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Urinary N-Acetyl-β-D glucosaminidase (uNAG) levels as an early marker for diabetic nephropathy in children with type 1 diabetes

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Abstract

Background: Diabetic nephropathy is considered a major complication among patients with type 1 diabetes. In the present study, we aimed to evaluate urinary N-acetyl-beta-D-glucosaminidase levels (uNAG) in patients with type 1 diabetes mellitus (DM).

Methods: This cross-sectional study of 60 patients with type 1 DM, was categorized into two groups (normoalbuminuria and microalbuminuria) and 30 healthy controls. uNAG was measured in all cases and controls.

Results: Patients with type 1 DM showed increased mean uNAG values compared to controls. Interestingly, increased NAG levels were found in diabetic patients without early signs of glomerular damage (normoalbuminuric). The severity of renal disease, poor glycemic control, and duration of diabetes were all proportional to uNAG increased levels.

Conclusions: NAG measurement is a useful, noninvasive tool for assessing renal involvement in children with diabetes and for early diagnosis of developing nephropathy.

Keywords: Diabetic nephropathy, Urinary N-acetyl-beta-D-glucosaminidase, Type 1 diabetes mellitus

Background

Diabetic nephropathy (DN), as one of the most common complications of diabetes mellitus, dramatically affects the patient's quality of life and life expectancy. The chronicity of this complication is accompanied by a higher risk of end-stage renal failure, together with cardiovascular disease and eventually premature death [5].

The earliest sign of diabetic nephropathy is moderately increased albuminuria (formerly known as microalbuminuria), which is persistent urinary albumin excretion between 30 and 300 mg/day. If not well managed, moderately increased albuminuria may progress to gross proteinuria (formerly known as macroalbuminuria), defined as persistent albumin excretion > 300 mg/day. The prognosis of diabetic nephropathy has dramatically improved with better glycemic control and the use of angiotensin-converting enzyme (ACE) inhibitors. It is a major cause of morbidity and mortality. Among young adults with type 1 diabetes, diabetic nephropathy is a major concern concerning morbidity and mortality [16].

For an extended, screening of DN has been basically by microalbuminuria (MA) assessment which may be found in 12–16% of adolescents with type 1 diabetes mellitus (T1D). Many researchers worldwide have challenged the value of MA screening in DN. Lately, other biomarkers are being studied aiming at the early identification of diabetic renal lesions [15].

Recently, many tubular damage markers have been studied in terms of their clinical value as markers for the development and progression of DN. Some studies have shown that these tubular damage markers are elevated in

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patients with diabetes, even before developing microalbuminuria, representing early markers of normoalbuminuric DN with good sensitivity and specificity. Among these markers is N-Acetyl-beta-D-glucosaminidase (NAG) [17].

N-acetyl-beta-D-glucosaminidase (NAG) is a hydrolytic lysosomal enzyme with a molecular weight of 150,000 Da found mainly in proximal tubules. It is considered a good marker of renal tubular damage in various conditions involved with renal injury or dysfunction [1].

Contrary to other urine biomarkers such as β2-microglobulin and α1-microglobulin, which are filtered through the glomeruli, NAG [coming only from tubular cells) increase in urine reflects tubular dysfunction [13].

We aimed to evaluate the level of urinary N-acetyl-beta-D-glucosaminidase (NAG) in urine as a marker of tubule-interstitial damage in children with type 1 diabetes concerning the level of microalbuminuria and renal function in order to explore the potential role of NAG as an early predictor for the development of nephropathy in type 1 diabetic patients.

Methods

This study is a cross-sectional study that included 90 children and adolescents aged more than 5 years. These patients were recruited from regular attendance of Diabetic Endocrine Metabolic Unit (DEMPU), Children Hospital, Cairo University, over the period of 8 months from May 2018 till December 2018. They have divided into three groups: group 1, including 30 patients with type 1 DM for more than 2 years with microalbuminuria, group 2, including 30 patients with type 1 DM for more than 2 years without microalbuminuria and group 3, including 30 children and adolescents, age- and sex-matched, served as a control group.

Children with type 1 DM of both sex, more than 5 years with a disease duration of more than 2 years, were included in the study.

Patients with urinary tract infection, patients on glucocorticoid or anti-hypertensive treatment, patients with renal disease, and any other chronic illness were excluded from the study. Also, diabetic children less than 5 years and children with duration of DM less than 2 years were excluded from the study.

The ethical committee approval was obtained for our study at the Faculty of Medicine, Cairo University.

All patients were subjected to the following:

1. *Record of detailed history* including demographic data (age, sex, and residency), age of onset of diabetes mellitus, duration of the disease, honeymoon period, insulin therapy (type, basal to bolus ratio, the dose of insulin IU/kg/day, and frequency), and other medications like angiotensin-converting enzyme inhibitors (ACEI) and corticosteroids. Also, history of complications obtained according to the patient's medical records, including occurrence and frequency of hypoglycemia or diabetic ketoacidosis at the diagnosis, history of ocular complications (retinopathy) documented by fundus examination, cardiovascular complications such as left ventricular hypertrophy (LVH) documented by echocardiography examination, renal complications including hypertension and microalbuminuria, and eurological complications including peripheral neuropathy were recorded. History suggesting other associated diseases such as autoimmune thyroiditis, Addison's celiac disease, and diet history were also recorded.
2. *Clinical examination* including measurements including body weight with standard deviation score (SDS), height with SDS, and body mass index (BMI) with SDS using Egyptian Growth Curves [8]. Vital signs include the body temperature, respiratory rate, and blood pressure, systemic examination (cardiac examination (by auscultating the heart), abdominal examination (by inspection and palpation to exclude organomegaly), and neurological examination (by inspection and palpation to exclude organomegaly examination for detecting peripheral neuropathy and weakness.
3. *Laboratory investigations* including urine analysis for detection of urinary tract infections, mean reading of blood glucose in last 3 months, complete blood count (CBC) with differential using CELL-DYN Ruby hematology analyzer, celiac screen including tissue transglutaminase antibody (tTG), deaminated gliadin peptide antibodies (DGP), anti-endomysial antibodies (EMA), anti-reticulin antibodies (ARA) and immunoglobulin A (Total IgA), microalbuminuria in urine by immunometric enzyme immunoassay, serum creatinine using Beckman Coulter AU 480 chemistry analyzer, lipid profile including total cholesterol (TC), serum high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-c) using Beckman Coulter AU 480 chemistry analyzer and thyroid profile including Free T4 measured with Radioimmunoassay (RIA) methods and serum TSH with immunoradiometric assay (IRMA) method.

Mean values of the last 3 HbA1c (glycosylated hemoglobin HbA1c) in the last 9 months before the study with Quantitative Colorimetric Determination of Glycohemoglobin in whole blood were recorded from the files.

Controls were subjected to the following:

1. *Record detailed history*, including demographic data (age, sex, and residency), and confirm that there is no history suggesting any diseases or infections.
2. *Clinical examination* including measurements including body weight with standard deviation score (SDS), height with SDS, and BMI with SDS using Egyptian Growth Curves [8], vital signs, and systemic examination.

Quantitative assessment of urine NAG

Urine NAG level was assayed using Human N-acetyl-β-D-glucosaminidase (NAG) ELISA Kit catalog number 95685 manufactured and distributed by: Glory Science Co., Ltd. 2400 Veterans Blvd. Suite 16-101, Del Rio, TX 78840, USA.

The 1st urine sample of the day (mid-stream) was aseptically collected, voided directly into a sterile container, centrifuged to remove particulate matter, aliquoted, and stored at ≤ 20 °C till the time of assay.

Quantitative assessment of microalbuminuria

Quantitative determination of micro-albumin in urine using Immunometric Enzyme Immunoassay kits manufactured and distributed by: ORGENTEC Diagnostika GmbH Carl-Zeiss-Straße 49-51 55129 Mainz, Germany.

Statistical analysis

Statistical Package of social science (SPSS) version 15.0 was used to analyze data. Data were summarized as mean and SD. Non-parametric test (Mann-Whitney *UI*) was used to analyze two quantitative data. The chi-square test was used for the analysis of qualitative data. One-way ANOVA test was used to analyze more than two quantitative data, followed by post hoc test to detect significance. Pearson's correlation was also done. *r* was considered weak if < 0.25, mild if > 0.25–< 0.5, moderate if > 0.5–< 0.75 and strong if > 0.75. Cut-off of NAG is calculated as the mean ± SD of the controls. *P* value was considered significant if < 0.05.

Results

The mean age was 10.05 ± 2.55 years. The mean duration of diabetes was 6.3 ± 2 years, and their sex distribution was 46.6% males (28 patients) and 53.3% females (32 patients). The healthy children and adolescents (control group) had a mean age of 10 ± 2.4 years, 40% of them were males (12 patients), and 60% were females (18 patients)

In this study, we divided the patients with type 1 diabetes into two groups according to urinary albumin creatinine ratio: group 1: normoalbuminuric patients (< 30 mg/g) including 30 patients from a total of 60 patients (50%), their mean age was 9.9 ± 2.5 years, 50% were males (15 patients), and 50% were females (15 patients) and group 2: microalbuminuria patients (≥ 30 mg/g) including 30 patients from total 60 patients (30%), their mean age was 10.2 ± 2.6 years, 43% of them were males (13 patients), and 57% were females (17 patients).

Demographic data of diabetic patients and controls are shown in Table 1. Baseline demographic data were similar in healthy and diabetic children ($P > .05$) except for BMI, where highly statistically significant differences were found ($P = 0.008$). Diabetic children with microalbuminuria had significantly lower BMI than healthy children ($P = 0.009$).

Table 1 Demographic data of diabetic and healthy children

Characteristics	Diabetics		Group 3 (Control) *n = 30*	*P* value
	Group 1 *n = 30*	Group 2 *n = 30*		
Age (years)	9.9 ± 2.5	10.2 ± 2.6	10 ± 2.4	0.89
Sex				
Boys	15 (50%)	13 (43%)	12 (40%)	0.73
Girls	15 (50%)	17 (57%)	18 (60%)	
BMI (kg/m^2)	18.1 ± 1.6	17.8 ± 2.1	18.9 ± 1.7	**0.008***
BMI-for-agezscores (BMIAZ				
Normal (*z* scores < − 1)	22 (73%)	25 (83%)	20 (67%)	0.26
Risk of overweight (*z* scores > + 1)	8 (27%)	4 (13%)	7 (23%)	
Overweight (*z* scores > + 2)	0 (0%)	1 (3%)	3 (10%)	

Data were presented as mean ± standard deviation, number (percentage)

BMI Body mass index

*Significant as *p* value < 0.05

Table 2 shows a comparison between both groups regarding duration of diabetes, diabetic history, different comorbidities, and laboratory findings. Children with diabetes with microalbuminuria significantly had longer DM duration than those with normal uACR ($P = 0.008$). There are statistically significant differences regarding infection, hypertension, and neuropathy between both diabetic groups ($P = 0.001$, $P = 0.011$, and $P < 0.001$, respectively). Other comorbidities were not significantly different. There are statistically significant differences between the two diabetic groups regarding HbA1c, serum HDL, serum creatinine level, uACR, and serum triglycerides.

Table 3 shows clinical examination of healthy and diabetic children regarding vital signs, systemic examination, and Tanner staging. There are statistically significant differences between the three groups regarding critical signs, but not systolic blood pressure ($P = 0.025$). Abdominal and neurological examinations were statistically significantly different in diabetic and healthy children ($P = 0.001$ and $P = 0.003$). Microalbuminuria children had a statistically significantly higher diastolic blood pressure than normoalbuminuric and healthy children ($P < 0.001$). No significant difference between normoalbuminuric and healthy children was noted ($P = 0.14$). Microalbuminuria children had a statistically significantly lower respiratory rate than healthy children ($P = 0.01$), but other group differences were not statistically significant ($P > 0.05$). Normoalbuminuric children had a statistically significantly higher heart rate than healthy children ($P = 0.006$). Similarly, normoalbuminuric children had a statistically significantly higher heart rate than microalbuminuria children ($P = 0.002$). No significant difference between microalbuminuria and healthy children was noted ($P = 0.71$).

There is a highly statistically significant difference between the studied diabetic and healthy children ($P < 0.001$) regarding urinary NAG. Microalbuminuria children had a statistically significantly higher NAG (1313 ± 464.6) than normoalbuminuric (913.2 ± 69) and normal children (697.7 ± 66.9) ($P < 0.001$). Similarly, normoalbuminuric children had a statistically significantly higher NAG than normal children ($P < 0.001$).

Table 4 shows correlations between urinary NAG and different baseline characteristics and diabetic history of the studied diabetic children. There was a statistically significant positive correlation between urinary NAG and duration of diabetes ($P = 0.009$, $r = 0.334$).

Family history was positive in 51.6% of all diabetic children compared to 48.4% of them who had a negative family history.

In our study, we studied the correlations between urinary NAG and other laboratory investigations (HbA1c, lipid profile, liver functions, kidney functions, and thyroid

Table 2 Comparison between normoalbuminuric and microalbuminric patients regarding duration of diabetes, diabetic history, comorbidities, and laboratory findings

Characteristics	Diabetics		*P* value
	Group 1 *n* = 30	Group 2 *n* = 30	
Duration of DM (years)	5 ± 1.8	7.6 ± 2.2	< 0.001
Consanguinity			
Negative	15 (50)	18 (60)	0.18
Positive	15 (50)	12 (40)	
Family history			
Negative	14 (47)	15 (50)	0.25
Positive	16 (53)	15 (50)	
Perinatal history			
Normal	26 (87)	25 (83)	0.13
NICU	4 (13)	5 (17)	
Honeymoon			
No	16 (53)	16 (53)	> 0.99
Yes	14 (47)	14 (47)	
Admission			
PICU	15 (50)	13 (43)	0.61
Ward	15 (50)	17 (57)	
Compliance of measurements			
No	12 (40)	17 (57)	0.20
Yes	18 (60)	13 (43)	
Infection	10 (33)	23 (77)	0.001
Celiac disease	0 (0)	3 (10)	0.24
Other diseases hypothyroidism	2 (7)	2 (7)	< 0.99
IBD	1 (3)	0 (0)	
Lipodystrophy	13 (43)	15 (50)	0.61
Hypoglycemia	16 (53)	13 (43)	0.44
Hypertension	0 (0)	7 (23)	0.011
Neuropathy	11 (37)	12 (40)	0.8
Retinopathy	12 (40)	10 (33)	0.6
Nephropathy	0 (0)	30 (100)	< 0.001
Arthropathy	11 (37)	15 (50)	0.3
HbA1c (%)	8.8 ± 1.5	11.2 ± 1.8	< 0.001
Cholesterol (mg/dL)			
Mean ± SD	165.4 ± 19.6	170.8 ± 30.5	0.42
Triglycerides (mg/dL)			
Median (range)	95 (42–176)	64.5 (12–161)	0.002
LDL (mg/dL)	97.4 ± 23.7	103.4 ± 39.5	0.48
HDL (mg/dL)	48.1 ± 11.4	57.7 ± 16	0.010
ALT (IU/L)	22 ± 8.9	23 ± 12	0.72
AST (IU/L)	24.6 ± 14	24.63 ± 12	0.99
Serum creatinine (mg/dL)	0.82 ± 0.14	0.93 ± 0.18	0.006
uACR (mg/g)	6.2 ± 1.8	73.2 ± 27.9	< 0.001*
Free T4 (ng/dL)	1.4 (0.6–21.6)	1.3 (0.8–17.1)	0.95
TSH (μIU/mL)	1.9 (0.5–8.2)	1.7 (0.35–7.4)	0.59

Data were presented as mean ± standard deviation, number (percentage) and median (interquartile range)

HbA1c Glycosylated hemoglobin, *LDL* Low-density lipoprotein, *HDL* High-density lipoprotein, *ALT* Alanine transaminase, *AST* Aspartate transaminase, *uACR* Urinary albumin creatinine ratio, *Free T4* Free thyroxin, *TSH* Thyroid stimulating hormone

*Significant as *p* value < 0.05

Table 3 Clinical findings in healthy and diabetic children

Clinical findings	Diabetics		Group 3 (Control) *n* = 30	*P* value
	Group 1 *n* = 30	Group 2 *n* = 30		
Systolic blood pressure (mmHg)	108 ± 6.5	111 ± 6.7	108 ± 6.2	0.25
Diastolic blood pressure (mmHg)	70 ± 4.4	75 ± 3.8	69 ± 5.1	**< 0.001***
Respiratory rate (cycle/min)	20 ± 1.7	19 ± 1.6	21 ± 1.5	**0.03***
Heart rate (bpm)	99 ± 6.5	94 ± 6.3	94 ± 5.6	**0.003***
Cardiac examination,				
High S1	0 (0)	0 (0)	2 (7)	0.10
Murmur	0 (0)	0 (0)	1 (30)	
Normal	30 (100)	30 (100)	27 (90)	
Chest examination				
Rhonchi	2 (7)	2 (7)	3 (10)	0.2
Crepitation	1 (3)	6 (20)	1 (3)	
Normal	27 (90)	22 (73)	26 (87)	
Abdominal examination				
Tenderness	1 (3)	6 (20)	0 (0)	**0.001***
Distension	4 (13)	0 (0)	0 (0)	
Normal	25 (83)	24 (80)	30 (100)	
Neurologic examination				
Neuropathy	11 (37)	12 (40)	0 (0)	**< 0.001***
Normal	19 (63)	18 (60)	30 (100)	
Tanner stage				
T1	1 (3)	9 (30)	9 (30)	0.4
T2	4 (13)	14 (47)	18 (60)	
T3	2 (7)	7 (23)	3 (10)	

Data were presented as mean ± standard deviation, number (percentage)

*Significant as *p* value < 0.05

Table 4 Correlations between urinary NAG and demographic data and diabetic history of the studied diabetic children

		Test	*P* value
Age (years)		***r*=** 0.095	0.47
BMI (kg/m^2)		***r*=** − 0.062	0.64
Sex		Independent samples *t* test = 1.2	0.25
Boys (*n* = 28)	**Girls** (*n* = 32)		
1051.6 (179.6)	1167.3 (499.3)		
BMI-for-agezscores (*BMIAZ*)		***r*=** − 0.042	0.75
Duration of DM (years)		***r*=** 0.334	**0.009***
Honeymoon		Independent samples *t* test = 0.5	0.61
No (*n* = 32)	**Yes** (*n* = 28)		
1137.4 (500.1)	1085.8 (193)		
Admission		Independent samples *t* test = 1.2	0.26
PICU (*n* = 28)	**Ward** (*n* = 32)		
1174.5 (521.4)	1059.8 (201.9)		

BMI Body mass index, *PICU* Pediatric intensive care unit

*Significant as *p* value < 0.05

profile), respectively. These correlations are presented in Table 5. There were highly statistically significant positive correlations between urinary NAG and HbA1c, uACR, and serum creatinine. Also, there was a significant positive correlation between urinary NAG and serum cholesterol.

Urinary NAG has an important predictive ability in diabetic nephropathy and helps differentiate between normoalbuminuric and microalbuminuria diabetic children. This is best shown in Fig. 1. Urinary NAG can well differentiate between normoalbuminuric and microalbuminuria diabetic children with an area under the curve (AUC) of 1.0 for urinary NAG (95% CI 0.92 to 1.0, $P < 0.001$). The optimal sensitivity and specificity were (96.7% and 96.7%, respectively, at a cut-off expression value > 1020 U/L).

Discussion

Urinary NAG is an essential predictor of diabetic nephropathy. Increased urinary NAG excretions may be detected in patients with early stages of diabetes mellitus even before any clinical evidence of kidney involvement [9].

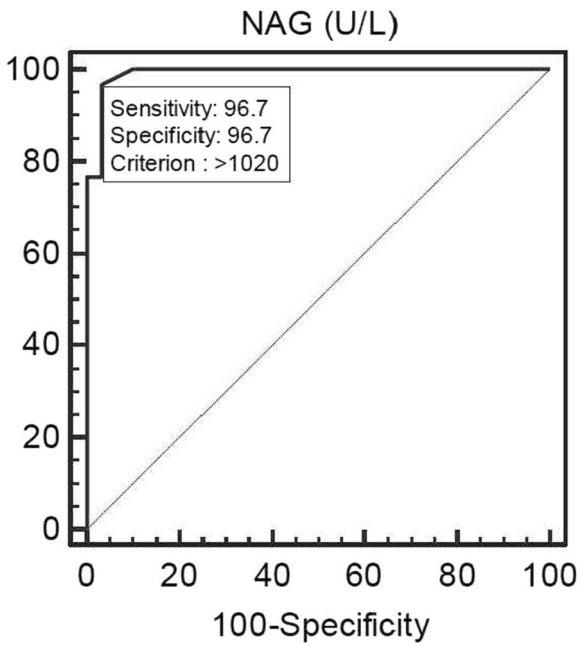

Fig. 1 Receiver operating characteristic (ROC) curve of urinary NAG in diabetic nephropathy

Table 5 Correlations between urinary NAG and other laboratory investigations in the studied diabetic children

Lab. Investigations		Urinary NAG (U/L)
HbA1c (%)	***r*-**	0.443
	P	**< 0.001***
Cholesterol (mg/dL)	***r*-**	0.288
	P	**0.026***
Triglycerides (mg/dL)	***r*-**	− 0.120
	P	0.360
LDL (mg/dL)	***r*-**	− 0.133
	P	0.311
HDL (mg/dL)	***r*-**	0.253
	P	0.051
ALT (IU/L)	***r*-**	− 0.113
	P	0.39
AST (IU/L)	***r*-**	− 0.149
	P	0.254
Serum creatinine (mg/dL)	***r*-**	0.355
	P	**0.005***
uACR (mg/g)	***r*-**	0.473
	P	**< 0.001**
Free T4 (ng/dL)	***r*-**	0.011*
	P	0.94
TSH (μIU/mL)	***r*-**	− 0.121
	P	0.36

r Pearson correlation, *LDL* Low-density lipoprotein, *HDL* High-density lipoprotein, *ALT* Alanine transaminase, *AST* Aspartate transaminase, *uACR* Urinary albumin creatinine ratio, *Free T4* Free thyroxin, *TSH* Thyroid stimulating hormone, *HbA1c* Glycosylated hemoglobin,

*Significant as *p* value < 0.05

In our study, the demographic data of both diabetic and healthy children were recruited, and they were similar ($P > 0.05$) except for body mass index (BMI), where diabetic children with microalbuminuria had significantly lower BMI than healthy children ($P = 0.009$).

This observation was not in concordance with other researchers [7], who found that BMI was slightly higher but statistically non-significant in all groups of patients as compared to healthy controls. Also, another study found that there is no significant difference in BMI between diabetic and healthy groups [6].

Our study revealed that diabetic children with microalbuminuria had a longer DM duration than diabetic children with normal uACR ($P = 0.008$) significantly.

This is in accordance with the results reported by [11]. In their study, the disease duration was significantly longer in a group of patients with microalbuminuria and a group of patients with macroalbuminuria than in a group of patients with normoalbuminuric.

However, another study reported no significant difference between microalbuminuria and normoalbuminuric patients regarding the duration of diabetes ($p = 0.12$ and $p = 0.406$, respectively) [14].

In the present study, normoalbuminuric and microalbuminuria children have statistically significant differences in hypertension and neuropathy ($P = 0.011$ and $P < 0.001$, respectively).

The same result was reported in another study that systolic blood pressure (SBP) was significantly higher in microalbuminuria than in normoalbuminuric patients ($P < 0.001$) [10]. Moreover, another study found that SBP was higher among those with microalbuminuria than normoalbuminuric subjects ($P = 0.001$) [12].

On the other hand, researchers found no statistically significant difference between both normoalbuminuric and microalbuminuria groups regarding blood pressure [2].

Our study confirmed that microalbuminuria diabetic children had significantly higher HbA1c levels than normoalbuminuric diabetic children ($P < 0.001$).

This agrees with another study that showed a significantly higher HbA1c level in macroalbuminuric and microalbuminuria diabetic patients than in normoalbuminuric diabetic patients, and healthy controls ($P = 0.006$) proved that poor glycemic control is a well-known risk factor for diabetic nephropathy [11].

Also, a study reported that microalbuminuria patients had significantly higher HbA1c than normoalbuminuric patients and healthy controls ($P < 0.001$) [10].

On the contrary, researchers found no statistically significant differences between microalbuminuria and normoalbuminuric groups regarding HbA1c [2].

In the present study, microalbuminuria diabetic children had significantly higher serum creatinine levels ($P = 0.006$) and urinary albumin creatinine ratio ($P < 0.001$) compared to normoalbuminuric diabetic children.

Similarly, a study showed that microalbuminuria T2DM patients with DKD had significantly higher serum creatinine and uACR than normoalbuminuric T2DM patients without DKD ($P < 0.001$ and $P < 0.001$, respectively) [12].

As shown in Bouvet et al. [2], the reported microalbuminuria group showed higher levels of ACR compared to the normoalbuminuric group ($P < 0.001$). They also found that there are no statistically significant differences between both groups regarding serum creatinine levels or eGFR.

In our study, microalbuminuria diabetic children had significantly higher serum HDL levels ($P = 0.01$) but substantially lower serum triglycerides levels ($P = 0.002$) compared to normoalbuminuric diabetic children.

Other studies analyzed different parameters of lipid profile; Suh et al. [14] reported that the lipid profiles did not differ between the normoalbuminuric and microalbuminuria groups, except for the total cholesterol level, which was significantly higher in the microalbuminuria group than in the normoalbuminuric group ($P = 0.02$).

In our study, uNAG was measured in the urine samples of all 90 children, where it was found that uNAG was significantly higher in all patients compared to the controls ($P < 0.001$).

This is in accordance with the results reported by Omozee et al. [6]. In these studies, the mean values of uNAG were higher in all patients with diabetes compared to the controls.

Both the mean and the median of uNAG were found to be significantly higher in the microalbuminuria group in comparison to the normoalbuminuric group ($P < 0.001$), and the mean and the median of uNAG level was higher in normoalbuminuric patients compared to controls ($P < 0.001$). Therefore, uNAG increased in parallel with the severity of renal disease, reaching higher levels in patients with manifest diabetic nephropathy.

Similarly, Mahfouz, Assiri, and Mukhtar [4] reported that the mean uNAG values in the microalbuminuria group were significantly high compared with normoalbuminuric patients. Also, Kim et al. [3] said that the uNAG level was higher in normoalbuminuric patients compared to controls.

In our study, there is a statistically significant positive correlation between urinary NAG (uNAG) and the duration of diabetes ($P = 0.009$, $r = 0.334$). This means that the more duration of diabetes, the higher the values of uNAG.

Similarly, Kim et al. [3] demonstrated that uNAG was positively correlated with the duration of diabetes and negatively correlated with BMI.

Patel and Kalia [7] reported that urinary NAG activity showed a non-significant increase in the early years of T2DM (0–5 and 5–10 years), and then it was significantly increased by 8 and 10 folds in 10–15 and 15–20 years of diabetes duration, respectively.

Recently, Siddiqui et al. [12] showed that NAG activity was increased with diabetes duration and with the progression of DKD.

Our study showed a highly statistically significant positive correlation between urinary NAG and HbA1c, uACR, and serum creatinine ($P < 0.001$, $P < 0.001$, and $P = 0.005$, respectively). Therefore, a strong relationship was reported between values of uNAG as a marker for diabetic nephropathy and HbA1c as a measure of glycemic control of diabetes. It is well described that poor glycemic control is a risk factor for most diabetic complications, among these is diabetic nephropathy.

In accordance with our study, Sheira et al. [11] typically revealed a significant positive correlation between urinary NAG level and albumin/creatinine ratio, serum creatinine, and HbA1c.

Similarly, Kim et al. [3] showed that urinary NAG had a positive relationship with urinary ACR ($r = 0.458$, $P < 0.001$). Also, uNAG and uACR were strongly correlated with HbA1c and serum creatinine. Also, Bouvet et al. [2] reported that there was a significant positive correlation

between the NAG and urinary albumin/creatinine ratio ($r = 0.74$, $P < 0.001$).

Our study found a positive correlation between uNAG and serum cholesterol in the studied diabetic children ($P = 0.026$). The same was reported by Sheira et al. [11], who found a statistically significant correlation between uNAG and serum cholesterol. On the contrary, Sharifi et al. [10] did not find any correlation between uNAG and serum cholesterol.

Our results revealed a strong positive correlation between urinary NAG and urinary albumin creatinine ratio (uACR), where urinary NAG levels increase dramatically with an increase in uACR.

The positive correlation between uNAG and uACR was observed by many researchers [2, 3, 11]. On the other hand, Omozee, Okaka, and Edo [6] did not show any correlation between urinary microalbumin concentrations and urinary NAG concentrations.

Our results reported that the optimal sensitivity and specificity of uNAG were 96.7% and 96.7%, respectively, at a cut-off expression value > 1020 U/L. Urinary NAG helps differentiate between normoalbuminuric and microalbuminuria in diabetic children and has an essential predictive ability in diagnosing diabetic nephropathy.

Similarly, Patel and Kalia [7] revealed that the sensitivity and specificity of uNAG were 85.3% and 96%, respectively.

In our study, we elicited gender prevalence in uNAG values, where we found significantly higher uNAG in female patients than in male patients ($P = 0.025$). Similarly, a higher prevalence of microalbuminuria was observed in females (60%) compared to males (40%). A selection bias could not be excluded as female patients in our study represented 55.6% more than male patients, who represented only 44.4%, possibly due to the higher attendance rates of females than males in the DEMPU during the research period.

This is in accordance with the results reported by Sheira et al. [11], who revealed that the number of females with microalbuminuria (60%) was more than males (40%).

To summarize, we observed that uNAG positively correlates with ACR, duration of diabetes, HbA1c, serum creatinine, and serum cholesterol. Also, positive uNAG results were found even in normoalbuminuric patients, so it can be concluded that we can use uNAG as an early biomarker for diabetic nephropathy in normoalbuminuric patients, especially those with long-standing diabetes, uncontrolled diabetes, and dyslipidemia.

Our study has some limitations. First is the small number of the study. Second is the lack of follow-up of the uNAG after glycemic control and control of albuminuria.

Our study revealed that uNAG levels are significantly higher in microalbuminuric patients compared to normoalbuminuric patients and healthy controls. Also, in normoalbuminuric patients, there are high urinary levels of NAG. Our study also revealed a strong positive correlation between uNAG levels and the duration of diabetes. Measuring uNAG level is an easy and non-invasive biomarker for early detection of DN, especially for patients with type 1 diabetes with more than 2 years of uncontrolled diabetes. More extensive studies are recommended to correlate uNAG with other glomerular and tubular biomarkers of diabetes nephropathy.

Conclusion

Our study revealed that uNAG levels are significantly higher in microalbuminuric patients in comparison to noromoalbuminuric patients and healthy controls. Also, in normoalbuminuric patients, there are high urinary levels of NAG. Our study also revealed a strong positive correlation between uNAG levels and duration of diabetes. Measurement of uNAG level is an easy and non-invasive biomarker for early detection of DN, especially patients with type 1 diabetes more than 2 years durations associated with uncontrolled diabetes. Larger studies to correlate uNAG with other glomerular and tubular biomarkers of diabetes nephropathy are recommended.

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Authors' contributions

Authors' contribution and participation were as following: Emad E Ghobrial participated in search in the literatures, followed the results, prepared the final manuscript, and is the corresponding author. Amal M Said participates in choosing the issue of the study, followed the results, and prepared the final manuscript. Gerges F Abd El Shaheed participated in collecting data of the patients, followed the results, and searched in the literatures. Youmna M Shaalan participated in choosing the issue of the study, followed the results, and prepared the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All the data and materials are submitted in supplementary files in the submission system and available.

Declarations

Ethics approval and consent to participate

This study was approved by the ethical scientific committee in the Cairo University hospital and was conducted in accordance with the University bylaws for human research. It conforms to the provisions of the Declaration of Helsinki in 2000. All caretakers have given their informed consent.

Consent for publication

All authors have contributed significantly and all authors are in agreement with the content of the manuscript.

Competing interests
The authors declare that they have no competing interests.

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