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Screening of Siblings of Diabetic Children for Prediction of Developing T1DM by Use of Urinary C-Peptide Creatinine Ratio

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Abstract

Background: Diabetes Mellitus is considered an epidemic with increasing incidence over years. UCPCR above 0.2nmol/mmol is suggestive of remaining pancreatic reserve and is also safe and noninvasive. Screening for type 1 diabetes in siblings of children with T1DM is recommended.

Aim of Study: To screen the siblings of children with T1DM for risk of development of diabetes by using Urinary c-peptide creatinine ratio.

Patients and Methods: A prospective case control study was carried out on 43 child who were classified into two groups: 21 children as a control group and 22 siblings of children with T1DM. These diabetic children were diagnosed and followed-up at Endocrinology Unit, Pediatric Department, Tanta University Hospitals. The duration of the study was one year.

Results: Study showed that one child had a diabetic level of fasting blood glucose. It forms 4.5% of siblings of diabetic children. UCPCR for siblings ranged between 0.052-1.393 with a mean 0.23 and SD ±0.29, while it ranged between 0.159-1.69 with a mean 0.49 and SD ±0.35 in control. The UCPCR was statistically significant (p=0.009*). Negative correlation between fasting blood glucose and UCPCR had a cut off 0.17 with 60% sensitivity and 95% specificity.

In Conclusion: C-peptide is a useful indicator of beta cell function, and production of insulin. Urinary C-peptide creatinine ratio may be used as screening test to predict childrendeveloping type 1 diabetes mellitus.

Key Words: *Type 1 diabetes mellitus – Screening – Urinary C-Peptide Creatinine Ratio.*

Introduction

TYPE-1 Diabetes Mellitus (T1DM) is a multisystem disease with both biochemical and anatomic/structural consequences. It is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin, which results from the marked and progressive inability of the pancreas to secrete insulin because of autoimmune destruction of the beta cells [1].

Type-1 DM involves both genetic predisposition and an environmental component although the genetic aspect of Type-1 DM is complex, with multiple genes involved; there is a high sibling relative risk. Extragenetic factors also may contribute. Potential triggers for immunologically mediated destruction of the beta cells include viruses (e.g., enterovirus, mumps, rubella, and coxsackievirus B4), toxic chemicals, and exposure to cow's milk in infancy, and cytotoxins [2].

Type-1 DM can occur at any age. It is most common in children but can also develop in adults, especially in those in their late 30s and early 40s. Unlike people with type 2 DM, those with Type-1 DM usually are not obese and usually present initially with Diabetic Ketoacidosis (DKA) Type-1 DM is associated with a high morbidity and premature mortality. More than 60% of patients with Type-1 DM do not develop serious complications over the long term, but many of the rest experience blindness and End-Stage Renal Disease (ESRD). The morbidity and mortality associated with diabetes are related to the short-and long-term complications. Such complications include hypoglycemia from management errors,increased risk of infections, microvascular complications (e.g., retinopathy and nephropathy), neuropathic complications andmacrovascular disease [3] the insulin precursor, preproinsulin, is produced in the rough endoplasmic reticulum of pancreatic beta-cells and is later cleaved to proinsulin and transported to the Golgi apparatus, where is packed into secretory granules. During maturation of this granules, proinsulin is cleaved into 3 peptide chains-insulin (2 chains, A and B) and C-peptide. C-peptide is a peptide composed of 31 amino acids.

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It is released from the pancreatic beta-cells during cleavage of insulin from proinsulin. It is mainly excreted by the kidney, and its half-life is 3-4 times longer than that of insulin [4].

Screening for Type-1 diabetes in asymptomatic low-risk individuals is not recommended. However, in patients at high risk (e.g., those who have first-degree relatives with Type-1 diabetes), it may be appropriate to perform annual screening for anti-islet antibodies before the age of 10 years, along with one additional screening [5].

Although, historically, C-peptide was considered to have no biologic activity; recent studies suggest that C-peptide may improve capillary blood flow in the feet, decrease urinary albumin excretion, and improve nerve function in individuals with Type-1 diabetes [6].

The urinary C-peptide/creatinine ratio (UCPCR) can assess beta-cell function in clinical practice. Single urine C-peptide creatinine ratio is a stable, reproducible measure that is well correlated with serum C-peptide following meal stimulation, even if there is moderate renal impairment. Urine C-peptide creatinine ratio therefore has potential for use in clinical practice as a measure of insulin secretion, but practicalities of collection limit its routine clinical use [7].

Urine C-peptide creatinine ratio is a non-invasive alternative that is stable for at least 3 days at room temperature in boric acid preservative. The Urinary C-Peptide Creatinine Ratio (UCPCR) result is best measured on a post prandial sample taken approximately two hours after a meal stimulus [8].

Validation of a single-sample urinary C-peptide creatinine ratio is a reproducible alternative to serum C-peptide in patients with Type 2 diabetes [9].

The aim of this study was to screen the siblings of children with T1DM for risk of development of diabetes by using urinary C-peptide creatinine ratio.

Patients and Methods

The present study is a prospective case control study which was carried out on 43 child who were classified into two groups:

- *Group 1 (study group):* This group included 22 siblings of children with T1DM (13 family). Their age ranged from 3-17 years. The diabetic children were diagnosed and followed-up at Endocrinology Unit, Pediatric Department, Tanta University Hospitals. The duration of the study was 12 months from March 2018 till March 2019.
- *Group 2 (control group):* It included 21 children as a control group, who were age and sex matched to the study group with no symptoms of DM and this were confirmed by finding fasting plasma glucose to be non-diabetic.

Inclusion criteria:

- All available siblings (males and females) of the known diabetic children (T1DM) were included in the study.
- Children with ages below 18 years old.

Exclusion criteria:

- Siblings of type 2 diabetes mellitus or monogenic diabetes.
- Siblings above 18 years or under corticosteroid treatment.

The details of the investigations done, technique and complications were explained to the patients and an informed consent was obtained. Approval by the Ethical Committee for Research in Tanta Faculty of Medicine was obtained before initiating this study.

All children in this study were subjected to the following:

1- History taking: Including the personal history, risk factors, family history and history of drug intake as corticosteroid.

2- Clinical examination with special emphasis on Anthropometric measurements: Weight, height, body mass index.

3- Laboratory investigations: Fasting blood glucose, serum creatinine, urinary creatinine and 2hr post prandial urinary c-peptide (urinary c-peptide concentration was measured using DRG C-Peptide ELISA).

Statistical analysis:

Quantitative data were presented as mean ± standard deviation. Independent *t*-test was used to compare the means of the two groups. Qualitative data were presented as count and appropriate proportion. Chi-square test was used to compare the two independent proportions. Pearson's correlation coefficient was used to test association between two variables. Significant results were considered with $p \leq 0.05$. However, a p-value <0.10 and >0.05

should be viewed as suggesting a true difference that may be masked by the relatively small number of candidates. SPSS (Statistical Package for Social Sciences) Version 22.0 was used in data entry and analysis. (IBM Corporation, 2013).

Results

Table (1): Descriptive data of siblings of diabetic children and control.

	Patient's siblings (n=22)	Control (n=21)	*t*-test	*p*-value
Age (years):				
Range	3-17	3-17	0.057	0.812
Mean	9.16	8.81		
S.D	4.80	4.78		
Weight (Kg):				
Range				
Mean	34.63	32.41	0.141	0.709
S.D	20.73	17.81		
Height (Cm):				
Range	94.5-175	95-175	0.044	0.834
Mean	129.89	131.54		
S.D	25.85	25.45		
BMI (%):				
Range	12.53-30.02	12.28-23.18	1.204	0.279
Mean	18.62	17.25		
S.D	4.93	2.99		

Table (1): Descriptive data of siblings of diabetic children and control (continue).

	Patient's siblings (n=22)		Control (n=21)		χ^2	*p*-value
	N	%	N	%		
Gender:						
Male	10	45.5	11	52.4	0.206	0.650
Female	12	54.5	10	47.6		
Consanguinity:						
Negative	16	72.7	16	76.2	0.068	0.795
Positive	6	27.6	5	23.8		
Total	22	100	21	100		

Table (2): Fasting blood glucose in siblings of diabetic children and control.

	Patient's siblings (n=22)	Control (n=21)	*t*-test	*p*-value
Fasting Blood Glucose (mg/dl):				
Range	57-130	70-99	0.008	0.931
Mean ± S.D	84.95±15.24	84.62±8.90		

Table (3): Serum creatinine in siblings of diabetic children and control.

	Patient's siblings (n=22)	Control (n=21)	*t*-test	*p*-value
Serum creatinine (mg/dl):				
Range	0.3-0.8	0.4-1.1	0.015	0.904
Mean ± S.D	0.61±0.14	0.62±0.15		

Table (4): Post prandial urinary creatinine, post prandial urinary c-peptide and urinary c-peptide creatinine ratio in siblings of diabetic children and control.

	Patient's siblings (n=22)	Control (n=21)	*t*-test	*p*-value
Urinary creatinine (mg/dl):				
Range	1.9-8.5	0.8-7.5	1.833	0.183
Mean	4.26	3.18		
S.D	1.74	1.94		
Post prandial urinary C -peptide (ng/dl):				
Range	0.251-0.781	0.428-3.8	14.780	0.001 *
Mean	0.54	1.30		
S.D	0.18	0.87		
Ratio:				
Range	0.052-1.393	0.159-1.69	7.455	0.009*
Mean	0.23	0.49		
S.D	0.29	0.35		

Table (5): Correlation coefficient between each of UCPCR and FBG.

	Ratio	
	r	*p*
Fasting Blood Glucose	–0.453	0.024*
Post prandial urinary C-peptide	0.555	0.007*

Table (6): Sensitivity and specificity of UCPCR in siblings of diabetic children.

	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Ratio	0.17	0.565	60	95	93	69	77

Discussion

Diabetes mellitus is a disease with significant burden on health care systems all over the world. Unfortunately this burden is continuing to increase. According to the international diabetes federation estimates for 2014, Egypt tops the countries of the Middle East region for prevalence of DM **[10]**.

Type 1 DM is one of the two major forms of this disease which, in contrast to type 2DM is

characterized by a childhood onset, presence of autoantibodies, pancreatic β-cells destruction and lack of insulin [11].

C-peptide is a useful and widely used method of assessing pancreatic beta cell function [12]. Urinary C-Peptide (UCP) is a non-invasive test, which can be performed in an outpatient setting [13]. When collected in boric acid UCP is stable at room temperature for up to 3 days. In patients with normal renal function, UCP quantity is reflective of 5-10% of total C-peptide secreted by the pancreas. The 24h urinary C-peptide sample collection (24h UCP) is a more time-consuming method, which is inconvenient for the patient [14].

The present study was conducted to question the validity of urinary C-peptide creatinine ratio as a screening test to siblings of children with T1DM for risk of developing diabetes. Subjects were divided into two groups (a siblings of diabetic children group and a control group).

Fasting blood glucose in siblings of diabetic children ranged from (57-130mg/dl) with a mean ± SD of (84.95± 15.24) mg/dl. This was compared to the normal non diabetic range of the control group (70-99mg/dl) with a mean ± SD of (84.62± 8.9) p=0.931.

One male of siblings of diabetic children group (4.5%) had high level of fasting blood glucose 130mg/dl.

In this study, post prandial urinary C-peptide was markedly lower in the siblings of diabetic children group 0.251-0.781ng/dl with a mean ± SD of (0.54±0.18) which was significantly lower than the control group range 0.428-3.8ng/dl with a mean ± SD of (1.3±0.87). p-value=0.001.

In 2009, McDonald, et al. [15], conducted a study to demonstrate that UCPCR is a stable and reproducible measure that correlates well with 24h UCP in nondiabetic subjects. The volume of urine over the 24-h collection was recorded. Second-void fasting UCPCR was highly correlated with 24-h urinary C-peptide (r=0.8, p=0.00006).

In 2011 Besser, et al. [8], demonstrated that UCPCR testing is a sensitive and specific method for detecting insulin secretion. UCPCR may be a practical alternative to serum C-peptide testing, Urine for UCPCR was collected at 120min and following a home evening meal. UCPCR median (IQR) was nmol/mmol 0.06 (0.01-0.43) Equivalent UCPCR cut-off for serum C-peptide was >0.2nmol/mmol 0.21. Sensitivity/specificity was (%) 86/96.

In 2011 Jones, et al. [16], correlated the gold standard of a stimulated serum C-peptide in a mixed meal tolerance test with fasting and stimulated (mixed meal tolerance test, standard home meal and largest home meal) urine C-peptide creatinine ratio in 51 subjects with insulin treated diabetes. That study showed Home urine C-peptide creatinine ratio after standard breakfast was 0.73. Correlation with 90min post mixed meal tolerance test serum C-peptide and Optimal urine C-peptide was <0.1nmol/l, optimal UCPCR <0.4nmol/mmol.

While the present study showed the mean value of UCPCR for siblings of diabetic children was 0.23±0.29ng/mg which was significantly lower than the mean of the control group 0.49 ±0.35ng/mg (p=0.009*).

In 2013 Oram, et al., [17]. Conducted a study to use UCPCR to assess insulin resistance and insulin production in people without diabetes. In healthy volunteers UCPCR strongly correlated with serum insulin (rs=0.69, p<0.0001), C-peptide (rs=0.73, p<0.0001) and HOMA2-IR (rs=–0.69, p<0.0001). 120min post-OGTT UCPCR correlated strongly with C-peptide and insulin area under the curve. In patients with CKD, UCPCR did not correlate with serum C-peptide, insulin or HOMA2-IR.

Similarly, a negative correlation was found between fasting blood glucose and UCPCR and it was statistically significant (p=0.024*) and r= –0.453.

In this study, we examined use of UCPCR to predict insulin deficiency and developing T1DM with cut off 0.17ng/mg that reflict the decrease in insulin production in 13 of siblings of diabetic children who had lower value while nine cases was above the cut off value. With sensitivity of 60% and specificity of 95% only one of control was with lower value but the remaining 20 child were above the cut off value.

In 2018 Shields, et al. [18]. Study showed that it would take 0.6 years for those diagnosed <=10.8 years to reach the clinically important threshold of absolute insulin deficiency (0.2nmol/mmol (equivalent to 200pmol/L), compared with 2.7 years in the older group diagnosed >10.8y.

In 2008 Karaguzel G, et al. [19] study showed screening of diabetes in a sample of Turkish children with type 1 diabetes and their siblings. In siblings, GAD65 was the most common positive autoantibody (53.9%) among diabetes-related autoantibodies. Percentage of both IAAs-and ICAs-

positive subjects were significantly higher in patients with DM1 than that of siblings ($p<0.001$ for both). Among the 89 siblings, 3.4% had all three diabetes-related autoantibodies, 18.0% had two of them, and 36.0% had one diabetes-related antibody.

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فحص آشقاء الآطفال المرضى بالسكرى لتوقع حدوث السكر من النوع الآول بإستخدام نسبة ال سى ببتايد إلى الكرياتينين فى البول

يعد مرض البول السكرى وباء يصيب آعداداً متزايدة من المرضى مع مرور الآعوام، حيث يصيب ٣٦٦ مليون شخص فى جميع آنحاء العالم، ومن المفترض آن يرتفع هذا الرقم إلى ٥٥٣ مليون شخص بحلول عام ٢٠٣٠.

يقارب تركيز ال سى ببتايد إلى ٥–١٠٪ من إفراز البنكرياس اليومى، بينما يمثل تركيز الآنسولين ٠.١٪ فقط من الآنسولين المفرز فى نفس الفترة. وتبقى نسبة ال سى ببتايد إلى الكرياتينين فى البول مستقرة لمدة ٢٤ ساعة فى درجة حرارة الغرفة ولمدة ٣ آيام إذا تم تبريده حتى +٤ درجة مئوية دون مواد حافظة. تم الإبلاغ عن إنخفاض بنسبة ١٨٪ عند تخزينه لمدة ٩٠ يوماً عند درجة حرارة –٢٠ درجة مئوية. ومع ذلك، إذا تم تخزينه عند –٨٠ درجة مئوية. فلن يشاهد آى إنخفاض فى معدل نسبة ال سى ببتايد إلى الكرياتينين فى البول وترتبط النتائج جيداً بعينات البول على مدار ٢٤ ساعة.

قد يصبح السى ببتايد آداة فحص لتقليل الحاجة إلى إختبارات الجسم المضاد التى يتم إجراؤها فى بعض المرضى لتآكيد آو إستبعاد التشخيص.

نسبة ال سى ببتايد إلى الكرياتينين فى البول فوق ٠.٢نانومول/مليمول يوحى بوجود إحتياطى البنكرياس المتبقى وهو آيضاً آمن.

الهدف من هذه الدراسة: هو فحص آشقاء الآطفال الذين يعانون من مرض السكرى من النوع الآول بإستخدام نسبة ال سى ببتايد إلى الكرياتينين فى البول.

النتائج: كشفت هذة الدراسة آن هناك فرق كبير فى نسبة ال سى ببتايد إلى الكرياتينين فى البول بين مجموعة الدراسة ومجموعة التحكم. كانت القيم آقل بكثير فى آشقاء مرضى السكرى من تلك التى تقاس فى ومجموعة التحكم. وآظهرت كل من العينات علاقة سلبية مع مستوى السكر الصيامى فى الدم.

الخلاصة: يمكن إستخدام نسبة السى ببتايد إلى الكرياتينين فى البول كإختبار فحص للتنبؤ بحدوث داء السكرى من النوع الآول، بالإضافة إلى الفحوصات الآخرى لتآكيد التشخيص.