LIPOPROTEIN(A) AS A STRONG RISK FACTOR FOR CORONARY ARTERY DISEASE IN IRANIAN POPULATION

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SUMMARY: Lipoprotein(a) [Lp(a)] a cholesterol rich particle in human plasma, is a risk factor for coronary artery disease (CAD). The aim of present study was to determine and compare serum Lp(a) in the CAD patients with normal healthy volunteers in Iranian population.

This investigation included 117 patients (25 females, 92 males) with different degrees of coronary stenosis and 103 normal subjects (51 females, 52 males). Blood samples were collected in the morning after an overnight fasting and sera were stored at -20°C until the Lp(a) assay. The lipids and lipoproteins were measured by enzymatic methods and Lp(a) assay was done by electroimmunodiffusion.

The results showed that the serum Lp(a) did not correlate significantly with age, sex and other lipid risk factors. The Lp(a) levels in CAD group with a mean of 41±40 mg/dl were significantly higher than controls with a mean of 25.5±28 mg/dl ($p<0.001$).

We conclude that Lp(a) is an independent risk factor for atherosclerotic coronary artery disease in the Iranian population.

Key Words: Coronary artery disease, lipoprotein(a).

INTRODUCTION

Lipoprotein(a) [Lp(a)] was first discovered by Berg in human plasma in 1963 (1). It is structurally similar to low density lipoprotein (LDL), with an additional disulfied linked glycoprotein termed apolipoprotein(a) [apo(a)] (2). Apo(a) shares extensive structural homology with plasminogen but varies in size, which is due to the variation in the number of Kringle 4-like domain of plasminogen. Due to the size heterogeneity apo(a) exhibits a genetic size polymorphism with apparent molecular weights of isoforms ranging from 300 to 800 KDa (2, 3). There is a wide inter-individual variability in plasma concentration of Lp(a). In contrast intra-individual levels are relatively stable. Furthermore there are considerable differences in the mean of plasma Lp(a) concentrations between different populations and ethnic groups (5). On the basis of results of most studies, plasma Lp(a) levels are inversely related to apo(a) isoform size (6). Many epidemiological and case-control studies have shown that, when Lp(a) is present in high

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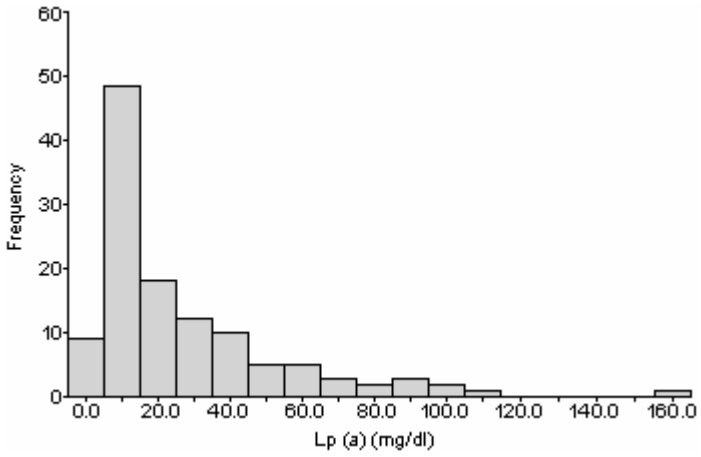
Figure 1: Frequency distribution of Lp (a) concentration in healthy controls.



level in the plasma, it is recognized as an independent risk factor for premature coronary artery disease (CAD) and also myocardial infarction (MI) (7-9). On the basis of the results of some perspective studies, plasma Lp(a) levels did not correlate significantly with atherosclerosis and CAD (10). Black races in the United States (US) and Africa have much higher levels of plasma Lp(a) than Whites, but they have however lower risk for atherosclerosis than Whites (11,12). These observations suggest that in addition to plasma levels, atherogenicity of Lp(a) could be affected by other factors. These factors include genetic background, race, phenotype, lipids risk factors and probably some environmental conditions. The major aim of the present study was to compare plasma Lp(a) levels in a group of CAD patients with healthy controls which might enable the diagnostician to evaluate this lipoprotein as a risk factor for atherogenic CAD in the Iranian population.

MATERIALS AND METHODS

Subjects and blood sampling

We studied 103 controls and 117 CAD patients. The CAD group included 25 females, 92 males, with a mean age of 54 years, ranging from 34 to 76 years. They had various degrees (at least 50%) of stenosis in one or more of the main branch of coronary artery documented by coronary angiography. The controls included 51 females, 52 males, with a mean age of 52 years, ranging from 19 to 72 years. They consisted of 40 normal subjects who proved to be healthy by health screening and 63 subjects who had no obstructions in the coronary artery by angiography. Patients with acute MI, Diabetes mellitus and chronic renal failure were excluded from the study group.

Blood samples were collected in the morning by venipuncture after an overnight fast and were allowed to clot at room temperature for about 1 h. Sera were separated from erythrocytes by centrifugation at 1500 Xg for 10 min. Each serum was divided into two aliquotes one of which (0.5 ml) was immediately stored at -20°C for a maximum of six months until the Lp(a) measurement. The other was kept at 4°C (2 days maximum) for lipids and lipoproteins analysis.

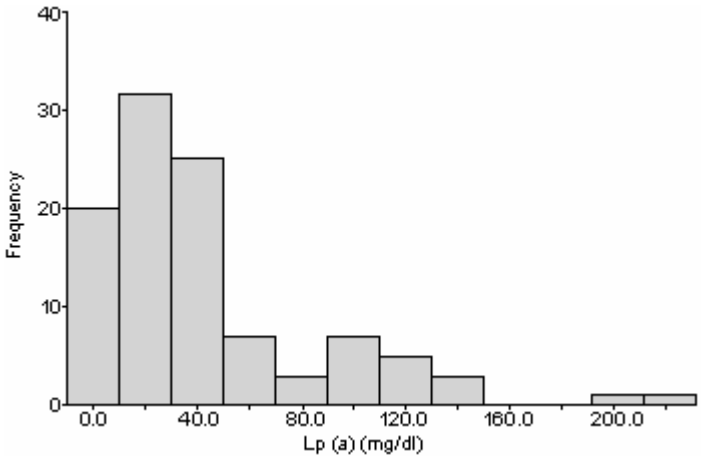
Lipids and lipoproteins analysis

Serum total cholesterol (TC) and triglycerides (TG) concentrations were measured using routine enzymatic methods, cholesterol oxidase and glycerol oxidase respectively. High density lipoprotein-cholesterol (HDL-C) in serum was determined by the same enzymatic method after precipitation of betalipoproteins by dextransulfate -$MgCl_2$. These methods are automated on Technicon RA-1000 Autoanalyser and total coefficient of variations (C.V) for TC and TG were < 4% and < 6% respectively. Low density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula when the concentration of TG was below 400 mg/dl (13).

Lp(a) assay

The concentration of serum Lp(a) was measured by electroimmunodiffusion method (14). Lp(a) primary standard and controls

Figure 2: Frequency distribution of Lp (a) concentration in CAD patients.



were obtained from Immuno (Vienna, Austria) and specific anti-Lp(a) antibody was prepared by immunization of rabbits with extracted Lp(a) from pooled Lp(a)-rich plasma and purified by affinity chromatography (15). The limit of detection for Lp(a) was considered to be 1 mg/dl and an intra-assay C.V of 6% (n=20) was obtained at 25 mg/dl.

Statistical methods

For statistical analysis SPSS software was used. Mann-Whitney U-test was used to compare Lp(a) in two groups. Other lipids and lipoproteins were compared using student's t-test. Pearson's test was used to evaluate correlation between Lp(a) level and other variables. A p value of < 0.05 was considered to be significant.

RESULTS

The frequency distribution for serum Lp(a) concentrations in healthy control group was highly skewed (Figure 1), but less skewness was found in CAD group (Figure 2). The serum Lp(a) level in CAD group with a mean of 41 ± 40 mg/dl and a median of 29.5 mg/dl was significantly higher than healthy control group with a mean of 25.5 ± 28 mg/dl and a median of 16 mg/dl ($p<0.001$) (Table 1). The serum Lp(a) levels in each group and in combined study population did not correlate significantly with sex, age and other lipid risk factors. There were no significant differences in the serum Lp(a) concentrations between males with the mean value of 34 mg/dl and females with the mean value of 31 mg/dl. 25% of the healthy control and 48% of CAD group had serum Lp(a) levels higher than 30 mg/dl. Some of the serum lipid levels were significantly different between CAD and control groups (Table 2).

DISCUSSION

The results of the present study indicate that plasma Lp(a) levels in Iranian population is higher than most other Caucasians and it is similar to Turkish population (16). In addition mean plasma Lp(a) levels in control group is about two-fold higher than CAD group (Table 1). Similar to most other populations in our study group plasma Lp(a) level was not correlated significantly with age, sex and other lipid risk factors.

Lp(a) is an atherogenic lipoprotein and it is known as an independent risk factor for CAD (17). However, similar to varying plasma levels in different populations and ethnic groups, Lp(a) atherogenicity could also differ between various populations (18,19).

According to plasma Lp(a) levels, populations and races can be divided to several categories. Chinese with a mean plasma Lp(a) level of 7 mg/dl have the lowest levels. Caucasians in Europe and US with a mean of 12 to 17 mg/dl have relatively low plasma Lp(a) levels. Indians, Turks and some others with a mean of 20 to 25 mg/dl have relatively high plasma Lp(a) levels (16). Blacks in Africa and US with a mean of about 45 mg/dl are in high Lp(a) level groups (20). It has been reported that blacks in the US have much higher levels of plasma Lp(a) than whites, but they

Table 1: Lipoprotein(a) levels (mg/dl) in CAD patients and control groups.

	Control (n=103)	CAD (n=117)	P value *
Mean	25.5	41	< 0.001
Median	16	29.5	
SD	28	40	
Minimum	1	1	
Maximum	159	229	

* Mann-Whitney U-test.

Table 2: Lipids and lipoproteins levels in control and CAD groups.

Parameters (mg/dl)	Control (n=103) Mean ± SD	CAD (n=117) Mean ± SD	P value *
Cholesterol	214 ± 42.4	236 ± 51.5	0.001
Triglycerides	199 ± 96.7	265 ± 136.5	< 0.001
HDL - C	42.2 ± 605	39.8 ± 7.4	0.023
LDL - C	131 ± 34	146 ± 40	0.18

* Student's t-test.

have lower risk for atherosclerosis (11). According to WHO reports, prevalence of atherosclerosis and MI in developed countries are higher than developing countries (21), while mean plasma Lp(a) levels in countries such as Turkey, India and also in our study in Iranians are higher than US and Sweden (16,22). These observations indicate that genetic background and some environmental factors could affect the atherogenicity of Lp(a). In this study 25% of controls and 48% with CAD had plasma concentrations above 30 mg/dl. This level is considered to make double the risk of developing premature CAD. It also has been proposed as a threshold value in the assessment of the risk of developing premature atherosclerosis (7). According to this cutoff point the percentage of high risk in the Iranian population is higher than most other Caucasians. On the basis of our finding, one should consider that the Iranian population has a greater risk for CAD than most other populations. On the basis of the report of epidemiological study that has been conducted in the central area of Iran, the prevalence of CAD is high (9.3%) in the Iranian population (23). On the other hand, lipid risk factors in Iranian population are also relatively high (24), and a strong correlation between plasma apolipoprotein-B concentration and atherosclerotic CAD is also reported (25). These observations with our finding indicate that in the Iranian population elevated apo-B containing lipoproteins, including Lp(a), may contribute to the high prevalence of CAD in this country.

In overall, plasma Lp(a) levels in Iranian population are relatively high in comparison to other Caucasians. This lipoprotein is an important independent risk factor for CAD in Iranians. Lp(a) and other apo-B containing lipoproteins may co-operate for development of atherosclerosis in Iranians. Further studies should be done to elucidate the frequency distribution of apo(a) phenotype and precise role of Lp(a) in atherosclerotic CAD in the Iranian population.

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