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Lipoprotein (a) in an immigrant Indian population sample in Australia

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People from the Indian subcontinent (South Asia) show a very high incidence of coronary heart disease (CHD).[1] This is seen both in India and in countries to which Indians have migrated in large numbers, such as the UK, USA and Trinidad.[2] Large differences in disease rates are seen for people of Indian origin when compared with the host population: myocardial infarction (MI) occurs at a lower age and mortality from MI is ten times higher.[2]

In a large survey of Indian subjects in South Africa, important risk factors noted were hypercholesterolaemia, diabetes and smoking in men, and hypercholesterolaemia and smoking in women.[3] In another study, dyslipidaemia was higher in Indian physicians living in the USA, compared with US-born physicians.[4] However, an earlier study reported normal to low levels of total cholesterol in Asian Indians, and McKeigue *et al*.[1] believed the excess cardiac mortality observed in this population could not be fully explained by conventional risk factors.

Insulin resistance, leading to a cluster of metabolic factors, is thought to explain the early onset of CHD[5] in Asian Indians and this has been demonstrated in some Asian Indian groups.[6] However, while insulin resistance may be implicated in the early occurrence of risk factors and CHD in Indians, there is some evidence to suggest that other aspects of the lipid profile, such as the lipoprotein (a) (Lp[a]) level, influence risk in Indian heart patients.[7] One study found Lp(a) to be an independent risk factor for CHD in non-insulin-dependent diabetes mellitus (NIDDM) patients in South India.[8]

Berg first identified Lp(a) in 1963[9] as a cholesterol-rich lipoprotein similar to low-density lipoprotein cholesterol (LDL-C) in physical and chemical properties, with apolipoprotein B100 as its major protein constituent. Studies have identified increased levels of Lp(a) – usually regarded as >30 mg/dL – as an important independent risk factor for CHD.[10] A meta-analysis of 27 studies shows a clear association between Lp(a) and CHD.[11] Increased LDL-C, or increased Lp(a), increases the risk of CHD two-fold; but if the patient has both then the risk increases six-fold.[12] Most studies show that Lp(a) is largely under genetic control and is resistant to dietary or lipid lowering intervention,[13,14] other than treatment with niacin[15] or hormone replacement therapy.[16]

Levels of Lp(a) range from <0.1 mg/dL to >100 mg/dL[17] and are known to vary markedly with ethnicity.[18] Only limited studies have been carried out on Lp(a) levels in people of Indian origin, and include Indian groups in Singapore, the USA and in India itself. The values obtained range from means of 8.7 mg/dL and 9.2 mg/dL in the USA and India, respectively, to 20.1 mg/dL in Singapore and 34.1 mg/dL for one small group in the USA. Clearly, the distribution of Lp(a) in Indian population groups needs further investigation.

The aim of this study is to investigate the level and distribution of Lp(a) in an Indian immigrant population in Australia and to determine the stability of this Lp(a) after 12-months' storage at -80˚C.

The study sample consisted of 50 volunteers (25 men, 25 women; age range: 23-75 years) who had migrated to Australia from India over the past 20 years. Anthropometric measurements (weight, height, hip and waist circumference) were taken without shoes and over light clothing.

Following overnight fasting, venous blood samples were collected into EDTA tubes and centrifuged at 1500 rpm for 10 min. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides were analysed on a Reflotron reflectance photometric analyser (Boehringer Mannheim, Germany) within 2 h of collection. LDL-C was estimated using the Friedewald formula.[19] Immuno-turbidimetric analyses of apoprotein A1 (apo A1) and apoprotein B (apo B) were carried out on plasma using a Turbitime system (Behring Diagnostics, Australia).

Lp(a) was determined by a sandwich enzyme-linked immunosorbent assay (ELISA).[20] Nunc-Immuno microplates (Nalge Nunc International, Denmark) were coated with 150 mL sheep anti-human Lp(a) antiserum (7.6 µg/mL; Immuno AG, Vienna) in phosphate-buffered saline (PBS; pH 7.4) overnight at room temperature (RT) with gentle shaking. Unbound antiserum was tipped out and the wells washed (x4) with PBS containing 5 g/L Tween 20. All the free sites were blocked by treating the wells with 200 µL 5 g/L bovine serum albumin (BSA) in PBS for 1 h. The wells were then washed as before and then incubated for 2 h with controls and plasma samples (100 µL) diluted 1 in 10 000 with dilution buffer (PBS containing 5 g/L Tween 20 and 5 g/L BSA) and the appropriate standards. After 2 h the wells were washed as before and 100 µL rabbit anti-human Lp(a) serum (Dade Behring, Germany), diluted 1 in 1250 with dilution buffer, was added. The plates were incubated for 2 h at RT and then washed (x4). To each well was added 100 µL diluted (1 in 5000) horseradish peroxidase (HRPO)-conjugated goat anti-rabbit-IgG (Sigma-Aldrich, USA) and the plates were incubated for 2 h at RT.

After washing as before, the reaction was developed by adding 100 µL substrate (0.4 mg/mL OPD [Sigma-Aldrich, USA] in 0.05 mol/L phosphate citrate buffer [pH 5.0] containing 0.03% sodium perborate [Sigma-Aldrich, USA]) per well. The reaction was allowed to proceed in the dark for

Table 1. Anthropometric and lipid variables by gender (mean ± SD)

	Male (n = 25)	Female (n = 25)	P value
Age (years)	40.8 ± 5.3	36.8 ± 4.7	0.01*
BMI (kg/m^2)	24.8 ± 3.7	25.6 ± 3.8	0.51
WHR (cm/cm)	0.91 ± 0.07	0.87 ± 0.07	0.05
TC (mmol/L)	5.04 ± 0.66	4.53 ± 0.80	0.02*
HDL-C (mmol/L)	1.01 ± 0.23	1.14 ± 0.28	0.08
LDL-C (mmol/L)	3.75 ± 0.62	3.16 ± 0.69	0.0028*
TG (mmol/L)	1.39 ± 0.58	1.14 ± 0.61	0.14
Apo A1 (mg/dL)	94 ± 11	99 ± 19	0.28
Apo B (mg/dL)	98 ± 18	83 ± 23	0.01*
Median Lp(a) (mg/dL)	19.3	27.4	
Values >30 mg/dL (%)	40	44	
Mean Lp(a) log transformed	1.35	1.41	

*P<0.05

a maximum of 15 min, by which time the plate had reached a golden yellow colour, before being terminated by adding 100 µL 2.5 mol/L H_2SO_4 per well.

Absorbance (A) was measured at 490 nm in a BioRad 3550 microplate reader. The calibration data was log transformed and concentration calculated using BioRad Microplate Manager v2.2. The samples were retested by the same operator after approximately 12-months' storage at -80˚C, using the same method and antibodies.

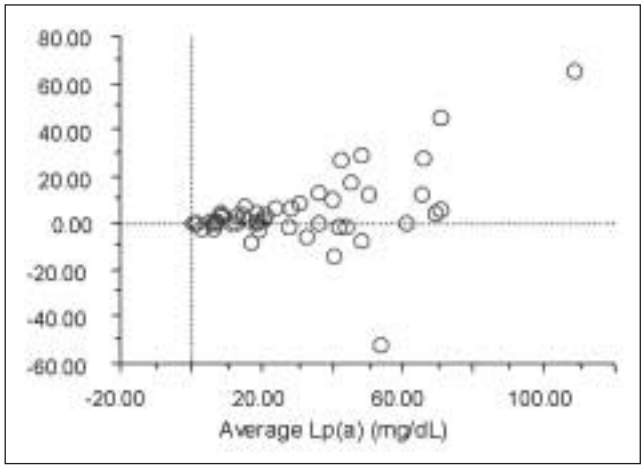
Variables were examined for normality. Log transformation was performed for variables that were not normally distributed. Differences between males and females were determined by Student's t-test for continuous variables or by Mann-Whitney for non-parametrics. Spearman rank correlation was performed to determine the difference between baseline and frozen samples. All statistical analyses were performed using Statview 5.0. P values <0.05 were considered significant.

Anthropometric and lipid variables of the group displayed by gender are shown in Table 1. All variables were normally distributed except for Lp(a), for which the median value is shown. Lp(a) distribution was positively skewed (4.16), hence the data was log transformed. No significant gender differences were found by Student's t-test or Mann-Whitney U test (non-parametrics). There was one Lp(a) outlier (331.3 mg/dL) and results were analysed both with and without this value.

There were no differences between males (mean age 40.8 years) and females (mean age 36.8 years) with regard to body mass index (BMI) or waist/hip ratio (WHR). 46% of the subjects were overweight (BMI >25 kg/m^2), with 9% regarded as obese (BMI >30 kg/m^2).

Blood lipids (TC, TG, HDL-C, LDL-C, apo A1 and apo B) were within the normal range (Table 1). HDL-C, TG and apo A1 showed no differences by gender but TC, LDL-C and apo B were higher in men. 42% of the subjects (10 men; 11 women) had Lp(a) >30 mg/dL. Four of the men in this group displayed an atherogenic lipoprotein profile consisting of increased Lp(a) (>30 mg/dL), increased LDL-C (>3.5 mmol/L) and decreased HDL-C (<1.0 mmol/L). Two women and one man had increased LDL-C and Lp(a) but normal HDL-C levels.

Fig. 1. Bland and Altman plot for Lp(a). Difference against average for baseline and frozen samples (outlier omitted).



Samples were retested after 12-months' uninterrupted storage at -80˚C. Correlation between baseline and frozen samples was highly significant (Spearman rank correlation: r = 0.93, P < 0.01). A Bland and Altman[21] type analysis (Figure 1) showed considerable agreement between the baseline and frozen values; however, some discrepancy was seen at the higher Lp(a) values.

Samples were split into two groups based on initial Lp(a) concentration: Lp(a) <30 mg/dL (n=29) and Lp(a) ≥ 30 mg/dL (n=21). A decrease in Lp(a) level on storage was seen in samples with original values >30 mg/dL (P=0.003) but no difference was seen with original levels <30 mg/dL (P=0.24).

A high incidence of CHD continues to be observed in Asian Indians worldwide.[1] The high rate of cardiac mortality in this group is not fully explained by conventional dyslipidaemia[2] and other risk factors have been identified, one of which is increased Lp(a).

In the present study, the mean Lp(a) value in this group of Asian Indians was 32.5 mg/dL – a value higher than that reported in most other studies. Lp(a) distribution was skewed towards lower concentrations, and this agreed with other reports of Asian Indian populations. Mean Lp(a) reported in other studies of Asian Indians were 20.1 mg/dL in Singapore,[22] 8.7 mg/dL in the USA,[23] 9.2 mg/dL in India[23] and 34.1 mg/dL in another US study.[24] The last result is closest to the current study (mean Lp[a]: 32.5 mg/dL, Lp[a] >30 mg/dL: 42%) but represents a sample size of only 30. In addition, assay methodology should be considered, as this varies from ELISA[23,24] – as used in the current study – to electroimmunodiffusion.[22]

Different storage conditions also are factors that produce variability in Lp(a) measurement. The samples in this study were retested after 12-months' storage at -80˚C, and this showed a decline of 21% in higher values but no difference in lower values. Previous studies of the effect of storage on Lp(a) have been contradictory: one showed a decrease of 46% after six months' storage,[25] while another showed no change over 8.5 years.[26] Lp(a) values after storage could have been affected by apo(a) heterogeneity, which accounts for 23% of Lp(a) concentration. There is an inverse relationship between apo(a) isoform size and Lp(a) concentration. Lp(a) loss on storage was found to be negligible within the middle range of isoform size but significant at both low and high

molecular weights,[27] thus the stability of Lp(a) appears to be affected by isoform size. Kronenberg *et al*.[28] confirm this, finding a marked post-storage decrease in Lp(a) containing low molecular weight apo(a) isoforms. Although isoform size was not determined on the samples in the current study, it is likely that the high Lp(a) values represent low molecular weight apo(a) isoforms, but this needs to be verified.

There is already evidence that Lp(a) may be higher in people from the Indian subcontinent when compared to other groups. In a study comparing seven ethnic groups, Indians were found to have the second highest Lp(a).[22] Values obtained in the current study of Indians were higher than for other ethnic groups (Tibetans, Koreans, Chinese, Belgians and Nigerians).[29] Mean and median Lp(a) values in the current study of a population group in Australia in which age span was wide show a higher prevalence of elevated Lp(a) and a higher median value than previously found in any Indian group other than one small US-based study.[24]

Elevated Lp(a) levels were found in a group of CHD patients in Pakistan, suggesting a role in atherogenesis and CHD.[30] High levels of Lp(a) have correlated with the development and progression of coronary atherosclerosis in Asian Indians in other studies. When combined with other cardiovascular risk factors, Lp(a) could help to explain the presence of premature CHD in Indian population groups.

The results presented here need to be confirmed in a larger study of Asian Indians in which both Lp(a) level and isoform size are determined. However, it is worth noting that this study confirms previous reports that high Lp(a) values can be masked by storage and that samples should be assayed as soon as possible. This is especially true for population groups in which Lp(a) levels may be high. □

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