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Factor analysis of risk variables associated with iron status in patients with coronary artery disease

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ABSTRACT

Objectives: Epidemiological evidence concerning the role of iron, a lipid peroxidation catalyst, in atherosclerosis and coronary artery disease (CAD) is inconsistent.

Design and methods: Exploratory factor analysis was used to examine the potential clustering of variables known to be associated with CAD using data from 188 patients with angiographically-approved disease. The resulting factors were then tested for their association with serum ferritin and soluble transferrin receptor (sTfR) as indicators of body iron status.

Results: Factor analysis resulted in a reduction of a variable number from the original 15 to 5 composite clusters. These factors were interpreted as (1) "proatherogenic factor" with positive loadings of TC, LDL-C, apoB and TG; (2) "inflammatory factor" with positive loadings of hsCRP, fibrinogen and MDA; (3) "antiatherogenic factor" with positive loadings of HDL-C and apoA-I; (4) "obesity factor" with positive loadings of weight and waist; and (5) "antioxidative status factor" with positive loadings of SOD and age and negative loading of superoxide anion. "Inflammatory", "obesity" and "antiatherogenic" factors predicted high ferritin values and the "proatherogenic factor" predicted high sTfR values. We compared the ability of the "proatherogenic factor" with that of a multivariable logistic model that included the "proatherogenic factor" and sTfR values in predicting significant stenosis in patients. The area under the ROC curve was 0.692 vs. 0.821, respectively.

Conclusions: "Inflammatory", "obesity", "antiatherogenic" and "proatherogenic" factors were associated with increased parameters of body iron status. The measurement of sTfR improves the prediction of CAD based on clustered cardiovascular risk factors.

Introduction

The most plausible mechanism by which iron may promote the formation of the atherosclerotic plaque lies in its well-known ability to catalyze the production of reactive oxygen species, lipid peroxidation and LDL-oxidation. In 1981 Sullivan proposed the "iron hypothesis", suggesting that greater levels of stored iron in men and postmenopausal women may explain the higher incidence of heart disease in these groups [1]. Since then, numerous studies have assessed the association between iron status and the risk of coronary heart disease, but findings have been inconsistent [2–4]. Various laboratory parameters have been used to assess body iron stores and iron availability when attempting to establish a link between iron and coronary artery disease [5]. Among, them ferritin and sTfR are considered to be the most reliable estimates of iron status; ferritin is considered the best measure of body iron stores whereas sTfR is considered to reflect the functional iron compartment.

Most *in vitro* studies have indicated that iron has prooxidant/proatherogenic properties but such properties have not always been confirmed. Evidence from several prospective epidemiological studies does not support the iron theory [6–8]. Moreover, many studies have failed to find a positive association before and after adjusting for a wide range of cardiovascular risk factors.

Increased serum ferritin levels have been shown to represent significant risk factors of myocardial infarction and atherosclerosis owing to iron-mediated oxidative damage [9]. Ferritin is also an acute-phase reactant and its concentration may be increased by myocardial damage

Abbreviations: CAD, coronary artery disease; CVD, cardiovascular disease; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; Lp(a), lipoprotein(a); hsCPR, high sensitivity C-reactive protein; MDA, malondialdehyde; $O_2^{\cdot-}$, superoxide anion; SOD, superoxide dismutase; sTfR, soluble transferrin receptors.

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and inflammation [10]. It is possible that ferritin plays a role through other risk factors such as blood pressure and cholesterol [11]. Further studies, particularly investigating interactions/synergies between serum ferritin and other risk factors, are required to establish a role for ferritin in development of CAD.

The inconsistency from these studies may be the result of the different parameters that have been utilized for measuring tissue iron stores, differences in samples sizes and recruitment biases. A crucial question that remains to be answered is whether serum ferritin and sTfR are independent atherosclerotic risk factors, or, whether the increased risk attributed to these iron status parameters is actually a result of their complex interactions with other traditionally recognized risk factors. So in this study we evaluated the influence of other biochemical parameters on iron status parameters using factor analysis. Factor analysis reduces a large number of intercorrelated variables to a smaller subset of underlying "independent" variables (factors). The factors represent statistically independent and physiologically distinct phenotypes and may reveal unifying communalities between physiological domains [12]. Although previous studies [9,11] have described the individual contribution of iron status parameters to atherosclerosis and CAD, the potential role of clustering cardiovascular risk factors has been little explored.

To investigate the value of iron status parameters in coronary risk assessment in a Serbian population with a high prevalence of atherosclerosis [13], we evaluated the relationship between serum ferritin, sTfR and the presence of CAD. We used exploratory factor analysis to examine potential clustering of variables known to be associated with atherosclerosis and CAD. The resulting factors were then tested for their association with iron status parameters. We also explored whether there was an independent association between iron status parameters and CAD after adjusting for different confounding "clustered factors". In addition, we tested the accuracy of iron status parameters and clustered variables in CAD detection.

Materials and methods

Subjects

The patient group consisted of 188 subjects who were undergoing coronary angiography for suspected coronary artery disease at the Institute of Cardiovascular Disease located in the Clinical Centre of Serbia in Belgrade. All patients with myocardial infarction within the last 6 months, those with unstable angina who had angina pain at rest within one month, or those with a history of prior coronary revascularization were excluded. All patients had a history of stable angina defined on the presence of chest pain that did not change its pattern during the preceding 2 months. Two cardiologists unaware that the patients were enrolled in the study reviewed all the angiograms. Patients were categorized into one of two groups based on the extent of their CAD, as assessed by coronary angiography. One hundred forty-eight patients had significant stenosis (≥50% in one or more coronary arteries), CAD(+) and 40 patients had no stenosis in any artery, CAD(−). All enrolled patients completed a questionnaire that incorporated numerous risk-related issues. For every patient, weight and waist circumference were recorded. We excluded patients with a history of recent clinical infection, concurrent major renal, hepatic or malignant disease, surgery or major trauma during the month prior to study entry. Diabetic patients (patients with a fasting glucose level ≥7.0 mmol/L or patients who were receiving oral hypoglycemic agents or insulin medication) and patients receiving any anti-hyperlipidemic medication were excluded from the study. We excluded individuals with hsCRP ≥10 mg/L, a level considered to be indicative of a clinically relevant inflammatory condition [14].

All patients gave informed consent prior to their enrolment in the study. The study was planned according to ethical guidelines following the Declaration of Helsinki. The institutional review committee approved our study protocol, thereby following local biomedical research regulations.

Methods

Blood samples were obtained from the patients after overnight fasting. Peripheral venous blood was drawn into collection tubes containing ethylene diamine-tetraacetic acid (EDTA), citrate or serum separator gel. Lipid, apolipoprotein and oxidative stress status parameters were determined in EDTA plasma, high sensitivity serum C-reactive protein (hsCRP), ferritin and sTfR in sera and fibrinogen in citrate plasma. All the assays were performed blindly.

Most of the biochemical markers were measured using an ILAB 600 analyzer (Instrumentation Laboratory, Milan, Italy). Total cholesterol (TC) and triglycerides (TG) were assayed using routine enzymatic methods. HDL-C was measured using the same enzymatic method after precipitation of the plasma with phosphotungstic acid in the presence of magnesium ions and LDL-C was calculated using the Friedewald formula. Apolipoprotein A-I (apoA-I), apolipoprotein B (apoB), ferritin and sTfR were measured using immunoturbidimetry (Dialab, Vienna, Austria). The concentrations of serum hsCRP were also measured using immunoturbidimetry (BIOKIT, Barcelona, Spain). Fibrinogen was measured by the Clauss method employing ACL 200 Instrumentation laboratory and original reagents. Plasma malondialdehyde (MDA) concentration was measured using the thiobarbituric acid-reactive substances (TBARS) assay, previously described by Girotti [15]. In our hands the intra-assay CV was 4.8% and the inter-assay CV was 7.2%. The rate of nitroblue tetrazolium reduction was used to measure the level of O2•−, as described by Auclair and Voisin [16]. The intra-assay CV was 5.6% and the inter-assay CV was 9.5%. Plasma SOD activities were measured according to the previously published method by Misra and Fridovich [17]. One unit of SOD activity is defined as the activity that inhibits the auto-oxidation of adrenalin by 50%. The intra-assay CV was 6.3% and the inter-assay CV was 9.2%.

Statistical analysis

Because the distributions of TG, Lp(a), hsCRP and ferritin values were skewed, logarithmic transformation of the data was performed. The results are expressed as arithmetic mean (X) ± standard deviation (SD) for normally-distributed variables and as geometrical mean and 95% confidence interval (CI) for the mean for TG, Lp(a), hsCRP and ferritin. Statistical differences were evaluated according to the Student t-test for continuous variables, whereas proportions were compared using the chi-square test for contingency tables. TG, Lp(a), hsCRP and ferritin values were compared after logarithmic transformation.

Factor analysis was conducted using the FACTOR procedure of MedCalc for Windows statistical software Version 9.6.3 (Mariakerke, Belgium) and SPSS 18 statistical software (SPSS Inc, USA). We used principal component analysis, a technique for reducing a number of original variables into fewer summary factors or principal components [18]. The Kaiser–Meyer–Olkin test was used to examine sampling adequacy. Only factors with eigenvalues >1 were extracted for the subsequent orthogonal (varimax) rotation of the factor matrix. Eigenvalues measure the amount of the variation explained by each factor. An eigenvalue greater than 1 indicates that factors account for more variance than accounted by one of the original variables in standardized data. Variables that shared ≥25% variance with a summary factor were used for interpretation (corresponding to a loading factor of ≥0.50) [12,16]. Factor scores, representing individual subjects' predicted eigenvalues for each factor, were calculated and modeled as independent variables in a multiple logistic regression model in which high ferritin and sTfR concentrations, defined as upper tertile, were the dependent variables. Using multivariate logistic regression analysis we examined the risk for development of significant stenosis in subjects with high ferritin and sTfR concentrations. After that, we calculated the area