Original Article

Serum Ferritin and Obstructive Coronary Artery Disease: Angiographic Correlation

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Summary

Objective: **To verify the possible association between the levels of serum ferritin and the degree of obstructive coronary artery disease.**

Methods: **115 patients with coronary arteriography and concomitant evaluation of serum ferritin were studied. The adopted cut-off values were 80 ng/ml for women and 120 ng/ml for men.**

Results: **The mean ferritin levels for males and females were 133.9±133.8 ng/ml and 214.6±217.2 ng/ml, respectively (p=0.047). It was observed that 44.1% of the women had normal serum ferritin levels in comparison to 30.9% of the men (p=0.254). In the patients without obstructive coronary artery disease or with less severe obstructions (group A) the serum ferritin level was 222.3±325 ng/ml. On the other hand, for those with moderate (group B) and severe obstructions (group C) the levels were 145.6+-83.7 ng/ml and 188.9±150.6 ng/ml, respectively. There was no correlation between the degree of coronary artery disease and the mean level of serum ferritin. Regarding the cut-off value, the number of women with serum ferritin level > 80 ng/ml in groups B+C or only C was significantly higher than the number of women in group A (ODDS RATIO 9.71 with 95%CI from 1.63 to 57.72). For males there was no significant difference between the number of cases above or below the cut-off values (ODDS RATIO 0.92 with 95%CI from 0.28 to 2.95).**

Conclusion: **It was verified that women with serum ferritin levels > 80 ng/mL presented more severe obstructive coronary artery disease than women with lower levels. In men, the serum ferritin level was not a predictor element of the degree of obstruction.**

Key Words: **Ferritin; coronary artery disease; coronary arteriography.**

Introduction

Cells of nearly all forms of life require well-defined amounts of iron for survival, replication and expression of differentiated processes. Iron has a central role in erythropoiesis and several intracellular processes. It is the fourth most abundant chemical element on Earth and the most abundant transition metal in living organisms. It is involved in oxygen transportation, transference of electrons, DNA synthesis, oxidation processes by oxygen and hydrogen peroxide (H_2O_2) and in many others, keeping the normal structure and function of virtually all mammal cells[1]. The total body iron content is approximately 50 mg/kg of weight in normal adult men and 35 mg/kg of weight in women. Most of this content, approximately 70% of it, is found in the heme compounds (markedly in hemoglobin and myoglobin). Around 30% remains as iron stores as ferritin and hemossiderin and only 0.1% or less is found in plasma, bound to the transport protein transferrin[1].

Many transition metals have variable valences, such as Fe^{2+} and Fe^{3+} or Cu^+ and Cu^{2+}. The variation between

the states of valence involves the acceptance or donation of electrons and thus, transition metals are markedly good promoters of free radical reactions. Copper and iron ions can convert the superoxide and the hydrogen peroxide into the highly harmful hydroxyl radical, through Fenton's reactions. The binding of iron to ferritin prevents its participation in Fenton's reaction, as the capacity of storage of this protein is much higher than what is theoretically necessary; however, there is no absolute certainty about that. The superoxide radical can also react with hydrogen peroxide (H_2O_2) in the presence of metals such as iron and copper, characterizing the so-called Haber-Weiss Reaction, where oxygen is produced, as well as the hydroxyl ion and the hydroxyl radical. This reaction is of great clinical importance, as the hydroxyl radical is around 5×10^8 more reactive than the superoxide and can attack almost all molecules of living cells. It is also noteworthy, due to its importance, the capacity of the superoxide radical in transforming oxidized iron (Fe^{3+}) into its reduced form (Fe^{2+}).

As previously mentioned, only 0.1% of the total iron is found in plasma (transferrin-bound). There is a strong need to keep it bound to the heme group or as iron stores, as it can lead to highly deleterious effects when it is free. Iron can lead to lipid peroxidation, *in vitro* as well as *in vivo*, promoting ischemic myocardial injury in animal models.

Ferritin is the intracellular iron storage molecule and detoxification with a molecular weight of approximately 460,000 Daltons. The intracellular ferritin is synthesized by the cell smooth endoplasmic reticulum whereas the normal plasma ferritin is synthesized by the rough endoplasmic reticulum, which is mostly glycosylated (70%) before being secreted by the cell, apparently by the Golgi complex. This plasma ferritin differs from the intracellular one by containing smaller amounts of iron. There is, however, an association between the stored iron, intracellular, and the secreted one, in a way that the plasma ferritin concentration reflects the amount of ferritin inside the cell. It is estimated that 1 μg/l of serum ferritin is equivalent to approximately 10 mg of stored iron[1].

It has been reported that iron chelants protect the tissues against the reperfusion injury. This raises the supposition that the iron is released in plasma or becomes accessible to the tissues in order for the reactions of oxidation and reduction to take place, and in this case, the source of this iron would be ferritin. Thus, ferritin would be an element at the same time pro- and anti-oxidant. By regulating the "pool" of free intracellular iron, ferritin controls the generation of reactive-oxygen species catalyzed by iron, thus working as an anti-oxidant. On the other hand, Cairo et al[1] showed, in rats submitted to oxidative stress by the administration of phorone, a glutathione-depleting drug, that initially there is a decrease in the amount of ferritin, indicating that part of the iron participating in the free "pool" derives from ferritin itself, which under these circumstances works as a pro-oxidant. Actually, the initial degradation contributes to the expansion of free intracellular iron, which later activates multiple molecular mechanisms to reconstitute ferritin, therefore limiting the pro-oxidant effect.

According to Wigginton (1995)[1] ferritin, in addition to functioning primarily as an iron storage protein, can also act in the modulation of the immune function: it can mask or depress the expression of molecules on the cell surface that are important for the activation of T cells and other effector functions. These interactions could become pathologically significant when there is accentuated hyperferritinemia.

The results of literature have been conflicting regarding the association between ferritin and atherosclerosis, with some studies confirming[2,3,4,5], and others denying this possible deleterious effect [6,7,8] .

The determination of plasma ferritin levels is a convenient iron balance assessment method. The hepatocytes, the reticulum-endothelial system and presumably, all the other cells that synthesize ferritin secrete a significant amount in the plasma. The measurement of plasma ferritin allows a more general evaluation of iron stores than the bone marrow aspiration to measure hemossiderin and has been widely used to estimate body iron stores, particularly in the detection of lack or overload of this metal[3].

The objective of the present study was to analyze a group of patients submitted to the measurement of serum ferritin simultaneously to the performance of a coronary arteriography aiming at determining whether there is a correlation between laboratory values and the degree of coronary disease.

Methods

Patients - A total of 115 patients participated in the study, whose single inclusion criterion was being submitted to cardiac catheterism for diagnostic coronary arteriography with simultaneous blood collection for serum ferritin measurement. The study was approved by the Ethics Committee on Research in Humans of the Institution. Of the 155 patients studied, 81 (70.4%) were males. Age ranged from 37 to 81 years with a mean age of 59.2±10.8 yrs.

Blood collection - A blood sample was collected from all patients, immediately before the coronary angiography was started, but with the peripheral vessel already catheterized, for the serum ferritin measurement. Based on the study by Custer and cols. (1995)[9], the cutoff values adopted were 120ng/ml for men and 80ng/ml for women.

Angiographic analysis - The coronary atherosclerotic disease was assessed based on the classification characterized by three levels of complexity or severity, as follows:

A - Normal or with mild obstructive coronary disease (stenosis of less than 50% of the lumen of any of the main branches);

B - Moderate, with stenosis of up to 70% of the lumen of 1 or more main branches, in any segment, or more than 70% as long as it is in distal segments;

C - Severe, with stenosis of more than 70% of the lumen in the proximal and mid-third portion of 1 or more main branches.

Statistical analysis - The ODDS RATIO with a 95% confidence interval was evaluated for the association between the degree of coronary disease and serum ferritin levels. For the comparative statistical study of continuous quantitative variables, Student's *t* test was employed with Welch correction. For categorical variables, Fisher's exact test was used. An alpha error of 5% was adopted and p values ≤ 0.05 were considered significant.

Results

The 34 women presented ferritin levels of 133.9±133.8 ng/ml (mean ±SD) ranging from 6 to 579ng/ml, whereas the 81 men had levels of 214.6±217.2 ng/ml ranging from 3 to 1,541ng/ml. The comparison between the mean values obtained for the female and male genders showed a statistically significant difference at Student's *t* test (p=0.0172). Considering the cutoff of 80ng/ml for women and 120ng/ml for men, it was observed that 15 of the 34 women (44.1%) had ferritin levels within the normal range, whereas 25 of the 81 men (30.9%) presented normal ferritin levels. Here, the statistical study by Fisher's bicaudal exact test shows that there was no significant difference between the genders regarding the percentage of cases with normal ferritin levels in the two groups (p=0.2011).

For the patients characterized as having Degree A of coronary disease (normal or with slight lesions) the mean and SD were 222.3±325.0 ng/ml. For the Degrees B and C, they were respectively 145.6±83.7ng/ml and 188.9±150.6 ng/ml. There was no association between the degree of coronary disease and the serum ferritin regarding mean ferritin levels.

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Regarding the cutoff values, the calculation of ODDS RATIO for the number of patients with or without coronary disease according to the sex and the cutoff level of ferritinemia (80ng/ml for women and 120ng/ml for men) were as follows:

Male sex - Degrees (B+C) *versus* A: 0.92 (95%CI: 0.28 - 2.95)

Male sex - Degree C *versus* A: 0.97 (95%CI: 0.29 - 3.25)

Female sex - Degree (B+C) *versus* A: 9.71 (95%CI: 1.63 - 57.72)

Female sex - Degree C *versus* A: 8.57 (95%CI: 1.43 - 51.36)

As it can be observed, it was possible to observe a significant correlation only for the female sex. Thus, the number of women with serum ferritin > 80 ng/ml that were found to present Degrees B+C or only C was significantly higher than the number of women with Degree A (ODDS RATIO 9.71 with 95%CI of 1.63 to 57.72). This is not true for the male sex, as there were similar degrees of coronary disease with ferritin levels above or below the cutoff value of 120 ng/ ml (ODDS RATIO 0.92 with 95%CI of 0.28 to 2.95).

Discussion

Salonen et al[10] following 1,931 unselected males, aged 42, 48, 54 or 60 years without symptoms of coronary heart disease in the beginning of the study, observed that 51 presented acute myocardial infarction during a mean follow-up period of 3 years. The patients with serum ferritin levels $\geq$ 200 mg/l had a 2.2-fold increased risk of infarction compared to those presenting lower levels. The association was stronger in those who presented levels of LDL-cholesterol $\geq$ 5.0 mmol/l, i.e., 193 mg/dl. The high consumption of iron also showed a significant association with the risk of coronary disease. The scope of the present study did not include the associated analysis of cholesterolemia and fractions and dietary habits. It is possible that these elements are coadjutants in the eventual deleterious effect of ferritin.

Regarding the existence or not of moderate or severe obstructive coronary disease depending on the higher levels of ferritin, we could only demonstrate this association in women, as in the group with mean ferritinemia > 80 ng/ml there was a significantly higher number of cases with severe obstructive coronary disease, whereas in men, this association was not confirmed. The literature is also controversial regarding this issue[6,8,11]. Rauramaa et al[11] tried to establish the association of conventional risk factors and the body iron status with the prevalence of carotid atherosclerosis in Finnish middle-aged men. They studied 206 patients aged 50 to 60 years, with the help of high-resolution US and determined the carotid artery intima and media thickness. They did not observe an association between the levels of ferritin, transferrin or dietary iron consumption with the occurrence of atherosclerosis at the carotid artery bifurcation.

Moore et al[11] did not find any association between serum ferritin and the carotid artery intima and media thickness, either. They used a cutoff value of 143 micrograms of ferritin per liter of blood. These data seem to confirm our findings, although they refer to the carotid artery and not to the coronary artery.

You et al[12] used proteomics to identify protein expressions in diseased coronary arteries and demonstrated that light-chain ferritin, which mediates iron stores in the cells, showed a higher expression in the coronary arteries of patients with an atherosclerotic process when compared to that of normal arteries (p=0.01). It is speculated that this increased expression could contribute to the pathogenesis of coronary artery disease by modulating the oxidation of lipids in the vessel wall by generating oxygen-reactive substances. However, the possibility that this is only a consequence of the atherosclerotic process and not actually its cause has not been ruled out.

Bozzini et al[13], in a study that was similar to the present one, assessed 849 individuals, with 546 of them having angiographically documented coronary artery disease, measuring the levels of serum ferritin. They observed that the levels of ferritin were slightly more elevated in patients with coronary disease, but this difference disappeared after being adjusted for sex and C-reactive protein levels, concluding that their results did not confirm that iron stores are predictors of a higher coronary risk.

Finally, Haidari et al[14] were able to demonstrate in a young population of 400 patients submitted to coronary angiography with diagnostic objectives, that serum ferritin was more elevated in men with obstructions (121 μg/l; 56-258 μg/l) than in men without significant obstructions (73 μg/l; 32-138 μg/l; p<0.002), with ferritinemia being an independent risk factor (p <0.01); this association was more prominent in men aged 50 years or less (OR = 2.65; 95%CI: 1.35-5.51; p <0.003). Interestingly, no association between serum ferritin levels and the presence of coronary disease was found in women, which is exactly the opposite of what was demonstrated by the present study. It is noteworthy that those authors mention the fact that their study was carried out with Iranian patients, which involves possible population variations with the influence of genetic and environment factors on the occurrence of alterations.

The present study, despite the limitation of the small sample size and the fact that the ferritin level assessment was based on values that have not been specifically standardized for the Brazilian population, allows us to conclude that that, in a transversal analysis, serum ferritin levels correlated with the existence of significant obstructive coronary artery disease only in women. Hormonal, genetic or population factors might be involved and additional multicenter studies with a prospective design must be carried out in an attempt to clarify this issue.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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