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Determination of urinary N-acetyl-β-D glucosaminidase (NAG) levels in experimental blunt renal trauma

Deneysel künt böbrek travmasında idrar N-Asetil-β-D glukozaminidaz (NAG) düzeylerinin belirlenmesi

Alpaslan HANBEYOĞLU,[1] Ahmet KAZEZ,[1] Bilal ÜSTÜNDAĞ,[2] Nusret AKPOLAT[3]

BACKGROUND

We evaluated the applicability of urinary N-acetyl-beta-D glucosaminidase (NAG) levels in the diagnosis and follow-up in blunt kidney injury.

METHODS

Twenty Sprague-Dawley rats were studied. In the Sham group, left kidney exploration was made. In the Trauma group, after left kidney exploration, a 20 g weight was dropped onto the kidneys. Urine was collected for analysis with strip and determination of urinary NAG and creatinine (Cr) levels at baseline and 0-6, 12-24, 24-36 and 36-48 postoperative hours. Mann-Whitney U and Kruskal-Wallis tests were used.

RESULTS

Macroscopic examinations of traumatized kidneys revealed grade II and III injury, and histopathological examinations showed relevant changes. Macroscopic hematuria was observed in all traumatized rats. Urinary NAG/Cr levels in the Trauma group were found to be significantly higher than their base levels at 0-6, 12-24, 24-36, and 36-48 hours. In the Sham group, only the level of NAG/Cr at 0-6 hours was significantly higher. The increase in NAG/Cr levels at 0-6 hours was significantly higher in the Trauma group than in the Sham group.

CONCLUSION

After isolated blunt renal trauma, urinary NAG levels increase in the early stage. However, more detailed clinical studies are needed to develop NAG levels as a criterion in the follow-up of blunt renal trauma.

Key Words: NAG; renal injury; trauma.

AMAÇ

İdrar N-asetil-β-D glukozaminidaz (NAG) düzeyinin, künt böbrek yaralanmasının tanı ve takibinde kullanılabilirliği araştırıldı.

GEREÇ VE YÖNTEM

Yirmi adet Sprague-Dawley cinsi genç-erişkin sıçanla çalışıldı. Sham grubunda sol böbrek eksplorasyonu yapıldı. Travma grubunda sol böbrek eksplore edildikten sonra 20 gr ağırlık böbrek üzerine düşürüldü. İşlem öncesi, işlem sonrası 0-6., 12-24., 24-36. ve 36-48. saatler arasında strip ile idrar tetkiki, idrar NAG ve kreatinin (Cr) düzeyleri için örnekler alındı. İstatistiksel analiz Mann-Whitney U ve Kruskall Wallis testleri ile yapıldı.

BULGULAR

Travma oluşturulan böbreklerin makroskopik incelemelerinde grade II ve III yaralanma olduğu, histopatolojik incelemelerinde de beklenen patolojik değişiklikler görüldü. Travma grubundaki sıçanların hepsinde makroskopik hematüri izlendi. Travma grubunda 0-6., 12-24., 24-36. ve 36-48. saatler arasındaki idrar NAG/Cr düzeyleri işlem öncesi kontrol değerlere göre anlamlı olarak yüksek bulundu. Sham grubunda ise sadece 0-6. saatteki idrar NAG/Cr düzeyleri kontrol değerlere göre anlamlı olarak yüksekti. Sıfır-6. saatteki NAG/Cr düzeylerindeki artış travma grubunda anlamlı olarak daha fazlaydı.

SONUÇ

İzole künt böbrek yaralanmalarından sonra erken dönemde idrar NAG düzeyleri anlamlı olarak yükselmektedir. NAG düzeylerinin künt böbrek yaralanmalarında bir ölçüt olarak kullanılabilirliği için daha kapsamlı ve klinik çalışmalarla geliştirilmesi gerekmektedir.

Anahtar Sözcükler: NAG; böbrek yaralanması; travma.

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Departments of [1]Pediatric Surgery, [2]Clinical Biochemistry, [3]Medical Pathology, Fırat University Faculty of Medicine, Elazig, Turkey.

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Fırat Üniversitesi Tıp Fakültesi, [1]Çocuk Cerrahisi Anabilim Dalı, [2]Klinik Biyokimya Anabilim Dalı, [3]Tıbbi Patoloji Anabilim Dalı, Elazığ.

Correspondence *(İletişim):* Ahmet Kazez, M.D. Fırat Üniversitesi Fırat Üniversitesi Hastanesi, Çocuk Cerrahisi Kliniği, 23119 Elazığ, Turkey.
Tel: +90 - 424 - 233 35 55 / 2907 e-mail *(e-posta):* akazez@firat.edu.tr

Although mortality and morbidity due to trauma have declined in children, injuries remain an important health problem. Most of the intraabdominal organ injuries in children occur due to blunt traumas, and approximately 10% of these injuries affect the genitourinary system.[1-4] The kidneys are the most frequently injured organs when genitourinary system injuries are considered, and renal injury is recorded in approximately 1-5% of all the injuries.[5,6] Clinical findings are not manifest in 25% of the cases with a serious renal injury.[7]

Although N-acetyl-β-D glucosaminidase (NAG; 2-acetamidodeoxy-β-glucoside acetamidodeoxy glucohydrolase) has been used for the identification of renal diseases and injuries, its application in renal traumas remains an unknown issue. NAG is a stable glycolytic enzyme excreted in the urine with a molecular weight of 130,000 Dalton. It is particularly found at a high level in the lysosomes of proximal tubule cells. It cannot be filtrated through the glomerulus due to its high molecular weight; thus, the increase in the urine concentration is indicative of proximal tubular damage and loss of lysosomal integrity.[8,9]

Having considered the fact that urine NAG levels can show significant changes depending on renal parenchyma and tubular damage after blunt trauma, these levels may be used as a guiding parameter in patients with suspected renal trauma.

In this study, it was aimed to investigate the availability of urine NAG levels for the diagnosis and follow-up of renal trauma in rats.

MATERIALS AND METHODS

This study was carried out in a University Experimental Research Center after the approval of the local Animal Ethics Committee. Young adult, male, Sprague-Dawley rats, 3 months of age and weighing 220-300 g, were included in the study.

The rats were fed with standard rat food ad libitum and tap water in metabolic cages in a controlled room (temperature 20 to 25°C; humidity 70% to 80%; 12-hour (h) light/dark cycle).

The study was conducted on 2 groups of 10 rats each.

Group 1: Rats underwent renal exploration only (Sham)

Group 2: Rats were exposed to isolated blunt renal trauma (Trauma)

Because basal NAG levels before the procedure would be measured, no separate control group was formed. An experimental procedure was carried out in aseptic conditions under general anesthesia of 50 mg/kg intramuscular ketamine hydrochloride (Ketalar®, Eczacıbaşı, İstanbul, Turkey) and 4 mg/kg xylazine hydrochloride (Rompun®, Bayer, İstanbul, Turkey).

Fig. 1. The trauma set prepared for the study: a 30 cm metal tube with a spoon mechanism at its lower tip and cylinder-shaped metal weights of 100, 75, 50, 40, 30, 25, 20, 15, 10 g, respectively.

(Color figure can be viewed in the online issue, which is available at www.tjtes.org)

Preliminary Study: Building an Experimental Isolated Renal Trauma Model

A preliminary study was conducted on two rats to obtain standard renal injury. Under general anesthesia, each of the weights, 50 and 40 g respectively, was allowed to fall from a metal tube of 40 cm (i.e. from 40 cm height) in a set designed especially by the researchers (Fig. 1) onto an isolated kidney. It was observed that both of the kidneys were completely crushed and shattered (Grade V injury) for these height and weight values. The height was then lowered to 30 cm and the weight reduced to 20 g to obtain milder injuries that did not require resuscitation and surgical operation following the trauma. It was recorded that an appropriate injury model was acquired as a result of the trial performed on both kidneys applying the latter values. Both rats were sacrificed with a high dose of anesthetics after the preliminary study.

Experimental Study

a) Sham group: A midline laparotomy was performed under anesthesia after shaving and cleaning with antiseptic solution. The left kidney was identified and isolated from the perirenal adipose tissue and replaced into its location. Five ml of saline solution was injected into the peritoneum and the abdominal wall was closed with 4/0 continuous atraumatic silk sutures in two layers. Rats were placed into their metabolic cages, fed by standard food and water and followed-up.

b) Trauma group: After the left kidneys were separated from the perirenal adipose tissue, they were lifted from lateral sides, and the "*spoon*" portion of the specially designed device for inducing trauma was inserted under the kidney. The parenchyma was targeted without causing damage to the renal pedicle, and a cylindrical metal weight of 20 g was allowed to fall once onto the kidney from a 30 cm height in vertical axis. The device was removed, and the kidney was replaced into the renal bed. Injury was observed macroscopically. Five ml of warm saline solution was injected into the abdomen, the abdomen was closed, and the rat was replaced into the metabolic cage. The same follow-up and care criteria were applied for both rat groups following procedures. Urine samples were acquired 6 h after the operation and at the intervals of 12-24, 24-36 and 36-48 h. Rats in both groups were sacrificed with high-dose anesthetic agents at the end of 48 h. The abdomen was opened, and both kidneys were removed and placed into 10% formalin solution for histopathological examination.

Collecting and Evaluating Urinary Samples

Urine samples accumulating in metabolic cages were taken from both groups before the procedure and were analyzed using URS-10 urine stripes (Teco Diagnostics, Anaheim, USA). Urine samples were taken to measure NAG and creatinine (Cr) levels in the urine and to calculate normal basal values before the procedure. Basal values made up the control group of the study.

Urine strip examinations were carried out in urine samples of the groups that were obtained in the first 6 h following the operation and between the intervals of 12-24, 24-36 and 36-48 h. Urine samples were obtained for NAG and Cr. Urine samples were centrifuged in 3000 rpm for 5 minutes (min). Acquired supernatants were placed into Eppendorf tubes for measurements and stored at -80°C.

The NAG index was calculated to eliminate the changes in urine samples that would arise due to urine volume.

Urinary NAG Index (U/g): The urinary NAG index was calculated as Urine NAG activity / Urine creatinine concentration.

3-cresol-sulphophthalein method was applied to measure the NAG activity in spot urine.[10] MCP-NAG was used as substrate and the absorbance of 3-cresol-sulphophthalein revealed through NAG hydrolysis was measured by Techcomp 8500 II UV/VIS spectrophotometer (Techcomp Ltd, Shanghai, China) in 580 nm. NAG 875406 kit (Roche Diagnostic GmbH, Manheim, Germany) was applied for measurements. Cr in urine was measured by Olympus AU600 autoanalyzer (Olympus Optical Co Ltd, Japan) using Olympus brand UV-kinetic kit and applying Jaffe method.[11]

Histopathological Examination

After the kidneys were fixed in 10% formalin solution 48 h after the procedure, samples that passed through the long renal axis and pelvis were taken. Hematoxylin eosin (H-E), Masson's trichrome (MAS) and periodic acid-Schiff (PAS) stains were applied to the samples.

Sections were evaluated with respect to capsule rupture, interstitial hematoma, cortical laceration, medullary laceration, glomerular damage (hematoma), tubular rupture, arcuate artery rupture, and infarction. Changes were rated between 0 and 3 for tubular rupture, subcapsular hematoma and interstitial hematoma. Accordingly, 0 expressed no pathology; 1 mild changes; 2 moderate changes; and 3 severe pathological changes. The other parameters were calculated as 0 (no pathological changes) and 1 (presence of pathology).

Statistical Analysis

Data acquired from the groups were expressed as mean value ± standard derivation (Mean ± SD). The Mann-Whitney U test was applied for the periodic evaluation of groups, Kruskal-Wallis test was used for period comparisons in each group, and Mann-Whitney U test was applied for dual comparisons. Lowest statistical significance level was accepted as $p<0.05$.

RESULTS

All the rats survived until the end of the study. Grade II or III renal damage was observed in all the rats in the Trauma group. Following the procedure in the Trauma group, macroscopic hematuria was seen in all rats.

Urinary Strip Analyses

There were no pathological findings with regard to urine strip examinations in either group before the procedure. Further, no pathological findings were identified in urine samples received from the Sham group after the operation between the intervals of 0-6, 12-24, 24-36, and 36-48 h. Macroscopic hematuria, 2+ (80 erythrocyte/µl) and 3+ erythrocyte (200 erythrocyte/µl) were observed in the strip test in urine samples acquired from all the rats of the Trauma group following the procedure in the interval of 0-6 h. Between 12-24 h, macroscopic hematuria in 3 of the rats and 1+ (25 erythrocyte/µl) or low-level hematuria in all the rats were still present. No macroscopic hematuria was recorded in any of the rats between 24-36 h. No macroscopic hematuria was observed between 36-48 h, whereas low-level hematuria was identified in 4 rats in the strip examination.

NAG Levels

Control NAG values before the procedure were found to be 3.213±1.392 U/g on average in

Table 1. Average NAG levels in groups

Groups	NAG (U/g) Control	NAG (U/g) 0-6 h	NAG (U/g) 12-24 h	NAG (U/g) 24-36 h	NAG (U/g) 36-48 h
Sham group	3.213±1.392	69.366±51.618*	13.413±13.179	3.805±2.590	2.439±1.313
Trauma group	2.659±0.840	113.00±45.109*[a]	14.703±13.962*	10.027±10.362*[a]	8.253±7.294*[a]

*: p<0.05 between the control and time interval values in each group.
[a]: p<0.05 between the corresponding values of the two groups.

the Sham group. It was recorded that NAG levels (69.366±51.618 U/g) measured 0-6 h after the procedure were increased compared to control NAG values before the procedure. Further, NAG levels (13.413±13.179 U/g) measured between 12-24 h were increased compared to control values before the procedure. The increase in NAG levels was statistically significant compared to control values between 0-6 h ($p<0.05$), whereas the increase between 12-24 h was not significant. No significant difference was found in NAG levels (3.805±2.590; 2.439±1.313 U/g) between the intervals of 24-36 and 36-48 h compared to control values ($p>0.05$) (Table 1).

Control NAG values before the procedure were found to be 2.659±0.840 U/g on average in the Trauma group. The NAG level was measured to be 113.00±45.109 U/g for this group between 0-6 h and was higher than all the other groups. These values were measured as 14.703±13.962, 10.027±10.362, and 8.253±7.294 U/g, respectively, between the intervals of 12-24, 24-36 and 36-48 h. It was determined that all NAG levels measured between the intervals of 0-6, 12-24, 24-36, and 36-48 h were observed to be increased significantly compared to control levels before the procedure ($p<0.05$) (Table 1).

When the Sham group and Trauma group were compared, no significant difference was found between NAG control levels and the values between 12-24 h, whereas the increase between the intervals of 0-6, 24-36 and 36-48 h was significant in the Trauma group compared to the Sham group ($p<0.05$) (Table 1, Fig. 2).

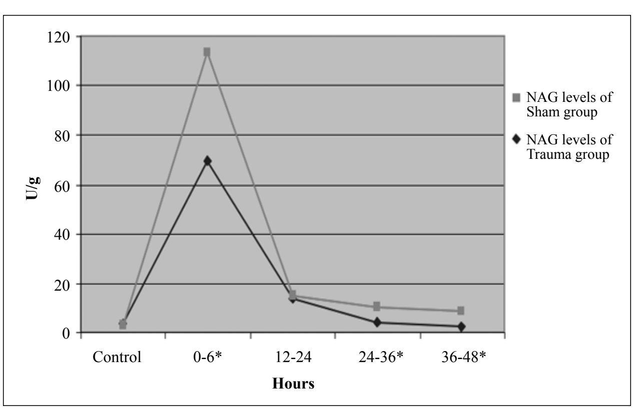

Fig. 2. Comparison of NAG levels of the groups.
*p<0.05: When the same periods of the groups were compared.

Histopathological Changes

No pathologic findings were observed in the Sham group on macroscopic and histopathological examination of the kidneys.

In the macroscopic examination of the kidneys exposed to trauma, capsule damage, parenchymal laceration, bleeding, and perirenal bleeding were identified. Grade III injury was determined in 6 rats, whereas Grade II injury was noted in 4 rats in the Trauma group macroscopically.

Pathological changes due to trauma were recorded for all the rats and are summarized in Table 2.

DISCUSSION

The measurement of the urine enzymes to diagnose kidney diseases and to reveal renal damage has a wide range of clinical application.[8,12,13] NAG, as a noninvasive test, has special importance in the diagnosis of renal damage in the early period, which occurs due to toxins or diseases, and in the follow-up of the progressive disease.[8] NAG determination is a sensitive test to measure the severity of the renal damage before renal functions regress.[14] However, there is not enough information in the literature about the changes in urine enzymes due to renal damage caused by trauma directly.

The easiest means of identifying whether the genitourinary system is affected in cases with general body trauma is to analyze the urine sample of the patient and to determine whether or not hematuria has developed.[15] Sixty-five percent of the severe renal damages have macroscopic hematuria in the beginning and 32.7% of those have microscopic hematuria; no hematuria may be recorded in only 1.7% of the cases. The reason for no hematuria in these cases is either renal pedicle damage or ureteropelvic rupture, both of which are rarely observed injuries.[16] No correlation has been found between the degree of hematuria and the severity of the renal injuries. Thirty-one percent of the minor renal damages have macroscopic hematuria and 65.5% of those have microscopic hematuria; no hematuria may be recorded in 3.4% of the cases.[16-19] Macroscopic hematuria is a more significant finding

Table 2. Histopathological examination of the kidneys in the trauma group

	A	B	C	D	E	F	G	H	I
T1	2	0	2	0	1	1	0	1	0
T2	2	2	2	1	1	1	0	0	0
T3	1	1	3	1	1	0	0	1	0
T4	3	2	1	1	1	1	0	0	0
T5	3	2	3	1	1	1	0	1	1
T6	3	0	1	1	1	1	0	0	0
T7	2	0	2	1	1	0	0	1	1
T8	3	3	3	0	1	1	1	0	1
T9	2	1	3	1	1	1	0	0	0
T10	2	0	1	1	1	0	0	0	0

A: Tubular Rupture*; **B:** Subcapsular hematoma*; **C:** Interstitial hematoma*; **D:** Capsule rupture; **E:** Cortical laceration; **F:** Medullary laceration; **G:** Glomerular damage hematoma; **H:** Arcuate artery rupture; **I:** Infarction.
In the first three parameters (*): 0: none, 1: mild, 2: moderate, 3: severe; other parameters are evaluated as 0: absent, 1: present.

when renal injuries are taken into account; however, major renal injury was identified in only 32% of the patients with macroscopic hematuria.[20] In this study, macroscopic hematuria was recorded in the early period in all rats of the Trauma group exposed to grade II-III renal injuries. No hematuria was determined in rats in the Sham group in either the macroscopic or strip examinations performed in the postoperative period.

Although various indicators (serum Cr and urea, Cr clearance, etc.) are used widely in routine laboratories to evaluate the glomerular damage in kidneys, biochemical indicators for the evaluation of tubular damage are limited. For his purpose, ALP (alkaline phosphatase), GGT (gamma-glutamyl transferase), LAP (leucine aminopeptidase), AAP (alanine aminopeptidase), GAL (beta galactosidase), NEP (neutral endopeptidase), and NAG measurements are used in urine. However, the stability of the molecule to be evaluated and the difficulty of collecting urine for 24 h restrict the usage of most of these indicators. Among these indicators, NAG has become prominent in the identification of renal damage.[14,21-24]

N-acetyl-β-D glucosaminidase (NAG) is the most frequently applied enzyme for the evaluation of renal tubular damage. It was reported in the literature that the stability of NAG enzyme excreted through the urine was higher and that it maintained its activity for a long period by freezing. Moreover, it was reported that this enzyme might be used in routine clinical practice by proportioning urine NAG activity to Cr and working with spot urine.[14,23,24] We also investigated the changes in urine NAG levels in the early period due to blunt renal injuries by applying the NAG/Cr ratio in order to eliminate the effects of volume changes.

In clinical studies and in an experimental study conducted on rats, it was indicated that NAG levels increased just after ESWL (extra-corporeal shock wave lithotripsy), urine NAG levels returned to normal levels in one week, and that ESWL might cause renal damage that recovers rapidly.[25-27] In an experimental study carried out on dogs by Fortes et al.,[28] it was recorded that urine NAG levels increased 12 h after ESWL, and were decreased after 24 h. A second ESWL applied 24 h later did not increase NAG levels again. However, a NAG-related study on blunt renal trauma was not found in the literature.

There are many study models in the literature on blunt renal injuries. Trauma models are used in which a cylinder-shaped weight attached to a pendulum in vitro is released to fall by extending it towards a side and crash to the kidney. Postmortem swine kidneys were used in other studies and biomechanics of the trauma and the final lesions were mostly emphasized.[29-31] No isolated blunt renal trauma model was found in the literature. Thus, a standard injury model was aimed to be obtained with a preliminary study. A metal tube was designed by the researchers to be used in the model. A spoon mechanism at its lower tip that could be placed under the kidney (for renal isolation) and a cylinder-shaped weight with smooth surface measuring 1 cm in diameter (for trauma) were used. The aim of the pre-study was to obtain a standard and acceptable renal injury that did not require any posttraumatic resuscitation or surgical intervention. It was found that Grade III injury, among the five- degree renal injury scale accepted by the AATS (American Association of Trauma Surgeon)[32] was observed by dropping a 20 g weight from a height of 30 cm. This trauma model was applied in rats in the Trauma group. It was determined both macroscopically and histopathologically that 4 of the rats had grade II and 6 had grade III renal injuries.

In this study, urine NAG levels, which were recorded at 0-6, 12-24, 24-36, and 36-48 h following isolated blunt renal trauma, were found to be significantly higher with respect to urine NAG levels before the trauma. It was noted that an apparent increase was found in urine NAG levels in the first 6 h particularly and that these high levels decreased in 48 h. An increase in urine NAG levels recorded at 0-6 and 12-24 h was observed in the Sham group. The increase at 0-6 h was statistically significant. No significant difference was found for this group when urine NAG levels at 12-24, 24-36 and 36-48 h were compared to control values. In the study of Fortes et al.,[28] intravenous thionembutal was administered to dogs under anesthesia with Pentrane inhalation. Intravenous contrast material was given during the procedure and a second ESWL was applied at 24 h in the same manner. In that study, urine NAG levels before ESWL were compared to NAG levels at 12, 24, 36, and 48 h after ESWL; however, no separate control group was formed to evaluate the effects of anesthesia or contrast material. In the present study, a Sham group, which received anesthesia using ketamine and xylazine, was formed in addition to the group exposed to trauma. The apparent increase in urine NAG levels in the Sham group recorded in the first 6 h was remarkable. It was thought that this increase might be related to the anesthesia or surgical operation. In the study carried out by Fortes et al.,[28] in which ESWL was applied at 24 h with a second anesthesia and contrast administration, no increase in NAG levels was observed. This again supports the fact that the increase in urine NAG levels in the Sham group, including in our study, may be due to the surgical exploration. Additionally, when urine NAG levels of both the Trauma and Sham groups recorded at 6 h were compared, it was found that the increase in urine NAG levels of the Trauma group was significantly higher.

In conclusion, urine NAG levels increase in the early period due to posttraumatic tissue damage in the kidneys. This increase is more apparent in the first 6 h and continues decreasingly to 48 h as well. The results should be supported by similar studies, and normal NAG values of various age groups should be determined for the clinical applications.

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