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SERUM HAPTOGLOBIN AND CERULOPLASMIN CONCENTRATIONS IN DOGS WITH VARIOUS DISEASES

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Farklı hastalıklı köpeklerde serum haptoglobin ve seruloplazmin düzeyleri

Özet: Akut faz proteinleri infeksiyon, inflamasyon veya travmayı takiben kanda konsantrasyonları artan plazma proteinleridir. Bu çalışmada, farklı hastalıklı köpeklerde serum haptoglobin ve seruloplazmin düzeyleri, farklı hastalıklarda prognoz ve diyagnozdaki potansiyel önemlerini belirlenmek için incelenmiştir. Bu çalışmada 2-7 yaşlarında karışık ırk ve cinsiyette, farklı hastalık ve travmalı, toplam 97 köpek kullanılmıştır. Bu çalışmanın sonucunda köpeklerde çeşitli hastalıklarda serum haptoglobin ve seruloplazmin konsantrasyonlarının yükseldiği gözlenmiştir. Bu çalışmanın sonucunda serum haptoglobin ve seruloplazmin düzeylerinin bir inflamatorik ya da infeksiyöz durumun varlığında tanı amaçlı kullanılabileceği görüşüne varılmıştır.

Anahtar Kelimeler: Akut faz proteinleri, haptoglobin, seruloplazmin, köpek.

Summary: Acute phase proteins are plasma proteins which increase in concentration following infection, inflammation or trauma. In this study serum haptoglobin and ceruloplasmin levels were determined in dogs with various diseases in order to determine their potential value in the diagnosis and prognosis. Totally 97 dogs with various diseases and traumas, 2-7 years old, mixed breed and mixed sex were used in this study. According to the results of this study serum haptoglobin and ceruloplasmin concentrations in dogs with various diseases were changed to compare with the control group. The results of this study it seems that serum haptoglobin and ceruloplasmin levels are raised in dogs with variety of disease. As a result of this study it thought to be serum haptoglobin and ceruloplasmin levels should prove to be valuable diagnostic tool providing evidence of an inflammatory or infectious process.

Key Words: Acute phase proteins, haptoglobin, ceruloplasmin, dog.

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Introduction

The acute phase response is part of the innate defense system of an animal against trauma, inflammation, and infection **(5, 8, 11, 13)**. During the response, there is an increased rate of synthesis and release of certain plasma proteins, such as C reactive protein (CRP), ceruloplasmin, and haptoglobin along with as a decrease in the synthesis of other plasma proteins, such as albumin **(5, 11)**. Quantification of plasma or serum concentrations of these proteins can provide valuable diagnostic information in the detection, prognosis, or monitoring of disease **(5)**.

Haptoglobin is a haemoglobin binding protein present in the plasma and its belived to participate in haemoglobin transport from blood to the liver and recycling of heme iron **(3)**. Haptoglobin is synthesized and secreted during acute phase response caused by conditions such as early inflammation or stress. Its synthesis is regulated by glucocorticoids and cytokines **(3)**. In dogs, haptoglobin is considered to be a moderate acute phase protein, as it increases aproximately two to three fold in inflammation, infection or trauma **(17)**. Ceruloplasmine is an α-2 glycoprotein transporting the copper, and it is needed for wound healing and protection of cells and tissues against oxidant compounds **(4)**. It is also one of the positive acute phase proteins, which are produced primarily in the liver **(3, 4)**.

Measurements of APPs are potentially useful clinical tool in veterinary medicine but more studies are required to asses their value in particular disease, because of the acute phase response varies in different species and different pathological processes **(11, 13, 16)**. The prime purpose of this study was to respectively evaluate serum concentrations of haptoglobin, and ceruloplasmin in dogs with various diseases and in order to determine their potential value in the diagnosis and prognosis of these situations.

Material and Method

Blood samples were collected as part of initial diagnostic investigations from canine cases referred to the Adnan Menderes University Clinics of Veterinary Faculty. Samples were also obtained from healthy animals as part of routine vaccination. The animals had been classified into twelve groups: Group 1 dogs with various skin problems (n=18); group 2 dogs with fracture for extremities (n=16); group 3 dogs with gastritis (n=10); group 4 dogs with enteritis (n=5); group 5 dogs with various trauma

(n=8); group 6 dogs with pneumonia (n=6); group 7 dogs with malignant neoplasia (n=6); group 8 dogs with leishmaniosis (n=4); group 9 dogs with babesiosis (n=4); group 10 dogs with hepatozoonosis(n=7); group 11 dogs with otitis (n= 3); and group 12 healthy control dogs (n=10). Dogs within the control group had no clinical and pathological evidence of inflammatory disease. Viral and bacterial infections were diagnosed from the clinical impression but isolation of virus and bacteria were not carried out. WBC levels were determined immediately. Serum samples were frozen at -20^{0}C until analyzed.

Haptoglobin and ceruloplasmin were measured using colorimetric methods, all previously validated for use in dogs. Haptoglobin concentrations were determined to haemoglobin binding capacity in plasma samples spectrophotometrically *ad modum* Bathchelor *et al*. **(1)**. This method is based on the principle that cyanmethaemoglobin is protected from acid denaturation when complexed to haptoglobin. Serum ceruloplasmin concentration was estimated in serum *ad modum* Sunderman and Numato **(20).** A colored oxidation product is formed from ceruloplasmin and p-phenylendiamine and the rate of formation of this product is proportional to the concentration of serum ceruloplasmin. These measurements were made on a UV-spectrophotometer (Schimadzu, UV-1601). A haemocytometer with improved Neubaer rule was used to determine total leukocyte counts (WBC) by standard methods **(12)**.

A Kruskal-Wallis analysis of variance was used to asses differences in plasma haptoglobin, ceruloplasmin concentrations and WBC among the groups. Posthoc Mann-Whitney tests were applied to identify between group differences, correcting for multiple testing were appropriate.

Results

The results of the analysis for haptoglobin concentration in serum samples from the 97 dogs are shown in Table 1, with the distribution shown in Figure 1. Mean plasma Hp levels were 0.84g/l in control groups. Increased concentrations of Hp were observed in all groups except the babesiosis and hepatozoon groups, while statistical significance were observed only in the fracture, malignant neoplasia and leishmaniasis groups among all of the diseased dogs ($P < 0.001$), (Table 1). Maximum rise was in 6 dogs with malignant neoplasia (Figure 1).

The results of the analysis for ceruloplasmin concentration in serum samples from the 97 dogs are shown in Table 1, with the distribution shown in Figure 2. Increased concentrations of Cp were observed in all groups of diseased dogs, but statistical significance only was observed in groups of trauma, leishmaniasis, babesiosis

and dermatitis ($P < 0.01$). Maximum rise was seen in dogs with various traumas (Figure 2).

The results of WBC counts in blood samples from the 97 dogs are shown in Table 1, with the distribution shown in Figure 3. In this study, statistical signifiqance were observed in WBC counts only in the malignant neoplasia group among all of the diseased dogs ($P < 0.01$).

Table1: Mean serum concentrations of haptoglobin (Hp), ceruloplasmin (Cp) and WBC count in dogs with various diseases.

Tablo 1: Farklı hastalıklı köpeklerde ortalama serum haptoglobin (Hp), seruloplazmin (Cp) konsantrasyonları ve WBC sayıları.

Group	Number of dog	Condition	Hp (g/l) X (Min-Max)	Cp (mg/dl) X (Min-Max)	WBC(x10^3/µl) X (Min-Max)
1	18	Skin problems	1.41^{ab} (0.91-2.79)	13.15^{bc} (5.4-32.94)	8279^{a} (5983-14442)
2	16	Fracture	2.98^{cd} (0.33-5.71)	11.76^{abc} (3.31-22.02)	12394^{a} (7850-18750)
3	10	Gastritis	2.24^{bcd} (1.10-4.68)	12.37^{abc} (9.17-14.02)	7578^{a} (5450-9376)
4	5	Enteritis	1.60^{abcd} (0.56-2.69)	6.37^{ab} (2.84-12.32)	9320^{a} (5450-13575)
5	8	Trauma	1.69^{abcd} (0.79-5.65)	15.07^{c} (5.68-25.22)	11157^{a} (5750-18000)
6	6	Pneumonia	1.76 ^{abcd} (0.45-4.54)	7.87^{abc} (6.87-9.95)	12279^{a} (9500-15675)
7	6	Malignant neoplasia	3.11 ^{d} (1.1-4.73)	11.17^{abc} (3,14-19,67)	24450^{b} (9350-58750)
8	4	Leishmaniasis	2.81 ^{cd} (0.80-4.87)	14,66^{c} (10.19-19.67)	12136^{a} (11160-13000)
9	4	Babesiosis	0,315 ^{a} (0.262-0.385)	13,29^{bc} (7.58-21.33)	9675^{a} (8500-11250)
10	7	Hepatozooanosis	0,86 ^{ab} (0,25-2,76)	9,85^{abc} (5.10-14.66)	9922^{a} (8750-11650)
11	3	Otitis	1.93 ^{abcd} (0.45-4.24)	11.05^{abc} (5.5-21.51)	7778^{a} (7000-8500)
12	10	Control	0.84 ^{abc} (0.45-1.77)	5.31 ^{a} (4.33-6.63)	8031^{a} (5983-12000)
			***	**	***

***: p< 0.001

**: p<0.01

a,b,c,d: Means with different superscripts in the same column are significantly different.

a,b,c,d: Aynı kolonda farklı harf taşıyan gruplar arası istatistiki fark önemli.

Figure 1: Mean serum haptoglobin levels in dogs with various disease.
Figure 1: Farklı hastalıklı köpeklerde ortalama serum haptoglobin düzeyleri

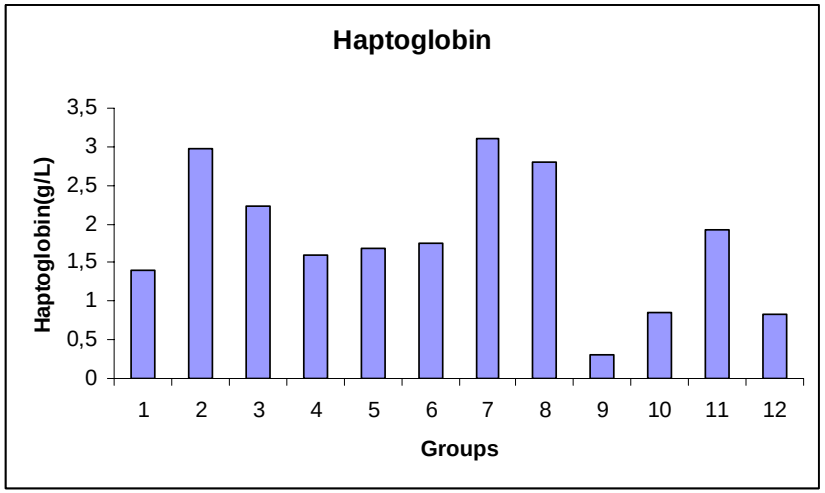
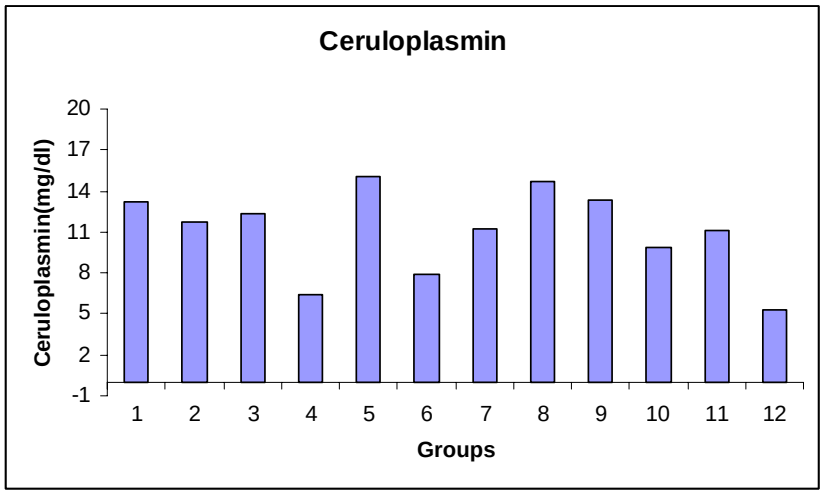


Fig 2: Mean serum ceruloplasmin levels in dogs with various disease.
Fig 2: Farkı hastalıklı köpeklerde ortalama serum seruloplazmin düzeyleri



Discussion

It is important to recognize that haptoglobin and ceruloplasmin concentrations are elevated in animals with many different diseases **(3, 11)**. It is likely that increased concentrations of haptoglobin and ceruloplasmin are consistent with the cytokine-stimulated inflammatory response. The acute phase response is stimulated by the release of cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor–α **(5).** Increased concentrations of haptoglobin were observed in all groups except the babesiosis and hepatozoon groups. Mean concentrations of haptoglobin increased over between 2-6 fold mean values of healty dogs. In this study we do not found the very high rises compare with the other studies **(6, 15, 17)**. This could be related to severity of disease **(7, 19)**. In contrast, a marked decrease in serum haptoglobin concentration was found in dogs with babesiosis. In this context, it is important to note that haptoglobin binds to free hemoglobin, and the complex is cleared by the reticuloendothelial system, resulting in a decrease in serum haptoglobin concentrations **(10)**. Low levels of haptoglobin in dogs with *B. canis* infection might be the result of an imbalance between the synthesis and the clearance of this protein from the circulation, and in particular may relate to the magnitude of hemolysis. Haptoglobin is particularly useful to support or exclude the clinical diagnosis of haemolysis.

Ceruloplasmin is one of the major positive acute phase proteins in dogs **(4, 5)**. The increaseing is higher and evident earlier than in humans. In this study, increased concentrations of ceruloplasmin were observed in all groups of diseased dogs. Similarly, serum ceruloplasmin concentrations were increased in dogs with tissue damage, leishmaniasis and babesiosis **(6, 15, 21)**. It is likely that inflammatory mechanisms in the diseases of in our study are similar to other conditions that lead to systemic inflammatory response which are cytokine mediated conditions. So measurements of this protein provide valuable information on the inflammatory status to clinicians in canine practice.

It is important to determine whether the diagnostic value of the assays of acute phase proteins is higher than that of haematological tests in the general population encountered in canine practice, where the assays could be used as primary diagnostic aids to identify inflammation. In this study, statistical signifiqance were observed in WBC counts only in the malignant neoplasia group among all of the diseased dogs ($P<0.01$). It is taught that the cause of this rises due to the important increase of WBC counts in one dog. Ceruloplasmin and haptoglobin assays are up to 6 times more sensitive than leukocyte counts in detecting inflammation and are increased in cases in which total and differential WBC counts show no changes **(5, 19)**. In addition, APPs concentrations are of value for detecting inflammation in animals which the bone marrow can not respond normally to an inflammatory stimulus, such as those with myelosuppresion attributable to treatment with chemotherapeutic agent or those with leukemia. APPs have longer analytic stability. APPs are more stable than the cellular

components of blood, and assays can be performed on frozen serum or plasma samples **(19)**.

It is reported that serum CRP and serum amyloid A (SAA) are shown maximum increase during the acute phase response in dogs with various diseases and inflammatory condition. There are a lot of studies to investigate in these proteins in different pathologic status **(2, 3, 15, 18, 22, 23)**. Commercial ELISA kits is available specific for canine CRP **(5, 14)** and SAA analyses is performed to sandwich ELISAs using anti canine **(23)** but these test kits are expensive and technical improvements are needed. In our study we determined serum haptoglobin and ceruloplasmin concentrations using spectrophotometric methods. The determination of haptoglobin method is based on the principle that cyanmethaemoglobin is protected from acid denaturation when complexed to haptoglobin **(9)**. Method that used in this study is advantage due to rapid and economically. In edition, results of this study are resembled to previous studies. In human medicine, methods for ceruloplasmin determination based on the oxidation of different compounds have become widely adopted for routine use in clinical chemistry laboratories. In canine practice used to only manual methods based on PPD-oxidase assay. Ceron *et al*. **(3)** adopted this method automated biochemical analyzer. The methods using in this study for determination concentrations of haptoglobin and ceruloplasmin are rapid and inexpensive.

It is clear from the results of this study serum haptoglobin and ceruloplamin levels are raised in dogs with variety of disease. Serum haptoglobin and ceruloplasmin levels should prove to be valuable diagnostic tool providing evidence of an inflammatoric or infectious process. The results of this study show that haptoglobin and ceruloplasmin assays are highly sensitive and relatively inexpensive to perform as well as providing reasonably rapid results.

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