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World J Gastroenterol 2004;10(9):1369-1371
World Journal of Gastroenterology
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• BRIEF REPORTS •

Diagnosis of intestinal acariasis with avidin-biotin system enzyme-linked immunosorbent assay

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Received: 2003-08-02 **Accepted:** 2003-09-18

Abstract

AIM: To explore the value of avidin-biotin system enzyme-linked immunosorbent assay (ABC-ELISA) in diagnosis of intestinal acariasis.

METHODS: Mite-specific IgG levels in serum of 48 patients with intestinal acariasis were measured with ABC-ELISA. The sensitivity of this method was compared with that of staphylococcal protein A enzyme-linked immunosorbent assay (SPA-ELISA).

RESULTS: The positive rate of mite-specific IgG detected with ABC-ELISA and SPA-ELISA was 89.58% (43/48) and 56.25% (27/48), respectively. The positive rate with ABC-ELISA was statistically higher than that with SPA-ELISA (χ^2=13.50, P<0.01).

CONCLUSION: ABC-ELISA is an effective method for the diagnosis of intestinal acariasis.

Zhang RB, Huang Y, Li CP, Cui YB. Diagnosis of intestinal acariasis with avidin-biotin system enzyme-linked immunosorbent assay. *World J Gastroenterol* 2004; 10(9): 1369-1371
http://www.wjgnet.com/1007-9327/10/1369.asp

INTRODUCTION

Mites could live in intestinal tract and cause intestinal acariasis with most frequent symptoms of abdominal pain, diarrhea and pyohemofecia[1-3]. Intestinal acariasis is traditionally diagnosed by identification of adult or larval mites, eggs or hypopus in either single or multiple fecal specimens under microscopy. The detection of the parasites is increased on examination of multiple fecal samples obtained on three different days. However, it is not easy to detect mites technically. Thus, the present study intended to develop and evaluate an enzyme-linked immunosorbent assay for mite-specific antibody detection in serum samples using *Dermatophagoides farinae* extract.

MATERIALS AND METHODS

Serum

A total of 78 serum specimens were collected from 48 patients with intestinal acariasis and 30 health blood donors. All of the subjects investigated were asked to provide stools for detection of mites by saturated saline floatation method. The 48 patients with mites in stools were grouped as experimental group, while the health blood donors without mites as control group.

Reagents

Reagents for ABC-ELISA and SPA-ELISA were provided by Shanghai Institute of Biological Products (Batch No. 990004 and 990012). *Dermatophagoides farinae* extract was made according to NIBSC82/518 approved by World Health Organization (WHO) in 1984. The mites were cultured in the initial medium for several months. A 48-h maceration in a borate buffer (pH 8.5) was centrifuged. The supernatant was neutralized and precipitated with a series of acetone. The precipitated fraction at 800 mL/L acetone was isolated, washed and dried. This purified extract was lyophilized or stored as a solution in the presence of 500 mL/L glycerol and 50 mL/L phenol[4-7].

Methods

ABC-ELISA procedure Initially 0.1 mL *Dermatophagoides farinae* extract with protein concentrations of 62.5 μg/mL was added in each well on a 40-well plate coated with enzyme, and the plate was placed at 4 ℃ overnight. Then the plate was washed with PBS (pH=7.4), sera of the patients with intestinal acariasis were diluted at 1:40 and added in duplicated wells, and incubated in water bath at 37 ℃ for 60 min. Following washing of the plate, bio-SAH IgG at the concentration of 1:40 was added and incubated at 37 ℃ for 60 min, avidin-HRP at the concentration of 1:20 was added for reaction at 37 ℃ for 30 min, and the substrate OPD-H_2O_2 was added and incubated at 37 ℃ for 30 min. Lastly, 2 mol/L H_2SO_4 was added to terminate the reaction. Sera of the control group were detected with the same procedure[8,9].

SPA-ELISA procedure Concentrations of antigen and sera dilution in SPA-ELISA method were similar to those in ABC-ELISA. SPA-ELISA was performed as routine. Values of optic densities (OD) in each well were determined, the value of 2.1 times or more of the negative control was considered as positive.

RESULTS

General data

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

ABC-ELISA data

In ABC-ELISA, the mean OD value of mite-specific IgG in sera of the 48 patients was 0.358±0.124 (0.176-0.615), while that in 30 controls was 0.112±0.065 (0.085-0.253). The Absorbent values in 43 of 48 patients were at least 2.1 times of 0.112, the positive

rate of the patients detected by ABC-ELISA was 89.58%. Interestingly, the *A* value of one case in normal control group was 0.253, and the false positive rate was 3.33%.

SPA-ELISA data

In SPA-ELISA, the mean OD value of mite-specific IgG in sera of the 48 patients was 0.225±0.147 (0.133-0.574) and that in the control group was 0.078±0.047 (0.043-0.172). The *A* values in 37 patients were at least 2.1 times of 0.078, the positive rate of the patients detected by SPA-ELISA was 56.25%. However, the *A* values of 2 cases in control group were 0.168 and 0.172, respectively, which were higher than 0.1638. The false positive rate in SPA-ELISA was 6.67%.

Comparison between ABC-ELISA data and SPA-ELISA data

The positive rate of the patients detected by ABC-ELISA and SPA-ELISA was 89.58% (43/48) and 56.25% (27/48) with a significant difference (χ^2=13.50, P<0.01). Although only 25 cases were positive in both ABC-ELISA and SPA-ELISA, the positive number of patients was 45 (93.75%) by ABC-ELISA or SPA-ELISA (Table 1).

Table 1 Intestinal acariasis detected with ABC-ELISA and SPA-ELISA

Methods	ABC-ELISA method (+)	ABC-ELISA method (-)	Total
SPA-ELISA method (+)	25	2	27[b]
SPA-ELISA method (-)	18	3	21
Total	43[b]	5	48

[b]: χ^2=13.50, P<0.01.

DISCUSSION

ABC-ELISA and SPA-ELISA were used to detect mite-specific antibody IgG in serum samples from 48 patients with intestinal acariasis, and the positive rate in ABC-ELISA and SPA-ELISA was 89.58% and 56.25%, respectively. ABC-ELISA had high specificity in diagnosis of intestinal acariasis. Moreover, ABC-ELISA in detection of mite-specific antibody was easy to perform, inexpensive, and numerous samples could be performed simultaneously. The test could be carried out quickly for the diagnosis, particularly for individuals who suffered from recurrent diarrhea, chronic abdominal pain, malabsorption and stunting due to infection.

The occurrence of false positive in two methods in diagnosis of intestinal acariasis might be associated with mites' invasive locus, stool examination techniques and dilution of sera. In addition to gastrointestinal tract, acaroid mites could infest other organs of the human body such as respiratory tract and urinary tract, no matter where they were parasized, mite-specific antibody would occur in peripheral blood[10-18]. Although saturated saline floatation method is useful in examination of feces, some large and heavy mites may be missed because of difficult drift. Sera dilution at 1:40 in the present study was lower than that in routine ELISA with dilution at 1:100 to 1:200. It was suggested that much more times repeating stool examination should be carried out in the normal control group to avoid false positive detection[19-24].

Our study showed 8 species of mites in human stools. We used *Dermatophagoides farinae* extract as coating antigen only, which might decrease the detectable rates of mite-specific antibody, because common antigen might exist in *Dermatophagoides farinae* and other seven species of mites[25-28]. In addition, the number of mites in intestinal tract might affect the levels of mite-specific antibodies and detectable rates. In conclusion, ABC-ELISA method is effective in diagnosis of intestinal acariasis.

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Edited by Ren SY and Wang XL **Proofread by** Xu FM