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Expressions of CD36: Predictor marker of the early Kidney Injury in Type 2 Diabetic Mellitus

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Abstract
Background: *Patients with uncontrolled diabetes prone to end stage renal disease, require kidney transplantation, haemodialysis or peritoneal dialysis which adds psychological distress. Early prediction of kidney injury by evaluating expressions of CD36, a trans-membrane protein of the class B scavenger receptor family, in type 2 diabetic mellitus minimizes the risk of diabetic nephropathy.*
Methods:*This study includes 241 subjects (118 male, 123 women, and age ranges 30-70 years) were included after screening for type 2 diabetic mellitusas per International Diabetic Federation guidelines. Subjects were groupedinto two groups after written consent and enrolled as per inclusion/exclusion criteria. Equal numbers of healthy volunteers enrolled in control group. Blood samples were processed for other renal parameters & rt -PCR to check expressions of CD36.*
Results: *In study groups all renal and lipidparameters found withinnormal range except albumin/creatinine ratio (p >.012), e-GFR (p >.00) and total cholesterol (p >.00). Descriptive analysis showed that genetic expressions of CD36 found highly significant (p >.00) in microalbuminuria subjects*
Conclusion: *Renal biochemical parameters are not enough to prevent diabetic nephropathy even in microalbuminuria due to its interpretive limitations. Early detection of gene expressions of CD36 may predict risk of kidney injury. Early management of disease progression may prevent morbidity & mortality of kidney due to diabetic nephropathy.*
Keywords: *Diabetes mellitus, microalbuminuria, gene expressions, risk prediction.*

I. Introduction

About 10 to 40 % Type 2 diabetic mellitus (T2DM) and 30 % Type 1 diabetic mellitus (T1DM) suffer from kidney failure increases huge financial burden for care for patients. (1) CD36 is a transmembrane protein of the class B scavenger receptor family and is involved in multiple biological processes. (2).Abnormal lipoprotein metabolism noted by Hirano which stated that increased CVD risk lead to cause dyslipidemia is multifactorial and complex. (3) Literature review showed that Ravid et al.(1998),Chaturvedi et al.(2001), and Bonnet et al.(2000), suggested that an abnormal lipid levelsmay cause nephropathy in both type 1 and type 2 diabetic patients.(4-11) Single-gene (Mendelian) disorders with large effects are the most dramatic examples of the genetic contributions to lipid deposition in arteries. (12) 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(13), andLDL receptor (LDLR). (14)

Previous studies documented that all multiple lipoprotein abnormalities described in diabetic patients with nephropathy become more accentuated with increasing urinary albumin excretion. (15-17) Abnormalities in plasma concentrations of lipoproteins and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion. Hyperglycaemia-induced synthesis of CD36 protein in macrophages has been associated with increased uptake of ox-LDL by macrophages and foam cell formation in atherosclerotic lesions in people with diabetes. While diabetic cardiovascular complications are closely linked epidemiologically with albuminuria and DN, a role for CD36 in DN and renal pathophysiology has not to our knowledge been described to date. (2) High ambient glucose has been shown to induce CD36 protein synthesis in macrophages.(18) A link between diabetes and atherosclerosis: Glucose regulates expression of CD36 at the level of translation. Because CD36 protein was markedly increased in proximal tubules in human DN (19), examined the effects of high ambient glucose on CD36 mRNA and protein expression in the human proximal tubule epithelial cells line human kidney -2 .

II. Material And Methods

Present research conducted at Department of Biochemistry, Dr D. Y. Patil University, Navi Mumbai. Patients referred to Diabetic clinic enrolled & distributed into 3 different groups; subjects of T2DM between ages 18-45 years; subjects of T2DM between 46-70 years and healthy volunteers (Non-diabetic) between 18-70 years.T2DM of diabetes age between 3-5 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 microalbuminuria (MALB) were included in this study. Subjects satisfying above criteria but suffering with chronic conditions were excluded from the study. Renal parameters (blood urea, serum creatinine, urine creatinine calcium and uric acid was measured and e-GFR, albumin-creatinine ratio were calculated from previously collected serum & urine samples. 3 ml whole collected for gene expressions separately. All biochemical renal parameters were measured by Dade Dimension dry chemistry auto-analyser (Roche Diagnostics). Gene expressions were measured by using rt-PCR commercial kit manufactured by Qiagen, Germany.

III. Results And Discussion

Glycosylated haemoglobin (A1C) is an average blood glucose (ABG) level for the past 2 to 3 months. It gives an idea about diabetes prognosis of the individual. In this study there was a significant difference between control and study groups (p>.00). There is no significant difference within study groups of T2DM. Measurement of glycosylated haemoglobin is useful parameter in diabetic nephropathy for monitoring T2DM treatment and its prognosis. In this study, on applying post hoc test within study groups significant difference between control and study groups in their FBS and PBS was found. (<45 years and>45 years; p>.00 and .00).

Study done by Nobuko Harita et al showed that lower serum creatinine increased the risk of type 2 diabetes.(20) Skeletal muscle is major target tissue of insulin and its resistance leads to the development of T2DM.(21) 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.,(20) 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 and .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 45age and above 45age.These observations does 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 fond that ACR is an important marker in diagnosis of DN but values reported in this study does not support.

Serum calcium concentrations are maintained in the normal range in healthy individuals. Very few studies have been done to understand the role of calcium in DN. In this study the level of calcium in control and study groups was practically the same and within the normal range and its role in prognosis of disease was not clear. It was recommend by the American Diabetes Association and the National Institutes of Health 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 (22). In this study e-GFR was calculated by MDRD (Modification of diet in renal disease) study group equation (23). 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 indicator in monitoring diabetic nephropathy associated with T2DM. All above statements were shown in table no 1 & 2.

Table1. Descriptive analysis of renal parameters, Microalbumin (MALB), Glycosylated Hemoglobin (HbA_1C) and Albumin-Creatinine ratio (ACR) within groups.

Groups / Parameters	Control		45 years and less		More than 45 years	
	Mean	SE	Mean	SE	Mean	SE
Glycosylated Haemoglobin	5.6	0.052	8.0	0.157	8.0	0.129
Blood glucose (F)	96	0.806	147	4.638	156	5.941
Blood glucose (PP)	108	0.921	175	4.242	197	7.696
Microalbumin	14.13	0.401	235.28	5.970	263.37	9.462
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
Calcium	0.94	0.064	9.6	0.069	9.6	0.057
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

Table2.P Value of Post Hoc Tests of renal 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
HbA_1C	.00	.00	.00	.948	.00	.948
Urine creatinine	.00	.00	.00	.822	.00	.822
Serum Creatinine	.034	.074	.034	.00	.074	.00
ACR	.008	.420	.008	.186	.420	.186
e-GFR	.00	.012	.00	.00	.012	.00
Calcium	.048	.146	.048	.871	.146	.871
BUN	.751	.040	.751	.197	.040	.197

Table3: Descriptive statistics of lipid parameters within groups and between the groups.

Parameters	Groups					
	Control		45 years and less		More than 45 years	
	Mean+ SD	SE	Mean+ SD	SE	Mean+ SD	SE
Cholesterol	177 ± 24.4	2.731	178 ± 53.1	5.933	191± 42.1	4.684
Triglyceride	138 ± 33.1	3.701	139 ± 71.1	7.948	161± 97.7	10.859
HDL mg/dL	46 ± 6.3	0.704	39 ± 6.3	0.711	41± 9.4	1.047
LDL mg/dL	101 ± 9.4	1.061	113 ± 39.5	4.418	131± 33.2	3.694
LDL:HDL ratio	3.6 ± 0.92	0.103	3.0 ± 0.94	0.106	3.3±1.1	0.120

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.

In this study descriptive statistics (table1).within groups showed significant difference only for HDL (P>.00) & HDL/LDL ratio (P>.00). All other lipid parameters p-values are non-significant. High density lipoprotein cholesterol (HDL-C) is protective against the development of coronary artery disease (CAD) and microalbuminuria. (21)

In this study it was found similar results in HDL & HDL/LDL ratio, other parameters like cholesterol, triglyceride, LDL and VLDL agreed with the outcomes of V.V. Khot et al.(2017), study on diabetic nephropathy. (25)

Table4. Post hoc test between study groups and CD36 gene 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 CD36	.00	.00	.00	.00	.00	.00

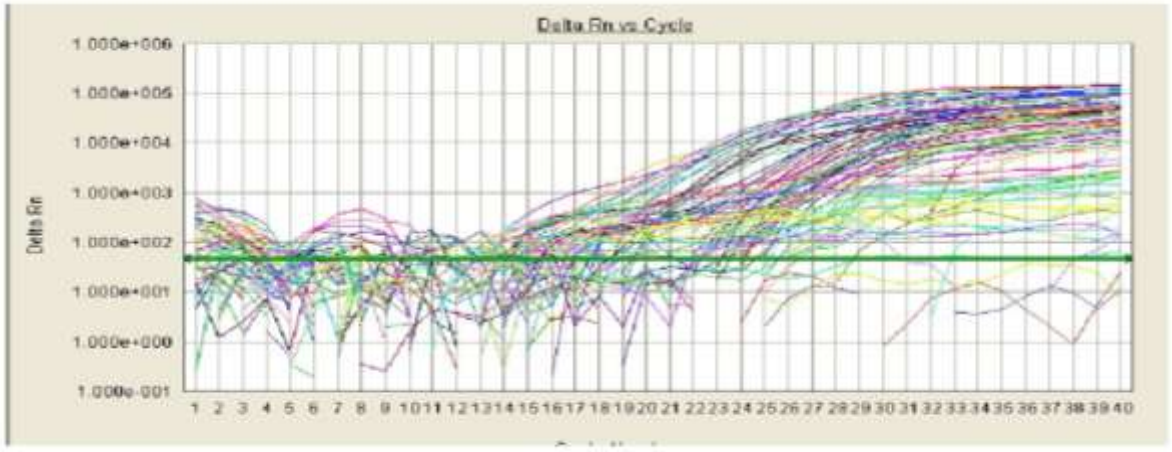
CT: threshold cycle quantification by RT-PCR. (CD36 amplification plot is shown below in fig 1)

CD36 is intimately involved in lipid metabolism and homoeostasis and has been strongly implicated in pathological conditions associated with metabolic dysregulation, including obesity, insulin resistance, diabetes, diabetic nephropathy and atherosclerosis.(2, 19, 25) Circulating form of CD36 was identified in human plasma as a novel biomarker for type 2 diabetes mellitus (T2DM) (24).Hyperglycaemia-induced synthesis of CD36 protein has been associated with increased uptake of LDL lead in atherosclerotic lesions in people with diabetes. (2, 18).Katalin et al. (2005), reported a new functional role for CD36 scavenger receptor in tubular epithelial apoptosis associated with tubular degeneration and progression of DN (19).Thus CD36 could have a central role in triggering diabetic nephropathy which is one of the observations of this study. (26) Despite immediate clinical implications for the treatment of people with kidney problems, this research may help in understanding how hyperglycaemia damages the kidney. In particular, it highlights how important it is to keep blood glucose levels within reference range.

IV. 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 CD36 and output of this exercise may help in confirmation of diagnosis. This observation strongly support risk prediction of DN. Early measurement of gene expression of CD36 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.

Figure 1. CD36 rt-PCR Amplification plot



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Competing interest

Nil

Author's contribution

[1]Khot V, analytical work, data entry, IEC presentation & manuscript writing.

[1*]Yadav KS, topic guidance, study design, protocol design & supervision of work, critical comments, finalizing manuscript draft.

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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