

# Urinary N-acetyl-beta-D-glucosaminidase in children with diabetes as an early marker of diabetic nephropathy

S. Abdel Shakour,<sup>1</sup> H. El-Hefnawy,<sup>1</sup> M. Y. El-Yamani<sup>1</sup> and Y. Azmi<sup>1</sup>

الاستفادة من N-أسيتيل-بيتا-D-غلوكونز أمينيداز (نأغ) في بول الأطفال السكريين كواسم باكرو للاعتلال الكلوي السكري

سامح عبد الشكور، محمد هشام الحفناوي، محمد يحيى اليماني وياسر عزمي صفر

المخالصة: تم استقصاء أسيتيل غلوكونز أمينيداز (نأغ) في بول الأطفال السكريين والمصابين بالسكري من النمط الأول باعتبارها واسماً باكراً للإصابة بالتهرب الشبكي، ودراسة علاقته بينة الميكروألبومين وبالسيطرة على سكر الدم. وقد تم تعيين نسبة نأغ إلى الكرياتينين، وبيبة الميكروألبومين وجليكوزيل الهيموغلوبين (Hb A1c)، وبيبة الميكروألبومين وقيم ضغط الدم الانقباضي والانساطي. وقد وُجد أن 59.5% من الأطفال السكريين لديهم نأغ إيجابي في البول، وأن 38.1% منهم لديهم بيبة ميكروألبومينية إيجابية.

**ABSTRACT** We investigated urinary N-acetyl-beta-D-glucosaminidase (NAG) levels in children with type 1 diabetes as an early marker of tubular damage and studied its correlation with microalbuminuria and glycaemic control. The study group comprised 42 children with type 1 diabetes and 20 healthy children as control. Urinary NAG to urinary creatinine ratio, microalbuminuria, glycated haemoglobin (Hb A1c), blood urea and serum creatinine were estimated. Urinary NAG levels in the children with diabetes were significantly higher than those of controls. There were positive correlations between urinary NAG levels and microalbuminuria, Hb A1c and systolic and diastolic blood pressure values. We found that 59.5% of diabetic children were positive for urinary NAG, while 38.1% of them were positive for microalbuminuria.

**La N-acétyl-βD glucosaminidase (NAG) urinaire chez des enfants diabétiques en tant que marqueur précoce de la néphropathie diabétique**

**RESUME** Nous avons examiné le taux de NAG urinaire chez des enfants atteints de diabète de type 1 en tant que marqueur précoce de lésion tubulaire et avons étudié sa corrélation avec la microalbuminurie et le contrôle glycémique. Le groupe étudié comprenait 42 enfants atteints de diabète de type 1 et 20 enfants en bonne santé en tant que témoins. Le rapport N-acétyl-βD glucosaminidase urinaire-créatinine urinaire, la microalbuminurie, l'hémoglobine glycosylée (HbA1c), l'urée sanguine et la créatininémie ont été estimés. Les taux de NAG urinaire chez les enfants diabétiques étaient significativement plus élevés que chez les témoins. Il y avait une corrélation positive entre les taux de N-acétyl-βD glucosaminidase urinaire et la microalbuminurie, entre l'hémoglobine glycosylée et les valeurs de la pression artérielle systolique et diastolique. Nous avons trouvé que 59,5 % des enfants diabétiques étaient positifs pour la N-acétyl-βD glucosaminidase urinaire, tandis que 38,1 % d'entre eux étaient positifs pour la microalbuminurie.

<sup>1</sup>National Institute of Diabetes and Endocrinology, Cairo, Egypt.

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## Introduction

Diabetic microangiopathy denotes widespread microvascular abnormalities that occur frequently in longstanding diabetes and affect many body organs. In renal affection, the lesions involve glomerular capillaries, as well as the afferent and efferent arterioles. Afferent arterioles usually exhibit gross thickening of their walls. The renal tubules show similar changes to those found in persistent glomerular nephritis, and very occasionally the proximal tubules contain large quantities of glycogen [1]. It was proven by Yaqoob et al. in 1995 that diabetic nephropathy is associated with comparable degrees of increased serum creatinine and albuminuria as markers of glomerular damage [2]. In addition, increased urinary excretion of leucine aminopeptidase, N-acetylglutaryl transferase, retinol binding protein and beta2-microglobulin as markers of tubular damage. Moreover, the measurement of N-acetyl-beta-D-glucosaminidase (NAG), a lysosomal enzyme localized in the proximal tubules, served as an additional parameter for the assessment of tubular damage [3].

NAG is a lysosomal enzyme originating in the renal tubules, and its urinary excretion is elevated in various renal disorders. Gibb et al. suggested that the tubular abnormalities are present early in the course of type 1 diabetes and that the early increase in urinary excretion of albumin may be, at least partly, tubular in origin and may be influenced by glycaemic control [4]. He also suggested that elevated urinary transferrin and NAG excretion rates may precede elevation of albumin excretion in children with type 1 diabetes.

## Methods

The aim of this study was to investigate the level of urinary NAG in children with diabetes in comparison with urinary micro-albumin as an early marker of diabetic nephropathy and to study their correlation with glycaemic control.

The study was carried out on 42 children with type 1 diabetes mellitus who attended the paediatric clinic of the National Institute of Diabetes and Endocrinology in Cairo, Egypt (NIDE). Their ages ranged between 5 and 15 years, and there were 22 females and 20 males.

Another 20 healthy children of the same age range were included in the study as a control group. There were 11 females and 9 males.

Both groups were subjected to the following:

- Full history taking, including duration of diabetes, methods of treatment, daily insulin requirements and any symptoms or signs of diabetic complications.
- Complete physical examination, both general and systemic, and observation for any manifestations of complications. Particular attention was given to blood pressure measurement.
- Laboratory investigations, which included:
  - complete blood picture
  - blood urea and serum creatinine
  - fasting plasma glucose.

Glycated haemoglobin (Hb A1c) was estimated using the chromatography method on a DCA 2000 apparatus [5]. The ratio of urinary NAG index in random morning urine sample was estimated. This

ratio is considered as an indicator for urinary NAG level in 24 hour urine. The upper reference limit for NAG was 8.15 U/g cr using the colorimetric technique [5].

The ratio of urinary albumin in mg to urinary creatinine (cr) in g (albumin index) in a random morning urine sample was estimated and considered an indicator for microalbumin level in a 24 hour urine collection. Children were classified as normoalbuminuria (if urinary albumin < 30 mg/g cr), microalbuminuria (if urinary albumin fell between 30–300 mg/g cr) and macroalbuminuria (if urinary albumin > 300 mg/g cr). This was done using the chromatographic DCA 2000 method [5].

To exclude any causes other than diabetes that might be responsible for renal abnormalities, all children positive for microalbuminuria or NAG excretion were subjected to urinalysis with culture and sensitivity and abdomino-pelvic ultrasonography.

Statistical analysis of the results was carried out using standard computer programs.

## Results

Urinary NAG levels in the diabetic group (42 patients) ranged between 2.6 and 80.4 U/g cr (mean =  $19.9 \pm 18.9$  U/g cr). Of these, 25 patients showed positive figures for NAG excretion (59.5%) (Figure 1). Urinary microalbuminuria was positive in 16 patients only (38.1%), all belonging to the NAG-positive group. The microalbumin level in the children positive for NAG excretion ranged from 5.8 to 120.8 mg/g cr (mean =  $23.4 \pm 25.3$  mg/g cr).

Table 1 shows that there was no significant difference found between the children with diabetes and the control children regarding age, sex, systolic and

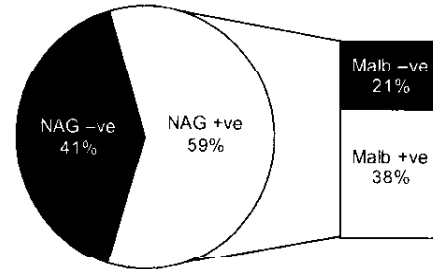


Figure 1 Percentage of children with diabetes with positive urinary NAG and those with positive microalbuminuria in the patient group under study

diastolic blood pressure values, blood urea, serum creatinine, serum cholesterol and serum triglyceride levels ( $P > 0.05$ ). However, a significant difference was found between both groups as regards urinary NAG levels (mean =  $19.9 \pm 18.9$  U/g cr for cases and  $3.66 \pm 2.59$  U/g cr for controls) ( $P < 0.01$ ). There was also a significant difference ( $P < 0.01$ ) found with respect to microalbumin levels in both groups (mean =  $23.43 \pm 25.32$  mg/g cr for cases and  $10.23 \pm 5.12$  mg/g cr for controls) and to glycated Hb A1c (mean =  $8.24 \pm 2.05\%$  for cases and  $5.19 \pm 0.67\%$  for controls) ( $P < 0.01$ ).

Table 2 shows the presence of positive correlation between urinary NAG levels in the children with diabetes and other parameters, namely the level of urinary microalbumin ( $P < 0.01$ ), glycated Hb A1c ( $P < 0.01$ ), duration of diabetes ( $P < 0.01$ ), and systolic and diastolic blood pressure values ( $P < 0.05$ ). No correlation ( $P > 0.05$ ) was found between urinary NAG levels in diabetics and age, blood urea, serum creatinine, serum cholesterol, serum triglycerides and fasting plasma glucose levels.

**Table 1 Characteristics and blood and urine measurements of children with diabetes compared with control children**

Data	Cases (n = 42)		Controls (n = 20)		P
	Mean	s	Mean	s	
Age (years)	10.0	3.5	9.9	3.6	>0.05
Systolic blood pressure (mm Hg)	107.5	19.5	106.7	20.7	>0.05
Diastolic blood pressure (mm Hg)	69.5	8.1	67.9	7.9	>0.05
Blood urea (mg/dL)	22.1	5.1	21.9	4.6	>0.05
Serum creatinine (mg/dL)	0.83	0.15	0.81	0.21	>0.05
Serum cholesterol (mg/dL)	184.3	36.2	179	29.1	>0.05
Serum triglycerides (mg/dL)	102.3	55.0	100.9	62.1	>0.05
Fasting plasma glucose (mg/dL)	205.3	82.6	75.9	10.1	<0.01
Urinary NAG (U/g cr)	19.97	18.96	3.66	2.59	<0.01
Urinary microalbumin (mg/g cr)	23.43	25.32	10.23	5.12	<0.01
Glycated Hb A1c (%)	8.24	2.05	5.19	0.67	<0.01

The female:male ratio in the cases was 0.523 and in the controls it was 0.545, a non-significant difference.

P < 0.05 was considered significant.

s = standard deviation.

NAG = N-acetyl-beta-D-glucosaminidase.

**Table 2 Correlation (r) of urinary NAG levels in children with diabetes to different factors**

Data	r	P
Urinary microalbumin (mg/g cr)	+0.460	<0.01
Hb A1c (%)	+0.749	<0.01
Duration of diabetes (years)	+0.473	<0.01
Age (years)	+0.034	>0.05
Systolic blood pressure (mm Hg)	+0.323	<0.05
Diastolic blood pressure (mm Hg)	+0.352	<0.05
Blood urea (mg/dL)	+0.045	>0.05
Serum creatinine mg/dL)	+0.121	>0.05
Serum cholesterol (mg/dL)	+0.097	>0.05
Serum triglycerides (mg/dL)	+0.084	>0.05
Fasting plasma glucose (mg/dL)	+0.094	>0.05

P < 0.05 was considered significant.

NAG = N-acetyl-beta-D-glucosaminidase.

## Discussion

We investigated the level of urinary NAG and urinary microalbumin in children with diabetes. Urinary NAG was regarded as an indicator of tubular affection, while microalbuminuria denoted glomerular affection. The analytes were studied in respect to their sensitivity in detecting early renal affection in the study group. In addition, the correlation of these analytes to glycaemic control was investigated.

The results of this study showed that 59.5% of the children with diabetes were positive for urinary NAG levels, while only 38.1% of them were positive for microalbuminuria. These latter patients were a subgroup of those patients found to be positive for urinary NAG levels. In ad-

dition, a positive correlation between urinary NAG levels and microalbuminuria ( $P < 0.01$ ) on the one hand, and Hb A1c ( $P < 0.01$ ) on the other hand was clearly identified.

Many studies have investigated the issue of nephropathy among diabetic patients, trying to understand the sequence of pathological events and to discover sensitive markers for early renal involvement. One feature of diabetic microangiopathy is endothelial dysfunction [6,7], which in type 1 diabetes precedes the onset of microalbuminuria [8].

Hsiao in 1996 examined 31 children with diabetes without any clinical evidence of nephropathy for urinary NAG and microalbuminuria [9]. He concluded that urinary NAG is a sensitive indicator of renal tubular injury (due to any cause or disease) and may be increased in children with diabetes even before the onset of microalbuminuria. Also, the urinary NAG was significantly correlated with plasma Hb A1c levels.

In 1996, Platonova et al. studied the urinary enzymes in type 1 diabetes and reported that the degree of epithelial cell lesions in the renal tubules could be assessed from the urinary activities of enzymes at various sites such as NAG which could be an early marker of proximal nephritic tubules in type 1 diabetes [10].

In 1997, Caliskan et al. concluded that urinary NAG levels in children with diabetes are significantly correlated with microalbuminuria and Hb A1c [11]. They also concluded that tubular dysfunction and/or damage occurs in type 1 diabetes earlier than glomerular affection.

Moreover, in 1997, Hirai M et al. concluded from a study on people with type 2 diabetes that the NAG index and albumin index in random spot urine samples may

serve as early functional indicators of diabetic nephropathy in people with diabetes [5]. They found that the NAG index positively correlated with systolic blood pressure, duration of diabetes, Hb A1c and albumin index. The study was done, however, on individuals with type 2.

In 1998, Kordonouri et al. reported the findings of a 7-year follow-up study of children and adolescents with type 1 diabetes with normal albumin excretion rate [12]. They maintained that urinary NAG excretion could develop before microalbuminuria. The investigators observed that patients with consistently low NAG levels did not progress to microalbuminuria. In contrast, patients with increased NAG levels were found to develop microalbuminuria at a later stage.

In 1999, Uehara et al., in an experimental study on diabetic rats, noticed that changes in glucose metabolism were associated with proteinuria and an increase in urinary excretion of NAG [13]. Furthermore, in 1999, Kordonouri et al. investigated the prevalence of incipient renal dysfunction in two groups with identical duration of type 1 diabetes but with either childhood or adult onset of the disease [14]. The pattern of glomerular (albumin) and tubular (alpha1-microglobulin and NAG) urinary protein excretion was studied in 97 patients. The results suggested that there was no difference concerning the prevalence of incipient diabetic glomerulopathy between both groups. However, a more frequent impairment of tubular function was observed in young patients with diabetes onset in childhood. The investigators attributed this finding to non-optimal glycaemic control in this population. Patients with diabetes onset in childhood showed significantly higher excretion of NAG compared to those with diabetes

onset after the age of 16 years. The excretion of tubular markers correlated significantly with Hb A1c values in both groups. In multiple regression analysis, tubular proteinuria and diabetes duration correlated significantly to microalbuminuria.

It is obvious from all of the above that our results stand in agreement with most of the results of other investigators. Thus, we can conclude that tubular dysfunction

occurs earlier than glomerular dysfunction in children with diabetes. Positive correlation of urinary NAG levels with glycaemic control was established. The finding that urinary NAG is an earlier marker of nephropathy than microalbuminuria makes it feasible to recommend that all children with diabetes have urinary NAG levels measured regularly for early detection of diabetic nephropathy.

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