

Short Communication

Plasma paraoxonase, sphingosine-1-phosphate, total sialic acid, and heat shock protein-27 in the liver of the sheep naturally infected with *Cysticercus tenuicollis*: Evidence on pathological changes

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ABSTRACT

The present study aimed to investigate whether sphingosine 1 phosphate (S1P), paraoxonase (PON), total sialic acid (TSA), and heat shock protein-27 (HSP27) are altered in the sheep during infection of the liver with *Cysticercus tenuicollis*. This study was conducted on 40 healthy sheep and 40 sheep with *Cysticercus tenuicollis* infection. The infected and non-infected animals were selected based on the observation of severe *Cysticercus tenuicollis* infection in the liver and absence of any hepatic cysts, respectively. All parameters were measured in serum and plasma. The results revealed a significant decrease ($P < 0.01$) in PON, TSA, and albumin (Alb) in the infected group, compared with those in the healthy one. Furthermore, the infected sheep had a significant increase ($P < 0.01$) in S1P, HSP-27, malondialdehyde (MDA), total bilirubin, and unconjugated bilirubin as compared with those in their non-infected counterparts. Moreover, no significant change was observed in total plasma protein level in the infected animals in comparison to that in the healthy ones. The low levels of TSA and Alb revealed liver damage in the infected sheep. Moreover, the PON reduction might have resulted from hepatic steatosis and MDA enhancement. Meanwhile, S1P elevation could be attributed to the activation of platelets. In addition, HSP-27 increase was ascribed to the disease-induced stress conditions.

Keywords: Paraoxonase, Sphingosine-1-phosphate, Total sialic Acid, Heat Shock Protein-27, *Cysticercus taenioculis*

Paraoxonase plasmatique, Sphingosine-1-phosphate, Acide sialique total et Protéine 27 de choc thermique chez les ovins atteints de *Cysticercus taenioculis* hépatique naturellement infecté : preuves de changements pathologiques

Résumé: Cette étude a pour but d'évaluer les modifications rencontrées dans le foie du mouton lors d'une infection naturelle de *Cysticercus taenioculis*, notamment au niveau de la sphingosine 1 phosphate (S1P), la paraoxonase (PON), l'acide sialique total (TSA) et la protéine de choc thermique 27 (HSP-27). Dans ce but, 40 moutons parasités et 40 moutons sains ont été respectivement sélectionnés sur la base de la présence de formes kystiques sévères dans le foie et de l'absence de parasite sanguin sans conformation kystique dans la carcasse. Tous les paramètres ont été mesurés et comparés dans le sérum et le plasma. Les résultats ont révélé une diminution significative ($p < 0,01$) du PON, du TSA, et de l'albumine (Alb) ainsi qu'une augmentation significative ($p < 0,01$) du S1P, du HSP-27, du malondialdéhyde (MDA, aldéhyde malonique), de la bilirubine

totale et de la bilirubine non-conjuguée dans le groupe infecté comparé aux groupes sains. Aucun changement considérable n'a été observé au niveau de la protéine plasmatique totale (TPP) dans le groupe de la cysticercose par rapport aux sujets sains. Les faibles niveaux de TSA et Alb chez les moutons atteints révèlent des dommages au foie. De plus, la réduction du PON pourrait être due à un changement du taux de graisse dans le foie et à une augmentation du MDA. Parallèlement, l'augmentation des taux de S1P et de HSP-27 chez les moutons contaminés peut être respectivement attribuée à l'activation des plaquettes et aux conditions de stress induites par la maladie.

Mots-clés: Paraoxonase, Sphingosine-1-phosphate, Acide sialique total, Protéine de choc thermique-27, *Cysticercus taeniaculis*

INTRODUCTION

Cysticercus tenuicollis is known as larval stage (metacystode) of the canine tapeworm, called *Taenia hydatigena* (Soulsby, 1986). The adult stage of *Cysticercus tenuicollis* (*Taenia hydatigena*) is observed in the small intestine of dogs, cats, mice, and wild carnivores, including wolves and foxes, throughout the world. The sheep and goats are most commonly considered as intermediate hosts that are infected by ingesting proglottids or eggs passed in the feces of the dogs in the pastures or feeding areas. In addition, cysticerci of *T. hydatigena* causes high degree of morbidity and mortality in the livestock. The adult parasites does not have a high pathogenicity in the definitive hosts. However, the grown cysticerci can migrate simultaneously in the liver of the intermediate hosts, and there by results in hemorrhagic and fibrotic tracts known as ‘‘hepatitis cysticercosa’’, which is similar to acute fasciolosis in gross pathology, that is fatal in most of the cases (Soulsby, 1982). This infection can be diagnosed based on the observation of cysts and implementation of serological methods (e.g., enzyme-linked immunosorbent assay). The acetylated derivatives of neuraminic acid are referred to as sialic acids, which are widely distributed in the mammal tissues and body fluids. The sialic acids are classified in to three groups, namely protein-bounded sialic acid (PBSA), lipid-bounded sialic acid (LBSA), and free form. These acids are located at the end chain of many acute phase proteins. Therefore, the determination of

sialic acid may be a valuable indicator for the diagnosis and prognosis of inflammatory diseases. The sialic acid values have been analyzed in the cattle in many infectious diseases, such as kerato conjunctivitis, leptospirosis, pneumonia, theileriosis, anaplasmosis, and traumatic reticulo-peritonitis. The linkage between sialic acid and infectious diseases makes the sialic acid susceptible to get involved in the cellular and molecular interrelationships and participate in lipoproteins and lipid metabolism. The glycosylation and sialylation of the lipids and proteins are carried out in the liver (Chrostek et al., 2011). There is evidence indicating the substantial role of the changes in the sialylation of proteins and lipids in the pathogenesis and development of various liver diseases. Sphingosine-1-phosphate (S1P) is known as a novel bioactive lipid mediator, belonging to sphingolipids group. This mediator abundantly exists in plasma and other body fluids and participates in both physiological and pathophysiological pathways. The S1P acts in an autocrine or paracrine manner and affects the function of the fundamental cells (Nofer, 2008). Erythrocytes and platelets are considered as fundamental sources of S1P, which store S1P and release it into the blood. In addition, S1P, as a major bioactive molecule, contributes to the exhaustion of lymphocyte from the secondary lymphoid tissues into the lymph. Therefore, S1P has fundamental effects in vivo and plays the pathophysiological role of a circulating paracrine mediator (Nofer, 2008). It should be noted that the S1P has been shown to have the ability to modulate

fibroblasts migration; as a result, it plays a substantial role in the fibrosis of different tissues (Takuwa et al., 2013). Paraoxonase (PON; arylalkylphosphatase [EC.3.1.8.1]) is a glycoprotein with 355 amino acids, which is synthesized by the liver and considered as a calcium-dependent esterase. This enzyme was initially identified by its involvement in the hydrolysis of the lipid peroxides in the oxidized lipoproteins and its antioxidant property (Sarandol et al., 2005). The PON protects the tissues and cells from oxidative stress and reduces oxidative stress in them.

In this regard, PON reduction has been reported in various abnormalities involved with elevated oxidative stress, such as diabetes, hypercholesterolemia, chronic renal failure, and cardiovascular diseases. It is worth mentioning that PON1 activity has been demonstrated to reduce during the chronic hepatic disease (e.g., chronic hepatitis and cirrhosis), which is related to the degree of liver damage (Ferre et al., 2002). Reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot), are continuously generated during the metabolic processes in the cells. Excessive ROS provokes a deleterious chain reaction that can cause cell damage or death. Oxidative stress affects the polyunsaturated fatty acids of the cell membrane lipids and stimulates lipid peroxidation, which is used as the determinant of oxidative stress and cellular injury indicator. Malondialdehyde (MDA), one of the lipid peroxidation byproducts, is considered as the most abundant and reliable biomarker for the assessment of lipid peroxidation. Furthermore, different studies have ascertained the presence of oxidative stress in the humans and animal parasitic diseases (Saleh et al., 2009). Heat shock proteins (HSPs), as ubiquitous molecules, were initially identified in different *Drosophila* tissues during the transient and sublethal enhancement of the normal core body temperature. All cells of all life forms generate HSPs. The HSPs have increased synthesis in many other undesirable conditions, such as ischemia, hypoxia, stress factors

like heavy metals, endotoxins, and exposure to organic solvents and reactive oxygen species. These proteins are expressed in the cell and are involved in the quality control of protein folding, whereas other proteins of this group are robustly increased in response to unfavorable conditions (Mymrikov et al., 2011). The small HSPs comprise a large number of proteins with monomer molecular mass within the range of 12-43 kDa. The HSP27 (HSPB1) belongs to small HSP family and is associated with α -crystallin proteins (Ferns et al., 2006). In 2014, (Bamorovat et al.) reported the alterations of some hematological and biochemical parameters in *Cysticercus tenuicollis* infection in the sheep. Nonetheless, to the best of our knowledge, the present study was the first attempt to investigate the alterations of the hematological and biochemical parameters in cysticercosis among the sheep.

MATERIALS AND METHODS

This study was conducted in Urmia, Iran, during 2014-2015. Both infected and non-infected animals were separated based on the observation of severe *Cysticercus tenuicollis* infection in the liver and absence of any cysts in the liver, respectively. The inclusion criteria were no blood parasite and lack of pathology in the carcass. Thereupon, 10 mL blood was collected via the jugular vein from the infected sheep (8-13 months). Blood samples were also obtained from the healthy sheep admitted for slaughtering at the abattoir of Urmia, Iran. The microscopic examination of the blood smears staining in the oil-immersion objective (X100) revealed no parasite in the infected and healthy sheep. After blood sampling, 5 mL of each blood sample was anticoagulated with ethylene diaminetetra acetic acid (EDTA), and the remained sample was transferred to non-EDTA-containing tubes for serum preparation. All samples were centrifuged at 6,000 g for 10 min at ambient temperature and kept frozen at -25°C until the implementation of the analysis. The plasma HSP27 and S1P levels were determined using an RA-1000 auto analyzer based on enzyme-linked

immunosorbent assay by means of AMS biotechnology (Switzerland) and ELISA kits (East Biopharm Co, Hangzhou, China), respectively. The total sialic acid (TSA) was estimated in serum by Sydow's method (spectrophotometer, model Spekol 1500, Germany). In addition, the PON level was measured in plasma by Furlong's method. Total bilirubin, unconjugated bilirubin, total plasma protein, and albumin (Alb) levels were measured in serum colorimetrically by an auto analyzer (Hitachi-917, Japan). Finally, serum MDA was determined using the Satoh's method (spectrophotometer, model Cecil, Italy).

Statistical analysis. The data were statistically analyzed. The Student's t-test was employed to estimate the mean, standard deviation, and variation between the data. Data analysis was performed in SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA). P-value less than 0.05 was considered statistically significant.

RESULTS

All of the altered parameters are presented in Table 1. The infected group had a significant increase in S1P, MDA, HSP27, total bilirubin, and unconjugated bilirubin levels, compared to the healthy group ($P < 0.01$). On the other hand, the TSA, PON, Alb, and high-density lipoprotein (HDL) levels were significantly lower in the infected group than those in the non-infected group ($P < 0.01$). Furthermore, there was no significant difference between the two study groups in terms of total plasma protein concentration. The pathologic evaluations of microscopic sections revealed some cellular changes, including severe fibrosis, hepatocytes destruction, and formation of island-like structures by hepatocytes (Figure 1A). Furthermore, some other changes were observed in another area of hepatic tissue that was affected by the parasitic cyst. These changes included fatty change, pyknotic nuclei (that is indicative of cell degeneration), and lymphocytic infiltration (Figure 1B). The histologic section of the parasitic cyst was also diagnosed (Figure 1C).

Table 1. Alterations of total sialic acid, adenosine deaminase, malondialdehyde, and heat shock protein-27 levels in the healthy and infected groups

DISCUSSION		
Parameters	Control Group	<i>Cysticercus tenuicollis</i> group
TSA (mg/dL)	41.36±3.14	9.52±4.62 [†]
MDA (nmol/mL)	2.25±0.06	7.76±0.22 [†]
S1P (ng/L)	72.18±8.57	182.19±17.12 [†]
HSP27 (ng/mL)	4.61±0.27	29.34±1.35 [†]
Alb (g/dL)	5.13±0.1	3.69±0.08 [†]
TPP (g/dL)	7.1±0.8	6.9±0.4
PON (U/L)	36.15±4.05	15.28±3.29 [†]
HDL (mg/dL)	19.58±2.31	8.84±1.67 [†]
Total bilirubin (mg/dL)	0.31±0.011	1.59±0.01 [†]
Unconjugated bilirubin (mg/dL)	0.06±0.001	0.85±0.06 [†]

Data are expressed as mean±SD[†] Significantly different from the control group ($P < 0.05$).

TSA: total sialic acid, ADA: adenosine deaminase, MDA: malondialdehyde, HSP27: heat shock protein-27, S1P: sphingosine 1 phosphate, Alb: albumin, TPP: total plasma protein, PON: paraoxonase, HDL: high-density lipoprotein

The S1P has been demonstrated to have substantial effects on the function (i.e., maturation and migration) of T and B lymphocytes. In the present study, we observed a significant elevation in S1P concentration. We could not find any evidence regarding the alteration of S1P in the cysticercosis of sheep. In line with our findings, in a study conducted by (Li et al.) in 2009, S1P concentration was observed to increase in carbon tetrachloride-mediated hepatic fibrosis in rats. However, (Ikeda et al.) in 2010 reported that S1P was reduced in the patients with liver fibrosis. Platelets are known to be one of the main sources of S1P in the plasma during sphingosine phosphorylation by sphingosine kinase. Accordingly, platelets reserve S1P,

and release it into the plasma after getting activated (Toghill and Green, 1983).

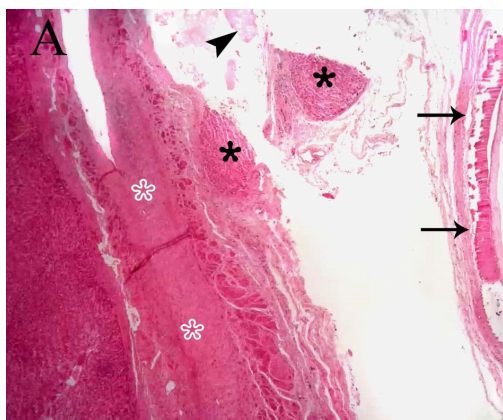
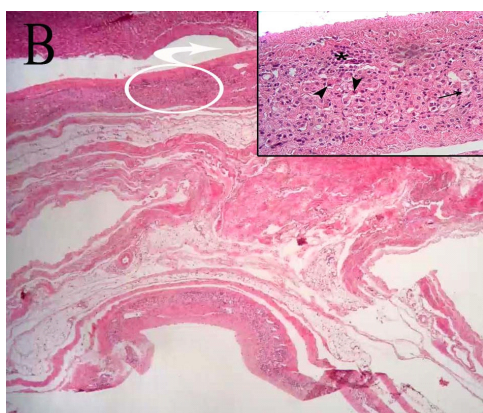
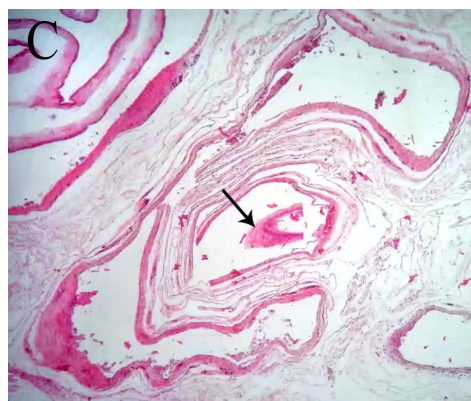


Figure 1. A. Pathologic effects of ovine cysticercosis on hepatic tissue. Parasitic cyst (arrow) caused pressure atrophy in the liver with the destruction of some hepatocytes specified as debris (arrowhead), island-like areas of hepatocytes (black asterisk), and severe fibrosis (white asterisk) in the hepatic tissue (H&E, X40)



1B. Cellular changes (specified area) caused by *Cysticercus tenuicollis* in another area of hepatic tissue (main figure, H&E, X40). Higher magnification for cellular changes. Fatty change (arrow), pyknotic nuclei of some hepatocytes (arrowhead), and infiltration of lymphocytic inflammatory cells (asterisk) (inner figure, H&E, X400).

Regarding platelets, Togill et al. in 1983 reported the hyperactivity of platelet during chronic hepatic disease. Moreover, cysticercosis causes hepatic damage in the sheep.



1C. *Cysticercus tenuicollis* with parasitic section (arrow)(H&E, X40).

Consequently, platelets might be activated in the sheep with liver cysticercosis, which could lead to the elevation of S1P. Furthermore, plasma S1P may enhance due to unknown reasons, which needs further clarification. In the current study, the group infected with *Cysticercus tenuicollis* had lower levels of TSA and Alb than the healthy sheep. Sialic acid, plasma proteins, glycoproteins, and lipids are mostly synthesized in the liver (Chrostek et al., 2011). Sialic acid is linked to the non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids. We could not find any information regarding sialic acid alterations in the cysticercosis of the sheep liver. In 2011, Chrostek et al. demonstrated a low level of lipid-bound sialic acid in non-alcoholic cirrhosis and attributed it to the liver diseases that affect the serum level of lipids and lipoproteins, and also the level of sialic acid bounded with these compounds. In addition, the occurrence of hepatic failure has been already revealed in the cysticercosis of the sheep. Since the synthesis of sialic acid as well as sialylation and desialylation of lipids and proteins are carried out in the liver and because PON and Alb have a significant decrease, the reduction of TSA may be associated with liver damage in the infected group. The PON plays a vital role in xenobiotic biotransformation and protects against lipid peroxidation. Many studies reported PON activity in human (Turk et al., 2004); nevertheless, no

comprehensive studies have been performed regarding PON activity in cysticercosis. In this study, PON concentration was low in the infected group, compared with that in the healthy ones, which is in accordance with the findings of other studies (Mido et al., 2012). Various mechanisms might involve in serum PON activity. First, PON activity can be reduced as a result of liver damage owing to the main role of the liver in PON synthesis and the high concentration of serum and hepatic pro-inflammatory cytokine (TNF- α) in the cysticercosis of sheep liver (Bobe et al.). Second, the low activity of PON in the infected sheep may be ascribed to HDL structure and composition because PON is known as a HDL-associated protein. The biosynthesis of HDL is reduced in a damaged liver, which in turn results in the reduction of PON level in the HDL structure. In the present study, the infected group had high level of MDA. Furthermore, PON participates in the hydrolysis of MDA and other lipid peroxides, and then get inactivated. The Alb is a negative acute-phase protein, which plays the role of an important extracellular antioxidant (Halliwell, 1988). In the present study, the low level of Alb can be suggestive of an oxidative stress. Among its different biological functions, Alb strongly encompasses antioxidant activity, which is mainly associated with the scavenging of free radicals and binding to metal ions. In addition, it hinders free radical production through the inhibition of the Fenton reaction (Loban et al., 1997). In this study, the decrease in Alb is attributed to either decrement in hepatic synthesis or its role in the reduction of free radicals. All the above-mentioned remarks are in accordance with the present study. The generation of ROS and occurrence of oxidative stress increase during some parasitic infections, such as *Fasciola hepatica* and *Dicrocoelium dendriticum* in the sheep. Our study demonstrated a considerable increase in MDA (oxidative stress marker) and a significant decrease in antioxidant (PON and albumin) concentrations in the sheep with *Cysticercus tenuicollis* infection, compared to the healthy ones. This finding highlighted the overproduction of ROS along with oxidative stress in

the liver infected with *Cysticercus tenuicollis*. The HSP-27 is known as a stress protein that possesses antioxidant effect reducing iron quantity in the cell and elevating the intracellular glutathione level (Ferns et al., 2006). Different conditions are involved in the high production of HSP-27, such as apoptosis, vascular diseases, hyperthermia, and various types of cancers. There is no literature investigating HSP-27 values in the sheep infected with *Cysticercus tenuicollis*. The HSP-27 is a biomarker in various diseases; accordingly, the expression of this protein can be utilized in the early diagnosis and progression of hepatocellular carcinoma (Fawzy et al., 2013). It is worth mentioning that in Ergonul and Askar (2009) reported a high level of HSP-27 in theileriosis of cattle. Accordingly, this might have been the case in this study as the high levels of HSP-27 in the parasitized sheep may be attributed to the elevation of protein synthesis in the cells under stressful conditions (e.g., fever and inflammation) for defending against disease. In pathologic findings, the pyknotic nuclei of hepatocytes is known as a significant sign of degenerating procedures toward necrosis. Regarding the fatty change, the low level of HDL might be justified based on this issue. Since one of the HDL components is apoprotein, which carries lipid from the liver to the blood, the reduction of HDL may account for the fatty change.

In conclusion, the low levels of TSA, PON, and HDL may indicate liver damage as sialic acid and PON are mostly synthesized in the liver. Furthermore, the elevation of MDA can be attributed to oxidative stress. In addition, the significant enhancement of S1P reveals the activation of platelet during the infection. The high level of HSP-27 may be ascribed to its inhibitory effect on stress and/or the stimulatory effect of parasite HSPs on the host immune system.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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