LIPOPROTEIN(A) DETERMINATION AND RISK OF CARDIOVASCULAR DISEASE IN SOUTH AFRICAN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA

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Objective. A raised plasma level of lipoprotein(a) (Lp(a)) is an established genetic risk factor for coronary heart disease (CHD), particularly in patients with concomitant elevation of low-density lipoprotein (LDL) cholesterol. The current study focused on the comparison of two commercially available Lp(a) assay kits to determine whether differences observed in measured Lp(a) levels could be deemed negligible in CHD risk assessment in familial hypercholesterolaemic (FH) patients.

Design. To compare results obtained on duplicate plasma samples using two commercially available Lp(a) measuring kits, the immunoradiometric assay (RIA) and the enzyme-linked immunoabsorbent assay (ELISA).

Setting. Division of Human Genetics, Department of Obstetrics and Gynaecology, University of Stellenbosch, Tygerberg, South Africa and the Institute for Medical Biology and Human Genetics, University of Innsbruck, Austria.

Subjects. Plasma samples were obtained from 146 family members of 65 molecularly characterised South African FH families for comparative analysis.

Results. Using the RIA method, 34 samples (23%) considered to be in the normal range by the ELISA technique, were placed in the high-risk group (> 30 mg/dl). Only one sample, considered to have a normal Lp(a) level with the RIA method, was categorised by the ELISA technique as high risk.

Conclusion. Our data demonstrate that measurements of Lp(a) using the RIA method (the only assay available in South Africa at the time of this study) differ significantly from those obtained by the reference ELISA technique, suggesting that misclassification could lead to inaccurate CHD risk assessment. This is an important consideration in Afrikaner FH families, where plasma levels of Lp(a) have been shown to be elevated significantly in FH patients compared with non-FH individuals.

S Afr Med J 2000; **90**: 374-378.

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Familial hypercholesterolaemia (FH) contributes significantly to the high mortality rate from coronary heart disease (CHD),[1] especially in the Afrikaner population of South Africa where the disease prevalence is increased owing to a founder effect.[2] FH is an autosomal dominant condition caused by mutations in the low-density lipoprotein receptor (LDLR) gene, and is characterised by elevated LDL cholesterol, xanthomas and premature CHD.[1] Raised plasma concentrations of lipoprotein(a) (Lp(a)), a subfraction of LDL, are also elevated in patients with heterozygous FH,[3,4] and are generally considered to be a feature of this disease. Sufficient evidence exists to suggest that a raised Lp(a) concentration is a strong independent risk factor for the development of CHD,[5] and may therefore confer additional risk of atherosclerosis in FH patients.

Phenotypic variability observed among FH heterozygotes with the same LDLR gene defect complicates accurate identification of individuals with the highest CHD risk.[6-8] Variation in the clinical expression of FH observed in a molecularly characterised South African Afrikaner family prompted an investigation into the possible allelic effects of apolipoprotein (apo) E and B polymorphisms, and elevated Lp(a) levels.[7] A severely affected 54-year-old proband had significantly higher Lp(a) levels than his relatively healthy 84-year-old father with the same LDLR gene mutation, implicating elevated Lp(a) concentration as a genetic factor contributing to CHD risk in the family. This family study was subsequently extended to include 30 FH families with one of the founder-related Afrikaner mutations D206E (FH 1), V408M (FH 2) or D154N (FH 3), for analysis of Lp(a) levels and possible effects of the apo(a) locus.[9] A significant effect of LDLR gene mutation

on Lp(a) concentration could be demonstrated, independent of allelic effects at the apo(a) locus. Given the interactive effect of high Lp(a) and elevated LDL on CHD risk, we deduced from this study that elevated Lp(a) may contribute to the CHD risk in (South African) FH patients.

The function and metabolism of Lp(a) is unknown, but it has been suggested that Lp(a) may aid in wound healing by binding fibrin at wound sites.[10] Subsequent to this Hoffmann *et al.*[11] have demonstrated that Lp(a) competes with LDL for LDL receptors, while Snyder *et al.*[12] showed that only 25% of Lp(a) is catabolised via the LDL pathway. Various contradictory reports have since been published on the significance of Lp(a) as a risk factor for CHD.[5] Two independent studies[13,14] indicated that for Lp(a) to exert its atherogenic effect, an elevation in LDL levels is essential, suggesting biochemical interaction between LDL and Lp(a). Various studies have supported this,[5] but the mechanism(s) of interaction still remains to be established.

The many contradictory reports in the literature with regard to Lp(a) are confusing and may lead to an underestimation of its significance as a risk factor for CHD. Berg[15] provided numerous reasons for different outcomes of studies on disease association, including technical problems encountered in measurement of Lp(a) concentration. It has been shown[16] that the design as well as the antibodies utilised for Lp(a) determination are factors that can greatly influence the outcome of an assay.

The current study focused on the comparison of two commercially available Lp(a) assay kits to determine whether differences observed in measured Lp(a) levels could be deemed negligible in CHD risk assessment in FH patients. The immunoradiometric assay (RIA) — the only Lp(a) assay available in South Africa at the time this study was initiated — is based on a direct sandwiching technique in which two monoclonal antibodies are directed against separate antigenic determinants on the apolipoprotein molecule. The second method is based on a double antibody enzyme-linked immunosorbent assay (ELISA), using an affinity-purified polyclonal apo(a) antibody for coating and horseradish peroxidase-conjugated antibody for detection.

MATERIALS AND METHODS

Subjects

Blood samples were obtained with informed consent from 146 members of 65 FH families. The proband in each family has been characterised at the molecular level with one of the Afrikaner founder-related LDLR gene mutations, D206E (FH1), V408M (FH 2) or D154N (FH 3).[6] Whole blood was kept on ice and centrifuged within 4 hours to recover the plasma. Duplicate samples were stored at –70 °C, while a single aliquot of each sample was sent on dry ice by air to Innsbruck for determination of Lp(a) levels and for comparison with

measurements obtained in a laboratory in South Africa.

Fifty-four index cases older than 25 years were further categorised into CHD-positive and CHD-negative FH groups (Table I). Patients were recorded as having CHD if they presented with angina pectoris or had suffered a myocardial infarction.

Table I. Characteristics of familial hypercholesterolaemia (FH) heterozygotes with and without coronary heart disease (CHD)

	CHD+*	CHD–[†]
Number of patients	22	32
Male/female ratio	10:12	10:22
Mean age (yrs)	50.3 (13.4)	45.3 (11.8)
Mean TC (mmol/l)	10.27 (2.54)	10.18 (1.84)
Mean TG (mmol/l)	1.67 (0.73)	1.42 (0.67)
Mean HDLC (mmol/l)	1.25 (0.36)	1.24 (0.38)
Mean LDLC (mmol/l)	8.25 (2.55)	8.29 (1.74)
FH 1 mutation (*N*)	10 (45.5%)	25 (78.1%)
FH 2 mutation (*N*)	7 (31.8%)	4 (12.5%)
FH 3 mutation (*N*)	5 (22.7%)	3 (9.4%)

* '+' indicates the presence of CHD.
† '–' indicates the absence of CHD.
TC = total cholesterol; TG = triglyceride; HDLC = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol;
FH 1 - 3 = Afrikaner founder-related LDLR gene mutations.

Apo(a) solid phase two-site immunoradiometric assay (RIA)

Antibodies, standards and reagents were obtained as test kit components from Pharmacia Diagnostics AB (Uppsala, Sweden). The RIA assay was performed according to the manufacturer's instructions and calibrated using the provided standards. The method uses two monoclonal antibodies directed against separate antigenic determinants on the apo(a) molecule. During incubation, apo(a) in the sample reacts with ^{125}I-anti-apo(a) antibodies and anti-apo(a) antibodies bound to Sepharose-particles. The antigen/antibody sandwich is separated from excess tracer by centrifugation and decantation, and the radioactivity in the pellet is counted. Results were reported in mg/dl; according to the manufacturer 1 U/l would correspond with 0.1 mg/dl.

Double antibody enzyme-linked immunosorbent assay (ELISA)

The ELISA assay uses an affinity-purified polyclonal apolipoprotein (a) antibody for capturing (coating) and the horseradish peroxidase-conjugated monoclonal antibody IA2 (Boehringer Mannheim, Germany) for detection.[9] This antibody does not cross-react with plasminogen.[17] Lp(a) concentrations were reported in mg/dl.

Statistical analysis

A mixed-model analysis of variance (ANOVA) was applied to log transformed Lp(a) levels. Independent variables, namely age, gender and familial dependence, were considered in the analysis. To determine whether agreement existed between the two techniques, a Bland-Altman method[18] was used for comparison. McNemar's chi-square test was used to determine whether the difference observed was statistically significant.

RESULTS

Plasma Lp(a) levels were determined in 146 individuals from 65 FH families using both an internationally accepted ELISA technique and an RIA method, widely used at the time the study was initiated in South Africa, to establish whether values observed with the two methods were compatible. Table II demonstrates results of the 146 samples classified according to Lp(a) levels measured using the two different techniques. The RIA method placed 34 samples in the high-risk group (> 30 mg/dl), whereas using the ELISA technique these were considered to be within the normal range. The ELISA technique indicated only a single sample in the high-risk group, which was considered normal by the RIA method. A statistically significant difference was detected between the two methods (P < 0.0001) using the McNemar chi-squared test.

Analysis of the data showed a negatively skewed distribution with a mean of –22.2 mg/dl (standard deviation (SD) = 30.93) (Fig. 1). The negative mean indicates that the RIA method preferentially overestimates Lp(a) levels, and would thereby include more false-positives compared with the ELISA technique. Using a paired t-test, a statistically significant difference was observed between the two methods (P < 0.0001).

To determine whether Lp(a) levels differ in FH patients with and without CHD, we analysed 54 index cases older than 25 years using the least squares method. No significant association with CHD was observed with either the ELISA or the RIA method (P = 0.55 and 0.49, respectively) after adjusting for age and gender (Table III). Plasma cholesterol concentration did not differ significantly between FH patients with and without
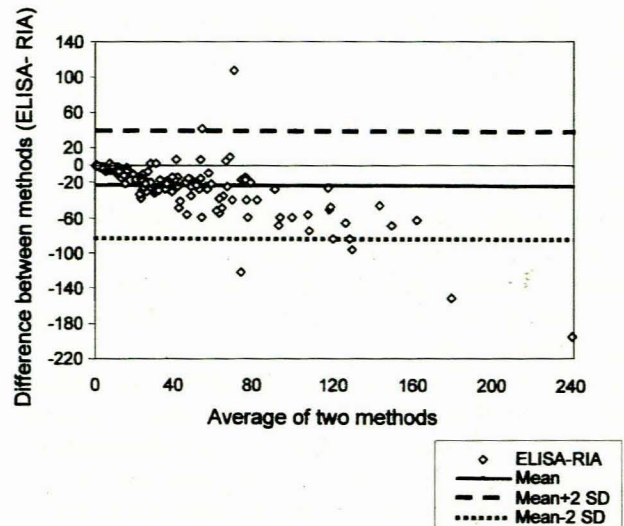

Fig. 1. Bland-Altman's plot of agreement. Data have been plotted to show the difference between the two methods (ELISA-RIA) against the average measurement.

CHD, despite the fact that the receptor-negative FH2 mutation was more prevalent (31.8% v. 12.5%) in the former group (Table I). Previous studies[6,19] have shown that the FH 2 mutation was associated with significantly higher cholesterol levels than the receptor-defective FH 1 or FH 3 mutations.

DISCUSSION

This comparative study was prompted by an earlier suggestion by Berg[15] that contradictory results obtained in the analysis of Lp(a) as a risk factor for CHD may be a consequence of technical problems rather than lack of association. The main objective of the study was to determine whether the RIA method, used routinely in South Africa at the time of the study, would provide similar results to the internationally accepted double-antibody ELISA technique. We collected duplicate plasma samples of 146 family members from 65 Afrikaner FH

Table II. Classification of 146 samples according to Lp(a) concentrations obtained with the immunoradiometric assay (RIA) and the enzyme-linked immunosorbent assay (ELISA)

		RIA	
		< 30 mg/dl (N = 59) (normal range)	> 30 mg/dl (N = 87) (high risk)
ELISA	< 30 mg/dl (N = 92) (normal range)	58	34
ELISA	> 30 mg/dl (N = 54) (high risk)	1	53

Table III. Comparison of two methods in determining risk for coronary heart disease (CHD) in familial hypercholesterolaemia (FH) heterozygotes using the least square means

Method	Least square mean CHD + (N = 22)	CHD − (N = 32)	Standard error CHD + (N = 22)	CHD − (N = 32)	Range CHD + (N = 22)	CHD− (N = 32)	P-value
ELISA	2.3348	2.2546	0.0997	0.087	2.5350 - 2.1347	2.4293 - 2.0799	0.55
RIA	2.6561	2.5674	0.0951	0.083	2.8471 - 2.4651	2.7341 - 2.4007	0.49

Values represent log transformation of Lp(a) concentrations (mg/dl).

families for Lp(a) analyses, initially for a sib-pair approach based on genotype information for both the LDLR and apo(a) genes,[9] and subsequently to determine whether differences observed in measurements using the ELISA and RIA methods could be considered negligible in patient management. This proved to be an important consideration in Afrikaner FH families, since the previous study[9] of 30 informative FH families demonstrated significantly elevated Lp(a) levels in FH individuals compared with non-FH relatives. This finding provided evidence for the presence of additional CHD risk in FH individuals, making accurate Lp(a) determination essential in South African patients.

Statistical comparison of the ELISA and RIA methods utilised in this study indicated that the degree of variation observed would be totally unacceptable for clinical purposes. For accurate identification of individuals likely to develop CHD, it should be borne in mind that the RIA method is likely to include many more false-positives than the ELISA technique. The same observation was made by März et al.[16] when comparing the RIA method with two other commercially available assays for Lp(a) determination. In this respect it is important to take into account the fact that, to date, no Lp(a)-lowering drugs are available, although various studies have proposed that lowering LDL in individuals with high plasma Lp(a) levels may reduce CHD progression.[5]

Since FH may be associated with an increase in plasma Lp(a), elevated levels could be regarded as characteristic of this disease. Plasma Lp(a) levels in 22 of the Afrikaner FH index patients who suffer from CHD were, however, not significantly different from 32 FH cases without CHD using either the ELISA or the RIA method, after adjusting for age and gender. At a molecular level the allelic distribution at the apo(a) locus[9] was similar in the two FH groups (P > 0.5) (data not shown). In an attempt to increase our sample size, we included an additional 11 FH family members above the age of 25 years (N = 65), but still no association could be demonstrated between Lp(a) concentration and CHD (data not shown). These results may reflect the small sample size, the composition of the FH patient cohort (e.g. younger individuals may still develop CHD) and/or the difficulty in demonstrating the effect of a

minor gene in the presence of a major gene such as the LDLR gene, known to be responsible for the disease in our study population. It has been suggested[20,21] that failure to demonstrate an association between raised Lp(a) levels and CHD in FH patients may be a consequence of either asymptomatic coronary disease that is common in FH, or a high mortality rate in affected individuals owing to gene-gene interaction. Comparative studies of CHD risk among FH patients can therefore be misleading and prospective studies are needed to address properly the issue of the degree of CHD risk imposed by raised Lp(a) levels in this group of patients.

In summary, our results have demonstrated that the RIA method for determination of Lp(a) concentration differs significantly from the reference ELISA technique. This may hinder the identification of individuals with a high risk of CHD in the South African population. Consequently, the continued use of the RIA method for measurement of Lp(a) should be reconsidered in order to facilitate a more effective patient management regimen. Candidates for Lp(a) measurement should include patients with early coronary disease in the absence of conventional risk factors and those with a family history of premature CHD. Reduction of modifiable risk factors in families with elevated Lp(a) should be a priority until an acceptable means of reducing Lp(a) levels is found.

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TO WHAT EXTENT DOES SOUTH AFRICAN MENTAL HEALTH AND SUBSTANCE ABUSE RESEARCH ADDRESS PRIORITY ISSUES?

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Objective. To investigate the extent to which South African mental health research addresses priority issues.

Design. Cross-sectional survey of conference presentations.

Setting. The most recent conferences of the following professional societies: the Epidemiological Society of Southern Africa, the Psychological Society of South Africa, the Society of Psychiatrists of South Africa, and the South African Association for Child and Adolescent Psychiatry and Allied Disciplines.

Main outcome measures. Whether the presentations addressed priority areas as defined in the list 'Selected priority areas of research' compiled by the Mental Health and Substance Abuse Thrust of the Medical Research Council, and if so which priority areas were addressed.

Results. There were 627 presentations, with 267 (43%) on mental health or substance abuse. Seventy-eight papers (29%) reported original research in a priority area identified by the Medical Research Council. Of these, 73 (94%) were on mental health and 5 (6%) were on substance abuse. Of the 73 papers on mental health, 33 (45%) addressed topics in health problem research, 7 (10%) aetiology research, 22 (30%) intervention research, and 11 (15%) operational and health systems research.

Conclusion. Much South African mental health research does not address priority issues. There is a particular dearth of research addressing substance abuse research priorities. Funding mechanisms and research capacity development initiatives could help to rectify the situation.

S Afr Med J 2000; 90: 378-380.

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