Učinak trajanja šećerne bolesti tipa 1 na aktivnost N-acetil-β-D-glukozaminidaze u mokraći djece i adolescenata

Effect of duration of diabetes mellitus type 1 in children and adolescents on urinary N-acetyl-β-D-glucosaminidase excretion

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Sažetak

Cilj: Cilj je bio ispitati ovisi li izlučivanje NAG u mokraći djece i adolescenata s šećernom bolesti tipa 1 o trajanju bolesti.

Metode: Katalitičke koncentracije N-acetil-β-D-glukozaminadaze određivale su se spektrofotometrijski u slučajnim uzorcima mokraće 66 djece i adolescenata s šećernom bolesti tipa 1 68 ispitanika iz kontrolne skupine. Bolesnici iz skupine dijabetičara bili su podijeljeni s obzirom na trajanje bolesti: skupina I manje od tri godine (N=17); skupina II tri do pet godina (N=19); skupina III pet do deset godina (N=19); skupina IV više od deset godina (N=11). Vrijednosti NAG izražene su u odnosu na koncentraciju kreatinina u mokraći radi isključivanja razlika u koncentraciji mokraće.

Rezultati: Izlučivanje NAG mokraćom kod dijabetičara bilo je statistički značajno povišeno u odnosu na kontrolnu skupinu. (p<0,001). U sve je četiri skupine dijabetičara statistički značajno bilo povećano izlučivanje NAG mokraćom u odnosu na kontrolnu skupinu (skupina I, p=0,001; skupine II i III, p<0,001 i skupina IV, p=0,004) no nije nađena statistički značajna razlika među skupinama dijabetičara (skupina I *vs*. II, p=0,716; skupina I *vs*. III, p=0,899; skupina I *vs*. IV, p>0,10; skupina II *vs*. III, p=0,549; skupina II *vs*. IV, p>0,10; skupina III *vs*. IV, p>0,10). Nije nađena korelacija između vrijednosti NAG u mokraći dijabetičara i trajanja bolesti (r=–0,017, p=0,892)

Zaključak: Izlučivanje NAG mokraćom povećano je kod djece i adolescenata s šećernom bolesti tipa 1, ali ne ovisi o trajanju bolesti.

Ključne riječi: N-acetil-β-D-glukozaminidaza, šećerna bolest tipa 1, dijabetička nefropatija

Abstract

Objective: The aim of this study was to examine whether there are differences between urinary NAG activity between children and adolescents with diabetes mellitus type 1 and healthy subjects and examine whether urinary NAG activity changes in relation to duration of the disease.

Methods: N-acetyl-β-D-glucosaminidase (NAG) activities were determined spectrophotometrically in random urine samples children and adolescents (N=66) with type 1 diabetes mellitus and control subjects (N=68). Patients in the diabetic group were divided according to the duration of diabetes in four groups: group I, less than three years (N=17); group II, three to five years (N=19); group III, five to ten years (N=19) and group IV, more than ten years (N=11). To exclude interference due to urine concentration differences, urinary NAG activities were referred to the level of urinary creatinine.

Results: Urinary NAG excretion in diabetic patients was significantly increased compared to the controls (P<0.001). All four subgroups had significantly higher activities of urinary NAG compared to the controls (group I, P=0.001, groups II and III P< 0.001 and group IV, P=0.004) but there was no significant difference among diabetic groups (group I vs. II, P=0.716; group I vs. III, P=0.899; group I vs. IV, P>0.10; group II vs. III, P=0.549; group II vs. IV, P>0.10 and group III vs. IV, P>0.10). There was no correlation between urinary NAG activities in diabetic patients and duration of diabetes (r= – 0.017, P=0.892).

Conclusion: Urinary NAG excretion was elevated in children and adolescents with diabetes mellitus type I but it did not depend on duration of the disease.

Key words: N-acetyl-β-D-glucosaminidase, diabetes mellitus type 1, diabetic nephropathy

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Uvod

Bubrežno oštećenje je ozbiljna komplikacija u šećernoj bolesti. Ako je dijabetička nefropatija dijagnosticirana

Introduction

Renal damage is serious complication in diabetes mellitus. If diabetic nephropathy is diagnosed by microalbumi-

na temelju mikroalbuminurije, proteinurije ili sniženoga klirensa kreatinina, vrlo je malo moguće postići da bi se spriječilo zatajenje bubrega (1). Stoga postoji potreba za ranijim pokazateljima strukturnih i funkcionalnih promjena u bubregu koje bi bilo moguće spriječiti strogom glikemijskom kontrolom.

U nekoliko je studija ukazano da rana tubularna promjena nastala zbog peritubularne mikroangiopatije može prethoditi glomerularnim promjenama te imati važnu ulogu u pojavi dijabetičke nefropatije (2). Pojačano urinarno izlučivanje enzima koji potječu iz proksimalnih bubrežnih tubula utvrđeno je u bolesnika sa šećernom bolesti tipa 1 kao posljedica tubularne ozljede; ti enzimi su N-acetil-β-D-glukozaminidaza (NAG), alanin-aminopeptidaza, alkalna fosfataza, urinarna dipeptidil-aminopeptidaza IV (3). Aktivnost nekolicine od tih tubularnih biljega povišena je u mokraći i prije pojave mikroalbuminurije.

NAG (EC 3.2.1.30) je lizosomski enzim velike molekulske mase, široko rasprostranjen u mnogim tkivima i tjelesnim tekućinama. Mokraćna NAG je bubrežnoga podrijetla (5) jer ne može proći kroz glomerularnu membranu zbog svoje velike molekularne težine (130 000 do 140 000 D). Otpušta se iz stanica kao posljedica fiziološke egzocitoze (6) ili raspada stanica (7). NAG je raspodijeljen uzduž čitavog nefrona no uglavnom se nalazi u proksimalnim bubrežnim tubulima i možemo ju smatrati ranim neinvazivnim biljegom tubularnog oštećenja bubrega. Aktivnosti NAG u mokraći su povišene u pijelonefritisu (8), refluksnoj nefropatiji (9), hipertenziji (5) i reumatskom artritisu, kod izloženosti nefrotoksičnim lijekovima, zagađivačima okoline poput žive (10), kadmija (11), te kod radnika koji su tijekom rada izloženi trikloroetilenu (12). NAG može biti korištan parametar u bolesnika s tubulointersticijskim nefropatijama za razlikovanje akutnih od kroničnih poremećaja. Aktivnost NAG veća od 20 U/L (15 U/g kreatinina) može se mjeriti kod akutnoga oštećenja tubularnog sustava (13).

NAG u mokraći je rani pretkazatelj razvoja dijabetičke nefropatije; u šećernoj bolesti tipa 1 povišen je prije pojave mikroalbuminurije (14).

Schultz i sur. (15) su dokazali da su povišene aktivnosti NAG u mokraći ispitanika mlađih od 16 godina povezane s trajanjem šećerne bolesti tipa 1 i glikiranoga hemoglobina (HbA_{1c}). Mocan i sur. (16) su ispitali 54 odrasle osobe sa šećernom bolesti tipa 2 i zapazili da aktivnost NAG počinje rasti tijekom treće godine bolesti, dostiže najvišu razinu između 3.–10. godine, te ubrzano raste nakon 10. godine.

Kavukçu (17) je dokazao da je aktivnost NAG u mokraći povezana s koncentracijom glukoze u krvi, a Kordonouri i sur. (18) su ustanovili da su oni ispitanici kod kojih je šećerna bolest nastupila u djetinjstvu imali značajno više aktivnosti NAG u mokraći u usporedbi s bolesnicima kod kojih je bolest nastupila nakon 16. godine života, te su češća oštećenja bubrega.

nuria, proteinuria or decreased creatinine clearance, little can be done to prevent the progressive course of renal failure (1). There is a need for earlier indicators of renal structural and functional changes which may be prevented by strict glycemic control.

A number of studies have shown that early tubular alteration due to peritubular microangiopathy may precede glomerular cha nges and play a role in the initiation of diabetic nephropathy (2). Increased urinary excretion of enzymes that originate from proximal renal tubules were found in patients with diabetes mellitus type 1 as result of tubular injury: N-acetyl-β-D-glucosaminidase (NAG), alanine-aminopeptidase, alkaline phosphatase, urinary dipeptidyl aminopeptidase IV (3). Activity of these tubular markers is increased in urine before the appearance of microalbuminuria (4).

NAG (EC 3.2.1.30) is a high molecular-weight lysosomal enzyme. It is widely distributed in many tissues and body fluids. Urinary NAG is of renal origin (5) as it cannot pass through glomerular membrane due to its high molecular weight (130 000 to 140 000 D). It is released from cells as a consequence of physiological exocytosis (6) or breakdown of cells (7). NAG is distributed along the whole nephron but mainly in renal proximal tubules and it is considered to be a non-invasive early marker of renal tubular damage. Urinary NAG activities are elevated in pyelonephritis (8), reflux nephropathy (9), hypertension (5) and rheumatoid arthritis, exposure to nephrotoxic drugs, environmental pollutants like mercury (10), cadmium (11) and in workers occupationally exposed to trichloroethylene (12). It can be a useful parameter in patients with tubulo-interstitial nephropathies to distinguish acute from chronic disorders. In acute damage of the tubule system, NAG activity of more than 20 U/L (15 U/g creatinine) can be measured (13).

Urinary NAG is an early predictor of development of diabetic nephropathy, it increases before the appearance of microalbuminuria (14) in diabetes mellitus type 1.

Schultz et al. (15) have shown that raised activity of urine NAG in subjects under sixteen years of age are related to duration of diabetes type 1 and concentration of glycated hemoglobin. Mocan et al (16) examined 54 adults with diabetes mellitus type 2 and observed that NAG activity begins to rise in the third year of diabetes, reaches a plateau between 3–10 years, and rapidly increases after the 10^{th} year.

Kavukçu (17) showed that urinary NAG activity is related to glucose level in blood and Kordonouri et al. (18) found that patients with diabetes type I onset in childhood showed significantly higher activities of NAG compared to those with diabetes onset after the age of sixteen years, and concluded that more frequent impairment of tubular function was observed in young patients with

tećenja tubularne funkcije u mlađoj dobi pripisali neodgovarajućoj glikemijskoj kontroli u toj populaciji.
Cilj je ovog istraživanja bio ispitati postoji li razlika u aktivnosti NAG u mokraći između djece i adolescenata sa šećernom bolesti tipa 1 i zdravih ispitanika. Nadalje, željeli smo ispitati mijenja li se aktivnost NAG u mokraći u ovisnosti o trajanja bolesti.

Materijali i metode

Ispitanici

Istraživanje je uključivalo 66-ero djece i adolescenata sa šećernom bolesti tipa 10 praćenih tijekom tri godine u Dječjoj bolnici Kantrida, Rijeka. Ukupno je bilo 37 muških i 29 ženskih ispitanika s medijanom dobi 14,5 (3,5–25) godina. Kontrolnu je skupinu činilo 68 ispitanika (29 muških i 39 ženskih) bez bubrežne bolesti i šećerne bolesti, s medijanom dobi 11,0 (0,5–27). Bolesnici sa šećernom bolesti podijeljeni su u četiri skupine ovisno o trajanju bolesti (Tablica 1.).

Uzorci

Nasumični uzorci mokraće bez aditiva prikupljeni su od svakog ispitanika i centrifugirani 10 minuta na 800 o/min. Supernatanti su čuvani na –20 °C do određivanja aktivnosti NAG koja se provodila jednom tjedno. Aktivnost NAG je postojana tijekom 7 dana na 4 °C i –20 °C (19).
Svježi uzorci mokraće korišteni su za određivanje koncentracije kreatinina i albumina u mokraći.
Puna krv je prikupljana s EDTA kao antikoagulansom radi određivanja HbA_{1c}.

Metode

Aktivnost NAG u mokraći određena je spektrofotometrijski komercijalnim testom (Boehringer Mannheim, Njemačka) s 3-krezolsulfonftaleinil-N-acetil-β-glukozaminidom kao supstratom (20). Koncentracija kreatinina u mokraći

diabetes onset in childhood maybe due to non-optimal glycemic control in this population.
The aim of this study was to examine whether there are differences between urinary NAG activity between children and adolescents with diabetes mellitus type 1 and healthy subjects. Beside that, we want to examine whether urinary NAG activity change in relation to duration of the disease.

Materials and methods

Subjects

The study included 66 children and adolescents with diabetes mellitus type 1 monitored during three years in Kantrida Children's Hospital, Rijeka. There were 37 males and 29 females; median age: 14.5 (3.5–25). In the control group, there were 68 individuals (29 males and 39 females) without renal disease and diabetes; median age 11.0 (0.5–27). The diabetic patients were classified in four groups according to the duration of diabetes. (Table 1).

Samples

Random urine samples were collected from each subject without additives, centrifuged 10 min at 800 g and supernatants were kept at –20 °C until NAG activity analysis which was performed once a week. NAG activity is stable over 7 days at 4 °C and –20 °C (19).
Fresh urine samples were used for determination of urinary creatinine concentration and albumin.
Whole blood was collected with EDTA as anticoagulant for determination of HbA_{1c}.

Methods

Urinary NAG activity was determined spectrophotometrically by commercial test kit (Boehringer Mannheim, Germany) with 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminide as substrate (20). Urinary creatinine concen-

Tablica 1. Značajke skupina bolesnika sa šećernom bolesti

Table 1. Characteristics of groups of diabetic patients

Group	Duration of disease (years)	Number of patients N (male/female)	Age (years) (median; min–max)
I	<3	17 (10/7)	15.5 3.5–21.0
II	3–5	19 (11/8)	13.0 7.0–19.0
III	5–10	19 (7/12)	14.5 11.5–18.5
IV	>10	11 (9/2)	16.0 12.0–25.0

Tablica 2. Aktivnosti NAG u mokraći bolesnika i kontrolne skupine

Table 2. Urinary NAG activities in diabetic patients and controls

Group	Patients (N)	NAG (kU/mol creatinine)			
		Median	min–max	1st quartile	3rd quartile
I	17	0.65*	0.14–2.72	0.27	1.20
II	19	0.76*	0.38–1.57	0.53	1.02
III	19	0.66*	0.15–3.42	0.37	1.13
IV	11	0.52**	0.18–4.55	0.32	1.21
All patients	66	0.69*	0.14–4.55	0.38	1.15
Controls	68	0.24	0.06–1.98	0.17	0.47

Patients vs. controls: *P<0,001; **P=0,004

mjerena je izmijenjenom Jaffeovom reakcijom (21). Kako bi se smanjio učinak diureze na izmjerenu aktivnost NAG u mokraći, rezultat smo izrazili kao omjer enzimske aktivnosti i koncentracije kreatinina u mokraći (kU/mol kreatinina).
Koncentracija HbA_{1c} određena je ionskom izmjenjivačkom kromatografijom (Bio Systems, Španjolska).
Koncentracija albumina u mokraći mjerena je radijalnom imunodifuzijom (Behring, Njemačka).

Statistička analiza

Za ispitivanje normalnosti raspodjele rabili smo Kolmogorov-Smirnovljev statistički test. Za ispitivanje razlika u aktivnosti NAG u mokraći između skupine bolesnika i kontrolne skupine rabili smo Mann-Whitney statistički test. Za usporedbu četiri dijabetičke skupine i kontrolne skupine rabljen je Kruskal-Wallisov test (P<0,05) te zatim Mann-Whitneyev test (P<0,005).
Izračunat je Spearmanov koeficijent korelacije aktivnosti NAG u mokraći u bolesnika i trajanja bolesti.
Pomoću Kruskal-Wallisovog testa uspoređene su razlike u koncentraciji albumina u mokraći i koncentracije HbA_{1c} između četiri dijabetičke skupine (P<0,05).
Vrijednosti određivanih parametara prikazani su kao medijan (minimum-maksimum).

Rezultati i rasprava

Aktivnosti NAG u mokraći u četiri skupine dijabetičkih bolesnika te u kontrolnoj skupini navedene su u tablici 2. Statistički značajno su više vrijednosti utvrđene za sve četiri dijabetičke skupine u odnosu na kontrolnu skupinu (skupina I, P=0,001; skupine II i III, P<0,001; skupina IV, P=0,004 u usporedbi s kontrolnim ispitanicima). Najniži medijan aktivnosti NAG zabilježen je u skupini IV (0,52; 0,18–4,55 kU/mol kreatinina) u kojoj su bili bolesnici s najduljim tra-

tration was measured using modified Jaffe reaction (21) by home made reagent. In order to minimize the effect of urine flow rate on urinary enzyme activity of NAG, results are expressed as the ratio of enzyme activity to urine creatinine concentration (kU/mol creatinine).
Glycated hemoglobin concentration was determined by ion exchange chromatography (Bio Systems, Spain).
Urinary albumin concentration was determined by radial immunodiffusion (Behring, Germany).

Statistical analysis

The Kolmogorov-Smirnov test was applied to test for a normal distribution Comparison of urinary NAG activity between entire diabetic group and controls was made by unpaired Mann-Whitney test. Comparison between four diabetic groups and control group was made by Kruskal-Wallis test (P<0.05) and post hoc by unpaired Mann-Whitney test (P<0.005).
Spearman's correlation coefficient between urinary NAG activity in diabetic patients and duration of disease was calculated.
Comparison between four diabetic groups for urinary albumin concentration and glycated hemoglobin concentration was made by Kruskal-Wallis test (P<0.05).

Results and discussion

Urinary NAG activities in four groups of diabetic patients and in the control group are shown in Table 2. Significantly higher values were found in all four diabetic groups compared to the control group (group I P=0.001, groups II and III P<0.001 and group IV P=0.004 compared to controls). The lowest median for NAG was in group IV (0.52, 0.18–4.55 kU/mol creatinine) which comprised patients with the longest duration of diabetes mellitus, and the highest median for NAG was established in group II (0.76, ran-

janjem bolesti, a najviši medijan za NAG je utvrđen u skupini II (0,76; 0,38–1,57 kU/mol kreatinina). Međusobne razlike u izlučivanju NAG između pojedine skupine ispitanika nisu bile statistički značajne. Shema Box-Whisker prikazuje medijan, kvartile, minimum, maksimum te vrijednosti koje odskaču u svakoj skupini. Raspodjela aktivnosti NAG u mokraći u četiri podskupine bolesnika i kontrolnoj skupini prikazana je na slici 1.

Aktivnost NAG u mokraći u sve četiri skupine bila je statistički značajno viša u usporedbi s kontrolnim ispitanicima bez obzira na trajanje šećerne bolesti. S druge strane, međutim, nije nađena korelacija između četiri dijabetičke skupine i trajanja bolesti (r=–0,017, P=0,892).

Hsiao i sur. (22) su ispitali 31 dijete sa šećernom bolesti tipa 1 te utvrdili značajno višu aktivnost NAG u mokraći u četiri dijabetičke skupine (skupine su podijeljene na isti način kao i u našem istraživanju, tj. prema trajanju bolesti) u usporedbi s kontrolnom skupinom, no bez statistički značajne razlike između bilo koje od tih četiri skupina. Lorini i sur. (23) i Mungan i sur. (24) također nisu otkrili korelaciju između aktivnosti NAG u mokraći i trajanja šećerne bolesti tipa 1 u mladih dijabetičkih bolesnika.

NAG u mokraći je povezana s koncentracijom glukoze u krvi (17) te može odražavati metaboličku kontrolu bolesnika sa šećernom bolesti, aktivnost bolesti, te težinu bubrežnog oštećenja.

Naši su bolesnici u sve četiri dijabetičke skupine imali slične koncentracije HbA_{1c}, što je upućivalo na slične razine

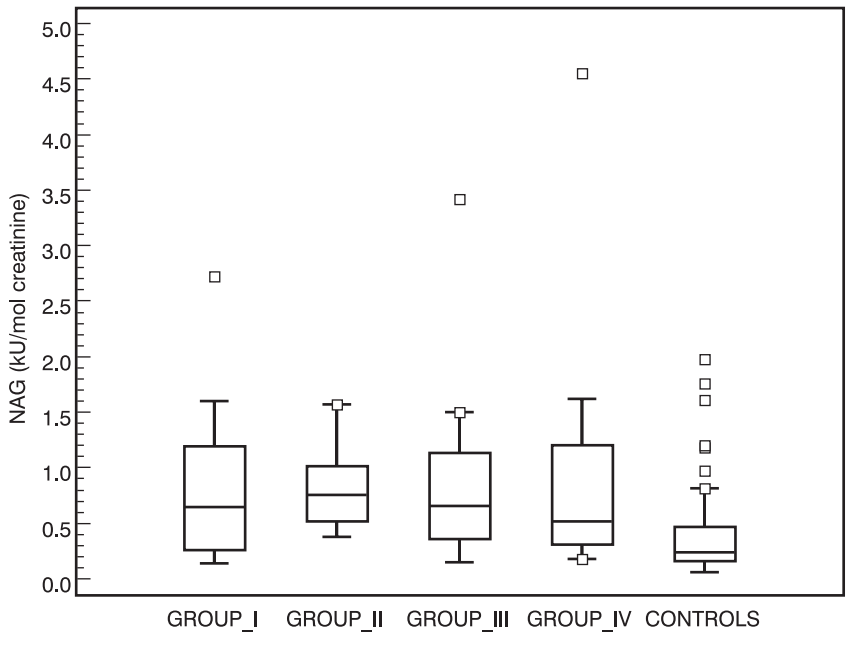
ge 0.38–1.57 kU/mol creatinine). There was no significant difference in NAG excretion among any diabetic groups. Box-Whisker plot presents median, quartiles, minimum, maximum and outliers of each group. Distribution of urinary NAG activities in four subgroups and controls is presented in Figure 1.

Urinary NAG activity of all four groups was significantly higher compared to controls regardless of the duration of diabetes. On the other hand, there was no correlation between the four diabetic groups and disease duration (r= –0.017 P=0.892).

Hsiao et al. (22) examined thirty-one children with diabetes mellitus type 1 and found significantly higher urinary NAG activity in four diabetic groups (the groups were divided in the same manner as ours, according to duration of diabetes) compared to the control group, but there was no statistically significant difference among any two of these four groups. Lorini et al. (23) and Mungan et al. (24) also did not find correlation between urinary NAG activity and duration of diabetes type 1 in young diabetic patients.

Urinary NAG is related to glucose concentration in blood (17) and may reflect metabolic control for patients with diabetes mellitus, activity of the disease and the severity of damage.

Our patients from all four diabetic groups had similar concentrations of glycated hemoglobin, which indicated that they had similar levels of glycemia. There was no significa-



Slika 1. Grafički prikaz aktivnosti NAG u mokraći kontrolnih ispitanika i podskupina bolesnik

Figure 1. Box-Whisker plots of urinary NAG activities in controls and patient subgroups.

Tablica 3. Koncentracije albumina u mokraći dijabetičara

Table 3. Urinary albumin concentration in patients

Group	Patients (N)	Urinary albumin (g/mol creatinine)			
		Median	min–max	1st quartile	3rd quartile
I	11	3.3	0.9–7.2	1.7	4.6
II	7	4.7	1.1–30.1	1.6	14.6
III	7	6.1	0.1–9.2	2.2	7.0
IV	6	2.7	1.9–20.5	2.3	9.9

Tablica 4. Koncentracija glikiranog hemoglobina u podskupinama bolesnika

Table 4. Glycated hemoglobin concentration in patient subgroups

Group	Patients (N)	Glycated hemoglobin (%)			
		Median	min–max	1st quartile	3rd quartile
I	9	9.6	6.7–12.8	8.0	9.9
II	8	8.9	6.8–16.3	8.4	11.4
III	7	10.8	7.8–12.0	8.9	11.5
IV	5	10.5	9.6–12.1	10.2	11.8

glikemije. Statistički značajne razlike nije bilo između bilo koje dvije od četiri dijabetičkih skupina u glikiranom hemoglobinu (P=0,574), što je prikazano u tablici 3. Statistički značajno više aktivnosti NAG u mokraći u djece i adolescenata sa šećernom bolesti tipa 1 u usporedbi s kontrolnim ispitanicima ukazuju da su ti dijabetički bolesnici imali tubularno oštećenje koje bi moglo biti posljedica slabe kontrole glikemije (medijan koncentracija glikiranog hemoglobina bio je 8,9% ili viši u sve četiri skupine).
Nije utvrđena statistički značajna razlika u koncentraciji albumina u mokraći između pojedinih podskupina bolesnika. Tablica 4. sadrži koncentracije albumina u mokraći za četiri podskupine dijabetičkih bolesnika.
Postoji mogućnost da razlike između skupina u izlučivanju NAG u mokraći nije bilo zbog slične razine glikemije te, posljedično, sličnoga stupnja bubrežnoga oštećenja. NAG u mokraći odražava metaboličku kontrolu bolesnika sa šećernom bolesti, aktivnost bolesti, te težinu tubularnog oštećenja, a ne samo trajanje bolesti.
Kako postoje mnogi drugi čimbenici koji dodatno mogu utjecati na izlučivanje NAG u mokraći, nije moguće donositi konačne zaključke o uzročno-posljedičnoj vezi povećane aktivnosti NAG i tubularnog oštećenja u šećernoj bolesti. Te će odgovore dati neka buduća ispitivanja na većem broju ispitanika.

Zahvale:

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nt difference between any two of the four diabetic groups in glycated hemoglobin (P=0.574) as presented in Table 3. Significantly higher activities of urinary NAG in children and adolescents with diabetes type 1 compared to controls indicate that these diabetic patients had tubular damage which was possibly a consequence of poor glycemic control (the median of glycated hemoglobin levels was 8.9% or higher in all four groups).
There was no statistically significante difference in urinary albumin concentration between diabetic groups. Table 4 presents urinary albumin values in four groups of diabetic patients.
It is possible that there was no difference between groups in urinary NAG excretion because of their similar level of glycemia and, as a consequence, similar degree of renal damage.
There are many factors that affected urinary NAG excretion so it is impossible to make definitive conclusions about reason consequence relation between increased NAG activity and impairment of tubular function in diabetes mellitus. Further studies on larger study groups are needed to provide those answers.

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