SERUM Lp(a) IN DIABETICS WITH AND WITHOUT EVIDENCE OF CLINICAL NEPHROPATHY- A PRELIMINARY STUDY

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ABSTRACT

Type 2 diabetes is associated with a marked increase in the risk of coronary artery disease. Dyslipidaemia is believed to be a major cause of this increased risk. Recently, elevated levels of lipoprotein (a), Lp(a), have been reported to be associated with an increased risk. However there is very little data regarding Lp(a) concentrations and type 2 diabetes from India. The objective of the study was to assess serum Lp(a) levels in type 2 diabetics with and without evidence of clinical nephropathy. We estimated serum Lp(a) levels in 30 control subjects, 30 diabetics without evidence of clinical nephropathy and 30 diabetics with evidence of clinical nephropathy. Statistical analysis showed that Lp(a) levels were increased in diabetic patients with nephropathy (mean 46.3 ± 17.6 mg/dl). The Lp(a) levels however did not differ significantly between control (mean 20.2 ± 15.9 mg/dl) and diabetics without nephropathy (mean 22.6 ± 13.1 mg/dl). Thus diabetes per se seems to have little or no influence on serum Lp(a) levels, however elevated levels were seen in patients with nephropathy.

KEY WORDS :

Diabetes, nephropathy, Lp (a), plasminogen, and atherosclerosis

INTRODUCTION

Diabetes is the most common endocrine disorder characterized by hyperglycemia and a predisposition to chronic complications like retinopathy, nephropathy, neuropathy and macrovascular diseases (1). Among the established risk factors, derangement in lipid metabolism plays an important role. Virtually every lipid and lipoprotein is affected in Type 2 diabetes (2). Since its discovery by Berg K in 1963, Lp (a) has become a focus of research interest owing to the results of case control and prospective studies linking elevated plasma levels of this lipoprotein with the development of coronary artery disease (3). The clinical interest in Lp (a) arose when Dahlen and coworkers recognized a higher frequency of Lp(a)

positive subjects among men with coronary heart disease as compared to the controls (4).

Lp(a) contains an LDL like moiety, in which the apolipoprotein, apoB-100, is covalently linked to the unique glycoprotein, apolipoprotein (a), apo(a) (5). Sequencing of apo (a) at the protein level has revealed a high degree of homology with plasminogen (6). Plasminogen is a plasma serine protease of the fibrinolytic system. It contains a signal sequence followed by five so-called kringle structures (K1 to KV) and a protease domain. Apo (a) differs from plasminogen in that kringles K1 to K11 are not present in apo(a). KV is present once, as is the protease domain. Apo (a) is much larger than plasminogen due to the amplification of one of the plasminogen like kringles K1V in apo (a). This is also responsible for the size heterogeneity of apo(a) (7,8).

Plasma apo(a) is secreted primarily by the liver (9). It is unclear where Lp(a) is assembled. Assembly from LDL and apo(a) in plasma has been demonstrated in mice transgenic for the

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human apo(a) gene (10). The mechanism and site of Lp(a) catabolism are unknown. The LDL receptor pathway seems to play a minor role, if any (7). Lp(a) has been detected in atherosclerotic lesions suggesting it may be atherogenic and because of the homology with plasminogen, it has been proposed that Lp(a) may provide a link between atherogenesis and thrombus formation (5). Several investigators have confirmed that a high Lp(a) concentration is an independent risk factor for development of ischaemic heart disease (11). The situation is less clear in diabetes mellitus. Increase in Lp(a) has been reported in Type 1 diabetic patients with microalbuminuria (12). No elevation of Lp(a) was found in type 2 diabetics and no effect of glycaemic control was found (13).

MATERIALS AND METHODS

Source of data

The study was undertaken at St. Johns Medical College Hospital during the years 1998-1999. The controls consisted of 30 normal subjects. The cases were selected by random sampling and consisted of 60 patients diagnosed to have type 2 diabetes and attending SJMCH.

The controls and cases were grouped as follows

Controls:

Comprised of 30 normal individuals

Group I :

Comprised of 30 patients with type 2 diabetes without evidence of diabetic nephropathy. Diabetes was diagnosed by history, clinical evaluation and fasting serum sugar of 140 mg/dl or more.

Group II :

Comprised of 30 patients with type 2 diabetes with evidence of clinical nephropathy. Diabetic nephropathy was diagnosed based on 24 hour urine protein estimation ($>0.5\text{gm/day}$), presence of retinopathy and sonological evidence of enlarged or normal kidneys.

Individuals belonging to other age groups, post menopausal women, smokers and alcoholics, subjects with history of primary hyperlipoproteinaemia, subjects with history of other known secondary causes of hyperlipoproteinaemias (like thyroid disorders, liver

disorders, cholestasis, etc.), subjects on drugs affecting lipid metabolism (beta blockers, oestrogens, steroids, cimetidine, etc.), patients with evidence of other renal disorders, ESRD (end stage renal disorder) and those on dialysis were excluded from this study.

Method of collection of data:

History was obtained from both the controls and cases and also from medical records section. Samples were drawn after an overnight fast, from the cubital vein at the out patient sample collection room of SJMCH. Serum was separated by centrifugation at 1200 rpm and analysed immediately.

The sample was then analysed for fasting serum sugar (glucose oxidase-peroxidase), serum creatinine (Jaffe's method) and lipoprotein(a) (ELISA method). All chemicals used for the estimations were of analytical grade. They were supplied by companies like Sigma, E-Merck, BDH, etc. The Lp(a) kit was obtained from immunogenetics.

The statistical analysis was done using students "t" test.

RESULTS

All data are presented as mean $\pm$ S.D. Data was analysed using independent 't' test. The statistical significance of difference between mean values was determined by the 't' test for equality of mean. The cases and controls for the study were selected by random sampling. The diabetics without nephropathy had a shorter duration of diabetes compared to non-nephropathy cases. They were under good glycaemic control and had good renal function as indicated by the serum creatinine levels. The nephropathy cases were under relatively poor glycaemic control (table 1).

Diabetics without nephropathy were found to have a mean Lp(a) level of 22.6 ± 13.1 mg/dl with range of 6-54 mg/dl whereas the nephropathy cases had a mean Lp(a) level of 46.3 ± 17.6 mg/dl and a range of 15-85 mg/dl as against the controls who had a mean value of 20.2 ± 15.9 mg/dl and a range of 6-50 mg/dl. Thus the Lp(a) values in non nephropathy cases were comparable ($p > 0.05$) to the controls. The nephropathy cases however had serum Lp(a) levels significantly elevated than the controls and non nephropathy cases ($p < 0.001$) (table 2).

DISCUSSION

Since its discovery Lp(a) has been implicated as an independent risk factor for atherosclerotic vascular disease. Lp(a) consists of an LDL molecule linked by a disulfide bond to a unique glycoprotein, apo(a). Because of the remarkable homology between apo(a) and serine protease zymogen, plasminogen(6), it has been speculated that Lp(a) may interfere with the normal fibrinolytic process there by providing a link between lipoproteins and thrombosis. Our study showed that serum Lp(a) levels in diabetics did not differ from controls ($p > 0.05$). The mean Lp(a) being 22.6 ± 13.1 mg/dl as against the controls who had a mean value of 20.2 ± 15.9 mg/dl. Although the mean Lp(a) levels were comparable between diabetics without nephropathy and controls, the proportion of cases with value above 30mg/dl was higher (25%) in diabetics without nephropathy compared to controls (20%). The mean Lp(a) value in nephropathy cases was 46.3 ± 17.6 mg/dl which were significantly raised ($p < 0.001$) when compared to non nephropathy cases and controls. Similar findings were reported by Haffner *et al* (1992) Warner *et al* (1993), Geethanjali F.S., 1999, who showed that Lp(a) levels did not differ significantly between diabetics and controls but increased levels were seen in patients with proteinuria and diabetic nephropathy(13,14,15).

The increased Lp(a) seen in nephropathy

could be due to increased hepatic synthesis, impaired catabolism or both. The kidney could have an indirect influence on the synthesis of Lp(a) in the liver. This might be indicated by a factor that is secreted by the kidney and regulates Lp(a) synthesis. Studies by Black I.W and Wilcken D.E.L have shown increased Lp(a) levels in ESRD patients which returned to normal levels following renal transplantation (16), indicating that kidney has a direct metabolic function and degrades Lp(a), which is altered in renal diseases. Thus the potential importance of increased Lp(a) in patients with proteinuria is three folds. Firstly Lp(a) may contribute to the increased CHD risk in these patients. Secondly it could also be thrombogenic in large vessels other than the coronary arteries, for example the renal veins. Finally it could also have a role in the progression of renal disease (17).

CONCLUSION

From the study it can be concluded that although diabetes is a secondary cause of hyperlipidaemia, diabetes per se seems to have no influence on serum Lp(a) levels. The significantly elevated levels of Lp(a) seen in cases of diabetic nephropathy suggests a possible role of kidney in the metabolism of Lp(a). Elevated Lp(a) could thus be an additional risk factor for coronary artery disease in diabetic nephropathy.

Table-1

Characteristics of study groups

Characteristics	Controls	Group-I	Group-II
Age (years)	48 ± 9.6	52.7 ± 5.9	58.4 ± 8.6
Male: Female	20:10	17:13	19:11
Duration of diabetes (Years)		6.5 ± 2.9	11.5 ± 6.3
Serum sugar (mg/dl)	90 ± 3.5	121.8 ± 47.4	169.7 ± 52.8
Serum creatinine (mg/dl)	0.8 ± 0.2	0.9 ± 0.2	1.2 ± 0.9

Table-2

Serum Lp(a) in study groups			
Study groups	n	Lp(a) mg/dl (mean $\pm$ SD)	p-value
Controls	30	20.2 $\pm$ 15.9	>0.05 *
Group-I	30	22.6 $\pm$ 13.1	<0.001**
Group-II	30	46.3 $\pm$ 17.6	<0.001**

* Control versus Group-I

** Control versus Group-II

*** Group-I versus Group-II

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