A practical method to determine the "atherogenic lipoprotein phenotype"

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Submitted: 2 February 2008
Accepted: 10 March 2008

Arch Med Sci 2008; 4, 2: 215–217

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Increasing evidence suggests that the "quality" rather than only the "quantity" of low density lipoproteins (LDL) exerts a great influence on cardiovascular risk [1]. Higher plasma triglyceride levels and decreased HDL cholesterol concentrations are usually accompanied by the presence of small, dense LDL in the so-called "atherogenic lipoprotein phenotype" (ALP) or lipid triad [1]. This phenotype is highly atherogenic and its prevalence may suggest higher overall burden of atherosclerotic disease than that associated with hypercholesterolaemia. As stated by the National Cholesterol Education Program Adult Treatment Panel III [2], there is evidence that each component of the lipid triad is individually atherogenic but the relative contribution of each component cannot be easily determined. For this reason, it has been suggested to consider this trait as a whole as a "risk factor". This is supported by data from epidemiological studies considering high-risk populations, which showed that the contribution to cardiovascular risk of each individual component of atherogenic dyslipidaemia cannot be dissected from the sum of all lipid risk factors [2].

Yet, treatment plans have not suggested so far how to exactly measure ALP in clinical practice. For this reason, several and contrasting criteria have been followed to assess ALP; for instance, regarding triglycerides it has been suggested to consider the highest quartile [3] or concentrations >75th or >90th percentile according to age and gender [4], as well as levels >1.5 mmol/l [5]. Regarding HDL cholesterol it has been proposed to consider the lowest quartile [3] or concentrations <25th percentile according to age and gender [4], as well as levels <1.1 mmol/l [5]. The predominance of small, dense LDL has been measured by levels of LDL3 subclass (density 1.044-1.060 kg/l) >40% of total LDL particles, as isolated by density gradient ultracentrifugation [5], or by apolipoprotein B concentrations in LDL5+LDL6 subclasses >25 mg/dl, as isolated by density gradient ultracentrifugation too [6].

We propose here a practical method to calculate ALP in whole plasma. According to the National Cholesterol Education Program Adult Treatment Panel III [3], and as confirmed by the recent guidelines of the joint American Heart Association/National Heart, Lung, and Blood Institute on "Diagnosis and management of the metabolic syndrome", we considered low HDL cholesterol levels as those <1.03 mmol/l (e.g. <40 mg/dl) in men and <1.29 mmol/l (e.g. <50 mg/dl) in women and elevated triglyceride concentrations as those >1.69 mmol/l (e.g. >150 mg/dl). Levels of small,

dense LDL (e.g. percent of LDL3+LDL4) were considered to be increased in those patients who presented with values greater than mean ±2SD of the values of control subjects, matched for age, gender and body weight (Figure 1).

LDL size and subclasses were assessed by 2-16% non-denaturing polyacrylamide gradient gel electrophoresis of whole plasma at 10-14°C [7]. Since the quality and the reproducibility of the polyacrylamide gradient gel electrophoresis method may affect future results, we suggest using the method of Krauss et al., which has been tested and validated worldwide in a very large number of studies in the last 20 years. Using this method, strong correlations have been found with the results obtained by analytical and standard ultracentrifugation and electron microscopy. In order to produce reproducible results, gels are also commercially available.

Gels were subjected to electrophoresis for 24 hours at 125 V in tris borate buffer (pH 8.3) as described elsewhere [7], were fixed and stained for lipids in a solution containing oil red O in 60% ethanol at 55°C, and were then placed on a light source and photographed with a Canon G3 digital camera. Migration distance for each absorbance peak was determined and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of size standards of known diameter, which includes carboxylated latex beads (Duke Scientific, Palo Alto, CA), thyroglobulin and apoferritin (HMW Std, Pharmacia, Piscataway, NJ) having molecular diameter of 380, 170 and 122 Å, respectively, and lipoprotein calibrators of previously determined particle size. With this method it is possible to identify up to seven subclasses of LDL (e.g. LDL I, IIA, IIB, IIIA, IIIB, IVA and IVB) as a percentage of total LDL [7]. The following were the coefficients of variations for our methodology: the inter-assay variation was within ±0.7 Å and the intra-assay variation was within ±0.4 Å. The percentage values of small, dense LDL (e.g. LDL3+LDL4) were calculated in patients and controls and were considered increased in individuals with values greater than mean +2 standard deviations of the values of controls (matched for age, gender and body weight).

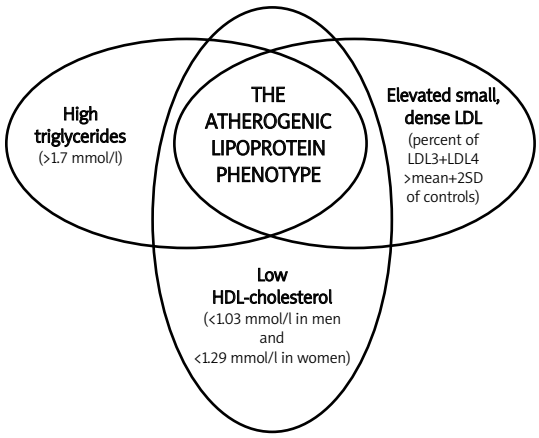

Figure 1. A model for the atherogenic lipoprotein phenotype (ALP)

We recently applied such methodology to determine the prevalence of ALP in healthy subjects as well as in different categories of patients at high cardiovascular risk, such as those with coronary and non-coronary forms of atherosclerosis (e.g. with coronary heart disease, aortic abdominal aneurysms, peripheral arterial disease) or metabolic diseases (e.g. with polycystic ovary syndrome and growth hormone deficiency) [8-10 and unpublished observations].

In conclusion, we propose a practical method to determine ALP in whole plasma. Since the definition of ALP has so far been somewhat controversial, this method may represent the basis for performing future studies with consistent criteria. In addition, this method may be more easily applied to clinical practice than those suggested in the past; in fact, beyond the measurement of plasma lipids, we propose the analysis of LDL subclasses by whole plasma electrophoresis [11], a much easier technique than the isolation of lipoprotein subfractions by density gradient ultracentrifugation.

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