

Changes in the Activity of Aspartate- and Alanine Aminotransferase in Dogs with Experimentally Induced *Staphylococcus aureus* Infection

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Summary

The main purpose of this study was to evaluate the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) plasma concentrations in dogs with experimentally-induced *Staphylococcus aureus* infection. Correlations between AST, ALT and Respiratory Rate (RR), Pulse Rate (PR) and Internal Body Temperature (IBT) were also calculated. Bacterial suspension with density of 3.1×10^9 cfu/mL was subcutaneously injected to 9 mongrel 2 years old male dogs whereas 6 other dogs served as negative controls. The concentrations were determined using commercial kits before application (0 h), 6, 24, 48, 72 h and 7, 14, 21 days after. The aminotransferase concentrations were higher in infected dogs than in the controls - AST peaked on days 7 and 14, and ALT - at the 72nd h. Strong positive correlations were recorded between ALT and AST concentrations and between RR and IBT. It was observed that the transaminases activities were slightly affected by the experimentally induced staphylococcal infection in dogs.

Keywords: *Staphylococcus aureus*, AST, ALT, Clinical signs, Dogs

Köpeklerde Deneysel Oluşturulan *Staphylococcus aureus* Enfeksiyonunda Aspartat ve Alanin Aminotransferaz Aktivitelerindeki Değişimler

Özet

Bu çalışmanın amacı köpeklerde deneysel *Staphylococcus aureus* enfeksiyonunda aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT) plazma konsantrasyonlarını değerlendirmektir. AST, ALT ve solunum oranı (RR), nabız oranı (PR) ve vücut iç sıcaklığı (IBT) arasındaki ilişki ayrıca değerlendirildi. Bakteri süspansiyonu (of 3.1×10^9 cfu/mL) 9 adet melez 2 yaşlı erkek köpeğe subkutan yolla enjekte edilirken 6 adet diğer köpek negatif kontrol olarak kullanıldı. Konsantrasyonlar 0, 6, 24, 48 ve 72 saat ile 7, 14, 21. günlerde uygulamadan önce ticari kitleler kullanılmak suretiyle belirlendi. Aminotrasferaz konsantrasyonları kontrole oranla enfekte köpeklerde daha yüksekti. AST 7. ve 14. günlerde, ALT ise 72. saatte en yüksek konsantrasyonlarda belirlendi. ALT ile AST konsantrasyonları arasında ve RR ile IBT arasında güçlü ilişki belirlendi. Transaminaz aktivitelerinin köpeklerde deneysel olarak oluşturulan Syafilokokal enfeksiyonu ile az derecede etkilendiği gözlemlendi.

Anahtar sözcükler: *Staphylococcus aureus*, AST, ALT, Klinik Belirtiler, Köpek

INTRODUCTION

Serum aminotransferases are enzymes that are often used to assist in the diagnosis of liver disease in domestic animals. Variable amounts of both ASA and ALT occur in

the apo-enzyme form (inactive protein portion), which has no catalytic activity, and needs to be converted to an active holoenzyme form by the addition of pyridoxal 5-phosphate



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(P5P), the bio-active metabolite of dietary vitamin B₆ [1]. The hepatic function was evaluated by measuring AST and ALT enzymes, which reflect cell damage and more specifically are indicators of acute and chronic injury, respectively. The plasma half life of ALT in dogs is 4-72 h and the half-life of AST is 5 h [2].

Staphylococcus aureus (*S. aureus*) is often associated with suppurative infections and is recognized as an inherent member of the microflora of the skin of humans and dogs [3]. This infection was chosen because *S. aureus* is commonly found in various animal species including dogs, horses, cats and pigeons. Toxin-mediated diseases caused by *S. aureus* include range from cutaneous infections to infections of wounds, osteomyelitis, endocarditis, bacteremia with metastatic complications, toxic shock syndrome [4]. The bacterial components and secreted products that affect the pathogenesis of *S. aureus* infections are numerous and include surface-associated adhesins, exoenzymes, exotoxins [5]. According to some authors [6,7], the key elements in *S. aureus* are peptidoglycan (PepG) and lipoteichoic acid (LTA), which are a component of cell wall, synergize to cause shock and organ dysfunction. However, few studies on the biochemical changes in dogs infected with this bacteria exist. Thus, this study aimed to investigate some of the enzyme changes in dogs experimentally infected with *S. aureus*, during the acute phase of the infection. Therefore, we studied the clinical signs and changes in the AST and ALT concentrations in dogs during an experimental infection caused by subcutaneous application of *S. aureus*.

MATERIAL AND METHODS

Experimental Animals and Protocol Design

The experiment was approved by the Ethic Committee at the Faculty of Veterinary Medicine, Stara Zagora (Licence No 2/2009 issued by National Veterinary Medicine Office). The study was performed on 15 mongrel male dogs, 2 years old, weighing 12-15 kg, provided by the municipality of Stara Zagora. Prior to the experiment, the animals were vaccinated with vaccine Nobivac® (Intervet International B.V) and orally treated against internal parasites (Caniverm®, Bioveta, A. S. Czech Republic, 1 tablet/10 kg B.W.) and external parasites (Bolfo® Puder, Bayer, Germany). Dogs were housed in metal cages and exposed to a 12 hours light-dark cycle at room temperature (20-22°C). They were fed with a commercially available diet of dog pellet twice daily and had free access to water. Among them, 9 were inoculated in the lumbar region subcutaneously with a *S. aureus* ATCC 15564 suspension (5 mL, density 3.1x10⁹ cfu/mL) and constituted the experimental group, whereas the other dogs (n = 6) were injected with the same volume of saline solution and served as controls. Dynamics of internal body temperature (IBT) (°C), respiratory rate (RR) and pulse rate (PR) in control dogs and in dogs with *S.*

aureus infection were also recorded.

Biochemical Analyses

Blood samples were collected from the puncture of the *v. cephalica antebrachii*. Blood samples were collected into heparinised tubes before inoculation (hour 0), then at 6, 24, 48, 72 h and 7, 14 and 21 days after *S. aureus* inoculation or saline injection. Heparinised blood samples were centrifuged (1500 x g, 10 min at room temperature) within 30 min after collection. Plasma was immediately separated and stored at -20°C until analysis. Plasma ALT and AST concentrations were determined with commercial kits (Human-GmbH, Germany).

Statistical Analysis

The statistical analysis was performed using one way analysis of variance (ANOVA). The results were processed with software Statistica v.6.1 (StatSoft Inc., 2002). All results are presented as mean and standard error of the mean (Mean ± SEM). The statistical significance of parameters was determined in the LSD test at P<0.05.

RESULTS

The changes in the AST and ALT concentrations after bacterial injection are shown in [Table 1](#) and [Table 2](#). In the experimental and control groups, the activities were followed during a period of 21 days. The experimental staphylococcal infection in dogs was accompanied with swelling, painfulness and high temperature of the tissues at the site of the injection since the 1st day post-inoculation. At the site of bacteria injection, hair loss and tissue erosions occurred on days 14. Skin abscesses were evidenced at the 7th day in 5 inoculated dogs. A reduced appetite, impaired motor activity and enlargement of the

Table 1. Plasma concentrations of aspartate aminotransferase (U/L) in healthy dogs (n = 6) and in dogs with experimental *Staphylococcus aureus* infection (n = 9) according to time after subcutaneous inoculation

Tablo 1. Subkutan inokulasyon sonrası zamana bağlı olarak sağlıklı (n=6) ve *Staphylococcus aureus* ile enfekte (n=9) köpeklerde plazma aspartat aminotransferaz (U/L) konsantrasyonları

Time	Inoculated Group mean ± SEM	Control Group mean ± SEM
0 h	31.56±1.89	29.95±2.36
6 h	31.54±1.70	30.20±2.21
24 h	31.82±1.66	30.70±2.00
48 h	33.24±1.41	30.85±2.10
72 h	35.34±1.43	31.33±1.90
7 days	37.48±1.04 ^{ab}	30.40±1.80
14 days	35.02±1.09 ^a	29.86±1.92
21 days	31.70±1.42	30.16±1.88

Superscript "b" indicate significant differences (P<0.01) according to time within a same group. For a given biochemical parameter: * (P<0.05) and ** (P<0.01) indicate significant differences between *S. aureus* inoculated and control dogs

inguinal lymphatic nodes in the limb which was injected was also noted the 1st day post staphylococcal inoculation. Furthermore, fever and purulent conjunctivitis eye infection were also recorded in 9 and 1 infected dogs (after 48th h), respectively. One of the experimental dogs had oedema on the scrotum.

In the experimental group, initial levels (before inoculation) of AST were 31.56±1.89 U/L and of ALT-29.78±3.23 U/L. At 48 hours after this, AST levels began to rise and on the 7th day they peaked significantly (P<0.01) to 37.48±1.04 U/L compared to control values. At the same time, in the experimental group the values of AST were significantly higher (P<0.01) than the initial levels. The concentrations of ALT reached significant values at the 72nd h-38.44±2.11 U/L compared to the baselines (P<0.05). However, these values are in the normal reference range for the dogs. On the 21 days, the enzyme activities restored their initial levels. The results indicated that these enzymes are slightly influenced by the experimentally induced staphylococcal infection in dogs.

Table 2. Plasma concentrations of alanine aminotransferase (U/L) in healthy dogs (n = 6) and in dogs with experimental *Staphylococcus aureus* infection (n = 9) according to time after subcutaneous inoculation

Table 2. Subkutan inokulasyon sonrası zamana bağlı olarak sağlıklı (n = 6) ve *Staphylococcus aureus* ile enfekte (n = 9) köpeklerde plazma alanin aminotransferaz (U/L) konsantrasyonları

Time	Inoculated Group mean ± SEM	Control Group mean ±SEM
0 hour	29.78±3.23	35.25±4.62
6 hours	30.28±2.42	36.35±4.58
24 hours	31.26±2.12	36.21±4.87
48 hours	35.50±1.60	36.15±4.59
72 hours	38.44±2.11 ^a	34.25±4.04
7 days	37.37±2.18	33.61±3.78
14 days	33.37±2.40	33.50±3.10
21 days	30.52±2.42	32.36±3,23

Superscript "a" indicate significant differences (P<0.05) according to time within a same group

Dynamics of internal body temperature (IBT), respiratory rate (RR) and pulse rate (PR) in healthy dogs and in artificially infected dogs are presented in *Table 3*. The results indicated that IBT is significantly increased from 24th h to 72nd h and RR from 48th to 72nd h. It was also observed that PR enhanced at 24th h, 48th h and on day 7.

As shown in *Table 4*, strong positive associations were observed between ALT and AST concentrations (r = 0.86, P<0.05) and between RR and IBT (r = 0.84, P<0.05). In addition, ALT concentrations were moderate positively associated with RR and IBT (r = 0.5 and r=0.4 respectively, P<0.05), and weakly with PR (r = 0.2, P<0.05). The AST were also weakly positive coupled to the clinical parameters.

DISCUSSION

Infection accompanied by local and general systematic signs-enhanced fever, increase heart and respiratory rates at 24th h after inoculation of bacteria which are indicators for non-specific response and signs of inflammation (*Table 3*). Similar clinical symptoms during infection were observed by Georgieva et al.^[8].

Aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) are enzymes found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys. The plasma activities of ALT and AST are useful indicators of hepatocellular injury. These markers are not specific for primary liver disease, because their enhancing can be induced by disease in other tissues, drugs, or liver injury secondary to another primary disease. The magnitude of their elevation may be proportional to the number of hepatocytes affected, so the absolute concentrations of the aminotransferases and their temporal elevation provide useful clinical clues to the cause of the liver disease. Increases in serum ALT activity are not liver specific, as increased serum ALT has been reported following muscle necrosis in dogs^[9]. We

Table 3. Dynamics of internal body temperature (IBT) (°C), respiratory rate (RR) and pulse rate (PR) in healthy dogs (n = 6) and in dogs (n = 9) with experimentally induced *S. aureus* infection. Results are expressed as means ± standard errors of the means (SEM)

Table 3. Subkutan inokulasyon sonrası zamana bağlı olarak sağlıklı (n = 6) ve *S. aureus* ile enfekte (n = 9) köpeklerde vücut iç sıcaklığı (IBT), solunum oranı (RR) ve nabız oranı (PR). Sonuçlar ortalama ± ortalamanın standart hatası (SEM) olarak verilmiştir

Time After Treatment	IBT Control Dogs	IBT Inoculated Dogs	RR Control Dogs	RR Inoculated Dogs	PR Control Dogs	PR Inoculated Dogs
0 hour	38.63±0.16	38.95±0.17	28.66±2.81	37.33±2.496	87.33±5.35	118.22±6.11
6 hours	38.80±0.18	39.06±0.18 ^b	35.00±2.35	38.77±2.66	86.33±5.14	122.88±6.10
24 hours	38.81±0.19	39.85±0.20 ^b	35.00±4.17	41.33±4.06	82.33±4.46	123.55±3.50 ^{**}
48 hours	38.66±0.17	40.13±0.31 ^{***c}	38.66±2.90	48.88±3.02 ^{***b}	85.33±3.78	133.55±6.43 ^{***}
72 hours	38.68±0.12	39.94±0.20 ^{***c}	34.00±4.47	44.55±4.16 ^{**a}	82.00±4.20	106.22±8.38
Day 7	38.60±0.17	39.03±0.18	34.33±3.87	38.88±2.58	81.16±3.22	132.22±10.10 ^{***}
Day 14	38.76±0.18	39.34±0.29	35.50±4.42	35.11±2.94	78.50±5.25	105.77±7.15
Day 21	36.65±0.19	39.18±0.17	28.66±1.33	37.44±2.04	80.00±4.25	94.88±6.33

For a given biochemical parameter: * (P<0.05), ** (P<0.01) and *** (P<0.001) indicate significant differences between infected and control dogs. Different superscripts a, b, c indicate significant difference (P<0.05 or more) according to time within the experimental group (*S. aureus* inoculated dogs)

Table 4. Correlations between aspartate- and alanine aminotransferase (AST, ALT) concentrations and respiratory rate (RR), pulse rate (PR) and internal body temperature (IBT) (°C), in dogs with artificial *S. aureus* infection. Correlations were calculated from all time points (from 0 h to the 21st day) and significant associations ($P < 0.05$) were in bold

Tablo 4. *S. aureus* ile enfekte köpeklerde aspartat ve alanin aminotrasferaz konsantrasyonları, solunum oranı (RR), nabız oranı (PR) ve vücut iç sıcaklıkları (IBT) (°C) arasındaki ilişkiler. İlişkiler tüm zaman dilimlerinde (0. saatten 21. güne kadar) hesaplandı ve anlamlı ilişkiler ($P < 0.05$) kalın yazılarak gösterildi

	ALAT	ASAT	RR	PR	IBT
ALAT	-	r=0.86 P<0.05	r=0.51 NS	r=0.20 NS	r=0.46 NS
ASAT			r=0.06 NS	r=0.16 NS	r=0.05 NS
RR				r=0.44 NS	r=0.84 P<0.05
PR					r=0.14 NS
IBT					

NS: not significant

can conclude that low enhanced activity of ALT in the dogs in this study may be associated with skeletal muscle damage at the site of bacterial inoculation without hepatocellular injures. It has to be taken into account that in dogs increased activities on AST, especially accompanied by elevated levels of CK talking about muscle damage [10]. In this respect, in experimental group the creatine kinase activity (data not shown) were significantly higher compared to the control dogs [11]. These changes may be due to progression of inflammation caused by injection, destruction of fascia, as well as proteolytic enzymes of accumulated leukocytes at the site of injection.

No significantly changes in total activities of AST of experimental groups were observed and this shows that it is possible permeability of liver cells plasma membrane to slightly increased. During the study the alanine and aspartate aminotransferase concentrations are in normal reference range for the dogs. Costa et al. [12] observed that ALT levels were higher on day 20 after parasitical infection in dogs. According to them, increased ALT levels in dogs are often associated with hepatocellular injury as a result of infectious or toxic inflammatory processes, which promote enzyme release from the cytoplasm of hepatocytes, even though these levels did not exceed the reference values, they were 41% greater than on day 0. In our study the concentrations were 29.1% greater than on day 0.

The increase in AST levels in the infected group was more than 50%, compared with day 0 [12]. The increase in AST in this study was lower-18.7% than the baselines. Nevertheless, the reference values were not exceeded in both studies. In dogs normal values for AST and ALT are between 1-37 U/L and ALT-3-50 U/L [2,13] and according to Hines [14] - 5-55 U/L and 5-107 U/L, respectively. Ezeokonkwo et al. [15] show that infection (parasitic) in dogs caused a significant increase in the activities of AST and ALT on day 7, which coincides with our study. According to Quinn et al. [4], liver enzyme activities (AST, ALT) showed only mild

to moderate increases during bacterial infection in dogs. In addition, the results displayed moderate positively correlation between ALT and RR and IBT, whereas AST associated weakly with the observed clinical parameters.

As a conclusion, these results indicate that AST and ALT activities are slightly influenced by *S. aureus* inoculation in dogs. In the course of this study the concentrations of AST (on day 7 and 14) and ALT (at the 72nd h) in the experimental group has little changed and could not be used as parameters with diagnostic value of experimental induced staphylococcal infection in dogs.

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