Original Article

EVALUATION OF ENZYME- LINKED IMMUNOSORBAENT ASSAY FOR THE SERODIAGNOSIS OF STRONGYLOIDOSIS

Koosha S [1], Fesharaki M [2] & Rokni MB [3]

ABSTRACT

Objective: To evaluate an enzyme- linked immunosorbaent assay using filariform larvae of Strongyloides stercoralis to detect specific antibodies in patients with strongyloidosis.
Design: Serum samples obtained from individuals infected with strongyloidosis and other parasitic diseases as well as normal people were analyzed by IgG-ELISA using crude antigen of filariform larvae.
Setting: School of Public Health Serum Blood Bank, Tehran University of Medical Sciences, volunteers' people and selected patients.
Subjects: Serum samples were obtained from 46 individuals infected with strongyloidosis, 37 from normal individuals and 379 cases from others with different parasitic infections.
Main outcome measures: The sensitivity, specificity, positive and negative predictive values of the test.
Result: The cut-off point was 0.537. The sensitivity of the test was 93.47%, whereas the specificity was 96.15%. The positive and negative predictive values were 72.88% and 99.25% respectively. Three individuals with hydatidosis, one with ascariasis and 12 with toxocariasis had antibodies that were reactive against larval antigen.
Conclusion: ELISA method using filariform larval antigen provides a sensitive and specific diagnostic assay.

KEY WORDS: Strongyloidosis, IgG-ELISA, Strongyloides stercoralis

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1. Dr. S. Koosha PhD
Assistant Professor,
Department of Parasitology,
School of Paramedical,
Shaheed Beheshti University of Medical Sciences,
Tehran, Iran.
2. Dr. M. Fesharaki PhD
Associate Professor,
Department of Statistic and Mathematic,
School of Public Health,
Iran University of Medical Sciences,
3. Dr. M.B. Rokni PhD
Assistant Professor
Department of Medical Parasitology & Mycology,
School of Public Health,
Tehran University of Medical Sciences,

Correspondence:

Dr. M.B. Rokni PhD
Assistant Professor
Department of Medical Parasitology & Mycology,
School of Public Health,
Tehran University of Medical Sciences,
P.O. Box: 14155-6446, Tehran, Iran.
Email: roknimoh@sina.tums.ac.ir

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INTRODUCTION

Strongyloidosis is an intestinal parasitic disease of human caused by the nematode Strongyloides stercoralis[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[3].

The definitive diagnosis of strongyloidosis usually depends on the demonstration of *S. stercoralis* larvae in the feces or duodenal fluid[4]. However, strongyloidosis in a majority of uncomplicated cases, the intestinal worm load is often very low and the output of larvae is minimal[5]. It has been shown that a single stool examination fails to distinguish larvae in up to 70% of cases[2]. Because it is crucial to examine multiple stool samples to make a correct diagnosis, it is imperative to note that failure to

detect larvae in a stool examination does not necessarily indicate the unambiguous absence of the infection[1,6]. Hence, there is a need for a highly specific and efficient serodiagnosis test for *S. stercoralis* that has the potential to be used even in multiple helminthes infection. Several immunodiagnostic assays have been tested over the years with limited success. Serologic tests to detect antibodies of *S. stercoralis* by ELISA test have been challenged and at present are available only at specialized centers[6-9]. In some previous studies the sensitivity of ELISA test has been reported as 88%[10], 97%[11] and 95%[12], while the specificity of the test reported by aforementioned researchers, had been 99%, 99% and 94.6% respectively.

This study describes the detection of the specific antibodies in human strongyloidosis by the ELISA method.

MATERIALS AND METHODS

Clinical samples

Blood samples were collected from individuals infected with S. stercoralis, diagnoses based on coprological analysis for *S. stercoralis* rhabditiform larvae using formel-ether method. Only individuals that were coprologically positive (46 individuals) and presented with a history of the disease were included in the present study. Serum samples obtained from patients infected with hydatidosis (n=50), toxocariasis (n=50), amoebiasis (n=10), entrobiasis (n=35), ascariasis (n=13), trichuriasis (n=44), hymenolepiasis (39), fascioliasis (n=40), giardiasis (n=37) and toxoplasmosis (n=47) were obtained from the Tehran School of Public Health Serum Blood Bank. Control serum samples were obtained from 37 volunteers at Tehran University of Medical Sciences, Iran. The humans' ethics committee at the School of Public Health, Tehran University of Medical Sciences, approved the study.

Preparation of antigens

Filariform larvae of *S. stercoralis* obtained from cultures of human feces containing rhabditiform larvae of the parasite using different media (charcoal, activated charcoal and agar plate) in different temperatures (20, 25, 30 and 33ºC). The suitable media for preparing the larvae was conventional charcoal media at 25ºC[13]. 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[14]. After separation, larvae were concentrated by centrifugation at 600 g for 15 min at 4ºC. Then they were washed six times by centrifugation at 150 g for three minute at 4ºC 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 5mM pepstatin[15], followed by sonication (Tomy Seiko model UP-200P, Tokyo), and centrifugation at 16000 × g at 4º C for 30 minutes. The supernatant was collected and delipidized with ether. After dialysis against distilled water at 4ºC overnight, the output used as the final antigen.

Protein content of the preparation was determined using Lowery method[16]. The obtained antigens were aliquoted and stored at –20ºC.

ELISA test

The immunodiagnostic assay was performed as previously described by Rokni et al.[17], with some modifications. It was performed in two procedures (without and with the use of preincubation of sera with extracts of different parasites including *Ascaris, Toxocara* and hydatid protoscolices in concentration of 50 µg/ml for each. Serum samples and extracts were mixed 1:50 at 37ºC for one hour). Briefly, 100 microliters of *S. stercoralis* crude antigen (2µg/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 30 minutes at 37ºC. After the wells were washed three times with PBS /Tween 20, 100µl of a serum sample (diluted 1:200) was added to each plate and incubated for 60 min at 37ºC. Following another washing step, 100µl of peroxidase-conjugated goat anti-human IgG (diluted 1:400) was added to each well and the plates incubated for a further 60 minutes at 37ºC. Following a final washing step 100µl 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 minutes by adding 50µl of 12.5% H2S04. 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, specificity, and the predictive values were calculated using the method of Galen[18]. Statistical analysis was carried out using SPSS for Windows, version 10.

RESULTS

Serum samples obtained from 46 individuals that were coprologically positive for strongyloidosis were analysed by ELISA for total antibody responses against crude antigen of *S. stercoralis*. Using above-mentioned formula the cut-off point was detected as 0.537. Therefore absorbance readings greater than the cut off value were considered to be seropositive for strongyloidosis. Accordingly, before absorption of the sera, 42 individuals that showed clinical manifestations of strongyloidosis were also seropositive, while after absorption 3 cases of false negative were detected. Therefore, the sensitivity of the test, before and after pre-incubation of sera, was 91.3% and 93.47%. There was no significant difference in absorbance readings between genders and no age group within the sample population demonstrated a tendency to have a higher incidence of infection (data not shown).

The mean absorbance and standard deviation for each group of individuals that were infected with diseases other than strongyloidosis 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 ($P<0.001$). However, before absorption, 4 individuals with ascariasis, 19 with toxocariasis and 6 with hydatidosis had antibodies that were reactive against *S. stercoralis crude* antigen, while after absorption, these values decreased to 1, 12 and 3 cases respectively (fig. 1). For the present data set, therefore, the specificity of crude antigen was calculated as 93.02% and 96.15%, before and after absorption, respectively. In this regard the positive predictive value of the test, before and after absorption, was calculated as 59.15% and 72.88%, whereas this value for negative predictive value was 98.97% and 99.25% correspondingly.

DISCUSSION

Strongyloides stercoralis is the most parasitic infection to diagnose[19]. There is a great need for a sensitive and rapid immunodiagnostic procedure to detect chronic, a symptomatic, and low-level of strongyloidosis infection in man. It is obvious that parasitological confirmation of the light to moderate infection with the parasite is frequently difficult[20].

A number of attempts have been made to develop an immunological test for the diagnosis of strongyloidosis including skin test, complement fixation test, indirect immunofluorescence analysis of fixed larvae, radio allergosorbent testing for specific IgE and gelatin particle agglutination[2].

Immunodiagnosis of strongyloidosis,

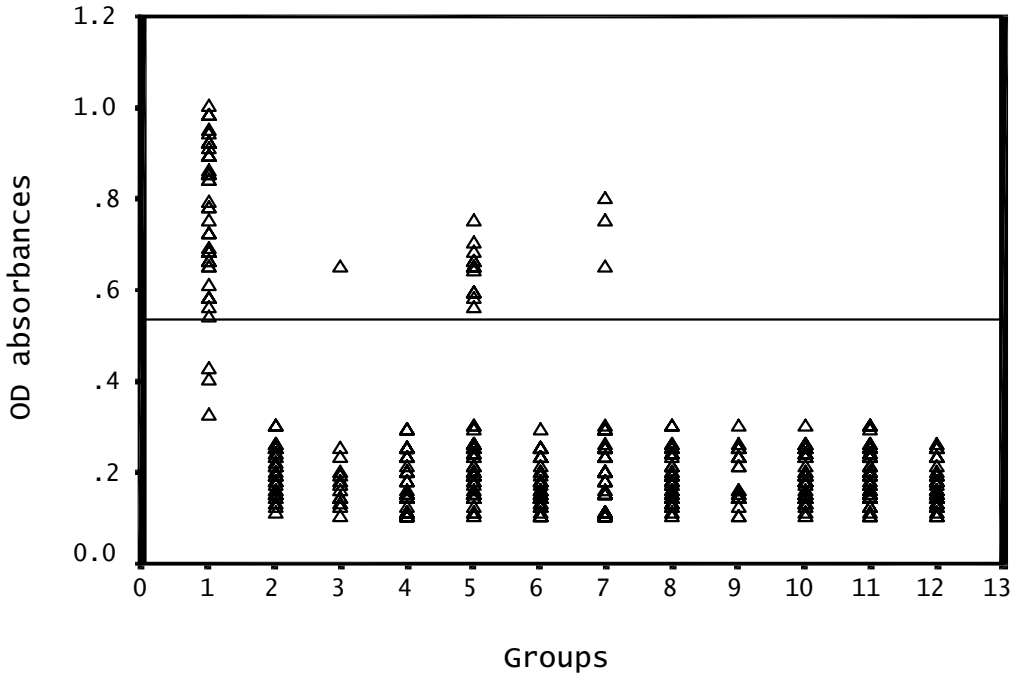

Fig. 1: Analysis of sera from patients with various single infections by IgG-ELISA using *S. stercoralis* filariform larvae crude antigen. Serum samples obtained from patients with strongyloidosis (46, lanes 1), entrobiasis (35, lanes 2), ascariasis (13, lanes 3), trichuriasis (44, lanes 4), toxocariasis (50, lanes 5), hymenolepiasis (39, lanes 6), hydatidosis (50, lanes 7), fasciolosis (40, lanes 8), amoebiasis (24, lanes 9), giardiasis (37, lanes 10), toxoplasmosis (47, lanes 11) and control human sera (37, lanes 12).

primarily by ELISA, has been reported by different investigators[10-12]. All these reports indicate approximately 88-97% sensitivity of the test. We have demonstrated that the sensitivity and specificity of the test could be improved when the serum samples are pre-incubated with aforesaid parasites antigens. Lindo et al[8] and Conway[15] could improve the sensitivity and specificity of ELISA using presoaking of sera with *Onchocerca* antigen.

As is shown in figure 1, after pre-incubation of sera, antibodies in the sera of some patients infected with hydatidosis, ascariasis and toxocariasis were reactive against *S. stercoralis* crude antigen. 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[21]. It is reported that *Strongyloides* antibody shows cross-reactivity with other helminth infection, including filariasis, ascariasis and acute schistosomiasis[22,23].

It is concluded that the ELISA may be an excellent assay to diagnose the hidden and disseminated strongyloidosis.

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