

# High risk levels of lipoprotein(a) in Pakistani patients with type 2 diabetes mellitus

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## ABSTRACT

**Objective:** To find the prevalence of high risk levels of lipoprotein(a) [Lp(a)] and the ratio between low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in patients with type 2 diabetes mellitus (DM) as evidence has been provided that Lp(a) and LDL can act additively in the development of atherogenesis.

**Methods:** This cross sectional study was carried out at the Department of Chemical Pathology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from February 2001 to May 2001. The patients participating in the study were diagnosed cases of type 2 DM. Fasting blood samples were analyzed for Lp(a), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucose and glycosylated hemoglobin. The data was analyzed by computer software SPSS 10.

**Results:** The data was analyzed by considering Lp(a) levels

<30 mg/dl as desirable level and >30mg/dl as the high risk level. It was found that in the control group 73.3% of individuals had desirable levels of Lp(a) while 26.7% had high risk levels. In diabetic patients with good glycemic control 56.6% of patients had desirable levels of Lp(a) while 43.4% had high risk levels. The same data was also analyzed by taking Lp(a) levels of <20 mg/dl as desirable levels and the same pattern was observed.

**Conclusion:** Diabetic patients have elevated levels of serum Lp(a) as compared to healthy subjects and the frequency of high risk levels of Lp(a) is also higher in diabetics compared to non-diabetic subjects. The increased prevalence of high risk levels in patients with type 2 DM may be due to increased prevalence of low molecular weight isoforms of apoprotein(a) [apo(a)].

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A large number of studies have shown that cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients suffering from type 2 diabetes mellitus (DM) as compared to normal individuals. The complications of DM are both micro and macroangiopathy. Microvascular complications of DM include retinopathy, nephropathy, peripheral neuropathy and autonomic neuropathy. While macrovascular complications of DM are myocardial infarction, stroke and peripheral vascular disease.<sup>1,2</sup>

Diabetes mellitus is one of the most common causes of secondary dyslipidemias.<sup>3</sup> Atherogenic dyslipidemia in diabetic patients is often called diabetic dyslipidemia. It is characterized by elevated levels of low-density lipoprotein (LDL), very LDL and lower levels of high-density lipoprotein (HDL) often referred to as lipid triad.<sup>4</sup> An issue of considerable interest is the relative contribution of each component of atherogenic dyslipidemia to coronary artery disease (CAD) risk. Growing evidence suggests that all the components of

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lipid triad are independently atherogenic.<sup>5</sup> Evidence has been provided that lipoprotein(a) [Lp(a)] and LDL can act additively in the development of atherogenesis. Within population the plasma Lp(a) levels can vary from <0.5 mg/dl to >200 mg/dl.<sup>6</sup> The cutoff Lp(a) value to classify subjects as being at increased risk for CAD varies greatly among studies and ranges from 20-40 mg/dl. Given the uncertainty related to Lp(a) cutoff value, it has been suggested that clinicians should use a conservative Lp(a) value of 20 mg/dl, particularly in patients with concomitantly elevated LDL cholesterol.<sup>7,8</sup> A meta-analysis of 27 prospective studies with information on 5436 CAD cases observed during mean follow up of 10 years provided the most reliable assessment of the association between plasma Lp(a) and CAD.<sup>9</sup> The relative risk of myocardial infarction has been reported to be 1.75 fold higher when Lp(a) levels are >30 mg/dl.<sup>10</sup> In another study, Lp(a) was suggested to be one of the important risk factors for venous thromboembolism during childhood.<sup>11</sup> Lipoprotein(a) continue to emerge as a potent CAD risk factor in whites and determination of Lp(a) levels may provide an important contribution to the clinical assessment of individuals at high risk for CAD or of patients with existing CAD.<sup>12</sup> The aim of this study was to find the prevalence of high risk levels of Lp(a) and the ratio between LDL and HDL in patients with type 2 DM as evidence has been provided that Lp(a) and LDL can act additively in the development of atherogenesis.

**Methods.** This study was carried out at the Department of Chemical Pathology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan. Sixty-two patients suffering from type 2 DM and 40 healthy individuals were studied. The subjects were divided into 3 groups as following: 1) group A - control subjects, 2) group B - patients with poor glycaemic control (HbA1c >7.5%) and 3) group C - patients with good glycaemic control (glycosylated hemoglobin [HbA1c] <7.5%). The patients participating in the study were diagnosed cases of type 2 DM. Thirty-five patients were males and 27 were females. Their height was measured in centimeters and weight in kilograms. Body mass index (BMI) was calculated by the following formula: BMI = body weight in kilograms / height (square meters). All the patients were in a stable metabolic condition. History was taken regarding any disease that could affect the metabolic status of the body and the parameters studied such as nephrotic syndrome, acute or chronic renal failure, thyroid disorders, acute infections, diabetic ketoacidosis and non-ketotic hyperosmolar diabetes. Patients having any of the above mentioned disorders were excluded from the study. Those patients giving history of familial hypercholesterolemias, ischemic heart disease or myocardial infarction were also excluded from the study. The history of medication was recorded and the patients taking lipid lowering agents, oral contraceptives and steroids were also excluded. Finally 62 patients were selected for this study. The subjects included in the control group were healthy individuals. They were not

suffering from any acute infection or any metabolic or psychological disorder. They had no family history of hypercholesterolemias or DM. Their lipid profile and fasting blood glucose were estimated. They had normal lipid profile and fasting blood glucose level <6.1 mmol/l (110 mg/dl). All the tests were run in duplicate and the average of the 2 readings was taken as the final result. Glucose was estimated by glucose oxidase phenyl ampyrone (GOD-PAP) method, an enzymatic colorimetric method with the kit supplied by Linear Chemicals (Cat No. GL-5083). The instrument used was Selectra 2 autoanalyzer. Ion exchange resin separation method was used for estimation of glycosylated hemoglobin. The kit was supplied by Stanbio Glycohemoglobin [Pre-Fil]. Serum HDL and LDL were measured by cholesterol oxidase phenol ampyrone method, an enzymatic colorimetric method, using kits of Merck System. Lipoprotein(a) was estimated by enzyme linked immunoabsorbant assay. The kits used were supplied by Innogenetics Biotechnology for Health Care, Belgium. The wells of polystyrene microplate strips had been coated with a mouse monoclonal anti Lp(a) [antibody to Lp(a), which constitutes the solid phase antibody]. The test sample was incubated in such a well. Lipoprotein(a) present in the sample, standards and controls, was bound to the solid phase antibody. Unbound substances were removed by washing the plate. Subsequently a sheep anti-apo B polyclonal antibody [antibody against apoprotein B-100], which had been labelled with the enzyme horse-radish peroxidase (HRP), was added. This labelled antibody was bound to any solid phase antibody, Lp(a) complex previously formed, since it could bind the apo B moiety of the Lp(a) complex. Incubation with the enzyme substrate produced a blue color in the test well, which turned into yellow when the reaction was stopped with sulphuric acid. The amount of color produced in the wells was proportional to the concentration of Lp(a) originally present in the sample or standard solution.

The data was analyzed by computer software program SPSS 10. The test used for significance was Student's t test. The relative percentage distribution of individuals in different groups with desirable and high risk levels of Lp(a) was determined.

**Results.** Clinical characteristics and LDL/HDL ratio is given in **Table 1**. The BMI was significantly higher in diabetic patients irrespective of glycaemic control ( $p < 0.001$ ). It was also found that LDL/HDL ratio was found to be significantly higher in diabetics as compared to normal subjects. When this ratio was compared between control subjects and diabetics with good glycaemic control the difference was non significant. There was a significant difference in LDL/HDL ratio when diabetics with good and poor glycaemic control were compared ( $p < 0.01$ ). The relative percentage distribution of subjects falling into desirable and high risk level categories was calculated in various groups and all diabetics as a whole group (**Table 2**). We

analyzed the data by taking 30 mg/dl of Lp(a) as the cutoff level for high risk cases. Any subject with Lp(a) 30 mg/dl was taken as high risk level. It was found that in the control group 73.3% of individuals had desirable levels of Lp(a) while 26.7% had high risk levels. In diabetic patients with good glycemic control 56.6% of patients had desirable levels of Lp(a) while 43.4% had high risk levels. Similarly, the percentages in diabetics with poor glycemic control were 43.3% and 56.7%. When data was analyzed for all diabetics together, it was found that 48.3% had high risk levels. As some of the scientists are of the view that 20 mg/dl of Lp(a) should be taken as the cutoff value for high risk levels<sup>13</sup> therefore, we also compared the data accordingly. The same trend was found with some difference in percentage values (**Table 3**).

**Discussion.** The major clinical objective in the management of DM is to control hyperglycemia and the long term objective is to prevent microvascular and macrovascular complications.<sup>14</sup> Glycemic control improves and may even normalize triglyceride and HDL-C levels in type 1 DM patients.<sup>15</sup> We also found a significantly higher levels of LDL/HDL in diabetics as compared to healthy subjects. An interesting finding was the observation of a non-significant difference of lipid profile between control subjects and diabetics with good glycemic control. The positive improvement in lipid profile with glycemic control is evident from many studies. Epidemiologic analysis of the United Kingdom Prospective Diabetes Study (UKPDS) data has shown that a continuous relationship exists between the risk of microvascular complications and degree of glycemic control.<sup>16</sup> Wide differences in Lp(a) values may be both method and population dependant and constitute a serious obstacle to clinicians in the interpretation of patient values and in the correct assessment of risk. The first report of Lp(a) concentrations in DM was given by Scherthaner et al.<sup>17</sup> These investigators suggested that Lp(a) concentrations were similar in diabetics and non-diabetic subjects. However, 14% of diabetic patients had Lp(a) concentrations >20 mg/dl compared with only 5% of control subjects. In this study, no distinction was made between type 1 and type 2 DM. Another cross sectional study<sup>18</sup> reported relatively increased Lp(a) levels in type 1 and type 2 diabetics when compared to normal glycemic control subjects. These investigators found no relation between HbA1c and Lp(a) concentrations. To determine the influence of improvement in glycemic control with intensive insulin therapy on Lp(a) concentrations in type 1 diabetic patients Perez et al<sup>19</sup> studied 105 poorly controlled type 1 diabetic patients without diabetic complications. After 3 months of intensive therapy, all patients exhibited improved glycemic control. However, although a more favorable lipoprotein profile was obtained, no significant changes in Lp(a) concentrations were observed in the whole group of patients or in patients with baseline Lp(a) levels of  $\geq 30$  mg/dl or <30 mg/dl. These data demonstrated that the

improvement of glycemic control does not influence plasma Lp(a) concentrations in type 1 diabetic patients.<sup>19</sup> In an interesting study by Takayama et al,<sup>20</sup> it was found that the concentration of Lp(a) in serum was dependent on size of apoprotein(a) [apo(a)] isoforms in normal subjects and has an inverse relationship with the molecular weight of apo(a) isoforms. They found significantly raised levels of Lp(a) in diabetics. Takayama et al<sup>20</sup> reported that high risk levels of Lp(a) (>25 mg/dl) in diabetics were 39% while 15% in control subjects. Our study reveals similar results with different percentages. Further supporting evidence in favor of the present study is the finding of Ribault et al<sup>21</sup> who found that the frequency of apo(a) isoforms was significantly different in type 1 and type 2 DM and a higher prevalence of isoforms of low molecular weight was observed in type 2 DM.<sup>21</sup> So most probably our patients have small size isoforms of apo(a), which result in higher levels of Lp(a).

In another study, no difference in Lp(a) concentrations was observed between diabetics and control subjects.<sup>22</sup> The prevalence of high risk cases in this study was 6% in type 2 diabetics and 12% in control. These findings are in contrast with our study. Racial and geographical variation in the levels of Lp(a) may be one of the reasons for their findings. The findings of Francis et al<sup>23</sup> support our data. They observed increased levels of Lp(a) in type 2 diabetics with raised prevalence of high risk levels of Lp(a) (>25 mg/dl) and the effect of glycemic control had a positive trend on Lp(a) levels but it did not reach the level of significance. Moreover, similar to our findings they also observed positive correlation of Lp(a) with total cholesterol and LDL-C but not with triglycerides and HDL-C. The results of the present study are in accordance to Ramirez et al<sup>24</sup> who found elevated Lp(a) levels in a combined group of type 1 and type 2 diabetic subjects relative to non diabetic subjects. They found that the levels of Lp(a) were similar in the control group and the diabetics with good glycemic control (HbA1c < 8%). When the diabetics with poor glycemic control (HbA1c) were compared with well controlled diabetics, a significant rise was found in poorly controlled diabetics. They noticed a proportionately higher percentage of patients (61%) with high risk levels of Lp(a) (>20mg/dl) in the poorly controlled diabetics while there were only 30% in the well controlled diabetics and 31% in the control group. One possibility for increased concentration of Lp(a) in diabetics could be explained by understanding the assembly of apo(a) with Apo-B 100 moiety of LDL apo(a) is primarily synthesized in the liver and is poured into the circulation, where LDL moiety is attached to it.<sup>25</sup> Thus, if higher number of LDL molecules are present in the circulation, there would be more union between LDL and apo(a) and thus higher concentrations of Lp(a). Therefore, the results of the present study are related accordingly. But this cannot be the sole explanation for this association since the metabolic pathways of LDL and Lp(a) are quite different. Further studies are needed in this regard. Despite

Table 1 - Clinical characteristics and lipid profile of control, DM patients with good glycaemic control (HbA1c &lt;7.5%), poor glycaemic control (HbA1c ≥ 7.5%) and all diabetic patients.

Clinical profile	Control N=40	DM patients with HbA1c < 7.5% N=32	DM patients with HbA1c ≥ 7.5% N=30	All DM patients N=62
Gender (male/female)	23/17	18/14	17/13	35/27
Age (years)	44.66 ± 1.12	49.53 ± 2.18	51.14 ± 1.71	50.38 ± 1.43
BMI (kg/ m <sup>2</sup> )	24.01 ± 0.38	27.46 ± 0.91*	26.29 ± 0.72 *	26.88 ± 0.58 *
Serum LDL/HDL ratio	2.26 ± 0.09	2.77 ± 0.27†	4.34 ± 0.35 *	3.56 ± 0.24 *

Data is expressed as mean ± SEM, \*p < 0.001 as compared to control, †p < 0.01 as compared to diabetics with poor glycaemic control, BMI - body mass index, LDL - low-density lipoprotein, HDL - high-density lipoprotein, DM - diabetes mellitus, HbA1c - glycosylated hemoglobin

Table 2 - Relative percentage distribution of control, DM patients with good glycaemic control, DM patients with poor glycaemic control and all DM patients into desirable [Lp(a) &lt; 30 mg/dl] and high risk level categories [Lp(a) ≥ 30 mg/dl].

Risk categories	Normal control subjects	DM patients with good glycaemic control	DM patients with poor glycaemic control	All DM patients
Lp(a) <30 mg/dl	73.3	56.6	43.3	48.3
Lp(a) ≥ 30mg/dl	26.7	43.4	56.7	51.7

Lp(a) - lipoprotein(a), DM - diabetes mellitus

Table 3 - Relative percentage distribution of control, DM patients with good glycaemic control, DM patients with poor glycaemic control and all DM patients into desirable [Lp(a) &lt; 20 mg/dl] and high risk level categories [Lp(a) ≥ 20 mg/dl].

Risk categories	Normal control subjects	DM patients with good glycaemic control	DM patients with poor glycaemic control	All DM patients
Lp(a) <20 mg/dl	63.3	46.6	23.3	35
Lp(a) ≥ 20mg/dl	36.7	53.4	76.7	65

Lp(a) - lipoprotein(a), DM - diabetes mellitus

intense research it does not seem to be cost-effective at this point to add the determination of apo(a) isoforms to CVD risk assessment. Clinicians should decide on an individual basis whether the determination of apo(a) isoforms is necessary to generate a more complete risk profile. Lipoprotein(a) continues to be a focus of intense research and new exciting data is continuously being produced.

In conclusion, diabetic patients have elevated levels of serum Lp(a) as compared to healthy subjects and the frequency of high risk levels of Lp(a) is also higher in diabetics than non diabetic subjects. The increased prevalence of high risk levels in patients with type 2 DM may be due to increased prevalence of low molecular weight isoforms of apo(a) in these patients.

## References

1. Chaudhary GM. Relative risk of hypertension and coronary artery disease in diabetes mellitus. *J Coll Physicians Surg Pak* 2001; 11: 239-241.
2. Bell DSH. Stroke in the diabetic patient. *Diabetes Care* 1994; 17: 213-218.
3. Haffner SM, Morales PA, Stern MP, Gruber MK. Lp(a) concentrations in NIDDM. *Diabetes* 1992; 41: 1267-1272.
4. Gruddy SM, Benjamin IJ, Burke GL. Diabetes and Cardiovascular Disease. A statement for health care professionals from the American Heart Association. *Circulation* 1999; 100: 1134-1146.
5. Grundy SM. Small LDL, Atherogenic Dyslipidemia, and the Metabolic syndrome. *Circulation* 1997; 95: 1-4.
6. Utermann G. The mysteries of lipoprotein(a). *Science* 1989; 246: 904-910.
7. Maher VM, Brown BG, Marcovina SM, Hillger LA, Zhao XQ, Albers JJ. Effects of lowering LDL cholesterol on the cardiovascular risk of lipoprotein(a). *JAMA* 1995; 274: 1771-1774.
8. Marcovina SM, Albers JJ, Jacobs DR. Lipoprotein(a) concentrations and apolipoprotein(a) phenotype in Caucasians and African Americans: the CARDIA study. *Arterioscler Thromb* 1993; 13: 1037-1045.
9. John D, Collins R, Peto R. Lipoprotein (a) and Coronary Heart Disease, Meta-Analysis of Prospective Studies. *Circulation* 2000; 102: 1082-1085.
10. Kostner GM, Avogaro P, Cazzolato G, Marth E, Bitollo BG, Quinci GD. Lipoprotein(a) and the risk of myocardial infarction. *Atherosclerosis* 1981; 38: 51-54.
11. Nowak GU, Ralf J, Marion H, Koch HG, Munchow N, Assmann G et al. Increased lipoprotein(a) is an important risk factor for venous thromboembolism in childhood. *Circulation* 1999; 100: 743-748.
12. Qari FA, Akbar DH. Diabetic foot presentation and treatment. *Saudi Med J* 2000; 21: 443-446.
13. American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Clinical Practice Recommendations. *Diabetes Care* 2000; 21 (Suppl 1): S5-S19.
14. National Cholesterol Education Program. Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *Circulation* 1994; 89: 1333-1445.
15. Ruotolo G, Micossi P, Galimberti G, Librenti MC, Petrella G, Marcovina S et al. Effects of intraperitoneal versus subcutaneous insulin administration on lipoprotein metabolism in type I diabetes. *Metabolism* 1990; 39: 598-604.
16. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; 321: 405-412.
17. Scherthaner G, Kostner GM, Dieplinger H, Prager R, Muhlhauser I. Apolipoproteins (A-I, A-II, B): lipoprotein(a), lipoprotein and lecithin cholesterol acyl transferase activity in diabetes mellitus. *Atherosclerosis* 1983; 49: 277-293.
18. Guillausseau PJ, Peynet J, Chanson P, Legrand A, Altman JJ, Poupon J et al. Lipoprotein(a) in diabetic patients with and without chronic renal failure. *Diabetes Care* 1992; 15: 976-979.
19. Perez A, Carreras G, Caixas A, Castellvi A, Caballero A, Bonet R et al. Plasma lipoprotein(a) levels are not influenced by glycaemic control in type 1 diabetes. *Diabetes Care* 1998; 21: 1517-1520.
20. Takayama S, Yasumuro Y, Kim JH, Ishikawa M, Tsujino D, Matsuo S et al. An application of apo(a) isoforms for the clinical assessment of Lp(a). *J Clin Lab Anal* 2000; 14: 53-58.
21. Ribault A, Durou MR, Letellier C, Wojcik F, Poirier JY, Ruelland A. Determination of lipoprotein(a) concentrations and apolipoprotein(a) molecular weights in diabetic patients. *Diabetes Metab* 2000; 26: 107-112.
22. Chico A, Perez A, Caixas A, Ordonez J, Pou JM, Leiva A. Lipoprotein(a) concentrations and non-insulin-dependent diabetes mellitus: relationship to glycaemic control and diabetic complications. *Diabetes Res Clin Pract* 1996; 33: 105-110.
23. Heller FR, Jamart J, Honore P, Derue G, Novik V, Galanti L et al. Serum lipoprotein(a) in patients with diabetes mellitus. *Diabetes Care* 1993; 16: 819-823.
24. Ramirez LC, Arauz-Pacheco C, Lackner C, Albright G, Adams BV, Raskin P. Lipoprotein(a) levels in diabetes mellitus: Relationship to Metabolic Control. *Ann Intern Med* 1992; 117: 42-47.
25. White AL, Rainwater DL, Lanford RE. Intracellular maturation of apolipoprotein(a) and assembly of lipoprotein(a) in primary baboon hepatocytes. *J Lipid Res* 1993; 34: 509-517.