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Research Article

Diabetes

Comparative analysis of biochemical parameters and gene expressions of novel markers in type II Diabetes mellitus

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Background: Diabetic nephropathy (DN) is the most common cause of end stage renal disease (ESRD). Early detection of the disease and treatment of this chronic complication which would reduce the medical and economic burden. Early detection of kidney injury by evaluating gene expressions of Il-6, Il-10, LDLr, and CD36 in T2DM with pre-ESRD microalbuminuria minimizes the risk of DN. **Methods:** Present research work conducted at the Department of Biochemistry, School of Medicine, Navi Mumbai. This study includes 241 subjects (118 male, 123 women, and age ranges 30-70 years) were included after screening for T2DM by measurement of blood glucose in fasting, post-prandial, glycosylated haemoglobin. Microalbumin in urine and e-GFR is measured to eliminate patients of ESRD. Subjects were recruited after written consent and enrolled as per inclusion/exclusion criteria. Categorization of subjects in three study groups; group I (30-45 years), group II (46-70 years) were done on the basis of T2DM duration 3-6 years, glycosylated haemoglobin level (HbA1c) ≥ 7.0% with fasting blood glucose ≥126 mg/dl) and microalbuminuria (30-300 mg/dl) in study group, equal numbers of healthy volunteers enrolled in control group. Blood samples were processed for other renal parameters and RT-PCR to check expressions of novel genes **Results:** In study groups all renal, lipids parameters are within normal range except albumin/creatinine ratio ($p < 0.012$), e-GFR ($p < 0.00$) and cholesterol ($p < .00$). Descriptive analysis showed high significance ($p < .00$) of delta CT gene expressions, parameters in pre-ESRD microalbuminuria subjects. **Conclusion:** Screening biochemical renal parameters are not enough to prevent DN even in microalbuminuria. Early detection of gene expressions of novel biomarkers predicts risk of kidney injury. Early intervention may prevent morbidity and mortality of kidney due to diabetic nephropathy.

Keywords: Diabetes mellitus, Microalbuminuria, Gene expressions, Risk prediction

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Introduction

About 10 to 40% Type 2 diabetes (T2DM) and 30% Type 1 diabetes (T1DM) suffer from kidney failure increases huge financial burden for care for patients [1]. Conventional biomarkers for kidney damage include glomerular filtration rate (GFR), serum/plasma creatinine, blood urea nitrogen (BUN), urinary micro-albumin excretion and several urinary findings such as proteinuria and haematuria. *IL-6* mRNA expression and insulin resistance were found to have a significant correlation and increased plasma IL-6 levels with higher risk of T2DM [2-5]making it an appealing candidate gene. Mesangial cells are the major local source of IL-10 in the normal adult kidney and elevated levels found in diabetic patients [6-7].

CD 36 protein was markedly increased in proximal tubules in human DN [8]. An increased accumulation of cholesterol through the dysregulation of LDL receptor in human mesangial cells has vital role in the progression of renal dysfunction [9]. Though these biomarkers played vital for early detection of DN extensive research in pre-ESRD microalbuinuria in needed. In order to advance these efforts, genetic expressions of novel plasma biomarkers IL-6, IL-10, LDLr and CD 36 were needed for early detection of renal injury and to identify risk for progression of renal dysfunction towards ESRD.

Material and Methods

Place of study: Present research conducted at Department of Biochemistry, Dr D. Y. Patil University, Navi Mumbai.

Sampling method: Patients referred to diabetic clinic were recruited in this study.

The enrolled patients were 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 age between 3-6 years, HbA1c ≥ 7.0%, pre-prandial blood glucose (FBS) ≥ 6.0 mmol/L (126 mg/dl), post-prandial glucose (PPBS) ≥ 8.0 mmol/L (200 mg/dl) and micro-albuminuria (MALB) ≥ 300 mg/dl were included in this study.

Inclusion criteria:

- Subjects satisfying above criteria were included in the study but

Exclusion criteria:

- Subjectssufferings with chronic co-morbidities were excluded from the study.

Renal and lipid parameters (blood urea, serum creatinine, urine creatinine and low-high density lipoprotein's, cholesterol and triglycerides) were measured and e-GFR, albumin-creatinine ratio were calculated. 3 ml whole blood collected for gene expressions of selected genes separately. All biochemical renal parameters were measured by Dade Dimension dry chemistry auto-analyser (Roche Diagnostics) and gene expressions measured by RT-PCR.

Consent- All authors declare that 'written informed consent was obtained from patients for publication of outcome of this study' copy of written consent may retrieve from us, if required.

Ethical approval- The topic was approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.'

Statistical analysis: Biochemical & gene expression data was analysed by R software & P value or descriptive value was calculated. Institutional ethics committee granted permission for this research work.

Results

Table-1: Descriptive statistics for age, duration and gender distribution of study population.

Descriptive statistics for age and duration of diabetes							
Parameters		N	Mean	SD	SEM	Min	Max
Age	Control	81	46.26	9.400	1.051	29	68
	30-45 years	80	40.01	3.892	0.435	32	45
	46-70 years	80	59.38	8.278	0.919	46	70
Duration of diabetes	Control	NA	NA	NA	NA	NA	NA
	30-45 years	80	3.49	0.729	0.082	3	6
	46-70 years	80	6.67	1.213	0.157	3	5.9
Gender Group cross tabulation							
Parameters			Control	30-45	46-70	Total	
Gender	Male	Count	33	38	47	118	
		Percentage	40.7	47.5	58.7	49	
	Female	Count	48	42	33	123	
		Percentage	59.2	52.5	41.2	51	
Total count			81	80	80	241	

Table-2: Post hoc statistical analysis (P-value) of lipid profile in study population.

Dependent variable	Control group I (30-70 yrs)		Study Group II (30-45 yrs)		Study Group III (46-70 yrs)	
	30-45 years	46-70 years	Control	46-70 years	Control	30-45 years
Cholesterol(T)	0.070	0.070	0.070	0.070	0.070	0.070
Triglyceride	0.086	0.086	0.086	0.086	0.086	0.086
LDL	0.00	0.00	0.00	0.00	0.00	0.00
HDL	0.00	0.00	0.00	0.071	0.00	0.071

Gender wise distribution of subjects shown in Table 1. There is marginal percentage difference in all groups since it is cross sectional subject recruitment. Table 2 represented no significant p-value for any lipid parameters.

Since criteria of subjects is pre-nephrotic condition and duration of diabetic is 1-3 years, may be majority of population could be health conscious or possibilities of lipolysis for energy sustainability. In the present study, nutritional and BMR assessment was not performed, otherwise authors might have been able put light on this observation.

Discussion

In lipid parameters we have observed significant p value of LDL in both the study groups. Study published by Sanas S [10] and Essam Abd-Allha [11] states that LDL is correlated with the incidence and severity of diabetic nephropathy in T2DM patients.

Author expressed that outcome of their study should be considered as a potential risk factor and as a diagnostic biomarker to be used in conjunction with other biochemical markers for early diagnosis, assessment.

In the post hoc analysis of gene expressions and lipid in study population (Table 2) showed similar results documented by Hirano T, [12] in their study detected high prevalence of LDL in type II diabetic patients with nephropathy. In1994, Sekizuka [13] reported that serum levels of IL-6 were significantly higher in patients with T2DN than the levels observed in diabetic patients without nephropathy [14].

These observations suggests that IL-6 cytokine may play a role in the pathogenesis of DN. Similar findings have been noted in the present study which showed IL-6 gene is expressed in the study group with high significance (Figure 1).

Fig-1: RT- PCR Quantification of IL6

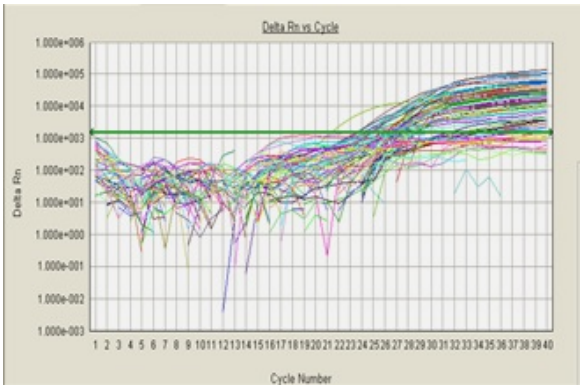

Table-3: Correlation of glycosylated hemoglobin (</=5.7%) and microalbumin with other novel gene and biochemical parameters.

Parameters	Statistical test	HbA1C	Microalbumin
Delta CT of IL6	Pearson correlation	-0.604**	-0.193**
	p-value	0.000	0.003
Delta CT of IL10	Pearson correlation	-0.568**	-0.177**
	p-value	0.000	0.006
Delta CT of CD36	Pearson correlation	-0.534**	-0.150*
	p-value	0.000	0.02
Delta CT of LDLr	Pearson correlation	-0.076	-0.026
	p-value	0.24	0.691
Urine creatinine mg/dl	Pearson correlation	0.311**	0.721**
	p-value	0.000	0.000
Blood creatinine	Pearson correlation	0	-0.041
	p-value	1	0.522
e-GFR	Pearson correlation	0.076	0.028
	p-value	0.239	0.665

Table 3 represented significant correlation between all the parameters except LDLr. Comparison of glycosylated hemoglobin with all genes showed significant correlation in all parameters except LDLr. A microalbumin comparison with gene expressions of IL-6, IL-10, CD36 and LDLr shows significant P-value (Table 3 and Figure1-4).

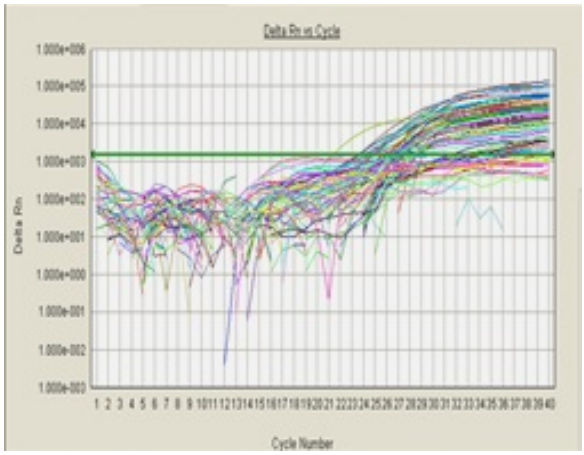

Figure-2: RT- PCR Quantification of IL10

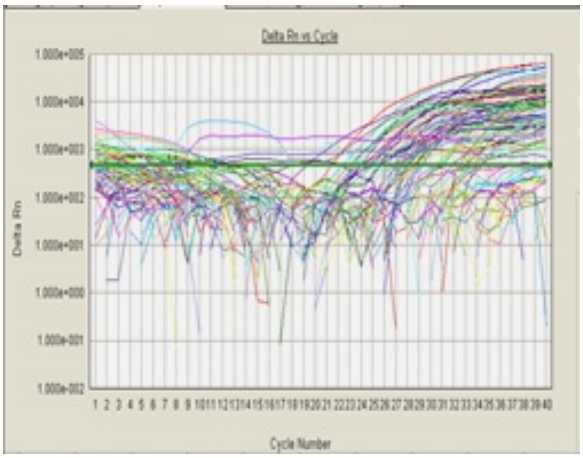

Figure-3: RT- PCR Quantification of CD-36

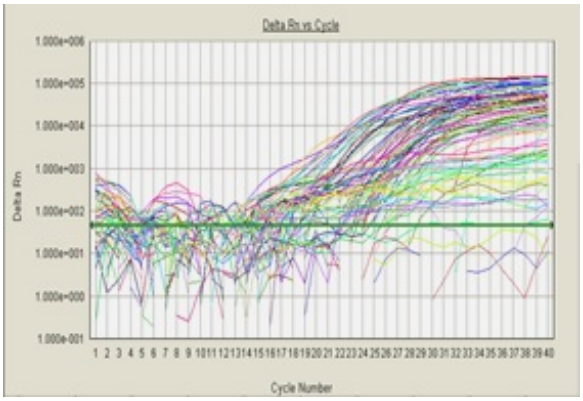

Figure-4: RT- PCR Quantification of LDLr

Authors Parisa Behzadi [15], Nakhjavani [16], Khot VV [17] studied two groups of diabetic patients consisting of patients with microalbuminuria and those with normal albumin excretion. In care of renal parameters there is no significant correlation found except microalbuminuria.

Conclusion

In conclusion, the present results suggested that for early detection of renal injury by analysing routine renal investigations (blood urea nitrogen and serum/ urine creatinine) are not enough to understand the progression of the disease.

Though GFR, micro albumin and urine albumin they play important role in diagnosis, reversion of renal dysfunction at early stage is impossible. Due to lethal conditions more than 60-70 % uncontrolled T2DM subjects demonstrate some degree of renal dysfunction.

The novel gene markers studied showed that their expressions alarm damage to kidney. Early evaluation of these gene expressions by RT-PCR may prevent morbidity and mortality.

Author's contribution

Dr. Varsha Khot: Research topic design, approval.

Dr. J.M. Kulkarni: Conducted experiments.

Dr. KS Yadav: Supervision, protocol setup and data analysis and writing of manuscript done.

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