Original Article
Evaluation of Iron Status in Cats With Hypertrophic Cardiomyopathy With and Without Congestive Heart Failure

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ABSTRACT

Background: All organisms need iron for their survival and metabolic activity, and the healing process of patients depends on this element. Hence, its deficiency can negatively affect patients’ quality of life and cause disorders. Although iron deficiency is proven an important comorbidity in human and canine patients with heart failure, no research has been published on the role of iron in feline hypertrophic cardiomyopathy.

Objectives: This research aimed to determine and compare the iron status of cats with hypertrophic cardiomyopathy with and without congestive heart failure.

Methods: Based on laboratory, radiographic, and echocardiographic findings, 45 client-owned cats were studied and divided into three groups: control, hypertrophic cardiomyopathy (HCM) without congestive heart failure, and hypertrophic cardiomyopathy with congestive heart failure. Iron and ferritin concentrations, total iron-binding capacity (TIBC), and serum transferrin saturation (TSAT) percentage were measured and compared in all cats. Statistical nonparametric testing was used to analyze the data.

Results: No groups illustrate any statistically significant difference for iron concentration (P=0.3), ferritin concentration (P=0.853), TIBC (P=0.1), and TSAT (P=0.639). The highest iron concentration and the lowest transferrin level and the transferrin saturation percentage were observed in the HCM group with congestive heart failure. Also, cats without congestive heart failure had the lowest TIBC compared to other groups.

Conclusion: Unlike previous studies in dogs and humans, our study did not show a significant difference between cats with hypertrophic cardiomyopathy regarding iron status.

Keywords: Hypertrophic cardiomyopathy, Iron deficiency, Iron status, Feline
1. Introduction

Iron deficiency anemia in dogs and cats is usually caused by decreased intake, impaired absorption, or chronic bleeding (Dev and Babitt, 2017; Naigamwalla et al., 2012). The most common anemia following iron deficiency is inflammatory anemia (after chronic diseases). Various mechanisms may be responsible for this type of anemia, including alterations in iron homeostasis, erythropoietin synthesis, and reduced red blood cell life cycle. Furthermore, the production of cytokines such as interferon omega, tissue necrosis factor (TNF), interleukin-1 (IL-1), IL-6, and IL-10 are responsible for changes in the body’s access to iron (McCown and Specht, 2011).

Unlike the various ways iron enters the heart cells, cardiomyocytes have only one way to release iron. This limitation makes heart cells prone to iron accumulation (Kazory and Ross, 2009). A study on mice showed that a deadly dilated cardiomyopathy occurs by inhibiting iron removal from the heart. In other words, the iron level in the heart cells increased compared to the systemic iron level (Opasich et al., 2005). A significant factor in the auto-regulation of systemic and cardiomyocyte iron levels is hepcidin, produced by cardiomyocytes. Unlike systemic hepcidin, cardiac cells’ hepcidin levels increase due to iron deficiency (to maintain intracellular iron) (Van der Meer et al., 2004). Certain drugs (angiotensin-converting inhibitors and antplatelet drugs), dietary iron deficiency, insufficient intestinal absorption of iron (due to inflammation of the intestinal mucosa), and increased hepcidin expression could be the causes of absolute iron deficiency in the context of heart failure (Von Haehling et al., 2019). The prevalence of iron deficiency is reported to be 18.5% in dogs with chronic mitral valve disorders with no congestive symptoms (Savarese et al., 2018). Moreover, studies have reported iron deficiency in human patients with acute heart failure, and its clinical signs will improve with intravenous iron administration (Anker et al., 2009; Kang et al., 2017).

If the causes of left ventricular hypertrophy, such as systemic hypertension, hyperthyroidism, and congenital aortic stenosis, are ruled out in tests, primary hypertrophic cardiomyopathy (HCM) is diagnosed by increased heart size due to ventricular hypertrophy (Fox, 2003). It is the most prevalent cardiac disease in cats, with a prevalence of 14%-16% (Paige et al., 2009). Arterial thromboembolism, congestive heart failure, left atrial dilatation, systolic dysfunction of the atrium and left ventricle, and left ventricular hypertrophy are various prognostic factors for this disease that have been reported so far (Payne et al., 2015; Roderick et al., 2017).

In previous studies, measurement of iron and its associated factors in cats with chronic renal failure and gastrointestinal disease showed that these cats had functional iron deficiency (Hunt and Jugan, 2021; Javard et al., 2017).

Based on what we know, there is no study on iron levels in cats with cardiomypathy. Here, we compare the iron levels of cats with HCM with and without congestive heart failure and evaluate the possible relationship between the levels of iron and its related factors with the occurrence of HCM.

2. Materials and Methods

This cross-sectional observational study included cats owned by clients presenting to a small animal hospital between September 2019 and April 2020. A complete physical examination by a veterinarian was performed for each cat. Obtaining blood was done with the informed owner’s permission. We enrolled 45 client-owned cats and divided them into three equal groups: control, HCM with congestive heart failure (group A), and HCM without congestive heart failure (group B) (15 cats per group). Cats with unremarkable physical examination findings were included in the control group. Additionally, the cats should not have dental disease, a history of an underlying illness, or clinical or biochemical abnormalities on previous CBC and biochemistry tests within the last 30 days. These cats were not treated with any medication except for worming and vaccination.

Cats with hypertrophic cardiomyopathy had one of the clinical signs of arrhythmia, murmur, tachypnea, muffled heart sound, cardiogenic pulmonary congestion (edema), or pleural effusion. Thoracic radiography and echocardiography were performed for suspected cats. Based on the results, cats with HCM echocardiographic characteristics (thickness of the left ventricular wall or interventricular septum greater than 6 mm) were included in the patient group. Radiographic and echocardiographic manifestations of cardiogenic pulmonary edema (alveolar-interstitial lung pattern) or pleural effusion with moderate to severe left atrial dilatation (left atrium to aorta ratio greater than or equal to 1.8) or furosemide-responsive tachypnea associated with moderate to severe left atrial dilatation (ratio of the left atrium to aorta greater than or equal to 1.8) were considered the signs and symptoms of congestive heart failure (Luis Fuentes et al., 2020; Rauch et al., 2020). The exclusion criteria...
included evidence of systemic hypertension (systolic blood pressure greater than 180 mm Hg or greater than 160 mm Hg with ocular manifestation), hyperthyroidism, acromegaly, and aortic stenosis. Other exclusion criteria were systemic illness (kidney, liver, and endocrinopathies), previous supplementation with iron, and recent blood transfusion. A Doppler ultrasound device (EICKEMEYER) was used to measure blood pressure.

The collection of blood samples, an iron panel, and a full complete blood count were done at the same time. One milliliter of blood for complete blood count (CBC) was obtained in an EDTA tube, and the test was done with an auto-analyzer (Celtac alpha, MEK-6550J/K). Five milliliters of whole blood were drawn and then collected in a tube that separates serum and immediately centrifuged to determine the biochemical test, serum iron concentrations, total iron binding capacity (TIBC), and ferritin concentrations. The samples were separated, frozen, and preserved at 80°C. Biochemical tests, serum iron concentration, and TIBC were evaluated routinely (Pars Azmoun company kit) by Vitalab selectra E auto analyzer. Ferritin concentration was assessed with a quantitative enzyme-linked immunosorbent assay using anti-ferritin monoclonal antibodies in a sandwich arrangement (Gest et al., 2015).

Transferrin saturation (TSAT)% was estimated according to this information (Equation 1):

\[
1. \text{Serum iron (µg/dL)/TIBC (µg/dL)} \times 100
\]

MyBioSource kit (MBS705772 made in the USA) was used through the immunoassay method to measure plasma thyroxine levels in healthy cats older than 7 years and all patient cats.

To statistically analyze the data, SPSS software, version 18 was used. P≤0.05 was considered statistically significant. After confirming the normality of the data by the Shapiro-Wilk test (P>0.05), an ANOVA test was used to evaluate the differences between study groups. The Kruskal-Wallis test for iron and ferritin and unilateral analysis of variance for TSAT and TIBC were performed, as the distribution in these groups was not normal.

3. Results

In the control group, 15 cats were investigated. Their mean age (based on the month) was 89.73 months (range 60-146 mo). Of the 15 cats in this group, 10 were male. The cats were Persian (12), DSH (2), and Siamese (1) breeds. Fifteen cats were included in group A (with congestive heart failure). Their mean age was 108.80 months (range: 65-164 mo). Ten cats in group A were male and included Persian (9), DSH (3), and Maine Coon (1) breeds. Also, the mean age of group B (without congestive heart failure) was 101.66 months (range: 67-154 mo). Of the 15 cats in group B, 11 were male and included Persian (11), DSH (3), and Himalayan (1) breeds. The age difference between these three groups was not significant (P>0.05)

Tables 1 and 2 present the radiographic and echocardiographic findings of the three groups. The three groups’ hematocrit, heart rate, and blood pressure were recorded. All cats were nonanemic, with a hematocrit greater than 28.7%. One cat in the control group and one in group A had tachycardia without arrhythmia (considered to be due to stress). No significant difference was found between cats regarding their hematocrit (P=0.077), heart rate (P=0.371), and blood pressure (P=0.057).

<table>
<thead>
<tr>
<th>Radiographic Demonstrations</th>
<th>Mean±SE</th>
<th>Control</th>
<th>(A)*</th>
<th>(B)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHS</td>
<td>7.3±0.41</td>
<td>8.3±0.4</td>
<td>8.8±0.49</td>
<td></td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>0</td>
<td>10.15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>0</td>
<td>6.15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td>0</td>
<td>2.15</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

VHS: Vertebral heart score, (A)*HCM group with congestive heart failure, (B)*HCM group without congestive heart failure.
Figures 1 and 2 show the results of iron and ferritin measurements. These two values did not follow the normal distribution. Iron (P=0.3) and ferritin (P=0.853) showed no statistically remarkable difference between the study groups. Iron concentration ranges were 55-143, 34-148, and 55-148 µg/dL for the control, A, and B groups, respectively. All cats had normal iron concentrations. Ferritin concentration ranges were 137-624, 129-580, and 105-505 ng/mL for the control, A, and B groups, respectively. In the control group, one cat had an elevated level of 624 ng/mL. Three cats from group A showed increased ferritin concentrations, including 490, 524, and 580 ng/mL, and two cats in group B had increased ferritin concentrations of 485 and 505 ng/mL.

Figures 3 and 4 show the results of TSAT and TIBC measurements. These two indices followed the normal distribution, and after analysis by ANOVA test, among study groups, no observable differences were found (P>0.05). TIBC concentration ranges were 165-345, and 203-395 µg/dL for the control, A, and B groups, respectively. Only one cat in each group had increased TIBC. Also, one cat in group A had decreased TIBC concentration of 165 µg/dL. Transferrin saturation percentage ranges were 15%-49%, and 18%-46% for the control, A, and B groups, respectively.

4. Discussion

Iron deficiency has been reported in human patients with severe heart failure (Hunt and Jugan, 2021). Improvement of clinical signs with iron supplementation in these patients has also been demonstrated (McCown and Specht, 2011; Acierno et al., 2020). According to the present study, no significant relationship was seen in the iron panel among the study groups.

The Mean±SD value of TIBC was the lowest for group A (268.27±15.16 versus 311±11.33 and 314.07±21.19 µg/mL for group B and control, respectively). The inflammatory processes are among the most common causes of decreased TIBC.

# Table 2. Echocardiographic findings in the control and HCM groups with and without congestive heart failure

<table>
<thead>
<tr>
<th>Index</th>
<th>Control</th>
<th>(A)*</th>
<th>(B)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval wall thickness (mm)</td>
<td>4.25, (2.9-5.7)</td>
<td>7.3, (6.4-9.5)</td>
<td>6.5, (6.1-7.8)</td>
</tr>
<tr>
<td>Left ventricular wall thickness (mm)</td>
<td>4.3, (1.3-5.5)</td>
<td>7.4, (6.6-10.1)</td>
<td>6.7, (6.3-8.3)</td>
</tr>
<tr>
<td>The ratio of the left atrium to the aorta</td>
<td>1.2, (0.9-1.45)</td>
<td>1.81, (1.5-2.3)</td>
<td>1.45, (1.1-1.63)</td>
</tr>
<tr>
<td>Largest left atrium diameter in right longitudinal view (mm)</td>
<td>11.8, (9.5-12.6)</td>
<td>17.8, (15-23.2)</td>
<td>12.6, (11.1-14.5)</td>
</tr>
<tr>
<td>The presence of spontaneous echo contrast</td>
<td>0</td>
<td>3.15</td>
<td>1.15</td>
</tr>
</tbody>
</table>

(A)*HCM group with congestive heart failure.

(B)*HCM group without congestive heart failure.

1. Iron reference interval: 33-157 µg/dL
2. Ferritin reference interval: 90-300 ng/mL
3. TIBC reference interval: 169-325 µg/mL
TIBC in patients with chronic diseases. The presence of congestion and an inflammatory process in the group A cats may be the reason for the low TIBC compared to other groups. According to previous studies in cats with renal failure and the significant difference between the patients and the control group, other factors may have effectively reduced this value in HCM cats (Gest et al., 2015).

Decreased appetite and gastrointestinal absorption of iron due to inflammation of the gastrointestinal tract are the main reasons for low iron concentration levels. No significant difference was detected among all groups for iron concentration; nevertheless, Mean±SD of iron concentration was the lowest for group A (76±9.36 versus 90.6±8.28 and 92.13±6.91 µg/dL for group B and control, respectively. In our study, group A cats were in the acute phase of the disease and had no appetite for at least a day. It may cause decreased iron concentration in the more severe phase of this disease. With increasing erythropoiesis following a decrease in intracellular iron, serum iron decreases, so it is impossible to obtain accurate information about the total amount of iron in the body just by evaluating the serum iron level. Since iron concentration is considered nonspecific, it should not be applied to assess total iron body content. Our research found no difference between study cat groups in iron concentration, confirming its inability to determine total iron body stores.

Despite no significant difference among groups, the highest Mean±SD value of ferritin concentration was observed in group A. The best way to identify an iron deficiency in humans without performing a bone marrow biopsy is to measure ferritin concentration, which strongly associates with total body stores. Serum ferritin levels are the best predictor of whole iron body stores in cats when measured with serum iron levels (Gest et al., 2015). No association was detected between tissue iron stores and total iron binding capacity or serum iron concentration (Andrews et al., 1994). Pro-inflammatory cytokines such as IL-1, IL-2, and TNF-α enhance ferritin release, an acute phase protein (McCown and Specht., 2011). Increased ferritin concentration was observed in 63.2% of cats with inflammatory disease (Freedman et al., 1983). Three cats in the control group had increased ferritin concentration. All imaging and laboratory findings were normal in these cats, and no historical evidence of systemic disease was described; thus, the significance of these findings is unclear.

It is difficult to distinguish between true and functional iron deficiency. A decrease in iron and ferritin and an increase in total iron-binding capacity are defined as true iron deficiency. In functional iron deficiency, ferritin is normal or even increased, and iron-binding capacity is reduced (Anker et al., 2009; Bohn, 2013).

According to the present study, functional iron deficiency can be seen more in the advanced stages of heart disease, such as congestive heart failure, than in the early stages of the disease and the control group.

TAST is a calculated value according to TIBC and determines the transferrin amount that has bound iron. Due to insufficient iron reserves, TSAT is normal to low in inflammatory disease-related anemia and is often low in absolute iron deficiency anemia. Although there is no reported reference index for cats, in human patients, a normal range is between 25% and 45%. Usually, because of insufficient iron storage and anemia, the TSAT index decreases in absolute iron deficiency. As iron content in group A was lower than in the other two groups, transferrin saturation was also reduced.
Functional iron deficiency has been demonstrated in cats and dogs with renal insufficiency, and injecte iron supplements (iron dextran 50 mg/cat, intramuscular) are recommended. The inject e form of the drug is preferable to the oral form (Payne et al., 2015). With this in mind, one study could examine the effects of iron intake in cats and dogs with congestive heart failure.

The obtained results may have been affected by some limitations in our study. Firstly, no gold standard exists for noninvasive estimation of iron status in cats to diagnose iron deficiency. Iron storage in the feline liver and spleen has been studied; however, its evaluation is impossible due to the invasive assessment method. The second limitation of this study was the number of samples. More accurate results can be obtained with a larger sample. Also, this study did not measure acute phase proteins such as hepcidin and serum amyloid A. Measurement of these factors can accurately show the inflammatory process’s effect on the iron panel. Moreover, symmetric dimethylarginine could rule out kidney disease more definitively.

5. Conclusion

In this study, despite the lack of significant differences between the patient and control groups, it can be concluded that there is functional iron deficiency in patients. More studies are warranted to assess iron’s effect on improving clinical conditions.

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References


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