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TUBULAR MARKERS IN CHILDREN WITH INSULIN – DEPENDENT DIABETES MELLITUS*

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The aim of the present study was to investigate the prevalence of tubular dysfunction and to assess the clinical significance of low-molecular-weight proteinuria and enzymuria in children with insulin-dependent diabetes mellitus (IDDM). N.acetyl-beta-D-glucosaminidase (NAG) and beta-microglobulin (β_2M) excretion was determined in 52 children with insulin-dependent diabetes mellitus and 28 controls. Patients were grouped according to the duration of diabetes: group 1 (n = 7): less than one year; group 2 (n = 27): one to five years; groups 3 (n = 18): greater than five years. Both parameters were significantly increased in groups 2 and 3 compared to controls. Urinary β_2M levels correlated significantly with albuminuria and HbA_{1C}, while urinary NAG levels correlated only with HbA_{1C}.

Two to four samples were obtained from 35 of 52 diabetic patients in the study group at one-month intervals. Of these, 23 patients had elevated NAG levels, and 22 patients increased β_2M excretion. However, only six patients displayed persistent enzymuria, and nine low-molecular-weight proteinuria. The mean (SD) of coefficients of variation of each patient was 50.45 (± 28.24) for NAG and 68.25 (± 42.57) for β_2M excretion.

We concluded that early tubular dysfunction and/or damage occurs in IDDM but is not established in the majority of children. *Key words: beta$_2$-microglobulin, insulin-dependent diabetes mellitus, n-acetyl-beta-D-glucosaminidase.*

Nephropathy is a serious microvascular complication of insulin-dependent diabetes mellitus (IDDM). Numerous studies have been carried out in order to predict the occurrence of diabetic nephropathy. It is generally accepted that microalbuminuria precedes macroalbuminuria[1] and has a predictive value in IDDM, but a long-term study failed to demonstrate this[2].

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It has been suggested, however, that peritubular vasculopathy may be the cause of renal failure in diabetic nephropathy[3]. In experimental diabetes mellitus, peritubular capillaries[4] and the tubular epithelium[5] are involved. In a recent study concerning macroalbuminuric patients, a greater increase in low-molecular-weight (LMW) proteinuria was observed in patients with IDDM compared to those with primary glomerulonephritis[6]. On the other hand, tubular dysfunction has been reported early in IDDM[7-12]. The clinical significance and predictive value of early tubular involvement in diabetic children remain to be clarified.

The aim of the present study was to investigate the prevalence of tubular dysfunction and to assess the clinical significance of low-molecular-weight proteinuria (beta$_2$-microglobulin, β_2M) and enzymuria (n-acetyl-beta-D-glucosaminidase, NAG) early in IDDM.

Material and Methods

Fifty-two patients with IDDM and 28 healthy children were admitted to the study. Diabetic patients were divided into three groups according to the duration of diabetes: group I: less than one year; group 2: one to five years; group 3: greater than five years, Demographic and clinical data of the patients and controls are presented in Table I. None of the patients had an infection or ketoacidosis.

Table I: Clinical Data in Diabetic Children

	Group 1 < 1 Year (n = 7)	Group 2 1-5 Years (n = 27)	Group 3 > 5 Years (n = 18)	All Groups (n = 54)
Sex	4M/3F	16M/11F	8M/10F	28M/24F
Age (years)	6.4 (1.7-8.8)	11.3 (4.2-17)	14 (7-20.2)	11.3 (1.7-20.2)
High systolic blood pressure[a]	–	2 (7%)	2 (11%)	4 (7%)
High diastolic blood pressure[a]	1 (14%)	5 (19%)	4 (22%)	10 (19%)
Retinopathy	–	1 (4%)	3 (17%)	4 (7%)
HbA_{1c} (%)	9.4 (7.6-15.1)	10 (8-15.8)	10.5 (7.6-12.8)	10.2 (7.6-15.8)
Albuminuria (µg/mmol creatinine)	0.6 (0.2-2.4)	1.3 (0.1-11)	1.5 (0.3-19)	1.3 (0.1-19.0)

Figures represent median (ranges) or number (%).
[a] > 2 SD of normal population[15].

A total of 124 samples were collected from the diabetic children. From each of 17 patients only one urine sample was available, whereas two to four samples were obtained from the remaining 35 children at one-month intervals (follow-up group). Ten β_2M values tested in acidic urine were excluded from the study.

Urine samples were collected early in the morning. Each sample was divided into two parts, which were stored separately at + 4 °C (to test albumin) and -20 °C (to test other parameters). Urinary creatinine and NAG were determined photometrically by a Hitachi 717 automatic analyzer. Urinary creatinine was

measured according to the Jaffe method. Crezolesulfonftalein-glucosaminide was used as a substrate in measuring NAG (Boehringer-Mannheim)[13]. Urinary β_2M was determined by radioimmunoassay (DPC). Microalbumin was measured by double-antibody radioimmunoassay (DPC). Inter-assay coefficients of variation were 8.4 percent for NAG, 10.6 percent for β_2M, and 9.5 percent for albumin. Urinary NAG, β_2M and albumin were evaluated by determining the NAG: creatinine, β_2M: creatinine and albumin: creatinine ratios. The upper limit of normal was defined as the mean + 2 SD of controls for NAG (0.92 U/mmol creatinine) and β_2M (18.0 µg/mmol creatinine) and higher than 3.5 mg/mmol creatinine for albumin, as previously reported[14].

All patients underwent a fundoscopic examination every six months. Microaneurysm confirmed by angiography was accepted as retinopathy. Measurement of the glycosylated hemoglobin (HbA_{1C}) level was done every three months. The one-year average value was utilized in statistical calculations. The blood pressure of all patients was measured. The standard deviation score was calculated for each patient according to values obtained from healthy Turkish children[15]. Parental consent was obtained in every case.

Non-parametric statistical tests were utilized because the data were not normally distributed even after logarithmic transformation (Mann-Whitney U test for comparing two groups, Sperman's Rho for correlation). Group 1 (n = 7) was not included in the statistical analysis. Within-individual variability was compared by coefficients of variation (CV). The CV of each parameter was calculated in each subject. Mean and SD were determined among subjects for each parameter. For statistical tests other than CV, the median value of all urine samples was utilized. Statistical tests were performed using the SPSS PC program.

Results

Age and sex distribution were not significantly different between the diabetic and control groups. Urinary excretion of NAG and β_2M was significantly higher in groups 2 and 3 compared to controls (Table II). There was a positive correlation between NAG and β_2M excretion in groups 2 and 3. Both parameters correlated with HbA_{1C} levels in group 3. β_2M excretion also correlated with albuminuria in group 3 (Table III).

Table II: Excretion of Urinary NAG and β_2M in Normal and Diabetic Children

	Group 1[a] < 1 Year (n = 7)	Group 2 1-5 Years (n = 27)	Group 3 > 5 Years (n = 18)	Controls (n = 28)
NAG (U/mg creatinine)	6.5 (1.2-8.7)	8.1* (0.1-31.6)	10.2** (2.8-20.5)	3.5 (2-9)
β_2M (µg/mg creatinine)	329 (24-4164)	142* (1-2202)	184* (16-1539)	85 (16-157)

* $p < 0.05$, ** $p < 0.001$ vs controls.
[a] Not included in the statistical analysis.
Figures represent median and ranges.

Table III: Correlation of Urinary NAG and β_2M Levels with Albumin Excretion, HbA_{1C}, Systolic and Diastolic Blood Pressure in Diabetic Children

	NAG		β_2M	
	Group 2 1-5 Year	Group 3 > 5 Years	Group 2 > 1-5 Years	Group 3 > 5 years
Albumin	NS	NS	NS	$r_s = 0.6$ ($p = 0.009$)
HbA_{1C}	NS	$r_s = 0.72$ ($p = 0.001$)	NS	$r_s = 0.75$ ($p < 0.001$)
Systolic blood pressure[a]	NS	NS	NS	NS
Diastolic blood pressure[a]	NS	NS	NS	NS
β_2M	rs = 0.40 (p = 0.024)	rs = 0.52 (p = 0.027)		

Figures represent median (ranges) or number (%).
[a] > 2 SD of normal population[15].

Of four retinopathic children, one had only microalbuminuria, one had microalbuminuria with enzymuria and LMW proteinuria, another had enzymuria and LMW proteinuria without microalbuminuria, and the last one had neither microalbuminuria nor LMW proteinuria and enzymuria.

The frequencies of increased excretions of each parameter in the follow-up group are shown in Table IV. The CVs of NAG, β_2M and albumin excretions are reflected in Table V. The CV of NAG was lower than those of albumin and β_2M, but the difference was insignificant.

Tablo IV: Frequencies of Increased Levels of NAG, β_2M and Albumin Excretions in the Follow-up Group.

	NAG n = 35 (%)	β_2M n = 34 (%)	Albumin n = 33 (%)
Elevated level in at least one sample	23 (66)	22 (65)	12 (39)
Elevated levels in at least two samples	15 (43)	13 (38)	4 (12)
Elevated levels in all samples	6 (17)	9 (26)	4 (12)

Table V: Variation of NAG, β_2M and Albumin Excretion in the Follow-up Group

	CVs (Mean ± SD)
NAG	50.5 ± 28.2
β_2M	68.2 ± 42.6
Albumin	61.5 ± 34.6

CVs: Coefficients of variation.

Discussion

Our results confirm that early tubular dysfunction and/or damage occurs in IDDM. Large CVs and the fluctuation of tubular marker levels observed in the follow-up group demonstrated that tubular involvement was not established in the majority of patients. This finding is similar to Ginevri et al.'s[12], who observed that all increased urinary levels of brush border antigen and LMW protein returned to normal limits in children with IDDM. One possible explanation for this reversible tubular involvement is the toxicity of albuminuria. It is known that enzymuria and LMW proteinuria occur in proteinuric states[16, 17]. However, microalbuminuria did not seem to play a major role in our study group: firstly, not all microalbuminuric patients had LMW proteinuria and enzymuria or vice versa; secondly, there was no correlation between albuminuria and NAG. Another possible explanation is that metabolic changes may cause tubular toxicity. Glycosylated hemoglobin is a good parameter for judging metabolic status, as found in other studies[10-12, 18], HbA1c was correlated with NAG excretion LMW proteinuria in group 3. It is well known that HbA1c is a good predictor of microvascular disease. One can speculate that peritubular vascular changes may contribute to early tubular involvement in IDDM. Martin et al.[9] observed higher urinary NAG levels in adult type I diabetic patients with retinopathy and postulated that "once microvascular disease is established, the excretion rates of NAG and LMW proteins tend to be increased and unresponsive to metabolic control". However, in our study ony two of four patients with retinopathy had eleated urinary NAG and β_2M levels. Considering this finding and the "reversible" elevation of tubular markers, it seems unlikely that peritubular vascular changes are the major cause of early tubular involvement in IDDM. NAG is a lysosomal enzyme located in the proximal tubular epithelial cell. A number of factors causing proximal tubular perturbation produce urinary NAG elevation[20]. If the causative factor is eliminated, urinary NAG activity returns to normal limits. Hyperglycemia may alter tubular cellular metabolism, resulting in an increased loss of the enzyme into urine. Watanable et al.[21] have shown that altered blood sugar regulation causes elevation of urinary NAG levels.

The mechanism and the clinical significance of reversible tubular damage and/or dysfunction in early IDDM is not yet clear. Long-term follow-up studies are required to assess whether early reversible tubular involvement has a predictive value or is an only an epiphenomenon.

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