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RESEARCH ARTICLE

Investigation of Some Sera Biomarker Levels in Fascioliasis Patient

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Summary

Fasciola sp. that are generally known as liver trematode of sheep and cattle cause infections also in the human. In this study, we investigated in the sera of patients with fascioliasis how paraoxonase, total oxidant level, total antioxidant capacity, apolipoprotein A-I, apolipoprotein B, transferrin and nitric oxide levels are affected. For this purpose, 45 patients with fascioliasis and 38 healthy controls were enrolled in the study. Fascioliasis was diagnosed with ELISA IgG, stool examination and radiologic imaging. Number of females and males were determined as 34/11 and 30/8 in patient and control groups. Mean age was 38.1±11.7and 35.8±16.9 years in patient and control groups, respectively. A statistically significant difference was not detected between groups in terms of age, gender and body mass index (P>0.05). As the result of the study, paraoxonase (P<0.001), Apolipoprotein A-I (P<0.001), transferrin (P<0.001) and total antioxidant capacity (P<0.024) levels were found lower in patient group compared to control group and the difference was statistically significant (P<0.001). A difference was not detected between two groups in terms of apolipoprotein B levels. Lower paraoxonase, total antioxidant capacity, transferrin and Apolipoprotein A-I levels in patients with fascioliasis compared to controls and higher nitric oxide and total oxidant status may be guide for understanding pathogenesis and immunity of fascioliasis and for novel biomarkers that could aid for diagnosis.

Keywords: *Fascioliasis, Human, Sera biomarker*

Fascioliasisli Hastalarda Bazı Serum Biyomarkır Düzeylerinin Araştırılması

Özet

Çoğunlukla koyun ve sığırların karaciğer trematodu olarak bilinen *Fasciola* sp. insanlarda da enfeksiyon oluşturmaktadır. Bu çalışmada, fascioliasisli hastaların serumlarında Paraoxonase, Total oksidan seviye, Total antioksidan kapasite, Apolipoprotein A, Apolipoprotein B, Transferin ve Nitrik oksit düzeylerinin nasıl etkilendiği araştırıldı. Bu amaçla çalışmaya 45 fascioliasisli hasta ve 38 sağlıklı bireylerden oluşan kontrol grubu dâhil edildi. Fascioliasisli hastalar ELISA IgG, dışkı bakısı ve radyolojik görüntüleme ile teşhis edildi. Hasta ve kontrol grubunda kadın ve erkek sayıları sırasıyla 34/11ve 30/8 olarak belirlendi. Ortalama yaş hasta grubunda 38.1±11.7, kontrol grubunda ise 35.8±16.9 idi. İki grup arasında yaş, cinsiyet ve vücut kitle indeksi açısından istatistik olarak farklılık saptanmadı (P>0.05). Bu çalışma sonucunda fascioliasisli hastalarda Paraoxonase (P<0.001), Apolipoprotein A (P<0.001), Transferin (P<0.001) ve Total antioksidan kapasite (P<0.024) düzeyi kontrol grubuna göre daha düşük seviyede belirlendi ve istatistik olarak anlamlı görüldü. Nitrik oksit ve Total oksidan seviye fascioliasisli hastalarda kontrol grubuna göre daha yüksekti ve istatistik olarak anlamlı görüldü (P<0001). Apolipoprotein B düzeyinde ise iki grup arasında fark saptanmadı. Kontrollere göre, fascioliasisli hastalarda düşük seviyedeki Paraoxonase, Total antioksidan kapasite, Transferin ve Apolipoprotein A ile yüksek seviyedeki Nitrik oksit ve Total oksidan seviye düzeyleri insanlarda fascioliasisin patogenezini ve immunitesini daha iyi anlamamız ve teşhise yardımcı olabilecek yeni serum biyomarkırları için yol gösterici olabilir.

Anahtar sözcükler: *Fascioliasis, İnsan, Serum biyomarkır*

INTRODUCTION

Fascioliasis is a zoonotic infection caused by *Fasciola* sp. trematodes. The parasite has a wide host population including ruminants (especially sheep, goats and cattle), rodents and humans. Contamination of humans develops

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through raw consumption of metacercariae contaminated water plants and drinking waters [1,2].

Fascioliasis is a disease that may be acute or chronic due to changes in liver parenchyma (young parasites) and in bile ducts (mature parasites) and present with different clinical findings varying from asymptomatic infection to severe hepatic cirrhosis [3-5].

Migration of the parasite in liver parenchyma accounts for basic pathologic changes. Parasites' digestion of liver tissue results in significant hemorrhagic lesions, immunologic and inflammatory responses, parenchymal destruction. Reactive hepatitis and hemorrhage develop with traumatic effect of the parasite. Small infarction areas may also develop due to vascular injury. These small areas in liver parenchyma may regenerate or may change with fibrosis [6,7].

Liver enzymes may elevate in varying degrees depending on the severity of the disease. Liver tests are usually based on measurement of alkaline phosphatase and two amino transferase enzymes and intracellular enzymes may be evaluated as an indicator of cellular injury [8].

Lipoprotein metabolism may be affected from degenerative necrotic injury in hepatocytes. Serum triglyceride and very low density lipoprotein metabolism may be affected. Serum triglyceride and very low density lipoproteins (VLDL) may elevate [9].

A reduction may be anticipated in plasma albumin level and coagulation factors as their production would decrease due to hepatic injury. Anemia becomes more prominent in chronic phase. Low iron levels may be detected in chronic phase of the infection [5].

In this study, how this trematode causing aforementioned pathologic disorders affected paraoxonase (PON1), total oxidant status (TOS), total antioxidant capacity (TAC), apolipoprotein A-I (ApoA-I), apolipoprotein B (ApoB), transferrin and nitric oxide (NO) levels was investigated.

MATERIAL and METHODS

A total of 45 fascioliasis patients who were referred to Parasitology Laboratory of Dicle University Medical Faculty Research and Training Hospital from various outpatient clinics between November 2010 and June 2011 and 38 healthy controls were included in the study. Fascioliasis was diagnosed through ELISA IgG (DRG International Inc., USA), stool examination native and sedimentation [midi Parasep® Faecal Parasite Concentrator tubes (Diasys company)], radiologic imagings (ultrasonography, computed tomography), clinical and laboratory parameters.

Venous blood samples obtained from patients and controls after fasting during the night were put into ependorf tubes after centrifuged at +4°C at 4.000 rpm for 10 min. Prepared sera were stored at -80°C in deep freezer until the day of analysis and thawed by keeping in room temperature on the test day.

NO levels were examined with Griess method [10], TAC, TOS were examined using total antioxidant capacity method developed by Erel [11]. Serum PON1 level was measured with spectrophotometric assay using modified Eckerson method. Initial ratios of paraoxon hydrolysis were determined by measuring free p-nitrophenol at 405 nm and 37°C (0.0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co. London, UK) [12]. Nephelometric method was used for ApoA-I, ApoB, transferrin measurements and spectrophotometric method was used for measurement of cholesterol and High density lipoprotein (HDL).

SPSS 12 (SPSS Inc. Chicago,IL) statistical package program was used for statistical analysis. Distribution pattern of data was assessed by Kolmogorov-Smirnov test. Student's t test and Pearson's correlation were used for analysis.

RESULTS

Number of females and males were determined as 34/11 and 30/8 in patient and control groups, respectively. Mean age was 38.1±11.7 years in patient group and 35.8±16.9 years in control group. A statistically significant difference was not detected between groups in terms of age, gender and body mass index (P>0.05).

PON1, TOS, TAC, ApoA-I, ApoB, transferrin and NO levels of patient and control groups are shown in *Table 1*. Levels of PON1 (P<0.001), TAC (p<0.024), ApoA-I (P<0.001), and transferrin (P<0.001) were detected lower in fascioliasis group compared to control group and the difference was found statistically significant. There were positive correlation between ApoA-I levels with PON1 activity (r: 0.63; P<0.001) in patient group. NO and TOS were higher in fascioliasis group compared to control group and the difference was statistically significant (P<0.001). No difference was found between ApoB levels in both groups.

Demographic features, clinical and laboratory findings of fascioliasis patients are shown in *Table 2*. Of the patients, 27 were living in rural areas and 18 were living in city center. Fascioliasis was present in 34 of females and 11 of males and the difference between genders was statistically significant (P<0.022). Forty three of the patients had the history of eating water cress. The most common symptoms were abdominal pain, fever, nausea, weight loss and urticaria, respectively. Hypereosinophilia was present in 34 of the patients and Ultrasonography and Computed Tomography reports were consistent with fascioliasis in all patients. Ova were detected in stools of eight patients

Table 1. *Serum biomarker results in patient with fasciolisasis and control group*
Tablo 1. *Fascioliasisli hastalarda ve kontrol grubunda serum biyomarkır sonuçları*

Parameters	Fascioliasis n=45 Mean ± SD	Control n=38 Mean ± SD	P Value
Paraoxonase	59.1±31.7	95.0±47.8	P<0.001
Total antioxidant capacity	1.16 (0.27±0.04)	1.28 (0.21±0.03)	P<0.024
Total oxidant status	17.7±9.5	10.7±7.1)	P<0.001
Nitric oxide	31.5±14.9	22.4±7.1	P<0.001
Transferrin	214.7±59.2	267.3±54.4	P<0.000
Apolipoprotein A-I	107.3±37.6	142.4±34.5	P<0.000
Apolipoprotein B	77.8±18.5	84.3±23.9	P>0.166

Table 2. *Demographic, clinic and laboratory findings in patient with fasciolisasis*
Tablo 2. *Fascioliasisli hastalarda demografik, klinik ve laboratuvar bulguları*

Feature		Fascioliasis (Parameter/Total Patient Number)	
Gender (F/M)		34/11	
Age		10-63 (11.7±1.75)	
Rural/Urbant		27/18	
Water cress eating story		41/43	
Symptoms	Abdominal pain	43/45	
	Fever	30/45	
	Nausea	19/45	
	Weight loss	15/45	
	Urticaria	13/45	
Radiological Findings (USG and CT appropriate)		45/45	
Hypereosinophilia		34/45	
High AST- ALT		8/45 -14/45	
High ALP-GGT		14/45 - 15/45	
Eggs in stool		8/45	
ELISA titer Cut off = 10 11>Positive		12-20	6
		21-30	25
		31-40	14

ALT, *alanine aminotransferase;* ***AST,*** *aspartate aminotransferase;* ***ALP,*** *alkaline phosphatase;* ***GGT,*** *gamma glutamyltransferase*

in stool examination. AST, ALT, ALP and GGT were found elevated in 8, 14, 14 and 15 patients, respectively.

DISCUSSION

Oxidative stress may simply be defined as the imbalance between antioxidant defense of the body and free radical production [13-16]. Many metabolic and functional disorders develop if free radical formation exceeds antioxidant capacity [17]. Oxidative stress and increased lipid peroxidation have been related with liver destruction [18]. In some studies, free oxygen radicals formed as the result of lipid peroxidation product due to cell and tissue injury in cells of infected hosts by different parasite species were shown to increase [19]. Level of malondialdehyde that is one of the products of lipid peroxidation was found to be elevated in humans infected with *T. gondii, Enterobius, Kist hidatik and Fasciola* compared to control group [20-22]. Lipid peroxidation development in liver was revealed in liver of rats infected with *F. hepatica* [23]. There are many internal and external factors affecting TOS reduction. Especially increased intracellular oxidative radicals lead to reduction of antioxidant defensive mechanism and increase of oxidative stress. Increased oxidative stress leads to death especially in hepatocytes and this result in elevation of hepatic enzymes and fibrosis development [24-26]. Kaya et al.[20] found that serum malondialdehyde level was high, superoxide dismutase, catalase and glutathion peroxidase activities were low in chronic infection although it is not the evidence of trematode enzymes' penetration into human tissues and considered that these effects could be resulted from toxins' released by the parasite run into blood. According to our results, elevated TOS values and reduced

TAC level in fascioliasis confirm high oxidative stress in the cell and cell destruction together with necroinflammation.

Nitric oxide has been investigated in many organs as intracellular transmitter since 1980 it was discovered. It was initially defined as endothelial derived releasing factor. It is synthesized from arginin by three different NO synthase [27]. Reactive nitrogen oxide species formed as the result of nitric oxide oxidation may lead to nitrosilation and nitration of cellular molecules, DNA injury, destruction of membrane lipids and inactivation of proteins/enzymes [28]. NO levels have been measured in patients infected with *E. vermicularis* and *T. gondii* and have been reported to be significantly higher compared to control group ($P<0.05$, $P<0.001$, respectively) [21,22]. In this study, similarly to two others, NO level was found higher in fascioliasis patients compared to controls ($P<0.001$). This may be interpreted as a defense mechanism through stimulation of cellular immune system against harmful effects caused by the parasite in liver tissue.

mRNA of paraoxonase (PON1) has been shown to be present also in kidneys, heart, brain, small intestine and pulmonary tissues besides liver and PON1 was determined to be localized in endothelial layer with immunohistochemical methods [29,30]. PON1 prevents oxidation of low density lipoprotein (LDL) and high density lipoprotein (HDL). Lipid peroxidation develops not only in LDL but also in HDL under oxidative stress [31,32]. PON1 has been reported to prevent both LDL and HDL from oxidation [33]. In recent studies, oxidative stress has been shown to play an important role in pathogenesis of atherosclerosis. LDL in serum converts to oxidized LDL that is the atherogenic form by exposing to oxidation and foam cells are formed by accumulation of oxidized products in macrophages and fatty streaks develop in endothelium. Atheroma plaque develops consequently [34,35]. Serum PON1 activity was suggested to play a protective role in the initial phase of this process. Thus prevention of oxidative modification of LDL is primarily necessary for protection from atherosclerosis [33]. In our study, PON1 activity and ApoA-I levels were found lower in fascioliasis patients compared to controls and the difference was found statistically significant ($P<0.001$). ApoA-I appears of major importance in defining serum PON1 activity and stability [36]. It is stated that enhanced oxidative stress leading to HDL. ApoA-I or PON1 oxidation could entail the destabilization of the PON1 association to HDL or a direct inactivation of PON1 enzymatic activity [37]. In our study the positive correlation of ApoA-I levels with PON1 activity supports the previous studies and this condition may suggest that fascioliasis may increase atherosclerosis development in patients.

Transferrin is the major protein providing iron transport. Ionized iron is transferred in the blood binding to transferrin [38]. Erythropoiesis was shown to accelerate as the reaction against anemia developing during fascioliasis [39] and a negative correlation was stated to be between serum transferrin and hemoglobin in calves [40]. In another study, transferrin was shown to increase during iron deficiency anemia in calves [41]. Transferrin level, serum iron level and ferritin level may be measured in fascioliasis patients and the relationship between them may be interpreted.

Increased PON1, TOS, TAC, transferrin, ApoA-I and NO levels in fascioliasis patients compared to controls may be a guide for understanding the pathogenesis and immunity of human fascioliasis and for novel serum biomarkers that could aid diagnosis. In more detailed studies, increase in one of these biomarkers may be helpful for indication of parasite load in the host or the relationship between severity of infection.

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