Dicle Üniversitesi Veteriner Fakültesi Dergisi
Dicle University Journal of Faculty of Veterinary Medicine

e-ISSN:1308-0679
https://dergipark.org.tr/tr/pub/duvetfd

RESEARCH ARTICLE

Determination of Oxidative Stress and Sialic Acid Levels in Cattle Infected with Hydatid Cyst

Merve DOĞAN[1,a], Kadir BOZUKLUHAN[2,b,✉]

[1]Institute of Health Sciences, Kafkas University, Kars, TÜRKİYE
[2]Department of Internal Medicine, Faculty of Veterinary, Kafkas University, Kars, TÜRKİYE

ORCID: [a]0000-0001-9730-1042; [b]0000-0003-4929-5156

✉ Corresponding Author

Kadir BOZUKLUHAN
Department of Internal Medicine, Faculty of Veterinary, Kafkas University, Kars, TÜRKİYE

kbozukluhan@hotmail.com

Received
23.08.2024

Accepted
14.11.2024

Published
31.12.2024

DOI

10.47027/duvetfd.1537837

How to cite: Doğan M, Bozukluhan K (2024). Determination of oxidative stress and sialic acid levels in cattle infected with hydatid cyst. *Dicle Üniv Vet Fak Derg*., 17(2):173-177

Abstract

The aim of the study was to determine oxidative stress and sialic acid levels in cattle with hydatid cysts. In the study, 15 hydatid cyst infected and 15 healthy cattle were used. Total sialic acid (TSA), lipid-bound sialic acid (LBSA), protein-bound sialic acid (PBSA), nitric oxide (NO) and malondialdehyde (MDA) levels were determined colorimetrically in the animals. It was determined that TSA, LBSA, NO, and MDA levels were increased in the cattle with hydatid cysts compared to the control group, while PBSA level increased but was statistically insignificant. In conclusion, it was concluded that oxidative stress occurs in the cattle infected with hydatid cyst and TSA can be used as an indicator in the diagnosis of the disease.

Key Words: Cattle, hydatid cyst, oxidative stress, total sialic acid

Hidatik Kist ile Enfekte Sığırlarda Oksidatif Stres ve Siyalik Asit Düzeylerinin Belirlenmesi

Öz

Çalışmanın amacı hidatik kistli sığırlarda oksidatif stres ve siyalik asit seviyelerinin belirlenmesidir. Çalışmada, 15 adet kist hidatik ile enfekte ve 15 adet sağlıklı sığır kullanıldı. Hayvanlarda total siyalik asit (TSA), lipid bağlı siyalik asit (LBSA), proteine bağlı siyalik asit (PBSA), nitrik oksit (NO) ve malondialdehit (MDA) düzeyi kolorimetrik olarak tespit edildi. Hidatik kistli sığırlarda TSA, LBSA, NO ve MDA düzeylerinin kontrol grubuna göre yükseldiği, PBSA düzeyinin ise artmakla beraber istatistiksel olarak anlamsız olduğu belirlendi. Sonuç olarak, hidatik kist ile enfekte sığırlarda oksidatif stresin oluştuğu ve hastalığın teşhisinde TSA'nın bir indikatör olarak kullanılabileceği kanısına varıldı.

Anahtar Kelimeler: Kist hidatik, oksidatif stres, sığır, total siyalik asit

INTRODUCTION

Echinococcus granulosus is a parasitic zoonosis that is widespread worldwide and important in terms of public health, causing hydatid cysts in many mammalian species, especially ruminants (1). In farm animals, it causes significant economic losses due to decreased meat and milk yield, decreased wool quality, infertility, and destruction of cystic organs such as the liver and lungs. Infective eggs excreted with the feces of the final host are ingested orally or rarely through the respiratory tract, resulting in infection in intermediate hosts (2,3). Since the disease is chronic, the development of cysts in intermediate hosts takes a long time, and symptoms vary depending on the size of the cyst, the organ it is located in, its number, and the stage of development of the cyst. Cysts located in the liver cause persistent diarrhea, jaundice and liver enlargement in animals, while cysts in the heart cause heart failure and cysts in the lungs cause symptoms such as cough, dyspnea, wheezing and rapid breathing (2,4).

In the organs and tissues where the cyst is located, an acute phase response (APR) occurs depending on the cellular and humoral response, and changes occur in the synthesis of acute phase protein (APP) in the liver (5,6). Sialic acid, which is found in the structure of many APPs, consists of pyruvate and mannosamine. Sialic acid, which is found in large amounts in animal tissues and bacteria, is an important component of the cell membrane, which has important functions such as acting as a receptor in membranes and playing a role in the adjustment of cellular stimuli, and recognizing each other in pathogen and host interactions (7). Total sialic acid (TSA), whose levels increase in inflammation and infection, is a very important biomarker in the diagnosis of inflammatory diseases (8).

Tissue damage, inflammation and infections occurring in the organism activate many mononuclear cells such as monocytes and macrophages. As a result of this activation, mononuclear cells consume excessive oxygen and ultimately cause the formation of free radicals such as hydrogen peroxide and superoxide anion (9). Oxidative stress occurs due to the excessive formation of free radicals. As a result of oxidative stress, end products of lipid peroxidation such as MDA accumulate, causing damage to tissues and organs (10). It has been reported that parasitic infections in particular cause damage to cells and tissues due to the increase in free radicals in host cells (11). NO is a free radical produced as a result of the reaction catalyzed by nitric oxide synthase and mediates many physiological or pathological events such as regulating cellular transmission in the organism and acting as a receptor in the structure of membranes (12,13). It has been reported that NO, produced by macrophages, neutrophils and mast cells, has anti-inflammatory, antimicrobial and antitumoral functions and its concentration increases in many bacterial, viral and parasitic infections (14-16). For these reasons, our aim in this study was to determine oxidative stress and sialic acid levels in cattle with hydatid cysts.

MATERIAL AND METHODS

In the study, a total of 30 cattle (3-4 years old, Brown Swiss cattle) were used as the hydatid cyst infected group (n=15) and the control group (n=15). After routine clinical examination of the animals brought to the Department of Internal Medicine of the Faculty of Veterinary Medicine of KAU with complaints such as dyspnea, neck extension, cough, cyanosis in the mucous membranes etc., the disease was diagnosed radiographically. The diagnosis was confirmed after slaughter. The animals constituting the control group were composed of clinically healthy animals with the same care and feeding conditions.

Blood samples were taken from the *jugular veins* of the animals in tubes without anticoagulants. The samples taken in tubes without anticoagulants were centrifuged at 3000 rpm for 15 minutes and the serum samples were separated and stored at -20 ^{o}C until analysis. NO concentration was measured in a spectrophotometer according to the method reported by Miranda et al. (17). In this method, nitrate was converted to nitrite with vanadium (III) chloride. The reaction of nitrite with sulfanilamide in acidic medium with N-(1-Naphthyl) ethylene diamine dihydrochloride resulted in the formation of a complex diazonium compound. This colored complex was measured at 540 nm. After the nitrate and nitrite levels were determined separately, the sum of the two indicates the amount of NO. Serum MDA concentration was determined according to the method reported by Yoshioka et al. (18). The formed MDA forms a pink complex with thiobarbituric acid and the absorbance of this solution is measured spectrophotometrically at 535 nm to determine the degree of lipid peroxidation.

TSA is the sum of free, PBSA and LBSA (19). Serum TSA and LBSA analyses were measured colorimetrically (Epoch, Biotek, USA) using the methods described by Sydow (20) and Katopodis and Stock (21), respectively, and the absorbances obtained were evaluated from the standard curve prepared with N-acetyl neuraminic acid. PBSA concentration was calculated by subtracting LBSA from TSA.

Statistical Analysis

SPSS 20.0 package program was used to evaluate the study data. Independent Sample T-test was used to compare the groups.

RESULTS

Clinical examination revealed rumen atony, dyspnea, cough, shallow and rapid respiration and tachycardia in the heart (Table 1). In addition, auscultatory examination revealed wheezing in the lung sounds and widespread dullness in the lung percussion area. Radiographic examination revealed numerous cysts with regular borders showing opacities in the lungs. When the cattle infected with hydatid cysts and the control group were compared, it was determined that TSA, LBSA, MDA and NO levels increased compared to the control group, while PBSA levels increased but were insignificant (Table 2).

Table 1. Physical examination findings of hydatid cyst infected cattle and control group

Parameters	Control	Infected	P
Rectal temperature (°C)	38.12±0.06	38.67±0.12	NS
Breaths/min	22.97±1.72	32.05±2.53	P<0.01
Heart beats/min	64.69±5.29	92.24±3.50	P<0.01

NS: Non significant

Table 2. Sialic acid and oxidative stress parameter levels in clinically healthy and hydatid cyst infected cattle

Parameters	Control	Infected	P
TSA (mg/dL)	71.32±2.66	92.37±3.11	P<0.001
LBSA (mg/dL)	32.15±2.34	44.83±2.21	P<0.001
PBSA (mg/dL)	39.17±3.71	47.54±3.08	NS
NO (µmol/L)	23.64±1.44	18.45±1.66	P<0.05
MDA (µmol/L)	2.12±0.09	3.32±0.19	P<0.01

TSA: Total sialic acid, LBSA: Lipid-bound sialic acid, PBSA: Protein-bound sialic acid, NO: Nitric oxide, MDA: Malondialdehyde, NS: Non significant

DISCUSSION AND CONCLUSION

Hydatid cyst is a chronic zoonotic parasitic infection that causes economic losses such as destruction of consumable organs, especially the liver and lungs, decrease in the amount and quality of meat, milk, and wool, delayed growth, and decreased birth rate (2,22). Although the disease is seen all over the world, it is more common in countries where eradication programs and preventive medical services are inadequate (23).

APR is formed in the animal tissues where it is located, depending on the cellular and humoral response, and changes occur in APP synthesis in the liver. The level of sialic acid in the structure of APPs also increases in parallel with the increase in APP concentration (12). TSA, which has an increased concentration, is a very important biomarker in the diagnosis of inflammatory diseases (8). Studies have reported that TSA levels increase in diseases such as neonatal diarrhea (24), tuberculosis (25), anaplasmosis and theleriosis (26), echinococcosis (12), hypodermosis (8), aspiration pneumonia (27), leptospirosis (28) and botulinum (29). In the study, TSA levels also increased and the increase may probably be due to increased sialoprotein synthesis in the liver as a result of APR, which develops due to inflammation and tissue damage.

NO is a free radical that is produced as a result of the reaction catalyzed by nitric oxide synthase and mediates many physiological or pathological events such as regulation of cellular conduction in the organism and acting as a receptor in the structure of membranes. It has been reported that NO produced by macrophages, neutrophils and mast cells has anti-inflammatory, antimicrobial and antitumoral functions and its concentration increases in many bacterial, viral and parasitic infections (14-16,30). In two separate studies conducted on animals infected with foot and mouth (14,16), it was reported that NO concentration increased in animals with foot and mouth. In addition, Özkan et al. (31) found that NO levels increased in a study they conducted in cattle with traumatic pericarditis. In addition, Atakişi et al. (15) reported an increase in NO concentration in another study they conducted on cattle with traumatic reticuloperitonitis. The NO level also increased in the study and the reason for the increase may be due to the increased activity in mononuclear cells such as monocytes and macrophages stimulated in the disease.

Tissue damage, inflammation and infections occurring in the organism activate many mononuclear cells such as monocytes and macrophages. As a result of this activation, mononuclear cells consume excessive oxygen and ultimately cause the formation of free radicals such as hydrogen peroxide and superoxide anion. Therefore, oxidative stress occurs and products such as MDA, which cause damage to tissues and organs, accumulate as a result of the oxidative stress (9,10). Studies have reported that the oxidant-antioxidant balance is disrupted and oxidative stress occurs in bacterial/viral and parasitic diseases such as hypodermosis (8,32), pneumonia (33) and sheeppox (34,35). It has been reported that parasitic infections in particular cause damage to cells and tissues due to the increase in free radicals in host cells (11). In a study conducted on sheep infected with *Toxoplasma gondii*, it was reported that oxidative stress caused by the parasite may play a role in the pathogenesis of the disease (36). In addition, in a study conducted on cattle with cystic echinococcosis, they reported that serum MDA levels increased due to increased lipid peroxidation in infected cattle (37). In another study conducted on sheep infected with *Dicrocoelium dendriticum* and hydatid cysts, they reported that endoparasites may cause oxidative stress (38). In the study, serum MDA levels increased due to the increase in lipid peroxidation, and the reason for the increase may be due to the increased activity in mononuclear cells against the parasite.

In conclusion, when the data obtained from the study were evaluated, it was determined that TSA, LBSA, NO and MDA levels were increased in animals with hydatid cyst compared to the control group, while PBSA levels increased but were statistically insignificant. It was concluded that the determination of serum TSA concentration and oxidative stress parameters in the cattle infected with hydatid cysts could be used as auxiliary biochemical parameters in determining and monitoring the severity of inflammation.

ACKNOWLEDGEMENT

This study was summarised by the first author's Master Thesis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

KB took part in the study planning and MD sample collection. The writing of the study and final checks were carried out with the contributions of all authors.

ETHICAL STATEMENT

This study was started after receiving the ethics committee approval of Kafkas University Animal Experiments Local Ethics Committee (2021/011).

REFERENCES

1. **Gıcık Y, Arslan MÖ, Kara M, Köse M (2004).** Kars ilinde kesilen sığır ve koyunlarda kistik ekinokokkozisin yaygınlığı. *Turkiye Parazitol Derg.*, 28(3):136-139.
2. **Avcıoğlu H (2013).** Sığırlarda karaciğer ve diğer iç organlarda görülen helmint hastalıkları. (İçinde): Veteriner Hekimliğinde Parazit Hastalıkları. Özcel MA (editör). Baskı 1. *Meta basım*, İzmir, Türkiye., 204-207.
3. **Regassa B (2019).** Review on hydatidosis in small ruminant and its economic and public health significance. *Dairy and Vet Sci J.*, 11(2):555808.
4. **Gökce HI, Bozukluhan K, Uzlu E (2009).** Hydadit cyst case in a cow with chronic respiratory disorders. *Dicle Üniv Vet Fak Derg.*, 1:15-18.
5. **Merhan O, Bozukluhan K (2022).** Acute phase response and some acute phase proteins in animals. In: Current Multidisciplinary Studies in Veterinary Medicine I. Yıldız G, Baran MS, Kaplan O, Durna Aydın Ö (eds). *Iksad Publishing House*, Ankara, Türkiye., 3-33.
6. **Bozukluhan K, Merhan O (2023).** Clinical significance of some acute phase proteins in cattle. In: Cattle Diseases - Molecular and Biochemical Approach. Kükürt A, Gelen V (eds). *IntechOpen*, London, 1-13.
7. **Schauer R, Kamerling JP (2018).** Exploration of the sialic acid world. *Adv Carbohydr Chem Biochem.*, 75:1-213.
8. **Merhan O, Taşçı GT, Bozukluhan K, Aydın N (2020).** Determination of oxidative stress index and total sialic acid in cattle infested with *Hypoderma spp*. *Kafkas Univ Vet Fak Derg.*, 26(5):633-636.
9. **Sezer K, Keskin M (2014).** Serbest oksijen radikallerinin hastalıkların patogenezisindeki rolü. *FÜ Sağ Bil Vet Derg.*, 28:149-156.
10. **Apaydin Yildirim B (2020).** Evaluation of biochemical parameters and oxidative stress in native and crossbred cattle naturally infected with dermatophytosis. *GSC Biol Pharm Sci.*, 13:99-104.
11. **Gültekin M, Paşa S, Ural K, Balıkçı C, Ekren Aşıcı GS, Gültekin G (2017).** Visseral leishmaniasis'in farklı evrelerindeki köpeklerde oksidatif durum ve lipid profili. *Turkiye Parazitol Derg*., 41:183-187.
12. **Yarım GF, Umur Ş, Açıcı M, Beyhan YE (2010).** Kistik ekinokokozisli sığırlarda serum sialik asit düzeyleri. *Ankara Univ Vet Fak Derg.*, 57(1):61-63.
13. **Atakisi E, Merhan O (2017).** Nitric oxide synthase and nitric oxide involvement in different toxicities. In: Nitric Oxide Synthase - Simple Enzyme-Complex Roles. Saravi SSS (ed). *IntechOpen*, London, 197-214.
14. **Yarım GF, Nisbet C, Çenesiz S, Coşkuner A (2006).** Şap hastalıklı koyunlarda serum nitrik oksit düzeyi ve adenozin deaminaz aktivitesinin araştırılması. *Ankara Univ Vet Fak Derg.*, 53:161-164.
15. **Atakisi E, Bozukluhan K, Atakisi O, Gokce HI (2010).** Total oxidant and antioxidant capacities and nitric oxide levels in cattle with traumatic reticuloperitonitis. *Vet Rec.*, 167(23):908-909.
16. **Bozukluhan K, Atakişi E, Atakişi O (2013).** Nitric oxide levels, total antioxidant and oxidant capacity in cattle with foot-and-mouth-disease. *Kafkas Univ Vet Fak Derg.*, 19:179-181.
17. **Miranda KM, Espey MG, Wink DA (2001).** A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide: Biol Chem.*, 5(1):62-71.
18. **Yoshioka T, Kawada K, Shimada T, Mori M (1979).** Lipid peroxidation in maternal and cord blood and protective mechanism againist active-oxygen toxicity in the blood. *Am J Obstet Gynecol.*, 135:372-376.
19. **Merhan O, Özcan A (2004).** Kazlarda serum seruloplazmin ve total sialik asit düzeylerinin araştırılması. *Kafkas Üniv Vet Fak Derg.*, 10(2):139-142.
20. **Sydow G (1985).** A simplified quick method for determination of sialic acid in serum. *Biomed Biochim Acta.*, 44:1721-1723.
21. **Katopodis N, Stock CC (1980).** Improved method to determine lipid bound sialic acid in plasma or serum. *Res Commun Chem Pathol Pharmacol.*, 30:171-180.
22. **Moro P, Schantz PM (2009).** *Echinococcosis*: A review. *Int J Infect Dis.*, 13:125-133.
23. **Umur Ş (1995).** Hidatidozun (kist hidatik) önemi, korunma yolları ve eradikasyonu için bir öneri. *Vet Hekim Dern Derg.*, 65(4):18-22.
24. **Uzlu E, Karapehlivan M, Çitil, Gökçe E (2010).** İshal semptomu belirlenen buzağılarda serum sialik asit ile bazı biyokimyasal parametrelerin araştırılması. *Yuzuncu Yıl Univ Vet Fak Derg.*, 21(2):83-86.
25. **Merhan O, Kukurt A, Bozukluhan K, et al. (2022).** Sialic acid and some biochemical parameter levels in cattle with tuberculosis. *Fresenius Environ Bull.*, 31:2428-2431.
26. **Güzel M, Kontas Askar T. Kaya G, Atakısı E, Avcı GE (2008).** Serum sialic acids, total antioxidant capacity, and adenosine deaminase activity in cattle with *theileriosis* and *anaplasmosis*. *Bull Vet Inst Pulawy*, 52:227-230.
27. **Akyüz E, Merhan O, Aydın U, et al. (2022).** Neopterin, procalcitonin, total sialic acid, paraoxonase-1 and selected haematological indices in calves with aspiration pneumonia. *Acta Vet Brno.*, 91:115-124.
28. **Keleş İ, Ertekin A, Karaca M, Ekin S, Akkan HA (2000).** Sığırların leptospirozisinde serum sialik asit ve lipid-bağlı sialik asit düzeyleri üzerine araştırma. *Yuzuncu Yıl Univ Vet Fak Derg.*, 11:121-122.
29. **Aytekin İ (2020).** Botulismuslu ineklerde serum total sialik asit konsantrasyonunun ve bazı biyokimyasal parametrelerin değerlendirilmesi. *Atatürk Üniversitesi Vet Bil Derg.,* 15(2):151-155.
30. **Demir Merkit C, Merhan O (2021).** Dietilnitrozamin ile indüklenen tavşanlarda β-karotenin nitrik oksit ve malondialdehit düzeylerine etkisinin araştırılması. *Dicle Üniv Vet Fak Derg.*, 14(1):39-42.
31. **Özkan C, Altuğ N, Kaya A, Başbuğan Y (2012).** Perikarditis travmatikalı sığırlarda serum nitrik oksit düzeyleri. *Yuzuncu Yıl Univ Vet Fak Derg.*, 23(3):131-135.

32. **Merhan O, Bozukluhan K, Gokce HI (2017).** Acute phase proteins and biochemical and oxidative stress parameters in *Hypoderma spp*. infested cattle. *J Hellenic Vet Med Soc.*, 68:535-540.
33. **Bozukluhan K, Merhan O, Kiziltepe Ş, Ergin Egritag H, Akyuz E, Gökce HI (2021).** Determination of haptoglobin, some biochemical and oxidative stress parameters in calves with pneumonia. *Fresenius Environ Bull.*, 30(07A):9485-9489.
34. **İssi M, Gul Y, Yılmaz S (2008).** Clinical haematological and antioxidant status in naturally poxvirus infected sheep. *Rev Med Vet.*, 159:54-58.
35. **Bozukluhan K, Merhan O, Gökçe Hİ, et al. (2018).** Determination of some acute phase proteins, biochemical parameters and oxidative stress in sheep with naturally infected sheeppox virus. *Kafkas Univ Vet Fak Derg.*, 24(3):437-441.
36. **Al-Kennany ER (2009).** Oxygen free radicals released in placentae of ewes naturally infected with *Toxoplasma gondii*. *Al-Anbar J Vet Sci.*, 2:1-6.
37. **Nisbet C, Çenesiz S, Acici M, Umur Ş (2008).** Kistik ekinokokkozisli sığırlarda serum malondialdehit, seruloplazmin ve adenozin deaminaz düzeylerinin belirlenmesi. *Erciyes Üniv Vet Fak Derg.*, 5(1):1-4.
38. **Çınar M, Aydenizöz M, Gökpınar S, Çamkerten G (2018).** Evaluation of biochemical parameters and oxidative stress in sheep naturally infected with *Dicrocoelium dendriticum* and hydatid cysts. *Turk J Vet Anim Sci.*, 42(5):423-428.