Iranian J Parasitol

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Original Article

Autoantibodies in Patients with Fasciolosis

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(Received 5 Apr 2009; accepted 2 July 2009)

Abstract

Background: Antiself humoral immune responses have been detected not only in classical autoimmune diseases, but autoantibodies have also been found in sera of patients suffering from chronic parasitic diseases. We aimed to investigate the role of fasciolosis as a trigger factor of autoimmune reactivity by searching some antibodies related to hepatobiliary systems, in patients with fasciolosis.
Methods: Thirty-two patients (17 males, 15 females) with fasciolosis were included in this case-control study. Anti-nuclear antibodies (ANA) Screen (antigen mixture of dsDNA, histones, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1, ribosomal P-proteins, centromere) ELISA and single-antigen ELISAs for detection of some antibodies (dsDNA, Anti-M2, Anti- liver-kidney microsomes type 1 (LKM-1) and Myeloperoxidase (MPO) were carried out.
Results: ANA-screen, M-2, LKM-1, MPO and anti-dsDNA positivity were detected with ELISA in 7, 7, 4, 2 and 2 of 32 patients with fasciolosis, consecutively. No statistically significant difference was detected for any of the autoantibodies' frequency between patients with fasciolosis and control group. However, autoantibody positivity rate was significantly higher in patients with fasciolosis (50 %) than control group (12.5 %). Absorbance values of all autoantibodies in patients with fasciolosis were statistically significant higher than controls.
Conclusion: These results lent support to the role of fasciolosis as a trigger factor of autoimmune reactivity by the breakdown of tolerance. In spite of the extensive knowledge that has accumulated, the specific relationship between fasciolosis and autoimmunity is still obscure.

Key words: *Fasciolosis, Autoantibody, Autoimmunity*

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Introduction

Autoantibodies reflect the presence, nature, and intensity of a certain autoimmune response (1). Therefore, they may be potentially useful as markers for diagnosis, classification, disease activity and prediction of clinical courses in many immune-mediated diseases. Antiself humoral immune responses have been detected not only in classical autoimmune diseases such as myasthenia gravis or systemic lupus erythematosus, but autoantibodies have also been found in sera of patients suffering from chronic parasitic diseases including Chagas' disease, leishmaniasis, malaria, schistosomiasis, and onchocercosis (2-5). One current hypothesis that links infectious diseases with an autoimmune response is based on the concept of molecular mimicry, the cross-reactivity of parasite and host antigens (6, 7).

Fasciolosis is a zoonotic infection, which is caused by *Fasciola hepatica* and *F. gigantica* (8, 9). In acute phase of fasciolosis, parasites digest hepatic tissue causing extensive parenchymal destruction, immunological and inflammatory reactions. Chronic phase develops months or years after initial infection and consists of inflammation and hyperplasia of the epithelium caused by adult flukes settled in the bile ducts (10, 11). The diagnosis of this parasitic infection is difficult, especially in a non-epidemic area. When remaining undiagnosed, *F. hepatica* may persist in the bile ducts for years, resulting in the chronic stage of fasciolosis (9). In the course of fasciolosis, it is indicated that excretuar-secretuar (ES) antigens may cause antigenic stimulation, as they may also exert immune suppressive effects for years (10). There are a few studies in the literature demonstrating a relationship between fasciolosis and autoimmunity, but none of them provided sufficient extensive data about the autoantibody profile in these patients (12, 13).

In this study, it was aimed to investigate the role of fasciolosis as a trigger factor of autoimmune reactivity by searching some antibodies related to hepatobiliary systems, in patients with fasciolosis.

Materials and Methods

This study was carried out in Clinical Microbiology Laboratory of Faculty of Medicine, Suleyman Demirel University, between April 2005 and September 2006. Thirty-two patients (17 males, 15 females; mean age: 46.1$\pm$11.3 years) with chronic fasciolosis were included in this case-control study. For each subject the diagnosis of fasciolosis was established serologically using a modified ELISA prepared with ES antigens in our laboratory and/or by finding eggs of *Fasciola* spp. in stools. Subjects with symptoms <4 months were considered as having acute infection, and patients with symptoms for >4 months were deemed to have chronic infection. Besides duration of the disease, patients were determined as having chronic fasciolosis according to clinical, laboratory (liver enzymes, eosinophilia, eggs in stools), and radiologic findings. A control group consisted of 32 healthy individuals (16 males, 16 females; mean age: 40.7$\pm$11.2) who were seronegative by ELISA assay for fasciolosis, and hepatitis B and C viruses, as well as negative for intestinal parasites in stool examination.

Patients and controls without liver dysfunction, diabetes mellitus, cardiac or renal failure, and autoimmune disease were included in the study. This study was approved by Medical Faculty Ethics Committee of Suleyman Demirel University, and written informed consent was obtained from all study subjects and controls.

The *F. hepatica* adults were incubated in phosphate buffered solution (PBS) contain-

ing 0.8 mol/l phenylmethylsulfonyl fluoride, 400 U of aprotinin/ml and 0.1 mM dithiothreitol (one worm/5 ml) (Sigma Chemicals, St. Louis, USA) at 37 °C for 3 hours. The suspension containing ES antigen of *F. hepatica* was centrifuged at 4°C (13,000 µg) for 2 hours and was filtered through a 0.2 µm pore size filter. Excretory-secretory-ELISA antigen was coated onto immuno plates (Nunc-MaxiSorp Immunoplate, Roskislde, Denmark) at a concentration of 12.8 µg/ml. Human sera (100 µl) were used at 1:100 dilution and alkaline phosphates conjugated anti-human IgG (100 µl) (Sigma Chemicals, St. Louis, USA) was used at 1:10,000 dilution. One µg/ml of 4-nitrophenyl phosphate disodium salt (Merck, Darmstadt, Germany) was used as the substrate. Plates were read on a microplate reader (Bio-Tek Instruments, ultra microplate reader ELX 808, Winooski, USA) at an absorbance of 405 nm. Test serum, antigen and conjugate titrations were determined with checkerboard titration. The cut-off point was calculated as the average of the absorbance values of negative sera +3 SD.

ANA Screen (antigen mixture of dsDNA, histones, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1, ribosomal P-proteins, centromere) ELISA, as a screening test for predifferentiation of antibodies against cell nuclei (ANA) and cytoplasm components; Single-antigen ELISAs for detection of antibodies against cell nuclei and cytoplasm antigens (dsDNA); Anti-M2 ELISA for detection of mitochondrial antibodies (AMA); Anti-LKM-1 ELISA for monospecific determination of antibodies against soluble liver-kidney microsomes type 1 (LKM-1) and MPO ELISA for the detection of antibodies against granulocyte cytoplasm, were used. All ELISA assays were performed using a commercial kit (Euroimmun; Medizinische Labordiagnostika GmbH, Luebeck, Germany), according to the manufacturer's instructions.

Statistical significance was evaluated using Student's *t*, Mann-Whitney *u* test and chi square tests, with a SPSS (version 10.0; SPSS, USA) software package. The association between ELISA absorbance value of patients with fasciolosis and absorbance value of autoantibodies was analyzed using the Pearson's correlation test. A P value < 0.05 was considered statistically significant.

Results

According to age and gender distribution, no statistically significant difference between fasciolosis patients and control group were detected (P>0.05). Demographic properties and autoantibody positivity rates in fasciolosis patients and controls are shown in Table 1. ANA-screen, M-2, LKM-1, MPO and anti-dsDNA positivity were detected with ELISA in 7 (21.8 %), 7 (21.8 %), 4 (12.5 %), 2 (6.3 %) and 2 (6.3 %) of 32 fasciolosis patients, consecutively. ANA screen positivity was detected in all of the patients with anti-dsDNA positivity (Table 1).

ANA screen, LKM-1 and M2 antibodies were also detected to be positive with ELISA in 2 (6.2 %), 1 (3.1 %) and 1 (3.1 %) patient in control group. No statistically significant difference was detected for any of the autoantibodies' frequency between fasciolosis patients and control group (P>0.05). However, autoantibody positivity rate was significantly higher in fasciolosis patients (50 %) than control group (12.5 %) (P=0.003).

The mean ELISA absorbance value, as a sign of high-load infection, was 2.498 ± 376 (range 1.950– 3.000) in autoantibody-positive cases and 1.518 ± 487 (range 1.000–2.700) in autoantibody-negative patients. The ELISA absorbance values were significantly increased in the autoantibody-positive group compared with the negatives (P < 0.001).

The eosinophil count was 1643.75 ± 1301.52 in autoantibody-positive cases and 790.63 ± 758.11 in autoantibody-negative patients. The eosinophil counts were high in the autoantibody-positive group compared with the negatives, but this was not statistically significant ($P > 0.05$, Mann-Whitney *u* test). Absorbance values of autoantibodies in patients with fasciolosis and controls were given in Table 2. Absorbance values of all autoantibodies in patients with fasciolosis were statistically significant higher than controls. ELISA absorbance value of patients with fasciolosis showed significant positive linear correlation with absorbance value of ANA screen, M2, LKM-1, MPO and dsDNA (respectively, $r= 0.534$, $P= 0.002$, $r=0.523$, $P= 0.002$, $r= 0.628$, $P< 0.001$, $r= 0.376$, $P= 0.03$, $r= 0.362$, $P= 0.04$).

Table 1: Demographic features and autoantibody positivity rates in patients with fasciolosis and controls

Variable	Fasciolosis patients (n=32)	Controls (n=32)	*P* value
Age (years, means+SD)	46.1±11.3	40.7±11.2	>0.05+
Sex (female/male)	17/15	16/16	>0.05*
ANA screen	7	2	>0.05*
dsDNA	2	0	>0.05*
M-2	7	1	>0.05*
MPO	2	0	>0.05*
LKM-1	4	1	>0.05*
Total	16	4	0.003*

* :chi square, +: Student's *t* test

Table 2: Values of optic density of autoantibodies in patients with fasciolosis and controls

Autoantibodies	Fasciolosis patients	Controls	*P* value (*t* test)
ANA screen	0.423±0.201	0.230±0.112	0.0001
dsDNA	0.207±0.088	0.173±0.031	0.046
M-2	0.395±0.170	0.212±0.096	0.0001
MPO	0.221±0.077	0.157±0.030	0.0001
LKM-1	0.371±0.144	0.178±0.043	0.0001

Discussion

Parasitic infections are known to serve as a triggering factor in autoimmune reactivity through several mechanisms. One possibility is based on molecular mimicry, the evidence being that self-tolerance can be broken by exposure to a protein that shares homology with host antigenic determinants (2). An excessive immune response contributes to the pathogenesis, and its down-regulation becomes beneficial to the host (5). Autoantibodies are considered to be reflective of immune-mediated mechanisms, but they are not diagnostic, pathogenic, or even required for the diagnosis (7). Although the role of autoantibodies in immune response against helminth parasites is not well understood, an association between the detection of circulating autoantibodies or immune complexes has been demonstrated in parasitic infections such as malaria, schistosomiasis, Chagas' diseases and onchocercosis (14-17). In addition, Demirci *et al.* have reported that autoimmune thyroid diseases and thyroid autoantibodies were significantly high in patients with chronic fasciolosis (12).

In this study, we have demonstrated significantly high autoantibody positivity rates among patients with fasciolosis when compared with controls. Although fasciolosis is a liver disease, it may cause a variety of symptoms and signs related to other systems (9, 12, 18). In the course of fasciolosis, many ES antigens that have not yet been fully identified may cause antigenic stimulation. ES components of *F. hepatica* may also exert direct immune suppressive effects through the activity of proteinases on immunoglobulin molecules (19). It has been well documented that parasitic infection is frequently accompanied by down-regulation of cell-mediated immunity. Inhibition of lymphocyte proliferative responses has been reported during nematode and *F. hepatica* infections (20, 21). It was demonstrated that in fasciolosis, high levels of IL-4 and IL-10 are secreted in vitro and that secretion of IFN-gamma and IL-2 are completely suppressed in high-dose infection (22). Brady and colleagues demonstrated that fasciolosis induced a Th2 immune response and down-regulated protective Th1 responses to infection or vaccination (23). T-cell-mediated dominant control of self-reactive T cells is one mechanism for maintaining immunologic self-tolerance. Abrogation of the control can evoke potent autoimmunity (24). These data mentioned above shed light on our findings indicating high autoantibody positivity rates among patients with fasciolosis. In the light of these data, we can also suggest that in the prolonged course of fasciolosis, ES antigens of *F. hepatica* may cause antigenic stimulation via the cross-reactivity of parasite and host antigens and give rise to the increased autoantibody positivity.

Eosinophilia in fasciolosis is one of the most common signs and persists for months, as in our cases. It is unclear whether eosinophils are innocent bystanders or contributing to tissue injury. Eosinophils can lead to slow development of tissue damage in patients with CF, and cell cytoplasmic contents released over a long time may trigger autoimmunity (12). It has been suggested that down-regulation of the Th1 immune response and activation of the Th2 response over a long time may be followed by activation of autoimmunity, progressing to overt autoimmune disease in genetically predisposed individuals. Therefore, eosinophilia is also proposed to act as pathogenic facilitators in these immunologic abnormalities. In our study, as another supporting finding, eosinophil counts of autoantibody positive patients were higher than autoantibody negative ones.

In our study, the mean ELISA absorbance value of patients with fasciolosis, as a sign of high-load infection was significantly increased in the autoantibody-positive group compared with the negatives. These results also provide other evidence supporting the

role of fasciolosis as a triggering factor of autoimmunity. Absorbance values of all autoantibodies in patients with fasciolosis were statistically significant higher than controls. ELISA absorbance value of patients with fasciolosis showed significant positive linear correlation with absorbance value of ANA screen, M2, LKM-1, MPO and dsDNA.

The changing antigenic profile of the developing parasite while it migrates through distinct anatomical regions of the body may result in the stimulation of independent immune responses in the lymph nodes that drain these separate compartments. Furthermore, stimulation of these different lymphoid compartments may lead to different isotypic responses (10). Thus, the parasites may be protected from contending with a single immune effector mechanism that would otherwise become increasingly efficient as the parasite migrates.

It has been indicated that myeloperoxidase, silenced in mature neutrophils, becomes expressed in patients with vasculitis (25, 26). Anti-M2 specifically occurs in primary biliary cirrhosis cases, and rarely occurs in chronic active hepatitis patients (27, 28). Anti-LKM-1 is found in a subgroup of patients with autoimmune hepatitis (AIH) II and the presence of LKM-1 antibodies is one of the most important criteria for the diagnosis of AIH-II. However, anti-LKM-1 is not completely specific to AIH-II. LKM-1 antibody is also produced in the course of chronic hepatitis (29). As it is known, hepatic tissue is the major target and vasculitis may be seen in the course of fasciolosis. This target tissue similarity may be explained with the increased prevalence of these autoantibodies in fasciolosis. Thus, these data and our findings let us suggest that chronic parasitic infections like fasciolosis must not be underestimated when evaluating autoantibodies, especially of hepatobiliary origin. Leung et al indicated that AMA reactivity to each of the mitochondrial autoantigens remained rather constant from day 1-7, but decreased sharply by 12 months after acute liver failure (30). The rapid induction of AMA in acute liver failure subjects suggests that liver injury can trigger the transient production of AMA. Thus, we can suggest that a transient increase can be seen in autoantibodies of hepatobiliary origin, like M2 and LKM1, in patients with fasciolosis during acute stage of the disease due to hepatic damage.

In conclusion, these results lent support to the role of fasciolosis as a trigger factor of autoimmune reactivity by the breakdown of tolerance. In spite of the extensive knowledge that has accumulated, the specific relationship between fasciolosis and autoimmunity is still obscure. Additional studies investigating the autoantibodies before and after treatment may be more useful to clarify the relevance of fasciolosis and autoimmune diseases.

Acknowledgements

The authors declare that they have no conflicts of interest.

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