Kafkas Univ Vet Fak Derg
22 (4): 479-483, 2016
DOI: 10.9775/kvfd.2015.14508

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Pernicious Anemia Due to Cobalamin Deficiency in Dogs with *Helicobacter* Gastritis

Duygu DALĞIN [1] Yücel MERAL [1] Sibel UÇAK SEMİRGİN

[1] Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, TR-55200 Samsun - TÜRKİYE
[2] Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Nükleer Tıp Anabilim Dalı, TR-55200 Samsun - TÜRKİYE

Article Code: KVFD-2015-14508 Received: 13.10.2015 Accepted: 16.03.2016 Published Online: 16.03.2016

Abstract

Pernicious anemia due to cobalamin deficiency is a predominant finding in human *Helicobacter* infections, but is generally considered in exocrine pancreatic insufficiency and intestinal disease in veterinary medicine. The aim of this study is to investigate the cobalamin levels in dogs infected with *Helicobacter* spp. Material of the study were selected from 81 dogs referred to our clinics with probable gastrointestinal system origined clinical complaints including vomiting, neusea, epigastric pain and anorexia, and consisted of 36 female and 22 male (n:58) dogs in which *Helicobacter* spp. gastritis was diagnosed with Urea Breath test (^{14}C-UBT). To these dogs, gastroscopy, complete blood count, serum biochemistry including blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkalen phosphatase (ALP), lipase, amylase, total protein and albumin levels were performed. In 38 dogs (65.51%) microcytic anemia was observed in complete blood count. All animals were treated with metronidazole, amoxycillin and famotidine and dietary management was scheduled. ^{14}C-UBT test, complete blood count and cobalamine levels were reperformed after ceasion of the therapy. Cobalamin levels were analysed in 38 dogs with anemia. Low cobalamin levels were determined in dogs with gastric *Helicobacter* infection and anemia and after therapy-without any B_{12} supplementation- cobalamin levels and mean corpuscular volume (MCV) and Haemoglobin (Hgb) values elevated to normal reference limits were observed. This suggests that cobalamin level must be determined in dogs with *Helicobacter* infections and in deficiencies which are still present after therapy, essential supplementation would be clinically beneficial. In conclusion, this is the first study demonstrating that pernicious anemia must be a considered factor in *Helicobacter* gastritis with microcytic anemia in dogs and further investigation will be beneficial to demonstrate the cobalamin levels in dogs with or without microcytic anemia in *Helicobacter* gastritis.

Keywords: *Cobalamin, Dog, Helicobacter, Pernicious anemia, Gastritis*

Helicobacter Gastritisli Köpeklerde Kobalamin Eksikliğine Bağlı Pernisiyöz Anemi

Özet

Kobalamin eksikliğine bağlı pernisiyöz anemi insan *Helicobacter* enfeksyonlarında baskın bir bulgu olmakla birlikte, bu anemi veteriner hekimlikte genellikle ekzokrin pankreatik yetmezlik ve intestinal hastalıklarda göz önüne alınmaktadır. Bu çalışmanın amacı, köpeklerde *Helicobacter* spp. enfeksiyonlarında kobalamin seviyelerinin araştırılmasıdır. Çalışmanın materyalini kliniklerimize kusma, iştahsızlık, bulantı ve anoreksi gibi muhtemel gastrointestinal sistem şikayetleriyle getirilen 81 köpekten üre nefes testi (^{14}C-UBT) ile helikobakter enfeksiyonu tanısı konulan 58 (36 dişi ve 22 erkek) köpek oluşturmuştur. Bu köpeklere gastroskopi, tam kan sayımı, kan üre nitrojen (BUN), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), gama glutamil transferaz (GGT), alkalen fosfataz (ALP), lipaz, amilaz, total protein ve albumin seviyelerini kapsayan serum biyokimyasal tetkikler uygulanmıştır. Bu köpeklerden 38'inde (%65.51) mikrositik tipte anemi gözlenmiştir. Tüm hayvanlar metronidazol, amoksisilin, famotidin ve diyetsel düzenleme ile tedavi altına alınmış ve tedavinin tamamlanmasından sonra. ^{14}C-UBT test, tam kan sayımı ve kobalamin seviyesi ölçümü tekrarlanmıştır. Anemili 38 köpekte kobalamin seviyesi ölçülmüş ve gastrik *Helicobacter* enfeksiyonlu ve mikrositik anemi gözlenen bu köpeklerde kobalamin seviyelerinin düşük olduğu görülmüştür. Anemili köpeklerde tedaviden sonra (B_{12} desteği verilmediği halde) kobalamin seviyesi ile ortalama hücresel hacim (MCV) ve hemoglobin (Hgb) değerlerinin normal referans aralıklara döndüğü gözlenmiştir. Bu durumda, klinik pratikte *Helicobacter* enfeksiyonlu köpeklerde kobalamin seviyesi belirlenmesi ve tedavi sonrası normal değerlere ulaşmadığı durumlarda takviye yapılması gerekebilir. Sonuç olarak, bu çalışmada *Helicobacter* gastritisli ve kan tablosunda mikrositik anemi gözlenen köpeklerde kobalamin eksikliği saptanması bu alandaki ilk çalışma olup, köpeklerde mikrositik anemi olan ve/veya olmayan *Helicobacter* gastritis olgularında kobalamin seviyesini ortaya koyan ileri çalışmalar faydalı olacaktır.

Anahtar sözcükler: *Kobalamin, Köpek, Helicobacter, Pernisiyöz anemi, Gastritis*

İletişim (Correspondence)
+90 555 5128917
ymeral@omu.edu.tr

INTRODUCTION

Helicobacter spp. are gram-negative, microaerophilic, motile and curved-spiral bacteria found predominantly in the stomach and also intestines and liver of many species [1]. In dogs the isolated Helicobacter spp. from the stomach are *H. felis, H. bizzozeronii, H. salomonis, Flexispira rappini, H. bilis,* and *H. heilmannii* [2]. In humans, *H. pylori*, injures the gastric mucosal barrier, diminishes the parietal cell responsiveness and alters the gastric secretory procedure and results with gastric inflammation, atrophic gastritis, peptic ulcers, and also predisposes humans to the development of gastric cancer [3,4].

In dogs, gastric infection with *Helicobacter* has a prevalence of 67 to 100% in healthy pet dogs, and 74 to 90% in dogs presented with vomiting [5,6] and is characterized by the predominant mucosal cellular infiltrate as lymphocytic, lymphoplasmacytic, eosinophilic, or granulomatous glandular degeneration, enlarged canaliculi and pyknotic parietal cells resulting with gastritis, ulcerations [2,6] nearby development of canine inflammatory bowel disease due to enterohepatic Helicobacter spp. [7].

Cobalamin and iron deficiency anemias are predominant findings in human *Helicobacter* infections [8-10]. There are few suggestions on *Helicobacter* infections cause cobalamine deficiency; *H. pylori* is usually located on the surface of the gastric antral epithelial cells and directly effects the gastric mucosa by either preventing iron uptake and B_{12} or causing an increased loss of iron in the stomach [11]. Another way of pernicious anemia development is by loss of gastric parietal cells, either by autoimmune destruction which are responsible, in part, for the secretion of intrinsic factor, a protein essential for subsequent absorption of vitamin B_{12} in the ileum [9,11]. Severe food cobalamin malabsorbtion and low acid-pepsin secretion are other factors considered [8,12].

Cobalamin is essential for many metabolic functions. It has a major role in DNA replication, in the synthesis of red blood, and in maintaining the myelin sheath that surrounds nerve cells and deficiency of cobalamin leads primarily to central and peripheral neuropathies, immunodeficiency and gastrointestinal abnormalities [13].

Pernicious anemia due to cobalamin deficiency is generally considered in exocrine pancreatic insufficiency and intestinal disease in veterinary medicine. Pernicious anemia often goes undetected and there is a weak correlation between vitamin B_{12} deficiency and mean corpuscular volume (MCV), therefore subjects with low serum vitamin B_{12} level should not be absolutely anemic [14]. According to the literature, there is no research performed on cobalamine levels in *Helicobacter* gastritis in dogs. The aim of this study is to investigate the cobalamin levels in dogs infected with *Helicobacter* spp..

MATERIAL and METHODS

Material of the study were selected from 81 dogs referred to our clinics with probable gastrointestinal system origined clinical complaints including vomiting, neusea, epigastric pain and anorexia, and consisted of 36 female and 22 male (n:58) dogs weighing 15-25 kg, in which *Helicobacter* spp. gastritis was diagnosed with Urea Breath test (^{14}C-UBT). Breed distribution of the subjects was as follows; 33 mixedbreeds, 5 Golden Retrievers, 3 Labradors, 12 Terriers, 4 Turkish shepherd dogs and 1 German shepherd. The mean age of dogs was 4.5 years with a range of 3 to 6 years.

Each of 81 dogs was performed a detailed physical examination and ^{14}C-UBT test. To 58 dogs with grade 2 ^{14}C-UBT test *(Helicobacter* infection positive) gastroscopy, complete blood count, serum biochemistry including blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkalen phosphatase (ALP), lipase, amylase, total protein and albumin levels were performed. Complete blood counts were studied with Abacus Vet Junior Hematology Analyzer (Diatron, Austria). Serum biochemistry analyses were performed with Idexx Vet 8008 Analyser. All data were recorded into their individual protocols.

^{14}C-UBT test was applied to all subjects. Dogs were starved for 6 h, free from antibiotics and active acid inhibitors for at least a month. Before the test, the animals were made to swallow a ^{14}C- Urea capsule (HELICAP®)-with plenty of water. Following the ingestion of the capsule, 2 mg/kg of 2% Xylazine HCl was administered to the patient for sedation. Within 10 min breath collector was connected to the dry cartridge. Suitable endotracheal tube was applied to sedated patient. The breath is collected with the attached system until the membrane's color of the cartridge changed from orange to yellow within 20 min (approximately this period longs for 20 min). Then, dry cartridge system was scanned at an analyser (Heliprobe Analyser Nosterkibion System 2223- A™), and the results were received in 250 sec. The results evaluated as GRADE 0 meaning negative infection; GRADE 1 meaning suspicious; GRADE 2 meaning infected. Evaluation of the Heliprobe Analyzer Results are presented in *Table 1*. Fifty eight dogs with grade 2 results were included in the study.

Gastroscopy was performed to 58 dogs with grade 2 ^{14}C-UBT test results before and after the therapy, with

Table 1. *Evaluation of the heliprobe analyzer results*
Tablo 1. *Heliprob analizer ile alınan sonuçların değerlendirilmesi*

Grading	Infection Status	d (cpm)
0	Infection negative	d ≤ 25 cpm
1	Suspicious	25 cpm < d < 50 cpm
2	Infection positive	d ≥50 cpm

Olympus XQ20 model endoscopy equipment. Starting from the pharynx, in addition to the systematic examinations of oesophagus and antrum, the examination of the stomach was completed with the scrutinization along the angulus antrum and pyloric canal as a whole.

Serum cobalamin levels were investigated in 38 dogs with microcytic anemia and determined with Immulite 2000 Vitamin B_{12} Macro ELISA kit, according to the manufacturers instructions.

All animals were treated with metronidazole (10 mg/kg three times a day; po) for three weeks, amoxycillin (20 mg/kg three times a day; po) and famotidine (1 mg/kg twice a day; po) and dietary management was scheduled [15]. ^{14}C-UBT test, complete blood count and cobalamine levels were reperformed after ceasion of the therapy.

The assessment of the results has been done using T-test in statistical package program (SPSS, 12.0). The findings have been presented as average values and standard error. For this study, the ethical committee report numbered 2009-38 has been taken from OMU Animal Ethical Committee.

RESULTS

Serum biochemistry including BUN, ALT, AST, GGT, ALP, lipase, amylase, total protein and albumin levels of the dogs were within reference ranges. Complete blood count revealed microcytic anemia in 38 subjects (65.51%). The distributions of "d" values obtained via ^{14}C- UBT method used in the study are presented in *Table 2* [16]. Serum cobalamine levels and MCV and Hgb values of the anemic dogs are presented in *Table 3*. Endoscopy revealed ulcerative-atrophic gastritis in 11 dogs and antral nodules, red patches, erythematous, scabraus mucosal view in the rest of the Helicobacter positive subjects. Clinical and gastroscopic evaluations revealed complete recovery with the disappearance of vomiting, neusea, epigastric pain and anorexia and healthy gastric endoscopic observation.

DISCUSSION

In dogs, various reports had been published on the prevalance of gastric infection with *Helicobacter* spp. in a range of 67 to 100% in healthy pet dogs, and 74 to 90% in dogs presented with vomiting [5,6,17]. In the present study, from the 81 dogs presented with vomiting, epigastric pain, anorexia and neusea to our clinics, ^{14}C-UBT test referred 58 (71.6%) gastric infection with Helicobacter spp.

Urea breath test used in the present study, demonstrates the actual *Helicobacter* colonization, it is the preferred noninvasive method to document a successful eradication in humans and animals [18]. Sensitivity rate of 96.55% was reported for ^{14}C-UBT in dogs in the detection of the existence of spiral bacterium before the designation of species. Wong et al.[19] reported a 94.5%, where Ricci et al.[20] reported a 95-97% sensitivity. Urea test was found 65 times more reliable than Helicobacter staining method, twice as reliable as PCR analysis method, that it is a non-invasive method increases its suitability among other methods used for diagnosis [21]. Similarly, in the study of Kopanski et al.[22] it was reported that urea breath test as a non-invasive choice for detection of Helicobacter infections had higher sensitivity.

Table 2. *The distributions of "d" values obtained via ^{14}C- UBT method used in the study*

Tablo 2. Çalışmada uygulanan ^{14}C- UBT yöntemi ile elde edilen "d" değer dağılımları

^{14}C Isotope Values (cpm)	Group I Before Treatment (n=38) (cpm) (mean±SE)	Group II After Treatment (n=38) (cpm) (mean±SE)
d	97±36 [a]	11±4.1 [b]

** Groups with different letters are significant among themselves (P≤0.001)*

Table 3. *Serum cobalamine levels and mean corpuscular volume and haemoglobin values of the anemic dogs*

Tablo 3. *Anemik köpeklerde serum kobalamin değerleri ile MCV ve Hgb değerleri*

Parameter/Unit	Before Therapy (n=38) (mean±SE)	After Therapy (n=38) (mean±SE)	Range
Cobalamin (ng/L)	117±19[a]	231±41[b]	150-1000
MCV (fl)**	57.66±3.2[a]	63.17±2.1[b]	60-77
Hgb (g/dL)***	10.89±2.6[a]	12.12±0.8[b]	12.00-18.00

** Groups that are assigned a different letter have been found to be statistically significant at the level of P≤0.05; ** Mean corpuscular volume; *** Haemoglobin*

Cobalamin is an essential micronutrient that plays an important role in the differentiation, proliferation and metabolic stability of cells. Cobalamin has fundamental roles in CNS function at all ages, especially the methionine-synthase mediated conversion of homocysteine to methionine, which is essential for nucleotide synthesis and genomic and non-genomic methylation [23]. It has a major role in DNA replication, in the synthesis of red blood, and in maintaining the myelin sheath that surrounds nerve cells and deficiency of cobalamin leads primarily to central and peripheral neuropathies, immunodeficiency and gastrointestinal abnormalities [13,23].

Pernicious anemia is an important disorder caused by cobalamin deficiency and is usually underdiagnosed [24]. Megaloblastic anemia occurs because of impaired DNA synthesis that results from deficiencies of vit B_{12}, folic acid or both [23]. In complete blood counts of the subjects, anemia observed in 65.51% of the dogs was in microcytic character resembling an iron deficiency etiology. This is not unexpected, regarding the reports suggesting a weak correlation between vitamin B_{12} deficiency and MCV and that subjects with low serum vitamin B_{12} level are not

absolutely anemic [14,25]. A likely explanation for the microcytic anemia in the present study may be iron deficiency accompanying gastritis [13,15,26]. Since gastritis both impair dietary iron and cobalamin absorbtion, the factors determining its clinical presentation in the form of microcytic or macrocytic megaloblastic anemia are suggested as age, gender, duration, severity of the disease as the cobalamin stores last longer than iron [26].

In the present study, low cobalamin levels were determined in dogs with gastric *Helicobacter* infection and anemia and following therapy-without any B_{12} supplementation- cobalamin levels and MCV and Hgb values elevated to normal reference limits were observed similar to the literatüre [9]. In human medicine *H. pylori* is determined as the causative agent of vitamin B_{12} deficiency for the last two decades [3,8,9,24].

In the present study, low cobalamin level determined in dogs with gastric Helicobacter infection and anemia is unexpected, since stomach is not the major source of the intrinsic factor in dogs. This cobalamin deficiency may be due to the following factors;

Pepsinogen and gastric acid liberates the dietary cobalamin ingested from the dietary proteins in the stomach and cobalamin immediately bounds to gastric and salivary R-protein (haptocorrin) which transfers cobalamin to duodenum where pancreatic proteases breake down this complex and intrinsic factor bounds to cobalamin, and finally this cobalamin-intrinsic factor complex is readily absorbed from ileal mucosa enterocytes [13,27].

In human medicine one reason of pernicious anemia observed in Helicobacter infections is suggested as the decreased secretion of intrinsic factor by parietal cells [8,28]. This is not valid for the dog, as pancreas is the major source of intrinsic factor and only a minor intrinsic factor secretion is from the stomach [13,29].

Intrinsic factor theory do not explain the cobalamin deficiency in *Helicobacter* infected dogs and we guess this is why B_{12} deficiency is considered only in intestinal and exocrine pancreatic disorders in the dog. However intrinsic factor deficiency is not the only reason for cobalamin decrease in Helicobacter gastritis and especially in the last decade few other predominant factors are stated.

Low acid-pepsin secretion in gastritis results in decreased release of free vitamin B_{12} from food proteins [8]. This maldigestion decreases the cobalamine consumption. Another factor of maldisgetion is that *Helicobacter* antigenically cross react with antral mucosa and parietal cells in dogs [6] leading to food cobalamine malabsorbtion. Also decrease in pepsin secretion and hypochlorhydria due to decreased parietal cell function promotes overgrowth of bacteria that bind vitamin B_{12} for their own use in the hypochlorhydric stomach [30].

As mentioned, R-protein (haptocorrin) has a crucial role in cobalamin metabolism. Haptocorrin location is found in parietal cells in the stomach of rat, human and dog. Haptocorrin in gastric juice comes from both salivary glands and gastric mucosa [27]. The parietal cells located in the upper third of the fundic gland secrete hydrochloric acid, R-protein and intrinsic factor in dog [13] and therefore impairment of parietal cell function due to gastritis effects cobalamin metabolism.

Return to normal cobalamin levels after therapy without and cobalamine supplementation may be explained by the remission of the above factors.

This is the first study demonstrating cobalamin deficiency in *Helicobacter* infections in dogs to the authors' knowledge. Up to date, cobalamine deficiency is only considered in exocrine pancreatic insufficiency and intestinal disorders, perhaps due to pancreatic secretion of intrinsic factor in dogs. However there are other contributing factors as mentioned above, in the etiology of cobalamine decrease in *Helicobacter* gastritis and the predominant factor in dogs playing role in this deficiency needs further investigation. Cobalamin level must be determined in dogs with *Helicobacter* infections and in deficiencies which are still present after therapy, essential supplementation would be clinically beneficial. In conclusion, this study demonstrates that pernicious anemia must be a considered factor in *Helicobacter* gastritis with microcytic anemia in dogs and further investigation will be beneficial to demonstrate the cobalamin levels in dogs with or without microcytic anemia in *Helicobacter* gastritis.

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