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

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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 seçilmiştir, negatif kontrol olarak da D. dendriticum’un görülememiş sağlıklı 17 kuzu serumu kullanılmıştır. Neticede, enfekte ve kontrol grubu koyunlarda serum malondialdehid seviyeleri arasındaki fark istatistiksel olarak önemli bulundu (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 throughout 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. Karg and Fidancı (15) reported that free radical activities, especially MDA, are responsible for the etiology 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 slaughtering and then the organs of sheep for *D.dendriticum* and other helminths and infections carefully, especially liver bile ducts, and gall bladder were examined. After these inspections the positive serum samples were selected among from the sheep infected with *D. dendriticum*. These sheep considered as are 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 °C 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 occurred 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 ± Standard deviation (SD). Statistical analysis was performed by using SPSS software package (Version 11.0 for Windows). For comparison of two groups of continuous variables, independent 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.

| Table 1. 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, edema, emaciation, and in advanced cases, cirrhosis, scarring of the liver surface, and marked distension of bile ducts. Due to its buccal stiles, 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 stresses 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).

Chronic inflammation may be related to oxidative alterations. In particular, the antioxidant defence capability of the liver, in hamsters experimentally infected with Dicrocoelium metacercer, was increased 80-120 days p.i. In the meantime, a decrease in superoxide dismutase activity in both the cytosol and mitochondria was registered, indicating inefficient scavenging of reactive oxygen, leading to oxidative liver damage and an increase in alanine 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 substances concernt-ration. Lipid peroxidation resulting from oxidative stress produces MDA which forms DNA adducts and may cause cytokotoxic 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 occurrence of antioxidative stress and lipid peroxidation as a mechanism of liver damage in cases of D. dendriticum infection.
REFERENCES


