Correlation between LDLr and CD-36 with Lipids in Pre-phase of Diabetic Nephropathy

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Authors’ contributions

This work was carried out in collaboration between both authors. Author VVK designed the study, wrote the protocol, wrote the first draft of the manuscript, performed the analyses of the study and managed the experimental process. Author KSY managed the literature searches, approved of final draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Type 2 diabetes progression leads to microalbuminuria, eventually renal failure may progress End Stage Renal Disease. CD-36 is protein markedly increases in proximal tubules in diabetic nephropathy. Primary receptor such as LDLr regulates plasma LDL concentrations. In this study genetic expressions of the CD-36 and LDLr and lipids were measured to highlight their diagnostic value in early detection of diabetic nephropathy. An objective of study was to evaluate correlation between expressions of LDLr, CD-36 and Lipids in diabetic nephropathy.

Methods: Study includes 241 subjects enrolled as per as per principle of Helsinki, at the Department of Biochemistry, School of Medicine, Navi Mumbai (India). Subjects were screened for T2DM by measurement of glucose, (fasting & post-prandial), glycosylated haemoglobin, microalbumin in urine and lipids after overnight fast by photometric technique & gene expressions by rt-PCR. Statistical analysed performed by R software.

Results: LDLr and CD-36 showed high degree of expressions on rt-PCR (p<.00) in both the study.
(less than 45 years and more 45 years) groups. Cholesterol (total, HDL) and triglyceride are within normal reference range. LDL and LDL/HDL ratio rose in both study groups and showed significant p-value (p<.00).

**Interpretation and Conclusion:** At the early stage of diabetic nephropathy measurement of lipids suggests no hypercholestremia and triglyceridemia. Increased level of LDL (bad cholesterol) suggests that accumulation of lipids may take place in future course of diabetic nephropathy. LDLr and CD-36 highly significant markers can strongly predict the risk of diabetic nephropathy, at early stage; lipidogram values with marginal significance indicate kidney injury, which is suggested to prevent morbidity & mortality in diabetic nephropathy.

**Keywords:** Diabetes mellitus; diabetes nephropathy; CD-36; LDLr gene expressions; renal parameters.

**1. INTRODUCTION**

Diabetic nephropathy (DN) is an increasing cause of morbidity and mortality worldwide and the leading cause of chronic kidney disease (CKD). As per the reports published by World Health organization, 2014, 9% of adult's world population above 18 years was diabetes. Dyslipidemia in patients with Type 2 diabetes (T2DM) is a reversible risk factor for the progression of kidney disease and cardiovascular mortality [1-2]. Sustained hyperglycaemia in diabetes promotes FA synthesis and TG accumulation. Elevated serum TGs, FFAs, and modified cholesterol because ectopic lipid accumulation in non-adipose tissues, including the pancreas, heart, liver, and blood vessel walls [3-6]. This process, termed lipotoxicity, seems to play a role in other diabetic complications. Lipotoxicity and lipid accumulation cause podocyte dysfunction and apoptosis.

In T2DM nephropathy, fall of glomerular filtration rate (GFR) is usually rapid and appears to be linear with time. Thus, factors other than hyperglycaemia have been suggested to contribute to such progression. Hyperlipidemia has received attention as one of the factor incriminated in this process by participation in the progression of glomerular disease [7]. More rapid decline of renal function has been observed in diabetic nephropathy patients with hyperlipidaemia than those are without it [8].

Study done by some researchers Ravid et al. [9], Chaturvedi et al. [10] and Bonnet et al. [11] suggested that an adverse lipid profile might cause nephropathy in both type 1 and type 2 diabetic patients [9-16]. Single-gene (Mendelian) disorders with large effects are the most dramatic examples of the genetic contributions to lipid deposition in arteries [17]. 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 (CD-36), lectin-like oxLDL receptor-1 (LOX-1 or OLR-1 [18], LDL receptor (LDLR) [19]. CD-36 is a trans-membrane protein of the class B scavenger receptor family and is involved in multiple biological processes [20]. Abnormal lipoprotein metabolism noted by Hirano [21] which stated that increased CVD risk lead to cause dyslipidemia is multifactorial and complex.

Previous studies documented that all multiple lipoprotein abnormalities described in diabetic patients with nephropathy become more accentuated with increasing urinary albumin excretion [22-24]. Hyperglycaemia-induced synthesis of CD-36 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 CD-36 in DN and renal pathophysiology has not to our knowledge been described to date [20]. High ambient glucose has been shown to induce CD-36 protein synthesis in macrophages [25]. A link between diabetes and atherosclerosis: Glucose regulates expression of CD-36 at the level of translation. CD-36 protein was markedly increased in proximal tubules in human DN [26], effects of high ambient glucose on CD-36 mRNA and protein expression was examined.

CD-36 is intimately involved in lipid metabolism and homeostasis and has been strongly implicated in pathological conditions associated with metabolic dysregulation, including obesity, insulin resistance, diabetes, diabetic nephropathy and atherosclerosis [20,26]. Circulating form of CD-36 was identified in human plasma as a novel biomarker for type 2 diabetes mellitus.
Hyperglycaemia-induced synthesis of CD-36 protein has been associated with increased uptake of LDL promote atherosclerotic lesions in people with diabetes [20,25]. Katalin et al. [26] reported a new functional role for CD-36 scavenger receptor in tubular epithelial apoptosis associated with tubular degeneration and progression of DN. Thus CD-36 could have a central role in triggering diabetic nephropathy which is one of the observations of this study. 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 the importance to keep blood glucose levels within reference range.

2. MATERIALS AND METHODS

Present research conducted at Department of Biochemistry, D. Y. Patil University, Navi Mumbai. Patients referred to Diabetic OPD were recruited in this study. The enrolled patients were randomly selected & distributed into 3 different groups; subjects of T2DM between ages 30-45 years; 46-70 years duration and healthy volunteers (Non-diabetic) between 30-70 years. Ethical clearance was taken and consent form of participants documented before enrolment in this study.

T2DM 3-5 years duration, HbA1c ≥ 7.0%, pre-prandial blood glucose (FBS) ≥ 6.0 mmol/L (126 mg/dL), post-prandial glucose (PPBS) ≥ 8.0 mmol/L (156 mg/dL) and microalbuminuria (MALB) were included in this study. Subjects satisfying above criteria but suffering with chronic conditions were excluded from the study.

Other lipid parameters (cholesterol, triglyceride, LDL, HDL and VLDL) were measured and LDL: HDL ratio was calculated. All biochemical lipid parameters were measured by Dade Dimension chemistry auto-analyser (Roche Diagnostics) and LDLr and CD-36 expressions by rt-PCR (Qiagen & Takara Bio, Inc).

3. RESULTS AND DISCUSSION

3.1 Results

Screening parameters used for diagnosis of microalbuminuria & non-microalbuminuria showed significant P value. Results are expressed as means ± SD and standard error. Statistical calculations were performed using the R software package. Means in control and type 2 diabetic groups were compared.

In this study descriptive statistics (Table 1) within groups showed significant difference 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 [28]. In this study similar results were found in HDL & HDL/LDL ratio, other parameters like cholesterol, triglyceride, LDL and VLDL disagreed with the outcomes of KMA Aziz et al. [28] study on diabetic nephropathy.

In this study there was significant difference found P-value (<.000) in all study groups. High degree of significance was found in both LDLr and CD-36 (Table 2). Dyslipidimia is a risk factor in the development and progression of microalbuminuria. In this study estimated lipids showed values within reference interval but LDLr & CD-36 expressions were observed at an early stage of DN.

3.2 Discussion

CD-36 is a trans-membrane protein of the class b scavenger receptor family & is involved in multiple biological processes [20]. High ambient glucose has been shown to induce CD-36 protein synthesis in macrophages, because CD-36 protein was markedly increased in proximal tubules in human diabetic nephropathy [26]. In our study similar findings has been noted with significant level of expressions in

| Parameters                  | Groups                        | Control         | 45 years and less | More than 45 years |
|-----------------------------|-------------------------------|-----------------|-------------------|--------------------|
| Cholesterol (<200 mg/dL)    |                               | 177 ± 24.4      | 178 ± 53.1        | 191 ± 42.1         |
| Triglyceride (<150 mg/dL)   |                               | 138 ± 33.1      | 139 ± 71.1        | 161 ± 97.7         |
| HDL (3.3-4.4 mg/dl)         |                               | 46 ± 6.3        | 39 ± 6.3          | 41 ± 9.4           |
| LDL (100 mg/dL)             |                               | 101 ± 9.4       | 113 ± 39.5        | 131 ± 33.2         |
| LDL: HDL ratio (0.5-3.0)    |                               | 3.6 ± 0.92      | 3.0 ± 0.94        | 3.3±1.1            |

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. Post hoc test (P value) between study groups and gene markers (Tukey HSD)

| Dependent variable | Control group | <45 years group | >45 years group |
|--------------------|---------------|-----------------|-----------------|
| CT of LDLr         | <45 yrs       | >45 yrs         | Control         | >45 yrs         | Control         | <45 yrs         |
|                    | .00           | .00             | .00             | .00             | .00             | .00             |
| CT of CD-36        | .00           | .00             | .00             | .00             | .00             | .00             |

Fig. 1. CD-36 amplification plot

Fig. 2. LDLr amplification plot

the study group compared to control, which indicate proximal tubular injury in subjects. Glycosylated hemoglobin and albuminuria compared with CD-36 showed significant results which indicate that hyperglycemia in the blood circulation lead to progression of renal injury. CD-36 is intimately involved in lipid metabolism and has been strongly implicated in pathological conditions associated with metabolic dis-regulation, including obesity, insulin resistance, diabetes, diabetic nephropathy and atherosclerosis [20,26]. 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. [29]. Similar study published by Essam Abd-Allha et al. [30] states that small dense LDL is correlated with the incidence and severity of DN in type 2 diabetic patients. Small dense LDL is correlated with the incidence and severity of DN in type 2 diabetic 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. Similar results documented by Hirano T [21] in their study ‘High prevalence of small LDL particles in non-insulin-dependent diabetic patients with nephropathy.

4. CONCLUSION

It was concluded that early detection of renal injury with the help of routine biochemical parameters create dilemma in T2DM patients. But when these parameters results evaluated with gene expressions (CD-36 and LDLr) help in confirmation of diagnosis. This observation
strongly support risk prediction of DN in early stage. LDLr and CD36 may predict the risk of diabetic nephropathy, at the early stage of the disease; this will reduce the risk of 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.

CONSENT

All authors declare that written informed consent was obtained from patients.

ETHICAL APPROVAL

All authors here by declare 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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