Original Article

Serum megalin levels in type-2 diabetes mellitus with and without cardiovascular disease

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Abstract

Background: Low-density lipoprotein receptor-related protein-2 (LRP2), also called megalin, is a multi-ligand receptor of the LDL receptor gene family mediating reabsorption of ligands like Apo-A1. Type 2 diabetes mellitus (T2DM) may possibly disrupt megalin functions as it is found to be regulated by insulin. This might cause cardiovascular complications due to derangement in lipoprotein metabolism. The current study was carried out to assess the serum megalin levels among T2DM individuals with cardiovascular complications in the Indian population. **Methods:** This was a cross-sectional study involving 80 patients with T2DM. 40 T2DM patients with known cardiovascular disease were selected as cases and 40 of those without evidence of cardiovascular disease were selected as controls. Demographic details, DM duration and class of oral hypoglycemic agents (OHA) used were collected from medical records while details of lipid profile, fasting glucose, serum creatinine and urea were collected from laboratory information system. Serum megalin levels were estimated using left-over samples by ELISA. **Results:** The study groups showed no statistical significance in baseline laboratory parameters except for serum creatinine and low density lipoprotein cholesterol (LDL-c). Mean serum megalin levels were statistically insignificant between cases and controls (0.91 ± 0.78 ng/mL vs. 0.85 ± 0.69 ng/mL, *P* 0.74).The subgroup analysis of serum megalin levels based on OHA consumption among cases was also statistically insignificant (*P* 0.056). Pearson's correlational analysis was statistically insignificant between serum megalin and lipid profile parameters among cases ($P > 0.05$). **Conclusion:** Serum megalin alone may not serve as a biomarker for cardiovascular disease.

Keywords: Biomarker, cardiovascular risk, insulin resistance, LRP-2

Introduction

Type-2 diabetes mellitus (T2DM), characterized by dysregulation in carbohydrate, protein, and lipid metabolism, ranks as the ninth leading cause of mortality worldwide, as reported by the World Health Organization (WHO).[1] T2DM which was considered to be a disease of the opulent countries has attained epidemic proportions in India with 77 million people affected with the disease.[2] Among the complications associated with T2DM, cardiovascular disease accounts for nearly 60% of years of life lost from diabetes.[3]

Considering the association between T2DM and cardiovascular disease, the American Heart Association has even stated that diabetes is cardiovascular disease equivalent and this has even led to the hypothesis that the genetic and environmental factors are common for both these diseases.[4] The presence of a chronic

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state of hyperglycemia and hyperinsulinemia in T2DM leads to alteration in the cellular metabolism and intracellular signaling pathways. These changes in the cellular milieu provide a fertile ground for oxidative stress and increase the susceptibility to remodeling of extracellular matrix and ischemia.[5]

Low-density lipoprotein receptor-related protein-2 (LRP2, also called gp330/megalin), a member of the LDL receptor family was discovered as an auto-antigen in Heymann nephritis using an animal model.[6] It is a multi-ligand receptor of the LDL receptor gene family expressed primarily in the apical membrane of proximal tubule epithelial cells and plays a pivotal role in receptor mediated endocytosis. Apart from the kidney, megalin is also expressed in cells of the brain, intestinal brush border, gallbladder epithelium, thyroid follicles, uterus, fallopian tubes and lungs. Numerous physiologically important substrates are ligands for megalin including insulin, lipoprotein(a) (Lp(a)), albumin, haemoglobin, vitamin D-binding protein, retinol-binding protein and β2-microglobulin. Apart from these, megalin also mediates endocytosis of many toxic substances like advanced glycation end proteins. Megalin functions cooperatively by forming a complex with cubilin, a membrane protein co-expressed with megalin on the epithelial cells.[7,8] Several studies have demonstrated the role of megalin-cubilin complex in the reabsorption of ligands filtered across the glomeruli of the kidneys including Apo-A1. A genetic defect in this complex has been found to affect the plasma high density lipoprotein cholesterol (HDL-c) homeostasis and its likelihood to affect hepatic HDL-c synthesis.[9]

The role of megalin in various diseases and syndromes like diabetic nephropathy, Lowe syndrome, Dent disease, Alzheimer's disease, and gallstone disease has been studied in the recent past.[10] Studies conducted by Bryniarski MA *et al.*[11] has found the possible role of insulin in the regulation of megalin through the key regulatory proteins of insulin intracellular signalling namely phosphatidylinositol 3-kinase (PI3K), protein-kinase-B, and the mechanistic target of rapamycin (mTOR). This discovery opens a new avenue towards the possibility of disruption in megalin functions in diabetes mellitus which might lead to derangement in the metabolism of lipoproteins.

Many animal studies have demonstrated the upregulation of megalin in T2DM and its role in the development of atherosclerosis[11,12] but only a few human studies have been conducted exploring the role of megalin in T2DM. The current study was carried out to assess the serum megalin levels among T2DM individuals with cardiovascular complications in the Indian population.

Materials and Methods

A cross-sectional study was planned after Institutional Human Ethics Committee approval (IHEC/2018/Appr/Exp/099) and the study was conducted in accordance with the Helsinki Declaration of 1975, and its later amendments. A waiver of consent was granted due to anonymized data and the utilization of leftover samples for serum megalin analysis.

Study population

The study group included patients attending the medicine and endocrinology outpatient department in a tertiary care hospital in South India. T2DM patients with known cardiovascular disease were selected as cases and those without evidence of cardiovascular disease were selected as controls. A total of 80 participants, with 40 in each group were recruited.

Inclusion criteria

Cases- Adults above 18 years old diagnosed with T2DM with a duration of the disease exceeding 5 years and on oral hypoglycaemic agents with cardiovascular disease. Controls: Adults above 18 years old diagnosed with T2DM with a duration of the disease exceeding 5 years and on oral hypoglycaemic agents without cardiovascular disease.

Exclusion criteria

Patients with microvascular complications of DM, co-existing renal diseases, thyroid disorders, gall bladder diseases, acute illnesses, malignancies, type-1 DM, and patients on insulin therapy were excluded from the study.

Baseline demographic details such as age, gender, duration of DM, and class of oral hypoglycaemic agent (OHA) used were collected from medical records. Lipid profile parameters including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were collected from the laboratory information system. In addition, fasting plasma glucose, serum urea, and serum creatinine were also retrieved. The laboratory parameters were analyzed using dedicated kits and reagents in Roche Cobas 6000 auto-analyzer. Serum megalin levels were estimated by Enzyme-linked Immunosorbent Assay (ELISA) kits. (BIOCODON TECHNOLOGIES, Biocodon LLC, 6029 Broadmoore# 1006, Mission, KS 66201, USA). The study participants were rigorously selected to ensure that parameters such as age, gender, duration of diabetes mellitus, and class of OHA did not confound the results pertaining to megalin.

Statistical analysis

Statistical analysis was done using SPSS version 24. Mean ± SD was calculated for continuous variables and percentages for categorical variables. Student *t*-test was used to compare the mean values for continuous variables. Pearson's correlation analysis was done to find the association between continuous variables. One-way analysis of variance was done to compare the mean values of more than two groups of continuous variables. $P < 0.05$ was considered statistically significant.

Results

In this study, patients with T2DM with cardiovascular disease were included as cases and those without cardiovascular disease

served as controls. There were 27 males and 13 females each in the case and control group. The mean age of cases and controls was 58.08 ± 8.89 years and 55.33 ± 9.04 years respectively. Therefore, the study population was age and gender-matched.

The two study groups were matched in terms of the duration of DM. The cases exhibited duration of 10.33 ± 4.43 years, while the controls maintained duration of 11.10 ± 5.31 years (*P* 0.51). The OHA consumption pattern among study participants was as follows: Metformin and sulfonylureas, either in combination or alone, constituted the predominant group of OHAs consumed by both cases (100%) and controls (92.5%). Baseline biochemistry laboratory parameters of study participants are shown in Table 1. There was no statistically significant difference in the baseline biochemistry laboratory parameters in the study groups except for serum creatinine and LDL-c.

The mean serum megalin levels showed no statistically significant difference between cases and controls (0.91 ± 0.78 ng/mL vs. 0.85 ± 0.69 ng/mL, *P* 0.74).

Among the cases, the participants were divided based on their OHA consumption into 3 sub-groups – namely Metformin only (42.5%), Sulfonylureas only (27.5%), and Metformin and Sulfonylureas combination (30%). Megalin levels were compared among the 3 subgroups using ANOVA. The subgroup analysis of serum megalin levels was also statistically insignificant with a *P* 0.056. The mean serum megalin levels among these subgroups are tabulated in Table 2. Pearson's correlational analysis was done between serum megalin and lipid profile parameters among cases and the results are in Table 3.

Table 1: Biochemistry laboratory parameters of study participants

Parameter	Mean±SD		*P*
	Cases	Controls	
Fasting plasma glucose (mg/dL)	139.58±72.03	155.43±49.93	0.261
Serum Urea (mg/dL)	25.68±8.52	26.50±17.47	0.799
Serum Creatinine (mg/dL)	0.95±0.25	0.78±0.19	0.001*
Serum Cholesterol (mg/dL)	143.85±35.55	159.73±40.52	0.068
Serum Triglycerides (mg/dL)	145.35±96.11	141.65±74.85	0.849
Serum HDL-c (mg/dL)	40.20±10.02	43.75±12.11	0.159
Serum LDL-c (mg/dL)	92.90±32.66	109.88±39.41	0.040*

*$P<0.05$ – statistical significance

Table 2: Mean serum megalin levels among cases with different OHA consumption

Group of OHA	*n*	Megalin levels (ng/mL)	
		Mean	SD
Metformin only	17	1.25	0.72
Sulfonylurea only	11	0.75	0.79
Metformin & Sulfonylurea combination	12	0.58	0.76

Table 3: Correlational analysis between serum megalin and lipid profile parameters among cases

Variables	*r*	*P*
Serum total cholesterol (mg/dL)	-0.15	0.36
Serum triglyceride (mg/dL)	0.26	0.10
Serum HDL-c (mg/dL)	-0.16	0.33
Serum LDL-c (mg/dL)	-0.26	0.10

Discussion

Studies have shown deletion of muscle LRP2 causes insulin resistance and glucose intolerance.[13] Insulin resistance is a major risk factor for developing T2DM and is associated with plasma lipid and lipoprotein abnormalities. The predominant dyslipidaemias include reduced HDL cholesterol and elevated triglyceride levels with increased hepatic secretion of triglyceride rich very low density lipoprotein (VLDL). With this hypothesis, we sought to establish an association between serum megalin levels and lipid profile parameters. However, we found no significant correlation between lipid profile parameters and serum megalin levels in our study participants. This may be attributed to the limitation of our study with respect to statin usage history which was not collected from the participants. Although acknowledging its significant limitation, this study functions as a pilot investigation into serum megalin levels among individuals with diabetes mellitus. This preliminary exploration may pave way for future research endeavours aiming to elucidate potential associations between megalin and cardiovascular diseases.

In patients with polycystic ovary syndrome and insulin resistance, pioglitazone-induced improvement of insulin action is associated with an increase in muscle LRP2 expression. This study states a new endocrine circuit related to LRP2 is pivotal to the maintenance of normal glucose homeostasis and insulin sensitivity. Pioglitazone increases muscle LRP2 in humans with insulin resistance.[13] Hence, we also investigated the relationship between serum megalin levels and the class of OHA consumed. However, in our study, there was no significant difference in serum megalin levels in participants on different OHA.

Feiming Ye *et al.*[12] have demonstrated the regulatory role of megalin on angiotensin II (Ang II) through the renin-angiotensin system in the kidney. Hence, disruption in megalin functions due to T2DM may lead to dysregulation of the renin-angiotensin system. This could explain the development of atherosclerosis due to the effects of oxidative stress and endothelial dysfunction brought about by Ang II. This study serves as a pilot project to establish an association between serum megalin and cardiovascular risk in diabetic patients.

We conclude that serum megalin does not possess a high diagnostic value as a cardiovascular marker. Cardiovascular disease-related mortality and morbidity and the causal relationship could not be determined in this cross-sectional study. The serum megalin level was not significantly different in diabetic patients with and without cardiovascular disease. This might have been caused by the limited number of subjects included in the study.

Limitations

The limitation of serum megalin and its utilization as a diagnostic marker is predominantly a lack of cardio-specificity. Moreover, the absence of prior serum megalin level assessments in human studies poses challenges in aligning results with existing literature. Furthermore, constraints such as small sample size and omission of statin usage history among participants contribute to the complexity of data interpretation.

List of abbreviations

T2DM = Type-2 Diabetes Mellitus
DM = Diabetes mellitus
LRP2 = Low-density lipoprotein receptor-related protein-2
HDL-c = High density lipoprotein cholesterol
LDL-c = Low density lipoprotein cholesterol
OHA = Oral hypoglycaemic agent
VLDL = very low density lipoprotein
Ang II = Angiotensin II.

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Conflicts of interest

There are no conflicts of interest.

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