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Paraoxonase 1 Activities in First Trimester Miscarriages**Birinci Trimester Düşüklerinde Paraoksonaz 1 Aktiviteleri**Hasan Basri SAVAS¹Dilek ERDEM² Orcid ID: 0000-0001-8759-4507 Orcid ID: 0000-0001-6199-0333¹ Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Medical Biochemistry, Antalya, Türkiye.² Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Gynecology and Obstetrics, Antalya, Türkiye.**ÖZ**

Amaç: Düşükler günümüzde giderek daha önemli bir sorun haline gelmektedir. Üreme çağındaki kadınlarda ilk trimester gebelik kaybının sıklığı artmaktadır. Bu çalışmada, ilk trimester gebelik kayıpları ile PON1 ve ARY düzeyleri arasındaki olası ilişkinin gösterilmesi amaçlanmıştır.

Gereçler: 6-14 gebelik haftasında 25 gebe sağlıklı kadın (Grup 1) ve ilk trimester düşüklü (Grup 2) 25 kadın çalışmaya dahil edildi. Serum örneklerinde paraoksonaz 1 ve arilesteraz aktiviteleri, tam otomatik kolorimetrik yöntemler kullanılarak araştırıldı. Grupları karşılaştırmak için ANOVA testi kullanıldı.

Bulgular: PON1 ve ARY aktiviteleri, ilk trimesterde düşük yapan gebelerde Anova testi ile analiz edilen ilk trimesterdeki sağlıklı gebe kadınlara göre istatistiksel olarak anlamlı derecede düşüktür ($p < 0,05$).

Sonuç: Bu çalışmanın sonuçları, ilk trimester gebelik kayıplarında paraoksonaz 1 ve arilesteraz antioksidan enzim aktivitelerinin anlamlı prediktif klinik laboratuvar parametreleri olarak değerlendirilebildiğini göstermiştir. Bu çalışma daha büyük hasta gruplarıyla doğrulanırsa, araştırma sonuçlarımız değer kazanacaktır.

Anahtar Kelimeler: Gebeler, birinci trimester, düşük, antioksidan, paraoksonaz 1, arilesteraz.

ABSTRACT

Aim: Miscarriages are becoming increasingly important problem nowadays. First trimester pregnancy loss frequency in women of reproductive age is increasing. In this study, it is aimed to show the possible relationship between first trimester pregnancy losses and PON1 and ARY activities.

Material and Methods: 25 pregnant healthy women (Group 1) and 25 women with first trimester miscarriage (Group 2) in 6-14 gestational weeks were included in this study. Paraoxonase 1 and arylesterase activities of the sera samples were investigated using fully automated colorimetric methods. ANOVA test was used to compare the groups.

Results: PON1 and ARY activities are statistically significantly lower in pregnant women with first trimester miscarriage compared to healthy pregnant women in first trimester analyzed by Anova test ($p < 0.05$).

Conclusion: The results of this study showed that paraoxonase 1 and arylesterase antioxidant enzyme activities can be evaluated as significant predictive clinical laboratory parameters in first trimester pregnancy losses. If this study is confirmed with larger patient groups, our research results will gain more value.

Keywords: Pregnants, first trimester, miscarriage, antioxidant, paraoxonase 1, arylesterase.

INTRODUCTION

Pregnancy is a physiological condition. However, not every pregnancy result in health. Miscarriages are becoming an increasingly significant problem nowadays. An increasing frequency of first trimester pregnancy loss is observed in women of reproductive age. This loss rate may be in the range of 8-20%. In determining first trimester pregnancy losses, the values of routine biochemical parameters were investigated, but no significant routine parameters were found (1). Although

some causes such as genetic factors and coagulation disorders are blamed, the reason cannot be explained in most patients with first trimester pregnancy loss. Oxidative stress was shown as one of the underlying causes (2). Therefore, it is important to know the antioxidant capacity in first trimester pregnancy losses. Oxidative stress formation is a condition that constantly occurs during routine metabolic processes in the organism. Oxidative damage occurs in cells, tissues and organs as a result of increased oxidative stress. Oxidative damage is prevented, kept in balance, and survival is possible only with antioxidant activity. If the increase in oxidative stress is not ba-

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lanced by the antioxidant system, a large number of diseases may occur. The following conditions are triggered mainly due to oxidative stress increase and the effects of free radicals. These triggered conditions such as aging, atherosclerosis, cancer, ischemia-reperfusion injury and autoimmune diseases are the main causes of death nowadays (3-7). The paraoxonase (PON) multigene family is localized on the 7th chromosome in the human genome. It consists of three members, PON1, PON2 and PON3. PON1 is a calcium-dependent hydrolase with both arylesterase (ARY) and paraoxonase activity. Paraoxonase 1 and Arylesterase (ARY) are enzymes in the esterase group that are encoded by the same gene. The active centers of PON1 and ARY are similar. PON1 and ARY enzyme activities both show antioxidant effects and may be important for the diagnosis of vascular health-related diseases (8). Additionally, total oxidant status (TOS) and total antioxidant status (TAS) values provide information to evaluate the oxidative stress level and oxidative stress index. The oxidative stress index (OSI) can be calculated using the TOS / TAS ratio (3-7). There is no biochemical parameter that can predict first trimester pregnancy losses in routine. In this study, it is aimed to show the possible relationship between first trimester pregnancy losses and PON1 and ARY levels. Total oxidant status (TOS) and total antioxidant status (TAS) values were measured to evaluate the oxidative stress level and oxidative stress index. The oxidative stress index (OSI) was calculated using the TOS / TAS ratio. PON1 and ARY levels may be predictive routine biochemical tests for first trimester pregnancy losses in clinical practice.

MATERIAL AND METHODS

Patients and Ethical Issue

25 pregnant healthy women (Group 1) and 25 women with first trimester miscarriage (Group 2) in their 6th-14th gestational weeks were included this study. The blood samples that were residual from routine examinations were collected. The collected blood was centrifuged, and its serum was separated, portioned in Eppendorf tubes and stored at -80 °C. This study was conducted with the permission of Alanya Alaaddin Keykubat University Clinical Research Ethics Committee (ALKU-KAEK) dated 26.09.2019 and numbered 10/15. The research was carried out in accordance with the ethical principles in the Declaration of Helsinki, which was adopted by the World Medical Association. All women included in the study signed an informed consent form. Paraoxonase 1, arylesterase activities, total antioxidant status, total oxidant status of the serum samples were investi-

gated using fully automated colorimetric methods.

Paraoxonase 1 Activity

The paraoxonase 1 activity of the serum samples was investigated using a fully automated method improved by Rel Assay Diagnostics (Mega TNp, Gaziantep, Turkey). In accordance with this method, paraoxonase activity was measured in media without NaCl (basal paraoxonase activity) and with NaCl (salt-stimulated paraoxonase activity). Hydrolysis of the paraoxon (diethyl-p-nitrophenyl phosphate) was observed with the tag after the increase of absorbance at 37 °C and 412 nm. The quantity of p-nitrophenol resulting from the hydrolysis was figured out. The net rate with enzymatic activity was calculated by subtracting the basal activity rate from the salt-stimulated activity rate. The outcomes are expressed as unit per liter, which is equal to the hydrolysis of 1 micromole substrate in 1 minute and 1 liter (9).

Arylesterase Activity

The paraoxonase and arylesterase activity of the serum samples was determined using a fully automated method developed by Rel Assay Diagnostics (Mega TNp, Gaziantep, Turkey). In accordance with this method, phenylacetate was used as a substrate for estimation of arylesterase activity, and with the hydrolysis of phenylacetate, phenol and acetic acid were composed. The resulting phenol joined 4-aminoantipyrine and potassium ferricyanide and was determined with the colorimetric method. The outcomes are expressed as unit per liter, which is equal to the hydrolysis of 1 micromole phenylacetate in 1 minute and in 1 liter (10).

Total Antioxidant Status (TAS)

Commercially available kits were used for measuring TAS levels (Relassay Diagnostics, Turkey). The fiction automated method is dependent on bleaching of the characteristic color of a more stable ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has ideal tender values, which are lower than 3%. The outcomes are expressed as mmol Trolox equivalent/L (11).

Total Oxidant Status (TOS)

Commercially available kits were used for measuring TOS levels (Relassay Diagnostics, Turkey). In the new method, oxi-

dants present in the pattern oxidized the ferrous ion-o-dianisidine complex into ferric ion. The glycerol molecules, present in the reaction medium, boosted the oxidation reaction. The ferric ion procreated a colored complex with xylenol orange in the acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the pattern. The dissection was calibrated with hydrogen peroxide, and the outcomes are expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) (12).

Oxidative Stress Index (OSI)

The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). For sums, the resulting unit of TAS was transformed to $\mu\text{l/L}$, and the OSI value was figured out according to the following Formula: $\text{OSI (arbitrary unit)} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / \text{TAC } (\mu\text{mol Trolox equivalent/L})$ (13, 14).

Statistical Analyses

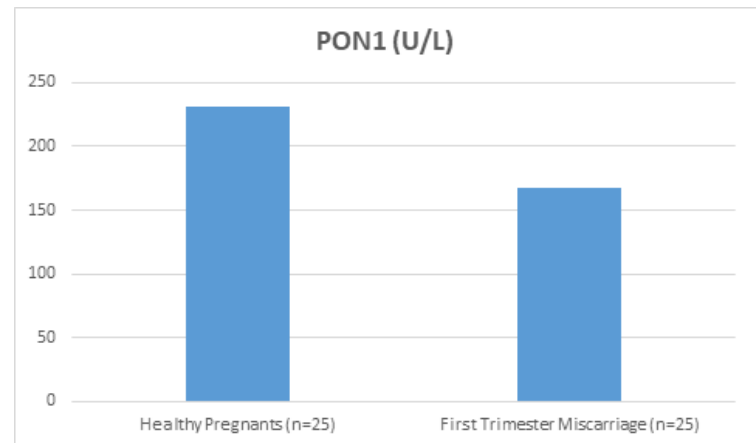
The results were statistically analyzed by the SPSS package software. ANOVA was used to compare the groups. There were two groups in total. For this reason, post-hoc tests were not needed after ANOVA. The significance level was accepted as $p < 0.05$.

RESULTS

The mean ages (mean \pm SD) were 28.04 ± 6.08 in the healthy pregnant group ($n=25$) and 30.20 ± 5.19 in the first trimester miscarriage group ($n=25$). The PON1 antioxidant enzyme activities were measured as mean \pm standard error in the healthy pregnant women and the pregnant women with first trimester miscarriage respectively as 230.69 ± 17.89 and 167.20 ± 9.84 (Unit: U/L). The PON1 activities were significantly lower in the pregnant women with first trimester miscarriage in comparison to the healthy pregnant women in the first trimester as analyzed by the ANOVA ($p=0.004$). The ARY antioxidant enzyme activities were measured as mean \pm standard error in the healthy pregnant women and the pregnant women with first trimester miscarriage respectively as 243.69 ± 17.08 and 192.76 ± 9.95 (Unit: U/L). The ARY activities were significantly lower in the pregnant women with first trimester miscarriage in comparison to the healthy pregnant women in the first trimester as analyzed by the ANOVA ($p=0.014$). The results of the PON 1 and ARY activities are shown in Figures 1 and 2 below. When the total

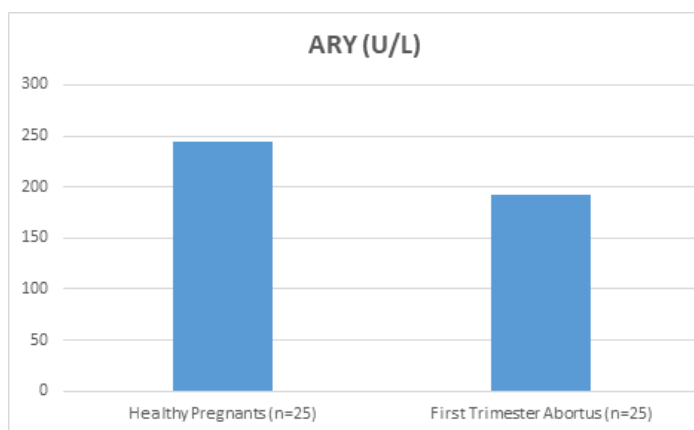
antioxidant status was compared, no significant difference was found between the groups. The TAS values were measured as mean \pm standard error in the healthy pregnant women and the pregnant women with first trimester miscarriage respectively as 1.02 ± 0.03 and 0.96 ± 0.02 ($p=0.08$). The TOS values were measured as mean \pm standard error in the healthy pregnant women and the pregnant women with first trimester miscarriage respectively as 31.29 ± 1.75 and 39.29 ± 2.04 ($p=0.005$). The OSI ratios were calculated as mean \pm standard error in the healthy pregnant women and the pregnant women with first trimester miscarriage respectively as 30.87 ± 1.76 and 42.25 ± 2.95 ($p=0.002$). In terms of the total oxidant status and oxidative stress index, significantly higher values were found in the first trimester miscarriage group in comparison to the healthy pregnant women ($p < 0.05$).

Figure 1. Comparison of PON1 Activities in Healthy Pregnant Women and First Trimester Miscarriage (Unit: U/L, and results are given as mean \pm standard error)



PON1 activities were significantly lower in the pregnant women with first trimester miscarriage in comparison to the healthy pregnant women in the first trimester as analyzed by the ANOVA ($p=0.004$).

Figure 2. Comparison of Arylesterase Activities in Healthy Pregnant Women and First Trimester Miscarriage (Unit: U/L, and results are given as mean± standard error)



Arylesterase activities were significantly lower in the pregnant women with first trimester miscarriage in comparison to the healthy pregnant women in the first trimester as analyzed by the ANOVA ($p=0.014$).

DISCUSSION

PON1 levels and activity are significantly impaired in diseases associated with the heart and liver, diabetes mellitus, obesity and vascular disorders. PON1 may be considered as a good marker in prevention of mortality and morbidity that may occur and in the follow-up of atherosclerotic patients. Lifestyle changes such as the Mediterranean diet may lead to an improvement in PON1 activity, reducing the devastating effects of diseases. Additionally, the effects of the treatments that are given on PON1 levels may be investigated. More studies are needed to solve the function of PON1 in healthy individuals and patients. These will also help discover new regulators of PON1 levels and activity to prevent mortality and morbidity in metabolic disorders. One of the accused cases of first trimester pregnancy losses is that oxidative stress becomes dominant due to an insufficient antioxidant system. Another possible important mechanism is various vascular pathologies that may occur in pregnant women, especially in the placenta. For these reasons, if PON1 and ARY activities are measured, and a possible relationship is found, it is considered PON1 and ARY activities may be used to detect first trimester pregnancy losses in advance (1-10). When we look at similar studies to compare our results, the relationship between idiopathic recurrent early pregnancy loss (REPL) and paraoxonase-1 (PON1) polymorphisms and the activities of the PON1 and ARY enzymes were investigated by Öztürk et al. At the end of their study, no rela-

tionship was found between PON1 activities or genotype and idiopathic REPL, and it was shown that ARY activity increased in early pregnancy. According to the results of our research, the activities of the PON1 and ARY enzymes were lower in those with first trimester pregnancy loss in comparison to those with healthy first trimester pregnancy. The authors' results were consistent with our research in terms of high ARY enzyme activities in those with healthy first trimester pregnancy (15). Considering PON1 and ARY enzyme activities in those with very early pregnancy loss, the PON1 and ARY activities were found to be significantly lower than the control group. When the results of their study are evaluated, the PON1 and ARY activities were consistent with our study, even though they examined a period about two weeks earlier than our study in terms of gestational weeks (16). In a study conducted in 2018, the relationship between low risk of pregnancy and PON2 and PON3 activities were examined. The PON2 and PON3 activities were found to be lower in the pregnant women who had low activity in comparison to the control group. In our study, a similar relationship was found for the PON1 activity. The PON1 enzyme has strong antioxidant activity and relationship with vascular diseases. For this reason, it is more meaningful to evaluate the activity of the PON1 enzyme in relation to miscarriage in comparison to PON2 and PON3 (17). In a study conducted in 2019, PON1 and ARY enzyme activities were not measured in pregnant women with habitual abortion, but the total antioxidant status was evaluated. Lower values were found in the pregnant women with habitual abortion in terms of the total antioxidant status. Although the gestational weeks and the examined parameters were different, consequently, their study was compatible with the results of our study in terms of the low antioxidant status in pregnant women who had miscarriage (18). Studies in the literature have shown that paraoxonase 1 levels are affected in vascular diseases including atherosclerosis, Type 2 Diabetes Mellitus, renal failure and patients receiving hemodialysis treatment (19-21). In addition, increased oxidative stress can lead to more than a hundred serious diseases (3). In this case, since the oxidant-antioxidant balance will be disrupted, PON1 activities may be affected. In our study, possible other effects on PON1 were tried to be eliminated by excluding those with additional diseases. Therefore, the change in PON1 levels may be a significant indicator in patients with first trimester miscarriages. The most common causes of first trimester miscarriages can be divided into six main categories: genetic causes (including molar pregnancies), infectious diseases, immunological

diseases, anatomic abnormalities of the uterus, implantation abnormalities, and endocrine disorders (22, 23). PON1 levels are likely to be affected by increased oxidative stress in most of these causes leading to first trimester miscarriages (24-26). Thus, it is possible that PON1 activities will be affected in first trimester miscarriages. Lower PON1 activities may be an important and valuable clinical laboratory marker in predicting first trimester miscarriages. The absence of a study in the literature the same as our study increases the unique value of our research. Our research results were compatible with similar studies. PON1 and ARY enzyme activities may be recommended as a predictive biochemical parameter in evaluation of first trimester miscarriages.

CONCLUSION

A natural, balanced and healthy diet with high content of vitamins, minerals, prebiotics, probiotics, vegetables and fruits and regular, moderate exercises may be beneficial in decreasing antioxidant capacity and the oxidative stress index for reducing first trimester pregnancy losses. In pregnant women, it is necessary to increase the antioxidant capacity to reduce the risk of first trimester miscarriage. In order to increase antioxidant capacity, a natural, balanced and healthy diet with high vegetable and fruit content may be recommended. If the correct diet is combined with regular exercises suitable for age and pregnancy and an active lifestyle, antioxidant activity will be increased. There is no routine biochemical parameter in the laboratory to show the risk of first trimester miscarriage. The PON1 and ARY enzymes are enzymes with strong antioxidant activity and known for protecting vascular health. Therefore, their relationship to the risk of first trimester miscarriage may be considered. According to the results of this study, the activities of the PON1 and ARY enzymes in pregnant women may be used as a determinant in showing the risk of first trimester pregnancy losses. These data need to be strengthened with new studies.

Note: There is no conflict of interest among the authors.

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