Research in Veterinary Science 198 (2026) 105993

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Serum iron levels as a biomarker for monitoring fracture healing in dogs: A longitudinal study

Ersoy Baydar [a,*], Ugur Aydogdu [a], Feyyaz Kaya [a], Muharrem Erol [b]

[a] Balikesir University, Faculty of Veterinary Medicine, Department of Veterinary Internal Medicine, 10145 Balikesir, Türkiye
[b] Balikesir University, Faculty of Veterinary Medicine, Department of Veterinary Surgery, 10145 Balikesir, Türkiye.

ARTICLE INFO

Keywords:
Iron
Dog
Inflammation
Fracture healing
Acute phase protein

ABSTRACT

Iron is an important mineral involved in various physiological processes and can be used as a biomarker, since its serum concentration changes during the inflammatory response. Eight crossbreed dogs with closed fractures in the antebrachium bone were included in the study. The fracture was treated with a closed reduction method and blood samples were taken initially at 0 h and subsequently at 7, 14, 21, 28, and 45 days. Iron, ferritin, hepcidin, tumor necrosis factor-alpha (TNF-α), C reactive protein (CRP), bone alkaline phosphatase (BALP), osteocalcin, total antioxidant status (TAS), total oxidant status (TOS) as well as routine hemogram and biochemistry analyses were performed in the blood samples taken. It was determined that the decrease in serum Fe levels reversed as the process progressed even though hepcidin, ferritin, osteocalcin, BALP, and TNF-α levels increased at the beginning of the healing process. As a result, it can be suggested that the analysis of serum Fe levels may be a useful biomarker in monitoring the fracture healing process.

1. Introduction

Iron (Fe) is a component of heme found in hemoglobin and myoglobin. Approximately 70 % of the body's iron is in heme; enzymes of the electron transport chain, cytochrome oxidase, ferredoxin, myeloperoxidase, catalase, and cytochrome P-450 enzymes require Fe as a cofactor. Fe is also required for photosynthesis, N_2 fixation, methanogenesis, H_2 production and consumption, TCA cycle, gene regulation, and DNA biosynthesis (Guyton and Hall, 1996; Reece, 1997; Shander et al., 2009). It is reported that serum Fe levels in humans and animals decrease rapidly within 24 h after the onset of the inflammatory response (Forsberg and Bullen, 1972; Kluger and Rothenburg, 1979; Quasim and Bedawi, 2023; Ratledge and Dover, 2000). This decrease may enhance the effectiveness of the nonspecific response against bacterial infections. (Kluger and Rothenburg, 1979). Inflammation, defined as a complex response that occurs in the body against damage to cells and tissues, is divided into two groups: acute and chronic. While the inflammatory response serves to dilute, eliminate, or isolate the factor causing damage in the body, it also initiates a cascade of events that promote the reconstruction and healing of the damaged cells and tissues (Baron and Lee, 2006; Kumar et al., 2000).

Hepcidin, the main regulator of iron metabolism, is a peptide hormone synthesized by hepatocytes and released into the blood as a result of high serum iron levels, infection, or inflammatory response. Hepcidin, whose serum levels increase during infection or non-infectious inflammatory processes, causes Fe storage in macrophages, hepatocytes, and enterocytes, thereby decreasing serum Fe levels (Ganz and Nemeth, 2009). Ferritin, which is widely used in the diagnosis of iron deficiency, is an intracellular protein that plays a key role in regulating iron homeostasis. Moreover, it is considered an acute-phase protein, and elevated ferritin levels are regarded as an indicator of inflammation. (Gabay and Kushner, 1999; Gillum, 2001; Jehn et al., 2004).

The synthesis of acute phase proteins that play an active role in the inflammatory process is regulated by endogenous glucocorticoids, cytokines, and proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) (Baydar and Dabak, 2014). In bone fractures, which are a type of injury, an inflammatory response is triggered that reaches its highest level 24 h after the injury and is completed within an average of 7 days (Cho et al., 2002). During this period, TNF-α levels, along with other cytokines, increase significantly in the first few days and have been reported to return to initial levels at the end of an average of 72 h (Gerstenfeld et al., 2003; Mountziaris and Mikos, 2008). On the other hand, C-reactive protein (CRP) is a positive acute phase protein

* Corresponding author.
E-mail address: ebaydar@balikesir.edu.tr (E. Baydar).

https://doi.org/10.1016/j.rvsc.2025.105993
Received 21 December 2024; Received in revised form 12 November 2025; Accepted 25 November 2025
Available online 25 November 2025

synthesized in the liver for inflammatory response in conditions such as infection and local tissue necrosis.The measurement of C-reactive protein (CRP), an important biomarker for identifying conditions such as inflammation, infection, and sepsis in dogs, aims to assess the severity of the systemic inflammatory response and to monitor the progression of the disease through serial measurements. (Marchetti et al., 2010; Nakamura et al., 2008).

In veterinary medicine, bone markers are used as a rapid and sensitive method to monitor the response to treatment or surgical intervention in many species of animals (Breur et al., 2004; Brankovic et al., 2022; Knych et al., 2023). Reference values for dog bone markers were reported before (Breur et al., 2004; Ekici et al., 2023). Four variants of alkaline phosphatase (ALP) have been identified in domestic animals: bone ALP (BALP), intestinal ALP, liver ALP, and corticosteroid-induced ALP in dogs (Sanecki et al., 1990). Bone ALP, which is considered one of the most sensitive markers of bone formation, is one of the biomarkers used in the monitoring of the fracture healing process in studies (Deftos et al., 1991; Vasileva et al., 2024). Osteocalcin (OC), also known as the vitamin K-dependent bone protein, is generated only by osteoblasts and megakaryocytes, then deposited in the bone matrix and plays a crucial role in its mineralization. Osteocalcin is not released during bone resorption; thus, its serum concentrations solely reflect osteoblastic activity (Eastell et al., 1988; Joffe et al., 1994). It, among the bone biomarkers, plays a role especially in the bone construction process (Vasileva et al., 2024).

The present study was conducted to evaluate and compare inflammatory markers, total oxidant-antioxidant status, hepcidin, and bone formation biomarkers in relation to serum iron levels during the processes of fracture formation and healing in dogs.

2. Material and methods

2.1. Animals and collection of blood samples

The study material consisted of male crossbreed dogs ($n = 8$) with a mean age of approximately 5 years brought to Balikesir University Faculty of Veterinary Medicine with fracture complaints. Dogs with radiologically detected closed fractures were examined in detail in the clinic, and only clinically healthy dogs (without any disease findings in other organs or systems) with fractures were included in the study. Patients within the first 24 h of the inflammation phase of the fracture were included in the study.

Dogs with fractures that could be treated by closed reduction were sedated. 0.1 mL/kg medetomidine (Domitor, Zoetis, Turkey) was applied intramuscularly for sedation. Dogs that showed excessive reactions or were aggressive during the procedure were administered 10 mg/kg Ketamine (Ketasol, Richter Pharma, Turkey) intramuscularly and short-term anesthesia was induced. Following sedation and anesthesia, the dogs were laid on the examination table and closed reduction was performed on the relevant long bone. To provide an example in the study, only dogs with closed fractures in the forearm antebrachium bone were included in the study. After the reduction of the bones, the extremity was routinely placed in a plaster bandage to immobilize the joint above the fracture.

Blood samples were taken from the dogs from the cephalic vein in 5 ml serum tubes for hematological analysis on days 0 (first sampling day), 7, 14, 21, 28, and 45, centrifuged at 700g, +4 °C for 10 min to extract the serum and stored at −20 °C until analyzed.

No invasive procedures were performed during the treatment and recovery period other than blood collection.

All dogs were fed a common, professional commercial diet. Each dog was given the amount of food recommended by the food manufacturer each day (Table 1). The dogs included in the study had native fecal examination and no parasites were found. No clinical finding was found in any dog during the clinical examinations performed regularly throughout the process. Furthermore, all dogs had complete abdominal and thoracic radiographic analyses, and there was no collection of blood in the body cavities.

Table 1
Ingredients of the commercial diet.

Crude Protein	23 %
Crude Fat	11 %
Crude Fiber	3 %
Crude Ash	%7
Calcium	%1,4
Phosphorus	%1,1
Sodium	%0,4
Vitamin A	16.250 IU/Kg
Vitamin D	1.820 IU/Kg
Vitamin E	143 mg/Kg
Vitamin C	104 mg/Kg

2.2. Biochemical analysis

Tumor necrosis factor-α, C-reactive protein (CRP), hepcidin, and bone formation biomarkers (B-ALP, osteocalcin) were determined from serum samples using species-specific ELISA kits (SunRed Biotechnology Company, Shanghai, China) according to the manufacturer's instructions. Total oxidant and antioxidant status were determined using the colorimetric method using commercial kits (Rel Assay Kit Diagnostics, Turkey). Optical density was determined using a spectrophotometer (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany). Serum biochemical examinations were performed to determine the levels of Fe, ferritin, blood urea nitrogen (BUN), glucose (GLU), total bilirubin (TB), albumin (ALB), creatine kinase (CK), alkaline phosphatase (ALKP), aspartate aminotransferase (AST) creatinine (Crea) and alanine aminotransferase (ALT) in the blood serum of animals using a biochemistry analyzer and device-specific test kits (RANDOX rx Monaco, UK).

2.3. Statistical analysis

Data are presented as mean and standard error (Mean ± SEM). ANOVA followed by Duncan's post hoc test was used to determine differences between the analyzed parameters. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using a software program (SPSS, Version 22).

3. Results

In the present study, serum Fe levels gradually increased over the course of healing. Statistically significant differences were found between days 0 and 28, days 0 and 45, days 7 and 45. In contrast, the ferritin levels gradually decreased during the process. Statistically significant differences were found between days 0 and 28, days 0 and 45, and days 14 and 45. No significant differences were determined in CRP levels during the process. However, its levels were decreased throughout the process. On the other hand, gradual decreases were observed in BALP, osteocalcin, hepcidin, and TNF-α levels during the process with statistically significant differences between time points. The time-dependent changes in hematological, biochemical and inflammatory biomarkers during fracture healing are given in Table 2, Table 3 and Table 4, respectively.

4. Discussion

It has been reported that the decrease in serum Fe levels during acute inflammatory response may be a good diagnostic and prognostic criterion for diseases with acute inflammation (Baydar and Dabak, 2014; Borges et al., 2007; Jacobsen et al., 2005; Neumann, 2003; Quasim and Bedawi, 2023). Because measuring serum Fe levels is practical and

Table 2
Time-dependent changes in hematological analysis results during fracture healing.

	Days					
	Day 0 X ± Sd	Day 7 X ± Sd	Day 14 X ± Sd	Day 21 X ± Sd	Day 28 X ± Sd	Day 45 X ± Sd
RBC (x10^3/μL)	8,07 ± 0,48	7,90 ± 0,43	7,14 ± 0,34	7,88 ± 0,39	7,64 ± 0,34	8,26 ± 0,54
WBC(/μL)	19,66 ± 3,02	14,76 ± 1,78	15,19 ± 2,60	13,81 ± 1,77	14,69 ± 3,26	12,78 ± 1,16
Hgb (g/dL)	16,97 ± 1,03	17,16 ± 1,02	15,06 ± 0,79	17,32 ± 0,99	16,15 ± 0,75	17,58 ± 1,34
PCV (%)	50,31 ± 2,96	49,41 ± 3,08	43,93 ± 2,21	48,61 ± 2,64	46,54 ± 2,34	51,67 ± 3,63
Lymphocyte (×10^3/mcL)	**1,50 ± 0,21^{b}**	**1,84 ± 0,30a^{b}**	**2,66 ± 0,40^{a}**	**2,04 ± 0,33^{ab}**	**2,03 ± 0,33^{ab}**	**1,61 ± 0,17^{b}**
Monocyte (×10^3/mcL)	0,80 ± 0,16	0,55 ± 0,09	0,62 ± 0,10	0,510,11	0,83 ± 0,29	0,60 ± 0,06
Neutrophil (×10^3/mcL)	16,49 ± 2,90	10,94 ± 1,64	10,67 ± 1,91	10,57 ± 1,53	11,29 ± 2,94	10,08 ± 1,20
Eosinophil (×10^3/mcL)	0,46 ± 0,18	0,40 ± 0,12	0,47 ± 0,15	0,52 ± 0,13	0,41 ± 0,08	0,33 ± 0,05
Basophil (×10^3/mcL)	0,16 ± 0,06	0,21 ± 0,07	0,09 ± 0,01	0,13 ± 0,02	0,09 ± 0,02	0,15 ± 0,03
MCV (fL)	62,37 ± 0,86	62,37 ± 0,99	61,37 ± 1,11	61,75 ± 1,25	60,87 ± 1,44	62,50 ± 1,56
MCH (pg)	21,06 ± 0,57	21,67 ± 0,33	21,07 ± 0,47	22,05 ± 0,49	21,66 ± 0,39	21,17 ± 0,44
MCHC (g/dl)	**33,83 ± 0,96^{b}**	**34,75 ± 0,22^{ab}**	**34,28 ± 0,26^{ab}**	**35,76 ± 0,46^{a}**	**35,67 ± 0,63^{a}**	**34,00 ± 0,48^{ab}**
PLT × 10^3/mcL	**389,0 ± 53,97^{a}**	**388,25 ± 56,35^{a}**	**212,25 ± 29,46^{b}**	**263,375 ± 38,44^{ab}**	**250,50 ± 56,10^{ab}**	**396,25 ± 75,11^{a}**

Erythrocyte (RBC) and total leukocyte counts (WBC), hemoglobin (Hgb) amounts, hematocrit values (PCV), differential leukocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and Platelet (PLT) values of dogs with fracture cases ($n = 8$). If the means within rows do not share the same superscripts such as "a,b,c" letters there is a statistically significant difference ($p < 0.05$). There is no difference between days share the same letter.

Table 3
Time-dependent changes in biochemical analysis results during fracture healing.

	Days					
	Day 0 X ± Sd	Day 7 X ± Sd	Day 14 X ± Sd	Day 21 X ± Sd	Day 28 X ± Sd	Day 45 X ± Sd
Glucose (mg/dL)	88,47 ± 7,06	74,87 ± 8,47	73,52 ± 4,22	82,75 ± 5,70	78,40 ± 6,09	96,35 ± 11,71
CK (U/L)	**637,62 ± 221,26^{a}**	**258,50 ± 35,24^{b}**	**249,62 ± 37,58^{b}**	**214,37 ± 28,03^{b}**	**208,50 ± 30,66^{b}**	**180,62 ± 19,95^{b}**
BUN (mg/dL)	9,77 ± 1,41	13,26 ± 2,33	14,22 ± 1,86	13,91 ± 2,89	16,72 ± 3,91	10,17 ± 1,01
Crea (mg/dL)	**1,01 ± 0,05^{ab}**	**0,99 ±,09^{b}**	**1,07 ± 0,05^{ab}**	**1,14 ± 0,05^{ab}**	**1,24 ± 0,10^{a}**	**1,08 ± 0,07^{ab}**
ALB (g/dL)	2,61 ± 0,13	2,45 ± 0,22	2,45 ± 0,15	2,57 ± 0,17	2,57 ± 0,20	2,50 ± 0,21
ALT (U/L)	33,95 ± 5,14	23,96 ± 5,41	26,10 ± 6,11	39,63 ± 16,06	35,00 ± 8,16	41,30 ± 8,71
AST (U/L)	**49,25 ± 9,27^{ab}**	**32,50 ± 2,92^{b}**	**35,62 ± 4,58^{ab}**	**37,62 ± 3,79^{ab}**	**52,00 ± 8,32^{a}**	**39,00 ± 3,92^{ab}**
ALP (U/L)	71,12 ± 12,75	69,25 ± 13,07	46,62 ± 5,82	56,25 ± 5,14	53,37 ± 6,98	57,12 ± 7,55
Ca (mg/dL)	9,46 ± 0,19	9.73 ± 0,24	9,56 ± 0,23	9,55 ± 0,38	9,35 ± 0,27	9,65 ± 0,47
P (mg/dL)	**5,13 ± 0,59^{ab}**	**5,15 ± 0,31^{ab}**	**5,06 ± 0,38^{ab}**	**5,37 ± 0,46^{a}**	**4,37 ± 0,54^{ab}**	**3,75 ± 0,58^{b}**

Glucose (GLU), Creatine Kinase (CK), Blood Urea Nitrogen (BUN), Creatinine (Crea), Albumin (ALB), Alanine Amino Transferase (ALT), Aspartate Transferase (AST), Alkaline Phosphatase (ALP), Calcium (Ca) and Phosphorus (P) values of dogs with fracture cases (n = 8). If the means within rows do not share the same superscripts such as "a,b,c" letters there is a statistically significant difference (p < 0.05). There is no difference between days share the same letter.

inexpensive, it is important to determine whether this test can serve as an indicator of inflammation in canine fracture cases.

It has been reported that serum Fe levels in animals and humans decrease rapidly within 24 h after the onset of inflammation (Kluger and Rothenburg, 1979; Lohuis et al., 1988a; Lohuis et al., 1988b; Quasim and Bedawi, 2023; Ratledge and Dover, 2000). It has been suggested that this may be important in increasing the effectiveness of nonspecific combat against bacterial infection (Kluger and Rothenburg, 1979; Sunder-Plassmann et al., 1999; Ward et al., 1996). Borges et al. (2007) investigated the diagnostic importance of serum Fe levels in systemic inflammatory diseases in horses. As a result of the study, they determined the mean serum Fe level as 123 μg/dl in horses with local inflammation, 64 μg/dl in horses with systemic inflammation, and 152 μg/dl in healthy horses. Again, serum Fe levels of 51 μg/dl were determined in horses with acute systemic inflammation, 75 μg/dl in horses with subacute systemic inflammation, and 67 μg/dl in horses with chronic systemic inflammation. In another study, it was emphasized that improvement in inflammation in horses after castration could be achieved very well by monitoring serum Fe levels (Jacobsen et al., 2005). Additionally, Jacobsen et al. (2009) determined that serum Fe levels decreased in all groups on the first day of the operation in a study conducted on horses requiring surgical intervention in 3 groups (Group 1: osteochondritic lesion, Group 2: laryngeal neuropathy and Group 3: ovarian tumor). In addition, Baydar and Dabak (2014) aimed to investigate the sensitivity of serum iron levels in determining acute inflammation in acute reticuloperitonitis traumatica and acute mastitis diseases in cattle. They determined significantly higher mean serum iron values (149.60 μg/dl) in the control group compared to the RPT group (33.50 μg/dl) and the mastitis group (43.70 μg/dl). On the other hand, in a study conducted on 44 cats and 50 dogs to investigate the effectiveness of serum Fe levels as an indicator of inflammation in cats and dogs, it was found that serum Fe levels decreased in 40 (90 %) of cats and 30 (60 %) of dogs (Neumann, 2003). Quasim and Bedawi (2023) examined hematological parameters and iron levels in 175 dogs to assess pathological and physiological changes. They found that the mean Fe concentration in 17 dogs with various inflammations (metritis, pneumonia, otitis, and urinary tract infection) was significantly lower (142.53 μg/dl) compared to clinically healthy dogs (227.83 μg/dl). Furthermore, Torrente et al. (2015) reported hypoferremia is a sensitive marker of systemic inflammation in dogs, and serum Fe levels are associated with a better prognosis than CRP in dogs with inflammatory disease processes. As a result of this study, it was determined that serum Fe values increased regularly on days 0, 7, 14, 21, 28, and 45 and that this increase was compatible with the fracture healing process and decreased inflammatory levels. It can be argued that the results obtained are consistent with previous reports.

Ferritin, which plays a key role in the regulation of iron homeostasis, is also used as an acute phase protein in monitoring inflammatory processes (Gabay and Kushner, 1999; Gillum, 2001; Jehn et al., 2004). Friedrichs et al. (2010) investigated ferritin levels in 20 dogs with various inflammatory diseases (blastomycosis, pancreatitis, pneumonia, septic pyothorax, pemphigus foliaceous, intestinal invagination, endocarditis, and severe enteritis) and found hyperferritinemia (6.58 ng/mL) in 40 % of the dogs. In parallel, Quasim and Bedawi (2023) conducted a

Table 4
Time-dependent changes in the analysis results of inflammation and bone healing biomarkers during fracture healing.

	Days					
	Day 0 X ± Sd	Day 7 X ± Sd	Day 14 X ± Sd	Day 21 X ± Sd	Day 28 X ± Sd	Day 45 X ± Sd
Fe (ug/dL)	**68,91 ± 10,26^{c}**	**78,67 ± 5,87^{bc}**	**94,26 ± 9,16^{bc}**	**111,83 ± 17,60^{abc}**	**142,11 ± 26,80^{ab}**	**168,00 ± 19,50^{a}**
Ferritin (ug/dL)	**1,87±,31^{a}**	**1,65±,31^{ab}**	**1,58 ± 0,26^{ab}**	**1,21 ± 0,27^{abc}**	**0,85 ± 0,19^{bc}**	**0,73 ± 0,18^{c}**
Hepcidin (ng/mL)	**303,66 ± 71,13^{a}**	**236,40 ± 46,74^{ab}**	**193,09 ± 34,41^{ab}**	**169,14 ± 27,52^{b}**	**145,56 ± 21,73^{b}**	**126,30 ± 13,19^{b}**
CRP (mg/L)	4,64 ± 1,59	3,63 ± 1,03	3,23 ± 0,80	2,93 ± 0,64	2,44 ± 0,42	2,11 ± 0,44
TNF-α (pg/mL)	**1,23 ± 0,34^{a}**	**0,91 ± 0,20^{ab}**	**0,80 ± 0,15^{ab}**	**0,68 ± 0,10^{ab}**	**0,62 ± 0,09^{b}**	**0,56 ± 0,07^{b}**
TAS (mmol Trolox Eq/L)	0,34 ± 0,04	0,34 ± 0,04	0,35 ± 0,03	0,37 ± 0,02	0,42 ± 0,06	0,40 ± 0,04
TOS (μmol H202 Equiv./L)	55,00 ± 17,32	41,56 ± 4,10	32,09 ± 7,94	42,98 ± 7,12	56,38 ± 9,79	34,31 ± 10,13
OSI (Arbitrary unit)	206,92 ± 72,84	149,78 ± 36,10	104,87 ± 33,64	115,31 ± 16,96	176,75 ± 47,53	92,89 ± 27,75
Osteocalcin (ng/mL)	**11,97 ± 3,01^{a}**	**9,87 ± 2,11^{ab}**	**8,58 ± 1,49^{ab}**	**7,36 ± 1,10^{ab}**	**6,96 ± 1,11^{ab}**	**5,82±,74^{b}**
BALP (ug/L)	**23,35 ± 6,85^{a}**	**18,11 ± 5,07^{ab}**	**16,20 ± 4,58^{ab}**	**12,90 ± 3,27^{ab}**	**9,92 ± 2,03^{ab}**	**5,89 ± 1,67^{b}**

Iron (Fe), C-Reactive Protein (CRP), Tumor Necrosis Factor Alpha (TNF-α), Total Antioxidant Level (TAS), Total Oxidant Level (TOS), Oxidative Stress Index (OSI), and Bone Alkaline Phosphatase (BALP) values of dogs with fracture cases (n = 8). If the means within rows do not share the same superscripts such as "a,b,c" letters there is a statistically significant difference ($p < 0.05$). There is no difference between days share the same letter.

study to determine hematological measurements and serum ferritin levels to evaluate pathological and physiological changes in 175 dogs and determined that the mean serum ferritin concentration (6.45 ng/dL) in 17 dogs with various inflammations (metritis, pneumonia, otitis, and urinary tract infection) was higher than in clinically healthy dogs (6 ng/dL). Similarly, Liu et al. (2022) reported a reverse correlation between survival rate and serum ferritin levels in humans with hip fractures. Consistent with the findings of previous studies, ferritin levels, which were significantly elevated in contrast to the decreased iron levels during the initial phase of fracture-induced inflammation, were found to decrease throughout the fracture healing process.

Hepcidin is a hormone released into the blood from hepatocytes in response to high serum iron levels or as a result of inflammation or infection. It is significantly induced during infection and inflammation and causes Fe retention in macrophages, hepatocytes, and enterocytes (Ganz and Nemeth, 2009). Sihler et al. (2010) investigated urinary hepcidin levels in human patients with trauma and inflammation and found a positive correlation between the severity of trauma/inflammation and hepcidin levels. Similarly, Salem et al. (2018) examined changes in hepcidin and clinical-pathological parameters in puppies infected with canine parvovirus enteritis and found a statistically significant increase in serum hepcidin levels (7.86 ng/mL) compared to the control group (5.34 ng/mL). In addition, Dörtkardeş and Şahinduran (2020) investigated hepcidin levels in respiratory tract infections caused by various viral agents in calves and revealed that hepcidin levels were significantly higher in the diseased group (0.833 ng/mL) compared to the healthy group (0.073 ng/mL). Therefore, it can be suggested that the results of studies reporting hepcidin levels in both inflammation and the healing process parallel the increases in hepcidin levels seen in fracture inflammation and a decrease in hepcidin levels as the fracture healing process progresses.

A bone fracture triggers an inflammatory response that peaks 24 h after the injury and generally resolves within the first week (Cho et al., 2002). During this period, a complex series of proinflammatory signals and growth factors are released in a time-dependent, controlled manner. The levels of various inflammatory mediators, including TNF-α, increase significantly in the first few days (Mountziaris and Mikos, 2008). TNF-α concentrations have been reported to peak 24 h after bone injury in mouse models and return to baseline levels within 72 h (Kon et al., 2001). On the other hand, Zhang et al. (2022) also determined increased TNF-α levels in the first 24 h after post-fracture. Sihler et al. (2010) determined a positive correlation between the severity of trauma, inflammation, and TNF-α levels in humans. In this study, the TNF-α level, which was found to be high on day 0, gradually decreased from day 7 onwards. These values detected in TNF-α levels were found to be consistent with the results of previous studies indicating an increase in TNF-α levels with the severity of inflammation (Cho et al., 2002; Kon et al., 2001; Sihler et al., 2010).

CRP levels increase approximately 4–6 h after the onset of infection or inflammation. Significant increases in CRP have been detected in dogs with pyometra, acute pancreatitis, malignant neoplasms, and especially in cases requiring orthopedic surgery (Kon et al., 2001). A prospective study conducted in orthopedics observed that CRP concentration peaked on the 1st day and gradually decreased in subsequent measurements (Kanno et al., 2019). In another study, Kumar et al. (2018) evaluated some biochemical parameters during fracture healing in 6 dogs with distal femoral diaphyseal fractures and found variable CRP levels. In this study, no statistically significant difference was found in terms of CRP concentrations between weeks. However, in parallel to previous studies, it was found that the CRP concentration was high at the beginning of the fracture cases and its levels gradually decreased. It can be suggested that the reason for the relatively high CRP concentrations at hour 0, unlike previous studies, was that the cases were accepted into the study for up to 48 h and that the inflammation process due to the fracture progressed during this period.

Paskalev (2010) reported statistically significant differences were found in BALP and osteocalcin levels in the experimental osteomyelitis group compared to the normal fracture healing group. In another study, Paskalev and Filipov (2005) investigated the changes in serum concentrations of some bone markers during the normal fracture healing process in 6 dogs that underwent intramedullary osteosynthesis. In the study, the increase in BALP levels before the operation and the decrease in the following weeks of fracture healing were found to be consistent with our study. On the other hand, although Taniguchi et al. (2003) reported reaching peak levels at different times in bone-specific ALP and osteocalcin, both of the levels of the parameters were increased after the fracture similar to our study. Similarly, Bhati et al. (2018) first reported an increase in BALP and then a decrease, during the fracture healing process. In our study, it was thought that the relatively high BALP levels determined before the operation could be explained by the average age of the dogs being under 2 years, and these results are consistent with the results of previous studies.

Joshi et al. (2022) investigated the serum Ca, P, and ALP levels during the healing of long bone fractures after surgery, and no significant change ($P < 0.05$) in these parameters was detected throughout the healing period. On the other hand, Paskalev and Filipov (2005) determined a significant increase in the inorganic P levels in dogs three weeks after intramedullary osteosynthesis. In this study, P levels were determined to be higher in the 3rd week (5.37 mg/dL) compared to the other weeks, similar to the results of previous studies.

Paskalev (2011) determined increased serum malondialdehyde and catalase concentrations in dogs with long bone fractures and reported increased oxidant status. In the present study, parallel to Paskalev (2011) TOS was at the highest point in the first blood sampling analysis

time, then gradually decreased.

5. Conclusions

In the initial period of fracture inflammation, the inflammation markers hepcidin, ferritin, osteocalcin, BALP, and TNF-α levels increased, whereas the decrease in serum Fe levels was observed during the healing process of the fracture. As a result, serum Fe measurement may be a useful biomarker for assessing fracture-related inflammation and healing in dogs.

CRediT authorship contribution statement

Ersoy Baydar: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis. **Ugur Aydogdu:** Validation, Supervision, Software, Project administration, Data curation. **Feyyaz Kaya:** Writing – review & editing, Resources, Investigation. **Muharrem Erol:** Validation, Supervision, Resources, Methodology.

Ethical approval

The experimental procedures were approved by the Committee of Animal Experiments of Balikesir University (Approval number:2019–039)

Declaration of competing interest

There is no conflict of interest among the authors.

References

Baron, S., Lee, C., 2006. General Pathology, Host Response to Injury, Acute Inflammatory Response, Lange Pathology Flash Cards, McGraw-Hill, New York, pp. 70–75.

Baydar, E., Dabak, M., 2014. Serum iron as an indicator of acute inflammation in cattle. J. Dairy Sci. 97, 222–228.

Bhati, S., Maheshwari, R., Kakkar, M., 2018. Evaluation of serum bone specific alkaline phosphatase levels in isolated closed diaphyseal fractures of long bones in relation to fracture healing. Int. J. Orthopedics Sci. 4 (1), 643–646.

Borges, A.S., Thomas, J.D., Tracy, S.O., Mohammed, O.H., 2007. Serum Iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. J. Vet. Intern. Med. 21, 489–494.

Brankovic, J., Leskovec, J., Šturm, S., Cerkvenik-Flajs, V., Šterpin, S., Osredkar, J., Pogorevc, E., Antolinc, D., Vrecl, M., 2022. Experimental exposure to bisphenol a has minimal effects on bone tissue in growing rams—a preliminary study. Animals 12, 2179.

Breur, G.J., Allen, M.J., Carlson, S.J., Richardson, D.C., 2004. Markers of bone metabolism in dog breeds of different sizes. Res. Vet. Sci. 76, 53–55.

Cho, T.J., Gerstenfeld, L.C., Einhorn, T.A., 2002. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J. Bone Miner. Res. 17 (3), 513–520.

Deftos, L., Wolfert, R., Hill, C., 1991. Bone alkaline phosphatase in Paget's disease. Horm. Metab. Res. 23, 559–561.

Dörtkardeş, A.B., Şahinduran, Ş., 2020. Determination of serum amyloid a, haptoglobin and hepcidin levels in calves with endemic viral pneumonia. Ankara Üniv. Vet. Fak. Derg. 67, 127–131.

Eastell, R., Delmas, P.D., Hodgson, S.F., Eriksen, E.F., Mann, K.G., Riggs, B.L., 1988. Bone formation rate in older normal women: concurrent assessment with bone histomorphometry, calcium kinetics and biochemical markers. J. Clin. Endocrinol. Metab. 67, 741–748.

Ekici, M., Koçkaya, M., Baş-Ekici, H., 2023. The influence of sex and age on bone turnover markers in the adult to geriatric Kangal shepherd dogs. Vet. Clin. Pathol. 52, 353–359.

Forsberg, C.M., Bullen, J.J., 1972. The effect of passage and Iron on the virulence of pseudomonas aeruginosa. J. Clin. Pathol. 25, 65–68.

Friedrichs, K.R., Thomas, C., Plier, M., Andrews, G.A., Chavey, P.S., Young, K.M., 2010. Evaluation of serum ferritin as a tumor marker for canine histiocytic sarcoma. J. Vet. Intern. Med. 24 (4), 904–911.

Gabay, C., Kushner, I., 1999. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340 (6), 448–454.

Ganz, T., Nemeth, E., 2009. Iron sequestration and anemia of inflammation. Semin. Hematol. 46 (4), 387–393.

Gerstenfeld, L.C., Cullinane, D.M., Barnes, G.L., Graves, D.T., Einhorn, T.A., 2003. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J. Cell. Biochem. 88 (5), 873–884.

Gillum, R.F., 2001. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men. The third National Health and nutrition examination survey. Int. J. Obes. Relat. Metab. Disord. 25 (5), 639–645.

Guyton, A.C., Hall, J.E., 1996. Red Blood Cells, Anemia and Polycythemia. "Medical Physiology" H Çavuşoğlu (Edit.). Nobel Medical Bookstores Ltd., Çapa-Istanbul, pp. 425–433.

Jacobsen, S., Jensen, J.C., Frei, S., Jensen, A.L., Thoefner, M.B., 2005. Use of serum amyloid a and other acute phase reactants to monitor the inflammatory response after castration in horses: a field study. Equine Vet. J. 37, 552–556.

Jacobsen, S., Nielsen, J.V., Kjelgaard-Hansen, M., Toelboell Fjeldborg, T.J., Halling-Thomsen, M., Martinussen, T., Thoefner, M., 2009. Acute phase response to surgery of varying intensity in horses: a preliminary study. Vet. Surg. 38 (6), 762–769.

Jehn, M., Clark, J.M., Guallar, E., 2004. Serum ferritin and risk of the metabolic syndrome in U.S. adults. Diabetes Care 27 (10), 2422–2428.

Joffe, P., Heaf, J.G., Hyldstrup, L., 1994. Osteocalcin: a non-invasive index of metabolic bone disease in patients treated by CAPD. Kidney Int. 46, 838–846.

Joshi, S., Venugopal, S.K., Nair, S.S., Sunilkumar, N.S., Martin, K.D.J., 2022. Evaluation of serum calcium, phosphorus and alkaline phosphatase levels during long bone fracture repair in dogs stabilized with string of pearls plating technique. J. Vet. Anim. Sci. 53 (1), 27–31.

Kanno, N., Hayakawa, N., Suzuki, S., Harada, Y., Yogo, T., Hara, Y., 2019. Changes in canine C-reactive protein levels following orthopaedic surgery: a prospective study. Acta Vet. Scand. 61 (1), 33.

Kluger, M.J., Rothenburg, B.A., 1979. Fever and reduced Iron: their interaction as a host defense response to bacterial infection. Science 203, 374–376.

Knych, H.K., Finno, C.J., Katzman, S., Ryan, D., McKemie, D.S., Kass, P.H., Arthur, R.M., 2023. Clodronate detection and effects on markers of bone resorption are prolonged following a single administration to horses. Equine Vet. J. 55, 696–706.

Kon, T., Cho, T.J., Aizawa, T., Yamazaki, M., Nooh, N., Graves, D., Gerstenfeld, L.C., 2001. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. J. Bone Miner. Res. 16 (6), 1004–1014.

Kumar, V., Cotran, R.Z., Robbins, S.L., 2000. Acute and Chronic Inflammation, "Basic Pathology" U Çevikbaş (Edit.). Nobel Medical Bookstores Ltd., Çapa-Istanbul, pp. 25–46.

Kumar, K.M., Prasad, V.D., Lakshmi, N.D., Raju, N.K.B., 2018. Evaluation of biochemical parameters for assessment of fracture healing in dogs. The Pharma Innovation J. 7 (3), 577–580.

Liu, M., Yang, C., Chu, Q., Wang, J., Wang, Q., Kong, F., Sun, G., 2022. High serum levels of ferritin may predict poor survival and walking ability for patients with hip fractures: a propensity score matching study. Biomark. Med. 16 (11), 857–866.

Lohuis, J.A., Verheijden, J.H., Burvenich, C., van Miert, A.S., 1988a. Pathophysiological effects of endotoxins in ruminants. 1. Changes in body temperature and reticulorumen motility and the effects of repeated administration. Vet. Q. 10, 109–116.

Lohuis, J.A., Verheijden, J.H., Burvenich, C., van Miert, A.S., 1988b. Pathophysiological effects of endotoxins in ruminants. 2. Metabolic aspects. Vet. Q. 10, 117–125.

Marchetti, V., Lubas, G., Lombardo, A., Corazza, M., Guidi, G., Cardini, G., 2010. Evaluation of erythrocytes, platelets, and serum iron profile in dogs with chronic enteropathy. Vet. Med. Int. 716040.

Mountziaris, P.M., Mikos, A.G., 2008. Modulation of the inflammatory response for enhanced bone tissue regeneration. Tissue Eng. Part B Rev. 14 (2), 179–186.

Nakamura, M., Takahashi, M., Ohno, K., Koshino, A., Nakashima, K., Steoguchi, A., Fujino, Y., Tsujimoto, H., 2008. C-reactive protein concentration in dogs with various diseases. J. Vet. Med. Sci. 70 (2), 127–131.

Neumann, S., 2003. Serum Iron level as an Indicator for inflammation in dogs and cats. Comp. Clin. Path. 12, 90–94.

Paskalev, M.D., 2010. Comparative investigation on blood bone markers in normally healing and infected bone fracture models in dogs. Bulg. J. Vet. Med. 13 (4), 235–244.

Paskalev, M., 2011. Relationship between blood malondialdehyde and catalase concentrations and the time of occurrence of non-fixed long bone fractures in dogs. Bulg. J. Vet. Med. 14 (4), 231–237.

Paskalev, M., Filipov, K.S., 2005. Changes in some serum bone markers after experimental fracture and intramedullary osteosynthesis in dogs. Trakia J. Sci. 3 (5), 46–50.

Quasim, N.A.A.K., Bedawi, N.M., 2023. The Haematological values and serum Iron profile in dogs with some pathological and physiological conditions. J. Survey Fish. Sci. 10 (3S), 737–745.

Ratledge, C., Dover, L.G., 2000. Iron metabolism in pathogenic Bacteria. Annu. Rev. Microbiol. 54, 881–941.

Reece, W.O., 1997. In: Cann, C. (Ed.), Blood and Its Function, "Physiology of Domestic Animals". Williams & Wilkins, Maryland. USA, pp. 139–141.

Salem, N.Y., Yehia, S.G., Farag, H.S., Soliman, S.M., 2018. Evaluation of Hepcidin level and Clinico-pathological modifications in canine parvovirus enteritis. Inter. J. Vet. Sci. 7 (2), 93–96.

Sanecki, R.K., Hoffmann, W.E., Dorner, J.L., Kuhlenschmidt, M.S., 1990. Purification and comparison of corticosteroid-induced and intestinal isoenzymes of alkaline phosphatase in dogs. Am. J. Vet. Res. 51, 1964–1968.

Shander, A., Cappellini, M.D., Goodnough, L.T., 2009. Iron overload and toxicity: the hidden risk of multiple blood transfusion. ISBT 97 (3), 185–197.

Sihler, K.C., Raghavendran, K., Westerman, M., Ye, W., Napolitano, L.M., 2010. Hepcidin in trauma: linking injury, inflammation, and anemia. J. Trauma 69 (4), 831–837.

Sunder-Plassmann, G., Patruta, S.I., Horl, W.H., 1999. Pathobiology of the role of Iron in infection. Am. J. Kidney Dis. 34, 25–29.

Taniguchi, T., Matsumoto, T., Shindo, H., 2003. Changes of serum levels of osteocalcin, alkaline phosphatase, IGF-1 and IGF-1 binding protein-3 during fracture healing injury. Int. J. Care Injured. 477–479.

Torrente, C., Manzanilla, E.G., Bosch, L., Fresno, L., Rivera del Alamo, M., Andaluz, A., Saco, Y., Ruiz de Gopegui, R., 2015. Plasma iron, C-reactive protein, albumin, and plasma fibrinogen concentrations in dogs with systemic inflammatory response syndrome. J. Vet. Emerg. Crit. Care 25, 611–619.
Vasileva, R., Chaprazov, T., Milanova, A., 2024. Effects of erythropoietin-promoted fracture healing on bone turnover markers in cats. J. Funct. Biomater. 15, 106.
Ward, C.G., Bullen, J.J., Rogers, H.J., 1996. Iron and infection: new developments and their implication. J. Trauma 41, 356–364.
Zhang, E., Miramini, S., Patel, M., Richardson, M., Ebeling, P., Zhang, L., 2022. Role of TNF-α in early stage fracture healing under normal and diabetic conditions. Comput. Methods Programs Biomed. 213, 106536.