Comparison of enzyme-linked immunosorbent assay and indirect immunofluorescence assay in the diagnosis of human strongyloidiasis

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Background: An enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) were evaluated for serological diagnosis of human strongyloidiasis. Methods: Serum specimens obtained from 46 individuals infected with Strongyloides stercoralis, 37 healthy persons and 381 persons with other parasitic infections were tested using an IgG-ELISA that used crude antigen of S. stercoralis filariform larvae and an IFA. Test sera were pre-incubated with antigens from Ascaris, Toxocara and hydatid protoscolices to remove non-specific antibodies. Results: The sensitivity, specificity, positive predictive value and negative predictive value for ELISA were 93.5%, 96.1%, 72.9% and 99.2%, respectively, and those for IFA were 87%, 90.1%, 49.4% and 98.4%, respectively. Both assays showed false positivity in hydatidosis, ascariasis and toxocariasis; however, this was less common with ELISA. Conclusion: ELISA method using filariform larval antigen may be a sensitive and specific test for human strongyloidiasis, and may be preferable to IFA. [Indian J Gastroenterol 2004;23:214-216]

Key words: Strongyloides stercoralis, ELISA, filariform larvae

Strongyloides stercoralis is an intestinal nematode that is widely distributed throughout the tropics and subtropics.1 Definitive diagnosis of strongyloidiasis usually depends on the demonstration of S. stercoralis larvae in the feces or duodenal fluid.2 However, in a majority of uncomplicated cases, the intestinal worm load is low and the larval output is minimal.3 A single stool examination thus fails to detect larvae in up to 70% of cases.4 Several immunological techniques, including skin test, complement fixation test, indirect immunofluorescence assay (IFA) using fixed larvae, radio allergosorbent testing for specific IgE, and gelatin particle agglutination, have been tried for the diagnosis of strongyloidiasis.4 Serologic tests to detect antibodies to S. stercoralis include enzyme linked immunosorbent assay (ELISA) and IFA.5-8

The sensitivity of ELISA has been reported as 88% to 97%, and its specificity as 94.6% to 99%.9,10,11 IFA is another sensitive and specific test for detection of IgG antibodies against Strongyloides antigen.12 In this study, we compared the value of ELISA and IFA in the diagnosis of human strongyloidiasis.

Methods

Blood specimens were collected from 46 persons (30 women) with S. stercoralis infection; the diagnosis was based on demonstration of rhabditiform larvae and absence of other parasites on examination of three stool specimens, using formol-ether method. Serum specimens from patients infected with hydatidosis (n=50), toxocariasis (n=50), amebiasis (n=26), enterobiasis (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. These patients had been diagnosed based on stool examination, ELISA, IFA as well as at surgery, as appropriate. Control serum specimens were obtained from 37 healthy subjects.

The Human Ethics Committee at the School of Public Health, Tehran University of Medical Sciences, approved the study. Informed consent was obtained from patients or their legal guardians.

Preparation of antigens

A crude antigen was prepared from filariform larvae of S. stercoralis that were obtained from cultures of human feces containing rhabditiform larvae of the parasite.13 In brief, human feces containing S. stercoralis rhabditiform larvae were mixed with distilled water and charcoal, and incubated at 30°C for 7 to 10 days.14 The resulting filariform larvae were then concentrated by centrifugation at 600 g for 15 min at 4°C, and washed 6 times by centrifugation at 150 g for 3 min at 4°C in sterile phosphate-buffered saline (PBS; pH 7.2) to remove bacteria. The larvae were homogenized in a small volume of 0.045 M PBS (pH 7.2) containing 1.7 mM phenylmethylsulfonyl fluoride, 5 mM EDTA, 5 mM EGTA and 5 mM pepstatin,12 using an electrical homogenizer (Homo 4/A; Edmund...
Buhler) followed by sonication (UP-200P; Tomy Seiko, Tokyo), and centrifugation at 16000 g at 4°C for 30 minutes. The supernatant was treated with ether to remove lipids and subjected to overnight dialysis against distilled water at 4°C. The resulting crude antigen was used as the final antigen. Its protein content was determined using the Lowry method, and it was aliquoted and stored at -20°C.

For the rest of the parasites tested, the crude antigens for pre-incubation were prepared as described previously.16

**IFA**

IFA was done as described previously. In brief, infective larvae of *S. stercoralis* were incubated with serial dilutions of test serum, washed, then incubated with fluoresceinated goat anti-human IgG, rewashed and observed for cuticular fluorescence.

**ELISA**

Test sera were pre-incubated with extracts of three parasites, including *Ascaris*, *Toxocara* and hydatid protoscolices, in concentrations of 50 g/mL each at 37°C for one hour.16

*S. stercoralis* crude antigen was dispensed into wells (2 g/mL; 100 L/well) of microtiter plates (Nuclon, Kamstrup, Denmark) and incubated overnight at 4°C. Excess binding sites were blocked with 200 L of bovine serum albumin (2% in PBS/0.1% Tween-20) and incubated for 30 min at 37°C. After washing thrice with PBS /Tween-20, 100 L of pre-incubated test sera (diluted 1:200) were added to the wells and the plate was incubated at 37°C for 60 min. Following another washing step, 100 L of peroxidase-conjugated goat anti-human IgG (1:400) was added to each well and the plates incubated at 37°C for 60 min. Following a final washing step, 100 L of o-phenylenediamine dihydrochloride (OPD) substrate (Sigma, Poole, UK) was added to each well; the reaction was stopped after 5 min by adding 50 L of 12.5% H2SO4, and optical density (OD) was measured using a Titertek (Helsinki, Finland) multiscan plate reader. All assays were run in triplicate and repeated twice.

**Statistical analysis**

OD values exceeding mean plus 3SD of those of the healthy group were considered positive. Statistical analysis was carried out using chi-squared test.

**Results**

The cut-off OD for ELISA was 0.537. The OD values on IgG-ELISA of sera from patients with various infections and controls are shown in the Figure. The number of false-positive tests in patients with parasitic infestations other than *S. stercoralis* was smaller with ELISA than with IFA (ascariasis 1/13 and 7/13 [p<0.05], respectively, hydatidosis 3/50 and 8/50 [p=ns], and toxocariasis 12/50 and 26/50 [p<0.01]). The sensitivity, specificity, positive and negative predictive values of ELISA were 93.5%, 96.1%, 49.4% and 98.4%, respectively. The corresponding values for IFA were 87%, 90.1%, 49.4% and 98.4%, respectively.

**Discussion**

In our study, ELISA was more sensitive (93.5% vs. 87%) and specific (96.1% vs. 90%) than IFA for the diagnosis of strongyloidiasis. The diagnosis of mild to moderate *S. stercoralis* infection is difficult. Thus, false-negative reactions have previously been reported with sera with low antibody titers or those in the early phase of infection. The use of purified parasite protein from filariform larvae of *S. stercoralis* in ELISA has been shown to increase the sensitivity and specificity of the test; we used such an antigen in our assay.

Strongyloides antibody shows cross-reactivity with other helminths, including filariae, ascaris and schistosoma. Helminths that contain cross-reactive antigens may persist in the host for a long time, producing circulating antibodies that can be detected for several years after exposure. Pre-incubation of sera with specific parasite antigens may reduce the false positivity and enhance the specificity of ELISA, as has been shown following pre-incubation with *Onchocerca* antigen. We used pre-incubation with antigens from ascaris, toxocara and hydatid protoscolices; this may have been
responsible for the lower false-positive tests in our ELISA as compared to the IFA; however, despite such pre-incubation some patients with hydatidosis, ascariasis and toxocariasis showed reactivity against *S. stercoralis* crude antigen.

IFA has been investigated by using digestion-fixation procedure on filariform larvae, with 100% positive reactions, but cross-reactions occurred with filariasis.\(^{16}\) Using living filariform larvae of *S. ratti* as antigen, a sensitivity of 98% for IFA was reported.\(^{22}\) In our study, we found some cross-reaction with hydatidosis, ascariasis and toxocariasis.

In conclusion, in our study, ELISA appeared to be a useful test for the diagnosis of strongyloidiasis. Thus, it may be possible to use this assay as a screening test in preference to other invasive and cumbersome methods such as duodenal aspiration or time-consuming tests like agar plate and Baerman-Moraes tests.

**References**


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