglycerides between the two groups, but there was no significant difference between LDL-c, HDL-c, total cholesterol or triglyceride levels. Some studies in pregnant women have proposed the triglyceride level as a prognostic factor for pre-eclampsia [12] and found more drastically reduced HDL levels and more significantly increased TG in cases of severe pre-eclampsia than in mild pre-eclampsia, with an effective lipid profile in endothelial dysfunction and pre-eclampsia incidence. Pre-eclampsia can be prevented in certain ways [44,6]. Other researchers indicate that there is no significant relationship between total cholesterol, HDL-c, LDL-c and triglycerides between the groups with and without pre-eclampsia in terms of the effect of the lipid profile on the aetiology of pre-eclampsia [16].

Conclusion:

Increased homocysteine can exacerbate oxidative stress effects in women with pre-eclampsia. Proper nutrition and a diet rich in antioxidants increases the rate of TAS, which can reduce oxidative stress’s harmful effects, as well as lipid peroxidation and its biomarkers, such as MDA. Moreover, the triglyceride level as well as homocysteine can also be used as a predictive factor for pre-eclampsia.

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Conflict of Interest:

The authors have declared no conflicts of interest.

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