AN EFFECTIVE INDIRECT FLUORESCENT ANTIBODY TEST FOR DIAGNOSIS OF INTESTINAL ACARIASIS

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Abstract. Adult mites' bodies of *Dermatophagoides farinae* were used as antigen in an indirect fluorescent antibody test (IFAT) to detect mite-specific IgG in sera of 48 patients with intestinal acariasis based on stool examination. Antibody titers with positive reaction ranged from 1:4 to 1:512 in 48 patients with intestinal acariasis. If antibody titers ≥1:16 is regarded as being positive, the positive rate of patients detected with IFAT was 92%.

INTRODUCTION

Although mite species, referred to collectively as house dust and storage mites, are recognized world wide as one of the most important indoor allergens responsible for atopic and bronchial asthma, rhinitis and dermatitis in persons with allergic diathesis (Nahm *et al*, 1995; Squillace *et al*, 1997; Moon and Choi, 1999; Barnes, 2000; Solarz, 2001), human acariasis with mites in gastrointestinal tract, lungs, urinary tract and other organs have not been well studied. Mites in various stages of development and their eggs were found in bile B, following duodenal intubation in the course of an acute febrile attack in a patient suffering from chronic nonlithiasis cholecystitis, and in the urinary sediment from seven cases (aged from 6 to 60 years) of primary infections, pyelonephritis and pyelocystitis (Pitariu, 1978, 1979). In the urine of a ten-year-old girl mites of the species *Tarsonemus minusculus* and *Cheyletus eruditus* were found (Bernhard *et al*, 1986). In China, an epidemiological survey on human pulmonary ascariasis suggested 5.3% subjects working in storehouses of grain or Chinese traditional medicines were found positive for mites in their sputum (Li and Li, 1990), the detectable rate of mite in stools collected in disposable feces boxes was 6.59%, male 6.81% and female 6.31% (Li and Wang, 2000). In Korea, a 23-year-old medical student showed a positive reaction on a skin test for *Paragonimus westermani*, and two *Tarsonemus floricolus* mites were subsequently found by sputum examination and identified morphologically (Ryu *et al*, 2003).

The most frequent symptoms of the intestinal acariasis are abdominal pain, diarrhea and pyohemofecia, and the mites living in intestinal tract may mechanically damage intestinal tissues with its gnathosoma, chelicera, feet, and other structures, and even intrude into mucous layer and deep tissues causing necroinflammation and ulcers (Li and Wang, 2000; Li *et al*, 2003a,b). Unfortunately, we can diagnose human acariasis only by detection of mites in stools at present. With small body size of about 1/4-1/3 mm and creamy white in color, mites may often go unnoticed in stools under a microscope, and some patients are misdiagnosed. The objective of this study was to develop a sensitive indirect fluorescent antibody test (IFAT) for diagnosis of intestinal acariasis.

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MATERIALS AND METHODS

Serum

Sera were collected from patients with intestinal acariasis and normal control group. All of the subjects investigated were asked to provide stools for detection of mites by saturated

saline floatation method (Cui *et al*, 2003). Patients with mites in stools were diagnosed as having intestinal acariasis, while the healthy blood donors without mites were used as control group.

Tissue section antigens from adult mites of *Dermatophagoides farinae*

All of the tissue section antigens were obtained from adult *Dermatophagoides farinae* as follows: *Dermatophagoides farinae* were cultivated in culture chambers with relative humidity of 75% and temperature at 25ºC. A culture medium comprising of yeast, wheat flour and rice was used. To isolate adult mites, the whole culture was taken and placed on glass plates, and after 30 minutes, the culture media were removed manually. The adult, larval mites and some media were collected in a small ceramic cup using a small writing brush. Under a lamp, the adults moved rapidly to the basal part of the ceramic cup. The adults were removed with a writing brush under a microscope. Subsequently, the adults were fixed with 10% formaldehyde solution, filtered through a filter paper, embedded in paraffin wax and sliced into tissue sections or about 8 μm. After drying at 54ºC, each glass sheet with four tissue sections was defatted with dimethylbenzene for three times, dehydrated with 100~50% alcohols, washed two times with phosphate buffer solution (0.01M, pH7.2), and dried.

Fluorescent antibody

Fluorescent SAH IgG, employed at a concentration of 1:8, was provided by Shanghai Institute of Biological Products (Batch No. 991001).

Procedures of IFAT in diagnosis of intestinal acariasis

Sera of the experimental and control group were serially diluted with phosphate buffer solution, and then added to the antigens on the glass sheet. After heating at 37ºC for 45 minutes, the glass sheet was washed with phosphate buffer solution (pH 8.0) and blotted with filter paper. Subsequently, fluorescent SAH IgG at dilution of 1:8 was added onto the glass sheet which was heated at 37ºC for 30 minutes and washed as described above. Glycerin was then added. The glass sheet was placed under a fluorescence microscope (Nikon E-400). When a brilliant green fluorescence was seen around the mites' body, positive result was judged.

RESULTS

A total of 48 patients (male 32 and female 16) with intestinal acariasis were recruited in this study, who were selected according to mites found in their stools. Of the 48 patients, 13 were workers in traditional Chinese medical storehouses, 22 in rice storehouse or mill, 8 in mines, 2 in machine factory and 3 in other occupations. The mites from stool samples included *Acarus siro, Tyrophagus putrescentiae, Dermatophagoides farinae, D. pteronyssinus, Glycyphagus domesticus, G.ornatus, Carpoglyphus lactis* and *Tarsonemus granaries*.

Results of IFAT in diagnosis of patients with intestinal acariasis

The antibody titers with positive reaction ranged from 1:4 to 1:512 in all of the patients with intestinal acariasis, while positive reaction was only seen in four health blood donors with titers of 1:4 and 1:8. Therefore, If antibody titers ≥1:16 was regarded to being positive, the positive rate of the patients detected with IFAT was 92% (44/48) (Table 1).

DISCUSSION

Physical examination has the virtue of being a definitive diagnosis of parasitic diseases, but its effect is not good for light infection, infection in earlier period, nonapparent infection, ectopic parasitism and parasite in tissues and splanchna organs. There is an urgent need to develop serological diagnosis of parasite infection. In the present study, adult mites of *Dermatophagoides farinae* were successfully separated from culture media of mites and made into tissue section antigens for indirect fluorescent antibody test of IgG in sera of subjects with intestinal acariasis. The outcome of the experiment showed that the detectable rate was 92% providing antibody titers of 1:16 or more was considered to be positive. When applied under field conditions, it will be useful in determining

Table 1
Results of IFAT in diagnosis of patients with intestinal acariasis.

Antibody titers	Experimental group		Control group	
	Number	Positive rate (%)	Positve number	Positive rate (%)
1:4	1	2	2	7
1:8	3	6	2	7
1:16	4	8	0	0
1:32	7	15	0	0
1:64	7	15	0	0
1:128	13	27	0	0
1:256	10	21	0	0
1:512	3	6	0	0
Total	48	100	4	14

the prevalence of intestinal acariasis and in making a timely diagnosis of infected individuals, particularly individuals who suffer from recurrent diarrhea, chronic abdominal pain, malabsorption and stunting as a consequence of infection.

The reasons why there were four individuals with positive reaction in the controls might be correlated with location of mites living in human body and techniques of stool examination for mites. As mentioned in the introduction, Acaroidea mites are able to live in lungs, urinary tract and other organs besides gastrointestinal tract, but mite-specific antibody must occur in peripheral blood wherever the mites exist. Although saturated-saline floatation allows living adult mites, mites eggs, larvae and dead mites to be isolated, some large and heavy mites may be missed. We suggest that more extensive examination of stools, urine and sputum should be conducted, if false negative detection is to be avoided.

There were eight species of mites in stools of the patients, while *Dermatophagoides farinae* only was used as antigens in IFAT, and this may diminish the detection rate in the present study, although common antigens exist between *Dermatophagoides farinae* and other seven species of mites (Hoog *et al*, 1991; Morgan *et al*, 1996; Munhbayarlah *et al,* 1998). Using a mixture of eight species of mites might raise the positive rate of IFAT. However, only *Dermatophagoides farinae* could be cultivated in this laboratory. In the future, we will make efforts to develop rearing techniques of other species of mites.

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