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Comparison of IMA, YKL-40, EN-RAGE, and AIM levels in maternal blood and cord blood in patients with preeclampsia

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ABSTRACT

Aim: Preeclampsia and severe preeclampsia are among the most significant causes of maternal mortality. Preeclampsia's pathogenesis is not fully understood, and it is a disease with early diagnosis and treatment possibilities. In this study, we aimed to investigate the levels of IMA, YKL-40, EN-RAGE, and AIM in maternal and cord blood. The results will ideally shed light on preeclampsia's pathogenesis and early diagnosis.
Methods: The study was conducted with the following three groups: a severe preeclampsia group (group 1), a preeclampsia group (group 2), and a control group (group 3). IMA, YKL-40, EN-RAGE, and AIM levels were measured in all patients across the groups using blood taken from the mothers before delivery and from the cords during delivery. Statistically descriptive analyses were performed. Specifically, a one-way analysis of variance was performed on group variables, and a Tukey test was used to determine the differences between the groups.
Results: The mean age was similar across all groups. The gestational week at delivery was low for the severe preeclampsia group (p=0.001). The IMA and YKL-40 levels in the maternal and cord blood were the same between the groups. The EN-RAGE levels in the maternal blood were found to be significantly higher in the control group (p=0.000). While the AIM levels in the maternal blood were high in the control group (p=0.001), they were significantly lower in the cord blood in the control group (p=0.029).
Conclusion: EN-RAGE and AIM levels are parameters that can be used in the early diagnosis of preeclampsia and severe preeclampsia.

Key words: Preeclampsia, IMA, YKL-40, EN-RAGE, AIM.

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Introduction

Preeclampsia (PE) is defined as high blood pressure that starts after the 20th week of pregnancy and that is accompanied by end-organ findings. Its incidence in pregnancy has been reported to be 2 to 8% worldwide [1]. Risk factors for PE include a previous history of PE, chronic hypertension, pre-pregnancy diabetes, multiple pregnancies, nulliparity, advanced maternal age, the use of assisted reproductive techniques, chronic kidney disease, and autoimmune diseases such as antiphospholipid syndrome and systemic lupus erythematosus

[2,3]. Its pathogenesis has not been fully elucidated. However, researchers believe that there is abnormal placental vascular development during early pregnancy. This results in insufficient placental perfusion, hypoxia, and ischemia, and antiangiogenic factors are released into the maternal circulation [4]. Subsequently, hepatic, renal, pulmonary, cardiac, and neurological symptoms emerge, referred to as end-organ dysfunction. Oxidative stress that develops after ischemia contributes to this damage [5]. Preeclampsia and severe preeclampsia (SPE) are serious diseases that can cause maternal and fetal morbidity and mortality. İn fact, they are responsible for 10 to 15% of direct maternal deaths worldwide [6]. It is stated that low-dose aspirin, which is started in the early period of pregnancy in patients with identified risk factors, reduces the risk of developing PE [7]. Patients can be protected from maternal and fetal morbidity and mortality if the risk of PE and SPE can be demonstrated during early pregnancy or before pregnancy. In our study, we examined the levels of IMA, YKL-40, EN-RAGE, and AIM within maternal and cord blood from patients with PE and SPE. These results may help us to understand the pathogenesis of PE and to diagnose the disease early.

Materials and metods

This prospective study was conducted with patients who gave birth in Dicle University Medical Faculty Hospital Gynecology and Obstetrics Clinic. The study was approved by the ethical committee of clinical research of the University of Dicle (Ethics Committee Decision Number: 2015-61). Informed consent was obtained before the study from each patient participating in the study. The authors would like to confirm that this study was conducted in accordance with the Declaration of Helsinki. A total of 117 patients were included in the study. The patients were divided into three groups: 40 patients with SPE (group 1), 36 patients with PE (group 2), and 41 patients in the control group (group 3). Diagnoses of PE and SPE were made according to the ACOG criteria [1]. PE was defined as encompassing patients with a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg based on at least two measurements at four-hour intervals. In addition to hypertension, 24-hour urine proteinuria (≥0.3 g), thrombocytopenia (<100000), elevated serum creatinine (>1.1), liver transaminase levels above 2x, pulmonary edema, headaches unresponsive to analgesics, and one visual sign (blurred vision, flashing lights, or scotoma) were required to be present. SPE was defined as encompassing a systolic blood pressure of ≥160 mmHg and/or a diastolic blood pressure of ≥110 mmHg, accompanied by one of the above findings. Pregnant women who also had hypertension, diabetes, heart disease, and hepatic, nephrotic, and autoimmune diseases were not included in the control group. While the maternal blood was taken immediately before delivery in patients with normal delivery and before anesthetic agents were administered in patients with cesarean sections, the cord blood was taken after cord clamping during delivery. The age and gestational week values of the patients were noted.

Ischemia-modified albumin (IMA) is the product of albumin that is continuously modified during oxidative stress. When albumin is modified, its metal-binding capacity is reduced, and free radicals cannot be captured [8].

YKL-40 is an anti-inflammatory marker related to extracellular matrix remodeling and angiogenesis. It is an inflammatory glycoprotein released from macrophages, and it increases in acute and chronic inflammatory processes [9].

EN-RAGE is an abbreviation for "extracellular newly identified RAGE-binding protein." Conversely, rage is referred to as the advanced glycation end product receptor and has been associated with various autoimmune and inflammatory processes [10].

AIM refers to "apoptosis inhibitor of macrophages" and is released from macrophages. It plays a regulatory role in intracellular physiological mechanisms, and its levels increase in autoimmune diseases, liver cancer, and acute kidney injuries [11].

Working Procedure: Samples were stored at -80. Before the study began, the temperature was increased to -20 and then +4 C, and the samples gradually dissolved. In the WiseTis Homogenizer brand device, ice was left in a separate container and homogenized in a phosphate buffer with a pH of 7.4 in ice (thus aiming to protect the molecules to be measured). The samples were centrifuged at 3,000 rpm at +4 C for 10 minutes in a Nüve brand centrifuge device, and the supernatant part was transferred to the Eppendorfs with a pipette made ready to work for ELISA. It was studied in accordance with the ELISA kit procedure. During the ELISA steps, the plate was washed in a BioTek ELX50 washing device. The study was completed by reading at 450 nm on the Triturus GRI FOLS brand device. For the results, the optical density values obtained from the standard solution and the STD concentration were taken, and the distribution graph was drawn in the Excel program. With the formula calculated according to the curve, the concentrations of the samples were calculated against their optical densities.

Statistical analysis

IBM SPSS 21.0 for Windows' statistical package program was used in the statistical evaluation of our research data. Measured variables were presented as mean ± standard deviation (SD), and categorical variables were presented as numbers and percentages (%). A one-way analysis of variance was performed on group variables. A Tukey test was used to determine the difference between groups. P ≤0.05 was accepted as a statistically significant result.

Results

The mean age of the patients was 31.6±6.3 in group 1, 29.6±7.7 in group 2, and 29.7±5.7 in group 3, and there was no significant difference between the groups.

The gestational week was 33.9±3.3 in group 1, 36.4±2.4 in group 2, and 35.7±2.7 in group 3. There was no significant difference between group 2 and group 3, but group 1 was significantly lower (p=0.001).

In terms of IMA levels, no significant difference was observed between the groups in the maternal and cord blood. Additionally, no difference was observed between the groups in terms of YKL-40 in the maternal and cord blood. While no difference was observed between groups in EN-RAGE (s100a12) cord blood, it was significantly higher in the maternal blood than in group 1 and group 2 in the control group (p=0.001).

In terms of AIM, it was significantly higher in the maternal blood in the control group (p=0.000). AIM levels in the cord blood were significantly higher in the PE and SPE groups (p=0.029). Demographic data and blood values are presented in Table 1.

Discussion

Preeclampsia is characterized by high blood pressure and end-organ disorders observed after the 20th week of pregnancy [1]. It can cause maternal and fetal morbidity and mortality. Particularly in early-onset PE, blood pressure becomes increasingly severe, and end-organ

Table 1. Demographic data and blood values.

Parameters	Group1 Mean ±SD	Group 2 Mean ±SD	Group 3 Mean ±SD	*p*
Age	31.6±6.3	29.6±7.7	29.7±5.7	*0.297*
Pregnancy week at birth	33.9±3.3	36.4±2.4	35.73±2.7	*0.001*
Systolic blood pressure	172.2±17.7	142.7±8.8	111.9±12.4	*0.000*
Diastolic blood pressure	103.7±13.3	89.1±9	70.2±8.8	*0.000*
IMA maternal blood	131.3±97.8	122.5±103.6	106.7±74.2	*0.522*
IMA cord blood	142.3±90.4	137.7±104.6	148.8±97.9	*0.899*
YKL-40 maternal blood	110.0±56.2	108.0±90.1	92.4±49.3	*0.618*
YKL-40 cord blood	110.0±56.2	89.2±47.6	644.6±422.3	*0.663*
EN-RAGE maternal blood	514.2±403.9	481.4±449.1	759.2±353.5	*0.001*
EN-RAGE cord blood	639.5±325.5	565.5±292.3	644.6±422.3	*0.597*
AIM maternal blood	107.2±105.9	95.4±103.9	197.5±88.2	*0.000*
AIM cord blood	179.8±94.6	184.8±102.6	131.0±95.2	*0.029*

damage becomes more evident. Chest pain, shortness of breath, and thrombocytopenia can be fatal findings [12]. Symptoms in patients mostly regress after delivery, and full recovery may require up to four weeks [13]. However, patients with PE have a high risk of developing cardiovascular disease, type 2 diabetes, hypertension, kidney diseases, and metabolic syndrome after a long period [14]. Its detection and treatment in early pregnancy highlight the importance of diagnostic studies.

In our study, patient ages were similar between groups. The gestational age in weeks at birth was significantly lower in the SPE group (p=0.001). Since blood samples were taken immediately before delivery, it was expected that patients with SPE would have an early delivery.

Ischemia-modified albumin is a product of albumin that is continuously modified during oxidative stress. When albumin is modified, its metal-binding capacity is reduced, and free radicals cannot be captured [8]. Although it is used as a marker of ischemia and hypoxia, especially in heart diseases, it has been reported that it may increase in healthy pregnancies [15,16]. In our study, no difference was found in terms of IMA levels in maternal blood and cord blood (p=0.522 and 0.899). In a study grouped according to the presence of PE in patients with fetal growth retardation, IMA levels were found to be significantly higher in the PE group [17]. We believe that the difference in the results arose in our study because the patients were not classified according to fetal growth retardation in our study. In a study examining serum and saliva IMA levels in preeclamptic patients, IMA levels were found to be significantly higher than in normal patients [18]. In another study examining IMA levels in PE and SPE, IMA levels increased as PE worsened [19]. The small number of patients in the groups in this study is the most important limitation of the study. In a meta-analysis investigating studies that examined maternal blood and cord blood IMA levels, the

authors concluded that measuring IMA levels from maternal and cord blood may be an easy and inexpensive method for PE. However, larger studies are necessary [20]. A study comparing IMA levels in normal and preeclemptic pregnancies stated that while IMA levels were found to be significantly higher in patients with preeclampsia, this difference did not remain when corrected with albumin, and IMA was not recommended as a PE marker [21].

YKL-40 is a glycoprotein released from neutrophils and macrophages. It is elevated in the serum of individuals with acute or chronic inflammatory diseases [9]. In a study investigating YKL-40 values in preeclamptic patients, blood values were investigated in all three trimesters, and no correlation was found between PE and YKL-40. However, the YL-40 value increased with increasing maternal age and body mass index [22]. In another study, YKL-40 levels were compared in PE and normal pregnant women. In this study, no significant difference was found between YKL-40 values in samples taken from maternal blood [23]. The small number of patients in the groups in the study is striking. In another study, YKL-40 levels in the early and late PE and control groups were found to be significantly lower only in the early-onset PE group [24]. In our study, no significant difference was observed between the groups in terms of YKL-40 levels measured from maternal and cord blood. The sample size and the fact that it was a single-center study may have been reflected in our results.

EN-RAGE is a proinflammatory protein that is secreted by activated granulocytes, macrophages, and lymphocytes and that binds calcium. When secreted extracellularly, it binds to RAGE and induces an inflammatory-immune response [25,26]. In a study in which early and late-onset pregnant women were compared with the control group, EN-RAGE was checked from maternal blood, and it was found to be high only in late-onset PE [27]. This study was the only EN-RAGE study conducted with preeclamptic patients in the literature, and it is the largest handicap to study 17 preeclamptic patients in total. In our study, EN-RAGE levels were found to be significantly higher in the SPE group (p=0.001). This appears to be consistent with the information in the literature.

AIM refers to a macrophage apoptosis inhibitor released from macrophages. Its level increases in autoimmune diseases, liver cancer, and acute kidney injuries [11]. In the literature, we could not find a previous study that examined levels of AIM in PE patients. In one study, AIM levels were found to increase with liver damage in patients with cirrhosis [28]. In another study examining AIM levels in patients with rheumatoid arthritis, AIM levels were found to be significantly higher than in healthy patients [29]. In our study, AIM levels in maternal blood were found to be significantly higher in the control group (p=0.001). Cord blood was significantly higher in the PE and SPE group than in the control group (p=0.029). In this case, it appears that AIM values may be higher in healthy pregnant women. In addition, high AIM levels in cord blood in preeclamptic patients indicate that there may be a placental origin. Further studies are clearly necessary to confirm this.

Conclusions

PE and SPE are serious conditions that can lead to maternal and infant death. Early diagnosis and treatment are important, and a common decision has not been reached in studies regarding early diagnosis in the literature. Thus, it is clear that a large number of multicenter studies should be conducted with large sample sizes.

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