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Evaluation of a model of latent pathologic factors in relation to serum ferritin elevation

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Abstract

Objectives: Serum ferritin increases in various disorders and clinical conditions. However, causal associations between the serum ferritin level and clinical factors that influence serum ferritin level are not well characterized. We report a model that quantitatively analyzes the causal relations between the serum ferritin level and clinical factors.

Design and methods: We analyzed the ferritin level and other laboratory data in the sera of 274 patients. Structural equation modeling was used to verify causal relations and the adequacy of latent factors.

Results: Three factors representing clinical status were identified: cell damage, hepatic function, and inflammation. Serum iron (SI) had the strongest effect on serum ferritin elevation. The effect of the cell damage factor on serum ferritin indicated cell destruction, and that of the hepatic function factor represented decreased serum ferritin clearance. The cell damage factor also indirectly increased the ferritin level via SI or the hepatic function factor. The total effect of the inflammatory status factor on ferritin level was very weak.

Conclusions: These causal relations may explain the mechanism of serum ferritin level elevation in various clinical conditions.

Keywords: Ferritin; SEM; Factor analysis; Hyperferritinemia; Structural equation modeling

Ferritin, an intracellular iron storage protein, is found in various species and plays important roles in iron metabolism and detoxification [1]. The ferritin molecule possesses an iron core of up to 4500 Fe(III) atoms as an inorganic complex [2]. Human ferritin is a 24-mer composed of two polypeptide chain subunits, the L subunit (19.5 kDa) and the H subunit (21 kDa), in different proportions [2,3]. H subunits catalyze the oxidation of Fe(II), and L subunits are necessary for storage of iron in the core [4,5]. Ferritin in the liver and spleen is rich in L subunits, whereas that in the heart and brain is rich in H subunits [1]. Ferritin in normal serum contains only trace levels of the H subunit. Ferritin synthesis is normally regulated by the intracellular iron concentration [1,2]. When cellular iron levels are low, iron regulatory protein (IRP) binds to the stem–loop structure of the 5′-untranslated region of ferritin mRNA and represses the translation of ferritin H and L chains [6–8]. The IRP binding site is termed the iron-responsive element (IRE). An IRE is also found in the 3′-untranslated region of transferrin receptor (TFR) mRNA. Under conditions of iron deficiency, IRP also binds to TFR mRNA to stabilize it [1]. Iron deficiency leads to decreased ferritin expression and increased iron uptake into cells via increased TFR expression. Under conditions of excess iron, IRP loses IRE binding activity [1], leading to derepression of ferritin synthesis and destabilization of TFR mRNA; production of ferritin is increased, and iron uptake is decreased [2].

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CFI, comparative fit index; CHE, cholinesterase; CP, ceruloplasmin; CRP, C-reactive protein; *df*, degrees of freedom; IRE, iron-responsive element; IRP, iron regulatory protein; LD, lactate dehydrogenase; P_{CLOSE}, significant probability of close fit; RMSEA, root mean square error of approximation; SEM, structural equation modeling; SI, serum iron; SSC, standardized structural coefficient estimate; TCHO, total cholesterol; TF, transferrin; TFR, transferrin receptor; TIBC, total iron-binding capacity.

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The serum ferritin level increases not only in patients with iron overload conditions such as hemosiderosis or idiopathic hemochromatosis but also in patients with forms of anaemia other than iron deficiency anaemia [9], malignancy (leukemia, Hodgkin's disease, cancer) [10–12], liver disorder [9,13], and inflammation [14,15]. Hereditary hyperferritinemia, caused by a single point mutation of the IRE of the ferritin H and L chains, has also been reported [16,17]. Mutation of the H chain and L chain induces iron overload and cataracts, respectively [18,19]. Thus, the serum ferritin level increases in various clinical conditions such as iron overload, tissue destruction, organ dysfunction, and inflammation and in response to gene mutation. However, causal relations between the increase in the serum ferritin level and these factors have not been well evaluated. In the present study, we used structural equation modeling (SEM) [20] to quantitatively analyze causal relations between serum ferritin elevation and pathologic factors and to elucidate the mechanisms of serum ferritin elevation.

Materials and methods

Subjects

We analyzed serum samples submitted to our laboratory for routine clinical testing from 274 patients (77 with malignancy, 75 with haematological disorder, 22 with autoimmune disease, 18 with cardiovascular disease, 15 with liver disorder, 12 with renal disease, 9 with diabetes mellitus, 6 with digestive disorder, and 40 with another disorder). Serum samples were randomly selected from a ferritin-normal group and from patients with hyperferritinemia so that the results did not depend on the spectrum of diseases or the number of patients in each disease category. We purged patient information other than sex, age, and diagnosis from the database to protect patient privacy. Whole blood samples were centrifuged after clotting at room temperature. Sera were stored at −80 °C.

Laboratory assays

Serum ferritin concentrations were measured with ADVIA Centaur Ferritin Assay Reagent and an ADVIA Centaur Analyzer (Bayer Diagnostics). Serum iron (SI) and total iron-binding capacity (TIBC) were measured with a Dimension clinical chemistry system (Dade Behring). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), cholinesterase (CHE), total cholesterol (TCHO), albumin (ALB), and C-reactive protein (CRP) levels were measured with a Hitachi 747-400 analyzer. Transferrin (TF) and ceruloplasmin (CP) levels were determined by nephelometric assay with a Behring Nephelometer II analyzer (Dade Behring). LD and TCHO were measured immediately before storage of sera at −80 °C.

Statistical analysis

Normality of distribution was assessed by means of the Kolmogorow–Smirnov test ($P>0.05$). Variables not normally distributed were power transformed to approximate normality. Statistical analyses were performed with SPSS for Windows 11.0J and AMOS 5.0 software (SPSS Inc.).

SEM with latent variable techniques and maximum likelihood parameter estimation was used to examine latent factors representing clinical status and to explain the relations between serum ferritin level and latent factors. SEM comprises a family of statistical techniques that integrates path analysis and factor analysis.

Model fit was assessed by Chi-square (χ^2) test, comparative fit index (CFI), root mean square error of approximation (RMSEA), and significant probability of close fit (P_{CLOSE}). Chi-square tests the null hypothesis that an unconstrained model fits the covariance/correlation matrix as well as the given model. If the hypothesis is correct, the obtained χ^2 value should be small. CFI provides an assessment of comparative fit independent of sample size and ranges from 0 to 1, with a value greater than 0.9 indicating a good fit to the data. RMSEA assesses closeness of fit. This is computed with the model χ^2, degrees of freedom (df), and number of subjects. Good model fit is indicated by RMSEA values less than or equal to 0.05. Adequate fit is indicated by values less than or equal to 0.08. P_{CLOSE} is the probability for testing the null hypothesis that the population RMSEA is no greater than 0.05. If P_{CLOSE} is less than 0.05, the null hypothesis is rejected. We accepted models in which RMSEA was less than 0.05 or P_{CLOSE} was greater than 0.05 and in which CFI was greater than 0.9.

Results

All variables except TIBC were power transformed to approximate normality. Median values and interquartile ranges for the results of laboratory tests are shown in Table 1. Mean ferritin concentrations in men and women were 1724 ng/mL and 1641 ng/mL, respectively. Table 1 also shows standardized partial regression coefficients between serum ferritin (dependent variable) and laboratory test results (independent variables) by multiple regression analysis. Because TIBC value is calculated from TF concentration and ALT strongly correlated with AST, ALT and TF were removed from the analysis to eliminate multicollinearity. SI, TIBC, AST, and LD were highly significant variables with respect to serum ferritin elevation.

We assumed that LD and AST, which increase in serum as a result of cell destruction, and ferritin depended on a latent variable that reflects cell damage and that TCHO, CHE, and ALB, which are synthesized in the liver and decrease as a result of hepatic dysfunction, depended on a variable that represents hepatic function. We also assumed that a factor reflecting inflammation determined CRP and CP, which are acute phase inflammatory markers, as well as ferritin. A path diagram for the relations between ferritin, SI, and latent factors is shown in Fig. 1 (model A). In this model, we assumed that ferritin was also determined by SI and hepatic function and that cell destruction produced hepatic dysfunction. Observed (measured) variables (rectangles) and residual error terms (circles) reflecting unexplained variance (effect of unmeasured variables) plus measurement error are shown. Latent variables (ovals) measured