

A critical evaluation of high density lipoprotein cholesterol as an index of coronary artery disease risk in Malaysians

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ABSTRACT

Fasting serum specimens from (a) 217 male and 46 female patients with coronary artery disease (CAD), aged 35-75 years, who had undergone angioplasty (PTCA) / coronary artery bypass graft (CABG), and (b) 160 apparently healthy controls (106 males, 54 females, aged 30-75 years), were assessed for serum lipid profile. Both sex and ethnicity significantly influenced the levels of serum high density lipoprotein cholesterol (HDLC); in the controls, females had higher HDLC levels than males (46.7 mg/dl vs 38.5 mg/dl, $p < 0.001$), while the Indian males possessed significantly lower HDLC values than the male Malay or Chinese. HDLC, triglycerides (TG) and the atherogenic index- LDLC/HDLC ratio were significantly different between the CAD patients and the healthy controls, while total cholesterol (TC) and LDLC did not seem to be of diagnostic value. Serum HDLC was lower in the CAD patients compared to the healthy controls in both sexes ($p < 0.001$), either expressed as HDLC *per se* or as % HDLC. This observation combined with the odds ratio (OR) values of 0.24 and 0.28 for HDLC and % HDLC respectively in males, firmly establishes HDL as a protective factor of CAD in Malaysians. Significance testing for the X^2 values associated with the OR values for the various lipid indices, together with the findings on the receiver-operating characteristics (ROC) curves, i.e. plots of sensitivity vs 1-specificity, indicated that HDLC, % HDLC and TQ were equally efficient as a means of risk assessment to CAD in Malaysians.

INTRODUCTION

In 1975 Miller and Miller emphasised the inverse relationship between plasma high-density lipoprotein cholesterol (HDLC) concentration and coronary heart disease (CHD), and since then prospective studies in several countries have confirmed this relationship and found HDLC to be independent of other risk factors (Gordon *et al*, 1977; Kannel, 1983; Castelli *et al*, 1986). However, there is probably cross-cultural differences on the degree of influence of HDL on the pathogenesis of CHD. This was amply demonstrated by the British Regional Heart Study which found that HDLC was not a major risk factor in the aetiology of ischaemic heart disease (IHD) in British men (Pocock *et al*, 1986).

In Malaysia, there are indications that the protective role of HDLC is in operation amongst the local ethnic groups. In 1991, Khoo *et al* reported that mortality due to CHD was twice as high in the Indians compared to that in either the Malays or the Chinese. Sub-sequently, Ng *et al* (1995) reported that the Indians not only had the lowest serum levels of HDLC, but also the highest

levels of lipoprotein(a) i.e. Lp(a) [the primary genetic risk factor for CHD] compared to the Malays and the Chinese. Higher levels of Lp(a) amongst the Indians compared to the Chinese were also reported by Utermann (1989) in Singapore.

Although Ng *et al* (1997) reported earlier that Lp(a) [OR = 4.48] was superior to apo-AI, apo-B and other serum lipid indices as an indicator of risk to CHD, they were not able to distinguish CHD patients from healthy controls (OR = 0.75, $p > 0.05$) on the basis of HDLC assays. These previous findings therefore were in direct contrast with earlier reports that HDLC was a powerful indicator of protection against CHD (Gordon *et al*, 1977; Gordon and Knoke, 1986).

The present study was conducted with particular reference to the clinical value of serum HDLC in the assessment of risk to CHD in Malaysians in view of recent conflicting reports on the index.

MATERIALS AND METHODS

Blood samples from patients

Fasting blood samples were obtained over a period of a year from 263 patients (217 males, 46 females; aged 35-75 years, mean = 54 years) who had been diagnosed angiographically as suffering from CAD and who subsequently underwent PTCA or CABG at the National Heart Institute (IJN), Kuala Lumpur. Overall, this CAD group was made up of 40% Malays and 30% each of Chinese and Indians. Patients with a history of acute myocardial infarction (AMI) were excluded from the study.

Non-CAD, healthy controls (106 males, 54 females; aged 30-75 years, mean = 43 years) matched for ethnic composition, consisted of fasting sera from apparently healthy urban executives who participated in a screening programme by IJN, as well as from staff of the IMR and other departments of the Ministry of Health Malaysia, who had no history of CHD including AMI, diabetes or any other disease known to affect serum lipid levels.

Biochemical determinations

Serum TC and TG were determined by enzymatic kits based on the "CHOD-PAP" and "GPO-PAP" reactions, respectively. In the HDLC assay, phosphor-tungstate-magnesium chloride was used as the precipitant for apo-B associated lipoproteins (LDL and VLDL); after centrifugation, an aliquot of the supernatant was analysed for cholesterol by the "CHOP-PAP" method. The Friedewald formula (1972) was used to estimate LDLC, while the atherogenic index-LDLC/HDLC ratio was calculated for all samples.

Humatrol (Boehringer Mannheim, Germany) was used as quality control (QC) material for all the serum lipid assays. In the case of the HDLC assay, 12 consecutive runs gave a precision as indicated by coefficient of variation (CV) of 5.0% and an accuracy (i.e. bias) of +10.0%, which bordered on the upper limit of acceptability (+25D) for the HDLC value cited. Each run

performed contained both patient and control samples to minimise errors arising from inter-batch variation.

Statistical analysis

Student's test was used to estimate the significance of the difference between two means. The one-way analysis of variance (ANOVA) as contained in the SAS Institute GLM programme was used to analyse differences among different means as well as the interaction between two variables.

Odds ratio for the various serum lipid indices was calculated using the following "cut-offs" for high risk: a) HDLC <30 mg/dl (males), <39 mg/dl (females); b) % HDLC <15.0; c) TC >240 mg/dl, d) LDLC >190 mg/dl, e) TG >200 mg/dl, and f) LDLC/HDLC ratio >5.0. The cut-offs chosen approximated mean + 1SD (for positive risk factors) or mean - 1SD (for protective factor) for the respective index in the controls. For the case of HDLC, the cut-off chosen coincided with that for the lowest quintile for HDLC distribution in males and females in the control group, while the cutoff of <15.0 for % HDLC has been used by the Division of Human Nutrition, Institute for Medical Research (IMR), Kuala Lumpur for the past two decades (Ng, 1990).

An OR <1.0 indicated negative risk that is, protective effect, while OR >1.0 meant increased risk with the exposure, that is, positive risk (WHO, 1992). The confidence interval for OR was reflected in the Mantel-Haenszel X^2 test applied to the data, using $p < 0.05$ to indicate significance.

ROC curves, that is, plots of sensitivity vs 1-specificity (Galen, 1982) were used to assess the clinical value of each of the indices measured. The nearer the curve of a particular index is to the respective axis, the better the index as a marker of CAD.

RESULTS AND DISCUSSION

Serum lipid levels

The levels of the various serum lipid indices in both the CAD group and the healthy controls are shown in Table 1. Serum TC and LDLC values were able to distinguish the CAD patients from the healthy controls only in the females but not in the males, confirming the limitations of these assays in the assessment of risk to CHD as were reported in a previous local study (Ng *et al*, 1997). Of the 6 indices measured, only 4 viz., HDLC, % HDLC, TG and the atherogenic index-LDLC/HDLC were sensitive enough to distinguish between the CAD patients and the controls. In this aspect, HDLC values per se (i.e. expressed as mg/dl) were just as efficient as "% HDLC" and superior to the LDLC/HDLC ratio.

It was noteworthy that in the controls, the Indian males had significantly lower ($p < 0.05$) HDLC levels than their Malay and Chinese counterparts (Table 2), thus reinforcing a similar observation

reported earlier in Malaysians (Ng *et al*, 1995). Within the patient and control groups, however, no difference in TG levels could be attributed to ethnicity in the present data set.

Table 1. Mean values of serum lipid indices in CAD patients and controls*

Group	N	HDLC (mg/dl)	% HDLC (mg/dl)	LDLC (mg/dl)	TC (mg/dl)	TG (mg/dl)	LDLC HDLC
Males:							
CAD	217	^a 32.1± 8.4	^a 16.0± 5.4	^a 136± 40	^a 209± 40	^a 206± 112	^a 4.57± 1.98
Control	106	^b 38.5± 10.3	^b 18.6± 5.6	^b 145± 27	^b 214± 31	^b 151± 89	^b 4.01± 1.20
Females:							
CAD	46	^a 34.1± 8.5	^a 15.3± 4.6	^a 162± 49	^a 231± 53	^a 175± 88	^a 5.02± 2.03
Controls	54	^c 467± 12.6	^c 228± 7.5	^c 143± 38	^c 213± 41	^c 118± 64	^c 330± 1.27

*Values given as mean ± SD; Values with different superscripts in the same column are significantly different (p < 0.05)

Table 2. HDLC and TG levels in males according to ethnicity in CAD patients and healthy controls

Ethnic group	CAD/ Controls	Number	HDLC (mg/dl)	TG (mg/dl)
Malays	CAD	95	31.3 ± 9.2	^c 196 ± 95
	Controls	46	*409 ± 10.9	^d 134 ± 73
Chinese	CAD	50	35.6 ± 7.5	^c 212 ± 111
	Controls	35	*385 ± 9.8	^d 173 ± 102
Indians	CAD	72	30.9 ± 7.4	^c 214 ± 133
	Controls	25	*331 ± 7.6	^d 152 ± 92
Combined	CAD	217	^a 321 ± 8.4	^c 206 ± 112
	Controls	106	^b 38.5 ± 10.3	^d 151 ± 89

Values given as mean ± SD; *33.1 < 38.5, 40.9 (p < 0.05); Values with different superscripts in the same column are significantly different (p < 0.05)

The markedly elevated TG levels in the CAD patients were unexpected as earlier findings from the same laboratory indicated that TG levels in both CHD patients and healthy controls were comparable (Ng *et al*, 1995). Early prospective studies have shown that serum TG is not an independent predictor of CHD after adjustment for classic risk factors (Tibblen *et al*, 1975; Heyden *et al*, 1980). However, the scenario on the TG-CHD issue is changing as recent reports based on the Paris Prospective Study by Cambien *et al* (1986), and the Helsinki Heart Study by Manninen *et al* (1992) have indicated that serum TG has clinical value in assessing CHD risk.

In the present study, the CAD patients had markedly elevated TG levels but reduced HDLC levels compared to the controls ($p < 0.05$). This inverse relationship between serum TG and HDLC levels in the CAD patients has been reported previously (Patsch, 1993) and is apparently caused by the metabolism of TG-rich lipoproteins on the HDL, particularly the subfraction HDL₂ which has a negative association with CAD risk (Miller *et al*, 1981).

Effects of sex

As expected, a sex effect was observed for HDLC levels in both patients and controls for all the three ethnic groups studied, with HDLC values significantly higher in females than males (Table 1). This observation accounts largely for the natural protection against CHD that females enjoy compared to their male counterparts. Gordon *et al* (1986) has estimated in a cohort of males that every 1 mg/dl HDLC increment from baseline levels was associated with a 4.4% risk reduction.

With comparable LDLC levels in both sexes, the lower HDLC values for males appearing in the denominator of the LDLC/HDLC ratio thus contributed to a significantly higher mean for this atherogenic index in males compared to in females (4.01 vs 3.30 mg/dl, $p < 0.001$). Females also had lower TG levels than in males (118 vs 151 mg/dl, $p < 0.02$) but this effect of sex was not apparent in the patient group.

Odds ratio analysis

The results of the assessment of risk to CAD by OR analysis are shown in Table 3.

Of the 6 lipid indices assessed, only HDLC, % HDLC and TG gave significant OR values in both the males and females in the combined data set. The OR for HDLC of < 1.0 i.e. 0.15 for combined sexes and 0.24 for males only, underscore the protective role of HDLC in the pathogenesis of CAD. Interestingly, the efficacy of the index in the assessment of CAD risk in the present study was about thrice that found in an earlier study comprising 561 adult males (Ng *et al*, 1997). It is noteworthy that the mean HDLC value for the male CAD patients in this study (32.1 mg/dl) was markedly lower, while the mean HDLC value for the healthy controls (38.5 mg/dl) was comparable, to the corresponding values reported in Ng's earlier study (1997). This apparent discrepancy in the former was not due to an underestimation of HDLC in the present study as the QC values reflected instead an overestimation approximating to 10%.

The impressive OR value of 2.55 (positive risk) in the male CAD patients for TG was unexpected as Ng's earlier study (1997) gave a value approximating 1.0, i.e. a lack of association with CHD risk. Although TG has been reported to exhibit the greatest biological variation, i.e. 35.7% diurnal variation among the serum lipid indices (Cooper *et al*, 1988), the inconsistent findings of our two separate studies remain unresolved.

Receiver-operating characteristics analysis

Plots of ROC curves, i.e. sensitivity vs 1-specificity, for the indices measured are shown in Figure 1. The clinically ideal curve represented a diagnostic test capable of 95% sensitivity and

95% specificity, while chance was indicated by a straight line at a 45° angle between the two axis. It must be emphasized that at any cut-off point selected to estimate sensitivity and specificity, one must sacrifice sensitivity for specificity and vice versa. In addition, sensitivity plus specificity must be greater than 100% if a test is to be better than chance.

Table 3. Odds ratio values obtained for the various lipid indices measured

Index	TG	LDLC HDLC	LDLC	TC	% HDLC	HDLC
	(..... Positive risk)				(... Negative risk ...)	
Overall data	3.01	2.44	1.40	1.24	0.42	0.15
P value for X ²	<0.0001	<0.001	ns*	ns	<0.001	<0.001
Males only	2.55	1.77	1.85	1.33	0.28	0.24
p value for X ²	<0.001	ns	ns	ns	<0.001	<0.001

*ns = not significant; OR value < 1.0 indicates negative risk (protective)

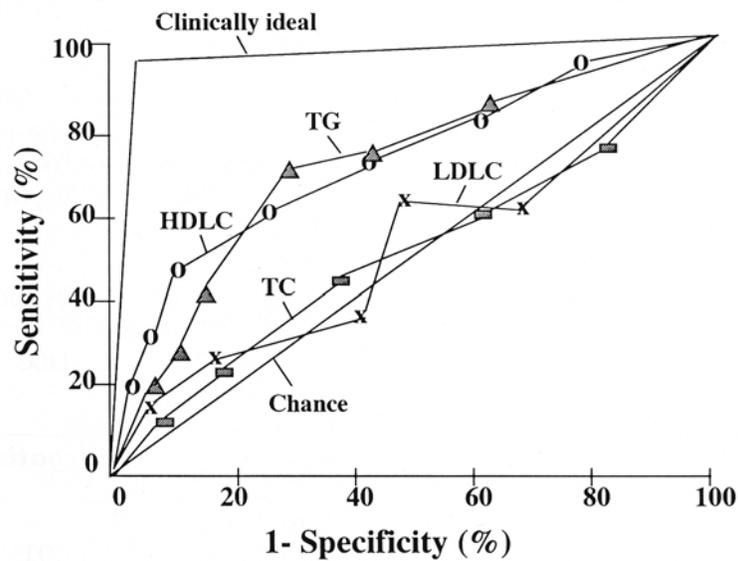


Figure 1. ROC curves for assessment of clinical value

Two important salient features in Figure 1 are: a) the TC and LDLC assays are of little clinical value in the diagnosis of CAD, and these observations agree with Ng's earlier study (1997), b) HDLC and TG assays, however, exhibited superior clinical value compared to TC and LDLC.

Caveats of the Study

All the PTCA and CABG patients from whom the sera samples were collected were not known to be on lipid-lowering drugs prior to hospitalisation at the IJN but this cannot be excluded in those patients who were referred from other states and it was not clear what proportion of them

were on lipid-lowering drugs. In this connection, it is noteworthy that the patient samples in this study had serum TG levels one-and-a-half times that of the healthy controls, which would support the contention that even if this confounding factor was in operation in some of the patients, it did not seem to have had a significant influence in this study.

It is also noteworthy that the common drugs which lower TG-rich lipoproteins, such as nicotinic acid, fibric acid and their derivatives, do not lower HDLC but may in fact raise it (Patsch, 1993). Thus, the lower HDLC levels observed in CAD patients in this study cannot be attributed to the lipid-lowering drugs.

The non-CAD healthy controls in the study were appreciably younger than the CAD patients (mean age, 43 years vs 54 years). Although this age difference might have affected marginally the TC and LDLC results, it probably did not have a confounding effect on the HDLC as Chong *et al* (1982) had previously demonstrated that age did not significantly affect HDLC levels in the Malaysian adults. Besides, analysis of a subset of 69 samples from the control group with ages >40 years gave means for TC and HDLC of 209 mg/dl and 38.4 mg/dl respectively (data not shown), which were comparable to the corresponding values reported for the control group in Table 1.

CONCLUSION

This study indicated that both the HDLC and TG assays may be regarded as valuable diagnostic aids for CAD and to estimate CAD risk. CAD patients possessed significantly higher TG but lower HDLC levels compared with healthy controls. In the case of HDLC, no advantage could be derived from the expression '% HDLCi or the atherogenic index-LDLC/HDLC ratio compared to the HDLC values per se. The results obtained here should dispel any lingering doubts on the diagnostic value of HDLC in the assessment of CHD risk.

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