Comparative study of postprandial hypertriglyceridemia in diabetic patients with occurrence of microalbuminuria

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ABSTRACT:
It has been demonstrated that Type 2 Diabetes Mellitus (T_2DM) patients, with the presense of microalbuminuria (MA) had higher postprandial triglyceride than those without MA. The present study further investigates this potential association and to elaborate the degree of dependence of T_2DM with MA condition on onset of high postprandial (PP) triglyceridemia in our setting. A total of 32 patients with T_2DM were included in the study during February 2007 and December 2008 and were divided into two groups according to the presence (n = 15, MA+ve) or absence of MA (n=17, MA-ve). Blood was drawn in the fasting state and at 2 and 6 h after the standard mixed breakfast test meal for biochemical analytes. Plasma ApoA, triglycerides, glucose, total cholestrol, HDL-cholesterol, LDL-cholesterol, creatinine and Glycosylated hemoglobin Alc (HbAlc) levels were determined using standard methods. 24 hr albumin and urinary microalbumin showed highly significant difference (*P<0.001)* in values in MA-ve and MA+ve groups, whereas glycosylated HbAlc 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 theory and observations that in patients with T_2DM and co-existence of MA, hypertriglyceridemia prevails, which further complicates the already co-morbid hyperlipidemic state in these patients.

Key words: Type 2 Diabetes Mellitus (T_2DM), microalbuminuria (MA), hypertriglyceridemia, hyperlipidemic, dyslipidemia.

INTRODUCTION

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 predominance of small, dense low density lipoprotein (LDL) cholesterol particles[1-3]. Furthermore, most of the patients with T_2DM exhibited altered postprandial lipemia after meals[1,4-6]. 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[1,7,8]. It is well documented that macrovascular complications are the leading cause of morbidity and mortality in patients with T_2DM[1,9,10]. One of the risk factor for macrovascular diseases in patients with T_2DM is microalhuminuria (MA) with prevalence rates of 10-48% [1,11,12]. Some of physiological and clinical anomalies that have been described in diabetic patients with MA, includes high blood pressure, dyslipidemia, insulin resistance, endothelial dysfunction, left ventricular hypertrophy, hypercoagulation, high plasma homocysteine and C-reactive protein levels, and leads to cardiovascular degradation[13]. Recently, an association between dyslipidemia and more specifically postprandial lipemia (triglycerdemia) has been recognized in patients with T_2DM with MA[1]. The study demonstrated that T_2DM patients with the presence of MA had higher postprandial triglyceride than those without MA. Therefore, the present study was undertaken to further investigate this potential association and to elaborate the degree of dependence of T_2DM with MA condition on onset of high postprandial (PP) triglyceridemia in our setting.

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MATERIALS AND METHODS

Patients:

Protocols of Tentoulouris *et al.,* 2007[1] was followed to standardized all procedures. A total of 32 patients with T_2DM were examined: Patients were recruited from the outpatient clinics of Government Lyari General Hospital and Liaquat National Hospital. Karachi during February 2007 and December 2008. 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[1,14,15]. 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.

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 insulin levels were determined by electrochemi luminescene (ECL) technology on Elesys 2010 (Roche Diagnostics, Basil).

Statistical Analysis

Dyslipidaemia was taken to be present when the total cholestrol 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 17 T_2DM patients without MA (MA-ve) and 15 T_2DM patients with onset of MA (MA+ve). The average age was 60.55 ± 5.9 yrs. and 63.50 ± 6.1 yrs., respectively. Male to female ratio was 58.8% and 41.17% in MA-ve and 60.0% and 40% in MA+ve groups (Table 1). Physical characteristics such as waist (cm) and waist to hip ratio was significantly different ($P< 0.002$ and $P< 0.05$, respectively) when compared in both groups; however other clinical characteristics shows no significant vatiations. Similarly 24 hr albumin and urinary microalbumin showed highly significant difference ($P<0.001$) in values in MA-ve and MA+ve groups, whereas glycosylated HbAlc and duration of T_2DM doesn't exhibit any significant difference. Biochemical characteristics in fasting and postprandial conditions showed variable levels of significance. For example glucose, total cholesterol and HDL-cholestrol exhibited mild ($P< 0.05$) to moderate ($P< 0.01$) significance when compared within the groups of MA-ve and MA+ve patients. However when same analytes were compared in fasting 2 hrs and 6 hrs postprandial conditions in their respective group, no significant difference was noted even at significance level of $P<0.05$. In this aspect, the other three lipoidal components, i.e. TG, LDL-cholestrerol 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.

DISCUSSION

It is reported that high postprandia lipemia is related to proatherogrenic conditions, and clinial studies provide evidence that exposure to postprandial lipoproteins is associated with cardiovascular diseases[1,7,8]. A variety of in vitro and clinical studies suggest that postprandial lipoidal components are associated with adverse effects on vascular endothelium[5]. In a recent study 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. Uptil now little is known about the effect of MA on postprandial lipemia, although lipid metabolism in diabetes and overt nephropathy has been examined extensively by reserchers over several decades[8,16,17]. 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[3-6]. TG derived from hepatics and intestine contributes to the exaggerated postprandial lipemia in individuals with T_2DM[5]. 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 intesitinally derived CM for clearance via the same lipolytic pathway[3-6]. 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[1,6,7]. Furthermore, high plasma TG levels are associated with changes in hemostatic factors that promote the risk for thrombotic events[(17)].

In present study, the 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]. In agreement with previous findings[1] our patients with MA exhibited higher insulin values than the patients without MA. It is postulated 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. It was stated earlier that emerging evidence strongly suggests that animal models with insulin resistance have increased prodution of the gighly atherogenic apoB by the intestine, which may explain the profound postprandial lipemia observed in insulin resistant[1,6,18]. Our study and the one reported earlier[1], 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 supports the idea that TG-rich lipoprotein accumulation and secretion in the intestine may be different in patients with MA. However, other mechanisms cannot be excluded that may also be involved in the exaggeration of postprandial lipemia associated with MA. The mechanism may be related to alterations in other apolipoproteins affecting lipid metabolism or decreased activity of lipoprotein receptors[1,6,18].

Several physicians, clinicians and researchers has attributed postprandial lipemia as an independent risk factor for coronaty artery disease and carried out case-control and cohort studies in this regard[19]. For example a recent review examined the effect of the medications used for the management of diabetes, obesity and dyslipidemia on postprandial lipemia. The authors hypothesized that type 2 diabtes mellitus and insulin resistance are associated with enhanced postprandial lipemia[19]. 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 short-acting human insulins. For example, acarbose emeliorates postprandial lipemia and reduces the atherogenic chylomicron and very low density lipoprotein remnants, whereas, metformin reduces both fasting and postprandial triglyceridemia, fasting and post-prandial free fatty acids and may increase the concentrations of the high density lipoprotein cholesterol. In additions, the repotrts noted that sulfonylureas reduce fasting and postprandial triglyceride levels while data on the effect on high density lioprotein levels are inconsistent, whereas, pioglitazone has additional beneficial effets on lipid metabolism because it reduces postprandial free fatty acids, fasting postprandial triglycerides and increases high density lipoprotein cholesterol levels[19].

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 (apo) which was identified as a modulator of triglyceride (TG) metabolism[20]. A study was conducted with 11 patients with Type 2 diabetes mellitus showed that postprandial apoAV was elevated in diabetic patients after ingeston of lipid-rich cream[20]. In recent past, not only hyper TG but also hyperglycemia have been identified as risk mrkers for cardiovascular disease in women as well[21]. Moreover, their complete mechanism and the parameters associated with these postprandial responses are largely undetermined until lately. Few studies have been conducted in near past seeking objectives to assess whether usually measured clinical and biochemical parameters can predict postprandial glucose and triglyceride responses and whether these responses are associated with each other. The reports site two groups of postmenopausal women, one with normal glucose metabolism (NGM) and other with type 2 diabetes mellitus (T_2DM)[21]. Both groups received two consecutive fat-rich meals and carbohydrate-rich meals on separate occasions[21]. Postprandial analysis showed that women with NGM, fasting triglycerides, hemoglobin A(1c), total cholesterol, and, inversely, high-density lipoprotein cholesterol were independently associated with triglyceride-iAUC whereas age and fasting triglycerides were independintly associated with glucose-iAUC. Howeveer in women with T_2DM, fasting triglycerides were independently associated with triglyceride-iAUC, where as hemoglobin A(1c) and fasting glucose were stronger than fasting triglycerides associated with glucose-iAUC. The researchers strongly argued that commonly measured clinical and biochemical parameters can partly explain postprandial glucose and triglyceride excursions[21].

In patients with myocardial infraction (MI), or cardiovascular complications, especially in elderly, postprandial triglycerride-rich lipoproteins (TRL) levels is a major problem and documented as a predictor for coronary atherosclerosis[22]. Comparison of fasting high density lipoprotein (HDL) cholesterol, plasma lipoprotein lipase (LPL) activity, and postprandial TRL were conducted between elderly survivors (65-85 years of age) of myocardial infarction (MI) and healthy controls. The patients were given a standard oral fat load with subsequent blood sampling over the next 8 h[22]. Multiple regression analysis revealed postheparin LPL activity as an independent predictor for postprandial TRL and fasting HDL cholesterol. Logistic regressions analysis revealed HDL cholesetol, triglycerides measured 2 h after the oral fat load, and postheparin LPL activity as independent predictors for MI. Their findings indicate that decreased fasting HDL cholesterol is associated with increased postprandial triglyceridemia which could be a target for life-style and therapeutic interventions in patients at risk for which could be a target for life-style and therapeutic interventions in patients at risk for cardiovascular disease[22].

In conclusion MA is associated with enhanced postprandial lipemia in normotriglyceridemic patients with T_2DM. Review of the literature and evaluation of past and recent studies provided substantial evidences which clearly suggests that postprandial lipemia is atherogenic; and the findinges of all related studies, including the present one, are noteworthy and may explain in part the excess cardiovascular disease risk in patients with T_2DM and MA. However, further studies are needed to examine whether reduction of MA restores the exaggerated postprandial lipemia in patients with T_2DM and MA ?. Furthermore, as stated eartlier[1], studies also required to elucidate the underlying mechanisms responsible for the abnormalities in lipid metabolism in the postprandial state in this group of patients.

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Table 1: Anthropometric and Clinical characteristics of the patients (n = 32) grouped according to the presence and absence of microalbuminuria.

Characteristics	Without MA (n = 17)	With MA (n = 15)	P
Male/female, n(%)	10 (58.8%) / 7 (41.17%)	9(60.0%)/6 (40.00%)	0.59
Age (years)	60.5±5.9	63.5 ± 6.1	0.40
Body mass index (kg/m2)	29.1± 4.9	31.29 ± 4.3	0.09
Waist (cm)	100±15.4	110.5 ± 0.10.9	0.002
Waist-hip ratio	0.91±0.08	0.99 ± 0.09	0.05
Systolic blood pressure (mmHg)	129.1±13.6	136.0 ± 12.4	0.34
Diastolic blood pressure (mmHg)	81.7±6.30	84.1 ± 7.55	0.38
Duration of diabetes (years)	8.5±2.15	8.0 ± 3.20	0.93
Glycosylated hemoglobin A1c(%)	7.12±1.15	7.4 ± 1.60	0.18
24 h urine albumin (mg/24 h)	7.35±1.21	72.6 ± 11.62	0.001
Uninary microalbumin	20.00±2.12	53.12 ± 10.29	0.001

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

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

Parameter	Fasting	2 hr	*6 hr*	*P*
Glucose (mg/dl)				
MA-	161 ± 5.11	194 ± 4.65	171 ± 4.67	0.30
MA+	191 ± 3.5	229 ± 5.43	198 ± 6.01	
Insulin (uU/ml)				
MA-	17.0 ± 2.13	59.2 ± 5.67	23.6 ± 2.65	0.03
MA+	28.1 ± 4.23	109.4 ± 12.5	50.2 ± 6.54	
Total cholesterol (mg/dl)				
MA-	181.5 ± 9.54	189.0 ± 14.22	197.5 ± 11.29	0.69
MA+	190.4 ± 12.21	199.4 ± 12.32	201.5 ± 10.43	
Total Triglyceride (mg/dl)				
MA-	107.4 ± 8.91	138.5 ± 9.45	136.8 ± 9.12	0.001
MA+	120.3 ± 11.09	182.1 ± 10.32	160.3 ± 11.45	
HDL cholesterol (mg/dl)				
MA-	38.6 ± 2.22	39.5 ± 3.43	39.6 ± 4.06	0.22
MA+	36.5 ± 3.32	36.7 ± 4.25	37.5 ± 4.21	
HDL cholesterol (mg/dl)				
MA-	136.5 ± 8.91	131.4 ± 10.54	137.4 ± 8.98	0.05
MA+	130.4 ± 5.67	132.7 ± 9.87	141.5 ± 9.11	
ApoA-1(mg/dl)				
MA-	125.8 ± 11.21	142.5 ± 7.32	129.6 ± 6.78	0.01
MA+	138.4 ± 9.22	178.7 ± 6.55	141.2 ± 8.67	

- 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 indicates the result of ANOVA, student's test for repeated measuements within each group and between the two groups (MA- vs MA+).