

CHANGES IN SERUM PARAOXONASE ACTIVITY, CALCIUM AND LIPID PROFILES IN PRE-ECLAMPSIA, A PRELIMINARY STUDY

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SUMMARY

Objective: To investigate serum paraoxonase (PON1) activity, serum calcium, and lipid profiles in pre-eclampsia.

Material and methods: Serum PON1 activity, calcium, and lipid profiles were measured in a cohort of 45 women with normal pregnancies (11 patients) and pre-eclampsia (34 patients).

Results: Triglyceride, total cholesterol, and LDL cholesterol concentrations were significantly increased in women with pre-eclampsia, compared with women with normal pregnancies. Serum HDL cholesterol, PON1, and calcium levels in pre-eclamptic patients were significantly lower than those in healthy pregnant women.

Conclusion: The significant decrease of PON1 activity in pre-eclampsia is related to lipid profiles and calcium.

Key words: calcium, lipid profile, Pre-eclampsia, serum paraoxonase (PON1).

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ÖZET

PREEKLAMPSİ'DE PARAOKSANAZ AKTİVİTESİ, KALSİYUM VE LİPİD PROFİLLERİNİN DEĞERLENDİRİLMESİ, ÖN ÇALIŞMA

Amaç: Preeklampitik ve normotansif gebelerde serum paraoksanaz (PON1) aktivitesi, serum kalsiyumu ve lipid profillerinin incelenmesi.

Gereç ve yöntemler: 34 preeklampitik ve 11 normotansif gebede serum PON1 aktivitesi, kalsiyum ve lipid profillerinin incelenmesi.

Bulgular: Preeklampitik gebelerde serum trigliserid, total kolesterol, LDL kolesterol seviyeleri, normotansif gebelerle kıyaslandığında anlamlı olarak daha yüksek bulunmuştur. Serum HDL kolesterol, PON1, ve kalsiyum seviyeleri ise normal gebelere kıyasla preeklampitik kadınlarda daha düşük olarak tespit edilmiştir.

Sonuç: Preeklampitik hastalarda PON1 aktivitesindeki anlamlı düşüş, lipid profili ve kalsiyum seviyesi ile ilişkilidir.

Anahtar kelimeler: kalsiyum, lipid profili, pre-eklampsi, serum paraoksanaz seviyesi

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INTRODUCTION

Pre-eclampsia is defined as pregnancy-induced hypertension (systolic blood pressure > 140 mm Hg and diastolic blood pressure > 90 mm Hg) with proteinuria (either ≥ 300 mg protein per day or an urinary protein/creatinine ratio ≥ 30 mg/mmol) occurring after 20 weeks of gestation. Although this definition uses readily measured clinical parameters, pre-eclampsia must be recognized as a multisystem disorder that may affect the brain, lung, kidney, and liver (1,2).

Pre-eclampsia affects between 0.4% and 2.8% of all pregnancies. This common disorder is associated with the highest maternal and fetal morbidity and mortality of all pregnancy complications(3). The cause of pre-eclampsia remains unknown, but endothelial cell dysfunction is a key feature of its pathogenesis. The cause of the endothelial cell injury is probably multifactorial, but poor placental perfusion plays a major role. In pre-eclampsia, characteristic pathological lesions in the placenta consisting of fibrin deposits, and acute atherosclerosis and thrombosis are present. Abnormalities in lipids are known to be strongly associated with atherosclerotic cardiovascular disease and have direct effects on endothelial function. Abnormal lipid metabolism seems important in the pathogenesis of pre-eclampsia as well(4).

Normal pregnancy is characterized by an elevation of the plasma levels of free fatty acids, triglycerides, cholesterol and low density lipoprotein (LDL) cholesterol(5-7). In pre-eclampsia, even higher levels of triglycerides and lipoproteins may be present(8,9). High levels of triglycerides generally lead to the formation of smaller LDL particles, which are more prone to oxidative modification(6). Oxidatively modified LDL has been shown to play a role in the impairment of vascular endothelial cell function during atherogenesis(9), and may also be involved in the development of pre-eclampsia.

Paraoxonase (aryldialkylphosphatase, E.C 3.1.8.1, PON1) is a serum esterase synthesized in the liver. The enzyme was originally found to be responsible for the hydrolysis of paraoxone, a toxin that irreversibly inhibits acetyl cholinesterase(10-14). PON1 circulates as part of high density lipoprotein (HDL) particles in the blood of humans. The enzyme is tightly bound to the hydrophilic N-terminal domain of Apo-AI. Recently,

PON1 has been shown to inhibit the oxidative modification of LDL(15), and it is considered to be an antioxidant enzyme with a protective role in atherosclerosis(16). PON1 can destroy active lipids in mildly oxidized LDL, thereby preventing the induction of inflammatory responses in endothelial cells. PON1 is strictly dependent on calcium for enzymatic activity (17-19).

In the present study, we investigated the relationship among serum PON1 activity, serum calcium concentration, and lipid profile in patients with pre-eclampsia.

MATERIAL AND METHODS

The study was conducted at the Department of Obstetrics and Gynecology of Dicle University Faculty of Medicine. Thirty-four women with pre-eclampsia and 11 women with normal, normotensive pregnancies were prospectively followed. All of the study related procedures were subjected to local ethics committee approval and the study protocol was approved by Ethical Committee of Dicle University Faculty of Medicine. Pre-eclampsia was diagnosed if blood pressures exceeded 140/90 mm Hg on two separate measurements taken 4 hours apart, after the 20 weeks of gestation, and if proteinuria of more than 300 mg of protein was excreted in 24 hours or if there was greater than 2+ protein present in a qualitative dipstick test on two samples of urine collected 4 hours apart in the absence of urinary tract infection. HELLP syndrome includes; platelet counts below 50000/microl; lactic dehydrogenase > or = 2000 IU/I; aspartate aminotransferase > or = 500 IU/I; alanine aminotransferase > or = 300IU/I and hematuria (20). In our study, we did not have any patients with HELLP syndrome.

All patients had neither pre-existing hypertensive disorders, nor any renal, hepatic or hematological diseases, and they did not receive any medication. None of them were smokers.

Blood samples (approximately 10 ml) were subjected to centrifugation at 4000 x g for 10 minutes at room temperature. Serum samples were collected and stored at -20°C for one week. PON1 activity was determined as described by Jureticé D et al(21). Initial rates of hydrolysis of paraoxone (o,o-diethyl-o-p-nitro-phenyl phosphate from Sigma, London, UK) was determined by measuring the absorbance at 405 nm at 37°C with

a spectrophotometer (Shimadzu UV-1208). This wavelength is the peak of the liberated p-nitrophenyl's absorption spectrum. The assay (reagent) mixture includes 2.0 mmol/L paraoxone and 2.0 mmol/L calcium chloride (CaCl₂) in 0.1 mol/L Tris buffer, pH 8.0. To 350 µl of the reagent mixture, 12 µl of serum was added. PON1 activity is expressed in units per liter (U/L) of serum. One unit of activity is defined as 1 mmol p-nitrophenol generated per minute.

Serum levels of AST, ALT, LDH, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and calcium were determined using enzymatic assays in an Abbot Aeroset autoanalyzer.

Mann-Whitney U test was used to identify differences between the groups. Data was given as mean ± S.D. Differences were considered significant when P < 0.05.

RESULTS

Table I shows the demographic characteristics of the patients. There were no differences in age, parity, and gravidity between the two groups. As expected, women with pre-eclampsia had significantly higher systolic (P < 0.001) and diastolic blood pressures (P < 0.001). Gestational age tended to be lower in women with pre-eclampsia.

Women with pre-eclampsia had significantly higher levels of hemoglobin, hematocrit, serum AST, ALT, and LDH than normotensive pregnant women (P < 0.001; Table II). Compared to normotensive women, women pre-eclampsia showed lower serum platelet counts (P < 0.01).

Triglycerides, total cholesterol, and LDL-cholesterol

Table I: Demographic and clinical characteristics of the study population.

| | Pre-eclamptic pregnant women (n=34) | Normal pregnant women (n=11) | P value |
|----------------------------------|-------------------------------------|------------------------------|---------|
| Maternal age (years) | 30.5 ± 6.1 | 30.0 ± 5.8 | NS |
| Gestational age (weeks) | 34.3 ± 3.7 | 37.5 ± 1.1 | <0.002 |
| Systolic blood pressure (mm Hg) | 155 ± 18.7 | 114 ± 9.3 | <0.001 |
| Diastolic blood pressure (mm Hg) | 97.9 ± 10 | 76.3 ± 6.7 | <0.001 |
| Gravida | 5.0 ± 3.3 | 3.6 ± 2.2 | NS |
| Parity | 3.8 ± 3.2 | 2.5 ± 2.3 | NS |

* Data are given as mean ± S.D.

Table II: Hematological and biochemical parameters.

| | Pre-eclamptic pregnant women (n=34) | Normal pregnant women (n=11) | P value |
|--|-------------------------------------|------------------------------|---------|
| Hematocrit | 36.13 ± 3.01 | 32.81 ± 2.67 | <0.001 |
| Hemoglobin (g/dl) | 12.10 ± 1.26 | 10.72 ± 1.34 | <0.02 |
| Platelet count (x 10 ³ /µL) | 146 ± 77.12 | 219 ± 62.93 | <0.001 |
| BUN (mg/dl) | 24.43 ± 7.61 | 14.45 ± 2.80 | <0.001 |
| Creatinine (mg/dl) | 9.36 ± 2.08 | 4.90 ± 1.22 | =0.16 |
| AST (U/L) | 92.93 ± 97.38 | 19.54 ± 12.20 | <0.001 |
| ALT (U/L) | 166.4 ± 242.8 | 24.90 ± 11.97 | <0.001 |
| LDH (U/L) | 783 ± 660 | 341 ± 225 | <0.001 |

* Data are given as mean ± S.D.

Table III: Serum levels of cholesterol, triglycerides, calcium, and paraoxonase.

| | Pre-eclamptic pregnant women (n=34) | Normal pregnant women (n=11) | P value |
|---------------------------|-------------------------------------|------------------------------|---------|
| Triglycerides (mg/dl) | 281 ± 101 | 204 ± 80 | <0.01 |
| Total cholesterol (mg/dl) | 245 ± 58 | 182 ± 42 | <0.0001 |
| HDL cholesterol (mg/dl) | 46.2 ± 8.2 | 62.0 ± 7.4 | <0.0001 |
| LDL cholesterol (mg/dl) | 162 ± 39 | 109 ± 33 | <0.0001 |
| Calcium (mg/dl) | 7.76 ± 0.5 | 9.30 ± 0.4 | <0.0001 |
| Paraoxonase (U/L) | 108 ± 63 | 161 ± 90 | <0.05 |

Data are given as mean ± S.D.

concentrations were significantly increased in women with pre-eclampsia, compared to normal pregnant women (Table III). Serum HDL-cholesterol, PON1 and calcium levels were lower in pre-eclamptic than in healthy pregnant women ($P < 0.05$).

In addition, normotensive women had no signs of any complications of pregnancy, and all gave birth to healthy infants.

DISCUSSION

Pre-eclampsia is one of the most serious complications of pregnancy. The causes of pre-eclampsia are unknown, but evidence suggest that it results from maternal vascular endothelial dysfunction⁽²²⁾. Since oxidized lipids can damage the vascular endothelium, elevated serum triglycerides, total cholesterol, and LDL-cholesterol levels have been suggested to play important roles in the pathogenesis of pre-eclampsia; Branch et al. and Votila et al. found increased serum concentrations of LDL in pre-eclampsia^(23,24). Wakatsuki et al. reported that, LDL and HDL particles were more susceptible to oxidative modification and plasma concentration of LDL particles but not of HDL particles was increased in pre-eclampsia⁽⁹⁾.

In our study, serum total cholesterol, LDL cholesterol, and HDL cholesterol levels were significantly different between pre-eclamptic and normal pregnant women. Triglycerides, total cholesterol and LDL cholesterol concentrations were significantly increased in women with pre-eclampsia, compared to women with normal pregnancies. This significant elevation in lipid levels in pre-eclampsia is consistent with several previous reports^(25,26). In addition, serum calcium levels were significantly lower in pre-eclamptic women. Because PON1 requires calcium ions for both activity and stability, it is tempting to speculate that low calcium might be the cause of decreased PON1 activity. However, there is no available data to support this hypothesis at present. Nevertheless, our findings confirm that low serum PON1 are present in pre-eclampsia.

Human pregnancy is associated with pronounced physiological hyperlipidemia and the normal gestational increase in triglycerides is associated with a change in LDL profile toward smaller, denser species⁽²⁷⁾. The elevated serum concentrations of triglycerides and

lower concentrations of HDL cholesterol found in pre-eclampsia patients are in good agreement with the results of other studies^(27,28). The elevated concentrations of serum triglycerides in pre-eclampsia can be explained by higher levels of free fatty acid in conjunction with reduced hepatic beta-oxidation, enhanced peripheral insulin resistance, and reduced catabolism of triglycerides⁽²⁷⁾. Hypertriglyceridemia in pre-eclampsia is proposed to include oxidative stress by promoting changes in the composition of LDL and consequently enhancing the formation of small, dense LDL. In a recent report, a case-control study of 50 women with pre-eclampsia and 101 women with uncomplicated term deliveries was conducted. Maternal serum was collected at 15 to 20 weeks and used to measure paraoxonase 1 activity using two substrates; paraoxon and phenylacetate (arylesterase activity). Paraoxonase 1 activity (oparaoxon) was significantly higher in women with pre-eclipse compared with controls (19.4 ± 9.4 versus 15.6 ± 8.0 change in absorbance per minute (dA/min), $p: 0.009$). When stratified by disease severity, paraoxonase 1 activity (paraoxon) was highest in women with severe pre-eclampsia (21.6 ± 9.1 versus 15.6 ± 8.0 dA/min, $p:0.002$). A trend was observed toward higher arylesterase activity in women with pre-eclampsia compared with controls ($0,343 \pm 0,07$ versus $0,323 \pm 0,06$ dA/min, $p: 0,06$). Midgestational paraoxonase 1 activity is higher in women with pre-eclipse before clinical signs of the disease were present⁽²⁹⁾.

Human paraoxonase-1 is thought to play a role in pre-eclampsia and atherosclerosis, mainly through a reduction in LDL oxidation. Oxidized LDL is very important in endothelial dysfunction of pre-eclampsia. Kim et al investigated the association between PON1 gene polymorphism and pre-eclampsia and determined the concentrations of serum lipid in pre-eclamptic patients. They also evaluated serum oxidized LDL levels in normal and pre-eclamptic patients. They reported no significant difference in PON1 genotype frequencies between the control and pre-eclamptic patients. The levels of serum cholesterol and high-density lipoprotein were significantly lower in pre-eclamptic patients compared with that of the control group ($p: 0,05$ and $p < 0,01$, respectively). The serum levels of oxidized LDL in pre-eclampsia patients were significantly higher than those in control women ($p: 0,001$)⁽³⁰⁾.

The results of our study suggest that an abnormal lipid profile and a decrease in the activity of lipophilic antioxidant paraoxonase may play a role in the pathogenesis of preeclampsia through increased susceptibility to lipid peroxidation. Further research is needed to clear the mechanism of this contribution.

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