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Research Article

CHEMISTRY

Role of Heparanase Urinary Level in Type 2 Diabetic Patients with and without Nephropathy

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KEY WORDS

Heparanase;

Heparan sulphate;

Diabetic nephropathy;

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ABSTRACT

End-stage renal disease is caused primarily by diabetic nephropathy (DN), which is the most serious and widespread diabetic kidney complication. Heparanase plays a role in the development and progression of DN.Heparan sulphate (HS) is particularly degraded by heparanase. The precise mechanism by which heparanase sustains the pathology of DN remains unknown. This study seeks to elucidate the role of heparanase urinary level in type 2 diabetic patients with and without nephropathy, as well as to assess its relationship with various clinical and biochemical parameters. This study involved (15 diabetics with normoalbuminuria, 15 with microalbuminuria, 15 with macroalbuminuria and 10 healthy volunteers as a control group).Urinary heparanase was significantly greater in patients with DN than in control subjects. The ratio of urinary albumin to creatinine was inversely correlated with the glomerular filtration rate, while it was positively correlated with the estimated glomerular filtration rate (e GFR). In type 2 diabetic patients, the urinary heparanase level can serve as a diagnostic biomarker for DN. The analysis was done using the Statistical Package for the Social Sciences (SPSS software version 12). Using the one-way ANOVA test, differences between groups were examined.

Introduction

Hyperglycemia, brought on by insufficient insulin secretion, ineffective insulin action, or both, characterises the metabolic disorders known collectively as diabetes. Chronic diabetes, which is associated with hyperglycemia, damages, impairs, and eventually destroys several organs, including the heart, eyes, kidneys, and nerves **(American Diabetes Association, 2017).** The conventional definition of DN is a gradual increase in urine albumin excretion (UAE) accompanied by a rise in blood pressure that ultimately leads to ESRD **(Sagoo and Gnudi, 2020).**

Heparanase is an endo—D-glucuronidase that degrades HS specifically. HS is a component of the extracellular matrix (ECM).Specifically, it acts as an enzyme by catalysing the cleavage of the β(1,4)-glycosidic bond between glucuronic acid and glucosamine residue. HS cleavage triggers ECM remodelling and controls the release of many HS-linked molecules, including growth factors, cytokines, and enzymes involved in inflammation, wound healing, and tumor invasion **(Kim, *et al*., 2011)**.

Diabetic nephropathy's pathophysiology is still up for dispute. Heparanase is one of many mechanisms that have a role in the development of DN. It is essential for the occurrence of both cancer and inflammation, and it plays a part in several proteinuric renal disorders, including DN. Furthermore, due to its large contribution to the biological pathway of renal fibrogenesis, HPSE may have a significant impact on the development of chronic kidney injury. This study was conducted to elucidate the role of heparanase urinary level and gene expression in type 2 diabetic patients with and without nephropathy and to evaluate its relation with various clinical and biochemical parameters.

Subjects and Methods

Human subjects:

The research study included 55 participants, including 45 patients with type 2 diabetes and 10 controls.15 diabetic patients were classified as having normoalbuminuria, 15 as having microalbuminuria, and 15 as having macroalbuminuria. They ranged in age from 50 to 59 years. The "Internal Medicine Department" at Tanta University Hospital served as a source for the patients. Patients and controls were informed of their consent after the Tanta University Local Ethical Committee approved the study. Approval code: 31945/11/17. This study excluded diabetic patients with uncontrolled hypertension, ESRD,

dialysis patients, hepatic disease, heart disease, and infection of urinary tract .A thorough history was taken, with special attention paid to the urinary symptoms, diabetic duration, history of any other associated diseases, symptoms of microvascular complications of diabetes, elevated blood pressure, smoking, elevated blood cholesterol, and therapeutic history.

Methods

Each subject's second morning urine samples were taken for complete urine analysis, estimation of the urinary albumin to creatinine ratio, and measurement of urinary heparanase level. Blood samples were collected for kidney function test (creatinine, urea and eGFR), fasting and postprandial blood glucose levels and glycosylated hemoglobin percentage. Glucose level in serum was assayed by using commercial kit that was supplied by Spinreact, from Egypt. Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H_2O_2), is detected by a chromogenic oxygen acceptor, phenol, 4–aminophenazone (4-AP) in the presence of peroxidase (POD) **(Trinder, 1969)**. Glycosylated hemoglobin in whole blood was assayed by using the NycoCard READER® supplied by (Axis-Shield, Oslo, Norway) (**Karami and Baradaran, 2014)**. Creatinine level in serum was assayed by using commercial kit that was supplied by BioSystems, from Egypt. Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex **(Rartels and Böhmer, 1971)**. Urea level in serum was assayed by using commercial kit that was supplied by BioSystems, from Egypt. Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry **(Searcy *et al*., 1967)**. Heparanase level was asseyed by using enzyme - linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology. Heparanase (HPA) was added to the wells, which are pre-coated with Heparanase (HPA) monoclonal antibody and then incubated. After that, anti HPA antibodies labeled with biotin was added to unite with streptavidin - HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Substrate A and B was added. Then the solution was turned blue and was changed into yellow with the effect of acid. The shades of solution and the concentration of Human Heparanase (HPA) are positively correlated **(Shafat *et al*., 2006).**

Analysis of statistics

The data were statistically analysed using Graph Pad Prism version 6.00 for

(Graph Pad Software Inc., San Diego, California USA). The descriptive data was presented as mean± SD. Using the one-way ANOVA test, differences between groups were examined. The association between the studied groups and the sex distribution was examined using the chi-squared test. A probability of less than 0.05 was considered statistically significant. The sensitivity of laboratory parameters in the diagnosis of DN was evaluated by computing the areas under the Receiver Operating Characteristic (ROC) curve. Multivariate regression analysis was applied to predict the dependency of a dependent variable to other expressive variables.

Results

Table (1) showed clinical and biochemical parameters in studied groups (distribution of age, BMI, diabetic duration, sex, kidney function tests, fasting and postprandial blood glucose levels, glycosylated hemoglobin percentage, ACR and urinary heparanase level). There is a statistically significant difference in body mass index and duration of diabetes between the groups, but not in age or sex. Urea, creatinine, and albumin/creatinine ratio were significantly increased in DN group as compared to control. While eGFR was significantly decreased in DN groupas compared to control. Table (2) showed that logistic regression coefficient of heparanase (β= 0.771, P= 0.001), ACR (β= 0.180, P = 0.003), eGFR (β= 0.201, P= 0.027) and diabetic duration (β= 0.119, P= 0.041).

Fig. (1) showed that diabetic groups with nephropathy had significantly higher urinary heparanase levels than control groups. Fig. (2, 3) showed that heparanase was positively correlated with ACR and negatively correlated with eGFR in diabetic patients. Fig. (4) showed that the area under the ROC curve of ACR is (0.869), the cut off of ACR was (0.99) which denoted sensitivity 87% and specificity 83%. As regard to eGFR, the area under the ROC curve of eGFR is (0.733), the best cut off of eGFR was (0.79) which denoted sensitivity 81% and specificity 78%. As regard to heparanase level, the area under the ROC curve is (0.978), the best cut off was (0.86) which denoted sensitivity 96% and specificity 93%.

Table (1): Clinical &biochemical parameters in the studied groups

Parameters	Control group(n=10) (I)	Type 2 diabetic patients (n=45)			P value
		DM with normo (n=15)(II)	DM with micro (n=15)(III)	DM with macro (n=15)(IV)	
Age (years) Range Mean± SD	50-59 53.7±2.9	51-58 55.4±2.9	49-60 53.2±3.4	45-62 53.4±4.6	0.353
Sex M/F (%)	4/6(40/60)	8/7(53/47)	8/7(53/47)	7/8(47/53)	0.9
BMI(kg/m^2) Range Mean± SD	20-26 22.5±2.2	25-30.2 27.8±1.8	27-31 28.8±1.4	28.3-32.4 29.7±1.3	0.001*
DM duration (years) Range Mean± SD		5-10 7.4±1.7	7-13 9.8±2.1	5-15 10.2±2.9	0.003*
FBG (mg/dl) Range Mean± SD	75-90 82.6 ± 4.9	157-180 168.07 ± 7.8	200-220 209.3 ± 5.4	220-255 237.8 ± 10.6	0.001*
PBG (mg/dl) Range Mean± SD	100-130 116.7± 10.3	224 -260 241.4±10.7	300-330 313.6 ± 7.6	310-350 330.6± 13.06	0.001*
HbA1c (%) Range Mean± SD	4.0-4.7 4.37 ± 0.20	6.0-10 8.0 ± 1.4	7.2 - 11.5 9.3 ± 1.6	8.0-11.9 10.3 ± 1.36	0.001*
Creatinine (mg/dl) Range Mean± SD	0.6-1.0 0.82±0.17	0.8-1.5 1.19±0.2	13-2.5 2.0±0.35	1.8-3.5 2.5±0.6	0.001*
Urea (mg/dl) Range Mean± SD	25-34 30.0±2.7	32-50 39.3±4.6	40-80 63.4±12.9	60-100 83.8±12.6	0.001*
eGFR (ml/min per 1.73 m^2) Range Mean± SD	74-136 104.7±24.8	55-94 72.08±11.7	34.58 43.8±6.9	23-37 29.7±3.6	0.001*
ACR (mg/gCr) Range Mean± SD	6-11 8.6±1.5	12-28 18.7±4.6	40-300 178.6±77.1	423-760 560.5±93.2	0.001*
HP level (ng/ml) Range Mean± SD	0.4-0.5 0.4±0.03	0.5-1.0 0.8±0.1	1.09-1.3 1.23±0.07	0.4-4.0 3.0±0.69	0.001*

Significant (*P*<0.05); eGFR, estimated glomerular filtration rate; ACR, albumin creatinine ratio

Table (2): Multivariate regression analysis of heparanase (HP) as a predictors of nephropathy among type 2 diabetic patients

Parameters	β	SE	EXP(B)	95%confidance interval for (B)		*P* value
				Lower limit	Upper limit	
Heparanase level (ng/ml)	**0.771**	**0.001**	**0.008**	**0.006**	**0.010**	**0.001***
ACR (mg/gCr)	**0.180**	**0.053**	**0.168**	**0.060**	**0.275**	**0.003***
eGFR (ml/min per 1.73m^2)	**0.201**	**0.214**	**0.489**	**0.004**	**0.046**	**0.027***
diabetic duration (year)	**0.119**	**0.012**	**0.021**	**0.010**	**0.275**	**0.041***

β = Logistic Regression Coefficient; SE= standard error of B; P=Significance; Significant (p<0.05)
EXP(B)=Estimated Odds Ratio

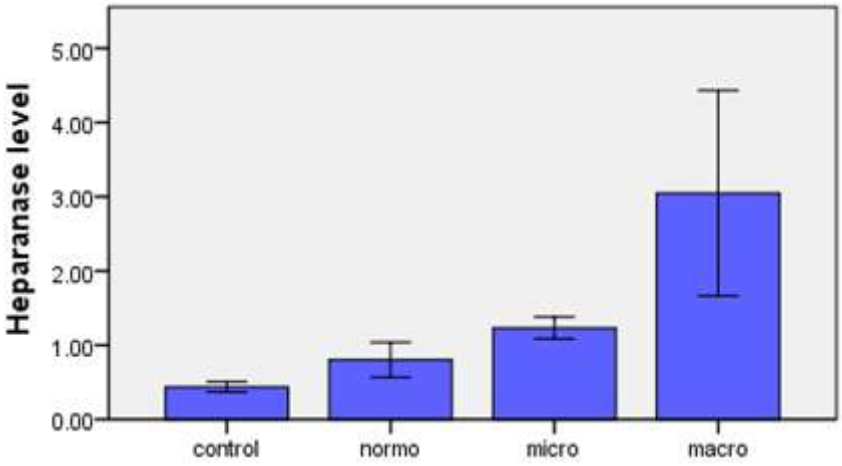

Fig. (1): Heparanase level in the studied groups

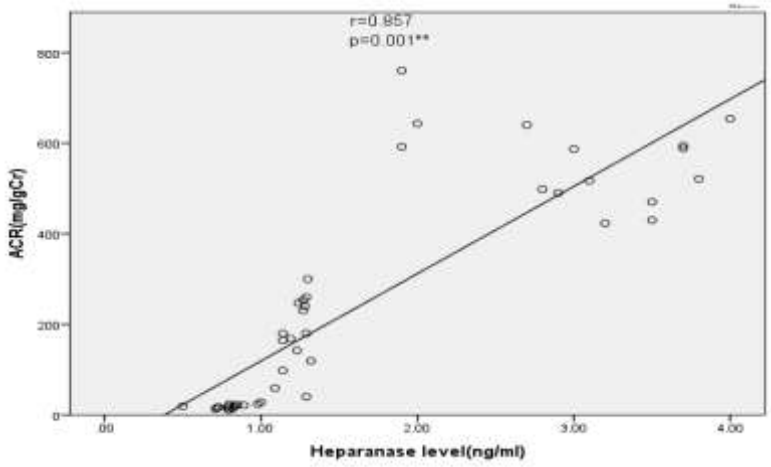

Fig.(2): Heparanase level and ACR

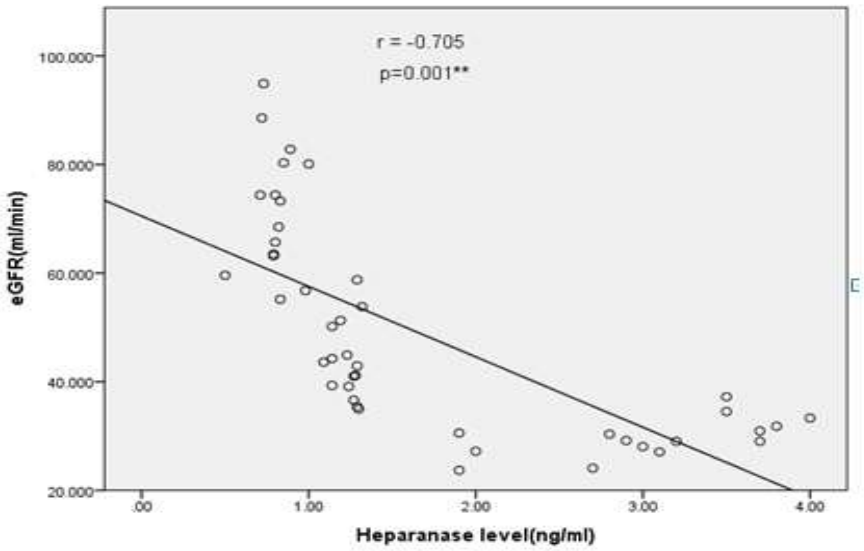

Fig. (3): Heparanase level and eGFR

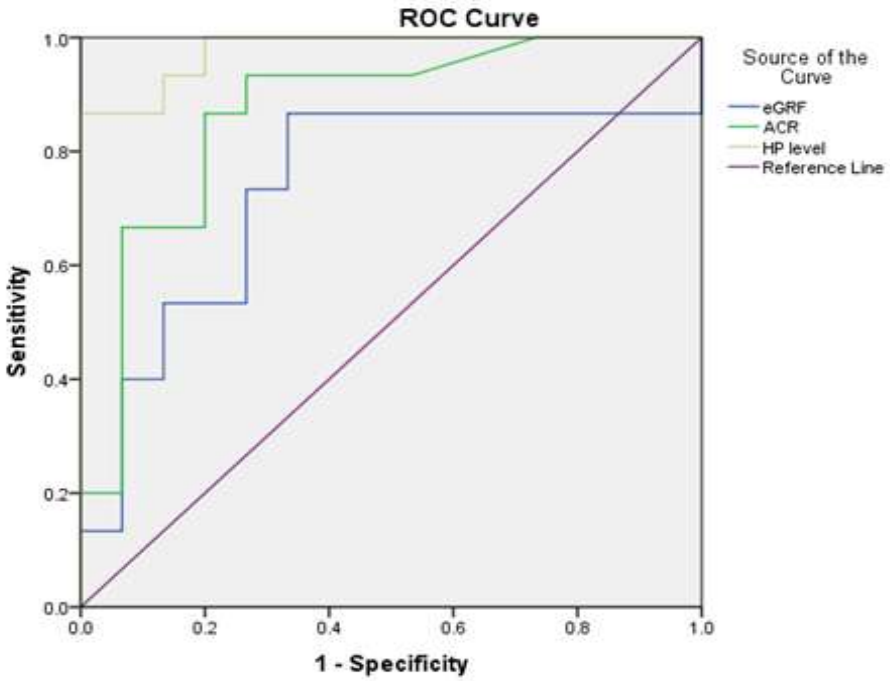

Fig. (4): Roc curve

Discussion

Increased rates of mortality and morbidity have been associated with DN, one of the most common and serious complications of diabetes mellitus **(Valencia *et al.*, 2017).**ESRD is the most common result of chronic kidney failure, with normo-albuminuria, micro-albuminuria and macro-albuminuria as its initial symptoms. Pathophysiologically, it is distinguished by an early stage marked by glomerular hypertrophy, hyperfiltration, and microalbuminuria that develops over time into an advanced stage marked by advancing glomerulosclerosis, proteinuria, and deterioration of renal function **(Parchwani1 *et al.*, 2012).**

A key element of ECM, HS is a negatively charged glycosaminoglycan that is linked to a core protein to form a heparin sulphate proteoglycan. **(Esko *et al.*, 2002).** HS in the glomerular basement membrane (GBM) is thought to be crucial in maintaining the glomerular capillary wall's charge-selective permeability by electrostatically repelling negatively charged serum proteins, particularly albumin. Heparanase's removal of GBM heparin sulphate increased the GBM's permeability to albumin **(Kanwar *et al.*, 1980)**.

The researchers wanted to determine if there was a difference in the role played by urinary heparanase levels between type 2 diabetics with and without nephropathy, as well as how those levels correlated with other clinical and biochemical parameters.

There was a significant difference between diabetics with micro- and macroalbuminuria in the current study's DN group compared to control and normoalbuminuria for ACR.

This finding was supported by **(Gluhovschi *et al.*, 2016)** who found micro albuminuria to be an effective biomarker in the diagnosis and follow-up of DN.

In the present study, heparanase levels were found to be significantly higher in diabetic groups with nephropathy compared to control. The levels of heparanase in diabetic patients were positively correlated with ACR and inversely correlated with eGFR.

The results of **(Rops *et al.*, 2011)**, who first linked renal dysfunction in diabetic patients with micro albuminuria to heparanase, agree with these results. There is a positive correlation between the ACR and the heparanase level in the urine, and a negative correlation between the heparanase level in the urine and eGFR.

Conversely**, Ezz *et al*., (2014)** reported that although urinary heparanase levels in diabetic patients were significantly higher than in controls, the biomarkers for kidney function had little to no change overall, with the exception of a significant drop in urinary creatinine.

This finding was supported by **(Shafat *et al*., 2012)** who reported that CKD patients' urinary heparanase levels were noticeably elevated and correlated with their levels of proteinuria **(Ezz *et al*., 2014)** who claimed that the parallel decreases in urinary eGFR in diabetic patients with microalbuminuria and increased urinary heparanase are significant.

In the current study, heparanase level was positively correlated with blood glucose in diabetic groups with nephropathy.

This finding was supported by **(Shafat *et al*., 2012)** who found that elevated levels of heparanase in urine and plasma were linked to elevated glucose levels in diabetic patients. Endothelial cells from diabetic patients may express heparanase when exposed to high glucose levels.

Conversely, **Masola *et al*., (2011)** found that high glucose levels, which have no effect on HS biosynthesis and turnover or HS proteoglycan expression in proximal tubular cells, may be responsible for the absence of a correlation between plasma glucose and serum heparanase in diabetic patients.

The following describes how heparanase and GFR are related: In the GBM and at cell surfaces, heparanase could first cleave HS, thereby altering the glomerular capillary wall's filtration characteristics. Second, the release of HS-bound growth factors like transforming growth factor by heparanase may cause mesangial matrix and GBM thickening. Thirdly, disruption of cell-matrix interactions may result from HS degradation by heparanase. Finally, diabetic nephropathy and cellular activation may result from the binding of heparanase to glomerular cells **(Rops *et al*., 2011).**

Heparanase levels in patients with DN had a sensitivity and specificity of 96% and 93%, respectively, in the current study, while the sensitivity and specificity of the heparanase gene had values of 91% and 87%, respectively. Thus, Heparanase level was the most sensitive among the studied markers for diagnosing diabetic nephropathy.

There has been a lot of interest in creating strategies that target heparanase action in treatment of DN, based on experimental evidence and clinical studies that heparanase plays a

significant role in the pathogenesis of DN. Even though albuminuria and heparanase activity in the urine were linked, in some patients with albuminuria, no heparanase activity could be found. Given that the use of angiotensin-aldosteronesystem) inhibitor-treated diabetics had lower urinary heparanase activity than those receiving other non-RAAS anti-hypertensive medications.

Vitamin D can control the expression of heparanase in glomerular endothelial cells directly. Vitamin D therapy led to increased heparanase and glomerular HS reduction. Heparanase activity is inhibited by vitamin D treatment because the vitamin D receptor (VDR) binds to the heparanase promoter.

ACEIs (angiotensin-converting enzyme inhibitor) or AT1 (angiotensin II Type 1) receptor blockers decreased the expression of glomerular heparanase in nephropathy. Urinary heparanase activity could be impacted by taking RAAS inhibitors.

Conclusion

Urinary heparanase level can be considered as a diagnostic biomarker for DN in type 2 diabetic patients.

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دور مستوى هيبارينيز فى البول فى مرضى السكرى من النوع الثانى المصحوب وغير المصحوب الإعتلال الكلوى

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يعتبر مرض السكرى من الأمراض الأيضية الناتجة عن عيوب في إفراز الأنسولين أو عمل الأنسولين أو كليهما. يرتبط مرض السكري المزمن بمضاعفات طويلة الأمد مثل اعتلال الكلية والأوعية الدمويه ومضاعفات القلب. ويعتبر الهيبارينيز له دور رئيسى فى ظهور امراض بروتينيه كلويه مثل اعتلال الكليه السكرى.

تهدف هذه الدراسة إلي توضيح دور مستوى هيبارينيز فى البول فى مرضى السكرى من النوع الثانى المصحوب وغير المصحوب بالاعتلال الكلوى. وقد إشتملت هذه الدراسةعلي أربع مجموعات.

المجموعه الأولي: تتكون من ١٠ اشخاص من الأصحاء للمقارنه ، المجموعه الثانية: تتكون من ١٥ مريضا بداء السكري من النوع الثاني وكمية الزلال في البول طبيعية، المجموعه الثالثة: تتكون من ١٥ مريضا بداء السكري من النوع الثاني وكمية الزلال في البول بسيطة والمجموعه الرابعة: تتكون من ١٥مريضا بداء السكري من النوع الثاني وكمية الزلال في البول كبيرة.وقد خضع جميع الأفراد المشاركون في الدراسة للفحوصات من تحليل بول كامل وقياس نسبة السكرفي الدم صائما وبعد الأكل بساعتين،قياس نسبة الهيموجلوبين السكري في الدم ،قياس وظائف الكلي (نسبة البولينا والكرياتينين في الدم ومعدل الترشيح الكبيبي المقاس) وقياس نسبة الألبيومين إلي الكرياتينين في البول وقياس مستوى الهيبارينيز فى البول.

وقد أظهرت هذه الدراسة إرتفاع مستوى هيبارينيز فى مرضى السكري بالمقارنة بالمجموعة الضابطة وتوجد علاقة تناسب عكسيه ذات دلالة إحصائية بين نشاط هيبارينيز البولى ومعدل الترشيح الكبيبي المقاس في مرضي السكري المصحوب بالإعتلال الكلوي وقد وجد ايضا علاقة تناسب طرديه ذات دلالة إحصائية بين مستوى الهيبارينيز البولى و نسبة الألبيومين إلي الكرياتينين في البول في مرضي السكري المصحوب بالإعتلال الكلوي.تم استنتاج ان تحديد مستوى الهيبارينيز قد يكون له أثر مستقبلى فى التشخيص المبكر وتحديث أساليب علاج الإعتلال الكلوي السكري.