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Research Article

Evaluation of Serum Haptoglobin, Ceruloplasmin and Pseudocholinesterase Levels in Cows with Botulism

İsmail AYTEKİN [1] Feyyaz KAYA [1] Hasan ATALAY [2]

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[1] Balikesir University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-10100 Balikesir - TURKEY
[2] Balikesir University, School of Veterinary Medicine, Animal Nutrition and Nutritional Diseases, TR-10100 Balikesir - TURKEY

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Abstract

The aim of this study was to determine serum pseudocholinesterase, rheumatoid factor, troponin I, C-reactive protein, caeruloplasmin, haptoglobin, urea, creatinin, creatinin kinase (CK), phosporus, calcium, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in cows with or without botulism. The study included 15 holstein cows aged two to five years as the botulism group and control group consisting of 10 healthy cows. The group included both pregnant and dry period cattle. Serum concentration of all the parameters were measured using an autoanalyzer and Nefelometre equipment BNII. Mice inoculation test was performed to make diagnosis and Clostridium Botulinum type C and D toxins were determined in silage and blood that cows with botulism. Serum troponin I, C-reactive protein, rheumatoid factor, creatinine, creatinine kinase, phosphorus, calcium and AST did not differ significantly between two groups. Biochemistry analysis of serum showed that in the botulism group haptoglobin, caeruloplasmin and urea were higher and that pseudocholinesterase and alanine aminotransferase were lower than in the control group. Serum haptoglobin, caeruloplasmin, urea, alanine aminotransferase and especially pseudocholinesterase concentrations may prove beneficial to the prognosis of botulism.

Keywords: *Botulism, Cow, Toxin, Pseudocholinesterase, Ceruloplasmin, Haptoglobin*

Botulismuslu İneklerde Serum Haptoglobulin, Seruloplazmin ve Pseudokolinesteraz Seviyelerinin Değerlendirilmesi

Özet

Bu çalışmada botulismuslu ve sağlıklı ineklerde serum pseudokolinesteraz, seruloplazmin, haptoglobin, romatoid faktör, troponin I, C-reactive protein, üre, kreatin, kreatin kinaz (CK), alanine aminotransferase (ALT) ve aspartate aminotransferase (AST), fosfor ve kalsiyum seviyeleri araştırıldı. Çalışma ve kontrol grubunu gebe ve kuru dönemde iki ile beş yaşları arasında değişen 15 adet botulismuslu ve 10 adet sağlıklı holştayn inek oluşturdu. Parametrelerin serum düzeyleri Nefelometre BNII ve otoanalizör kullanılarak ölçüldü. Botilusmuslu hayvanların kan serumlarından ve silajdan fare inokulasyon testi yapılarak clostridium botulinum tip C ve tip D toksini tespit edildi. Serum troponin I, C-reactive protein, romatoid faktör, kreatin, kreatin kinaz, AST, fosfor ve kalsiyum seviyeleri botulismuslu ve sağlıklı ineklerde önemli bir değişiklik göstermedi. Botulismuslu ineklerde haptoglobin, seruloplazmin ve üre yüksek çıkarken, ALT ve pseudokolinesterazın sağlıklı ineklere göre daha düşük çıktığı tespit edildi. Botulismuslu ineklerde serum üre, ALT, haptoglobin, seruloplazmin ve özellikle pseudokolinesteraz düzeylerinin belirlenmesi botulismusun prognozuna faydalı olabileceği sonucuna varıldı.

Anahtar sözcükler: *Botulismus, İnek, Toksin, Pseudokolinesteraz, Seruloplazmin, Haptoglobin*

INTRODUCTION

Botulism is caused by a neurotoxin produced by *Clostridium botulinum* that a gram positive, spore forming anaerobe microorganism. Botulinum NeuroToxin (BoNT) is an exotoxin that produced during growth and autolysis process of the organism under anaerobic conditions [1,2]. Eight different botulinum toxins, A, B, Ca, Cb, D, E, F and G have been identified. BoNT blocks acetylcholine release at neuromusculer junction [3]. Affected cattle shows many symptoms that includes loss of tongue tone, decreased upper eyelid and tail tone, loss of appetite, ataxia and decreased ruminal movements [4]. Many types of silages are used extensively in ruminants feeding [5]. However silage

İletişim (Correspondence)
+90 266 6136692/211
aytekin0331@gmail.com

may support *C. botulinum* growth and toxin production [6]. Primarily botulism caused by types C and D in cattle. Type C and D toxins produced by *Clostridium botulinum* in rotting material, silage that contaminated dead rodents, birds or reptiles [1,2,7].

Haptoglobin is an APP that free haemoglobin binding protein in blood [8]. Haptoglobin has also antioxidant role via iron stabilization and antiinflammatory activity during innate immun response [9]. Iron is important for bacteria to grow and haptoglobin makes the iron unavailable via binds free haemoglobin. In this way it shows bacteriostatic effect on bacteria such as *E. coli* [10]. Caeruloplasmin is an acute phase protein (APP) that contains copper and oxidizes ferrous iron to it's nontoxic ferric form [11]. It protects not only tissues from iron mediated free radical damage but also involved in various antioxidant and cytoprotective mechanisms [12].

Cholinesterase is a mammalian enzyme found in two forms. These are acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) [13]. Pseudocholinesterase or butrylcholinesterase is an enzyme synthesized in various tissues that include liver, brain, lungs and heart and the enzyme has many roles in tissues such as lipoprotein metabolism [14], myelin maintenance [15], neurogenesis and neurite growth [16]. Butyrylcholinesterase hydrolyses butyrylcholine at higher rate than acetylcholine and propionylcholine [17]. However until now many studies were performed physiological functions of the enzyme remains unknown [13].

Diagnosis of botulism is not so challenge but few of biochemistry analysis investigated. The aim of this study was to determine the serum pseudocholinesterase, haptoglobin, caeruloplasmin and some biochemical parameters in cows with and without Botulism.

MATERIAL and METHODS

Animals

This case occured via feeding musty silage to cows incidentally in a dairy farm in Edremit that is a province of Balikesir. The study included 15 Holstein dairy cattle aged to two to five years and mix stage of pregnancy as the Botulism group and a control group consisting of 10 healthy dairy cattle. There are two pregnant cows both botulism group and control group. All cattle were clinically examined before collecting blood samples. The study was approved by the Çanakkale 18 Mart University Ethics Committee (No: 2014/ 03-12).

Serum Biochemistry Analysis

Blood samples were collected from the jugular vein and kept for two hours at room temperature for proper clotting. The samples were centrifuged at 2.500 g at 4°C for 15 min and stored at -20°C until analysed. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), calcium, phosporus, urea, creatinine, troponin I and pseudocholinesterase levels were measured using commercially available kits as per manufacturer's recommendations using a biochemistry auto analyzer Cobas 8000 (Roche, Germany). C-reactive protein, rheumatoid factor, caeruloplasmin and haptoglobin levels were measured using commercially available kits as per manufacturer's recommendations using a Nefelometre equipment BNII (Siemens, Germany).

Statistical analysis was performed using SPSS 20 for Windows. All the cattle that with and without botulism included in the statistical analysis. Results were statistically analysed using the independent samples t test for ALT, haptoglobin, creatinin, pseudocholinesterase, urea and using Mann- Whitney U test for AST, creatinin kinase, calcium, caeruloplasmin and troponin I.

Toxin Isolation

Samples that includes blood serum, ruminal content and silage were sent to the Veterinary Control and Reserach Inst.

RESULTS

Clinical Findings

Anorexia, lethargy, loss of tongue tone, lameness, recumbency, decreased tail tone, reduced rumen contractions, some of them head turned back against flank and death were observed in botulism group. The cattle in control group were completely healthy.

Biochemical Findings

Biochemistry analysis of serum showed that in the Botulism group pseudocholinesterase ($P<0.05$), and ALT ($P<0.01$) were lower and that haptoglobin ($P<0.001$), caeruloplasmin ($P<0.001$) and urea ($P<0.008$) were higher than in the control group *(Table 1)*. Serum troponin I, C-reactive protein, rheumatoid factor, creatinine, creatinine kinase, phosphorus, calcium and AST did not differ significantly between two groups *(Table 1)*.

Mice Inoculation Test

Toxin types C and D were identified in corn silage and blood serum. Mice inoculation test was performed and results were positive.

DISCUSSION

Enzyme Linked Immuno-Sorbent Assay (ELISA) and mice inoculation test are used to diagnosis of botulism but it is lower sensitivity than mice inoculation test. Because of this reason mice inoculation test is the most reliable

Table 1. *Pseudocholinesterase, haptoglobin, caeruloplasmin and biochemical parameters in cows with botulism and healthy group*

Tablo 1. *Botulismuslu ve sağlıklı ineklerde pseudokolinesteraz, haptoglobin, seruloplazmin ve biyokimyasal parametreler*

Parameters	Healthy Cows (n=10)	BotulismCows (n=15)	P Values
ALT (U/L)	31.62±1.70	22.47±1.46	*
AST (U/L)	80.36±3.01	77.91±7.44	NS
C-Reactive Protein (mg/L)	<3.48	<3.48	NS
Phosporus (mg/dL)	5.82±0.37	5.50±0.35	NS
Haptoglobin (mg/dL)	6.37±0.08	7.59±0.19	**
Calcium (mg/dL)	9.55±0.21	9.72±0.36	NS
Creatine Kinase (U/L)	197.50±9.67	452.40±118.18	NS
Creatinine (mg/dL)	0.95±0.03	0.91±0.03	NS
Pseudocholinesterase (U/L)	94.80±15.59	57.06±5.95	***
RheumatoidFactor (IU/mL)	<11.5	<11.5	NS
Caeruloplasmin (mg/dL)	4.37±0.23	7.26±0.32	**
Troponin I (ng/mL)	0.10±0.00	0.11±0.01	NS
Urea (mg/dL)	12.03±1.06	15.84±0.80	****

** P<0.01, ** P<0.001, *** P<0.05, **** P<0.008,* ***NS-*** *Not Significant,* ***SEM-*** *Standart Error of Mean*

test for botulism [18,19]. However negative mice inoculation test results do not eliminate the disease due to the toxin may be present at below level of threshold of detection. Additionaly BoNT is rapidly biodegraded in rumen by rumen microflora [20,21]. Our test results are agreement with previous reports.

The common clinical findings in animals with botulism include loss of tongue tone, decreased upper eyelid and tail tone, decreased rumen motility, pupillar and anal reflexes, loss of appetite and ataxia [4,22] which were all observed in the present study.

Braun et al.[23] reported normal or increased levels of biochemical parameters in plasma that include alanine aminotransferase and aspartate aminotransferase in cattle with botulism. In parallel Cobb et al.[24] determined any abnormalities other from hyperglycaemia and neutrophilia in dairy cows with botulism. However Senturk et al.[4] and Senturk et al.[22] found aspartate aminotransferase levels in normal reference ranges, Senturk and Cihan [4] found slightly increased serum aspartate aminotransferase levels in cattle with botulism. In the present study alanine aminotransferase enzyme did differ significantly between the two groups, it's level is in normal reference ranges [25]. Our results agreement with the previous reports [22-24]. The findings highly suggest that hepatocyte integrity and function of the liver was not impaired severely in the animals with botulism.

Although Senturk and Cihan [4] found slightly increased serum creatinine kinase levels, they found increased creatinine levels in cows with botulism. However Senturk et al.[22] found creatinine levels in normal reference ranges, they found increased creatinine kinase levels in cows with botulism. We think that the differences of results are caused by time of sampling. In the present study our results agreement with previous reports [4,22].

Senturk and Cihan [4] found calcium and phosphorus levels in reference ranges. In the present study although calcium levels slightly decreased and phosphorus levels were in reference ranges in cows with botulism [26]. Our results are similar with previous report [4].

Acute phase proteins do not have sufficient specificity, however they are good indicators of inflammation [27]. In healthy ruminants its blood level is negligible but it increases over 100-fold on immune stimulation [28,29]. Kirbas et al.[30] found increased haptoglobin levels in cows with traumatic reticuloperitonitis and their results are in agreement with the previous reports. Sixfold increases in haptoglobin concentrations were determined in dairy cows that suffer from infectious and metabolic disease compared to animals with minor lesions [31]. Gerlach et al.[32] observed the increased levels of serum haptoglobin in chronic botulism with *C. botulinum* proliferation. Similarly in the present study haptoglobin levels were higher in botulism group as previously reported [27-32].

Caeruloplasmin remains less common compared the other acute phase proteins to make diagnosis. However there have been certain studies determined increased caeruloplasmin levels and ferroxidase activity is an indicator of infection in cattle [28,29,33-35]. Similarly Nisbet and Cenesiz [36], and Nazifi et al.[37], found increased caeruloplasmin levels in cattle infected with cystic echinococcosis and *Theileria annulata* respectively. In the present study high levels of caeruloplasmin were observed in botulismus group, which suggests that inflammation due to botulism.

Although C-reactive protein indicates health status of herd it does not consider a primer acute phase protein in cattle [38]. Similarly in the present study we found any statistical importance between the control and botulism groups.

Urea reference ranges is 6-27 mg/dl in cattles [26]. Additionally Saraiva [39], found serum urea reference ranges is 20-30 mg/dl in healthy Nelore cattles. Senturk and Cihan [4], and Senturk et al.[22] found elevated serum urea concentrations cattle with botulism type C and D. In the present study urea did differ significantly between the two groups, but it's level is in normal reference ranges. We think that our results are in reference ranges because blood samples taken in the first stages of infection.

Pseudocholinesterase's primary pharmacological and toxicologigal importance is hydrolyzing ester- containing drugs and scavenging cholinesterase inhibitors including potent organophosphorus nerve agents before they reach their synaptic targets [40]. Both in veterinary medicine

and human medicine cholinesterases take attraction as a bioscavenger drug, carbamate and organophosphate insecticides [40-43]. Some qualifications of the enzym that includes hydrolyzing carboxylic or phosporic acid ester containing compounds and attachment to certain aminoacids such as proline [44,45]. In non-toxic inflammatory diseases such as metabolic syndrome in humans, diabetes mellitus and obesity in both dogs and humans, elevated serum pseudocholinesterase concentrations were observed [46-49]. In case of systemic inflammation increased oxidative stress and decreased antioxidant status in blood levels were common in end-stage inflammatory disease and some kind of toxicity such as Cd in rats [50,51]. Similarly Aytekin et al.[52] found decreased antioxidant status caused by the elevated oxidant status in sheeps with Bluetongue. All the previous reports as stated above such as, qualifications of the enzyme, increased serum level of the enzyme in case of non toxic inflammatory diseases, decreased antioxidan status in inflammation and our results that include decreased serum pseudocholinesterase level in cows with botulism highly suggest that BoNT may be detoxified by the enzyme like potent organophosphorus nerve agents.

In conclusion, the literature includes many studies on the toxin's structural investigation, therapeutically uses in humans and blocking mechanism of the acetylcholinesterase in synaptic membrane; however few have investigated biochemical parameters such as pseudocholinesterase, haptoglobin, caeruloplasmin were investigated in the present study. Serum haptoglobin, caeruloplasmin, urea, alanine aminotransferase and especially pseudocholinesterase concentrations may prove beneficial to the prognosis of botulism.

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