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Serum lipoprotein (a) levels in type 2 diabetes mellitus patients

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Abstract
Objective: Compare the influence of diabetes control treatment on serum lipoprotein (a) concentrations.
Setting: Patients of know diabetics attending or admitted to GGS Medical College, Faridkot and Hospital.
Design: In this context lipoprotein (a) concentrations were compared with normal control group, a group of diabetic patients with glycated hemoglobin (HbA1c) less than 8.20%, and a group of diabetic patients with HbA1c of 8.0% or higher.
Patients: One hundred normal controls and 130 diabetic subjects (75 with insulin-dependent diabetes mellitus and 55 with noninsulin-dependent diabetes mellitus).
Results: Sixty patients with HbA1c levels of 8.0% or higher had higher (25 mg/dL) median levels of lipoprotein (a) when compared with either 100 normal controls (8.8 mg/dL) or 55 diabetic patients with HbA1c less than 8.0% (7.5 mg/dL) (P = 0.008 and P = *0.012,* respectively). A similar pattern of distribution of lipoprotein (a) levels according to extent of control was seen in patients with insulin-dependent diabetes mellitus and noninsulin-dependent diabetes mellitus. No difference in the lipoprotein (a) distribution was noted between diabetic men and women. No correlation was observed between lipoprotein (a) levels and total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels.
Conclusion: Lipoprotein (a) levels are elevated in poorly controlled diabetic patients. Increased levels of lipoprotein (a) may be a contributing factor to the high risk for atherosclerosis observed in diabetic patients.

Keywords: HbA1c, diabetic, lipoprotein, cholesterol, insulin, metabolic, glycated

Introduction

It was Susruta in the 15th century who described 'Honey Urine'(Madhu Meha) a term used for sweet urine. Mathew Dobson first established the presence of sugar in blood and urine of diabetic patients [1]. We investigated the changes in parameters of blood coagulation and the fibrinolytic system and in plasma levels of lipoprotein (a) (Lp a)) in 130 patients with type II diabetes mellitus and 44 healthy control subjects matched for age and body mass index (BMI) to determine whether hemostatic disturbances may lead to increased cardiovascular mortality. Median levels of fibrinogen ($P <$ 0.0001), thrombin-antithrombin III complex (TAT) ($P <$ 0.005), and plasminogen activator inhibitor-1 (PAI-1). The incidence and prevalence of type 2 DM is globally increasing and becoming a major public health problem for health care providers [2]. Currently the number of cases of diabetes worldwide is estimated to be around 150 million. This number is predicted to double by 2025 [3]. Phospholipids play an important role in stabilizing lipoproteins; they are synthesized by the liver and include lecithins and sphingomyelins. All these molecules do not circulate freely, as they are not soluble in the aqueous environment of the plasma, but are linked by hydrophobic bonds to transport proteins, called apoproteins, that keep lipids in solution in the form of micelles. This linkage between lipids and apoproteins result in the formation of Lipoproteins [7]. Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disorder characterized by chronic hyperglycemia caused by decreased production or sensitivity to insulin [1]. Chronic hyperglycemia results in a number of complications including cardiovascular disease (CVD). Diabetic patients have more than double the risk of CVD-related mortality when compared to age-matched controls [2]. The traditional cardiovascular risk factors associated with diabetes are not sufficient to explain the high rate of cardiovascular incidents in diabetic patients. Lipoprotein (a) (Lp(a)) is an emerging cardiovascular risk factor which is also associated with diabetes [3, 4] lipoprotein a is a low-density lipoprotein (LDL) particle with the glycoprotein apo(a) covalently bound to apo B-100 [5]. Apo(a) is a highly glycosylated hydrophilic apolipoprotein which is synthesized by the liver. Apo (a) is structurally homologous to the plasma protein plasminogen which is involved in the lysis of clots [6]. Like plasminogen, apo(a) is composed of two kringle domains and a serine protease domain. The size of one of the domains, kringle IV type 2, exhibits a wide variation and hence the size of apo(a) is heterogeneous among the general population [7]. The concentration of Lp(a) in plasma depends on the isoform of kringle IV type 2 which is genetically determined. The size of the apo(a) isoform inversely correlates with its plasma concentration and accounts for about 40% of its variation in plasma concentration [8]. Apo (a) by its similarity to plasminogen can competitively inhibit the functioning of this zymogens and hence increase the risk of atherosclerotic vascular disease. The similarity of Lp(a) to LDL and its ability to undergo oxidation are another reason why it has been implicated in atheroma development and has been suggested to be involved in foam cell formation, smooth cell proliferation, endothelial dysfunction, and vascular inflammation [9, 10]. Several epidemiological studies have found clear association between Lp(a) and CVD and have

suggested Lp(a) to be an independent risk factor for CVD [11, 12]. Guyton *et al*. observed racial differences in mean Lp(a) levels. In their study, they found that Black participants had almost double the amount of mean Lp (a) levels compared to Whites [13]. Differences in mean Lp (a) values among different ethnic groups were also noted by Sandholzer *et al*. [4] in past Lp(a) levels among Blacks concluded that higher Lp(a) levels did not contribute to any increased cardiovascular risk among the Black population [3, 5, 6]. However, Virani *et al*. in a 20-year follow-up study have shown that high Lp(a) levels are equally and positively associated with the incidence of CVD in both Blacks and Whites [7]. The relation between Lp(a) and T2DM is still not clear with some studies showing a higher level of Lp(a) in diabetics [18] and some showing a decreased level or no difference between diabetics and controls [2]. In African studies, increased Lp(a) has generally been observed in T2DM patients [2, 3].

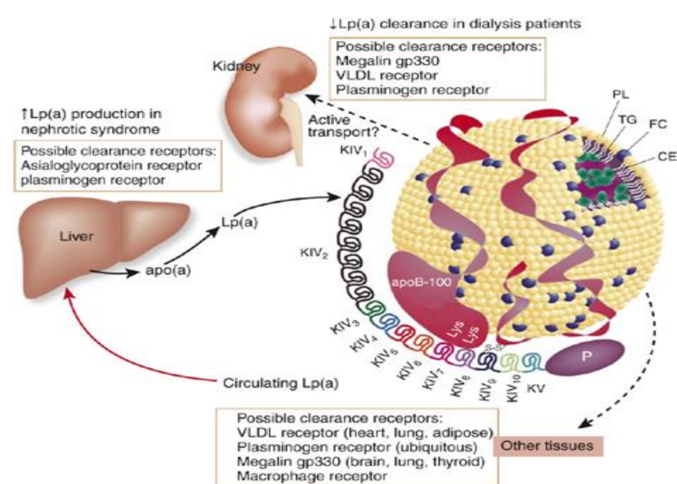

Fig 1: Lipoprotein

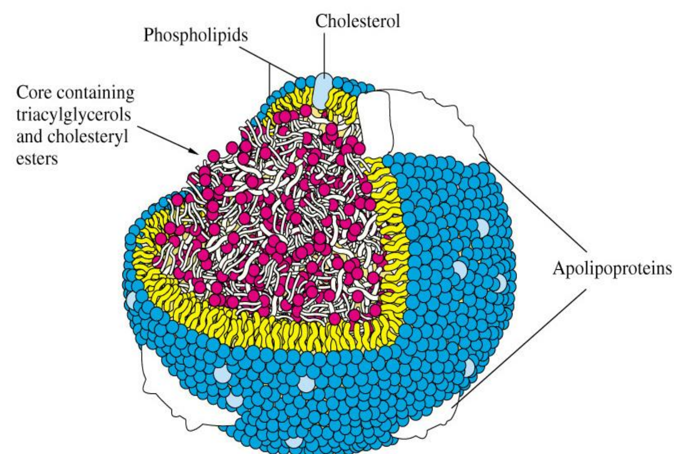

Fig 2: Typical Lipoprotein

Materials & Methods

A total of 230 participants (100 controls and 130 patients with type 2 diabetes) were recruited for the study. The mean age of the controls was 52± 6.3and that of the diabetic patients was 61 ± 5.6. The number of male participants was 70 (34 controls and 36 diabetics), and females were 60 (33 controls and 37 diabetics). Participants with a positive clinical history of T2DM were approached. The participants were excluded if they had any other chronic diseases apart from diabetes and hypertension. Participants with known diabetic complications (micro- and macrovascular complications) and those on insulin treatment and lipid-lowering medication were also excluded. Healthy control participants were selected from the local population. The participants were included in the study following informed consent. Interviewer administered questionnaires were used to obtain information on the duration of diabetes, medication, and lifestyle. The blood pressure and anthropometric measurements of all participants were also taken. The body mass index (BMI) was calculated as the weight (kg)/height (m) [2]. Blood and urine samples were collected from the participants following overnight fast into appropriate vacutainer tubes and sterile urine containers. The serum or plasma was removed within an hour and refrigerated at −80°C for analysis. Apart from a comprehensive metabolic panel, the samples were also used for analyses of urinary albumin and creatinine, insulin, and C-reactive protein (CRP), all of which were performed at the using the Roche Cobas 6000 autoanalyzer. Following evaluation of the results, participants with signs of liver disease, renal disease, and infection were excluded. Serum samples which were stored at −80°C were used in the determination of the Lp (a), total antioxidant (TAO) capacity, and oxidized LDL (ox-LDL) concentration, within a month. The serum TAO capacity was measured by a commercial kit from Sigma-Aldrich using the ABTS method. Lp(a) and ox-LDL were worked out by kits from Seimen's The absorbance was read using a analyzer at 450 nm. The ox-LDL assay uses murine monoclonal antibody mAb-4E6 directed against oxidized apo B, while the Lp(a) assay uses antibodies directed against epitopes of apo(a).The data was analyzed statistically using the IBM Statistical Package for the Social Sciences Beckman's, version 16). as mean ± standard deviation (SD) and the independent sample -test was done to check for the difference between groups. Data which did not exhibit normal distribution was expressed as median and interquartile range and the difference between groups was determined by Mann–Whitney test. Participants were divided into tertiles according to their serum Lp(a) levels for correlation analysis: tertile 1 (), Lp(a) < 320 U/L; tertile 2 (a), Lp(a) = 320–728 U/L; (n=28)and tertile 3 (35), Lp(a) > 728 U/L. Spearman's correlation tests were performed to analyze the correlation between the variables within the various groups. Kruskal-Wallis test was used to check correlations between other variables and Lp(a) across the tertiles was considered as statistically significant.

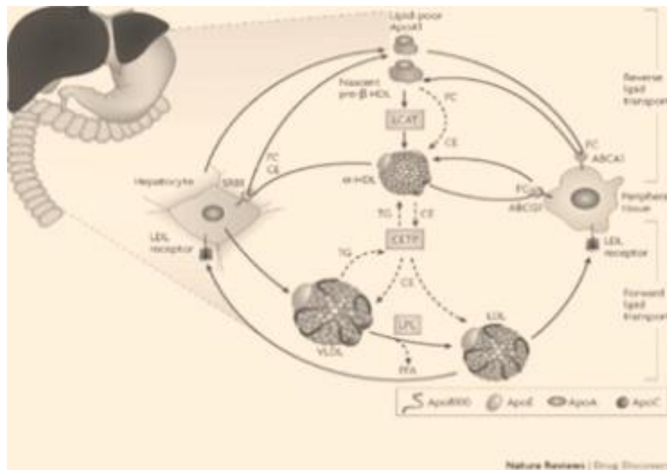

Fig 3: Lipoprotein metabolism

Discussion

This study revealed that there was a significantly higher level of Lp (a) among the T2DM patients when compared to normal people. Several researchers have reported similar findings in T2DM patients [1] 3.1 ± 0.88 3.1 ± 0.889,[2]. Studies on Lp (a) levels in Africa have also reported elevated levels in the T2DM population. Mohieldein *et al*. in 2014 reported that T2DM patients in Sudan had a significantly higher Lp (a) level [22]. This was similar to the finding of Ogbera and Azenabor in Nigeria [2, 3]. The high level of Lp (a) in diabetics can be because of the decreased clearance of Lp(a) from the serum due to glycation of its apolipoproteins [2, 5]. Kadkhodaei *et al*. in 2006 stated that the glycated Lp (a) in the diabetic population is significantly higher in the diabetic population [2, 6]. In this study, the patients with longer duration of diabetes had a higher level of Lp (a). This is similar to the findings of Chandni and Ramamoorthy [2, 7] the Link in Lp(a) levels and duration of diabetes is clinically important because the duration of diabetes confers a twofold increase in the risk of vascular complications [2, 8].

The Lp(a) levels in the diabetic population correlated significantly with the LDL cholesterol levels in this study. This correlation has been observed in other studies also [2]. This correlation can be attributed to the contribution of the Lp(a) cholesterol levels in the calculation of LDL cholesterol using the Friedewald formula. Lp(a) has been shown to be independent of or weakly associated with the concentrations of other lipoproteins in other studies [9, 3]. The Lp(a) levels showed a significant correlation with the amount of ox-LDL in the diabetic group. This correlation between Lp(a) and ox-LDL was not observed among the controls. In diabetics, there is increased oxidation of lipoproteins [2, 3] including Lp(a) because it is as prone as LDL to oxidation [3, 5]. Moreover, in vitro studies have shown the preferential transfer of oxidized phospholipids from ox-LDL to Lp(a) thereby increasing the oxidation of Lp(a) [6, 7]. The oxidatively modified Lp(a) can cross-react with the antibodies used in the ox-LDL analysis. The diabetic patients had increased levels of inflammation as shown by the increased CRP levels. The diabetic patients also had increased oxidative stress as evidenced by the increased ox-LDL levels and decreased TAO capacity. Some scientists stated that increased levels of oxidized phospholipids observed during inflammation and oxidative stress may cause the overexpression of Lp(a). The increased Lp(a) can bind and transport the oxidized phospholipids thereby ameliorating the inflammation and oxidative stress. However, in high concentrations, this beneficial Lp(a) molecule can become proinflammatory and atherogenic [3, 7]. This is similar to our observation among diabetic individuals: a negative correlation was observed between CRP and the first two tertiles of Lp(a). However, the third tertile of Lp(a) was positively and significantly correlated to CRP, TG, and ox-LDL levels. Increased levels of ox-LDL and Lp(a) have been found to be independently associated with a poor prognosis following acute myocardial infarction [3, 8]. Hence, the increase in both ox-LDL and Lp(a) among the diabetics in this study not only exposes them to an increased risk of a cardiovascular event but also can negatively impact the prognosis of these patients following such an event.

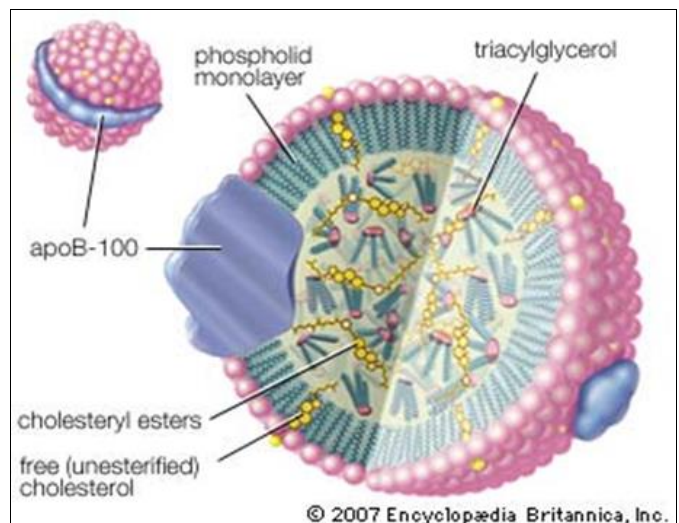

Fig 4: Schematic Diagram of Lipoproteins

Conclusion

T2DM is associated with increased levels of various cardiovascular risk factors including Lp(a) and ox-LDL. Very high levels of Lp(a) among diabetics are proinflammatory.

Table 1: Clinical and metabolic characteristics of type 2 diabetics and healthy controls.

Variable	Control	Diabetic	Diabetic
BMI (kg/m^2)	26.1 ± 5.2	31.4 ± 7	31.4 ± 7
Waist-hip ratio	0.78 (0.75 0.89)	0.82 (0.77–0.90)	0.82 (0.77–0.90)
Total cholesterol (mmol/L)	4.8 (3.9–5.4)	4.9 (3.9–5.3)	4.9 (3.9–5.3)
HDL (mmol/L)	1.4 (1.1–1.6)	1.2 (1.0–1.4)	1.2 (1.0–1.4)
Triglycerides (mmol/L)	1.1 (0.9–1.5)	1.3 (1.1–1.6)	1.3 (1.1–1.6)
LDL (mmol/L)	2.8 (2.2–3.2)	2.9 (2.0–3.4)	2.9 (2.0–3.4)
CRP (mg/L)	1.7 (1.0–4.2)	4.0 (2.3–7.7)	4.0 (2.3–7.7)
TAO capacity (mM)	0.6 ± 0.23	0.5 ± 0.24	0.5 ± 0.24

Data is shown as mean ± SD (standard deviation) or median (interquartile range). The mean difference is significant at. BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein

Data is shown as mean ± standard deviation. The median difference is significant at p >005.

Table 2: Median Lp (a) between the groups

CRP	−0.33	0.42	−0.67	51.8 ± 8.6	0.73	0.007
TG	0.11	0.79	0.32	7.8 ± 5.6	0.48	0.023
Tao	−0.55	0.87	−0.16	9.9 ± 3.3	−0.23	0.026
Ox-LDL	0.01	0.96	0.06	5.1 ± 1.0		0.048

Data is shown as mean ± standard deviation. The median difference is significant at p >005.

The median difference is significant at p >005

Table 3: Clinical characteristics of diabetic patients by Lp (a) tertiles.

	Tertile 1	**Tertile 2**	**Tertile 3**	**P value**
Age	46.4 ± 9.5	47.3 ± 8.2	51.8 ± 8.6	0.051
Diabetic duration	1.7 ± 2.9	4.0 ± 3.3	7.8 ± 5.6	0.038
HbA1c	8.3 ± 1.4	9.6 ± 2.6	9.9 ± 3.3	0.403
Total cholesterol	4.6 ± 1.1	4.7 ± 0.77	5.1 ± 1.0	0.12
LDL	2.7 ± 1.0	2.7 ± 0.79	2.7 ± 0.79	3.1 ± 0.88
Ox-LDL	87.3 ± 30	117 ± 24	3.1 ± 0.88	0.09
CRP	3.1 ± 1.9	5 ± 3.5	141.7 ± 26.6	0.048
TAO	0.64 ± 0.3	0.61 ± 0.3	7.7 ± 4.4	0.057
TG	1.45 ± 0.5	1.36 ± 0.3	0.53 ± 0.1	0.041

Data is shown as mean ± standard deviation. The median difference is significant at. <.oo5

Table 4: Correlation of different tertiles of Lp (a) with CRP, TG, TAO, and ox-LDL.

	Tertile 1		**Tertile 2**		**Tertile 3**	
	r	p	r	p	R	P
CRP	−0.33	−0.67	−0.67	0.87	0.73	0.007
TG	0.11	0.32	0.32	0.30	0.48	0.023
TAO	−0.55	−0.16	−0.16	0.46	−0.23	0.026
Ox-LDL	0.01	0.06	0.06	0.84	0.44	0.048

The median difference is significant at p >005.

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