

Original Research Article

# LDLr Gene Expressions: A Novel Biomarker in Risk Prediction of Renal Injury

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## ABSTRACT

**Background:** Patients with uncontrolled diabetes prone to ESRD, require kidney transplantation, haemodialysis or peritoneal dialysis which adds psychological distress. Early detection of kidney injury by evaluating gene expressions of LDLr in T2DM with microalbuminuria minimizes the risk of DN. Present research work conducted at the Department of Biochemistry, D.Y.Patil school of Medicine, Navi Mumbai, India.

**Methods:** This is cross-sectional analytical study includes 241 subjects (118 male, 123 women, and age ranges 30-70 years). Categorization of subjects in three study groups were done on the basis of T2DM duration 3-5 years, Glycosylated haemoglobin level (HbA1c)  $\geq 7.0$  % with fasting blood glucose  $\geq 126$  mg/dl) and microalbuminuria (30-300 mg/dl), equal number of healthy volunteers enrolled in control group. Blood samples were processed for other renal parameters by photometry assay & LDLr expressions by RT-PCR.

**Results:** All renal and lipids parameters are within normal range except albumin/creatinine ratio ( $p > .012$ ), e-GFR ( $p > .00$ ) and cholesterol ( $p > .00$ ). Descriptive analysis showed high significance ( $p > .00$ ) for LDLr in microalbuminuria subjects.

**Conclusion:** Screening biochemical renal parameters are not enough to prevent DN. Early detection of LDLr gene expressions predicts risk of kidney injury. Early intervention may prevent damage to kidney due to diabetic nephropathy.

**Key words:** Diabetes mellitus, microalbuminuria, LDLr gene expression, risk prediction.

## 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. <sup>[1]</sup> Diabetes has become a common global health problem that affects >170 million people worldwide. It is one of the leading causes of death and disability. World Health Organization estimated that by 2030, the number will rise to 366 million. DM is associated with long-term complications that affect almost every organ of the body, kidney, liver heart etc. T2DM is a complex polygenic disorder in which common genetic variants interact

with environmental factors to unmask the disease. Genetic factors are known to play an important role in the development of DN, atherosclerotic plaque. Ravid et al; Chaturvedi et al; and Bonnet et al. found that abnormal lipid profile might cause nephropathy in both type 1 and type 2 diabetic patients. <sup>[2-4]</sup> Single-gene (Mendelian) disorders with large effects are the most dramatic examples of the genetic contributions to lipid deposition in arteries. <sup>[5]</sup> Uptake of cholesterol, mediated by the low-density lipoprotein (LDL)-receptor, plays a crucial role in lipoprotein metabolism. Dysregulation of cholesterol

metabolism has also been linked to lipotoxicity and lipid accumulation in diabetes. Cholesterol influx into cells is mediated by several independent receptors, including scavenger receptor class A (SR-A1), class B (CD36), lectin-like oxLDL receptor-1 (LOX-1 or OLR-1, [6] and LDL receptor (LDLR). [7]

Low density lipoprotein receptor (LDLr) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), serving important functions in maintaining cholesterol uptake and synthesis, respectively, are regulated by SREBP-2 in the human mesangial cell line. [8,9]

Michel Herman, et al., stated that highly significant correlation between glomerular filtration rate, inflammation, and lipid metabolism genes, which supported possibility of abnormalities in lipid metabolism led to cause DN progression, which is supporting our observation too. [10]

Mesangial and glomerular epithelial cells express low-density lipoprotein (LDL) receptors and are capable of endocytosis of bound LDL. [11, 12]

Earlier studies documented that multiple lipoprotein abnormalities in diabetic nephropathy become more accentuated with increasing urinary albumin excretion. [13]

## **MATERIALS 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. The enrolled patients 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  $\geq$  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 were excluded from the

study. All biochemical 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.

## **RESULTS AND DISCUSSION**

Fasting blood glucose (FBS), Post-prandial blood glucose (PPBS), Glycosylated haemoglobin (A1C) and urinary microalbumin gives an idea about diabetes progression of individual. Since these are screening parameters for subject selection, there was a significant difference between control and study groups ( $p > .00$ ). In study group subjects no significant difference has been observed.

Study done by Nobuko Harita et al showed that lower serum creatinine increased the risk of T2DM. [14] Skeletal muscle is major target tissue of insulin and its resistance leads to the development of T2DM. [15] Creatinine is commonly used to determine GFR. In our study average serum creatinine reported within the normal range in control and study groups, similar findings were reported by Harita et al, [14] urinary excretion of creatinine was almost two fold higher in both the study groups against the control group. Further post hoc analysis within study groups ( $<$ 45 years and  $>$ 45 years) irrespective of gender showed significant P-value ( $p > .00$ ). In this study it was found that no significant difference in urine creatinine was observed between control and study groups.

Micro-albuminuria is a gold standard parameter in diagnosis of renal diseases. Albumin/ creatinine ratio (ACR) is greater than or equal to 2.5 (men) or 3.5 (women), or albumin concentration greater than or equal to 20 mg/L is significant observation in diagnosis of renal diseases. Literature survey reveals that early stage of kidney disease demonstrates an abnormal ACR. This study reported marginally significant difference of ACR between control and study groups. Further post hoc analysis also

showed similar observations between control, less than 45 years and above 45 years. These observations do not indicate any confirmatory outcome. So it was recommended to undertake study on a larger population to achieve final conclusion. After literature survey it was found that ACR is an important marker in diagnosis of DN but values reported in this study does not support.

It was recommended by the American Diabetes Association (ADA) and the National Institutes of Health (NIH) that in all the people with diabetes for detection of

kidney dysfunction, e-GFR must be calculated from serum Creatinine at least once a year. In this study e-GFR was calculated by modification of diet in renal disease (MDRD) study group equation. [16] There is significant difference between control and study groups ( $p > .00$ ). Further analysis by post hoc test within the study groups (<45 years and >45 years) and control showed the significant difference ( $p > .00$ ). Therefore measurement of e-GFR is useful in monitoring diabetic nephropathy associated with T2DM. All above statements are tabulated in table 1 & 2.

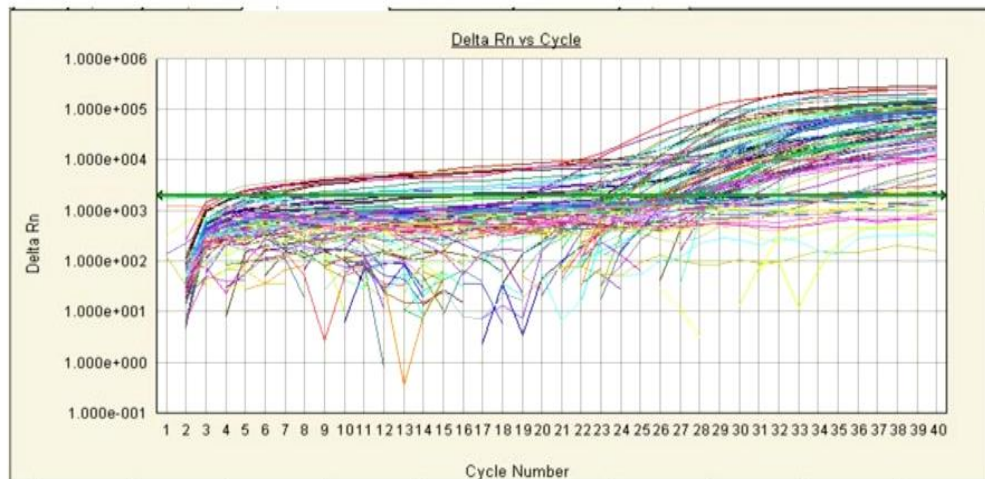
**Table1. Descriptive statistical analysis of renal parameters, and lipid parameters by R software within groups.**

Parameters	Control		45 years and less		More than 45 years	
	Mean	SE	Mean	SE	Mean	SE
Urine Creatinine	60.99	4.335	121.06	9.231	134.65	13.960
Albumin/ creatinine ratio	0.44	2.113	3.35	3.556	3.35	2.623
Blood urea nitrogen	10	0.284	10	0.237	11	0.335
Uric Acid	4.8	0.112	5.0	0.203	5.3	0.139
Serum Creatinine	0.79	0.02	0.716	0.019	0.854	0.023
e-GFR	100	2.46	94	114	90	2.077
Total Cholesterol	117	2.73	178	5.9	190	4.68
Triglyceride	137	3.7	139	7.9	160	10.8
HDL Cholesterol	46.22	0.70	38.80	0.711	41.42	1.0
Low density lipoprotein	101.22	1.061	113.21	4.41	130.5	3.69
CHOL:HDL ratio	5.040	0.121	4.64	0.135	4.79	0.14
LDL:HDL ratio	3.60	0.103	2.956	0.106	3.306	0.120
VLDL	27.59	0.704	27.828	1.59	32.10	2.17

**Table2. P Value of Post Hoc Tests of renal parameters and lipid parameters within groups and between the groups (Tukey HSD).**

Dependent Variable	Control group		<45 years group		>45 years group	
	<45 yrs	>45 yrs	Control	>45 yrs	Control	<45 yrs
Sr Creatinine	.034	.074	.034	.00	.074	.00
ACR	.008	.420	.008	.186	.420	.186
e-GFR	.00	.012	.00	.00	.012	.00
BUN	.751	.040	.751	.197	.040	.197
HDL Cholesterol	.000	.000	.000	.071	.000	.071
Triglyceride	.080	.086	.080	.089	.086	.089
LDL:HDL ratio	.000	.138	.000	.064	.138	.064

**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.



**Figure1: LDLr gene expressions by RT-PCR**

**Table3. Post hoc test between study groups and LDLr marker (Tukey HSD)**

Dependent Variable	Control		Less than 45 years		More than 45 years	
	<45 yrs	>45 yrs	Control	>45 yrs	Control	>45 yrs
CT of LDLr	.00	.00	.00	.00	.00	.00

CT: threshold cycle quantification by RT-PCR.

In this study descriptive statistics (table1) within groups showed significant difference only for HDL ( $P>.00$ ) & LDL/HDL ratio ( $P>.00$ ). All other lipid parameters p-values are non-significant. Further in post hoc analysis in table 2 HDL shows significant p value  $p=.00$  only between the groups but HDL remain non-significant within the group. High density lipoprotein cholesterol (HDL-C) is protective against the development of coronary artery disease (CAD) and microalbuminuria. [15]

Further post hoc analysis in table 3 showed highly significant p value ( $p>.00$ ) for LDLr gene expression in both the study groups.

Dyslipidaemia is a risk factor for development and progression of microalbuminuria. In this study estimated lipids showed values within reference interval but LDLr expressions were observed at an early stage of DN. High degree of significance was found in LDLr ( $P>.00$ ) also, which is shown in table 3.

The expressions of LDLr molecules involved in low-density lipoprotein receptor (LDLr) pathway and podocyte injury. The mean of LDL receptors observed expressed in this study, similar results were published by Laurence Duvillard, et al. [17] Y.Wu et al., has also found high level expression of mRNA in ORG (Obesity related glomerulopathy) patients. [18]

As per the previous studies Inflammation plays a central role in the progression of DN and induces marked changes in lipid and lipoprotein metabolism [19-23] in animal models & in human kidneys, suggesting a possible role for inflammation in causing altered lipid metabolism, even when the serum lipid profile is well controlled which is supported to our study observation.

## CONCLUSION

It was concluded that early detection of renal injury in T2DM patients with routine biochemical parameters create dilemma. But when these results evaluated with gene expressions of LDLr and output of this exercise may help in confirmation of diagnosis. This observation strongly support risk prediction of DN. Early measurement of LDLr may prevent morbidity & mortality. The present study was carried out in limited number of T2DM subjects. Further extensive research on large number of subjects with population diversity has been recommended.

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**Competing interest:** Nil

### Author's contribution

Khot V: analytical work, data entry, IEC presentation & manuscript writing.

Yadav KS: topic guidance, study design, protocol design & supervision of work.

### 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

'All authors are here by declared that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.'

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