

## CLINICAL NOTE

# ASSESSMENT OF SERUM LIPOPROTEIN (a) LEVELS IN AFGHAN IMMIGRANTS LIVING IN YAZD

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**Background-** Lipoprotein (a) [Lp (a)] is a cholesterol-rich and atherogenic particle in human plasma. Its elevation in plasma is an independent risk factor for coronary artery disease. The purpose of the present study was to determine the plasma level of Lp (a) and to evaluate its relationship to other serum lipids in Afghan immigrants.

**Methods-** Serum Lp (a) in a group of Afghan immigrants (81 males, 86 females) living in Yazd, was measured using the electroimmunodiffusion method. Specific anti-Lp (a) antibodies were prepared by immunization of rabbits with Lp (a) and purified by affinity chromatography.

**Results-** The mean Lp (a) value was  $0.43 \pm 0.45$  g/L (mean  $\pm$  standard deviation [SD]) and there was no difference between the sexes. Lp (a) levels did not correlate significantly with age, total cholesterol, triglycerides or high-density and low-density lipoprotein cholesterol. The frequency distributions of Lp (a) were skewed toward lower values.

**Conclusion-** In comparison to most other ethnic groups, this Afghan population had high plasma Lp (a) concentrations.

**Keywords** • Afghan • electroimmunodiffusion • lipoprotein (a)

### Introduction

Lipoprotein (a) [Lp (a)] was first described by Kare Berg in 1963 as a genetic trait found in human plasma.<sup>1</sup> It structurally resembles low-density lipoprotein (LDL) with an additional disulfide-linked glycoprotein termed apolipoprotein (a) [apo (a)].<sup>2</sup> Apo (a) varies in size, due to variation in the number of kringle 4-like domains of plasminogen.<sup>3</sup> Because of its heterogeneity in size, the molecular masses of Lp (a) have a range of 300 to 800 kDa.<sup>4</sup> Epidemiologic and case-control studies have shown that when present in high levels in the plasma, Lp (a) is an independent risk factor for premature coronary artery disease (CAD) and myocardial infarction (MI).<sup>5-7</sup> Plasma Lp (a) concentration can be affected in some other diseases such as renal failure,<sup>8</sup> diabetes mellitus<sup>9</sup> and dislipidemia.<sup>10</sup>

Although plasma Lp (a) concentrations are largely related to genetic background, a wide range of values is observed within each population. In addition, plasma Lp (a) concentrations are not influenced by diet or lipid lowering drugs and are generally insensitive to the effects of age, sex and lifestyle.<sup>11</sup>

Considerable racial differences in Lp (a) distribution have been shown. High levels of plasma Lp (a) have been reported mostly in Blacks from the US and Africa. However, African American Blacks have much higher levels of plasma Lp (a) than whites, but they have a lower risk for CAD and MI.<sup>12</sup>

The Lp (a) atherogenesis mechanism and parameters affecting this process is not yet fully elucidated. It is believed that genetic background, race and some other risk factors have considerable roles in atherogenesis. The aim of the present study was to determine the distribution of plasma Lp (a) levels in a group of Afghan immigrants living in Yazd, and to evaluate its relationship to other plasma lipids and lipoproteins.

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## Lipoprotein (a) Levels in Afghan Immigrants

### Materials and Methods

The study group consisted of 167 healthy Afghans (81 males, 86 females) who had no history of cardiovascular, hepatic, renal or endocrine disorders and whose ages were between 10 and 75 years (mean age, 25 years). The subjects were selected by simple sampling of patients referred from Yazd health centers. The experimental procedure was explained to all selected subjects to come to an agreement for blood sampling. Blood samples were collected in the morning after an overnight fast and were allowed to clot at room temperature for 1 hour. Serum was obtained by low speed centrifugation (1,500 x g for 15 minutes). Each serum sample was divided in two aliquots. One was immediately stored at -20°C for up to 6 months for Lp (a) assay and the other was used for cholesterol and triglyceride analyses at the same day that the sample was drawn. Serum cholesterol and triglycerides were measured using commercial kits on RA-1000 autoanalyzer (Technicon Instruments Corporation, Tarry town, New York, USA). High-density lipoprotein-cholesterol (HDL-C) was also determined after precipitation of Beta-lipoproteins by dextran sulfate and MgCl<sub>2</sub>. LDL-cholesterol (LDL-C) was calculated by using the Friedwald formula.<sup>13</sup>

Serum Lp (a) was determined by electroimmunodiffusion (EID) as described by Marz.<sup>14</sup> Lp (a) reference standard was obtained from Immuno AG (Vienna, Austria). Lp (a) antigen and antiserum were prepared as described by Abe et al.<sup>15</sup> Briefly, Lp (a) was prepared by ultracentrifugation of the dextran sulfate- and CaCl<sub>2</sub>-precipitated fraction from human Lp (a)-rich pooled plasma. Antibody against Lp (a) was produced in rabbits by immunization with crude

**Table 1.** Plasma lipids and lipoproteins by sex

	Males (n = 81)	Females (n = 86)	<i>p</i> *	Total (n = 167)
Cholesterol (mmol/L)	3.73 ± 0.75	4.05 ± 0.84	0.34	3.9 ± 0.81
TG (mmol/L)	1.25 ± 0.42	1.41 ± 0.68	<0.001	1.30 ± 0.57
HDL-C (mmol/L)	1.28 ± 0.27	1.31 ± 0.33	0.31	1.30 ± 0.30
LDL-C (mmol/L)	2.07 ± 0.59	2.23 ± 0.65	0.64	2.16 ± 0.63

\*According to student's *t*-test, TG = triglycerides; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol.

**Table 2.** Plasma lipoprotein (a) concentrations (g/L).

	Males (n = 81)	Females (n = 86)	<i>p</i> *	Total (n = 167)
Mean ± SD	0.48 ± 0.44	0.39 ± 0.44	0.14	0.43 ± 0.44
Median	0.280	0.235		0.250
Minimum	0.01	0.01		0.01
Maximum	10.38	2.24		2.24

\*According to Mann-Whitney, U-test.

Lp (a) fraction. To evaluate the specificity of prepared antibodies, in addition to observing a single rocket after serum loading on EID plates, pure LDL and plasminogen (Sigma Chemical Co, St. Louis, MO, USA), as the two most important potential cross-reactors, were run in separate wells on the same EID plate.

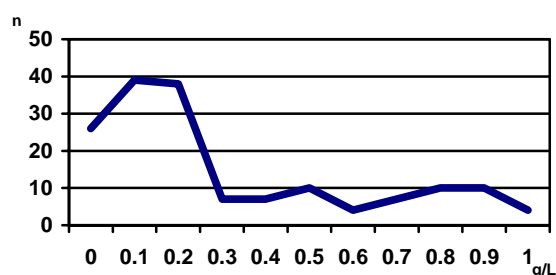
For statistical analysis, SPSS software (version 6) (SPSS Inc. Chicago, IL, USA) was used. The Mann-Whitney U-test was used for comparison of Lp (a) between the two sexes, student's *t*-test was used for comparison of cholesterol, triglycerides and low-density and high-density lipoprotein cholesterol between the two sexes. Pearson's correlation test was used to compare Lp (a) and other variables in the study population.<sup>7</sup>

### Results

The serum lipid profile and Lp (a) concentrations are summarized in Tables 1 and 2, respectively. Lp (a), cholesterol, HDL-C and LDL-C did not differ significantly between the sexes, whereas triglycerides differed significantly (*p* < 0.001). Serum Lp (a) did not correlate significantly with age, cholesterol, triglycerides, HDL-C or LDL-C. The distribution frequency of Lp (a) concentrations was skewed toward lower values (Figure). In 43% of males, 37% of females and 40% of both males and females, the serum Lp (a) concentrations were above 0.3 g/L. After staining of EID plates, no rocket formations were observed for LDL and plasminogen, which indicates that the antibodies were highly specific. Depending on the Lp (a) concentration (low, medium or high), the intra- and inter-assay (n = 20) coefficients of variation ranged from 5% to 7% and from 6% to 10%, respectively.

### Discussion

Serum Lp (a) level is known to be fairly constant throughout an individual's life, although



**Figure.** Frequency distribution of Lipoprotein (a) concentrations (g/L) in the Afghan population ( $n = 167$ ).

among different individuals, its serum concentrations may vary considerably. On the other hand, there are variable mean serum Lp (a) concentrations among various populations and ethnic groups.<sup>11</sup> Serum lipoprotein (a) levels are inversely related to apo (a) glycoprotein size.<sup>2,16</sup> Variability of mean serum Lp (a) in different populations is mostly due to different apo (a) allele distributions, but the physiologic significance of such distributions is not fully understood. According to plasma Lp (a) levels, populations and races can be divided into several categories. Chinese, with a mean plasma Lp (a) of 0.07 g/L, have the lowest levels. Caucasians in Europe and the United States, with a mean of 0.12 to 0.17 g/L, have relatively low plasma Lp (a) levels. Indians, Turks and other populations, with a mean of 0.2 to 0.25 g/L, have relatively high plasma Lp (a) levels.<sup>17</sup> Blacks in Africa and the USA, with a mean of about 0.45 g/L, have high Lp (a) levels.<sup>12</sup>

The results of the present study indicate that Afghans, with a mean serum Lp (a) concentration of 0.43 g/L, have levels similar to Blacks. In Afghans as in other populations, serum Lp (a) concentration had no association with age, sex, lipid profile or lipoprotein level. In our study population, the frequency distribution was skewed toward lower values. Among Caucasians, a serum Lp (a) concentration of 0.3 g/L is considered the cut-off point for high risk of CAD. Less than 30% of Caucasians are above this level.<sup>7</sup> In our study, 37% of females, 43% of males and 40% of combined population had serum Lp (a) levels above 0.3 g/L, which is a relatively high percentage compared to most other populations and ethnic groups. While we do not know the actual prevalence of CAD and MI among Afghans, according to the World Health Organization (WHO), the risk for such conditions in developed countries is higher than that in developing

countries.<sup>18</sup> Therefore, the prevalence of CAD in Afghans is probably lower than in European and American populations.

Unfortunately, we did not find any reports about the prevalence of CAD and MI in the Afghan population. If the WHO reports about the distribution of atherosclerotic risk factors in developing and developed countries included Afghanistan, we would conclude that the cutoff point of Lp (a) for atherosclerosis risk in the Afghan population is higher than in most other Caucasian populations. In addition, the existence of ethnic groups such as Blacks and populations such as Afghans with high plasma Lp (a) levels and yet a relatively low prevalence of CAD, indicate that the atherogenicity of Lp (a) is affected by genetic background and some other acquired environmental conditions. Further studies of the prevalence of CAD and distribution of other risk factors in Afghan populations are needed.

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