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ABSTRACT

Nephropathy is a serious microvascular complication of insulin-dependent diabetes mellitus (IDDM). Early detection of diabetic nephropathy by measuring some glomerular and tubular markers was our aim of this work. 75 IDDM patients (36 female and 39 males) aging from 4.9 to 18.2 years and having a mean duration of diabetes 6.9 years, were divided into 3 groups according to the duration: A (0-5 years), B (5-10 years) & C (> 10 years). 25 healthy subjects were selected as control "D". All subjects were investigated for glycated haemoglobin (HbA1c), urinary albumin creatinine ratio (ACR) and N-acetyl-beta-D-glucosaminidase (NAG). Serum samples for sialic acid, prorenin, nitric oxide (NO,as nitrite and nitrate), lipid profile, blood sugar and kidney function were assessed. The ACR had significant higher values in diabetic groups (P<0.01) than the control. It was correlated significantly with HbA1c, duration of diabetes, as well as, serum values of sialic acid, prorenin, NO and serum lipids. The level of urinary NAG showed significant increase (P<0.01) between diabetic and control groups, also there was a significant positive correlation between NAG and other glomerular and tubular markers. We concluded that there is tubular dysfunction in the early stage of IDDM children ever before there is any clinical evidence of nephropathy, and urinary ACR and NAG can be used as early markers of diabetic nephropathy, and they may also reflect glycemic control in such patients.

INTRODUCTION

Nephropathy is a serious microvascular complication of insulin-dependent diabetes mellitus (IDDM). Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/day), a rise in arterial blood pressure, and a relentless decline in glomerular filtration rate leading to end-stage renal failure[1].

Glomerular and tubular markers have been carried out in order to predict the occurrence of diabetic nephropathy. Microalbuminuria and urinary N- acetyl-beta- D- glucosaminidase (NAG) are sensitive indicators of renal tubular injury[2].

Chronic hyperglycemia is associated with an increased nitric oxide (NO) biosynthesis and action that contributes to generating glomerular hyperfiltration and persistent microalbuminuria[3].

Also, elevated plasma sialic acid concentration is strongly related to the presence of microvascular complications in IDDM especially retinopathy and nephropathy[4].

The aim of this study was to assess some glomerular and tubular markers including microalbuminuria & NAG excretion to detect cases of early diabetic nephropathy.

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SUBJECTS AND METHODS

The study included 75 children, adolescents and young adults (36 females and 39 males) with type I diabetes mellitus, who were drawn from the diabetes clinic at Benha University Hospital and Benha Children Hospital. They had a mean age of 12.17 ± 7.3 years (range 4.9 to 18.2), and a mean duration of diabetes 6.9 years (range 0.6 to 14.0). All patients were on conventional insulin therapy.

75 IDDM patients were divided into 3 groups according to the duration of disease (group A, B, and C). Group D was the control:

Group A: Comprised 25 patients with IDDM duration from 0-5 years.

Group B: Comprised 25 patients with IDDM duration from 5-10 years.

Group C: Comprised 25 patients with IDDM duration >10 years.

Group D: 25 healthy subjects, age and sex matched, were selected as controls. None of them had history of diabetes mellitus in a first degree relative, nor past history of any renal troubles.

Both patients and controls were subjected to:

1- Detailed history taking including onset and duration of diabetes and history suggestive of diabetic complications.

2- Full clinical examination including blood pressure measurements and signs of renal impairments.

3- Laboratory investigations:

a-Glycated haemoglobin (HbA1C) was measured chromatographically. The normal range was 5.8%-8%.

b-Urine collection for albumin/ creatinine ratio (ACR) and NAG using different spectrophotometric methods.

c-Serum samples for blood sugar, cholesterol, high density lipoprotein (HDL), urea and creatinine.

d-Blood samples were collected to measure nitric oxide (NO). As NO is labile compound that rapidly decomposes to nitrite and nitrate(NO2 / NO3) in biologic fluids, the later were measured in triplicate by conversion of NO2 to NO3 by commercially available kit based on the Griess reaction following the manufacturer's instructions[5].

e-Plasma sialic acid was assayed using an enzymatic method (Boehringer Mannheim, Lewes, Sussex, UK)[6].

f- Prorenin represents the inactive Precursor that we activate to renin vitro using exogenous trypsin, it was assayed using[7].

4- Statistical analysis data were analysed using Epi-info version.

RESULTS

Table (1) there was a significant difference ($P<0.01$) between each diabetic group and control as regarding glycated hemoglobin (HbA1c), fasting and random blood sugar and serum creatinine, and also between late diabetic (B & C) and control groups as regarding lipid profile and serum urea. There were also significant increased values between different diabetic groups A, B & C (ANOVA, $P<0.01$) as regarding all previous biochemical variables except for serum creatinine.

Table (2) both prorenin and nitric oxide (NO) were significantly different between each diabetic group and control ($P<0.01$), but inversely they have no significant differences between the 3 diabetic groups themselves ($P>0.05$). The mean values of sialic acid were

significantly different between late diabetic (B, C) and control groups (P<0.01), as well as between different diabetic groups (P<0.01).

Table (3) the ACR and NAG were significantly different between all diabetic & control groups (P < 0.01) as well as, between the 3 diabetic groups (P<0.05).

Table (4) the ACR had significant positive correlations with serum sialic acid, NO, prorenin, cholesterol, HDL and random blood sugar, as well as, urinary NAG and HbA1c.

Table (1): Plasma biochemical variables between different studied groups.

Investigation	Group A (No. = 25)	Group B (No. = 25)	Group C (No. 25)	Group D (No. = 25)	Test of Significance
Haemoglobin HbA1c-%	8.29 ± 1.69*	10.33± 1.60*	9.86± 1.16*	5.85± 1.13	F= 11.42 P 0.001
Fasting blood sugar (mg/dl)	181.36±54.4*	165.88±34.22 *	241.72±42.05 *	78.27±12.62	F= 20.41 P=0.001
Random blood sugar (mg/dl)	246.76±74.2*	252.28±59.63 *	317.76±39.43 *	96.53±11.46	F= 11.91 P 0.001
Serum cholesterol (mg/dl)	91.64± 11.14	114.6±14.9*	156.3±32.13*	82.87± 10.36	F= 8.65 P 0.001
High density lipoprotein (mg/dl)	29.92±9.38	37.84±9.55*	31.64±7.18*	23.87±6.2	F= 5.64 P 0.01
Serum urea (mg/dl)	19.28±7.88	25.40±7.39*	29.2±7.69*	12.6± 3.79	F= 16.57 P 0.001
Serum creatinine (mg/dl)	0.76± 0.04*	0.85± 0.04*	0.82± 0.05*	0.67± 0.04	F= 1.09 P 0.05

* Significant difference P< 0.01 in comparison to the control group D.
F = (or ANOVA) to test difference between diabetic groups A,B,C.

Table (2): The comparison of serum sialic acid, prorenin, and nitric oxide in different studied groups.

Investigation	Group A (No. = 25)	Group B (No. = 25)	Group C (No. 25)	Group D (No. = 25)	Test of Significance
Sialic acid (mmol/L)	51.3 ± 14.93	55.25± 15.1*	64.32 ± 22.09*	46.37 ± 8.05	F= 3.42 P 0.05
Prorenin (pg/ml)	2.43± 1.25	2.78±1.38	2.65±1.31	1.87 ± 0.35	F= 0.46 P 0.05
Nitric oxide (umol/L)	24.92± 15.09*	28.67 ± 15.66*	33.06±15.62*	13.63±5.52	F= 1.75 P 0.05

* Significant difference P< 0.01 in comparison to the control group D.
F = (or ANOVA) to test difference between diabetic groups A,B,C.

Table (3): The comparison of urinary albumin/creatinine ratio, and N-acetyl-beta-D-glucosaminidase in different studied groups.

Investigation	Group A (No. = 25)	Group B (No. = 25)	Group C (No. 25)	Group D (No. = 25)	Test of Significance
Albumin/creatinine ratio (mg/mmol)	0.18±0.08*	0.2± 0.1*	0.22 ± 0.1*	0.11±0.03	F= 3.53 P<0.05
N-acetyl-beta-D-glucosaminidase (U/g creatinine)	15.47 ± 8.5*	18.81± 11.47*	19.96 ±11.43*	7.77± 2.18	F= 3.77 P 0.05

* Significant difference P< 0.01 in comparison to the control group D.
F = (or ANOVA) to test difference between diabetic groups A,B,C.

Table (4): Correlation between albumin/ creatinine ratio (ACR) and other studied parameters in diabetic cases.

Correlation	Coefficient of correlation	Significance
Albumin/creatinine with:		
Sialic acid	0.86	HS
Nitric oxide	0.85	HS
Pro-renine	0.83	HS
NAG	0.78	HS
HbA1c	0.34	S
Random blood sugar	0.33	S
Fasting blood sugar	0.14	NS
Cholesterol	0.6	HS
HDL	0.33	S
Urea	0.11	NS
Creatinine	0.04	NS

HS = Highly significant S = Significant NS = Non significant

DISCUSSION

Diabetic nephropathy is an important complication of diabetes. Established nephropathy is preceded by a long silent phase of incipient nephropathy characterized by a subclinical increase in albumin excretion known as microalbuminuria (30-300 mg/day)[8].

Bakker[9] did a study to compare albumin-to-creatinine ratio (ACR) and albumin excretion for detection of microalbuminuria. He concluded that, the ACR performs better than the later in screening for microalbuminuria.

The current study showed that, the ACR had significant difference in diabetic groups in comparison to the control (D) and there were also significant differences in ACR between the different diabetic groups. ACR was correlated positively with HbA1c in all diabetic groups.

Similarly, ***Chiarelli et al.***[1] ***and Schultz et al.***[10] reported that microalbuminuria and/or ACR had significant higher values in their diabetic patients and could be used for early detection of diabetic nephropathy.

Thirty-three out of 75 diabetic patients (i.e. 44%) had a significant increase in ACR, that was higher than data (19.7%) reported by ***Moore and Shield***[11].

Urinary NAG is a sensitive indicator of renal tubular injury[12]. In our study the level of urinary NAG showed significant increase between the diabetic groups and control, also there was a significant correlation between NAG and HbA1c in all diabetic groups.

In the current study, serum values of sialic acid, prorenin and nitric oxide were significantly correlated with ACR in diabetic groups. They were also significantly higher in diabetic patients than controls, so they are useful indicators for diabetic nephropathy. Similar data have been reported by ***Spanda et al.***[13], ***Yokoyama et al.***[14] *and Stevens*[15].

Hypertension and lipid profile (cholesterol and HDL) are risk factors for the development of diabetic complications and in this work, lipid values were significantly higher in late diabetic than control group. Also, the duration of IDDM correlated positively with lipid profile.

CONCLUSION

- There was significant elevation of ACR in diabetic patients that emphasizing the importance of ACR as early marker of diabetic nephropathy.
- NAG was found in early diabetic patients, even before the significant increase of ACR level and can be used as a tubular marker in routine investigation of diabetic patients.
- Poor glycemic control is correlated with early development of diabetic nephropathy.
- Prorenin, sialic acid and NO were correlated positively with ACR in diabetic patients, so can be used for follow up of diabetic nephropathy.

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