Indian Journal of Experimental Biology
Vol. 61, July 2023, pp. 499-504
DOI: 10.56042/ijeb.v61i07.2214

Serum triacylglycerol: A putative early biomarker of disease severity of Type 2 diabetes mellitus compared to microalbuminuria

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Received: 27 April 2023; *Revised 23 May 2023*

Poorly controlled type 2 diabetes mellitus progresses to several complications including nephropathy. While glycated hemoglobin demarcates severity, urinary microalbumin indicates renal involvement. Considering nephropathy is a late manifestation of the disease, here, we explored whether serum triacylglycerol (TAG) can be used as an early disease severity biomarker. About 100 Type 2 diabetes mellitus patients were recruited and categorized as moderate (n=43) and severe (n=57) based on glycated haemoglobin (8%) level. Duration of the disease, BMI, systolic and diastolic BP, fasting and Post Prandial plasma glucose, glycated haemoglobin, serum lipid profile and urinary microalbumin were measured. Results obtained were compared between the groups and correlated. Taking glycated haemoglobin as reference, receiver operating characteristic curves were constructed for serum triacylglycerol and urinary microalbumin excretion to check their efficacy as classifier of disease severity. Significant differences (P <0.001) were recorded for plasma glucose, glycated haemoglobin, triacylglycerol and microalbuminuria but not for other parameters. Significant association (P <0.001) of glycated haemoglobin was displayed with triacylglycerol (r=0.67), fasting (r=0.0.71) and Post Prandial (r=0.82) plasma glucose and urine microalbumin levels (r=0.54). Serum triacylglycerol and urinary microalbumin levels also showed significant correlation (P <0.001, r=0.44). ROC curve analysis showed better performance of triacylglycerol (AUC=0.97) than microalbuminuria (AUC=0.88) to demarcate severity of diabetes. The results indicate that serum triacylglycerol is a better classifier of Type 2 diabetes mellitus than urinary microalbumin level, and may help in early assessment of the disease progression.

Keywords: Diabetic nephropathy, Glycaemia, HbA1c, Hyperglycemia, Hypertriglyceridemia, Post prandial plasma glucose, Receiver operating characteristic curve

Diabetes mellitus (DM) is a multifactorial metabolic disorder due to either true insulin deficiency in type 1 or decreased peripheral response to insulin in type 2 disease, characterized by persistent hyperglycemia. As the metabolism of carbohydrate, lipid and proteinare interrelated, such altered hormonal environment in DM results in metabolic derangements of each component[1]. As per the latest World Health Organization report global prevalence of diabetes mellitus (DM) is about 422 million, responsible for nearly 1.5 million deaths per year[2].

Studies have already established obesity as one of the cornerstones for the development of insulin resistance, resultant hyperglycemia and compensatory hyperinsulinemia in type 2 DM[1]. The term diabetic dyslipidaemia comprises a quartet of elevated fasting and postprandial triacylglycerol (TAG), decreased high density lipoprotein cholesterol (HDL-C), increased low density lipoprotein cholesterol (LDL-C) and excess of small, dense low-density lipoprotein (LDL) particles[2]. Quantitative and qualitative alteration of lipid profile along with hyperglycemia have always remained the major factors behind the development of complications associated with type 2 DM, including cardiovascular diseases, retinopathy, nephropathy and neuropathy[3,4].

Hypertriglyceridemia in type 2 DM is a combined result of increased hepatic secretion of very low density lipoprotein (VLDL) and delayed clearance of triglyceride rich lipoproteins. Increased lipid peroxidation results in free radical induced membrane damage and one of the principal reasons of type 2 DM associated complications[5]. Urinary albumin leakage is a manifestation of vascular damage[7]. For long microalbuminuria (urinary albumin excretion of 30-300 mg/24 h or 30-300 mcg/mg creatinine or 20-200 mcg/min on two out of three urine collections)

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has been described as an important prognostic indicator for progressive renal damage and higher cardiovascular risk[6]. Unfortunately, relatively little attention has been paid to the serum TAG as a biomarker of disease severity and its relation with disease progression towards development of nephropathy, characterized by microalbuminuria, especially where elevation of the former is a much early incidence compared to the later one. Evaluation of such elevated serum TAG could serve as a biomarker of disease severity of T2DM in terms of development of nephropathy as traditionally indicated by microalbuminuria. Here, we have explored whether serum triacylglycerol (TAG) can be used as an early and at least, equivalent marker of disease severity compared to microalbuminuria reflecting diabetic nephropathy in a setup of type 2 DM.

Material and Methods

Selection of participants

After obtaining ethical clearance from competent authority, the study was conducted at a state level tertiary care hospital. It was a hospital based, cross sectional, comparative, observational study. Study participants were selected from the patients attended General Medicine OPD of the hospital. Freshly diagnosed, treatment naïve patients of Type 2 DM with microalbuminuria (urinary albumin excretion of 30-300 mg/24 h)[6] were recruited for the study to avoid effects of treatments with regime and dose variability. Patients with renal dysfunction due to other pathologies, liver disease, known endocrinal disorders other than insulin resistance or on medications for any other diseases were excluded from our study. Pregnant and lactating women having altered metabolic profiles, were also not been considered for the study. Total 100 participants, irrespective of gender were recruited for the study with age group between 35 and 65 years. Written and informed consent was obtained from each of them prior their participation.

Based on glycated hemoglobin level (HbA1c) as grouping variable, they were divided into two groups; (i) 43 patients as Gr. I (control group) with moderately controlled glycaemia (HbA1c<8%); and (ii) 57 patients as Gr. II (case group) with poorly controlled severe glycaemia (HbA1c≥8 %)[8,9].

As nephropathy is one of the major complications of severe long term uncontrolled diabetics, therefore, we had compared between diabetic patients (under similar altered hormonal environment) rather disease free controls. HbA1c above or below 8% was considered to categorize poorly controlled (HbA1c ≥8%) and moderately controlled (HbA1c <8%) plasma glucose respectively to indicate apparent severity[10].

Method

After 12 h of fasting, 7 mL venous blood was collected from antecubital vein. Collected sample was divided into three parts: 2 mL was collected in fluoride-oxalate vial for estimation of fasting plasma glucose (FPG); 3 mL in vial containing clot activator, and both were processed for plasma and serum for further biochemical parameter assessments; remaining 2 mL blood was transferred to EDTA vial for measurement of glycated hemoglobin (HbA1c). Two mL blood was collected again after 2 h of oral ingestion of 75 g glucose for post prandial plasma glucose (PPPG) assay. Early morning, mid-stream urine sample was collected in sterile container for urinary microalbumin assay (MAlb). Samples collected were analysed as early as possible to minimize errors.

FPG, PPPG, serum cholesterol, TAG, HDL-C and MAlb were estimated using commercially available kit from Erba Mannheim. Friedewald Formula was followed for serum LDL-C estimation. As we did not measure LDL-C by direct method, a serum TAG level ≥400 were excluded to avoid error in VLDL-C estimation. Biochemical analyses were performed by random auto analyser (Transasia-ERBA XL-600). HbA1c was estimated by ion exchange resin method. Anthropometric measurements of the participants were noted for BMI calculation. Systolic and diastolic blood pressure was measured using standard sphygmomanometer.

Statistical analysis

Data analysis was performed by SPSS software package (version 16.0). Values are expressed as Mean ± SD (Standard deviation). Significance of the difference in mean between control and case group was assessed by the student's t test (for independent samples, 2-tailed). A '*P*' value <0.05 was considered significant.Pearson's correlation analysis (1-tailed) was performed between obtained parameters from control and case groups to evaluate existence of any significant correlation between them. ROC curves were constructed individually for urinary MAlb and serum TAG for finding suitable cut-off value for describing the disease severity of and their efficacy as biomarkers.

Results

This study included 100 patients irrespective of gender distribution with mean age 58 years (range 35-65 years). As shown in Table 1, at 95% confidence interval (CI), mean BMI (kg/mt^2) did not vary significantly between the case and control group. However, mean FPG, PPPG, HbA1c, serum TAG, serum VLDL-C and urinary albumin excretion were found significantly higher among the case group (P <0.001).

When Pearson's correlation (one tailed) was performed, we found that HbA1c had significant (P <0.001) positive correlations with FPG (r=0.71), PPPG (r=0.82), urinary MAlb (r=0.54), serum TAG (r=0.67). HbA1c also showed a weak (r=0.28) but significant (P <0.05) positive correlation with serum VLDL-C.

Correlation of serum TAG and urinary MAlb with HbA1c had ascertained that, both urinary albumin excretion and serum TAG have significant capability to estimate disease severity. Also interestingly, urinary MAlb was found to have significant positive correlation with serum TAG (P <0.001; r=0.44) and serum VLDL-C (P <0.05; r=0.37).

At 95% CI, Receiver operating characteristic curve (ROC) was prepared for serum TAG and urinary MAlb taking HbA1c as reference and the best cut off values were determined. The ROC curve analysis showed the Area under the curve (AUC) for serum TAG was 0.97 (Lower bound: 0.940, Upper bound: 0.999), (P <0.001) (Table 2). At the cut off value to 285 mg/dL, both sensitivity and specificity were 93%. The ROC curve for urinary MAlb showed AUC 0.88 (Lower bound: 0.812, Upper bound: 0.943) with (P <0.001).At the cut off value of 50 mg/L, it showed 93% sensitivity and 65% specificity. Thus, when sensitivity, specificity and AUC results were combined, TAG was found to be an excellent classifier of disease severity (Fig. 1).

Discussion

This study showed significantly higher level of FPG, PPPG, HbA1c, Serum TAG, VLDL-C and urinary albumin excretion among case (poorly controlled) group. At 95% Confidence Interval (CI) these parameters also showed significant positive correlation with HbA1c. Higher FPG, PPPG, HbA1c levels in case group and their positive correlations are easily understandable and are not emphasized further.

Table 1 — Comparison of mean between control and case group at 95% CI

Parameters	Control group (HbA1c<8%) n=43; CI=95%	Case group (HbA1c>8%) n=57; CI=95%
SBP (mm/Hg)	135±18	137±21
DBP (mm/Hg)	84±11	84±11
BMI (Kg/Mt^2)	23.71±3.2	24±3.9
Fasting plasma glucose (mg/dL)	147±23	229±85**
Post prandial plasma glucose (mg/dL)	253±40	394±106**
HbA1c %	7.7±0.47	11.1±1.74**
Total cholesterol	210.2±60.19	224.87±59.96
Serum TAG (mg/dL)	211±69	362±110**
LDL cholesterol	123.49±34.82	131.28±49.12
HDL cholesterol	56.86±23.9	64.49±24.09
VLDL cholesterol	42.3±13.91	72.47±22.05*
MAlb (mg/L)	43.86±14.99	80.49±30.41**

[*P <0.05 and **P <0.001]

Table 2 — Area under the curve (AUC) results of serum TAG & urinary MAlb (n=100)

Test result variable (s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
TAG	0.969	0.015	0.000	0.940	0.999
MAlb	0.878	0.033	0.000	0.812	0.943

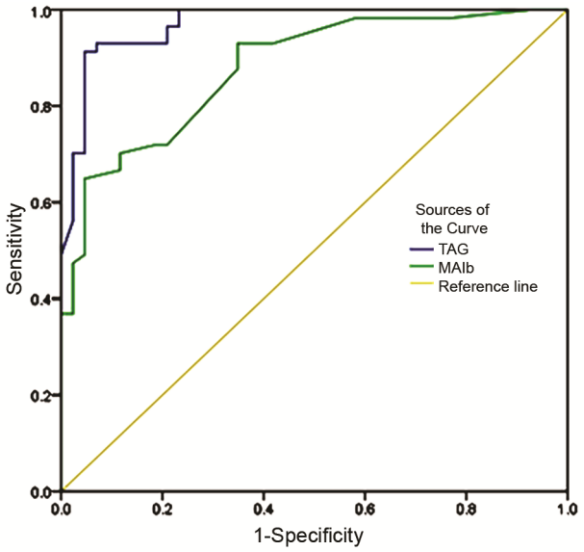

Fig 1 — ROC Curve comparison between serum triacylglycerol (TAG) and urinary microalbuminuria (mMAlb) in reference to HbA1c

Type 2 DM has two important arms, dyslipidaemia and DM specific complications including nephropathy. Relative insulin deficiency in Type 2 DM puts the system in a hyperglycemic metabolic environment. Decreased cellular glucose uptake and utilization force the system to use stored lipid (TAG) as the alternative source of energy. Glucagon, catechol amines and other lipolytic factors then take the leading role in metabolism. Increased lipolysis in

adipocytes by hormone sensitive lipase releases free fatty acids (FFA) in circulation which reach to the liver. Insulin inhibits the ribosomal synthesis of $ApoB_{100}$ and conversion of smaller and dense VLDL2 to larger and TAG rich VLDL1 in liver[11]. Insulin also activates lipoprotein lipase, essential for circulating TAG rich VLDL1 catabolism which releases FFA from VLDL1 for uptake by adipocytes. By down-regulating FOXO1, insulin suppresses hepatic Apo CIII expression. Free Apo CIII (especially monosialylated $apoC\text{-}III_1$) has been described as a major regulator for VLDL1 catabolism and thus an important modulator of plasma TAG level. Increased level of monosialylated apoC-III1 has been associated with decreased catabolism of VLDL112. Thus, insulin resistance promotes increased synthesis, secretion and decreased clearance of VLDL1 resulting in hypertriglyceridemia in type 2 DM.

However, other than lipolytic, glucagon and catechol amines also have neoglucogenic actions[13]. Thus, further addition of glucose from non-carbohydrate substances even worsens the hyperglycemia, if not intervened with oral hypoglycemic or insulin administration. Persistent hyperglycemia generates advanced glycation end products (AGEs) which are non-enzymatic and irreversible glycation of proteins which are pro inflammatory[14].

Many researchers have described the mechanisms behind oxidative stress and generation of reactive oxygen species (ROS) in type 2DM at molecular level[15-19]. In addition, advanced oxidation protein products (AOPP) are formed in type 2 DM due to oxidative stress by the hypochlorus acid (produced by myeloperoxidase in activated phagocytes). These compounds are dityrosine containing crosslinked proteins which have been implicated with vascular endothelial dysfunction and thus nephropathy[20]. Thus, persistent hyperglycemia and oxidative stress generate AGEs and AOPPs that synergistically act as pro inflammatory substances for vascular endothelial tissue which results in nephropathy and microalbuminuria in type 2 DM.

High level of circulating non-esterified fatty acids are taken up by albumin and delivered to the proximal tubular cells in a “Trojan horse” manner. Fatty acid accumulation and its metabolites then exhibit toxicity towards the host cells[21]. On the other hand, TAG rich circulating VLDL itself increases cytokine production e.g., monocyte chemotactic protein-1 (MCP-1) in mesangial cells that invokes increased monocytes adhesion[22]. Studies have suggested that oxidized LDL increases Lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) activity and inflammatory marker Intercellular adhesion molecule 1 (ICAM-1) expression in the rat renal epithelial cell line NRK52E[23]. Finally, saturated free fatty acid mediated apoptosis in mesangial cells and podocytes has also been implicated[24]. Thus, other than glycation end products, dyslipidemia and hypertriglyceridemia independently can exhibit toxicity towards renal vascular endothelial cells, mesangial cells and proximal tubular cells which results in nephropathy in type 2 DM, and such nephrotoxicity is mainly by fatty acid mediated. Injury to renal micro-vasculature and ultrastructural changes lead to disruption of glomerular filtration barrier which eventually leads to albumin leakage and microalbuminuria[25]. Thus, from above discussion it is evident that increased degree of insulin resistance and hyperglycemia results in corresponding increased serum TAG level.

We have studied both parameters by ROC and AUC analysis to evaluate their efficacy as biomarkers for disease severity. The result showed that at their best cut off values (50 mg/L for MAlb and 285 mg/dL for TAG), and TAG to be significantly much better classifier of disease severity than MAlb.

Metabolic alteration and hypertriglyceridemia develops much earlier in type 2 DM, far ahead of progression to nephropathy. Also having significant cytotoxic potency, it is one of the major causes behind its pathophysiology. On the other hand, microalbuminuria is the effect of nephropathy and it is not as early marker of disease severity as initially thought to be, and therefore search for an alternative is still on[26]. Further, its role in describing disease severity and predictability to disease progression has found to be limited[27,28].

Conclusion

Hypertriglyceridemia is an independent risk factor for kidney dysfunction in patients with Type 2 diabetes mellitus. Urinary albumin excretion, though is a direct marker of nephropathy, as the extreme effect, it can serve as a measure of the disease severity only after development of nephropathy. Having a major role in pathophysiology of nephropathy, it develops much early in a setup of insulin resistance than the disease progress to nephropathy, and thus Malb excretion. Our present study showed that TAG estimation as a biomarker of disease severity, may lead to early, easy and better prognostic outcome,

especially in a setup of Type 2 DM where lipid profile estimation is a very frequent added laboratory test. Furthermore, as it is relatively easy to control dyslipidemia, and thus the TAG level with lifestyle modification and medications to prevent or at least delay the progression to nephropathy. Due to time and other resource constraint, the study was performed within a limited time period and therefore, it was not possible to follow up the cases for a long-time scale. However, the promising results of the study further warrants a prospective cohort study with more number of participants of Type 2 DM induced nephropathy. Evaluation of serum TAG and urinary MAlb over the time along with HbA1c estimation, and thus the disease progression is required to draw a concrete conclusion.

Acknowledgement

This study is a primary work of Late Dr. Kanika Mandi Chowdhury, Ex Associate Professor, College of Medicine & Sagar Dutta Hospital, Kamarhati, Kolkata, West Bengal, India.

Conflict of interest

Authors declare no competing interests.

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