Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cukurova Med J 2017;42(2):344-350
DOI: 10.17826/cutf.322952

ARAŞTIRMA / RESEARCH

Urinary N-acetyl-β-D-glucosaminidase (NAG) and renal tubular injury in mild preeclampsia

Hafif preeklampside üriner N-asetil-β-D-glukozaminidaz (NAG) ve renal tübüler hasar

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Cukurova Medical Journal 2017;42(2):344-350

Abstract

Purpose: This study aimed to evaluate the amount of N-acetyl-β-D-glucosaminidase (NAG) in the 24 h urine output of patients with normotensive pregnancies (control) group with mild preeclampsia (study) group and to detect the presence of renal tubular injury in mild preeclampsia patients.

Material and Methods: The study was conducted among 13 patients study group and 15 patients control group. The 24 h urine output and protein, glomerular filtration rate, systolic and diastolic arterial pressures, serum creatinine, and 24 h urine NAG values of the pregnants were calculated.

Results: Statistical difference was not detected among gestational week, 24 h urine volume (mL), serum creatinine (mg/dL), and glomerular filtration rates of the control and the study groups. The differences of the 24 h urine protein (U/l), systolic and diastolic arterial blood pressures (mmHg), and 24 h urine NAG between groups were statistically significant. A significant correlation was found between the NAG values and 24 h urine protein.

Conclusion: This study shows that; evaluating the impact of protein assessment along with NAG, which indicates both glomerular and tubular injuries, is important, and that renal tubular injury occurs in patients with mild preeclampsia but it did not cause any changes of serum creatinine.

Key words: N-acetyl-β-D-glucosaminidase, mild preeclampsia, proteinuria.

Öz

Amaç: Bu çalışmada, normotansif gebe (kontrol) grubu ile hafif preeklampsi (çalışma) gruplarında 24 saatlik idrardaki N-asetil- β-D-glukozaminidaz seviyelerinin karşılaştırılması ve hafif preeklampsi hastalarında renal tubüler hasarın saptanması amaçlandı.

Gereç ve Yöntem: Bu çalışma, 13 hasta çalışma grubunda ve 15 hasta kontrol grubunda olmak üzere planlandı. 24 saatlik idrar çıkışları ve 24 saatlik idrarda protein, glomerüler filtrasyon hızı, sistolik ve diyastolik arter basınçları, serum kreatinin ve 24 saatlik idrarda NAG değerleri hesaplandı.

Bulgular: Çalışma ve kontrol gruplarında, 24 idrar volume (mL), serum kreatinin (mg/dL) ve glomerüler filtrasyon hızında istatistiksel olarak fark saptanmamıştır. Gruplar arasında, 24 saatlik idrarda protein (U/l), sistolik ve diyastolik arter basınçları (mmHg) ve 24 ssatlik idrarda NAG değerleri istatistiksel olarak anlamlı bulundu. NAG ve 24 saatlik idrarda protein değerleri arasında anlamlı korelasyon bulundu.

Sonuç: Bu çalışmada; hem glomerüler, hem de renal tubüler hasarı gösteren NAG ile birlikte protein değerlendirilmesinin etkisi önemlidir, ve hafif preeklampsi hastalarında renal tubüler hasar oluşurken serum kreatinin seviyelerinde değişikliğe sebep olmadığını bulunmuştur.

Anahtar kelimeler: N-asetil-β-D-glukozsaminidaz, hafif preeklampsi, proteinüri.

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Geliş tarihi/Received: 13.11.2016 Kabul tarihi/Accepted: 27.11.2016

INTRODUCTION

During pregnancy, renal blood flow and glomerular filtration rate increase. However, in patients with preeclampsia compared with normal pregnants, renal perfusion and glomerular filtration rate decreases[1]. Changes in the disease can be monitored with renal biopsies from patients with preeclampsia. In these patients, swelling in the glomerular capillary endothelium and the deposition of fibrinogen derivatives under and between the endothelial cells are observed. Spargo called this structure glomerular capillary endotheliosis[2]. Glomerulopathy causes the increased permeability of proteins with high molecular weight and the development of proteinuria.

The molecular weight of N-acetyl-β-D-glucosaminidase (NAG) is 130 kDa. NAG is present in lysosomes in the renal proximal tubule cells[3]. As NAG cannot be filtered through the glomeruli, the increase in urinary NAG activity is indicative of proximal tubular injury[4]. This system can easily be destroyed, and consequently the lysosomal enzyme NAG is excreted in the urine[5]. Alpha-1 microglobulin and beta-2 microglobulin may appear on the display of tubular injury, but NAG activity is a better predictor. As alpha-1 microglobulin and beta-2 microglobulin cannot be filtered through the glomeruli, they are partially reabsorbed by the tubules[6]. The determination of NAG is a sensitive test method to measure the severity of kidney injury during the period before a decline in renal functions occurs[5]. According to renal function tests, NAG increases in urine in the early stages[7].

However, this method is rarely used because accumulating urine for 24 h to measure NAG is difficult. Eliminating the variations created by urine volume is possible by comparing the NAG level with the urine creatinine levels in the measured urine. However, diuresis affects the urinary excretion of this enzyme. The presence of bacteria in urine does not affect the measurement results[5,8]. NAG can be used in various kidney-related situations because of these features.

The amount of proteinuria and the degree of hypertension correlate with the size of histologic changes. In severe preeclampsia, increased proteinuria and blood pressure values cause increased renal injury. Renal tubular injury is considered to lead to tubular injury after afferent arteriolar vasospasm and edema (renal ischemia and microinfarcts) occur[9].By measuring the amount of 24 h urinary NAG enzyme in patients with mild preeclampsia, we aimed to demonstrate that renal tubular injury could occur even without severe preeclampsia.

MATERIAL AND METHODS

The ethical committee of Gulhane School of Medicine (Etlik-Ankara, Turkey) approved the study. Informed consent was obtained from each subject. The study group consisted of 13 preeclampsia and 15 normotensive pregnant women. A total of 28 patients were monitored in our hospital between between February 2014 and January 2015. The 24 h urine output and protein, 24 h urine NAG, glomerular filtration rate, systolic and diastolic arterial pressures, and serum creatinine values of the pregnants were calculated.

Study design

All patients were chosen from pregnants between 31and 37 weeks to decrease the NAG measurement differences. The following patients were included in the study group: those who scored between 140/90 mm/Hg and 160/110 mm/Hg after a minimum of two times in a 6 h measurement, those whose diastolic blood pressure increased at least 15 mm/Hg and whose systolic blood pressure increased at least 30 mm/Hg compared with the measurements in the pre-pregnancy period, and those whose 24 h urine contained protein more than 300 mg and was lower than 5 g and diagnosed with mild preeclampsia. Westored 24 hoururine protein samplesfor NAG analysis from all pregnants. When we conclueded that patient had mild preeclampsia with clinical sign sandurine protein, westudied NAG in thesame 24 hour urine samples mean time. The control group was chosen from among healthy pregnants between 31-37 gestational week.

The following patients were excluded from the study: those with systematic diseases that could affect the study and patients who had severe preeclampsia (blood pressure over 160/110 mm/Hg, 24 h urine protein ≥ 5 gr proteinuria or ≥ 3+ after measurements made with dipsticks, oliguria ≤ 400 mL/24 h, cerebral and visual disturbances, epigastric pain, nausea, vomiting, pulmonary edema, cyanosis, thrombocytopenia, and increase in creatinine levels), those diagnosed with gestational

diabetes, those who had pre-existing renal disease, and pregnant women who used drugs (antihypertensives and thyroid medication).

NAG measurement

To obtain 24 h urine, patients were suggested to take the first morning urine out and then accumulate the next. They were also instructed to accumulate the first morning urine for the next day. For the evaluation of renal tubular injury occurring in preeclampsia, the measurements of both protein and NAG in patients' 24 h urine were obtained in the GATA Laboratory of Medical Biochemistry. The amount and volume of protein in the 24 h urine samples were calculated. The samples were centrifuged for 10 min. The urine remaining on the surface, which was separated from the cellular elements, was collected and allocated for NAG activity. Samples were kept at +4 °C until the study time. The spectrophotometric measurements were conducted using Cintra 303 spectrophotometer (GBC Scientific Equipment).

The NAG level in the 24 h urine collected from the study and control groups was measured according to the method established by Xu et al [10]. The results were expressed as U/L. Glomerular filtration rate (GFR) was calculated using the short Modification of Diet in Renal Disease (MDRD) formula [GFR=186 x $Pcr^{-1.154}$ x $age^{-0.203}$ x 1.212 (if black) x 0.742 (if female).

Table 1. The measurement of 24-hour urine output and protein. 24-hour urine N-acetyl-β-D Glucosaminidase. glomerular filtration rate. systolic and diastolic arterial pressures. maternal age and serum creatinine of thecontrol and study groups

	Control Group n:15 (min-max)	Study Group n:13 (min-max)	p
Maternal Age	28.9±3.7 (23-34)	29.1±4.1 (23-36)	NS
Gestational Week	33.4±1.7 (31-37)	33.6±1.5 (31-36)	NS
NAG (U/L)	9.23±6.24 (2.48-21.63)	17.95±18.7 (2.16-44.02)	p<0.05
24-hour Urine Protein (mg/day)	167±42 (91-261)	673±384 (308-1333)	p<0.001*
24-hour Urine Output (ml)	2206±846 (700-3800)	2800±753 (1900-4400)	NS
Serum Creatinine mg/dL	0.61±0.09 (0.5-0.8)	0.62± (0.5-0.8)	NS*
Glomerular filtration rate(GFR)	125.46±22.9(88-162)	123.28±20.7(91-161)	NS
Systolic arterial pressures (mmHg)	108±8(100-120)	143±5(140-150)	p<0.001*
Diastolic arterial pressures (mmHg)	69±8(60-80)	103±4(100-110)	p<0.001*

Mann-Whitney-U testi. *

Table 2. Correlation analysis of NAG activity with other parameters in the whole study participants

	Correlation with NAG(U/L)	
	r	p
Groups (Study and Control)	0.439	0.02
24-hour Urine Protein (mg/day)	0.424	0.025
Glomerular filtration rate(GFR)	-0.225	0.25
Serum Creatinine mg/dL	0.173	0.38
Systolic arterial pressures (mmHg)	0.344	0.073
Diastolic arterial pressures (mmHg)	0.321	0.096
24-hour Urine Output (ml)	0.216	0.271
Gestational Week	-0.085	0.667
Maternal Age	0.017	0.93
Pearson's correlation test		

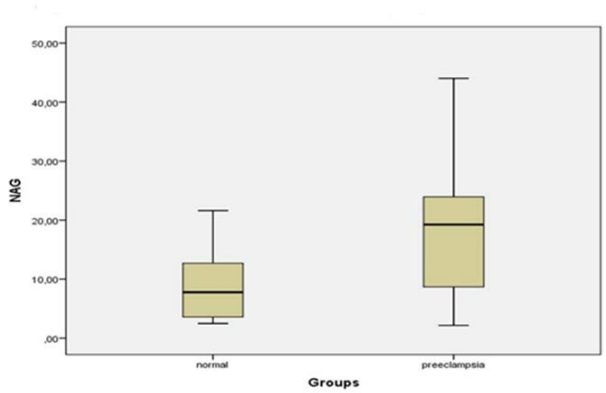

Figure 1. The measurement of 24-hour urine NAG (U/L)

Statistical analysis

Parameter values were given as the mean and standard deviation. Statistical analyses were conducted using SPSS version 15.0 (SPSS, Chicago, USA). Student's t-test was used to analyze age, gestational week, 24 h urine volume, GFR, and NAG value. Mann–Whitney U-test was used to analyze 24 h urine protein, serum creatinine, and systolic and diastolic arterial blood pressure values. Pearson's correlation was used for the analysis of NAG. The results were considered statistically significant when the p-value was ≤ 0.05. Linear regression analysis was conducted to identify the independent determinants of NAG activity in the whole population.

RESULTS

The average gestational age of the 31- to 37-week study group was 33.6. In the control group, the gestational weeks were 31 to 36 weeks and the average gestation was 33.4. The mean age of the study group was 29.1 and that of the control group was 28.9. No statistical difference was found between the groups on the basis of age and gestational weeks. No statistical difference was found between the average values of the control and the study groups' 24 h urine volume (mL) (2206±846 vs. 2800±7539), serum creatinine (mg/dL) (0.61 vs 0.62), and GFR (125.46±22.9 vs. 123.28±20.7) (Table 1).

A statistically significant difference was found between the average values of the control and the study groups' 24 h urine protein (U/l) (167±42 and 673±384 p<0.001) and 24 h urine NAG (U/L) level (9.23±6.24 and 17.95±18.7 p<0.05) (Figure 1 and Table 1).Correlation analysis was performed between NAG and the other parameters [24 h urine protein, the groups (mild preeclampsia + control) GFR, serum creatinine, age, gestational week, and systolic and diastolic arterial pressure]. A significant correlation was found between the NAG values and 24 h urine protein (r=0.424 p=0.025) and the groups (mild preeclampsia + control) (r=0,439 p=0,020). No correlation was observed among the GFR, serum creatinine, age, gestational week, and systolic and diastolic arterial pressure (Table 2).

Table 3. The results of linear regression analysis of 24-hour urine output and protein, 24-hour urine N-acetyl-β-D Glucosaminidase, glomerular filtration rate, systolic and diastolic arterial pressures, and serum creatinine values the whole study population

Variables	NAG (U/L)		
	Unstandardized coefficients (B) R2 0.180		p
24-hour Urine Protein (mg/day)	8.554		0.025

Linear regression analysis was conducted to further investigate if GFR, 24 h proteinuria, 24 h urinary volume, and systolic and diastolic blood pressure were independently associated with NAG activity in the whole population. The model, including all parameters, showed an R2 of 0.180. The analysis demonstrated that 24 h proteinuria was the main determinant of NAG activity. The linear regression

analysis results are shown in Table 3.

DISCUSSION

In pregnant women, urinary excretion of enzymes and other proteins varies significantly[11]. As changes in enzyme excretion may be related to the adaptation of renal functions to pregnancy, different values should be determined for the excretion of enzymes. The NAG value is low for healthy individuals who are not pregnant. Urine excretion increases during healthy pregnancy and varies during gestational weeks; however, studies performed with spot urine proved that no change occurred between 30 and 37 weeks[12]. Consequently, we chose our working groups from among 31- to 37-week pregnant women to prevent the NAG values from being affected. In healthy pregnants, NAG values between 31 and 37 gestational weeksdoes not change. So we used urinary 24 hour NAG calculation as a tubuler injury marker in mild preeclampsia patients regarding to normotensive pregnants[12].

The daytime changes in NAG activity have incurred different opinions. Urinary NAG enzyme excretion is a diurnal rhythm. Unlike the examination of first morning urine in the literature, examining 24 h urine is more suitable. However, the 24 h urine collection process is difficult to perform correctly. The first morning urine collection and determining the NAG activity in comparison with creatinine (urine NAG activity/urine creatinine) are preferred[13]. In their study, Rogers et al. showed that the NAG/creatinine ratio is a diurnal rhythm and that it could produce different values during the day[14]. This situation is considered to depend on the fluctuations in the amount of urine flow. It is minimized by correcting the serum creatinine while calculating the amount of NAG[15].

Goren et al. determined the values of healthy pregnant women in the group of preeclampsia women and connected these findings to the occasional fluctuations of the disease. In other words, they suggested that urine could be taken from pregnant women with preeclampsia in a period without vasospasm[3]. Adoga et al. argued that 24 h urinary measurement was the best method as urinary lysosomal enzymes have a diurnal rhythm[16]. Adoga et al. proved that measurement of 24 h urine NAG to prevent the fluctuations of the NAG value could be use[16]. We did not need to adjust the creatinine to remove such fluctuations as we calculated the NAG value from the 24 h urine.

Moreover, 24 h urinary NAG enzyme inactivation may occur during collection. To avoid this situation, patients are recommended to store urine samples at +4 °C after collection[17]. Correspondingly, we ensured that the urine samples were kept at +4 °C after collection.

Renal blood flow and glomerular filtration rate for pregnant women with preeclampsia and eclampsia decrease significantly compared with those for normal pregnant women in the same period. The reason for this decrease in renal blood flow is the construction of the afferent arterial system. It causes injury by increasing the permeability of the afferent glomerular vasoconstriction membranes for proteins. It increases the permeability and allows the passage of large molecular weight proteins such as transferrin and globulin. The typical renal lesion of preeclampsia or eclampsia is glomerular capillary endotheliosis. This defect occurs through the storage of fibrinous material in and among the endothelial cells. Renal vasoconstriction and decreased glomerular filtration rate in severe preeclampsia cause oliguria. The level of plasma creatinine increases because of a decrease in renal artery vasospasm and glomerular filtration rate[18,19]. Goren et al. reported that renal ischemia and microinfarctions occur together with tubular injury in patients with moderate and severe preeclampsia[3]. In literature there isn't any study about tubuler changes or damage in mild preeclampsia. With using NAG we want to describe that tubuler damage is occuring in mild preeclampsia.

Rogers et al. evaluated patients with preeclampsia using the NAG/creatinine ratio in 24 h urine in the second trimester and detected a significant increase[20]. They determined that the NAG/creatinine ratio in spot urine was higher than the NAG score in 24 h urine. Semczuk et al. also observed renal tubular injury and increased NAG in the morning urine of preeclampsia patients[21]. Hayashi et al. reported increased NAG excretion in urine in patients with preeclampsia but only during the 28 to 40 gestational weeks and without a distinction between mild and severe preeclampsia[22]. Likewise, Paternoster et al. found a high level of urine NAG enzyme but only in pregnant women 24 to 40 weeks and without separating the mild preeclampsia group; they did not consider the significance of the excretion of NAG enzyme in

urine before and after 30 weeks[23]. Unlike previous studies, our study evaluated pregnants 30 to 37 weeks, during which the elongation amount of urinary NAG was not different. Severe preeclampsia was excluded from the study. We detected a statistically significant difference between the NAG enzyme level of the control group and that of the study group in patients with mild preeclampsia.

Rustom et al. detected a statistically significant correlation between glomerulonephritis other than diabetic nephropathy, hypertensive nephrosclerosis, proteinuria associated with chronic pyelonephritis and urinary NAG enzyme. Patients with mild preeclampsia were not evaluated[24]. In our study, a significant correlation was detected between urinary NAG in patients with mild preeclampsia and 24 h urine protein. We also found no correlation between patients' blood pressure values and urinary NAG levels. Semczuk et al. proved that the NAG increase in preeclampsia did not correlate with the increase in blood pressure values[21]. This finding strengthens the probability of tubular injury with the increase in proteinuria, not with renal ischemia and microinfarcts.

GFR is the best measurement method used for evaluating renal function[25,26]. Serum creatinine, which can easily pass through the membrane glomeruli and cannot be reabsorbed by the proximal tubules, is also used for GFR estimation. The increase in serum creatinine indicates glomerular injury. Glomerular injury may cause renal tubular injury. The decrease in GFR causes an increase in NAG[27]. Junk et al. found that the variability of renal blood flow also affected the amount of NAG[28]. In our study, renal tubular injury could occur in patients with mild preeclampsia even without the decrease in GFR. We showed that, tubuler injury in preeclampsia patients could be occurred without GFR rising with 24 hour urine NAG assessment.

We determined that the increase in urinary NAG enzyme was correlated with proteinuria. Through linear regression analysis, we found that the urinary NAG values of pregnant women with mild preeclampsia and the control group increased independently from GFR, 24 h proteinuria, 24 h urinary volume, and systolic and diastolic blood pressure. This increase in urinary NAG was dependent and correlated with proteinuria.

A portion of the protein passes through the glomeruli but is reabsorbed in the proximal tubules. The increase in proteinuria, with its toxic effect, has been proposed to cause injury to proximal tubule cells[29,30]. In patients with mild preeclampsia, an increase in proteinuria and in the proximal tubule injury indicator NAG was observed. The increase was dependent on and correlated with each other, as shown in the regression analysis. No correlation was found between patients' blood pressure values and urinary NAG levels. This finding supports the fact that proximal tubule injury is more likely to result from proteinuria than from vasospasm in afferent arterioles.

Studies show that a minimal relationship exists between the quantity of Massive proteinuria urinary protein (greater than 5 g) and pregnancy outcome with preeclampsia. Massive proteinuria was eliminated because of severe preeclampsia[31]. There is no adverse perianatal outcomes in the normotensive pregnants and one preterm labor was occurred in mild preeclampsia group in 34. gestational week. Newborns were healthy in both groups..

Even in our study with a limited number of cases, we determined that renal tubular injury occurred in patients with mild preeclampsia. We think this injury is tolerated by kidney and does not cause oliguria or renal failure. We suggest that it should be considered in prospective studies as it progresses to severe preeclampsia when not tolerated. We believe that evaluating the impact of assessing protein along with NAG, which indicates both glomerular and tubular injuries, is important.

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