

Identification of infective larva (L3) proteins of *Strongyloides stercoralis* by immunoblot

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ABSTRACT

Background: *Strongyloides stercoralis* is prevalent in tropical and subtropical regions worldwide. This parasite is the only nematode with the ability to multiply in its host's body via autoinfection transmission. Larvae detection in feces is difficult partly because of low egg production and also irregular larvae excretion in feces. Serologic tests (ELISA, IFA) are also diagnostic, however *Strongyloides stercoralis* antigens are not available as a diagnostic tool. In the present study, we analyzed filariform larva (L3) proteins of *Strongyloides stercoralis* by the immunoblot technique.

Materials and methods: Stool samples were examined by direct smear, formalin-ether and agar plate method to identify infected patients. Sera were also obtained and stored at -20°C. Infective larvae were then obtained by agar plate culture, which was incubated for 6-7 days at 25°C, then frozen at -70°C. Finally, larvae were suspended at a concentration level of 12000 in 250µl PBS, containing protease inhibitors and then were sonicated. Protein level was measured by Bradford method. Proteins of *Strongyloides stercoralis* filariform larvae were separated by SDS-PAGE, blotted onto nitrocellulose paper. Western blot analysis of these antigens was achieved using infected human sera (0.1, 0.01, 0.001 dilution) with strongyloidiasis, toxocariasis, hydatidosis, amebiasis and normal human serum as control.

Results: Four immunodominant proteins (23, 28, 30, 41 kDa) were recognized with strongyloidiasis sera in 0.1 diluted serum. None of the proteins reacted to normal human and amebiasis serum, but some showed reaction with serum of hydatidosis and toxocariasis. Having increased the level of serum dilution, only 41 kDa protein was recognized by strongyloidiasis sera. Other serums did not represent any reaction to the parasite's proteins. Therefore, the 41 kDa protein presents as the most important immunodominant protein in this study.

Conclusion: The identification of immunodominant proteins adapted to the physiological and genetic conditions of the host is an appropriate diagnostic approach, which could be associated with improved sensitivity and specificity of serologic tests.

Keywords: *Strongyloides stercoralis*, Infective larva (L3), SDS-PAGE, Immunoblot.
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INTRODUCTION

Strongyloides stercoralis is a parasite prevalent in tropical and subtropical regions of the world. Infection occurs by penetration of filariform larvae

via skin. After migrating through lung, they reach to the small intestine where they become adults. *Strongyloides stercoralis* is the only nematode with the ability to multiply in its host's body via autoinfection transmission. Therefore, they can live for a long time in their host. Infection is asymptomatic in most cases, however, sometimes it causes mild stomach ache and occasional diarrhea.

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The unspecific symptoms are skin problems, digestive and lung disorders, due to the migratory nature of this parasite (1,2).

Disseminated strongyloidiasis occurs when the body's cell-mediated immune responses are deficient, often in patients with leukemia or other malignant diseases, on immune suppressive drugs, after renal and other organs transplantation or those suffering malnutrition (3,4). There are scanty data on prevalence of this parasite, however, there are some reports from different parts of Iran. Rouhani and Jalali reported a prevalence of 1.4% and 1.3%, respectively, in north of Iran (5,6), whereas Farahnak reported a prevalence of 6.9% in southern parts of Iran (7).

The presence of rhabditiform first-stage larvae in fecal sample and duodenal fluid or other fluids provide a positive diagnosis, but early diagnosis is difficult by parasitological methods because of low egg production and larvae excretion in feces irregularly. For these reasons, infection remained unknown for a long time in some cases. Thus, for prevention of disseminated strongyloidiasis, we require proper and specific method in patients with chronic diseases and in immunodeficient patients.

Several studies have been performed for analysis of filariform larva (L3) proteins of this parasite worldwide (8-11). These studies have revealed that antigens identification and production of filariform larvae and their recombinant protein could be suitable and effective for immunodiagnosis of strongyloidiasis. It could enhance sensitivity and specificity in serologic tests (8,10,11).

In the present study, we analyzed filariform larva (L3) proteins of *Strongyloides stercoralis* by immunoblot technique. To our knowledge this is the first study of its kind in Iran.

MATERIALS and METHODS

Stool samples of two females aged 30 and 34 years were examined by direct smear, formalin-

ether and agar plate methods (12) in Tehran and Behshahr (north of Iran). Sera stored at -20°C. Filariform larvae were obtained from agar-plate cultures of human feces containing rhabditiform larvae for 7 days in 25°C. Larvae were washed 3 times in PBS (phosphate-buffered saline), pH 7.3 for 20 min in 600g at 4°C. Then, they were suspended for 5 min in PBS with penicillin G and streptomycin, finally, washed 3 times in PBS and frozen at -70°C.

At the time of testing, the larvae were suspended at a concentration of ~12000 in 250ul PBS with protease inhibitors EDTA (ethylene diamine tetra acetic acid) and PMSF (phenyl methyl sulphonyl fluoride) in microtube, and sonicated for 2 min. Incubation were done for 1 hr at 4°C, the ruptured larvae were pelleted by centrifugation for 15 min at 4°C. Protein level was measured using Bradford method. The supernatant was stored at -20°C to be used as the soluble antigenic extract.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed as described by Sambrook and Russell (13). Briefly, equal volumes of antigenic soluble and sample buffer were heated in 100°C for 5min and were loaded at 12% gradient gel. Samples and molecular weight marker were electrophoresed until dye migration to the bottom. The proteins were transferred to a nitrocellulose paper. This paper was cut in to 4mm strips. The nitrocellulose strips were incubated with strongyloidiasis sera (2 sera), hydatidosis, toxocariasis, amoebiasis and uninfected control sera (one serum for each of them). Sera at 1:10, 1:100, and 1:1000 dilutions in PBS (for 90 min at 37°C) were used in this study. The strips were then incubated with peroxidase-conjugated rabbit anti-human immunoglobulin (1:1000) for 1-2 h. The strips were finally developed in the substrate solution (3, 3'-diaminobenzidine,) for 10 min. Strips were rinsed and transferred to distilled water. After drying, bands were visualized and scored.

This study was approved by ethical committee of Department of Parasitology and Cellular and Molecular Center of of Shahid Beheshti University M.C., Tehran, Iran.

RESULTS

The filariform larvae were observed in plate-agar culture after 6-7 days. This time was the best time for collecting the infective larvae. Numerous proteins in the size range of 10-90 kDa were recognized by SDS-PAGE. Major bands were 23, 25, 28, 30, 33, 38, 41, 48, 60, 66, 74 and 85 kDa (figure 1).

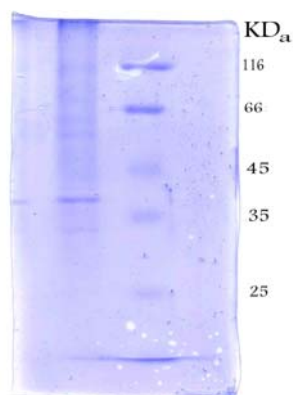


Figure 1. Electrophoretic analysis of antigen composition (SDS-PAGE patterns) of filariform larva (left) and molecular mass markers, kDa (right).

In 0.1 diluted sera, human sera with strongyloidiasis showed reaction with 23, 28, 30 and 41 kDa bands on Western blot. Human serum with toxocariasis reacted to 25, 30, 33 and 60 kDa and human serum with hydatidosis reacted to ~42, 33 and 30 kDa proteins on Western blot. No reaction was found with amebiasis and uninfected control sera (figure 2a).

In 0.01 diluted sera, human sera with strongyloidiasis showed reaction with 30 and 41 kDa bands and serum with hydatidosis reacted to only ~42 kDa band. There was no reaction with other sera (toxocariasis, amebiasis, uninfected) in this dilution of serum (figure 2b).

Finally, in 0.001 diluted sera, only 41 kDa protein was recognized by strongyloidiasis sera, but there was no reaction to other sera (figure 2c).

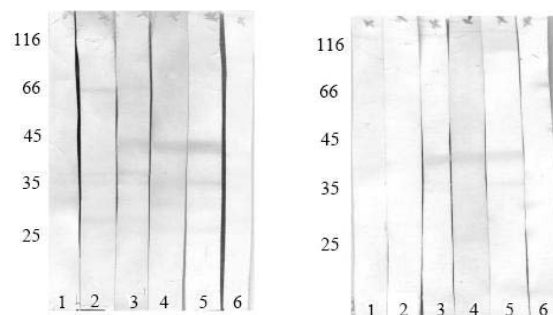


Fig. 2a

Fig. 2b

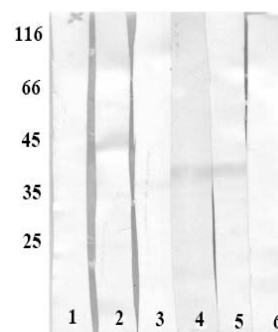


Fig. 2c

Figure 2. Western blot analysis of *Strongyloides stercoralis* filariform proteins with human sera, *Entamoeba histolytica* infection (lane 1), *Toxocara* infection (lane 2), hydatid cyst infection (lane 3), *Strongyloides stercoralis* infection (lanes 4, 5) and uninfected control (lane 6).

DISCUSSION

Several parasitological methods with variable sensitivities are used for the detection of *Strongyloides stercoralis* larvae in fecal samples such as Baerman (14,15), formalin-ether concentration (9,12) and agar plate culture (14,16).

The development of reliable immunological assays such as ELISA and immunoblot is an important alternative approach for the diagnosis of strongyloidiasis. The sensitivity of ELISA technique ranges from 85% to 95% while its specificity can reach up to 90% (11,17).

Immunoblot analysis was also introduced as an alternative method for strongyloidiasis immunodiagnosis, showing better sensitivity than ELISA (11,18). In previous studies, three immunodominant proteins of *Strongyloides stercoralis* L3 larvae with a molecular weight of 28, 31 and 41 kDa were recognized as useful antigens for specific immunodiagnosis of strongyloidiasis in serum samples by immunoblot (8,10). In one series, all suspected sera for strongyloidiasis recognized the 41- and 31-kDa bands and 93% recognized 28-kDa band (8). Two similar sized bands "28 and 41 kDa" were found in this study. The 31 kDa protein may correspond to the 30 kDa band described here. The results of the present study agree with those of previous studies except for 23 kDa band.

In 1999, Uparanukraw et al. described that only serum samples obtained from patients infected with *Strongyloides stercoralis* recognized several proteins of the filariform larvae (L3) by immunoblot. The major recognized proteins had molecular weight of 28, 31, 41 and 205 kDa, however, reaction with 41 kDa protein was more prominent (11). Similar results were reported by other investigators (8-10). Discrepancy in results of this study with others could be partly explained by the differences in the source, antigenic preparation, parasite polymorphism and immunoblotting methods (8,18-20).

In 1993, Conway et al. obtained *Strongyloides stercoralis* larvae by fecal culture from a patient with strongyloidiasis (8). In 1997, Siddiqui et al. obtained *Strongyloides stercoralis* larvae from culture of feces from a dog infected with the parasite (9). However, Silva et al. found *Strongyloides ratti* larvae from the feces of experimentally infected rats. They have recognized 11 immunodominant antigenic components by infected human sera. They believed that *Strongyloides ratti* larvae provide convenient and suitable antigens for the immunodiagnosis of human strongyloidiasis (21).

Several proteins on the surface of *Strongyloides stercoralis* filariform larvae, including one of 30kDa, and several excretory larval products, including those of 40, 30 and 25 kDa were identified by Brindley et al. (22). These proteins were recognized by antibodies from infected patients (22).

Excretory-secretory protein with a molecular weight of 30 kDa was similar to four immunogenic proteins in this study. The 40 kDa protein may correspond to the 41 kDa band described in the present study.

We observed reactivity to the 41 kDa molecular band in 0.1 dilution serum samples from patients positive for toxocariasis and hydatidosis. Having increased the level of serum dilution up to 0.001, only 41 kDa protein was recognized by strongyloidiasis sera.

A protein band of approximately 26 kDa presented a high frequency of reactivity with serum samples from the strongyloidiasis patients in Brazil. They also reported cross-reaction in ascariasis, taeniasis and hook worm infection (23).

Our results indicate that the 41 kDa band can be an important tool for the development of diagnostic technique for strongyloidiasis. Our findings could be used for future studies to obtain recombinant antigens, hence, avoiding the time consuming steps for parasite purification from patients stool (23).

In conclusion, the identification of immunodominant proteins from *Strongyloides stercoralis* filariform larvae in Iran, which have adapted themselves to the physiological and genetic condition of the host, is an appropriate diagnostic approach.

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REFERENCES

1. Adam C, Seeymiller MD, Rita M. Abdominal pain and leukocytosis in an immunosuppressed patient. *Lab Med.* 2004;35:669-71.
2. Kim J, Joo HS, Ko HM. A case of fatal hyperinfection Strongyloidiasis with discovery of autoinfection L3 larvae in sputum. *Korean J Parasitol.* 2005;43:51-55.
3. Coucha R, Harrington W. Intestinal strongyloidiasis: Recognition, management, determinant of outcome. *Clin Gastroenterol* 2005;39:203-24.
4. Muller R, Wakelin D, editors. *Worms and human disease.* 2nd edition. CABI Publishing, 2002;p:109-228.
5. Rouhani S, Kianian H, Athari A. Prevalence of intestinal parasite in rural area in Sari, north of Iran. *Journal of Zanjan University of Medical Sciences* 2001; 9:33-40. (In Persian)
6. Jalali M, Rezaiian M, Rokni MB. Relationship between serum IgE level and intestinal parasites. *Iranian Journal of Public Health* 2004;33:28-32.
7. Farahnak A. Survey on prevalence of parasites in the central area of Kouzestan. *Medical Journal of Tabriz University of Medical Sciences.* 2001;49:57-62. (In Persian)
8. Conway DJ, Bailey YW, Lindo JF, Robinson RD, Bandy DA, Bianco AE. Serum IgG reactivity 41, 31, 28 kDa Larval proteins of *Strongyloides stercoralis* in individuals with strongyloidiasis. *J Infect Dis* 1993;168: 784-87.
9. Siddiqui AA, Koenig NM, Sinensky M, Berk SL. *Strongyloides stercoralis*: identification of antigens in natural human infections from endemic areas of the United States. *Parasitol Res.* 1997; 83:655-58.
10. John F, Lindo JF. Prospective evaluation of enzyme-linked immunosorbent assay and immunoblot methods for the diagnosis of endemic *Strongyloides stercoralis* infection. *Am J Trop Med Hyg.* 1994; 51:175-79.
11. Uparanukraw P, Phonogsri S, Morakote N. Fluctuations of larval excretion in *Strongyloides stercoralis* infection. *Am J Trop Med Hyg.* 1999; 60:967-73.
12. Intapan PM, Maleewong W, Wongsaroj T, Sinthong S, Morakote N. Comparison of the quantitative formalin ethyl acetate concentration technique and agar plate culture for diagnosis of human strongyloidiasis. *J Clin Microbiol* 2005;43:1932-33.
13. Sambrook J, Russell D, editors. *Molecular cloning, a laboratory manual.* 3rd ed. New York, CSHL press. 2001;p:A8.40-A8.55.
14. Kaminsky RG. Evaluation of three methods for laboratory diagnosis of *Strongyloides stercoralis* infection. *J Parasitol* 1993;79:277-80.
15. Willcox HP, Coura JR. The efficiency of Lutz, Kato-Katz and Baermann- Moraes (adapted) techniques association to the diagnosis of intestinal helminthes. *Mem Inst Oswaldo Cruz.* 1991;86:457-60.
16. Koga K, Kasuya S, Khamboonruang C, Sukavat K, Nakamura Y, Tani S, et al. An evaluation of agar plate method for the detection of *Strongyloides stercoralis* in north Thailand. *J Trop Med Hyg.* 1990;93:183-88.
17. Schaffel R, Nucci M, Carvalho E, Braga M, Almeida L, Portugal R, et al. The value of an immunoenzymatic test (enzyme-linked immunosorbent assay) for the diagnosis of strongyloidiasis in patients immunosuppressed by hematologic malignancies. *Am J Trop Med Hyg.* 2001;65:346-50.
18. Sato Y, Inoue F, Matsuyama R, Shiroma Y. Immunoblot analysis of antibodies in human strongyloidiasis. *Trans R Soc Trop Med Hyg.* 1990; 84:403-6.
19. Sato Y, Inoue F, Kiyuna S, Shiroma Y. Immunoblot analysis of three antigens preparation from *Strongyloides stercoralis* larvae in human strongyloidiasis. *Jpn J Parasitol.* 1990; 139:258-66.
20. Genta RM, Gatti S, Linke MJ, Cevini C, Scaglia M. Endemic strongyloidiasis in northern Italy: clinical and immunological aspects. *Quart J Med.* 1988;68:679-90.
21. Silva LP, Barcelos ISC, Passos-Lima AB, Espindola FS, Campos DMB, Costa- Crus JM. Western blotting using *Strongyloides ratti* antigen for the detection of IgG antibodies as confirmatory test in human strongyloidiasis. *Mem Inst Oswaldo Cruz.* 2003; 98:687-91.
22. Brindly PJ, Gam AA, Mckerrow JH, Neva FA. The zinc endopeptidase secreted by inf *Strongyloides stercoralis*. *Exp Parasitol.* 1995;80:1-7.
23. Sudre AP, Siqueira RC, Barreto MGM, Peralta RHS, Macedo HW, Peralta JM. Identification of a 26-kDa protein fraction as an important antigen for application in the immunodiagnosis of strongyloidiasis. *Parasitol Res.* 2007;101:1117-23.