Urinary Cyclophilin: A New Marker for Diabetic Nephropathy

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Abstract

Background: Nephropathy is a significant cause of morbidity and mortality in patients with Diabetes Mellitus (DM). The condition is characterized by persistent albuminuria and may be decline in the Glomerular Filtration Rate (GFR). Urinary cyclophillin A has been proposed as a simple, accurate and rapid endogenous marker for DN.

Aim of Study: To assess the value of urinary cyclophillin A anew marker of diabetic nephropathy.

Subjects and Methods: Group I: 60 patients with type 2 diabetes mellitus which will be subdivided into: (A) 30 diabetic patients with diabetic nephropathy. (B) 30 diabetic patients without diabetic nephropathy. Group II: 30 healthy persons as a control group.

Results: There was significance association between urinary cyclophillin A and serum creatinine, blood urea, 24 h protein collection and ACR.

Urinary cyclophilin A found higher in diabetic nephropathy patients than diabetic patients without nephropathy so it is a new marker for diabetic nephropathy.

Conclusion: The results of this study suggest that urinary cyclophillin A new marker for diabetic nephropathy.

Key Words: Type 2 diabetes mellitus – Diabetic nephropathy – Urinary cyclophillin A.

Introduction

DIABETES is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

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Type 2 Diabetes Mellitus (DM) is the most common cause of end-stage renal disease [2].

The Joint committee of diabetic nephropathy is trying to develop a new classification of Diabetic Nephropathy (DN) combining both glomerular filtration rate and albuminuria systems [3]. There are several markers to predict onset and progression of DN. Albuminuria is the most commonly used marker. However, it lacks both sensitivity and specificity to detect early stage of DN [4].

Urinary Cyclophilin A (CypA) is an 18-kDa protein with ubiquitous characteristics. It is mostly distributed in the cytoplasm and facilitates protein folding and protein trafficking. It also acts as a cellular receptor for cyclosporine A (CsA) [5].

Secreted Cyp A (sCyp A) was reported to be correlated with Cardiovascular Disease (CVD), asthma, Rheumatoid Arthritis (RA), liver injury and inflammatory diseases [6]. It was also detected in diabetic patients' plasma and was shown to be secreted by monocytes in response to hyperglycemia [7].

Subjects and Methods

This study will be done from February 2016 to September 2016 at Tanta Hospitals ". An informed consent will be taken from all participants and the privacy of the data will be greatly considered.

The study will be carried out on two groups:

Group I: 60 patients with type 2 diabetes mellitus which will be subdivided into:

A- 30 diabetic patients with diabetic nephropathy.

B- 30 diabetic patients without diabetic nephropathy.

Group II: 30 healthy persons as a control group.

Study design:

It is cross sectional observational study.

Exclusion criteria:

- Patients suffer from infectious disease.

- Patients suffer from chronic inflammatory disease.

- Patients suffer from liver disease or malignancy.

- Pregnant women.

All patients included in the study will be subjected to:

- Through history taking.

- Complete clinical examination including: Body Mass Index (BMI) calculation.

- Laboratory investigations including:

    • Blood urea and serum creatinine.

    • Fasting and 2 hour post prandial blood glucose.

    • HB A1C.

    • Urine Albumin/Creatinine ratio.

    • Lipid profile (Triglycerides, Cholesterol, LDL and HDL).

    • Estimated glomerular filtration rate.

    • Erythrocyte Sedimentation Rate (ESR).

    • 24 hours urine collection for proteins.

    • Specific investigation: Estimation of urinary cyclophilin A by ELIZA.

- Imaging including: Ultra sonography abdomen, pelvis.

Statistical analysis:

For quantitative data, the Shapiro-Wilk test for normality was performed. For normally distributed data, values were expressed as mean $\pm$ standard deviation and independent samples *t*-test was performed for comparison between two groups while one way ANOVA was used for comparison between more than two groups. For data that were not normally distributed, median and interquartile range (expressed as 25th-75th percentiles) were calculated and Kruskal Wallis test and Spearman's rank-order correlation were used. For qualitative data, Pearson's Chi square test was used to examine association between two variables. Significance was adopted at $p < 0.05$ for interpretation of test results.

Results

Demographic data of all studied groups:

After Ethical Committee approval from Research Center in Tanta University and written consent from all subjects, this study was conducted in the Internal Medicine Department. The patients were recruited for the study on the basis of standard clinical and laboratory criteria for diagnosis of diabetes and kidney disease. They were selected from in-patients and out-patients clinics of Internal Medicine Department, Tanta University Hospital. This study was conducted on 90 subjects classified as follows:

Group IA: 30 diabetic patients with diabetic nephropathy. Their age ranged from 42 to 64 years with a mean value 58.3.

18 patients were males and 12 were females with a male to female ratio of 1.5:1.

Group IB: 30 diabetic patients without diabetic nephropathy. Their age ranged from 44 to 69 years with a mean value 56.7.

16 patients were males and 14 were females with a male to female ratio of 1.14:1.

Group II: 30 apparently healthy volunteers (as a control group), their age ranged from 43 to 68 years with a mean value 55.5. 15 of them were males and 15 were females with a male to female ratio of 1:1.

Table (1): Age, sex and BMI in the studied groups.

	Group			Tests of significance	
	Group IA	Group IB	Group II	Test statistic	*p*
Age:					
Minimum-maximum	42-64	44-69	43-68	F=	0.291
Mean	58.3	56.7	55.5	1.253	
Standard Deviation	6.0	7.7	6.7		
Sex:					
• Male:					
N	18	16	15	χ^2 ChS=	0.731
%	60.0	53.3	50.0	0.627	
• Female:					
N	12	14	15		
%	40.0	46.7	50.0		
BMI:					
Minimum-maximum	21-35	19-35	18-28	FWelch=	<0.001*
Mean	29	28	23	31.334	
Standard Deviation	3	5	3		

Post-hoc comparisons (Tukey's test).

Table (2): Urinary Cyclophilin A level in the studied groups.

Urinary Cyclophilin A	Group			Tests of significance	
	Group IA	Group IB	Group II	Test statistic	p
Minimum-maximum	9-19	3-6	0-2	ZKW= 79.140	<0.001*
Median	10	4	2		
IQR	10-12	3-5	1-2		
Mean ranks	75.50	45.50	15.50		

Pair-wise comparison (between group comparison).
Dunn-Bonferroni test
Group IA - Group IB.
p<0.001 * Group IA-Group II.
p<0.001 * Group IB-Group II.
p<0.001 *.

Table (3): Correlations between Urinary Cyclophilin A and blood urea, serum creatinine, albumin/creatinine ratio, estimated GFR and 24h protein in the studied groups.

	Urinary Cyclophilin A			
	All participants	Group IA	Group IB	Group II
Blood urea:				
r_s	0.746	0.878	0.080	−0.141
p	<0.001*	<0.001*	0.674	0.458
Serum creatinine:				
r_s	0.619	0.837	0.400	−0.088
p	<0.001*	<0.001*	0.028*	0.644
Albumin/creatinine ratio:				
r_s	0.627	0.921	0.681	0.123
p	<0.001*	<0.001*	<0.001*	0.518
Estimated glomerular filtration rate:				
r_s	−0.705-	−0.716-	−0.565	0.342
p	<0.001*	<0.001 *	0.001*	0.064
p	<0.001*	0.939	0.064	0.138
24h protein:				
r_s	0.743	0.949	0.987	0.782
p	<0.001*	<0.001*	<0.001*	<0.001*

Table (4): Correlations between Urinary Cyclophilin A and fasting blood glucose, 2h postprandial blood glucose, HbA1c and ESR in the studied groups.

	Urinary Cyclophilin A			
	All participants	Group IA	Group IB	Group II
Fasting blood glucose:				
r_s	0.697	0.498	0.331	−0.072
p	<0.001*	0.005*	0.074	0.704
2h Postprandial blood glucose:				
r_s	0.732	0.519	0.048	0.364
p	<0.001*	0.003*	0.802	0.048*
HbA1c:				
r_s	0.778	0.040	0.122	
p	<0.001*	0.834	0.521	
ESR:				
r_s	0.547	0.015	0.343	0.277
p	<0.001*	0.939	0.064	0.138

Discussion

Diabetes mellitus is a group of physiological dysfunctions characterized by hyperglycemia resulting directly from insulin resistance, inadequate insulin secretion, or excessive glucagon secretion. Type 2 DM (non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [8].

Type 2 DM results from interaction between genetic, environmental and behavioral risk factors.

Patients with diabetes mellitus are more vulnerable to various forms of both short-and long-term complications, which often lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type 2 DM [9].

Diabetic nephropathy represents the major cause of End-Stage Renal Disease (ESRD). One of the hallmarks of diabetic nephropathy is the development of proteinuria, which is usually followed by a progressive decline in renal function. The development of diabetic nephropathy is also a major risk factor for cardiovascular disease [10].

From an epidemiological, pathophysiological and clinical perspective, hypertension and poor glycaemic control are usually associated with diabetic nephropathy [11,12].

The aim of the present study to assess the value of estimation Urinary Cyclophilin A as a new marker for diabetic nephropathy.

This study was conducted in the Internal Medicine Department. The patients were recruited for the study on the basis of standard clinical and laboratory criteria for diagnosis of diabetes and diabetic nephrorathy. They were selected from inpatients and out-patients clinics of Internal Medicine Departments, Tanta University Hospital.

An informed consent will be taken from all participants and the privacy of the data will be greatly considered.

In this work it was found that there was no significance difference among all studied groups as regard age and sex, however it was observed that the percentage of male patients with diabetic nephropathy was increased as compared to that of female patients in the same group.

This was in agreement with Rossouw et al., (2002) [13] who reported that men are more likely

to develop coronary artery disease, stroke, micro-vasculopathy and other cardiovascular manifestation of atherosclerosis. This is due to the protective effect of female hormones (estrogens) so that heart disease risk for women rises dramatically after menopause.

Also Michelle, [14] reported that the incidence of coronary artery disease and kidney disease increases with age, men older than 45 and women older than 55 (or younger if they have premature menopause) are at greater risk of heart disease.

In this study, we propose that urinary CypA can be used as an early marker for identifying DN with a high sensitivity and high diagnostic power. Detection of urinary CypA is also very convenient because it is noninvasive. Now that urinary CypA appears to be capable of identifying DN in the silent stage.

In an extensive review conducted by Lee et al., urinary CypA was not mentioned as a potential biomarker for DN [15].

This is the first study to use urinary CypA in early DN detection. CypA was mostly studied in CVD and lung or liver injury [5].

Asthma and RA are associated with this new marker [16].

The present investigation is the 3 rd study to identify a correlation between CypA and DM. Furthermore, this is the 1 st study to verify the association between urinary CypA and DN.

Conclusions:

• Urinary cyclophilin A was significantly increased in diabetic patients with diabetic nephropathy than diabetic patients without nephropathy and normal (control groups).

• Urinary cyclophillin A is useful practical non invasive tool for early detection of diabetic nephropathy.

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السيليكوفيلين آ البولى كدلالة جديدة لإعتلال الكلى السكرى

المرض السكرى هو مجموعة من الأمراض الأيضية التى تتميز بإرتفاع السكر فى الدم الناتج عن خلل فى إفراز الأنسولين أو عمل الأنسولين أو كليهما ويترافق إرتفاع السكر المز من فى الدم بالضرر على المدى الطويل والعجز وفشل الأجهزة المختلفة وخاصة العينين والكليتين والأعصاب والقلب والأوعية الدموية. ويعد المرض السكرى من النوع الثانى من أكثر الأسباب شيوعا فى حدوث المرض الكلوى (الداء الكلوى بمراحله الأخيرة) وهناك دلالات عديدة للتنبؤ بظهور إعتلال الكلى لمريض السكر وأكثرها الزلال البولى ومع ذلك قد وجد أنه يفتقر الحساسية والخصوصية للكشف عن مراحله مبكرة للمرض ولذا إستمر البحث قائما لإكتشاف دلالات جديدة للكشف المبكر لإعتلال الكلى السكرى. السيليكوفيلين آ وهو بروتين ١٨ كيلو دالتون وله خصائص متميزة فى التركيب ويوزع فى الغالب فى السيتوبلازم. كما أنه يعمل على مستقبلات الخلايا للسيكلوسبورين. وقد وجد أن السيلكوفيلين آ المفرز له علاقة قوية بأمراض القلب والأوعية الدموية والربو وإلتهاب المفاصل الروماتيزمى وأمراض الكبد والأمراض الإلتهابية المزمنه كما أنه يفرز نتيجة لإرتفاع السكر بالدم.

الهدف من البحث: قيمة دراسة تقدير السيلكوفيلين آ البولى كدلالة جديدة للإعتلال الكلى السكرى.

المرضى وطرق البحث:

تم إجراء هذا البحث على مجموعتين من المرضى:

• المجموعة الأولى: ستون مريض بالمرض السكرى من النوع الثانى وسوف تقسم إلى مجموعتين:

- الأولى: ثلاثون مريض بالمرض السكرى مع إعتلال الكلى السكرى.

- الثانية: ثلاثون مريض بالمرض السكرى بدون إعتلال الكلى السكرى.

• المجموعة الثانية: ثلاثون شخص أصحاء لا يعانون من المرض كمجموعة ضابطة.

وتمت تلك الدراسة من فبراير ٢٠١٦ إلى سبتمبر ٢٠١٦. وتم إتخاذ الموافقة المسبقة من جميع المشاركين. وإستبعد من البحث: المرضى الذين يعانون من الأمراض المعدية، الأمراض الإلتهابية المزمنة، وأمراض الكبد أو الأورام الخبيثة والسيدات أثناء فترة الحمل.

النتائج: كان هناك إرتباط كبير بين مرض البول السكرى والمضاعفات الرئيسية لدى مرضى السكرى: الإعتلال الكلوى، إعتلال الشبكية، الإعتلال العصبى والأمراض القلبية الوعائية.

هناك علاقة بين مدة الإصابة بالسكرى وحدوث مضاعفات. كلما زادت المدة زادت المضاعفات. اليوريا والكرياتينين نسبتهم فى دم مرضى الإعتلال الكلوى السكرى أعلى من غيرهم. السيكلوفيلين آ البولى هام جدا فى التنبؤ بالإعتلال الكلوى السكرى وله علاقة طردية وقوية مع مستوى البولينا والكرياتينين فى الدم وتجميع البروتين فى البول لمدة ٢٤ ساعة.