DETERMINATION OF SERUM MALONDIALDEHYDE LEVELS IN SHEEP NATURALLY INFECTED WITH *DICROCOELIUM DENDRITICUM*

Sami ŞİMŞEK[1] Abdurrauf YÜCE[2] Armağan Erdem ÜTÜK[1]

[1]Fırat Üniversitesi, Veteriner Fakültesi Parazitoloji Anabilim Dalı , Elazığ – TÜRKİYE

[2] Fırat Üniversitesi, Veteriner Fakültesi Fizyoloji Anabilim Dalı, Elazığ – TÜRKİYE

Geliş Tarihi: 28.10.05 Kabul Tarihi: 02.03.2006

ABSTRACT

The aim of this study was to investigate the changes of serum malondialdehyde levels in sheep naturally infected with *Dicrocoelium dendriticum*. Serum malondialdehyde activity was measured in 20 sheep that were naturally infected with *D.dendriticum*. These sheep were selected to examination of bile ducts and gall bladder after slaughtering. Scores were obtained for the positives and 17 *D.dendriticum* negative healthy controls. The difference between malondialdehyde levels of sheep infected with *D.dendriticum* and control group was statistically significant (P<0.001). In conclusion that malondialdehyde levels clearly increase in sheep infected with *D.dendriticum*.

Key Words: *Dicrocoelium dendriticum*, *Malondialdehyde, Sheep, Lipid peroxidation.*

ÖZET

***Dicrocoelium Dendriticum* ile Doğal Enfekte Koyunlarda Serum Malondialdehid Seviyesinin Belirlenmesi**

Bu çalışmanın amacı, *Dicrocoelium dendriticum* ile doğal enfekte koyunlarda serum malondialdehid seviyesindeki değişikliklerin araştırılmasıdır. Serum malondialdehid aktivitesi *D.dendriticum* ile doğal enfekte 20 koyunun serumunda ölçülmüştür. Bu koyunlar, kesim sonrasında safra kanalları ile safra kesesinin muayenesine göre seçilmiş, negatif kontrol olarak da *D.dendriticum*'un görülmediği sağlıklı 17 kuzu serumu kullanılmıştır. Neticede, enfekte ve kontrol grubu koyunlarda serum malondialdehid seviyeleri arasındaki fark istatistiksel olarak önemli bulunmuş (P<0.001) ve *D.dendriticum* ile enfekte koyunlarda malondialdehid seviyesinin yükseldiği belirlenmiştir.

Anahtar Kelimeler: *Dicrocoelium dendriticum*, *Malondialdehid, Koyun, Lipid peroksidasyonu.*

INTRODUCTION

Dicrocoelium dendriticum, the lancet fluke, has a world-wide geographical distribution and parasitises in the liver of many species including animals and humans (1). This parasite is frequently present throughly Europe, Asia, North Africa, and North America (2). It is considered to be an important parasite in sheep in many countries in Europe and Asia (3, 4). The definitive host becomes infected by eating infected ants. Within the definitive host, metacercaria lose their protective envelope and the young flukes migrate from the intestine via the ductus choledocus or the portal blood system (5). In the first few days of infection there is angiectasis of the central veins and the portobiliary vessels. After the acute stage a chronic inflammation of the biliary ducts with a marked proliferation of connective tissue develops (1). *D.dendriticum* is a long-lived parasite and pathological changes (chronic irritation) increase in severity over time if left untreated. Long-term infection causes progressive hepatic cirrhosis, shortens the reproductive life of sheep and decreases wool production and lactation (6). The diagnosis of this parasitosis usually is based on the detection of eggs in the faeces of the infected animals (7), although pointed out that in sheep with <100 flukes that test typically is negative (8).

Lipid peroxidation is a well-established mechanism of cellular injury, and is used as an indicator of oxidative stress in cell and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and can decompose to form a complex series of compounds. These include reactive carbonyl compound, which is the most abundant malondialdehyde (MDA). Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in both human and animals (9, 10).

The effects of other trematode infections, such as fasciolosis, on liver function have been extensively studied. Changes in bile composition and alterations in liver phase I and phase II enzymes have been reported (11). There are several research about changes in the level of serum MDA in patients infected with some parasite infections. But no significant correlation was found between MDA levels of both females and males for *Giardia intestinalis*, *Taenia saginata* and *Blastocystis hominis* infection and control groups (12, 13, 14). This situation indicated that these infections have no effect on oxidative stress in cells and tissue and accordingly on cellular injury in human. Sometimes the serum MDA levels can elevate together with chronic organ disease. Kargın and Fidancı (15) reported that free radical activities, especially MDA, are responsible for the ethiology and pathogenesis of kidney diseases in dogs. Researchers (15) showed that there was a positive correlation between the intensity of the oxidative stress and the damage of kidney disease.

The aim of this study was to evaluate the hypothesis of decreased activity of defense system protecting tissues from free radical damage in sheep naturally infected with *Dicrocoelium dendriticum* by measuring the level of MDA (an end-product of lipid peroxidation) in sheep serum samples.

MATERIAL and METHOD

Infected Animals

In this group, studies were carried out on 20 sheep (Akkaraman) naturally infected with *D.dendriticum*. All sheep were one age. Sheep age was determined via oral herd records and dentition patterns. Blood samples were taken from sheep brought in for slaughtering at a local abattoir in Elazığ province of Turkey. The blood samples were immediately collected from all sheep after slaugtering and then the organs of sheep for *D.dendriticum* and other helminths and infections carefully, especially liver bile ducts, and gall bladder were examied. After these inspections the positive serum samples were selected among from the sheep infected with *D. dendriticum*. These sheep consideres as were healthy in that they had no viral or bacterial infections according to inspection.

Control Animals

Seventeen healthy Akkaraman lamb (mean age 6 months) were used as control. These clinically healthy animals were selected on the basis of a clinical investigation, coprological examination (flotation and sedimentation) and necropsy. At the examination of bile ducts and gall bladder, both *D.dendriticum* and *Fasciola hepatica* were not determined.

Blood Analysis

All blood samples were centrifuged at 500 g for 5 min. Sera were then removed and stored at -20 ºC until used. Serum MDA levels were measured by the double heating method (16). The principles of the method was based on the spectrophotometric measurement of the color occured during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substance was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex and expressed in nmol/ml.

Statistical Analysis

The values collected were stated as mean ±Standart deviation (SD). Statistical analysis was performed by using SPSS software package (Version 11.0 for Windows). For comparison of two groups of continuous variables, indipendent samples t-test was used. A probability value of $P<0.001$ indicated a statistically significant difference.

RESULTS

In the macroscopic examination of the organs and carcasses of positive sheep, there was no determined other parasite. All liver bile ducts were examined, and no *F.hepatica* was encountered. Malondialdehyde scores are given in Table 1.

Table1. MDA levels of sheep naturally infected with *Dicrocoelium dendriticum* and control group

	Infected	Control
n	20	17
MDA levels	4.10±0.67*	2.83±0.42*

*Statistically difference is significant ($P<0.001$), Data represent mean values± SD

The difference between MDA levels of infected and control group was statistically significant ($P<0.001$), (Table 1).

DISCUSSION

This study was aimed to evaluate and characterize the relationship between liver flukes infection of dicrocoeliosis, which can cause pathology and oxidative stress mechanism as a mediator of tissue damage concurrent with dicrocoeliosis infection.

Compared with fasciolosis, dicrocoeliosis produces mild symptoms in affected animals, however it causes severe economic losses, in terms of milk and meat production, due to liver function impairment. The disease can be fatal on rare occasions (17). Small ruminant dicrocoeliosis is common in sheep flocks with a prevalence of up to 100% in many European and Eastern countries (18).

The young flukes migrate directly up the biliary duct system of the liver without penetrating the gut wall, liver capsule or liver parenchyma as in fasciolosis. Clinical symptoms are not usually manifested, even in heavy infections, and therefore major lesions, due to liver impairment are detectable only at necroptic examination of the liver (1). Animals suffering from dicrocoeliosis may show anemia, oedema, emaciation, and in advanced cases, cirrhosis, scarring of the liver surface, and marked distension of bile ducts. Due to its buccal stilets, the small liver fluke irritates the bile duct surface, thus, causing proliferation and changes in the septal bile ducts of the lobular hepatic edges (17).

Although there were no detect any oxidative response in some parasite infections including *G. intestinalis*, *T. saginata* and *B. hominis* (12, 13, 14) we detected oxidative stress and lipid peroxidation at sheep naturally infected with *D.dendriticum* by measuring of MDA levels at sera.

MDA (lipid peroxidation) is a well-established mechanism of cellular injury and is used as an indicator of oxidative stres in cells and tissues (19). Oxidative stress and enhanced lipid peroxidation have been associated with several models of liver injury (20) and tissue injury in dicrocoeliosis might in part be mediated by the generation of reactive oxygen species (21).

In lamb liver, experimental fasciolosis induces a simultaneous increase in cytosolic calcium and a decrease in cytosolic glutathione by 8 weeks post-infection (p.i), when the liver-cell degenerative process is maximal (22). Similar alterations are detected in rats at 3 and 6 weeks p.i. such an oxidative cell injury has been suggested to occur in the course of fasciolosis as the consequence of tissue destruction produced by toxic secretions of the flukes (23).

Chronic inflammation may be related to oxidative alterations. In particular, the antioxidant defence capability of the liver, in hamsters experimentally infected with *Dicrocoelium* metacerceria, was increased 80-120 days p.i. In the meantime, a decrease in superoxide dismutase activity in both the cytosol and mitocondria was registered, indicating inefficient scavenging of reactive oxygen, leading to oxidative liver damage and an increase in alanin transaminase and aspartate transaminase activities (21). Sanchez-Compos et al. (21) suggested that experimental dicrocoeliosis also courses with oxidative stress and lipid peroxidation as indicated by the significant increase in liver thiobarbituric acid-reactive subtances concent-ration. Lipid peroxidation resulting from oxidative stress produces MDA which forms DNA adducts and may cause cytotoxic effects (24).

Kargın and Fidancı (15), determined that there was a positive correlation between the intensity of the oxidative stress and the damage and kidney disease. In this study also we detected a relation between lipid peroxidation and liver damage due to the dicrocoeliosis in sheep.

Levels of MDA were significantly increased in sheep infected with *D.dendriticum*. The results of our study strongly suggested that one of the main reasons for high MDA levels in sheep infected with *D.dendriticum* could be decreased activity of defense system protecting tissue from free radicals damage.

The presence of lipid peroxidation could contribute to hepatic injury and to the reduced capacity for handling of drugs and xenobiotics previously reported for this parasitosis (25).

As it is known that lipid peroxidation is a free radical-related process and may occur biological systems under enzymatic control, e.g., for the generation of lipid-derived inflammatory mediators, or non-enzymatically. This later form was associated mostly with liver damage as a result of oxidative stress, which also involved cellular antioxidants in this process. The high MDA concentration and the significant correlation strongly indicate the occurence of antioxidative stress and lipid peroxidation as a mechanism of liver damage in cases of *D.dendriticum* infection.

REFERENCES

1. Theodoridis Y, Duncan JL, MacLean JM, Himonas CA. Pathophysiological studies on *Dicrocoelium dendriticum* in sheep. Vet Parasitol 1991; 36: 61-66.
2. Soulsby EJL. Helminths, Arthropods and Protozoa of Domesticated Animals. 6th Edition, Bailliere and Tindall: London, 1968.
3. Jithendran KP, Baht TK. Prevalence of dicrocoeliosis in sheep and goats in Himachal Pradesh, India. Vet Parasitol 1996; 61: 265-271.
4. Terry DW. *Dicrocoelium dendriticum*: the life cycle in Britain. J Helminthol 1969; 43: 403-416.
5. Steele JH. Parasitic zoonoses. In:, Hillyer GV, Hopla CE. (Editors). Handbook Series in Zoonoses, Volume 3. CRC, Boca Raton, Florida,1982: 33-52.
6. Sanchez-Andrade R, Paz-Silva A, Suarez JL, Arias M, Lopez C, Morrondo P, Scala A. Serum antibodies to *Dicrocoelium dendriticum* in sheep from Sardinia (Italy). Prev Vet Med 2003; 57: 1-5.
7. Ferre I, Ortega-Mora LM, Rojo-Vazguez FA. Prevalance of *Dicrocoelium dendriticum* infection in Leon province (NW Spain). Prev Vet Med 1994; 21: 147-154.
8. Ambrosi M. La diagnostica coprologica nelle elmintiasi di allevamento caso delle distomatosi dei ruminanti. Praxis Vet 1991; 12: 17-21.
9. Rojo-Vazquez FA, Cordero del Campillo M, Diez N, Chanton-Schaffer M. Relationship existing between the number of eggs in the feces and parasitic charge during ovine natural *Dicrocoelium dendriticum* infestation. Revue Med Vet 1981; 132: 601-607.
10. Romero FJ, Bosch-Morell F, Romero MJ, Jareno EJ, Romero B, Marin N, Roma J. Lipid peroxidation products and antioxidants in human disease. Environ Health Perspect 1998; 106: 1229-1234.
11. Galtier P, Vandenberghe Y, Coecke S, Eeckhoutte C, Larrieu G, Vercruysse A, Differential inhibition of rat hepatic glutation S-transferase isoenzymes in the course of fascioliasis. Mol Biochem Parasitol 1991; 44: 255-260.
12. Kılıç E, Yazar S, Saraymen R, Yalçın Ş. Changes in the level of serum malondialdehyde in patients infected with Giardia intestinalis. Acta Parasitologica Turcica 2003; 27: 184-186.
13. Kılıç E, Yazar S, Saraymen R, Özbilge H. Serum lipid peroxidation level in patients with Taeniasis saginata. Acta Parasitologica Turcica 2004; 28: 91-93.
14. Kılıç E, Yazar S, Saraymen R. Lipid peroxidation level in patients with Blastocystosis. J. Inonu Univ. Med. Fac. 2003; 10: 1-3.
15. Kargın F, Fidancı UR. Kidney diseases and antioxidative metabolism in dogs. Tr J Vet Anim Sci 2001; 25: 607-613.
16. Placer ZA, Cushmann L, Johnson BC. Estimation of products of lipid peroxidation in biochemical systems. Anal Biochem 1966; 16: 359-364.
17. Wolff K, Hauser B, Wild P. Dicrocoeliosis in sheep: pathogenesis and liver regeneration after therapy. Berl Munch Tierarzt Woch 1984; 97: 378-387.
18. Manga-Gonzalez MY, Gonzalez-Lanza C, Del-Pozo P. *Dicrocoelium dendriticum* (Trematoda, Digenea) eggs in the faeces of lambs and ewes in Porma Basin (Leon, NW Spain). Ann Parasitol Hum Comp 1991; 66: 57-61.
19. Kılıç E, Yazar S, Saraymen R, Özbilge H. Serum malondialdehyde level in patients infected with *Ascaris lumbricoides*. World J Gastroenterol 2003; 9: 2332-2334.
20. Panazzo PM, Basso D, Balint L, Biasin MR, Bonvicini P, Metus P. Altered lipid peroxidation/glutathione ratio in experimental extrahepatic cholestasis. Clin Exp Pharmacol Physiol 1995; 22: 266-271
21. Sanchez-Compos S, Tunon MJ, Gonzalez P, Gonzalez-Gallego J. Oxidative stress and changes in liver antioxidant enzymes induced by experimental dicroceliosis in hamsters. Parasitol Res 1999; 85: 468-474.
22. Galtier P, Larrieu G, Tufenkji AE, Franc M. Incidence of experimental fascioliasis on the activity of drug-metabolizing enzymes in lamb liver. Drug Metab Dispos 1986; 14: 863-872.
23. Galtier P, Eeckhoutte C, Larrieu G. *Fasciola hepatica*: liver enzymes in rats and interaction with chemical inducers. Exp Parasitol 1987; 63: 189-194.
24. Chaudhary AK, Nokubo M, Reddy GR, Yeola SN, Morrow JD, Blair IA, Marnett LJ. Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science 1994; 265: 1580-1582.
25. Draper H, Hadley M. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotica 1990; 20: 901-907.

Yazışma Adresi:, Fırat Üniversitesi, Veteriner Fakültesi Parazitoloji Anabilim Dalı, 23119 Elazığ–TÜRKİYE
Tel: 0 424 237 00 00- 3967 e-posta: ssimsek@firat.edu.tr