# Determination of serum total sialic acid and ceruloplasmin concentrations in *Toxoplasma gondii* seropositive dogs

**Cevat NİSBET[1], Sena ÇENESİZ[1], Taraneh ÖNCEL[2], Gül Fatma YARIM[1], Gülay ÇİFTCİ[1], Erol HANDEMİR[3]**

*[1]Ondokuzmayis University Faculty of Veterinary Medicine Department of Biochemistry, Samsun; [2]Parasitology Laboratory Pendik Veterinary Control and Research Institute, Istanbul: [3]Parasitology Laboratory Konya Veterinary Control and Research Institute, Konya, Turkey*

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**Summary:** This study aimed to investigate serum total sialic acid (TSA) and ceruloplasmin (CP) levels and their possible correlation in *Toxoplasma gondii* seropositive dogs. The study was conducted on the dogs that had been kept in doghouses located in the province of Istanbul. Based upon indirect fluorescence antibody test (IFAT) results, 15 *T. gondii* seronegative dogs and 25 seropositive infected dogs were included in the study. Blood samples were collected from each dog and serums were obtained. Subsequently, TSA and CP concentrations were measured. For serum TSA concentration, no significant difference was found between control (17.20±0.55 mg/dl) and infected dogs (15.97±0.55 mg/dl) ($p>0.05$). On the other hand, serum CP concentration was significantly higher in seropositive dogs (16.49±1.11 mg/dl) compared to control dogs (10.28 ±1.63 mg/dl) ($p<0.01$). No correlation was found between serum TSA and serum CP. Absence of a significant difference between groups for serum TSA might be related to the fact that the disease was in chronic stage, and the severity of cellular damage might have been decreased. Elevation of serum CP in *T. gondii* infected dogs is most likely related to cellular damage as a consequence of infection. We suggest that serum CP can be used as an additional prognostic parameter in toxoplasmosis in support to other serologic parameters.

**Key words:** Ceruloplasmin, dog, sialic acid, *Toxoplasma gondii.*

## *Toksoplazma gondii* yönünden seropozitif köpeklerin serum total sialik asit ve seruloplazmin düzeylerinin belirlenmesi

**Özet:** Bu çalışmada, İstanbul'un çeşitli barınaklarında bulunan ve *Toxoplasma gondii* seropozitif olan köpeklerin serum sialik asit (TSA) ve seruloplazmin (CP) düzeylerinin araştırılması ve bu düzeylerin arasında olası ilişkinin incelenmesi hedeflendi. Bu amaçla indirekt floresan antikor testi (IFAT) sonuçlarına göre *T. gondii* seronegatif bulunan 15 sağlıklı köpek ile seropozitif bulunan 25 enfekte köpekten kan örnekleri alındı ve serumları çıkarıldı. TSA ve CP değerleri ölçüldü. Yapılan analizlerde hasta ve kontrol gruba ait TSA düzeyleri sırası ile 17.20±0.55 mg/dl ve 15.97±0.55 mg/dl olarak tespit edildi. Buna göre hasta ve kontrol gruba ait serum TSA düzeyleri arasında istatistiksel olarak önemli bir fark bulunmadı ($P>0.05$). Serum SP ortalamaları hasta ve kontrol gruplarda sırasıyla 16.49±1.11 mg/dl ve 10.28±1.63 mg/dl olarak bulundu. Hasta grubun serum CP düzeyleri kontrol grubuyla karşılaştırıldığında anlamlı olarak yüksek bulundu ($P<0.01$). Diğer taraftan hasta ve kontrol grubuna ait serum TSA ile CP düzeyleri arasında herhangi bir korelasyon bulunamadı. Gruplar arası serum TSA düzeyinin anlamlı bulunmaması hastalığın kronik döneminde olduğunu ve hücresel hasarın ortadan kalktığını düşündürmektedir. *T. gondii* ile enfekte köpeklerde yüksek serum seruloplazmin düzeyinin, bu enfestasyon sonucunda oluşan hücre hasarının bir sonucu olduğu ve serum seruloplazmin düzeyinin bu hastalığın serolojik bulgularına yardımcı olarak kullanılabileceği kanaatine varılmıştır.

**Anahtar sözcükler**: Köpek, seruloplazmin, sialik asit, *Toksoplazma gondii.*

## Introduction

*Toxoplasma gondii* is an intracellular parasite that causes infection in human and animals. The main hosts of the parasite are the felidae; however, a number of animal species including dog, cattle, species causing human disease needs rephrasing as poorly articulated (1, 21). In addition to fecal-oral route of infection, *T. gondii* is transmitted among definitive hosts and intermediate hosts by carnivorism, transplasental route, consumption of inadequately pasteurized milk, infected chicken eggs, infected soil, congenital route, blood tranfusion and via some antropodia (1, 22). Approximately 50% of canine toxoplasmosis is associated with clinical sings re-

***Yazışma adresi / Correspondance:*** *Cevat Nisbet, Ondokuz Mayıs University Faculty of Veterinary Medicine Department of Biochemistry, 55139, Kurupelit, Samsun, Turkey. E-posta: cnisbet@omu.edu.tr*

lated to the respiratory system. Other important signs include nervous and digestive systems disorders. In dogs, the disease is also associated with in appetence, weight loss, pneumonia (15, 23).

Acute phase proteins (APP) are important not only for diagnosis and prognosis of a number of diseases IN several animal species but also for determination of treatment modalities. Among the acute phase proteins is ceruloplasmin (CP) which is synthesized in the liver and recognized as copper carrier as it binds to 95% of plasma copper (8, 12). Due to its potential of scavenging reactive oxygen radicals, seruloplasmin is also recognized as a part of plasma antioxidant system (8). Sialic acid, which binds to glycoproteins and glycolipids in tissues and cellular fluids, is an acetylated derivative of neuronimic acid. Due to their acidic nature making the cell surface negatively charged, sialic acids take important roles in intercellular communication, cell-matrix interaction, and transfer of biological information (12, 14, 18).

In this study, due to its potential for scavenging activity, we measure TSA and CP concentrations in dogs with an IgG antibody titer of 1:64 or higher confirmed by IFAT, a diagnostic test comparable to Dye-Test, a reference test for toxoplasmosis and to investigate correlation between serum concentrations of TSA and CP.

## Material and Method

**Collection of samples:** A total of 150 stray dogs were sampled by random selection from various dog rehabilitation centre (DRC) and kennels of Istanbul and were examined for antibodies to *T. gondii*. Canine toxoplasmosis was diagnosed on the basis of history, results of serological examination, serum biochemical analyses, and clinical findings. In the study, 25 naturally infected *T. gondii* dogs confirmed serologically (see below) constituted the infected groups. Another 15 dogs with no seropositivity for toxoplasmosis and without any clinical disorders were included as control groups. All dogs tested were serogically negative for *Leishmania* infections. 10 ml blood samples were collected from each dog. Serum was obtained following centrifuging of blood samples at 3000 rpm for 10 min and kept at -80°C until assayed.

**Serological examination:** Sera were tested for anti-*T. gondii* antibodies using an indirect immunofluorescent antibody test (IFAT). This test was performed using standard procedures at the Parasitology Laboratory of the Konya Veterinary Control and Research Institute. *T. gondii* RH strain which were used as antigen for IFAT were obtained by 48 hours intraperitoneal passages from 3-5 weeks old white mice (Mus musculus variety albino). *Toxoplasma* tachyzoites were washed by centrifugation and then resuspended at a concentration of $2\times10^6$ $ml^{-1}$ in saline buffer. Sera samples were diluted from 1:16 until 1:4096 in phosphate buffered saline (PBS 0.015 M, pH 7.2). Ten microlitres of each diluted sera was pipetted in each delimited circle on the slides previously adsorbed with *T. gondii* antigen from tachyzoites. The slides were incubated at 37°C for 30 min in a wet chamber. Then they were washed three times in PBS, dried and were incubated for 30 min, at 37°C with a FITC conjugated anti-dog IgG antibody (Sigma F-4012) diluted 1:32 in PBS. The slides were washed and air dried. A drop of glycerol buffer was added and each slide was covered with a coverslip. Finally, the samples were observed under the immunofluorescent microscope (Olympus Mod BH2, Tokyo, Japan). Titres of 1:64 and above were accepted as positive (20). Positive and negative control sera were obtained from dogs previously tested by conventional serological assays (Sabin-Feldman dye test and IFAT).

**Biochemical analyses:** Serum TSA concentration was measured using the Warren method (26). Serum CP analysis was conducted by a spectrophotometric method, which included P-phenyldiamine dichloride (PPD) use (4).

**Statsitical analysis:** Data obtained from control and experiment groups were compared by One-way Anova test using a statistical software package program (SAS 82005). The correlation between serum TSA and serum SP was analyzed by Pearson correlation analysis test. $P<0.05$ was considered significant.

### Findings

Serum TSA and serum CP concentrations in dogs diagnosed either *T. gondii* negative (control) or positive (infected) are presented in Table 1. For TSA concentration, no statistical difference was found between seropositive dogs (17.20±0.55 mg/dl) and

control dogs (15.97±0.55 mg/dl) ($p>0.05$). On the other hand, serum CP was significantly higher in seropositive dogs (16.49±1.11 mg/dl) compared with control dogs (10.28±1.63 mg/dl) ($p<0.01$). There was no correlation between serum TSA and serum CP either in seropositive or control dogs.

**Table 1.** Serum total sialic acid and serum ceruloplasmin concentrations in *T. gondii* seropositive and control dogs.

| | Seropositive dogs (n=25) | Control dogs (n=15) | Level of significance |
|---|---|---|---|
| TSA (mg/dl) | 17.20±0.55 | 15.97±0.55 | >0.05 |
| CP (mg/dl) | 16.49±1.11 | 10.28±1.63 | <0.01 |

## Discussion and Conclusion

Toxoplasmosis is a zoonotic enfestation with a high prevalence. As *T. gondii* can localize to various organs and tissues, toxoplasmosis is associated with different clinical signs, depending on the organs and tissues affected. Thus, its differential diagnosis is often difficult as toxoplasmosis can be confused with a number of infectious and non-infectious diseases. Due to late diagnosis, toxoplasmosis can spread easily (17).

Serological detections of specific IgM and IgG antibodies are still the current diagnostic tools for toxoplasmosis; however, specific IgM antibodies may not be detected during the early phase of the infection. The advance techniques indicated that tests of IgM detection are not sufficient in diagnosis due to presence of either residual specific IgMs or naturally interfering IgMs in individuals having no acute infections. Besides, it has also been reported that no IgM elevations occurs in children with congenital toxoplasmosis and in individuals with a reactivated infection. Therefore, additional tests are needed in diagnosis as well as determination of ongoing status of toxoplasmosis (9). It has been reported that IgG concentration elevates towards the end of the first month of infection. Maintained as high titers for 6-8 months, IgG concentration declined during the subsequent 1-2 months (19).

*T. gondii* rapidly proliferates during the tachyzoite period in the intermediate host, and destroys the invaded cells. During this period, a large number of cells are destroyed. In the present study, serum concentration of TSA, indicative of cell damage, was investigated in *T. gondii* seropositive dogs. TSA is incorporated into the structures of glycoproteins, mucopolysaccharides and glycolipids in tissues and mucosal secretions, and its serum concentration is elevated during infection (18). Biochemical and morphological changes in the cell membrane caused by any agent during infections result in changes of serum TSA concentration. Increase in TSA concentration is a consequence of similarly resulted from sialidase enzyme activity, cleavage of serum glycoproteins by sialyltranferase, release of sialic acid from the cell membrane (12) and synthesis of sialoglycoproteins in the liver in response to some acute phase reactions and their release into circulation (14, 18, 25). It has been reported that serum TSA concentration is elevated during several infectious diseases, chronic liver diseases, arteritis, parasitic diseases and malignant tumors (5, 18). Serum TSA concentration is higher in *T. gondii* seropositive dogs; however, the difference is not large enough to create a statistical significance. Following its substantial release as a consequence of degenerations and structural changes in the cell membrane due to parasitic manifestation, sialic acid elevates in circulation. Therefore, serum sialic acid concentration is directly related to severity of the damage and extent of infection (16). Absence of a significant elevation in serum TSA concentration in seropositive dogs is most likely related to the fact that the infection was either in chronic phase or in recovery phase, during which the cellular damage and degenerations are minimized. Such data can indicate whether the animal is infected as well as the extent of infection and prognosis. Acute phase proteins are plasma proteins that are elevated in response to acute inflammation and play a role in immunoglobulin synthesis, tissue repair and complex immune reactions in response to hydroxyl effects of oxygen radicals (7, 10, 13). APP are important in clinical diagnoses and prognoses of animal diseases as well as determination of fundamentals of treatment (8). The mechanism of APP fluctuations during various inflammations and

tissue damages is not completely known; however, there are reports indicating a cytokine involvement in induction of APP (10, 11). APP concentrations during acute and chronic inflammation vary among animal species. For instance, the first response during bacterial infections in cattle is given by haptoglobulins while serum amyloid A is mildly affected (7). While C-reactive proteins and serum amyloid A protein are important infection and inflammation indicators in humans (7), ceruloplasmin is an acute phase protein that responses mildly to inflammation and tissue damage (12). Some researchers pointed out that there are positive correlations between acute phase reactants and the severity of lesions as well as prognosis (3, 11). In the present study, ceruloplasmin concentration was higher in *T. gondii* seropositive dogs compared to control dogs ($P<0.01$). The number of studies on APP response during parasitic diseases of the dog is limited; however, it was reported that serum seruloplasmin concentration was elevated in dogs during gastrointestinal nematode manifestations (2). The higher serum ceruloplasmin concentration in *T. gondii* seropositive dogs compared to control dogs was interpreted as if the seropositive dogs were not completely recovered from inflammation which was developed in response to *T. gondii* invasion. Besides, previous studies also support the notion that elevation of serum ceruloplasmin concentration can occur during the beginning and recovery phases of inflammation (11). Previous studies also indicated that elevation in serum TSA concentration was dependent on various factors. Especially sialyltransferase activity is much higher in tumorogenesis and metastatic events (24). Wilson et al., (27) reported that the enzyme sialidase is highly functional on sialic acid transfer during parasitic diseases and may play a crucial role in pathogenesis of parasitic diseases. Gungor et al. (12) reported that there is no meaningful relationship between APP and serum TSA. Other studies indicated that serum TSA concentration is elevated depending on several factors (14, 18, 25). In correlation analyses conducted in the present study found no correlation between serum TSA and CP in seropositive dogs.

In conclusion, absence of a significant difference between seropositive and control dogs for serum TSA concentration, a marker for cell damage, may indicate that the disease is in the bradyzoit phase during which bradyzoits are encapsulated by cysts and thus the cellular damage is minimum. Increased serum concentration of CP suggests that toxoplazmozis cases studied in the present study are in chronic stage and tissue damage is not completely repaired. Importantly, serum concentration of CP, a normally stable protein, may be a useful prognostic parameter in toxoplasmosis.

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