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Analysis of Postprandial Hyper-triglyceridemia in Type 2 Diabetes Mellitus (T_2DM) Patients with Presence of Micro-albuminuria

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Abstract It is postulated that in patients with Type 2 Diabetes Mellitus (T_2DM) and presence of microalbuminuria (MA) shows higher postprandial triglyceride than those without MA. The present study therefore analyze the potential association and to elaborate the degree of dependence of T_2DM with MA condition on onset of high postprandial (PP) triglyceridemia. A total of 49 patients with T_2DM were included in the study during January 2012 and December 2015 and were divided into two groups according to the presence (n = 22, MA+ve) or absence of MA (n = 27, MA-ve). Blood was drawn in the fasting state and at 2 and 6 h after the standard mixed breakfast test meal for biochemical parameters. Plasma Biochemical components such as Plasma ApoA, triglycerides, glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, creatinine, Insulin and Glycosylated hemoglobin A1c (HbA1c) levels and urinary components such as Urinary Albumin and micro-albumin were determined using standard methods on Cobas 6000 c501, Hitachi 912, Elecsys 2010 and Cobas e411. Twenty four hr urinary albumin and urinary microalbumin showed highly significant difference ($P<0.001$) in values in MA-ve and MA+ve groups, whereas glycosylated HbA1c and duration of T_2DM doesn't exhibit any significant difference. Biochemical constituents such as glucose, total cholesterol and HDL-cholesterol exhibited mild ($P< 0.05$) to moderate ($P< 0.01$) significance when compared within the groups of MA-ve and MA+ve patients in fasting and postprandial conditions. Comparatively highest level of constantly significant difference in values was noted only in triglycerides when MA+ve was compared with MA-ve, which remains high not only at 2 hrs postprandial ($P<0.001$) but also after 6 hrs under same conditions ($P<0.001$). The data strongly support the postulation that T_2DM patients with co-existence of MA have significant hypertriglyceridemia, which further complicates already existing co-morbid hyperlipidemic state in these patients.

Keywords Type 2 Diabetes Mellitus (T_2DM), microalbuminuria (MA), hypertriglyceridemia, hyperlipidemic, dyslipidemia

Introduction

Most of the patients with T_2DM exhibited altered postprandial lipemia after a standard traditional meals [1-6] and the data gathered by epidemiological reviews strongly suggest that high plasma TG levels, both in the fasting state and in postprandial condition, are associated with cardiovascular and macrovascular diseases in patients with diabetes [1, 7-10]. It is an already known fact that diabetic dyslipidemia is a known condition in Type 2 Diabetes

Mellitus (T_2DM) and is characterized by high levels of fasting triglycerides (TGs), low high density lipoproteins (HDL) cholesterol levels, and with high proportion of low density lipoprotein (LDL) cholesterol [1,3,4,11,12]. Moreover, it is well documented that macrovascular complications are the leading cause of morbidity and mortality in patients with (T_2DM) [1,11-14]. One of the risk factor for macrovascular diseases in patients with T_2DM is microalbuminuria (MA) with prevalence rates of 10–48% [1,15,16]. Several physiological and clinical anomalies have been described in diabetic patients with MA, which includes high blood pressure, dyslipidemia, insulin resistance, endothelial dysfunction, left ventricular hypertrophy, hypercoagulation, high plasma homocysteine and C-reactive protein levels, that ultimately leads to cardiovascular degradation [17]. Furthermore, an association between dyslipidemia and more specifically postprandial lipemia (triglycerdemia) has been established in patients with T_2DM with MA [1,2] demonstrating that T_2DM patients with the presence of MA had higher postprandial triglyceride than those without MA. Therefore, the present study described the analysis of several plasma and urinary components regarding association of T_2DM having MA with onset of high postprandial (PP) triglyceridemia in our setting.

Materials and Methods

Selections of Patients

Protocols of Tentoulouris *et al.*, 2007 [1] and Alam et al., [2] were followed to standardize all procedures. A total of 49 patients with T_2DM were examined. Patients were recruited from the outpatient clinics of Government Lyari General Hospital and Liaquat National Hospital, Karachi during January 2012 and December 2015. Patients with evidence of existing conditions that may cause dyslipidemia, macroalbuminuria, abnormal liver or thyroid function and those treated with medications affecting plasma lipids (statins, fibrates, ezetimide), urinary albumin excretion (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers), and LPL activity (heparin in the previous 3 months, glitazones) were excluded as per recommendation reported earlier [1,2,18,19]. Current smokers were also excluded to avoid potential effect of smoking on plasma lipid levels. Patients were divided into two groups according to the presence or absence of MA.

Research designs, Procedures and anthropometric measurements:

Each patient attended the OPD unit of hospitals in the morning after a 12–14 h fast. The antidiabetic medications were given at the end of the visits in the unit. Patients were permitted to consume only water during the study. Blood was drawn in the fasting state and at 2 and 6 h after the test meal for biochemical analytes. For postprandial determinations, patients received a standard mixed breakfast consisting of four slices of toast bread, around 225 g of a low-fat cheese, and 40 g of butter (total energy, 783 kcal: 52.5% as fat, 20% as protein, and 27.5% as carbohydrates, mainly as complex carbohydrates) as per recommended in an earlier study [1]. After processing plasma samples were stored in 2 ml aliquots at -20°C until assayed at the end of the study. All anthropometric measurements and procedures were performed as detailed earlier [1,2]. Briefly, weight and height were measured using standard methods. Furthermore, body mass index (BMI) and waist-to-hip ratio (WHR) were measured and calculated. In addition, records of myocardial infarction, heart diseases and from history of angina or coronary revascularization procedure were retrieved from HIS. Similarly, cerebro-vascular disease was evaluated by history, clinical examination, and hospital records of stroke, whereas arterial blood pressure was measured in the sitting position on three occasions with an interval of 1 min between determinations.

Analysis of Biochemical parameters

Plasma ApoA concentrations were measured at baseline and at the time of the peak concentration of plasma TGs (2 h and 6 h after the test meal) on Hitachi 912 (Roche Diagnsotics, Basil) (own reference) by immunoturbidimetric method. Plasma glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, creatinine and Glycosylated hemoglobin A1c (HbA1c) levels were determined on Hitachi 912 chemistry analyzer and Cobas 6000 c501. MA in urine samples was determined by Immunoturbidimetric method (Roche Diagnostics, Basil). Serum insulin levels

were determined by electrochemi luminescence (ECL) technology on Elecsys 2010 and Cobas e411 ((Roche Diagnostics, Basil).

Statistical Analysis

Dyslipidaemia was taken to be present when the total cholesterol was note to be >216.50 mg/dl and/or triglycerides >183.80 mg/dl, LDL >131.50 mg/dl, and/or HDL <35.20 mg/dl[1]. Fasting blood glucose was measured by glucose oxidase method and ranges recommended by American Diabetic Association were used as references. Glycosylated haemoglobin (HbA1C) value of less than 7% was taken to indicate good glycemic control. Statistical analysis was performed using programs available in the SPSS 13.0 statistical package (USA). Student's t-test was used to compare parameters between patients with and without MA. One-way ANOVA was used to assess differences in the tested variables, as well as for repeated measurements to test the timing effect of the studied parameters after the test meal. Paired Student's t-test was used for comparison of the differences of the values of the studied parameters in the postprandial and the baseline state. Comparisons in the values with and without MA were performed using Pearson's correlation. $P < 0.05$ (two-tailed) was considered statistically significant.

Results

The present study describes the postprandial biochemical parameters, especially triglyceride, in T_2DM patients with and without MA. After fasting blood sampling, a normal breakfast proceeds, followed by after 2 hrs and 6 hrs blood collection for the evaluation of blood chemistry in 27 T_2DM patients without MA (MA-ve) and 22 T_2DM patients with onset of MA (MA+ve). Anthropometric data shows male to female ratio at 62.96% to 37.03% in MA-ve group as compared to 59.09% to 40.98% in MA+ve group (Table 1). Other parameters such as Age (years) , Body mass index (kg/m2) , Waist (cm), Waist-hip ratio, Systolic blood pressure (mmHg), Diastolic blood pressure (mmHg), Duration of diabetes (years), Glycosylated hemoglobin A1c (%), 24hr urinary albumin (mg/24 hr) and Urinary microalbumin (mg/dl) were also assessed and except Waist (cm) ($P < 0.002$), 24hr urinary albumin (mg/24 hr) ($P < 0.001$) and Urinary microalbumin (mg/dl) ($P < 0.001$), all are noted to be non-significant.

Table 1: Comparative Anthropometric and Clinical characteristics of the patients (n = 49) grouped according to the presence (n = 22) and absence (n = 27) of microalbuminuria

Anthropometric and Clinical Characteristics	**Without micro-albuminuria (MA-ve) (n = 27)**	**With micro-albuminuria (MA+ve) (n = 22)**	***P***
Male/female, n (%)	17 (62.96%)/10 (37.03%)	13 (59.09%)/9 (40.98%)	*0.20*
Age (years)	57.45 ± 6.90	56.60± 9.35	*0.40*
Body mass index (kg/m2)	30.30 ± 5.20	32.40 ± 6.55	*0.08*
Waist (cm)	113.45 ± 25.35	119.30 ± 27.50	*0.002*
Waist-hip ratio	0.96 ± 0.08	1.09 ± 0.09	*0.08*
Systolic blood pressure (mmHg)	131.5 ± 12.45	138.0 ± 15.55	*0.21*
Diastolic blood pressure (mmHg)	88.55 ± 7.40	89.10 ± 7.60	*0.38*
Duration of diabetes (years)	7.5 ± 1.90	7.8 ± 2.45	*0.93*
Glycosylated hemoglobin A1c (%)	8.05 ± 2.25	8.90 ± 3.45	*0.18*
24 h urine albumin (mg/24 h)	7.80 ± 2.75	64.50 ± 13.55	*0.001*
Urinary microalbumin (mg/dl)	18.10 ± 3.00	59.35 ± 11.45	*0.001*

Data are shown as means ± SD or n (%).

Biochemical parameters were also assessed in both group of MA+ve and MA-ve T_2DM patients, such as Glucose (mg/dl), Insulin (uU/ml), Total cholesterol (mg/dl),Total Triglyceride (mg/dl), HDL cholesterol (mg/dl), LDL cholesterol (mg/dl) and ApoA-1 (mg/dl) (Table 2). In this aspect, the other three lipoidal components, i.e. TG, LDL-

cholesterol and ApoA, in addition to the hormone, insulin, exhibited inter-group significant difference varying from $P<0.05$ to $P<0.001$ and fasting and postprandial difference ranging from $P<0.03$ (insulin) to $P< 0.001$ (TG). Comparatively highest level of constantly significant difference in values was noted only in triglycerides when MA+ve was compared with MA-ve, which remains high not only at 2 hrs postprandial ($P<0.001$) but also after 6 hrs under same conditions ($P<0.001$). This strongly support our theory and observations that in patients with T_2DM and existence of MA, high triglyceride levels co-exists which further complicates the already present hyperlipidemic state in these patients.

Table 2: Comparative Fasting and postprandial (PP) characteristics of the biochemical parameters in patients with (MA+ve) and without (MA-ve) microalbuminuria

Biochemical parameters	Fasting levels	After 2 hr (PP)	After 6 hr (PP)	*P*
Glucose (mg/dl)				
MA-	145.40 ± 8.25	187.35 ± 6.70	165.55 ± 7.80	*0.04*
MA +	185.35 ± 10.55	234.65 ± 20.85	190.25 ± 15.40	
Insulin (uU/ml)				
MA-	15.05 ± 3.35	50.45 ± 7.50	20.15 ± 3.10	*0.03*
MA+	29.25 ± 6.45	98.30 ± 10.75	45.30 ± 5.40	
Total cholesterol (mg/dl)				
MA-	170.45 ± 19.95	195.05 ± 20.05	203.30 ± 24.10	*0.10*
MA+	200.10 ± 20.35	210.65 ± 22.15	201.50 ± 20.45	
Total Triglyceride (mg/dl)				
MA-	108.55 ± 10.65	141.60 ± 10.70	140.15 ± 12.20	*0.001*
MA+	126.45 ± 20.10	198.25 ± 20.10	180.10 ± 21.55	
HDL cholesterol (mg/dl)				
MA-	39.10 ± 6.25	41.50 ± 7.54	40.55 ± 4.80	*0.22*
MA+	35.10 ± 5.55	35.45 ± 6.70	36.20 ± 5.45	
LDL cholesterol (mg/dl)				
MA-	138.50 ± 10.91	132.45 ± 10.54	136.35 ± 10.95	*0.05*
MA+	134.10 ± 6.10	138.60 ± 10.85	151.45 ± 11.20	
ApoA-1 (mg/dl)				
MA-	121.15 ± 20.35	149.35 ± 10.40	132.45 ± 10.75	*0.01*
MA+	141.45 ± 16.65	183.40 ± 20.10	152.35 ± 21.35	

-Normal reference ranges: Glucose (random) = 80-160 mg/dl; Glucose (fasting) = < 100 mg/dl; Insulin = 2 -25 uU/ml; total cholesterol = ≤ 200 mg/dl; total triglycerides = 70-150 mg/dl; HDL- Cholesterol = ≥ 35 mg/dl; LDL-cholesterol = ≤ 130 mg/dl; Apo A-1 = 104-225 mg/dl.

-Data presented as means ± SD. *P (< 0.05)* indicates the result of ANOVA, student's t test for repeated measurements within each group and between the two groups (MA- vs MA+)

Discussion

In present study, we noted that postprandial elevation in Apo A was highest in patients with MA and lipemia. Previously similar state was noted and suggest of the fact that Apo-A mechanism for regulation of TG metabolism is not impaired in patients with MA [1,2]. In agreement with previous findings [1,2] our patients with MA exhibited higher insulin values than the patients without MA. It was documented that the clearance of TG-rich lipoproteins may not be impaired in subjects with MA, the enhanced postprandial lipemia in these patients may be attributable to an altered metabolism of TG-rich lipoproteins in the intestine [1,7,20]. Our study and the two reported earlier [1,2] showed that the subjects with MA had higher insulin resistance compared with subjects without MA, and the increase of postprandial lipemia was mainly attributable to the increase of intestinally derived TGs. Therefore, these findings support the idea that TG-rich lipoprotein accumulation and secretion in the intestine may be different in patients with MA.

Researchers and clinicians reported that high postprandial lipemia is related to proatherogenic conditions, as exposure to postprandial lipoproteins is associated with cardiovascular diseases [1,8,9,11,12] Moreover, it was noted that normotriglyceridemic patients with T_2DM and MA have an almost 3-fold higher postprandial triglyceridemia than patients without MA after ingestion of a mixed test meal. Studies showed that lipid metabolism in diabetes and overt nephropathy is a possibility [9,21,22]. However, previous studies have proved that patients with T_2DM had higher and more prolonged increments in plasma TGs after a mixed meal compared with healthy individuals [4-7]. It is reported that the abundant offer of free fatty acids, glucose, and chylomicron remnants to the liver in the presence of insulin resistance results in the overproduction of large very low density lipoprotein particles, which compete with intestinally derived CM for clearance via the same lipolytic pathway [4-7]. Furthermore in the postprandial state, similar to that of hypertriglyceridemic patients, there is abundant formation of atherogenic small, dense LDL particles and less formation of antiatherogenic large HDL2 particles [1,7,8]. Furthermore, high plasma TG levels are associated with changes in hemostatic factors that promote the risk for thrombotic events [22].

In past two decades, several authors hypothesized that type 2 diabetes mellitus and insulin resistance are associated with enhanced postprandial lipemia [23]. They also suggested that Insulin is effective in reducing both fasting and post prandial total triglyceride levels as well as triglycerides contained in the triglyceride-rich lipoprotein sub-fractions. Review of many rapid-acting insulin analogues showed that it seems to be more effective in the reduction of postprandial lipemia than the short-acting human insulin. Certain past reports specifically targeted postprandial hypertriglyceridemia as a risk factor for cardiovascular disease in Type 2 diabetes and studied its mechanisms and related biochemical determinants, such as that of apolipoprotein which was identified as a modulator of triglyceride (TG) metabolism [24]. Additionally, not only hyper TG but also hyperglycemia have been identified as risk markers for cardiovascular disease in women as well [25].

Conclusion

In conclusion MA is associated with enhanced postprandial lipemia in comparatively normo-triglyceridemic diagnosed with T_2DM. Review of the literature and evaluation of past and recent studies provided substantial evidences which clearly suggest that postprandial lipemia is atherogenic.

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