Abstract. – AIM: There are many studies evaluating the role of inflammation in the pathogenesis of preeclampsia. However, little is known about the relationship between the severity of inflammation and the severity of preeclampsia due to insufficient of studies reporting this matter. To investigate the maternal serum concentrations of IL-6, TNF-alpha and Neopterin in patients with mild preeclampsia and severe preeclampsia and HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome in preeclampsia and determine their association with the severity of the disease.

PATIENTS AND METHODS: Patients, hospitalized with the diagnosis of preeclampsia between October 2011 and March 2012, were included in the study. The patients with preeclampsia were divided into three groups as mild preeclampsia, severe preeclampsia and HELLP syndrome. The control group was comprised of normotensive and uncomplicated pregnant women. The serum levels of IL-6, TNF-alpha and Neopterin (NEO) were determined, using enzyme-linked immunosorbent assay. Spearman’s rank correlation tests were used for the correlations between the serum levels of inflammatory markers and the severity of preeclampsia.

RESULTS: There was no observed significant difference among mean serum TNF-alpha and IL-6 levels of four groups (p > 0.05). The median serum concentration of NEO in subjects with mild preeclampsia of 14.1 nmol/L and severe preeclampsia of 14.8 nmol/L was significantly higher than that of 10.3 nmol/L in normotensive controls (p = 0.013; p = 0.000 respectively). In addition, the median serum concentration of NEO was detected to be highest in subjects with HELLP syndrome. The serum levels of NEO was well correlated with the severity of preeclampsia (r = 0.533, p = 0.000).

CONCLUSIONS: The serum levels of NEO, an important marker of cellular immunity, associated with severity of disease in patients with preeclampsia.

Key Words: Preeclampsia, HELLP syndrome, IL-6, Tumor necrosis factor-alpha, TNF-α, Neopterin.

Abbreviations

NEO = neopterin
BMI = Body mass index

Introduction

As a systemic disorder unique to pregnancy, preeclampsia affects 3-14% of pregnant women. The disease is a major cause of maternal mortality (15-20% in developed countries), morbidities (acute and long-term), perinatal deaths, preterm birth, and intrauterine growth restriction. Redman et al first suggested that preeclampsia arises as a result of an excessive maternal inflammatory response to pregnancy. There are many studies evaluating the role of inflammation in the pathogenesis of preeclampsia. However, little is known about the relationship between the severity of inflammation and the severity of preeclampsia due to insufficient of reports investigating this matter. Additionally, none of those studies evaluated the patients with HELLP syndrome as a separate group.

Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α), which are an important marker of acute phase response, were secreted, by activated T lymphocytes and macrophages during inflammation, respectively. Neopterin (NEO), secreted by activated monocytes, is an important indicator of cellular immunity. The aim of this study was to determinate the maternal serum concentrations of IL-6, TNF-α and NEO in patients with mild preeclampsia and severe preeclampsia and HELLP syndrome and evaluate their association with the severity of the disease.
Materials and Methods

Study-Design

This study was carried out at Dicle University Hospital Department of Obstetrics and Gynecology. The study protocol was approved by the Medical Ethics Committee of the Dicle University. Dicle University Hospital is a 1400-bed referral Hospital in Diyarbakir city center, and it is the largest tertiary care health center serving five provinces in the southeast region including approximately 2.5 million people. Other hospitals in the region have taken part in the treatment of patients with preeclampsia, but the patients with severe preeclampsia and HELLP syndrome have referred to our Clinic. The patients, hospitalized with the diagnosis of preeclampsia between October 2011 and March 2012, were included following informed consent. Clinical findings and laboratory test results of voluntary subjects were also recorded.

Exclusion criteria were illicit drug use, preexisting medical conditions such as chronic inflammatory diseases (systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, etc.), acute inflammatory diseases (tonsillitis, urinary tract infections, chorioamnionitis, etc.), cardiovascular disease, diabetes mellitus and renal disease.

Preeclampsia, severe preeclampsia and HELLP syndrome were determined as defined by the American College of Obstetricians and Gynecologists guidelines. Preeclampsia was defined hypertension after 20 weeks of gestation and proteinuria. Proteinuria is defined as the presence of 0.3 g/L protein in a 24 hour urine specimen. Severe preeclampsia was determined based on the presence of one or more of the following criteria: high blood pressure (160 mm Hg systolic or higher or 110 mm Hg diastolic or higher), Proteinuria (5 g/L or higher in a 24 hour urine specimen or 3+ or greater on two random urine samples), Oliguria (500 mL/24 h), Cerebral or visual disturbances, Pulmonary edema or cyanosis, epigastric or right upper-quadrant pain, impaired liver functions, and fetal growth restriction. HELLP syndrome was diagnosed by laboratory abnormalities including hemolysis, elevated liver enzymes, and low platelet counts. The patients with preeclampsia were divided into three groups as mild preeclampsia, severe preeclampsia and HELLP syndrome. Patients who had mild preeclampsia (n = 22), severe preeclampsia (n = 20) and HELLP syndrome (n = 20) were included in this study.

The control group was comprised of 24 uncomplicated pregnant women matched for age, gestational, age, weight, and parity. All pregnant women in the control group had a singleton pregnancy and were normotensive and non-proteinuric throughout pregnancy, with intact membranes.

Sample Collection and Biochemical Analyses

Blood samples of all recruited subjects were collected just before starting treatment. All collected blood samples were immediately centrifuged at 4000 rpm and +4°C for 10 min and then collected sera were transferred into Eppendorf tubes. Samples were transferred on ice and kept at 70°C in deep freeze until they were analyzed. Serum neopterin, IL-6 and TNF-α levels were determined using enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Neopterin; DRG Diagnostics International, Inc., Mountainside, NJ, USA) (IL-6 and TNF-α; DIA Source, Nivelles, Belgium). Intra and inter assay coefficients of variation ranged from 3 to 7% for Neopterin, IL6 and TNF alfa.

Statistical Analysis

Means and standard deviations were used to describe numerical variables. Kolmogorov-Smirnov test was used to evaluate the distribution pattern of the data. Kruskal-Wallis test was used for comparison of four groups. Mann-Whitney U-test was used for comparison of two groups. The correlation coefficient was determined by the Spearman’s rank correlation test. The statistical package SPSS for Windows 15.0 (Statistical package for social sciences; SPSS Inc., Chicago, IL, USA) was used to analyze the data. The values of $p$ less than 0.05 were accepted as statistically significant.

Results

Demographic and clinical characteristics of the study patients are presented in Table I. No significant difference was found in the mean age, BMI, serum creatinine levels and gestational age at the time of diagnosis among the four groups ($p > 0.05$). Rates of nulligravidity were significantly lower in the control group than the other groups ($p < 0.05$).

The median serum concentrations and ranges of NEO, IL-6 and TNF-α in control and preeclampsia groups are shown in Table II. There was no sig-
**Table I.** Clinical and laboratory characteristics of the cases in the groups.

<table>
<thead>
<tr>
<th></th>
<th>Uncomplicated pregnant (n = 24)</th>
<th>Mild preeclampsia (n = 22)</th>
<th>Severe preeclampsia (n = 20)</th>
<th>HELLP syndrome (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.3 ± 6.9</td>
<td>30.3 ± 6.3</td>
<td>29.2 ± 5.8</td>
<td>28.1 ± 6.9</td>
</tr>
<tr>
<td>Gravida</td>
<td>4.3 ± 2.7</td>
<td>3.1 ± 1.7</td>
<td>4.2 ± 3.2</td>
<td>3.7 ± 2.9</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>4 (14.3)</td>
<td>7 (33.3)*</td>
<td>8 (40)*</td>
<td>9 (45)*</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>34.7 ± 4.1</td>
<td>34.3 ± 2.8</td>
<td>32.7 ± 3.6</td>
<td>32.4 ± 3.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 ± 2.8</td>
<td>31.1 ± 5</td>
<td>28.3 ± 4</td>
<td>27.55 ± 5.2</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.63 ± 0.10</td>
<td>0.58 ± 0.07</td>
<td>0.62 ± 0.13</td>
<td>0.66 ± 0.12</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>30.9 ± 42.2</td>
<td>19 ± 13.7</td>
<td>51.1 ± 38.6</td>
<td>160.7 ± 118.4**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>42.3 ± 82.1</td>
<td>27.6 ± 14.6</td>
<td>58.9 ± 58.3</td>
<td>239.5 ± 193.4**</td>
</tr>
<tr>
<td>Platelets (K/µL)</td>
<td>238.3 ± 68.5</td>
<td>266.1 ± 79.2</td>
<td>189.8 ± 73.9</td>
<td>121.1 ± 66.6**</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>290.5 ± 284.8</td>
<td>329.3 ± 111.4</td>
<td>467 ± 193.8</td>
<td>881.8 ± 476.5**</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76.9 ± 16</td>
<td>91.1 ± 9.2*</td>
<td>106.6 ± 7.7*</td>
<td>106.6 ± 7.2*</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3089 ± 668.5</td>
<td>2406 ± 838.4</td>
<td>2082 ± 904.0*</td>
<td>1877 ± 635.3*</td>
</tr>
<tr>
<td>Low Apgar scores (&lt; 6) at 5 minute (%)</td>
<td>0</td>
<td>22.2*</td>
<td>50*</td>
<td>53.3*</td>
</tr>
</tbody>
</table>

BMI = Body mass index; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; *p < 0.05 compared with uncomplicated pregnant; **p < 0.05 compared with the others groups.

significant difference in serum TNF-α and IL-6 levels of the four groups (p > 0.05). The median serum concentrations of NEO in subjects with mild preeclampsia of 14.1 nmol/L and severe preeclampsia of 14.8 nmol/L were significantly higher than that of 10.3 nmol/L in normotensive controls (p = 0.013; p = 0.000 respectively). In addition, the median serum concentration of NEO was detected to be highest in subjects with HELLP syndrome when compared to normotensive controls and patients with mild preeclampsia and severe preeclampsia (p = 0.000; p = 0.023; p = 0.045 respectively). The serum NEO levels of four patients with mild preeclampsia were found to be higher than the mean serum NEO levels of the cases with HELLP syndrome.

The serum levels of NEO was well correlated with the severity of preeclampsia (r = 0.533, p = 0.000) when the possible relationship between serum levels of inflammatory markers (IL-6, TNF-α and NEO) and the severity of the disease were evaluated. The distribution of serum levels of NEO in the groups and the relationship between serum NEO concentration and the severity of preeclampsia are presented in Figure 1.

**Discussion**

In this study, there were no significant differences in the serum levels of IL-6 and TNFα between the groups. The levels of TNF-α and IL6 in preeclampsia are controversial in literature. Vince et al12 reported elevated serum levels of IL6 and TNF-α in patients with preeclampsia. In another study, the high serum levels of IL-6 were detected only in patients with severe preeclampsia7. Afshari et al13 didn’t find a significant differ-

**Table II.** Serum levels of Neopterin, TNF-α, IL-6 in uncomplicated pregnancies and the patients with mild preeclampsia, severe preeclampsia and HELLP syndrome.

<table>
<thead>
<tr>
<th></th>
<th>Uncomplicated pregnant (n = 24)</th>
<th>Mild preeclampsia (n = 22)</th>
<th>Severe preeclampsia (n = 20)</th>
<th>HELLP syndrome (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin (nmol/L)</td>
<td>10.3 (6.5-15.1)</td>
<td>14.16 (7.2-27.9)*</td>
<td>14.8 (7.6-24.6)**</td>
<td>19.1 (8.5-31.5)***</td>
</tr>
<tr>
<td>TNF-α (ng/dl)</td>
<td>7.05 (3-24.4)</td>
<td>9.5 (4.1-35.5)</td>
<td>7.7 (3.4-20.3)</td>
<td>7.7 (3.7-41.5)</td>
</tr>
<tr>
<td>IL-6 (ng/dl)</td>
<td>44.3 (28.7-211.9)</td>
<td>50.2 (24.2-248.1)</td>
<td>55.7 (34.2-263.4)</td>
<td>52.4 (16.7-274.6)</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with uncomplicated pregnancy; **p < 0.01 compared with uncomplicated pregnancy; *p < 0.05 compared with mild preeclampsia or severe preeclampsia.
ence in the serum levels of TNF-α between the groups. In contrast, Founds et al. detected elevated the serum levels of TNF-α in the patients with preeclampsia. The significant difference between the two studies is due to the values of inter-assay coefficient of variation. The overall inter-assay coefficient of variation for TNF-α in those study were 6.9% and 13.4% respectively. In this study, intra and inter assay coefficients of variation ranged from 3 to 7% for IL6, TNF-α and NEO. Another important difference in this study is that the patients with HELLP syndrome were evaluated as a separate group.

Freeman et al. reported a study investigating long and short term changes in serum levels of inflammatory markers in patients with preeclampsia. In this report, no significant difference has been found in the levels of TNF-α between normal pregnant women and patients with preeclampsia in third trimester. However, the patients with preeclampsia in the first trimester had higher levels of inflammatory markers. In the pathogenesis of preeclampsia, humoral immune response is followed by cellular immunity triggered by the serum pro-inflammatory cytokine levels. Neopterin, an important marker of cellular immunity, is recommended for the recognition of chronic inflammatory diseases such as sarcoidosis, tuberculosis and brucellosis. However, in this chronic inflammatory disease, acute phase reactants (CRP, etc.) and pro-inflammatory cytokines (IL6 and TNF-α, etc.) doesn’t provide useful diagnostic and follow-up treatment. In this study, the relationship between high levels of serum neopterin and the severity of preeclampsia was determined. However, the same relationship was not found in the levels of serum pro-inflammatory cytokines (TNF-α and IL6). All of these results suggested that serum levels of pro-inflammatory cytokines in the pathogenesis of preeclampsia are transient but cellular immunity, triggered by cytokines, has continued.

Previously, von Versen-Hoeynck et al. had reported that patients with preeclampsia had higher serum NEO levels than healthy pregnant women. In this study, serum levels of NEO were statistically significant different between the groups of mild and severe preeclampsia and control group. Furthermore, patients with HELLP syndrome have the higher serum levels of NEO than patients with mild and severe preeclampsia.

It has been reported that serum NEO levels are influenced by some factors such as age, body mass index (BMI), serum creatinine levels, and gestational age. In this research, no significant difference was found among the four groups of patients.
when evaluated in terms of these factors. Based on these findings, it can be claimed that the data of this study was not affected by the factors affecting the metabolism of NEO.

The detection of severe inflammation as that of patients with HELLP syndrome in four patients with mild preeclampsia, and the detection of non-normal distribution of the serum levels of NEO between the groups have increased the value of our work designed according to clinical classification. Currently, the evaluation of patients with preeclampsia is recommended according to clinical findings. The detection of severe inflammation may contribute to clinical evaluation of patients with preeclampsia.

Redman et al conducted one of the first studies regarding significance of endothelial damage in the pathogenesis of preeclampsia. The endothelial damage is evident especially in patients with severe preeclampsia and HELLP syndrome. There are many studies regarding cytokines responsible for the endothelial damage in the pathogenesis of preeclampsia. However, there are a few studies investigating changes in serum levels of cytokines induced by the severity of preeclampsia.

The necessity of terminating the pregnancy is related with severity of findings preeclampsia. The decision of delivery can be taken easily in preeclamptic pregnant with gestational age greater than 34 weeks. However, in smaller gestational age, the timing of the decision to birth is important for the viability of the fetus. It should be noted that the patients with clinically mild preeclampsia may turn into severe disease.

Currently, unfortunately, there isn’t an effective method derived from the studies investigating the products (cytokines, etc.) of the histopathological changes in preeclamptic process may provide a more successful clinical follow-up of these patients.

Conclusions

The serum levels of NEO which reflects the cellular immunity is increased with the severity of preeclampsia.

Acknowledgements

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References


