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Evaluation of Enzyme- linked Immunosorbaent Assay, Using Somatic and Excretory-Secretory Antigens of *Strongyloides stercoralis* for the Serodiagnosis of Strongyloidosis

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Abstract

Strongyloidiasis, caused by a nematode parasite so-called *Strongyloides stercoralis* is one of the major human intestinal nematode infections. Considering that stool examination for *Strongyloides* larvae is not a sensitive method and immunodiagnostic methods are more applicable for this purpose, so the present study was conducted to compare the somatic (S) and excretory - secretory (ES) antigens of *Strongyloides stercoralis* in IgG-ELISA to diagnose human strongyloidiasis. Serum samples obtained from 50 individuals infected with *Strongyloides stercoralis*. Sera from healthy control individuals, not infected with any parasitic diseases (n= 30) and from others with different parasitic infections including hydatidosis (n=20), toxocariosis (n=18), ascariasis (n=2), trichostrongylosis (n=10), and hymenolepiasis (n=2) were examined as well. The cut-off point for (S) and ES was 0.48 and 0.36, respectively. Thirty eight and 42 out of 50 individuals infected with *Strongyloides stercoralis* were also seropositive using (S) and ES antigens, in that order, whereas 15 cases of false positive reactions for (S) and 10 for ES antigen were detected when non-strongyloidiasis sera were examined, therefore the sensitivity of the test was 80.6% and 86.2% for (S) and ES antigens, respectively. The specificity of those antigens was calculated as 84.2% and 88.2%, correspondingly. It was concluded that overall ES antigen showed a more convincing diagnosis in comparison with (S) antigen, although every interpretation of the results should be in accompany with clinical manifestations and a history of the disease.

Keywords: *Strongyloidiasis,* Strongyloides stercoralis, *Somatic antigen, ES antigen, IgG-ELISA, Iran*

Introduction

Strongyloidiasis, caused by a nematode parasite so-called *Strongyloides stercoralis*, is one of the major human intestinal nematode infections. A notable distinctiveness of the parasite is ability to multiply in its host via internal and external autoinfection transmission (1). It infects 30 million people in 70 countries (2). People are infected when the free-living filariform larvae in soil penetrate the intact skin and enter a venous or lymphatic channel, after an internal migration;eventually they enter the small intestine. The classic diagnosis of strongyloidiosis usually depends on the demonstration of *S. stercoralis* rabditiform larvae in the feces or duodenal fluid (3). However, this method as described by many investigators, is not sufficiently sensitive and not mention the stool exam for consecutive days, the chance of finding larvae using routine methods is low (4, 5). For this reason, a sensitive serological method enable to detect the disease even in chronic and uncomplicated cases, is highly necessary and a lot of surveys have been conducted in this

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regard. Given that taking some immunosuppressive drugs in infected patients with strongyloidiasis might result to a hyper infection and even a disseminated case, the need for evaluating and establishing a serological method stands logical. Serologic tests to detect antibodies of *S. stercoralis* by ELISA test have been conducted by some researches and the sensitivity of the test has been reported as 88%(6), 97%(7) and 95%(8), while the specificity of the test reported by aforementioned researchers, had been 99%, 99% and 94.6%, respectively.

The present study, tried to evaluate an IgG-ELISA test using somatic(S) and Excretory-Secretory (ES) antigens of SS to diagnose human strongyloidiasis.

Materials and Methods

Blood samples were collected from individuals infected with *S. stercoralis*, diagnosed based on coprologically analysis for *S. stercoralis* rhabditiform larvae using formel-ether method. Only individuals that were coprologically positive (50 individuals) and presented with a history of the disease were included in the present study. Serum samples obtained from patients in- fected with hydatidosis (n=20), toxocariosis (n=18), ascariasis (n=2), trichostrongylosis (n=10), hymenolepiasis (2), were included in the test. Control serum samples were obtained from 30 volunteers at Tehran University of Medical Sciences, Iran.

Preparation of antigens Filariform larvae of *S. stercoralis* obtained from cultures of human feces containing rhabditiform larvae of the parasite using agar plate and conventional charcoal media. To produce crude antigen of filariform larvae, infected feces were mixed with distilled water and charcoal, then incubated for 7 to 10 days at 30ºC before separation of larvae. Hence, only filariform larvae (third-stage) larvae were obtained from fecal cultures by the Baermann method (9). After separation, larvae were concentrated by centrifugation at 600 g for 15 min at 4º C, then were washed 6 times by centrifugation at 150 g for 3 min at 4º C in sterile phosphate-buffered saline, pH 7.2, each time, to remove additional bacteria. Afterwards, the larvae were homogenized with an electrical homogenizator (Edmund Buhler Co., model Homo 4/A mit uhr) in a small volume of 0.045 M PBS/pH 7.2 containing 1.7mM of phenylmethylsulfonyl fluoride (PMSF); 5mM EDTA; 5mM EGTA and 5nM pepstatin (10), followed by sonication (Tomy Seiko model UP-200P, Tokyo), and centrifugation at 16000×g at 4º C for 30 min. The supernatant was collected and delipidized with ether. After dialysis against distilled water at 4º C overnight, the output was used as the final antigen. ES antigen was collected as described earlier (10).

Protein content of the preparation was determined using Bradford method (11). The obtained antigens were aliquoted and stored at-20º C.

ELISA test The immunodiagnostic assay was performed as previously described (12), with some modifications. Briefly, 100 microliters of *S. stercoralis* crude antigen (3µg/ml) and ES Ag (3 ug/ml) was dispensed into the wells of microtiter plates (Nuclon, Kamstrup, Roskilde, Denmark) and then incubated overnight at 4° C. Excess binding sites were blocked with 200µl of bovine serum albumin (2% diluted in PBS/ 0.1% Tween 20) and incubated for 45 min at 37º C. After the wells were washed three times with PBS /Tween 20, 100 µl of a serum sample (diluted 1:800) was added to each plate and incubated for 60 min at 37º C. Following another washing step, 100 µl of peroxidase-conjugated goat anti-human IgG (diluted 1: 2000) was added to each well and the plates incubated for a further 45 min at 37º C. Following a final washing step 100 µl of O-phenylendiamine dihydrochloride (OPD) substrate (all from Sigma Chemical Co., Poole, Dorset, United Kingdom) was added to each well and the reaction stopped after 5 min by adding 50 µl of 12.5% H_2S0_4. The optical density (OD) of the samples was measured at 492 nm using a Titerteck (Helsinki,

Finland) multiscan ELISA plate reader. All assays were tested in triplicate and repeated twice.

Statistical analysis:

We used the mean plus 3.0 standard deviation OD value of the healthy group sera as the lower limit of positivity.The sensitivity and specificity were calculated using the method of Galen (13). Statistical analysis was carried out using SPSS for Windows, version 10.

Results

All serum samples were analysed by IgG-ELISA for total antibody responses against (S) and ES antigen of *S. stercoralis*. The cut-off point was detected as 0.48 and 0.36 for (S) and ES antigens, respectively. Therefore absorbance readings greater than the cut off value were considered to be seropositive for strongyloidiasis. In view of that, 38 and 42 out of 50 individuals infected with *Strongyloides stercoralis* were also seropositive using (S) and ES antigens, in that order, whereas 15 cases of false positive reactions for (S) antigen and 10 for ES antigen were detected when non-strongyloidiasis sera were examined (Fig. 1), therefore the sensitivity of the test was 80.6% and 86.2% for (S) and ES antigens, respectively. The specificity of those antigens was calculated as 84.2% and 88.2%, correspondingly.

The mean absorbance and standard deviation for each group of individuals that were infected with diseases other than strongyloidiasis was determined and shown to be not significantly different from those obtained for the negative control sera. Moreover, the absorbance readings from all samples from the *S. stercoralis* seropositive individuals were significantly higher than those obtained from patients that were seronegative or were infected with other parasites (P<0.001).

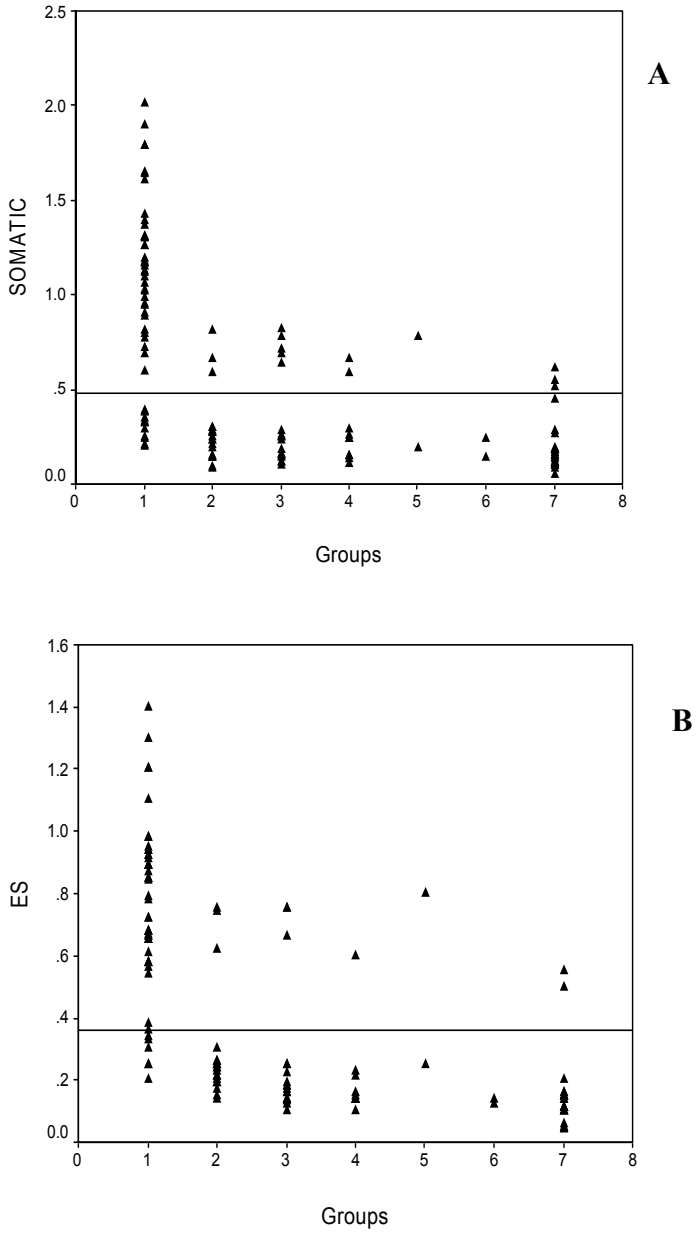

Fig. 1: Analysis of sera from patients with various single infections by IgG-ELISA using *S. stercoralis* Somatic antigen (A) and ES antigen (B). Serum samples obtained from patients with strongyloidosis (50, lanes 1), hydatidosis (20, lanes 2), toxocariasis (18, lanes 3), trichuriasis (10, lanes 4), ascariasis (2, lanes 5), hymenolepiasis (2, lanes 6) and control human sera (30, lanes 7).

Discussion

Immunodiagnosis of strongyloidiasis, primarily by IgG-ELISA, has been evaluated by different investigators (6-8). All these researchers indicate approximately 88-97% sensitivity of the test; most of them make use of crude filariform larvae as antigen. However, the present study shows in comparison, less sensitivity, that may be explained as not presoaking the sera before the test, by different parasites. Lindo et al (14) and Conway (10) could improve the sensitivity and specificity of ELISA using presoaking of sera with *Onchocerca* antigen. Also, Koosha et al, utilizing pre-incubation of sera with antigens from *Ascaris*, *Toxocara* and hydatid protoscolices could improve the sensitivity of the test and reduction of false-positive cases as compared to the IFA test (12).

As it is obvious from Fig. 1, some cross-reaction cases were detected in the test. These cases may have been exposed to cross-reactive antigen of *S. stercoralis*. It has been demonstrated that helminthes that contain cross-reactive antigens have the ability to persist long-term in the host and the tendency to produce circulating antibodies that can be detected for many years after exposure (15). It is reported that *Strongyloides* antibody shows cross-reactivity with other helminth infections, including filariasis, ascariasis and acute schistosomiasis (16, 17). It is worth mentioning that, to date, no cases of filariasis have been reported from Iran and schistosomiasis no longer is of any importance in the country. However, due to scanty of helminthic cases in Iran, further efforts should be conducted to evaluate more sera and to obtain a more authentic estimation of specificity of the test.

It is concluded that IgG-ELISA, preferably using ES antigen, might be a functional assay to help diagnosing human strongyloidiasis, especially when there is problem with diagnosis of the disease by routine stool examination procedures.

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