

Serum malondialdehyde level in patients with cystic echinococcosis

Ismail S. Koltas, PhD, Guzide Yucebilgic, PhD, Ramazan Bilgin, PhD, Cem K. Parsak, MD, Gurhan Sakman, MD.

ABSTRACT

Objective: To investigate the changes of serum malondialdehyde (MDA) level for the oxidative stress hypothesis in patients with cystic echinococcosis (CE).

Methods: The study was conducted on patients with CE before the surgical treatment. Anti-*Echinococcus granulosus* antibodies were determined by serological method. We assayed MDA activities measured of 57 subjects and matched in 45 healthy controls. This study took place in the Faculty of Medicine, University of Cukurova, Balcali Hospital in Adana, Turkey, between March 2004 and October 2005.

Results: The mean \pm SD of MDA levels of patients

with CE was 6.70 ± 1.66 and healthy controls was 2.53 ± 0.43 . The difference between MDA levels of patients and controls was statistically significant ($p < 0.001$).

Conclusion: The high infection/control ratio of MDA concentration and the significant correlation strongly indicate the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage in cases of CE. Our study highlights the usefulness of serum markers to investigate complex pathological situations, including distinct forms of chronic liver inflammation associated with CE.

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Echinococcosis is a cosmopolitan zoonosis caused by adult or larval stages of cestodes belonging to the genus *Echinococcus* (family *Taeniidae*). Larval infection (cystic echinococcosis, hydatid disease, hydatidosis) is characterized by long-term growth of metacestode (hydatid) cysts in the intermediate host. The 2 major species of medical and public health importance are *Echinococcus granulosus* (*E. granulosus*) and *Echinococcus multilocularis* (*E. multilocularis*), which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE).¹ Cystic echinococcosis is known to be the most important parasitic zoonosis in the Mediterranean region creating serious problems to the public health and national economy of most countries in this region.

High prevalence of CE is encountered in countries with large numbers of nomadic or semi-nomadic sheep and goat flocks. The species of the parasite prevailing in all countries of the Mediterranean region is *E. granulosus*, while *E. multilocularis* has been identified in Tunisia, the southern part of France, in northern Italy and Turkey. The ability of *E. granulosus* to adapt to a wide variety of host species and the repeated introduction of food and domestic animals from endemic areas to other parts of the Mediterranean region contributes to the broad distribution of the parasite. The chain of transmission of the infection to humans involves dogs, sheep, camels, goats, cattle and other herbivorous animals.² Cystic echinococcosis occurs in age groups from <1

From the Department of Parasitology (Koltas), Department of General Surgery (Parsak, Sakman), Medical Faculty and the Department of Chemistry (Yucebilgic, Bilgin), Arts and Science Faculty, University of Cukurova, Adana, Turkey.

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Address correspondence and reprint request to: Dr. Ismail S. Koltas, Department of Parasitology, Faculty of Medicine, University of Cukurova 01330, Balcali, Adana, Turkey. Tel. +90 (322) 3386060 Ext. 3486. Fax. +90 (322) 3386572. E-mail: koltas@cu.edu.tr

to >75 years. In some areas of endemic infection, most hospital cases are recorded in the age groups between 21 and 40 years, but the highest morbidity may also occur in younger individuals aged between 6 and 20 years.^{3,4} Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA). Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems.⁵⁻⁷ In previous studies, showed that serum MDA levels clearly increase in patients infected with *Ascaris lumbricoides* and *Plasmodium vivax*.^{8,9} The aim of the study was to investigate the levels of MDA determined in serum samples in patients with CE and controls.

Methods. This study was conducted on patients with CE before the surgical treatment from March 2004 to October 2005 at the Faculty of Medicine, University of Cukurova, Balcali Hospital in Adana, Turkey. Anti-*E.granulosus* antibodies were determined by Echinococcosis Western blotting, Euroimmun, Germany, according to the protocols established by the manufacturer. We assayed MDA activities of 57 subjects (33 men and 24 women) aged between 27-66 years. All patients were liver CE at least 2 years. None of them were smokers, had any known pathologies and taking steroids or medications and had any infectious disease such as hepatitis, HIV or parasitic diseases other than CE. Serum samples for controls were obtained from healthy subject. These individuals were free from infections. Their investigations were carried out by regular laboratory check-up analysis. All subjects fasted after midnight before blood collection the next morning. Fifty-seven patients and 45 controls were examined in this study. The mean age of the patients, which consisted of 33 men and 24 women were 43.3±12.4 years and 41.9±11.8 years. The mean age of the controls, which included 24 men and 21 women were 42.1±13.1 years and 40.8±11.7 years. All venous blood samples taken between 8 and 9 a.m. after 8 hours of fasting were collected in polystyrene tubes and vacutainers containing heparin. The tubes were centrifuged at 1500 rpm for 15 min. Sera were then removed and stored at -20°C until analysis. Lipid peroxidation was assessed by measuring MDA an end product of fatty acid peroxidation. The level of MDA was determined by measuring the color intensity of the complex formed between MDA and thiobarbituric acid at 532 nm according to the method of Ohkawa et al.¹⁰

Table 1 - Malondialdehyde levels of patients infected with cystic echinococcosis and controls.

Parameters	Age (years) Mean±SD	MDA levels (nmol/ml) Mean±SD
<i>Patients</i>		
Female (n=24)	41.9 ± 11.8	7.52 ± 2.13
Male (n=33)	43.3 ± 12.4	6.86 ± 1.62
Total (n=57)	42.5 ± 11.9	6.70 ± 1.66
<i>Controls</i>		
Female (n=21)	40.8 ± 11.7	2.57 ± 0.53
Male (n=24)	42.1 ± 13.1	2.41 ± 0.45
Total (n=45)	41.2 ± 11.7	2.53 ± 0.43

Statistical analysis was performed using SPSS software package (Version 11.0 for Windows). The data were expressed as mean ± standard deviation (SD). For comparison of 2 groups of continuous variables, independent sample t-test was used. A probability value of less than 0.05 indicated a statistically significant difference.

The study protocol was reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Cukurova, Adana, Turkey and informed consent was obtained for each participant.

Results. Table 1 shows the MDA levels of patients infected with cystic echinococcosis and controls. The difference between MDA levels of patients and controls was statistically significant both for women ($p<0.001$) and men ($p<0.001$), (Table 1). In patients and controls, no correlation was found between age and MDA levels ($p>0.05$) both in women and in men. In addition, no significant correlation could be found between MDA levels of both women and men for patients and controls ($p>0.05$).

Discussion. In this study, it was aimed to evaluate and characterize the relationship between MDA and CE, which can cause pathology and oxidative stress mechanism as a mediator of tissue damage concurrent with CE. Clinical signs at CE may occur after a highly variable incubation period of several months or years. Hepatic cysts can cause pain in the upper abdominal region, hepatomegaly, cholestasis, biliary cirrhosis, portal hypertension, ascites, and a variety of other manifestations. Cysts may rupture into the peritoneal cavity, causing anaphylaxis or secondary CE, or into the biliary tree, leading to cholangitis and cholestasis. Abscess formation is possible after bacterial infection of cysts. Chronic

cough, expectoration, dyspnea, hemoptysis, pleuritis, and lung abscess are selected symptoms caused by pulmonary cysts, and neurological disorders can be induced by cysts in the brain.^{3,4,11} This was the first study to characterize the relationship between CE and MDA (lipid peroxidation), which is a well-established mechanism of cellular injury in human, and is used as an indicator of oxidative stress in cells and tissues. In our study, we found increased levels of MDA in patients with CE when we compared their values with controls. During our literature survey, we could not find any study related to MDA levels of CE. On the other hand, Kilic et al¹² reported that no change in levels of MDA in patients infected with *Giardia intestinalis* as compared to controls. In another study, Kilic et al⁸ showed that serum MDA level were significantly increased in patients infected with *Ascaris lumbricoides*. The results of our study strongly suggested that one of the main reasons for high MDA levels in patients with CE could be decreased activity of defense system protecting tissues from free radical damage. However, in patients and controls, no correlation was found between age and MDA levels both in women and in men. In addition, no significant correlation could be found between MDA levels of both women and men for patients with CE and controls. These results for patients with CE could possibly be explained as that with high MDA activity in all ages. As it is known that lipid peroxidation is a free radical-related process that in biologic systems may occur under enzymatic control, for the generation of lipid-derived inflammatory mediators, or nonenzymatically. This latter form was associated mostly with cellular damage as a result of oxidative stress, which also involved cellular antioxidants in this process.⁷ The high infection/control ratio of MDA concentration and the significant correlation strongly indicate the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage in cases of CE.

In conclusion, our study highlights the usefulness of serum markers to investigate complex pathological

situations, including distinct forms of chronic liver inflammation associated with CE.

References

1. Zhang W, Li J, McManus D. Concepts in Immunology and Diagnosis of Hydatid Disease. *Clin Microbiol Rev* 2003; 16: 18–36.
2. Seimenis A. Overview of the epidemiological situation on echinococcosis in the Mediterranean region. *Acta Tropica* 2003; 85: 191-195.
3. Ammann RW, Eckert J. Cestodes: Echinococcus. *Gastroenterol Clin North Am* 1996; 25: 655-689.
4. Pawlowski ZS, Eckert J, Vuitton DA, Amman RW, Kern P, Craig PS, et al. Echinococcosis in humans: clinical aspects, diagnosis and treatment, In: Eckert J, Gemmell MA, Melsin FX, Pawlowski ZS, editors. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris, France: World Organisation for Animal Health; 2001. p. 20-66.
5. Draper HH, Hadley M. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. *Xenobiotica* 1990; 20: 901-907.
6. Nayak DU, Karmen C, Frishman WH, Vakili BA. Antioxidant vitamins and enzymatic and synthetic oxygen derived free radical scavengers in the prevention and treatment of cardiovascular disease. *Heart Dis* 2001; 3: 28-45.
7. 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.
8. Kilic E, Yazar S, Saraymen R, Ozbilge H. Serum malondialdehyde level in patients infected with *Ascaris lumbricoides*. *World J Gastroenterol* 2003; 9: 2332-2334.
9. Yazar S, Kilic E, Saraymen R, Ozbilge H. Serum malondialdehyde level in patients infected with *Plasmodium vivax*. *West Indian Med J* 2004; 53: 147-149.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1973; 95: 351-358.
11. Eckert J, Deplazes P. Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern. *Clin Microbiol Rev* 2004; 17: 107-135.
12. Kilic E, Yazar S, Saraymen R, Yalcin S. Changes in the level of serum malondialdehyde in patients infected with *Giardia lamblia*. *Acta Parasitol. Acta Parasitol Turcica* 2003; 27: 184-186.