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Arginase activity and total oxidant/antioxidant capacity in cows with lung cystic echinococcosis

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Summary

The aim of this study was to investigate arginase activity, total oxidant capacity (TOC), total antioxidant capacity (TAC), and oxidative stress index (OSI) in cows with lung cystic echinococcosis (CE). The subjects were 20 cows with lung CE (parasitized group) and 20 healthy cows (control group). A significant increase in arginase activity, TOC, and OSI, and a significant decrease in TAC were found in the parasitized group compared with the control group ($P < 0.001$). Histopathological examination revealed a fibrous tissue reaction with inflammatory cell infiltration surrounding the CE in the lungs. The study revealed that increased arginase activity, TOC, and OSI levels and decreased TAC levels occurred in lung CE in cows. Increased arginase activity may be an important parameter for pulmonary fibrosis associated with lung CE in cows. Oxidant production due to chronic inflammation, as evidenced by the increased OSI levels, might contribute to persistent lung injury.

Keywords: arginase, cystic echinococcosis, cow, oxidative stress

Echinococcosis is one of the most important parasitic zoonotic diseases in the world. This parasite depends on the dog–sheep cycle and is actively transmitted in all pastoral regions where sheep, cattle, and camelids predominate (3). In Turkey, the prevalence rates in cattle have reached 13.5% in Burdur (29), 14.17% in Kırıkkale (32), 29.47% in the Afyonkarahisar district (18), and 33.9% in eastern Turkey (27). Animal production losses due to cystic echinococcosis (CE) include loss of offal, carcass weight, productivity, and decreased hide value (3).

CE (mainly in the liver and lungs) is caused by the metacestode stage of various strains of *E. granulosus*. The metacestode consists of an internal cellular layer (germinal layer) and an outer acellular, laminated layer. This cyst gradually expands and induces a granulomatous host reaction, followed by a fibrous tissue reaction and the formation of a connective tissue layer (6). Pulmonary fibrosis is a common response to a variety of lung injuries, with the terminal stages characterized by fibrosis and the accumulation of collagen (19).

The induction of arginase, an enzyme that metabolizes L-arginine to urea and L-ornithine, is essential for collagen synthesis (21). L-ornithine can be metabolized by arginase to proline, the precursor for collagen production, and as such might contribute to pulmonary remodeling and fibrosis (11). Arginase is a key enzyme of the urea cycle in the liver, but it is also expressed in cells and tissues that lack a complete urea cycle, including the lungs. In the airways, both arginase I and II are constitutively expressed in bronchial epithelial cells, endothelial cells, (myo)fibroblasts and alveolar macrophages (16, 23, 30).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals, which are generated physiologically during oxidative phosphorylation. They have various physiological roles and are removed rapidly from the body. Their persistence can cause cell dysfunction and cell death. Defense mechanisms against oxidants involve enzyme and non-enzyme antioxidant systems (26). An imbalance between the generation of ROS/RNS and antioxidant defenses leads to a negative condition known as oxidative/nitrosative stress. Oxidative stress may affect lipids, DNA, carbohydrates, and proteins. ROS and RNS are produced mainly by phagocytes, as well as by polymorphonuclear, alveolar, bronchial, and endothelial cells. During inflammatory processes, activated macrophages and neutrophils can release a great amount of hydrogen peroxide and superoxide via the phagocytic isoform

of nicotinamide adenine dinucleotide phosphate oxidase (1). In addition, L-ornithine-derived polyamines can contribute to lung damage, as back-conversion of the higher order polyamines results in the formation of toxic compounds such as hydrogen peroxide (15), while reduced L-arginine availability to iNOS induced by arginase can result in the synthesis of both NO and the superoxide anion by this enzyme, thereby enhancing the production of peroxynitrite (23).

Recent studies have indicated that arginase and oxidative stress might play an important role in the pathogenesis of various pulmonary disorders (1, 23). To the best of our knowledge, arginase activity and oxidant/antioxidant status has not been studied in the lung CE in cows. Thus, this study aimed to investigate arginase activity, total oxidant capacity (TOC), total antioxidant capacity (TAC), and oxidative stress index (OSI) in cows with lung CE.

Material and methods

Twenty cows (6-8 years of age) with lung CE (parasitized group) and 20 healthy cows (control group) were selected from animals slaughtered at an abattoir in Erzurum province, Turkey. The parasitized animals were selected from animals with only lung CE. The healthy control animals had no pathology in the lung carcass detected by macroscopic and microscopic examination.

The CE and healthy lungs tissues were fixed in 10% buffered formalin solution, subjected to routine processing, embedded in paraffin, cut into 5 μm-thick sections, and stained with hematoxylin-eosine (HE). The sections were examined under light microscopy.

Specimens of the cow lung tissue were obtained, weighed, placed in empty glass tubes, and stored at –80°C until processing. Prior to biochemical assays, the tissue samples for arginase analysis were dehydrated between two filter papers, diluted with 2 mM $MnCl_2$ (1/6 w/v), and homogenized in Potter Elvehjem (glass–glass) homogenizer. The homogenate was centrifuged at 10 000 g for 12 min at 4°C, and the supernatant was used as an enzyme source. For TOC and TAC analysis, 10 mL of 140 mM KCl solution per 1 gram of tissue was added to tubes containing tissue samples, and homogenized in a motor-driven homogenizer. The homogenate was centrifuged at 2800 g for 10 min at 4°C, while the supernatant was used for the TOC and TAC analyses.

Arginase activity was detected by the measurement of urea produced by hydrolysis of L-arginine through arginase enzyme by the thiosemicarbazide diacetylmonoxime urea (TDMU) method (10). Protein was measured by the method of Lowry et al. (22). One unit of arginase activity is an expression in mg protein of enzyme activity producing 1 μmole of urea from L-arginine for 1 hour at 37°C.

The TOC and TAC levels in the CE and healthy lungs were determined by a commercial test kit (Rel Assay Diagnostics). TOC level was determined using a novel automated measurement method, developed by Erel (8). Oxidants in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. Glycerol molecules increase the oxidation reaction. A colored complex with xylenol orange in an acidic medium is produced by the ferric ion. The color intensity, which can be determined spectrophotometrically, is associated with the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide; the results are expressed as mmol H_2O_2 equivalent/g protein for lung tissue samples.

TAC levels were determined using a novel automated colorimetric measurement method developed by Erel (9). In this method, the hydroxyl radical is produced by Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon addition of the sample, the oxidative reactions started by the hydroxyl radicals in the reaction mixture are suppressed by the antioxidant components of the sample, preventing the color change and thereby providing an effective measure of the total antioxidant capacity of the sample. The assay has excellent precision values, lower than 3%. The results are expressed as mmol Trolox equivalent/g protein for lung tissue samples.

Oxidative stress index (OSI) was determined as the percent ratio of TOC to TAC (31).

The SPSS 16.0 program for Windows (SPSS Inc., Chicago, IL) was used. In the control of differences among groups independent t test was used. Results are given as mean ± SE (standard error). Differences were considered significant when P values were less than 0.05.

Results and discussion

The present study demonstrated that arginase activity was significantly higher in lung CE in cows than in the control group, possibly in conjunction with pulmonary fibrosis, as indicated by histopathological examination. Recent studies have indicated that arginase, which converts L-arginine into L-ornithine and urea, might play an important role in the pathogenesis of various pulmonary disorders (23). Increased arginase activity has been reported in bleomycin-induced fibrosis in mouse lungs (7), in lung allograft fibrosis in rats (21), and in herpes virus-induced lung fibrosis in mice (25), by leading to excessive collagen deposition. The induction of arginase, an enzyme that metabolises L-arginine to L-ornithine and urea, is essential for collagen synthesis (21). L-ornithine can be metabolized by arginase to proline, the precursor for collagen production, and as such may contribute to pulmonary remodeling and fibrosis (11). Thus, the results of this study indicate that increased arginase activity might be an important parameter for pulmonary fibrosis associated with lung CE in cows.

Pulmonary fibrosis is a common response to a variety of lung injuries, with the terminal stages characterized by fibrosis and the accumulation of collagen (19). Fibrous tissue reaction with inflammatory cell infiltration surrounding CE in the lungs was observed by histopathological examination in this study.

Oxidative stress is an important molecular mechanism underlying fibrosis in a variety of organs, including the lungs (2). Activated macrophages and

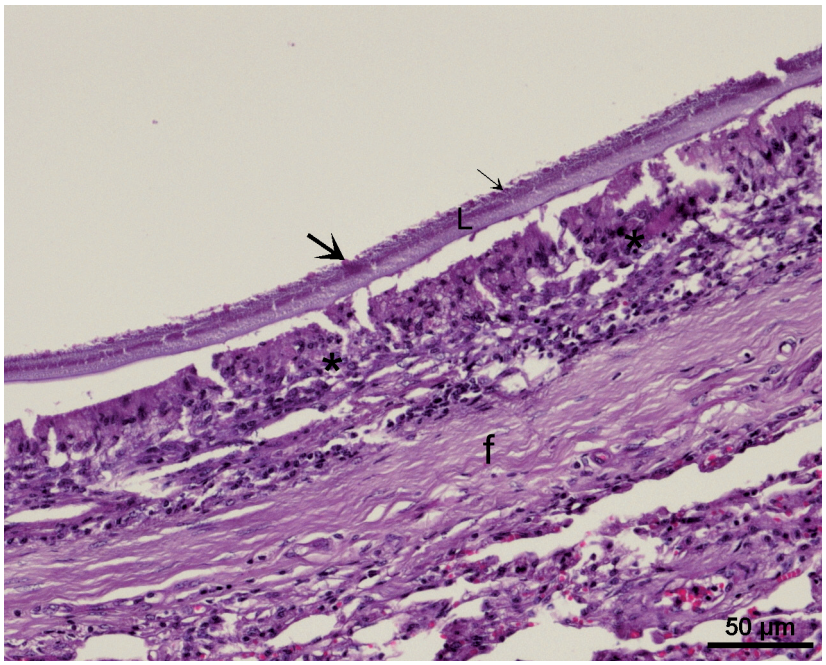

Fig. 1. Fibrous tissue around the CE (f); inflammatory cell infiltration consisting predominantly of histiocytes and lymphocytes (*); laminar layer of CE (L); germinal layer (thin arrow); and calcareous corpuscles belonging to CE (arrow).

Tab. 1. Arginase activity, TOC, TAC and OSI levels in the lungs of parasitized group and the control group

Parameters	Control Group	Parasitised Group	P *value*
Arginase (Unite/mg Protein)	1.64 ± 0.03	2.38 ± 0.05	0.001
TOC (µmol H_2O_2 Eq/g tissue)	9.09 ± 0.12	14.31 ± 0.23	0.001
TAC (mmol Trolox Eq/g tissue)	1.38 ± 0.03	0.84 ± 0.03	0.001
OSI (AU)	0.66 ± 0.02	1.73 ± 0.06	0.001

Explanation: Results are given as mean ± SE

neutrophils can release a great amount of hydrogen peroxide and superoxide via the phagocytic isoform of nicotinamide adenine dinucleotide phosphate oxidase during inflammatory processes (1), while L-ornithine–derived polyamines could contribute to lung damage, as back-conversion of the higher order polyamines results in the formation of toxic compounds such as hydrogen peroxide (15). Histopathological examination showed that the lung CE in this study consisted mainly of histiocyte and lymphocyte inflammatory cell infiltration. As a host reaction to the parasitic infection, this chronic inflammatory process could generate a great number of free radicals, with enhanced TOC levels, consistent with the report that free radicals, especially nitric oxide, play a key role in host defense against echinococcosis (28).

A significant increase in TOC level and decrease in TAC level may explain the occurrence of oxidative stress and the consumption of total antioxidants against oxidants in lung CE in cows. Thus, oxidant production due to chronic inflammation as evidenced by the increased OSI levels might contribute to persistent lung injury. Similarly, recent studies have shown that liver cystic echinococcosis is associated with oxidative stress and has a role in the injury of hepatocytes in camels, sheep and cattle (12-14). In addition, it is indicated in human chronic liver echinococcosis that oxidative stress occurrence and lipid peroxidation with MDA levels occur as a mechanism of tissue damage (17) and reduced glutathione peroxidase activity decreases (20). Parasitic infections affecting the lung, such as *Dictyocaulus viviparus*, *Nippostrongylus brasiliensis*, and *Trichinella spiralis* are also among the major causes of oxidative stress (4, 5, 24).

The lungs are protected from the negative effects of oxidant persistence in tissues by endogenous agents called antioxidants; non-enzymes such as glutathione, vitamins, beta carotene and uric acid; and enzyme proteins such as superoxide dismutases, catalases, and peroxidases (26). In the present study, TAC levels were significantly lower in the parasitized group compared to the control group. In this case, the lack of TAC levels against increased oxidants in the parasitized group compared to the control group might not have been able to prevent the lung injury. Immediately after the lung injury, transforming growth factor-β (TGF-β) exerts its proinflammatory and chemotactic activities by recruiting myofibroblasts (1). Therefore, increased fibrocyte activity around the CE in the present study might result from persistently elevated TGF-β levels, and might contribute to ROS production, given that TGF-β can induce over-production of ROS via H_2O_2 release from lung fibroblasts, mitochondrial ROS production and NAD(P)H oxidase activation, and decrease antioxidant defenses in the lungs; at the same time ROS can upregulate TGF-β (2).

In conclusion, this study revealed that increased arginase activity, TOC, and OSI levels and decreased TAC levels occurred in cows with lung CE. The increased arginase activity might be an important parameter in pulmonary fibrosis associated with lung CE in cows. Oxidant production due to chronic inflammation, as evidenced by the increased OSI levels, might contribute to persistent lung injury.

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