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Original Article

Impact -of Urinary Growth Norifonë/Insplin-Like Growth Factor-I on The Development of Nephropathy in Early-Onset and Long-Term Diabetic Children

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ABSTRACT

Background: Growth hormone' (Gil), insuImn-1mke growth factor-I (1G1'-1), 1GF-binding protein (GHJIGF/IGFBP) axis has been suggested bothţo maintain.normal renal function and to play an important role in the development of diabetic nephropathy (DN), in patients with type I diabetes (TI D). Nephromegaly **(NM) and** microalbuminuria (MA) are early markers of DN.

Objective: Study the role of urinary concentrations of GH and IGF-I in the development of DN, in children and adolescents suffering T I D.

Study design: A total of 50 children and adolescents with TI D of 6-14 years old were recruited **from the** Pediatric Diabetes Clinic at King Abdulaziz University Hospital, 23 were males and 27 were females. Subjects were divided into two groups; 20 with early-onset diabetes and 30 with long-term diabetes. Both groups were subjected to history-taking, clinical examination including body mass index (BM1), pubertal staging according to the rating of Tanner , assessment of glycemic control, measurement of kidney volume (KV), as a marker of glomerular hypertrophy, by ultrasonography (U/S).

Results: The overall rate of MA and NM accounted for 20% (10/50) and 26% (13/50), respectively, being more detected in long-term diabetics than that in patients with early-onset diabetes, with significant difference. Long-term diabetics had significantly higher albumin excretion rate (AER) and urinary GH and IGF-1 concentrations than that in early-onset diabetics. The mean **BMI,** pubertal duration, urinary GH **and** urinary IGF-1 were significantly higher among diabetics positive for MA than that among diabetics negative for hMA.

Conclusion: Our data, which reflect increased mean urinary GH/IGF-1 production, strengthen the evidence of an association between GH, MA, NM and also implicate urinary G1l/IGF-I in DN, particularly in children and adolescents with long-term diabetes, increased pubertal duration and poor glycemic control.

INTRODUCTION

Diabetes mellitus (DM), the most common endocrine metabolic disorder of childhood and adolescence, is characterized by chronic hyperglycemia and disturbances in carbohydrate, lipid and protein metabolism as a cDr'sëquence of decreased insulin secretion or activity or both. TiD, previously known as juvenile diabetes or insulin-dependant **DM (IDDM),** is the major form of diabetes in those under 10 years OIд`). In 1997; there were 11.5

million people with TID in the world, this figure is expected to rise to 33.7 million, in the year 2011 [3].

Type 1 diabetes, with chronic hyperglycemia is associated with microvascular complications (retinopathy, neuropathy and nephropathy). I-However, advances in treatment permit tight glycemic control which delays and slows progression of these complications. Despite all this positive development, epidemiological studies have demonstrated that during the past three decades, DN continues to occur in 15-40% of patients with TID with a peak incidence after 15 to 20 years of diabetes.

The development of MA is prognostically important in both T 1 D and T2D. While the "classical" histological changes of diabetic glomerulopathy (thickening of the glomerular basement membrane, mesangial expansion and arteriolar hyalinosis) are common in those with TID and renal dysfunction, the morphological lesions in those with T2D and renal dysfunction are more heterogenous[5].

The GI-I/IGF/IGFBP axis and related growth factor families [transforming growth factor-[3 (TGF-[3), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF)j demonstrate significant actions on the development of experimental diabetic kidney disease through defined intra-renal systems. These growth factors initiate the earliest renal changes associated with hyperglycemia. The majority of circulating IGF-I is produced by the liver and other tissues under the effect of GH and insulin. The kidney is a site for the production of IGF-I, which normally mediates its effect on renal growth and function. In cell cultures, IGF-I has been shown to induce mcsangial proliferation and the secretion of collagen[6].

In children and adolescents with "Γ1 D, Cummings et al. have reported an association between duration of puberty and the prevalence of MN & MA. GH, IGF-I, testosterone and prorenin are potential mediators of this effect. These observations suggest that the hormonal milieu of puberty may be involved in the pathogenesis of early DN".

AIM OF TILE WORK

Study the role of urinary concentrations of GH and IGF-I in association with microalbuminuria in early detection of DN, in children and adolescents suffering from early onset and long-term Type I diabetes.

PATIENTS AND METπOI)S

This study was carried out in the Pediatric Diabetes Outpatient Clinic at the King Abdulaziz University Hospital. during the year 2010, on 50 children and adolescents suffering TID. Patients selected were either suffering newly diagnosed Ti D-within one month of disease onset (20 patients), or suffering T10, with various disease durations (30 patients). Disease duration was defined as the day of initial diagnosis of diabetes to the day of blood collection in this study.

Methods

All study patients were subjected to the following:

A) History — taking

B) Clinical examination; including:

- Identification of patients with diabetic ketoacidosis (DKA) or hypoglycemic

coma. These patients were admitted to the Pediatric Intensive Care Unit for assessment and further management.

- General and systemic physical examination, including assessment of the nutritional status, using body mass index (BMI) calculated as kilograms per square meter (Kg/m`) and pubertal duration.

C) Investigations:

After the written informed parental consents were obtained, samples were collected from study children.

1. Routine investigations:

a. Urine analysis, with particular stress on glucosuria, ketonuria, microalbuminuria, pyuria, hematuria, and/or casturia. Cases with urinary tract infection (UTI), glomerulonephritis, systemic hypertension and nephrotic syndrome were identified and excluded from the study.

b. Complete blood count (CBC).

c. Blood urea nitrogen (BUN).

2. Investigations for glycemic control:

a. Fasting blood glucose determination, using dimension RXL auto analyzer (Siemens medical solutions diagnostic, Tarrytown, WY, USA).

b. Determination of glycated hemoglobin (HbA 1 c) percent, using chromatographic spectrophotometric ion exchange method (Bio Systems S.A. Costa Brava, Barcebna-Spain).

Glycemic control was considered "good to excellent" with I--**IbA 1 c** less than or equal to 8.0%, "fair" control 8.1-10.0% and "poor" control if HbA l c > 10.0%[8].

3. Research Investigations:

a. Measurement of urinary albumin excretion rate (AER) in 24 hr urine collections, by an enzyme immunoassay.

Normoalbuminuria was defined as an AER of < 20 }.Lg/min, in at least two separate urine specimens. MA was defined as an AER of 20-200 µg/min[9].

b. Urinary 01-I concentration was measured according to Butt and Sochettt[10]

c. Urinary IGF-I concentration was measured according to Gargosky et al. (1993) .

d. Measurement of KV:

KV (right + left) of each subject was measured by U/S. KV was calculated based on the formula for the volume of an ellipsoid [12]. Nephromegaly was defined as KV > 300 m1/1.73 m^2.

4. Statistical Analysis:

Data were collected, entered and checked to a SPSS, version 15. Data were expressed as mean + standard deviation (X ± SD) in quantitative variables, number and percentage for qualitative variables. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The duration of symptoms due to hyperglycemia, BMI and pubertal duration were significantly higher among patients with long-term diabetes, $p < 0.05$. In patients with early onset diabetes, the niost common presenting symptoms included history of infections (e.g., gastroentcritis and upper respiratory tract infections), enurcsis/nocturia, DKA and weight loss, while in long-term diabetics the most common presenting symptoms included oral/perincal candidiasis, infections and

enuresis nocturia. Table 1

Fable (2) presents the den uographic characteristics of long-term diabetics versus early-onset diabetics. The mean blood sugar of patients with early-onset diabetes was significantly hi`her than that in patients with long-term diabetes, $p < 0.05$. Mean-while the mean I ihA 1 c in long-term diabetics was significantly higher than that in patients with early-onset diabetes, $p < 0.05$. On other hand, ketonuria (+, -t--+-, or f +) was significantly more detected in children with early-onset diabetes than that in those with long-term diabetes, $p < 0.01$. NM & MA were significantly prevalent in children and adolescents with long-term diabetes than those with early-onset diabetes_ $p <- 0.05$.

The mean concentrations of urinary albumin. G11 and IGF-1 were significantly hieher in children and adolescents with bug-term diabetes than that in those with early-onset diabetes. Table 3.

Table (4) shows that the mean HMI, pubertal duration, glycated hemoglobin, urinary concentrations of (Hi and ЮГ-1 to be significantly higher in patients positive for MA (MA -1-ve) than that in MA -ve diabetic patients, $p < 0.05$.

Table 1: Clinical presentations of 30 long-term diabetic children versus 20 early-onset diabetic children.

Presentation (S)	Early-onset diabetics N = 20	Long-term diabetics N = 30	p value
Duration of symptoms (days)	21 ± 9.3	468.6 ± 96.4	< 0.001 (S)
Range	12-30	380 ± 715	
BMI (Kg/m^2)	18.27 ± 3.66	23.13 ± 2.81	< 0.01 (S)
Infections	10 (50%)	3 (10%)	< 0.01 (S)
Enuresis or nocturia	9 (45%)	3 (10%)	< 0.01 (S)
Diabetic ketoacidosis	8 (40%)	2 (6.7%)	< 0.01 (S)
Weight loss	4 (20%)	2 (6.7%)	< 0.05 (S)
Candidiasis	3 (15%)	4 (13.3%)	> 0.05 (NS)
Hypoglycemic seizure/coma	-	3 (10%)	< 0.05 (S)
Pubertal duration*, X ± SD, (years)	0.9 ± 0.6	5.1 ± 2.2	< 0.01
Range	1-3	2-6	

X ± SD: mean ± standard deviation N: number S: significant
NS: non significant.
* Pubertal duration was calculated as the following: prepubertal girls < 10 years and boys < 11 years of age.
BMI: body mass index Kg: kilogram m^2: meter square.

Table 2: Demographic characteristics of 30 long-term diabetic children versus 20 early-onset diabetic children.

Variable X ± SD	Early-onset diabetics N = 20	Long-term diabetics N = 30	p value
Age (years) Range	8.51 ± 3.9 6-10	11.95 ± 4.6 9-14	< 0.05 (S)
Gender (male/female)	9/11	14/16	> 0.05 (NS)
Blood glucose (mg/dl)	218 ± 86.2	183 ± 44.7	< 0.05 (S)
Current HbA1c (%)	8.6 ± 0.7	10.4 ± 1.5	< 0.05 (S)
Ketonuria, n & (%)$	13 (65%)	4 (13.3%)	< 0.01 (S)
BUN (mg/dl)	19.3 ± 8.8	16.4 ± 6.7	> 0.05 (NS)
Prevalence of nephromegaly*	3 (15%)	10 (33.3%)	< 0.05 (S)
Prevalence of microalbuminuria#	2 (10%)	8 (26.7%)	< 0.05 (S)

HbA1c: glycated hemoglobin BUN: blood urea nitrogen $: ketonuria (+, ++, or +++)
S: significant NS: non significant N = number
*: nephromegaly (NM) defined as kidney volume (KV) > 300 ml/1.73 m^2
#: microalbuminuria (MA) is defined as albumin excretion rate (AER) = 15-200 µg/min.
X ± SD: mean ± standard deviation

Table 3: Albumin excretion rate (AER), Urinary growth hormone (GH) and urinary insulin growth factor-I (IGF-I) in 30 long-term diabetics versus 20 early-onset diabetics.

	Early-onset diabetics N = 20	Long-term diabetics N = 30	p value
AER (µg/min)	26 ± 8.5	118 ± 77.3	< 0.001 (S)
Urinary GH (ng/day)	6.4 ± 0.7	17.8 ± 1.9	< 0.01 (S)
Urinary IGF-I (ng/day)	143 ± 18	395 ± 32	< 0.01 (S)

N: number S: significant X ± SD: mean ± standard deviation
ng: nanogram µg: microgram

Table 4: Body mass index (BMI), pubertal duration, glycated hemoglobin (HbA1c%), urinary growth hormone (GH) and urinary insulin growth factor-I (IGF-I) in diabetic children with microalbuminuria (MA +ve) and in children without MA (MA -ve).

	MA -ve N = 40	MA +ve N = 10	p value
BMI (Kg/m^2)	19.1 ± 1.2	22.3 ± 2.3	< 0.05 (S)
Pubertal duration (year)	1.2 ± 0.8	6.4 ± 1.7	< 0.05 (S)
HbA1c (%)	6.8 ± 1.2	11.1 ± 1.8	< 0.01 (S)
Urinary GH (ng/day)	5.1 ± 0.6	22.4 ± 2.6	< 0.01 (S)
Urinary IGF-I (ng/day)	293 ± 18	645 ± 110	< 0.05 (S)

N: number S: significant ng: nanogram
X ± SD: mean ± standard deviation

DISCUSSION

Type 1 diabetes (T1D), a disease that results from autoimmune destruction of the insulin-producing 0-cells in the pancreatic islets of Langerhans, is associated with accelerated atherosclerosis and predisposes to certain specific microvascular complications, including retinopathy, neuropathy and nephropathy[131]. Classically, the development of diabetic microangiopathy depends mostly on the duration of diabetes and on the degree of glycemic control. However, the development and progression of DN shows significant variation between individuals and, consecutively develops only in a fraction of diabetic subjects despite the inadequately controlled diabetes[14].

In this case-control study, 50 children and adolescents suffering TID, 20 early-onset diabetics and 30 long-term diabetics were recruited, to study the effect of urinary GH and IGF-I excretion on the development of DN, marked by MA and NM.

In this study, the most prevalent clinical presentations among early-onset diabetics included history of infections (50%), enuresis/nocturia (45%), DKA (40%), followed by weight loss and oral/perineal candidiasis. On other hand, the most common clinical presentations among long-term diabetics included infections, candidiasis, DKA, nocturnal enuresis, hypoglycemic seizure/coma and later weight loss. Similar results were reported by other studies who stated that younger children are more likely to present in DKA and that HbA1c and the loss of 0-cell function is insidious, even in children under age 6 years[1 s-17].

Although it is well known that Candida albicans thrives in adolescents with uncontrolled diabetes and frequently causes vulvo-vaginitis, our observations highlight

the association of oral and perineal candidiasis in young children with new-onset TID. Furthermore, Quinn et al. (2006) identified a significant association between the duration of candidal infection to I-IbA 1 c at diagnosis and underscored the importance of early recognition of candidiasis to detect the onset of TI D before it progresses to KDA. The significantly longer duration of candidiasis in young children who present in DKA (p = 0.004) justifies the inclusion of yeast infections as a sign of new-onset diabetes in this susceptible age group[18].

In the present study the mean blood glucose level and ketonuria at onset, among newly diagnosed children were significantly higher than that in long-term diabetics. On other hand, the mean value of HbA 1 c at entry to the study was higher in long-term diabetics than that in newly diagnosed cases. Similar results were obtained by Samuelson and Stenhammar[19].

Persistent MA is a strong predictor for DN and is diagnosed in 30-40% of adults and 15-25% of adolescents with Ti Meanwhile, Vasylyeva et al. (2007) reported a prevalence of MA in children with Ti D which accounts for 7 to 20%(6). The prevalence of elevated AER increases after 10-15 years of diabetes duration. In children and adolescents, the role of diabetes duration is, however, more controversial. Some pediatric and adolescent studies have shown a possible assoeiation[21].

In this study the overall rate of MA and NM (both are early markers of DN) accounted for 20°/o (10/50) and 26% (13/50), respectively, being more detected in patients with long-term diabetes than in patients with early-onset diabetes. Similar results were obtained by other studies[21]. There is considerable evidence that multidisciplinary care including psychosocial support and intensive therapy reduces the risk and progression of microvascular complications[4].

Diabetic nephropathy is characterized by specific renal morphological and functional alterations. Features of early diabetic renal changes are glomerular hyperfiltration, glomerular and renal hypertrophy, increased AER, increased basement membrane thickness, and mesangial expansion with the accumulation of extracellular matrix proteins such as collagen, fibronectin, and laminin[20].

In this study, daily AER, OH and IGF-I were determined in newly-diagnosed children and adolescents and in long-term diabetics. Long-term diabetic children and adolescents had significantly higher AER and urinary G1-1 and IGF-I concentrations than that in newly diagnosed cases. These Findings are consistent with some previous studies[7-12].

In this study, out of 50 diabetics, 10 patients and 13 patients were identified to have MA and NM, accounting for a prevalence of 20% and 26% in children and adolescently with TiD, respectively, being significantly higher in long-term diabetics more than that in early-onset diabetics.

The mean BMI, pubertal duration, urinary GH and urinary IGF-i were significantly higher in diabetics positive for MA (MA -+-ve) than that in diabetics negative for MA (MA -ve). A similar result was reported by Cummings et al., in T1 D of children[17], and by Suzuki et al. in a heterogenous group of mostly adults with

both type 1 and 2 diabetes[22]. Meanwhile, Scheijvere et al. reported that prepubertal microalbuminuric patients had higher levels of urinary GH and urinary and plasma IGF-I than normo-albuminuric diabetics and control subjects[10]. On other hand, Shestakova et al. reported non significant relationship between BMI and MA 14.

Although the association between urinary GH and MA that we report is consistent with animal studies that implicate GH in glomerulosclerosis and DN, there is less support for a role for IGF-1 in this process. GH-deficient rats with diabetes are relatively protected from the typical renal effects of diabetes seen in OH-sufficient rats, while transgenic mice expressing excess OH develop glomerular hypertrophy, albuminuria, and glomerulosclerosis, a sequence of events similar to the evolution of DN. Similarly, transgenic mice expressing excess IGF-1 binding protein have elevated GH levels and develop mesangial hypertrophy and glomerulosclerosia, despite a decrease in plasma IGF-1 levels[6].

OH excess in both the human and transgenic animal models is characterized by significant changes in blood pressure and renal function. The GH/GH receptor (GHR) axis is also implicated in the development of DN. Glomerular podocytes express functional GHRs and GH increases levels of reactive oxygen species and induces reorganization of the action cytoskeleton in these cells. These results provide a novel mechanistic link between OH's action and glomerular dysfunction in diseases such as acromegaly and diabetic glomerulosclerosis and DN[3].

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