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Serum Amyloid-A, Haptoglobin and Ceruloplasmin Levels in Dogs Infected with *Sarcoptes canis*[*]

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Abstract: This study was aimed to determine serum amyloid-a (SAA), haptoglobin (Hp) and ceruloplasmin (Cp) levels in dogs infected with *Sarcoptes canis*. The animal material of the study was consisted of 25 dogs with different age, gender and race (13 female, 12 male) which were bearing symptoms such as itching, rash and crusting and were diagnosed as *Sarcoptes canis* in the clinics of Internal Medicine Department of Veterinary Faculty, Kafkas University. 10 healthy dogs of different age, sex and race (4 female, 6 male) were used for the control group. Biochemical analysis revealed that the SAA and Hp values of the sick animals were statistically different than healthy animals (P<0.001). Similarly, when the serum Cp values of sick animals were compared with those of healthy animals, the difference was statistically significant (P<0.05). Obtained results showed that investigated acute phase proteins were found increased in dogs with *Sarcoptes canis* with local skin damage. It was concluded that acute phase proteins, SAA, Hp and Cp can be used as inflammatory indicators in sarcoptic mange in dogs.

Keywords: Ceruloplasmin, Dog, Haptoglobin, *Sarcoptes canis*, Serum Amyloid-A.

Sarcoptes canis ile Enfekte Köpeklerde Serum Amiloid-A, Haptoglobin ve Seruloplazmin Seviyelerinin Belirlenmesi

Öz: Bu çalışmanın amacı *Sarcoptes canis* ile enfeste köpeklerde serum amiloid-A (SAA), haptoglobin (Hp) ve seruloplazmin (Cp) seviyelerinin belirlenmesiydi. Çalışmanın hayvan materyalini, Kafkas Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı Kliniklerine, kaşıntı, deride döküntü ve kabuklanma şikayetleriyle getirilen, *Sarcoptes canis* tanısı konulan farklı yaş, cinsiyet ve ırkta (13 dişi, 12 erkek) 25 hasta köpek ve kontrol amaçlı yine farklı yaş, cinsiyet ve ırkta (4 dişi, 6 erkek) 10 sağlıklı köpek oluşturdu. Biyokimyasal analiz sonucunda hasta hayvanların SAA ve Hp değerleri ile sağlıklı hayvanların SAA ve Hp değerleri istatistiksel olarak karşılaştırıldığında farkın önemli olduğu (P<0.001) belirlendi. Aynı şekilde hasta hayvanların serum Cp değerleri ile sağlıklı hayvanların serum Cp değerleri istatistiksel olarak karşılaştırıldığında farkın anlamlı olduğu görüldü (P<0.05). Bu sonuca göre genellikle yangı mediatörü olarak kullanılan akut faz proteinlerin lokal seyreden ve deri hasarı şekillenen uyuz köpeklerde yüksek bulunması bu hastalıkta da SAA, Hp ve Cp'nin yangı indikatörü olarak kullanılabileceklerini göstermektedir.

Anahtar Kelimeler: Haptoglobin, Köpek, *Sarcoptes canis*, Seruloplazmin, Serum Amiloid-A.

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INTRODUCTION

Skin diseases are very common in dogs. These diseases are mainly caused by ectoparasites (1,2). The most frequently observed skin disease of dogs is scabies (2,3). The majority of canine mange cases are caused by *Sarcoptes scabiei (var. canis)* sarcoptic mange is a non-seasonal zoonotic disease, which is characterized by intense pruritus and observed in several mammalian species including humans. This disease is generally localized to areas with less hair, such as the ventral abdomen and the medial surface of the legs, and is also observed in the form of miliary dermatitis in the periocular skin, pinna, elbows and their medial surface, and causes severe pruritus (2,4-7).

Tissue damage results in an acute phase response (APR). APR plays a role in the elimination of tissue damage and pathogens (8). Acute-phase proteins (APPs) are synthesized in the liver in reaction to inflammation and the APR, even before clinical disease symptoms appear. These proteins have various functions and features. While APPs are found at very low levels in healthy animals, their levels rapidly increase with inflammation (9). Haptoglobin (Hp) is an APP, which is synthesized in the parenchymal cells of the liver and freely circulates in the blood. Hp levels increase approximately 8 hours after the generation of inflammatory stimuli (10,11). Plasma haptoglobin levels increase in the event of several conditions such as inflammation, trauma, tissue damage, and malignant proliferation. On the other hand, plasma Hp levels decrease with haemolysis and severe hepatocellular damage. In view of these alterations observed in Hp levels, it is suggested that this parameter could be used for diagnostic purposes and the evaluation of the success of treatment (12).

Serum amyloid-A (SAA) is synthesized in the liver during inflammation and is used for distinguishing between inflammatory and non-inflammatory diseases, as well as for the monitoring of diseases, the assessment of the success of the treatment applied and the prognosis of the disease (9,13,14).

Ceruloplasmin (Cp) is a protein responsible for the transport of copper in the blood plasma (8). Although known to be synthesized primarily in the liver, it is also produced in extrahepatic areas and the pulmonary epithelium (11,15,16). Cp catalyses the oxidation of toxic ferrous iron (Fe^{2+}) into the nontoxic ferric iron (Fe^{3+}), and thereby, protects tissues against free radical-induced damage (15).

This study was aimed at determining the levels of SAA, Hp and Cp, which are APPs that are used as indicators of inflammation, in dogs naturally infested with *Sarcoptes canis*.

MATERIALS and METHODS

Animal Material

The study was conducted after receiving approval from the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK, Investigation code: 2017/096, No 2017/112). The study material comprised 25 sick dogs (13 females and 12 males), which were of different age, sex and breed and were diagnosed with sarcoptic mange upon being admitted to the clinic of the Internal Medicine Department of Kafkas University, Faculty of Veterinary Medicine with signs of pruritus, papular rash and scaling, and 10 healthy dogs (4 females and 6 males), which were also of different age, sex and breed.

Five-ml blood samples were taken from the V. cephalica accecorius of the sick and healthy dogs and were centrifuged at 3000 rpm for 10 minutes for the extraction of serum. The serum samples were stored at -20 °C until being used for biochemical analyses.

Parasitological Diagnosis

For the purpose of parasitological diagnosis, deep skin scrapings were taken from the skin lesions and were maintained in 10% KOH for 20 minutes.

Subsequently, these scrapings were examined under a light microscope at a magnification of 10X40.

Measurement of Serum Amyloid-A Levels

Serum amyloid-A levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Tridelta Development Limited, Ireland). The SAA-specific kit included test strips, which were coated with monoclonal antibodies. Both the extracted serum samples and the reference samples with known SAA concentrations were added to the wells of the test strips together with anti-SAA monoclonal antibodies (conjugate). In this method, SAA, either captured by the antibodies coating the test strips or marked by the conjugate antibodies, accumulates in the wells. After the test strips were washed to eliminate unbound material, a tetramethylbenzidine (TMB) substrate solution was added. The resulting colour change was assessed spectrophotometrically by means of comparison with the SAA concentration in the reference samples.

Measurement of Haptoglobin Levels

Haptoglobin levels were measured spectrophotometrically using a haptoglobin assay kit (Tridelta Development Limited, Ireland). At low pH levels, free Hb is inhibited and displays peroxidase activity. Haptoglobin appears in samples combined with haemoglobin. The peroxidase activity measured is directly proportionate to the level of Hb in the sample.

Measurement of Ceruloplasmin Levels

Ceruloplasmin levels were also measured spectrophotometrically, at a pH level of 5.6 and at a wavelength of 546 nm, using the "*p-phenylenediamine oxidase activity method*" described by Richterich and Colombo.

Statistical Analysis

The data obtained in the present study were statistically analysed with the independent samples test (t-test), using the SPSS 20.0 software package. Statistical significance was set at $P<0.05$.

RESULTS

The sick animals included in the study showed clinical signs of severe pruritus, hair loss, skin scaling, crusting, and hyperkeratosis. The lesions were mainly localised to skin regions with less hair, but were also observed in other parts of the body.

The parasitological examination of the skin scrapings taken from the sick animals revealed the presence of *Sarcoptes canis* mites.

The SAA and Hp levels measured by biochemical analyses were found to significantly differ between the sick and healthy animals ($P<0.001$). Furthermore, statistical comparison showed that the serum Cp levels of the sick and healthy animals also significantly differed from each other ($P<0.05$). The SAA, Hp and Cp levels of the sick and healthy animals are presented in Table 1.

Table 1: The serum Hp, SAA and Cp levels of the sick and healthy dogs.
Tablo 1: Hasta ve sağlıklı köpeklerin serum Hp, SAA ve Cp seviyeleri.

Parameters	Patient X±SE (n=25)	Control X±SE (n=10)	P Values
Hp (g/L)	2.74±0.12	1.83±0.04	P<0.001
SAA (µg/mL)	18.11±0.84	5.8±0.71	P<0.001
Cp (mg/dL)	8.53±0.54	6.48±0.5	P<0.05

Hp: haptoglobin, SAA: serum amyloid-A, Cp: seruloplazmin.

DISCUSSION and CONCLUSION

Skin diseases are very common in pet animals and the most frequently observed skin disease of dogs is mange (1,2,17). Sarcoptic mange is a major skin disease characterized by severe pruritus, and is highly prevalent in Turkey and across the world. This disease occurs in several mammalian species, including humans, with no age, sex or breed predisposition (2,4). Sarcoptic mange is caused by mites, but predisposing factors are also highly influential on the occurrence of the disease (2). The most common skin disease of dogs is mange, and the

majority of cases are caused by the mite *Sarcoptes scabiei* var. *canis* (5). In the present study, all of the sick dogs were determined to be infested with *Sarcoptes canis*.

In dogs, sarcoptic mites mostly infest sites with less hair, such as the ventral abdomen and the medial surface of the legs. However, these mites may also be localised to the periocular skin, pinna, elbows and their medial surface, and cause miliary dermatitis and intense pruritus (2,4,5,18). At the early stage of infestation, the skin of the affected area presents with erythema and the formation of tubercles and vesicles. As the disease progresses, scaling and crusting occur, together with the formation of skin folds (4,18). Chronic cases may present with seborrheic miliary dermatitis and lymphadenopathy (1,2,4). In agreement with literature reports, the sick animals included in this study presented with severe pruritus, hair loss, scaling, crusting, tubercle formation and hyperkeratosis.

Haptoglobin is an APP, which is synthesized by the parenchymal cells of the liver and freely circulates in the blood. Its levels start to increase approximately 8 hours after the development of inflammation (2,10). Haptoglobin is also synthesized locally, and shows major antioxidant/antimicrobial activity in the alveolar fluid and on mucosal surfaces. Owing to these features, haptoglobin is involved in the repair of tissue damage and protection against infections. The plasma levels of haptoglobin increase with inflammation, tissue damage and malignant proliferation, and decrease in the event of haemolysis and severe hepatocellular damage. Thus, the measurement of plasma Hp concentrations is very important both for diagnosis and the evaluation of the success of treatment (12). In the present study, higher serum Hp levels having been detected in the sick animals, when compared to the healthy controls, suggests that despite inflammation being local in the scabies cases investigated, a systemic effect may have occurred.

Serum amyloid-A is composed of two apolipoproteins, namely, A-SAA and C-SAA. Of these, A-SAA increases with inflammatory processes. On the other hand, C-SAA concentrations undergo minimum alteration during the APR. The main site of production of SAA is the liver, yet extrahepatic synthesis also takes place (13,19). In the event of inflammatory processes, SAA is synthesized in the liver (14). SAA is made use of in the differentiation of inflammatory and non-inflammatory conditions as well as in the monitoring of diseases and the evaluation of the success of the treatment applied (9).

Significantly elevated levels of SAA are detected in bacterial and viral infections. In human medicine, SAA levels are measured to detect inflammatory activity and the extensity of inflammation, to monitor the prognosis of diseases, and to evaluate the efficiency of the treatment applied (13). Rahman et al. (20) reported a ten-fold increase in the SAA levels of Alpine mountain goats with sarcoptic mange. Similarly, in the present study, it was determined that SAA levels had increased in the sick dogs, as a result of the inflammation caused by an ectoparasitic disease.

Ceruloplasmin is an APP, which is mainly responsible for carrying copper in the blood plasma. It is synthesized in the liver and in extrahepatic regions, including the pulmonary epithelium (11,15,16). Owing to its role in the plasma transport of copper, ceruloplasmin can be used for the diagnosis of diseases that affect plasma copper transport. Ceruloplasmin enables the oxidation of toxic Fe2+ into non-toxic Fe3+, and thereby protects tissues against free radical-induced damage (15). Furthermore, as it aids in the elimination of superoxide and reactive oxygen, it also shows antioxidant activity (9,15,16,21). The results of the present study revealed that the serum Cp levels of the sick animals were higher than those of the healthy control animals, and thereby, demonstrated that inflammatory damage had occurred in the infested dogs.

In conclusion, the serum Hp, SAA and Cp levels of the dogs infested with *Sarcoptes canis* were

determined to be higher than the levels of the healthy dogs, and this difference was found to be statistically significant. Accordingly, the levels of these APPs, which are generally used as inflammatory indicators, having been detected to be higher in mangy dogs with local skin damage suggested that these parameters could also be used as inflammatory indicators in mange cases.

Conflict of interest

The authors declare that they have no conflict of interest.

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