Tubular Function in Pregnant Women with Preeclampsia¶

PREEKLAMPSİLİ GEBE KADINLARDA TUBULER FONKSİYON

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Abstract

Objective: Preeclampsia (PE) is a frequently occurred complication of pregnancy. PE often has renal involvement characterised by proteinuria, and decreased glomerular filtration. The aim of this study was to investigate the changes of urinary and fractional excretion (FE) of beta-2 microglobulin (β_2M) as indicator of tubular function in both preeclamptic and normal, healthy pregnant women as compared to healthy, non-pregnant women.

Material and Methods: Twenty third-trimester pregnant women with PE (group 1), 20 third-trimester healthy pregnant women (group 2), and 20 healthy, non-pregnant women (group 3) were included in the present study. The serum and urinary levels of beta-2 microglobulin were measured and fractional excretion (FE) of β_2M was determined in each group. The urinary value obtained for β_2M was then expressed per gram of creatinine (β_2M/Cr).

Results: The urinary β_2M/Cr ratio was statistically higher in both group 1 and group 2 when compared to group 3 ($p<0.001$), but there was no significant difference between group 1 and group 2 ($p>0.05$). The serum β_2M levels were significantly higher in group 1 when compared with those in group 2 and group 3 ($p<0.01$), and there was no significant difference between group 2 and group 3 ($p>0.05$). FE of β_2M increased significantly in group 1 and group 2 compared with that in group 3 ($p<0.05$), and there were no significant differences between group 1 and group 2 ($p>0.05$).

Conclusion: The results of our study indicate that urinary and fractional excretion of β_2M significantly increased in preeclampsia and in normotensive pregnancy as compared to healthy, non-pregnant women and these increases might indicate tubular dysfunction.

Key Words: Preeclampsia, β_2M, tubular dysfunction

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Özet

Amaç: Preeklampsi (PE) gebeliğin sık oluşan komplikasyonlarından biridir. PE, proteinüri ve glomerüler filtrasyonda azalmayla karakterize böbrek tutulumuna sıklıkla sahiptir. Bu çalışmanın amacı, preeklamptik ve normal gebelerde, gebe olmayan sağlıklı kadınlarla karşılaştırıldığında tubuler fonksiyonun göstergesi olan beta-2 mikroglobulin (β2M)'in idrar ve fraksiyonel atılımındaki değişimleri araştırmaktır.

Gereç ve Yöntemler: Bu çalışmaya, üçüncü trimesterde olan 20 preeklamptik (grup 1), 20 sağlıklı gebe (grup 2) ve kontrol grubu olarak da gebe olmayan 20 sağlıklı kadın (grup 3) katıldı. Her bir gruptaki kadınlarda serum ve idrar beta-2 mikroglobulin düzeyi ölçülerek, β_2M fraksiyonel eksresyonu hesaplandı. İdrar β_2M değeri, idrar kreatinin değerine oranlanarak verildi (β_2M/Cr).

Bulgular: İdrar β_2M/Cr oranı grup 3'le karşılaştırıldığında, grup 1 ve 2'de anlamlı olarak yüksekti ($p<0.001$), fakat grup 1 ve 2 arasında anlamlı bir farklılık yoktu ($p>0.05$). Grup 2 ve 3'le karşılaştırıldığında grup 1'de serum β_2M değerleri anlamlı olarak yüksekti ($p<0.01$) ve grup 2 ve 3 arasında anlamlı bir farklılık yoktu ($p>0.05$). Grup 3'le karşılaştırıldığında grup 1 ve 2'de β_2M 'in fraksiyonel eksresyonu anlamlı olarak artmıştı ($p<0.05$) ve grup 1 ve 2 arasında anlamlı bir farklılık yoktu ($p>0.05$).

Sonuç: Çalışmamızın sonuçları, preeklampsili ve normal sağlıklı gebelerde, gebe olmayan sağlıklı kadınlara göre β_2M'in idrar ve fraksiyonel atılımının önemli oranda arttığını ve bu artışın tubuler fonksiyon bozukluğunun bir belirtisi olabileceğini göstermektedir.

Anahtar Kelimeler: Preeklampsi, β_2M, tubuler fonksiyon bozukluğu

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Preeclampsia (PE) is the term used to describe a disorder of pregnancy characterised by hypertension, proteinuria, and often oedema, usually manifesting after 20th week of gestation. The incidence of PE is between 3% and 10% of pregnancies.[1,2]

Reduced organ perfusion and ischemia of the kidney, liver and brain can be detected in PE. In

Table 1. The clinical characteristics of the subjects including the study

	Group 1 X±SE	**Group 2** X±SE	**Group 3** X±SE
At sample collection	n=20	n=20	n=20
Age (years)	27.83±1.39	27.10±0.94	24.10±1.05
Gestational age (weeks)	33.87± 0.92[b**]	38.89±0.30	
Systolic blood pressure (mmHg)	153.88±3.80[a,**b**]	114.76±2.02	116.00±1.97
Diastolic blood pressure (mmHg)	102.22±2.86[a**,b**]	70.57±3.48	74.50±1.65
At delivery			
Gestational age (weeks)	34.55±0.96 [b **]	39.04±0.28	
Birth weight (g)	2726.37±237.20 [b *]	3610.11±127.82	
Perinatal mortality (%)	10 [b**]	0	

*p<0.05; **p<0.001; [a]:compared to group 3; [b]:compared to group 2.

Table 2. The values of some biochemical parameters of the study

	Group 1 X±SE	**Group 2** X±SE	**Group 3** X±SE
Serum BUN (mg/dL)	12.83±1.02	10.83±0.88	10.55±0.64
Serum Creatinine (mg/dL)	1.02±0.33[b*]	0.62±0.00	0.65±0.00
Serum uric acid (mg/dL)	6.00±032[a**,b*]	4.52±0.52	3.60±0.26
Serum AST (U/L)	41.43±9.34	19.38±1.38	21.75±2.10
Hemoglobin (g/L)	134±5.39	123±5.32	129±3.09
Platelets (10^9/L)	250.68±28.60	268.29±12.84	220.00±3.16
Serum β_2M (ng/mL)	116.22±15.23[a*,b*]	62.72±8.91	59.77±7.52
Urinary β_2M/Cr (μg/gr)	852.00±217.36[a**]	548.33±137.06[a*]	85.68±8.27
Urinary Prot/Cr (mg/gr)	4843.76±1210.60[a**, b**]	315.90±99.02	113.73±22.18
FE of β_2M (%)	9.94±3.87[a*]	9.47±2.59[a*]	1.43±0.34

*p<0.05; **p<0.001; [a]:compared to group 3; [b]:compared to group 2.

the kidney, the most typical anatomopathological lesion is glomerular endotheliosis but renal tubular damage was also demonstrated.[3] Many pathological conditions affecting the proximal renal tubule are characterised by an increased urinary excretion of low molecular weight plasma proteins.[4] Beta-2 microglobulin is low molecular weight protein of 11800 Dalton composed of 100 aminoacids with one disulphide bridge. β_2M is freely filtered at the glomerulus but is almost completely reabsorbed and degraded in the renal tubule.[5] Dysfunction of the proximal tubules with a normal glomerular filtration rate will be accompanied by a decreased tubular reabsorbtion and increased urinary excretion of β_2M.[6]

The present study was carried out to clarify changes of tubular function in both preeclamptic and normal pregnant women.

Material and Methods

Subjects

"World medical association decleration of Helsinki Ethical Principles for medical research involving human subjects" was accepted in the present study, and all subjects provided informed consent. The study was also approved by ethical committee.

Twenty third-trimester pregnant women with PE (maternal ages 20-38 years and gestational ages 29-40 weeks) (Group 1) and 20 third-trimester healthy pregnant women (maternal ages 22-37 years and gestational ages 27-41 weeks) (Group 2) were included in the present study. 20 healthy non-pregnant women (ages 18-35 years) (Group 3) were included in the study as control group**.** The following clinical data were recorded at sample collection; age, gestational age, parity, and blood

pressure. The following clinical outcomes were also recorded at delivery; gestational age, birth weight, and perinatal mortality. Blood pressure was measured three times 2 h apart in sitting position after 30 min rest. Blood pressure was assessed by auscultation of brachial artery using a sphyngomanometer. The appearance of the first Koratkoff sound was recorded as the systolic and the disappearance of the fifth sound was recorded as the diastolic blood pressure. Patients with previously known renal disease or other secondary causes of hypertension and before the administration of any drug known to affect blood pressure were excluded from this study.

Selection of Women with Preeclampsia

Patients were classified as mild preeclampsia as they fulfilled the standard criteria:[7] Systolic blood pressure > 140 mmHg and/ or diastolic blood pressure > 90 mmHg or an increase in systolic pressure higher than 30 mmHg or diastolic pressure of 15 mmHg compared with blood pressure obtained before 20th gestational weeks, proteinuria greater than 300 mg per 24 hours or > 30 mg/dl on a clean catch urine specimen, mild oedema and urine output >500 ml/24 hours.

Urine Samples

The first voided morning urine samples were collected in closed polystyrene vials. We immediately centrifuged 10 ml of urine samples for 10 min at 3000 rpm and activities of protein (prot) and creatinine (Cr) were measured. Thereafter 1 ml urine sample was buffered and stored –70 ^{o}C for the measurement of β_2M. This ensured minimal degradation of heat labile proteins such as β_2M. The values obtained prot and β_2M were then expressed per gram of Cr.

Analytic Methods

Serum aspartate transaminase (AST), uric acid, serum and urinary levels of Cr, blood urea nitrogen (BUN) and prot were measured using Roche kit by automatic analyser (Hitachi 747; Hitachi, Tokyo, Japan). Serum and urinary β_2M levels were measured using Immulite kit, which is a solid-phase two-site chemiluminescent immunometric assay, by automatic analyser (Immulite 1000; DPC, Los Angelas, U.S.A).

FE of β_2M was calculated as follows (8):

$$FE\ \beta_2M = \frac{\text{Urine } \beta_2M * \text{Serum Cr}}{\text{Serum } \beta_2M * \text{Urine Cr}} \times 100\%$$

Statistical Analysis

All data were expressed as mean ± SE values. Statistically comparison of parametric findings was analysed by ANOVA followed by Tukey test, and non-parametric findings was analysed by Kruskal-Wallis test followed by Benferroni test. Statistical comparison of gestational age between group 1 and 2 was analysed with Student's t test.

Results

Clinical Outcomes

The clinical characteristics and outcomes of the subjects are given in Table 1**.** There was a significant difference in gestational age at delivery between group 1 and group 2 ($p<0.001$). Significant low birth weight, and increased perinatal mortality was noted in-group 1 compared with that in-group 2 ($p<0.01$).

Levels of the Biochemical Parameters

As shown in Table 2; serum β_2M levels were significantly higher in-group 1 compared with that in groups 2 and 3 ($p<0.05$). The serum β_2M levels in-group 2 did not differ significantly compared with that in-group 3 ($p>0.05$). Urinary β_2M/Cr and prot/Cr ratio was significantly higher in groups 1 and 2 compared with that in-group 3 (respectively; $p<0.001$, $p<0.05$, $p<0.001$). Also, in-group 2, prot/Cr ratio did not differ significantly compared with that in-group 3 ($p>0.05$). There was no significant difference in urinary β_2M/Cr ratio between groups 1 and 2 ($p>0.05$).

FE of β_2M significantly increased in groups 1 and 2 compared with that in-group 3 ($p<0.05$). In-group 1, FE of β_2M did not differ significantly compared with that in-group 2 ($p>0.05$).

Discussion

PE is the most common medical complication of pregnancy.[9] The etiology and pathogenesis of the PE remain poorly understood. There is important evidence to suggest that the diverse manifestations of PE, including altered vascular reactivity, vasospasm and discrete pathology in many organ systems, are derived from pathologic changes within the maternal vascular endothelium.[1]

Normal human pregnancy is characterised by profound changes in the cardiovascular system, including decreased vascular reactivity and a reduced vascular tone. Also, an increase in reactivity and a reduction in relaxation capacity of resistance arteries occur with PE.[10] The hypertension increased blood pressure responsiveness to vasoconstrictors.[11] In the present study, mean urinary protein excretion rate was significantly higher in the pregnant women with PE than in the both normotensive pregnant women and non-pregnant women. Pregnancy is characterised by an increase in GFR of 40% to 80%. An increase in the transglomerular pressure gradient, which increases the rate of filtration, could cause to microalbuminuria. Later, depletion or modification of the negatively charged polyanion of the glomerular membrane leads to the basement membrane becoming a less effective electrostatic barrier to circulating polyanionic proteins such as albumin. Later still, as the architecture of basement membrane becomes abnormal, progressive enlargement of the pores occur and it becomes a less selective sieve and larger quantities of albumin enter the filtrate together with higher molecular weight proteins causing clinical proteinuria.[12-14]

Assays of low molecular weight proteins appear to have considerable potential for indicating tubular dysfunction at an early stage. Low molecular weight proteins are filtered quite freely through the glomerular basement membrane. The affinity of these sites is higher for lower molecular proteins such as β_2M. Low molecular weight proteins are thus nearly completely reabsorbed by the proximal tubular cells and less than 1% of the filtered load appears in urine.[5,15] In the present study, we have found that significantly increased both urinary β_2M excretion and FE of β_2M in both pregnant women with PE and normotensive pregnant as compared to healthy non-pregnant women. There were various reports concerning urinary measurement of β_2M for the prediction of tubular function in the PE. Sudan et al[16] have reported that FE of β_2M from a spot urine sample did not differ among the third trimester normal pregnancy, PE, and gestational hypertension. Yoshida et al[17] have reported that significantly higher β_2M excretion in patients with severe preeclampsia than normal pregnancy. These investigators[16,17] did not measure β_2M/Cr ratio that we used. Another study concerning serum levels of β_2M in PE and normal pregnancy was published by Oian et al[18] These investigators have found serum β_2M concentrations to be slightly but significantly elevated in preeclamptic patients compared with normal pregnant women. The paper regarding urinary β_2M levels relative to Cr excretion in PE and normal pregnancy has been recently published.[19] These investigators have found significantly higher β_2M/Cr ratio in PE and normotensive pregnancy compared with that in non-pregnant women. Our study confirms the results of Hayashi et al,[19] showing a considerably impaired renal tubular reabsorbtion in normal pregnancy and in PE. We thought that only determination of urinary β_2M excretion would allow one to know whether there is increased glomerular filtration, decreased tubular reabsorbtion, or both in pregnant women, compared with non-pregnant women. Therefore we have determined the FE of β_2M. Our present study's results showed significantly higher serum β_2M levels in PE compared with that in normotensive pregnancy and non-pregnant women. Also, preeclamptic and normotensive pregnant women had higher FE of β_2M as compared with those non-pregnant women, but FE of β_2M did not differ between the PE and normotensive pregnancy. With normal kidney function, serum β_2M is elevated only in patients with tumours or inflammatory diseases, representing in these cases increased production rather than reduced clearance.[5,20] It is possible that ischemic placenta may cause increased production of β_2M. Evidence

points to the placenta as key factor that leads to maternal endothelial cell dysfunction in preeclampsia. Placental lipid peroxidation products, tumour necrosis factor alfa (TNFα), and syncytiotrophoblast membrane fragments are candidate blood-borne agents with potential to cause endothelial cell dysfunction.[1,21]

In conclusion, both urinary and FE of β_2M were increased in pregnant women with preeclampsia and normotensive women and these results might indicate tubuler dysfunction.

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