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Research Article

EVALUATION OF GENE EXPRESSIONS OF KIDNEY INJURY MOLECULE -1 (KIM-1) & CONVENTIONAL RENAL PARAMETERS IN TYPE II DIABETES MELLITUS

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A B S T R A C T

Introduction: Approximately 40% Type 2 diabetes and 30 % Type 1 diabetes suffer from kidney failure. Uncontrolled diabetes were more prone to end stage renal disease which required kidney transplantation, haemodialysis or peritoneal dialysis adds psychological & financial burden. Early kidney injury can be prevented by evaluating gene pressions of kidney injury molecule-1 in Type 2 diabetes with microalbuminuria. ***Methodology:*** It is cross sectional study includes 241 subjects (118 male, 123 women, between age 30-70 years). Subjects were recruited after screening for Type 2 diabetes by easurement of blood glucose (FBS & PP), HbA1c and microalbumin in urine. Subjects with chronic clinical conditions and uncontrolled blood glucose level were excluded from the study. Equal numbers of healthy volunteers were enrolled in control group and expressions of kidney injury molecule-1 measured by rt-PCR. Results: In study groups all renal parameters found within normal range except albumin creatinine ratio ($p < .012$) & e-glomerular filtration rate ($p < .00$). Kidney injury molecule-1 showed high degree of ignificance ($p < .00$). ***Conclusion:*** Biochemical renal parameters are not enough to speculate nephropathy. Early detection of gene expressions of kidney injury molecule-1 may suggests the status of kidney functions & help to prevent progression of diabetic nephropathy.

INTRODUCTION

Recent estimates by the National Institutes of Health indicate that diabetes represents the single largest cause of end-stage renal disease.[1] Today it has become the single most common cause of end-stage renal disease (ESRD) in the entire world. Apart from the individual human suffering that cannot be expressed in numbers, patients with type 2 diabetes undergoing maintenance dialysis consume significantly more financial resources than those with non-diabetic ESRD. In addition, type 2 diabetic patients do poorly on dialysis and have an excess mortality. DN is typically defined by microalbuminuria, i.e., renal albumin excretion between 30-300 mg in 24 hrs collection or Microalbuminuria. There are several biomarkers in use to detect kidney damage. Conventional biomarkers for kidney damage include glomerular filtration rate (GFR), plasma creatinine, blood urea nitrogen (BUN), urinary micro-albumin excretion rate and several urine qualities such as proteinuria and hematuria as well as liver function test, glycated hemoglobin, RBC indices and urine routine. However these biomarkers are nonspecific and insensitive, hence new specific markers are being developed.[2]

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Routinely used (clinical) biomarkers for kidney injury are serum creatinine & blood urea has several disadvantages. Serum levels of creatinine may only change after about 50-60 % of the kidney function has been lost. Altered levels of serum creatinine take time to establish and make it impossible to detect acute kidney injury early. Poorly sensitive methods are failed to recognized early detection of diabetic nephropathy, these patients has greater chances of ESRD. Use of novel biomarkers has become necessary to detect early diabetic nephropathy progression. Several novel biomarkers of kidney injury have been shown to increase in the urine & plasma of individuals with diabetes at early stage of disease.[3-11]

KIM-1 is a type I cell membrane glycoprotein which contains, in its extracellular portion, a novel six-cysteine immunoglobulin-like domain, two *N* -glycosylation sites and a T/SP rich domain characteristic of mucin-like *O* -glycosylated proteins. There are many reasons to consider that KIM-1 may be released into the circulation after Kidney proximal tubule injury. With injury, tubular cell polarity is lost, such that KIM-1 may be released directly into the interstitium. Further, increased trans-epithelial permeability after tubular injury leads to back leak of tubular contents into the circulation. [12-13]

There is utmost need to know highly sensitive biomarkers for the early detection of diabetic nephropathy. Inadequate tools are failed to recognize early detection of diabetic nephropathy at an early stage. Literature search of KIM1 expression found that it is a promising biomarker for kidney damage and it may help for identifying risk of DN.[14] Also, altered micro vascular permeability is an important contributor to the pathophysiology of kidney injury.[15-18]

MATERIAL AND METHODS

Present cross sectional research conducted at Department of Biochemistry, Dr D. Y. Patil University, Navi Mumbai. Patients referred to Diabetic clinic OPD were recruited in this study. Study initiated after institutional ethics committee approval. The enrolled patients were randomly selected & distributed into 3 different groups; subjects of T2DM between ages 30-45 years; subjects of T2DM between 46-70 years and healthy volunteers (Non-diabetic) between 30-70 years. T2DM of diabetes duration between 3-5 years, HbA1c ≥ 7.0 %, pre-prandial blood glucose (FBS<126 mg/dl), post-prandial glucose (PPBS <200 mg/dl) and microalbuminuria (30-300 mg/dl) were included in this study. Subjects satisfying above criteria but suffering with chronic conditions like uncontrolled diabetic, non-diabetic kidney failure, tuberculosis, liver dysfunction, etc were excluded from the study. Untreated mild hypertensive's were included whereas hypertensive under treatment was excluded. Other renal parameters (blood urea, serum creatinine, urine creatinine calcium and uric acid) was measured and e-GFR, albumin-creatinine ratio were calculated from measured values. 3 ml whole for gene expressions were collected separately. All biochemical renal parameters were measured by Dade Dimension dry chemistry auto-analyser (Roche Diagnostics), isolation and amplification of m-RNA was performed by "One Step Prime Script RT-PCR (Perfect Real Time)" designed by using TaqMan® probe. This kit uses PrimeScriptTM RTase, which has excellent extendibility and can efficiently synthesize cDNA in short time period, and TaKaRa Ex TaqTM HS, high efficiency hot start PCR enzyme, and they are optimized for one step RT-PCR.

Principle of assay

One Step PrimeScriptTM RT-PCR Kit (Perfect Real Time) performs cDNA synthesis from RNA using reverse transcriptase, PrimeScriptTM RTase, and PCR amplification by TaKaRa Ex TaqTM HS within one tube continuously. PCR amplification products are detected with TaqMan® probe in real time monitoring.

TaqMan® Probe One Step Prime Script RT-PCR (Perfect Real Time) method

The TaqMan® method is based on a combination of TaqMan Technology and a real time PCR instrument. KIM-1 estimated from buffy coat. Buffy coat was isolated after centrifugation of whole blood. Supernant watery plasma discarded and WBC rich cloudy layer above pack cells which were aspirated for quantification of KIM1 gene expressions. Oligonucleotide modified with fluorescence substance (e.g.FAM) at 5'-end and with quencher (e.g.TAMRA) at 3'-end is added in a reaction system. Under the annealing condition, TaqMan® probe specifically hybridizes to template DNA, but the fluorescence is suppressed by quencher. In extension step, the 5' → 3' exonuclease activity of Taq DNA polymerase degrades the TaqMan® probe hybridized to a template. Accordingly, TaqMan® probe is released from the suppression with a quencher, resulting in emission of fluorescence which corresponds to its expression value in term of threshold cycle.

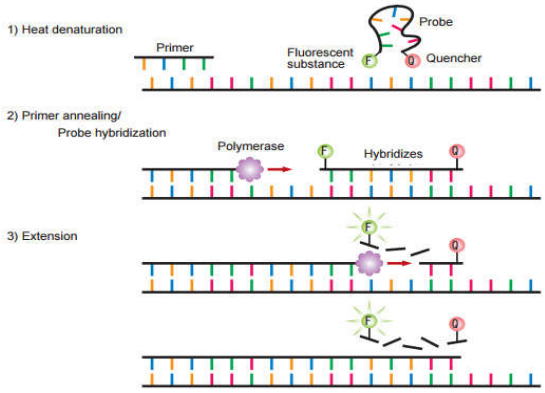

Figure1 Expressions plot of Kidney Injury Molecule-1 by rt-PCR.
(Source: www.takara-bio.com)

RESULTS

Screening parameters used for diagnosis of microalbuminuria & non- microalbuminuria showed significant P value. Results are expressed as means ± SD and standard error (SE). Statistical calculations were performed using the R software package.

Table2. P Value of Post Hoc Tests of renal parameters within groups and between the groups (Tukey HSD). Statistical analysis (descriptive and post hoc) of table 1 & 2 showed significant difference of HbA1c and urine creatinine. GFR is gold standard parameter for renal assessment but in early stage of DN it was non-significant.

Table 1 Descriptive analysis of renal parameters: Microalbumin (MALB), Glycosylated Hemoglobin (HbA_1C) and Albumin-Creatinine ratio (ACR) within groups.

	Groups					
	Control		≤ 45 years		≥ 45 years	
Parameters	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Glycosylated Haemoglobin (4.5-6.5 gm/ dL)	5.6	0.052	8.0	0.157	8.0	0.129
Blood glucose (F, ≤126 mg/dL)	96	0.806	147	4.638	156	5.941
Blood glucose (PP, ≤ 200 mg/dL in DM)	108	0.921	175	4.242	197	7.696
Micro-albumin (30-300 mg/dl)	14.13	0.401	235.28	5.970	263.37	9.462
Urine Creatinine (120–230 mcmol/kg body weight per 24 hr)	60.99	4.335	121.06	9.231	134..65	13.960
Albumin/Creatinine ratio (≥3.5 mg/mmol)	0.44	2.113	3.35	3.556	3.35	2.623
Calcium (8.6–10.0 mg/dL)	9.4	0.064	9.6	0.069	9.6	0.057
Blood urea nitrogen (6–20 mg/dL)	10	0.284	10	0.237	11	0.335
Uric Acid (mg/dL)	4.8	0.112	5.0	0.203	5.3	0.139
Sr. Creatinine (0.7–1.3 mg/dL)	0.79	0.02	0.716	0.019	0.854.	0.023
e-GFR (>60 mL/min/1.73m^2).	100	2.46	94	114	90	2.077

Abbreviations: SD: standard deviation, SE: standard error, PV: P value (Post hoc test). Data are mean ± SD with range in parenthesis or absolute number of patients.

Table 2 P Value of Post Hoc Tests of renal parameters within groups and between the groups (Tukey HSD). (Original data) f

Dependent Variable	Control group		≤ 45 years group		≥45 years group	
	≤ 45	≥45	Control	≥45	Control	≤45
HbA_1C (4.5-6.5 gm / dL)	.00	.00	.00	.948	.00	.948
Creatinine (U, 120–230 mc mol / kg body weight/ 24 hr)	.00	.00	.00	.822	.00	.822
Sr. Creatinine (0.7–1.3 mg/dl)	.034	.074	.034	.00	.074	.00
ACR (≥3.5 mg/mmol)	.008	.420	.008	.186	.420	.186
e-GFR (>60 mL/min/1.73m^2).	.00	.012	.00	.00	.012	.00
Calcium (8.6–10.0 mg/dL)	.048	.146	.048	.871	.146	.871
BUN (6–20 mg/dL)	.751	.040	.751	.197	.040	.197

Thus observation of e-GFR is supported by Nielsen SE, *et al* study.[18] Study done by Panduru *et al*., (2015) concluded that KIM-1 did not predict progression to end-stage renal disease independently of AER and added no prognostic benefit to current biomarkers. Nevertheless, the MR showed that the inverse association of increased KIM-1 levels with lower e-GFR is likely to represent a causal link.[19]

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Table3 Post hoc test (P value) between study groups of KIM1 gene (Tukey HSD). (Original data)

Dependent Variable	Control group		<45 years group		>45 years group	
	≤ 45	≥45	Control	≥45	Control	≤45
CT OF KIM-1	.00	.00	.00	.00	.00	.00

In this study it was found significant P-value in all study groups. High degree of significance was found in KIM1 (table 2) despite all routine renal parameters are within reference interval.

CT is nothing but threshold cycle. In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The CT (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold; simply genes get expressed at particular CT in this assay. Every single colour in amplification graph represents one sample. CT is calculated for group. It is software generated graph after rt-PCR run. In this study it was found significant P-value (<.00) in all study groups. High degree of significance was found in KIM1 (table 2) despite all routine renal parameters are within reference interval.

DISCUSSION

KIM1 is a type 1 trans-membrane protein which is exclusively and abundantly expressed in damaged kidney cells. The ectodomain of KIM-1 is shed into urine and easily detectable. According to Waanders F, *et al*.,[21] KIM-1 expressions are measurable within a day after the onset of kidney damage. It is a marker for acute and chronic kidney disease. It was observed raised level of blood KIM1 expressions in study groups. In contrast, blood KIM-1 was reported raised shortly after proximal tubular injury reported by Vaidya VS, *et al* .[22] Study published by Nielsen SE, *et al*., (2010)[19] states that urine-NGAL and urine-KIM1 (u-KIM1) are elevated in Type1 diabetic patients, with or without albuminuria, indicating tubular damage at an early stage. Study done by Buket Kin Tekce, *et al*[23] and Khot VV, *et al*.,[24] indicates urinary KIM-1 levels predict renal injury secondary because urinary KIM-1 was elevated despite normal urinary albumin excretion in the normoalbuminuria subgroups also. From this study it was concluded that urinary KIM-1 has great importance even in normoalbuminuria subjects suffering with T2DM.

Study conducted by Venkata S. Sabbisetti *et al*.,[20] identified blood KIM1as a marker of kidney injury in humans, where KIM1 levels are significantly elevated in AKI and CKD which predict progression of renal disease in a T1DM cohort. They further concluded that this biomarker may have potential utility as a sensitive and specific diagnostic and prognostic value for kidney injury.

Results of KIM1 in our study are supported by observations of Venkata S. Sabbisetti, *et al* study.

CONCLUSION

It was concluded that early detection of renal injury in T2DM patients with routine biochemical parameters create dilemma. Microalbuminuria is a gold standard tool to intervene early in the course of the disease but KIM-1 expressions measurement helps in pre-microalbuminuria which will help in early diagnosis of DN and progression of disease may be controlled. Early measurement of KIM-1 may help to prevent overt microalbuminuria. Further extensive research on large number of subjects with population diversity has been recommended.

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