

Determination of Iron Deficiency Anemia in Helicobacter Infected Dogs^[1]

Yücel MERAL¹  Duygu DALĞIN¹ Mehtap ÜNLÜ SÖĞÜT²

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¹ University of Ondokuz Mayıs, Faculty of Veterinary, Department of Internal Medicine, TR-55139 Atakum, Samsun - TURKEY

² University of Ondokuz Mayıs, Health School, TR-55139 Atakum, Samsun - TURKEY

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Abstract

Many studies demonstrated that Helicobacter infections in humans affects iron metabolism and decreases ferritin level resulting with iron deficiency anemia. The aim of this study is the investigation of a correlation between Helicobacter infection and iron deficiency anemia in dogs. The material of the study consisted of 42 Helicobacter (+) (determined with polymerase chain reaction-PCR- following upper gastrointestinal system endoscopy) dogs (Group I) (n=42) referred with vomiting after feeding, anorexia and epigastric pain in the abdominal palpation, and 21 (Group II) (n=21) dogs negative for Helicobacter with PCR and in the routine clinical examinations. Grup I were given therapy against Helicobacter gastritis for 21 days. Blood specimen were obtained from Group I at 0. and 21. days and Group II at 0. day for blood count and ferritin levels. Iron deficiency anemia was observed in 28 dogs of 42 (66.6%) infected with Helicobacter. In conclusion, iron deficiency anemia can be a probable evidence in frequently diagnosed Helicobacter infections in dogs and must be considered in the diagnostic and therapeutic plan. This is also the first study investigating iron deficiency anemia in Helicobacter infections of dogs as far as our knowledge.

Keywords: Helicobacter, Dog, Iron deficiency, Anemia, Serum ferritin

Helikobakter Enfeksiyonlu Köpeklerde Demir Yetmezliği Anemisinin Belirlenmesi

Özet

Helikobakter enfeksiyonunun insanlarda demir metabolizmasını etkileyerek ferritin seviyesinin düşüşüne neden olduğu ve demir eksikliği anemisi oluşturduğu çeşitli çalışmalarla ortaya konulmuştur. Bu çalışmanın amacı, köpeklerde helikobakter enfeksiyonu ile demir eksikliği anemisi arasında bir ilişki olup olmadığının araştırılmasıdır. Çalışma materyalini, yemeyi takiben kusma, iştahsızlık ve abdominal palpasyonda epigastrik ağrı şikayeti ile getirilen ve üst gastro intestinal sistem endoskopisi sonucu polimeraz zincir reaksiyonu (PZR) ile helikobakter (+) olduğu saptanan 42 adet (Grup I) (n=42) köpek ve rutin klinik muayeneleri ve PZR'yle pozitiflik saptanmayan 21 adet (Grup II) (n=21) köpek oluşturmuştur. Grup I, Helikobakter gastritisi yönünden 21 gün süreyle tedavi edildi. Grup I'den 0. ve 21. günler, Grup II'den 0.gün kan sayımı ve serum ferritin bakıldı. Helikobakter ile enfekte 42 köpeğin 28'inde (%66.6) demir eksikliği anemisi saptandı. Sonuç olarak, köpeklerde sıklıkla gözlenen helikobakter enfeksiyonlarında demir eksikliği anemisinin kuvvetle muhtemel bir bulgu olabileceği ve hastanın teşhis ve tedavi şeması dahilinde göz önüne alınması gerekliliği ortaya konmuştur. Bu aynı zamanda, araştırmalarımız çerçevesinde köpeklerde Helikobakter enfeksiyonlarında demir eksikliği anemisini inceleyen ilk araştırmadır.

Anahtar sözcükler: Helikobakter, Köpek, Demir yetmezliği, Anemi, Serum ferritin

INTRODUCTION

The existence of spiral bacterium in the stomach of humans and animals has been recognized since the beginning of 1800s^[1]. In addition to this, a relationship

between *H. pylori* and gastric diseases in humans have been discovered^[2,3].

At least 4 types of spiral organisms colonized in the stomach of cats and dogs are known. These are *H. felis*^[3], *H. salomonis*^[4], *H. bizzozeronii*^[5] and *H. heilmannii*^[2] also



İletişim (Correspondence)



+90 555 5128917



ymeral@omu.edu.tr

named *Gastropirillum hominis*. Many studies has been attempted for the detection of *H. pylori* in dogs, but only few reported positive results^[6,7], so it is very rare.

Recently, O' Rourke et al.^[8] have managed to distinguish *H. felis*, *H. salomonis*, *H. bizzozeronii* and *H. heilmannii* by conducting sequence analysis of a part of urease gene complex. In subsequent studies, it has been found out that *H. pylori* experimentally causes a similar disease in dogs^[7], but *Helicobacter* is a frequent infection an dogs^[9,10].

Although there are many studies^[11-16] on helicobacter infections and iron deficiency in human medicine, it has not been completely revealed with what mechanisms this condition occurs in humans with helicobacter infections. The explanation most commonly offered for this relationship is based upon the development of *H. pylori*-associated chronic pangastritis with resultant achlorhydria and reduced ascorbic acid secretion leading to reduced intestinal iron absorption. Other potential explanations for an association between iron deficiency and *H. pylori* include occult blood loss from erosive gastritis and sequestration and utilization of iron by the organism^[12]. In a retrospective study on 1294 patients diagnosed with iron deficiency, Ünal et al.^[15], performed upper gastrointestinal endoscopy in 205 patients and found out that 84 of these patients (41%) were helicobacter (+). In their study, Serin and Serin^[14], reported that *Helicobacter* infection should be definitely considered in patients with treatment-resistance iron deficiency anemia. Similarly, in the human literature, it is often stated that helicobacter infections may cause growth failure in children; however, this situation has not been clearly put forward. As ferritin is also an acute phase protein, the chances of inflammatory diseases, such as liver diseases and hemolytic diseases increase. In iron deficiency anemia, serum ferritin level decreases, and it increases in chronic disease anemia. Although there are many studies^[11,13,14] and discussions on it in human medicine, there are no authentic studies about it in veterinary medicine as far as our knowledge. Our study aims to reveal the relationship between helicobacter infections in dogs and iron deficiency anemia.

MATERIAL and METHODS

Animal material consisted of 42 dogs (Group I) which were referred to Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Internal Diseases between 2013-2014, due to vomiting after being fed, loss of appetite and epigastric pain in the abdominal palpation.

For the diagnosis of helicobacteriosis, gastro intestinal system endoscopy materials were analysed with PCR. DNA extraction of contents were conducted with commercially available DNA extraction kits (PureLink Genomic DNA Kits, Invitrogen, Canada) according to the manufacturers'

instructions. The 100 µl of extracted DNA was kept frozen at -20°C until molecular tests were carried out. Genus-specific PCR analysis was conducted as reported by Riley et al.^[17], and the observation of a 375 bpm band was considered as positive (Fig. 1). The DNA of *Helicobacter pylori*, found in culture collection of our faculties' microbiology department, was used as positive control in all PCR analyses.

The dogs, diagnosed *Helicobacter* spp. positive with PCR were treated for 21 days as stated by Aytuğ^[18]. On day 0 and 21, whole blood count and serum biochemistry were conducted. The group in which positivity was not detected with PCR (Group II) (n=21) was accepted healthy after routine clinical examinations and whole blood count and ferritin determinations performed on day 0.

While dogs in Group I (n=42) consisted of 22 crossbreeds, 2 labradors, 5 golden retrievers, 12 terriers, and 1 Turkish shepherd dog, Group II (n=21) consisted of 18 crossbreeds, 2 golden retrievers, and 1 German shepherd. There were 28 male and 14 female dogs in Group I (17-32 kg) and 17 female and 4 male dogs in Group II (15-25 kg). Average age of the animals were 3-7 years (5.4±1.5).

Via jugularis, 2 ml of blood was obtained from dogs into tubes with heparin and anticoagulant-free tubes. Blood samples with heparin were analysed in Abacus Vet Junior device and the results of the blood count (RBC, HGB, HCT, MCV, MCH, MCHC, RDWc, PLT) were saved separately for each animal. After anticoagulant-free blood samples were centrifuged 5.000 rpm/5 min, they were taken into eppendorf tubes with the help of serum micropipette extricated from blood. Serum was stored at -18°C for analysis.

In the serums obtained, ferritin was analysed with ELISA^[19,20]. Ferritin (FE) ELISA Kit (ABIN991700) was used and evaluated according the manufacturers instructions.

For the gastroscopic examination, dogs were not given any food for a day. H₂ receptor blockers, antiacidic treatment and antibiotic were not used for a week and in the last three hours intake of water was prevented. Dogs were anaesthetized before the procedure. For gastroscopic examination, preparations were made in line with the gastroscopic examination procedure for clinically sick and healthy dogs. For gastric endoscopy, a flexible endoscopy device with Olympos® XQ20 model working channel and cold light source was used. Following the examination of front gastrointestinal channel, PCR liquid was taken from the stomach where was stored at -18°C for analysis. PCR analysis was conducted according to the method reported by Riley et al.^[17] as described above. The assessment of the results have been done using one-way ANOVA and Duncan's multiple range tests in statistical package program (SPSS, 12.0). The findings have been presented as average values and standard error.

RESULTS

Iron deficiency anemia were diagnosed in 28 of 42 (66.6%) dogs in Group 1. Blood count and ferritin levels of the Group 1 dogs with iron deficiency are demonstrated in *Table 1*. As seen, results demonstrate microcytic-hypochromic anemia, characteristic of iron deficiency and low ferritin levels in dogs with *Helicobacter* gastritis. Species-specific PCR results are presented in *Fig. 1*.

and sequestration and utilization of iron by the organism. In their study, Serin and Serin ^[14], reported that helicobacter infection should definitely be considered in patients with treatment-resistant iron deficiency. While Bakır ^[21], explain helicobacter infections causing iron deficiency anemia in people with autoimmune gastritis, Michael ^[22], reports that hepcidin hormone, which is an inflammation mediator, prevents iron from being taken into the cell by getting on the protein that allows iron go in through the cell surface;

Table 1. Blood count and serum ferritin parameters of *Helicobacter* infected dogs with iron deficiency anemia

Table 1. Demir eksikliği anemisi gözlenen *Helicobacter* ile enfekte köpeklerin kan sayımı ve serum ferritin parametreleri

Parameter	Helicobacter Infected Dogs with Iron Deficiency, Group I (n=28)		Group II (n=21) (Healthy Group)	Range
	Day 0	Day 21		
RBC (10 ⁶ µl)	5.08±1.70 ^a	5.26±0.60 ^a	6.11±1.20 ^b	5.50-8.50
HGB (g/dl)	11.11±0.90 ^a	12.07±1.40 ^b	13.04±2.10 ^b	12.00-18.00
HCT (%)	30.66±3.56 ^a	31.12±2.70 ^a	38.21±4.32 ^b	37.00-55.00
MCV (fl)	58.27±3.30 ^a	62.32±2.50 ^b	62.41±5.20 ^b	60-77
MCH (pg)	21±6.40 ^a	23.82±40 ^b	22.02±4.80 ^a	22-24.50
MCHC (g/dl)	35.15±5.17 ^a	37.39±4.20 ^b	36.82±8.21 ^b	36.00-38.00
PLT (10 ³ µl)	596±116 ^a	289±141 ^b	421±132.40 ^c	200-500
Ferritin ng ml ⁻¹	27±61 ^a	158±36 ^b	171±44 ^b	36-220 ^[6,16]

Groups that are assigned a different letter have been found to be statistically significant at the level of $P \leq 0.05$



Fig 1. Species-specific PCR results . M: marker ; 1, 3, 4 and 8'th the examples are positive, the others negative

Şekil 1. Tür spesifik PZR sonuçları. M: Markır; 1, 3, 4 ve 8. örnekler pozitif, diğerleri negatif

DISCUSSION

Although helicobacter has been known for about 95 years, it was defined in real terms in the 20th century. Scientists have focused on the essential information that belongs to the bacterium for the last 20 years ^[8,14,17]. There are evidences on the fact that helicobacter infections cause iron deficiency ^[11-16]. The explanation most commonly offered for this relationship is based upon the development of *H. pylori*-associated chronic pangastritis with resultant achlorhydria and reduced ascorbic acid secretion leading to reduced intestinal iron absorption. Other potential explanations for an association between iron deficiency and *H. pylori* include occult blood loss from erosive gastritis

that the hunger for iron inside the cell causes iron stores to be emptied; that iron- which is abundant in circulation- is caught by macrophages and therefore iron deficiency anemia develops. In his study, on the other hand, Arrigo ^[9], reports that helicobacter gastritis reduces hydrochloric acid and ascorbic acid secretion and therefore iron malabsorption develops.

In veterinary medicine, a study regarding the relationship between *Helicobacter* infections and iron deficiency anemia has not been reported yet as far as our literature search. Only Gazyagci and Macun ^[23] reported microcytic anemia due to gastric ulcer in a dog in which ferritin level was at the bottom of the reference spectrum and elevated

after the therapy, and this can be due to blood loss from the gastric ulcer.

Due to the positive correlation between serum ferritin levels in animals and their body iron stores, ferritin gives the most accurate information about the condition of iron stores in the body [24]. In iron deficiency anemia, serum ferritin level decreases, and it increases in chronic disease anemia [19,20,24], because as ferritin is also an acute phase protein, an increase is expected in inflammatory diseases such as liver diseases and haemolytic an increase. As seen in *Table 1*, serum ferritin level decreased in dogs with helicobacter gastritis (27±61), similar to the reports of human the literature [12,14,16]. Although dogs were not provided with any iron deficiency treatment, and only received helicobacter gastritis treatment, in the measurement of serum ferritin levels done on day 21, a statistical rise in ferritin levels were observed (158±36) (*Table. 1*). In other erythrocyte index parameters in *Table 1*, on the other hand, iron deficiency anemia symptoms (microcytic-hypochromic type anemia) were detected as defined [24,25]. It has been reported that normocytic-normochromic-nonregenerative anemia appears in chronic disease anemia [24,25].

In conclusion, we had been determined a rate of 66.6% iron deficiency anemia in helicobacter infected dogs, so persistent iron deficiency anemia should definitely be considered in the diagnostic and therapeutic plan. Also, this is the first study demonstrating the relationship between iron deficiency anemia and Helicobacter infection in dogs.

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