Lipoprotein(a) is a superior serum marker for CHD risk compared with apoproteins and traditional lipid profile in Malaysian adult males

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Abstract

Out of 561 Malays, Chinese and Indian adult males, aged 25-79 years, screened at a cardiology clinic, 106 were identified as suffering from coronary heart disease (CHD) while the remaining 455 CHD-free males served as controls in the study. Body mass index (BMI) and waist-hip ratio (WHR) were recorded for all subjects while a fasting blood specimen collected from each subject was analysed for serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), apoprotein (apo) A-1, apo B, and lipoprotein(a) [Lp(a)]. The results of CHD risk assessment showed that the traditional serum lipid and lipoprotein risk factors namely, TC, LDLC, and TG had little diagnostic value in the present population (odds ratio, i.e. OR, all <1.5), while the negative risk factors, HDLC and apo A-1, were equally unimpressive (OR= 0.75 and 1.00, respectively). On the other hand, the clinical value of Lp(a) and apo B as serum markers for CHD risk appeared impressive, being signifcantly higher ($p<0.05$) in the CHD group compared to controls (25.8 vs 12.5 mg/dl and 107 vs 87 mg/dl, respectively). Comparatively high OR values for Lp(a) [4.48] and apo B (3.85), supported by results of receiver-operating characteristics (ROC) plots, indicate a strong positive association of these two risk factors with CHD. Overall, Lp(a) seemed by far, the most reliable of the biochemical markers for CHD risk in the present Malaysian subject population, and the use of the index in routine screening should be given serious consideration.

Key Words: Lipoprotein(a), apoproteins, serum lipids, coronary heart disease

Introduction

CHD has a multifactorial etiology, with hyperlipidaemia, cigarette smoking and hypertension as the recognised primary risk factors (Blackburn, 1980). However, editors of medical textbooks may have long overlooked a fourth primary risk factor - a genetically-linked trait referred to as "lipoprotein(a)" or simply Lp(a) for short, which bears a striking homology to plasminogen (McLean *et al.*, 1987), a protein responsible for the lysis of blood clots. Since its discovery by Berg (1963), Lp(a)'s role as a posirve risk factor in coronary artery disease (CAD) has received much attention. For example, Lp(a) has been reported to be positively associated with myocardial infarction (Kostner *et al.*, 1981; Rhoads *et al.*, 1986), stroke (Zenker *et al.*, 1986; Murai *et al.*, 1986), restenosis in arterial bypass grafts (Hoff *et al.*, 1988), and promote the proliferation of human smooth muscle cells (SMC) [Grainger *et al.*, 1993], which represents the beginning of a chain of SMC activity leading to the formation of the "neointima" and subsequently, the dreaded fibrofarty lesion that clots arteries (Schwartz, 1995).

Biotechnology in action has established that alleles at the apo(a) locus on chromosome 6 code for the different-size Lp(a) isoforms and that the size of the apo(a) glycoprotein is inversely related to the plasma levels of Lp(a) [Utermann *et al.*, 1987], which in turn determine the risk for CHD (Sanhoizer *et al.*, 1992).

Recently, Ng *et al.* (1995) highlighted the dispatity in the distribution of serum Lp(a) levels in the major Malaysian ethnic groups, viz., mean levels were 1.5 to 2.0 times higher in the Indians (21.6 mg/dl) compared to that in the Malays (16.3 mg/dl) and Chinese (11.1 mg/dl). This interesting observation agrees with an earlier report of higher Lp(a) levels in Singapore Indians versus Chinese (Utermann, 1989). Ng *et al.*'s finding above is also consistent with the CHD mortality data reported for these ethnic groups during the period 1975-1989 (Khoo *et al.*, 1991). The present report represents additional new data from Ng *et al*'s earlier study (1995), and compares the diagnostic value of serum Lp(a), apo A-1, apo B, and the traditional serum lipid and lipoprotein indices in screening CHD.

Materials and Methods

Subjects

A total of 561 male adult subjects (Chinese=294, Malays=157, Indians=110), aged 25-79 years, who attended a medical clinic were screened for coronary heart disease (CHD) using a questionnaire that probed into individual and family medical histories, a physical examination, resting/exercise electrocardiogram (ECG), and chest x-ray. Inclusion criteria for CHD patients (n=106) consisted of a past history of myocardial infarction (>3 months ago), a previous percutaneous transluminal coronary angioplasty (PTCA) or coronary

artery bypass graft (CABG). CHD-free controls (n=455) were subjects who underwent the same routine CHD screening, but had no past history of the above CHD events, and exhibited a negative ECG.

Anthropometric and Biochemical Indices

Weight, height, waist- and hip-circumference measurements were made from which the body mass index (BMI) and waist-hip-ratio (WHR) of the subjects were calculated. A fasting blood specimen was obtained from all subjects and serum TC, TG and HDLC were determined by enzymatic kits (Human, Germany), and LDLC by the Friedewald formula (Friedewald *et al.*, 1972), and Lp(a) was measured by an ELISA method [Macra Lp(a) System, Terumo Corporation, USA]. All 561 males, including the 106 CHD patients, were analysed for Lp(a) and lipid profile, but only the CHD patients and 119 randomly-selected, CHD-free age-matched controls were measured for apo A-1 and apo B by immunoturbidimetry at 340 nm (Sigma, USA).

Data Analysis

Between group means were assessed by the Student t test, using $p<0.05$ for significance. Odds ratio (OR) for the biochemical indices measured were calculated using the respective cut-off for "high risk" which corresponded to the 90th percentile for the index in the CHD-free groups. Confidence interval for OR is reflected in the Mantel-Haenszel X^2 test applied to the data, using $p<0.05$ to indicate significance.

The diagnostic values of the biochemical indices were also assessed by receiver-operating characteristics (ROC) curves, i.e. plots of sensitivity vs 1-specificity (Galen, 1982).

Results

The results obtained indicated that the mean values for BMI, WHR, TC, LDLC, and TGwere comparable in the CHD group and CHD-free controls (Table 1). This data set suggests that these indices would have little clinical value in assessing risk for CHD in Malaysians as supported by the respective unimpressive ORs shown in Table 2.

The negative risk factors, HDLC and apo A-1, were only marginally higher in the CHD-free controls compared to CHD patients, viz., 38.4 vs 36.5 mg/dl and 127 vs 120 mg/dl, respectively. The OR for HDLC is 0.74, indicating a protective effect but the result obtained here for this index is unimpressive which is unexpected, after earlier studies which proclaimed HDL as perhaps the most powerful of the lipid parameters in assessing or predicitng risk for CHD (Gordon *et al*, 1977). In addition, the comparatively low levels of HDLC compared to TC or LDLC, would require an analytical precision of 3% (CV) or lower, equivalent to a standard deviation of <2 mg/dl for HDLC, and it is unclear what proportion of clinical laboratories currently providing this routine test in the country has actually achieved this level of analytical performance.

Table 1. Anthropometric and biochemical indices in CHD patients and non-CHD controls.

Index	CHD (n=106) (Age: 54.1 ± 9.5 y)	Non-CHD (n=455) (Age: 51.0 ± 11.5 y)
BMI (kg/m²)	24.1 ± 3.0	24.4 ± 3.2
WHR	0.91 ± 0.036	0.910 ± 0.046
TC (mg/dl)	206 ± 44	207 ± 40
TG (mg/dl)	158 ± 93	161 ± 96
HDLC (mg/dl)	36.5 ± 10.1	38.4 ± 9.0
LDLC (mg/dl)	140 ± 41	136 ± 36
Lp(a) (mg/dl)	[a]25.8 ± 21.0	[a]12.4 ± 13.3
Apo A-1* (mg/dl)	120 ± 35	127 ± 28
Apo B* (mg/dl)	[b]107 ± 31	[b]87 ± 26

*A sub-sample of n=119 was ramdomly selected for the CHD-free group

Values with the same superscript are significantly different at $p<0.05$

Table 2. Odds ratio (OR) assessment of risk for CHD.

	Positive Risk Factors				Negative Risk Factors	
Index	Lp(a)	Apo B	LDLC	TC	Apo A-1	HDLC
OR	4.48	3.85	1.47	1.24	1.00	0.75
χ^2	44.13	15.10	1.45	0.42	0.0002	0.73
p	<0.0001	<0.0001	n.s.	n.s.	n.s.	n.s.

n.s. = not signiicant ($p>0.05$)

Serum Lp(a) and apo B were significantly ($p<0.05$) higher in the CHD group compared to controls (25.8 vs 12.5 mg/dl, and 107 vs 87 mg/dl, respectively). Using Lp(a) >30 mg/dl and apo B >120 mg/dl as cut-offs for "high risk", OR analysis of risk indicates a strong positive association with CHD for Lp(a) [OR=4.48] and apo B (OR=3.85) [Table 2]. These associations are supported by the ROC plots shown in Figure 1.

Discussion

Overall, the results obtained with the serum lipoprotein and cholesterol indices have been unimpressive and the limitations associated with their efficacy for use in screening CHD in Malaysians should be recognised by physicians and other health professionals involved in the request for these tests or interpreration of the test results. It would appear that the clinical value of these

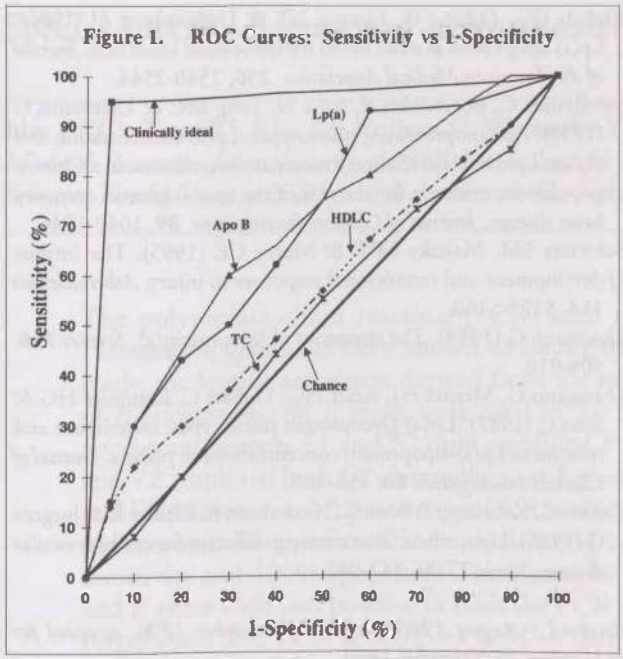

Fig. 1. ROC curves: sensitivity vs 1-specificity.

indices rests primarily on their use in the monitoring of hyperlipidaemic patients who are undergoing diet or drug therapy.

Another aspect of concern is the observation that while mean TC values for CHD-free subjects have risen marginally compared to the values reported by Chong & Khoo (1975) about 20 years ago, mean values for the protective HDLC in urban adult males have actually dropped from about 45 mg/dl 15 years ago (Chong *et al.*, 1982) to 38.4 mg/dl reported in this study.. The reason for this apparent HDLC drop is unclear but variation in analytical performace is unlikely as analysis of serum samples in both the studies were performed by the same laboratory, albeit the Technicon Autoanalyser II System was employed in the earlier study while the CHOD-PAP enzymatic method, in the present study.

The ROC plots indicated that the serum Lp(a) and apo B assays are inrermediate between chance and clinically ideal, but far superior in clinical value than TC, LDLC or HDLC. These findings reinforce the earlier report by Ng *et al.* (1995) on the great potential of serum Lp(a) as a marker for CHD risk in Malaysians. However, the OR of 4.48 obtained here for Lp(a) in the present analysis involving 561 males is much higher than the OR of 3.50 reported in the earlier data above involving 959 combined males and females. This observation would mean that Lp(a) is a more efficient index for CHD risk in Malaysian males than in females.

There are, however, two major limiting factors confronting the introduction of the serum Lp(a) assay for routine screening for CHD in Malaysia. Firstly, the ELISA immunological technique involved is not user-friendly and requires a skilled laboratory technician and a fairly sophisticated laboratory set-up. Secondly, and the more important of the two limiting factors, is the cost. At current prices, the total cost of the serum Lp(a) rest for single-assay using the 96-wells monoclonal-polyclonal double sandwich ELISA diagnostic kit which does nor entail prior dilution of serum sample, is estimated to be about RM60 (Table 3). This estimate only holds if each diagnostic kit is opened when 80 specimens are available, otherwise the assay run would have to put 'on hold' until this number of specimens has been received by the laboratory concerned. Anyway, the introduction of the Lp(a) assay in routine testing is estimated to increase the operational budget of the average clinical laboratory by at least 10-fold.

Table 3. The Ringgit and Sen of Laboratory Tests*.

Test	Lp(a)	Apo B	Apo A1	HDLC	TG	LDLC	TC
RM	60	40	40	15	15	**	10

* Based on single assay and total costing
** Included in serum lipid profile package (TC, TG, HDLC & LDLC) estimated to cost RM50

Conclusion

The study shows thar Lp(a) is a superior serum marker for CHD risk compared with apoproteins and the traditional lipid profile in rhe present population of Malaysian adult males. Lp(a)'s clinical value outweighs the comparatively expensive immunoassay involved, and the test should be included in routine CHD assessment.

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