Diarrhea and acaroid mites: A clinical study

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METHODS: Acaroid mites in fresh stools of 241 patients with diarrhea were separated by flotation in saturated saline. Meanwhile, skin prick test, total IgE and mite-specific IgE were detected in all patients.

RESULTS: The total positive rate of mites in stool samples of the patients was 17.01% (41/241), the positive rates of mites in male and female patients were 15.86% (23/145) and 18.75% (18/96), respectively, without significant difference (P>0.05). The percentage of skin prick test as "++++", "++", "+", "±" and "-" was 9.13% (22/241), 7.47% (18/241), 5.81% (14/241), 4.98% (12/241) and 72.61% (175/241), respectively. The serum levels of total IgE, mite-specific IgE in patients with and without mites in stool samples were (165.72±8.53) IU/ml, (132.44±26.80) IU/ml and (145.22±82.47) IU/ml, (67.35±45.28) IU/ml, respectively, with significant difference (P<0.01). The positive rate of mites in stool samples in staffs working in traditional Chinese medicine storehouses or mills (n=155) and staffs working in rice storehouses or mills (n=39), and control group (n=155) including miners (n=36), staffs of railway system (n=34), pupils (n=62) and others (n=23).

CONCLUSION: Acaroid mites cause diarrhea and increase serum levels of total IgE and mite-specific IgE of patients, and the prevalence of diarrhea caused by acaroid mites is associated with occupations rather than the gender of patients.

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INTRODUCTION

Grain or flour mite is a serious and widespread pest of stored foodstuffs, particularly grain and grain products[1-10]. Further studies have shown that some mites with strong vitality not only live freely, feeding on a wide variety of food, but also exist in animals or human intestines. After ingesting contaminated food by mites, like grain and grain products, individuals might have diarrhea, abdominal pain, burning sensation around anus and other symptoms of gastrointestinal tract[11]. The characteristics of diarrhea caused by acaroid mites were investigated in 241 patients with diarrhea in this study.

MATERIALS AND METHODS

Patients

Two hundred and forty-one patients with diarrhea (male 145 and female 96, aged from 6 to 58 years) were divided into experimental group (n=86) including staffs working in traditional Chinese medicine storehouses (n=47) and staffs working in rice storehouses or mills (n=39), and control group (n=155) including miners (n=36), staffs of railway system (n=34), pupils (n=62) and others (n=23).

Reagents

Horse anti-human IgE and standard working solution for IgE (17 000 IU/ml) were provided by Beijing Institute of Biological Products, and horse anti-human horseradish peroxidase-IgE was provided by Third Affiliated Hospital, Shanghai Second Medical University.

Methods

History-taking, separation of mites from stool samples, skin prick test and detection of total IgE and mite-specific IgE were carried out in all 241 subjects.

History-taking Detailed information of each subject was collected via telephone and personal interview, including age, gender, present history, anamnesis, symptoms (i.e. abdominal pain, cramps, diarrhea, urodynia, cloudy urine, and urination frequency), onset and duration of symptoms, personal hygienic habits, living conditions and the date of stool samples collected.

Stool examination The stool samples were collected, the mites were separated by flotation in saturated saline and identified under microscope. Stool examination was performed three times for each person, positive specimens were labeled once either adult or larval mite, egg, or hypopus was found.

Skin prick test Skin prick test was performed with the concentration of 1:100 (W/V). After skin was disinfected, a little of extract (about 0.01 ml) was dripped on skin of the right forearm flexor, then a sterile needle was pricked into the skin through the drop of the extract for about 0.5-1 mm in depth without bloodshed. About 5 cm distal and proximal of the prick site, normal saline and histamine were used as negative and positive control, respectively. The mean diameter of the wheals or areolae was measured 15~20 min after the test. The reactions of skin prick test with the mean diameter $\geq$ 1.5 mm, $\geq$ 2 mm, $\geq$ 3 mm, $\geq$ 5 mm and $\geq$ 10 mm were regarded as ±, +, ++, +++ and ++++, respectively. Otherwise, the reaction was judged as negative[12,13].

The test extract was prepared according to WHO approved document NIBSC 82/518 in 1984. The purified fraction was prepared as follows: the mites cultured in initial medium for survival for 7 days were collected via telephone and personal interview, including age, gender, present history, anamnesis, symptoms (i.e. abdominal pain, cramps, diarrhea, urodynia, cloudy urine, and urination frequency), onset and duration of symptoms, personal hygienic habits, living conditions and the date of stool samples collected.

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50% glycerol and 5% phenol[14-16].

**Detection of total IgE and mite-specific IgE.** To investigate humoral immune function in individuals with diarrhea caused by acarid mites, the levels of total IgE and mite-specific IgE in peripheral blood of mite-positive individuals were tested with ELISA. Peripheral venous blood of the subjects was withdrawn and saved in Eppendorf tubes, then the optical density (OD) value was tested on enzyme labeling meters. Positive and negative control tubes were included each time. When the OD value in the tested sample was 2.1 times that of the control, it was regarded as positive.

**Mites separated from environment.** Directicopy, waterenacopy and pullgren were used to separate mites from mill floor dust, stores of traditional Chinese medicine, and traditional Chinese herbs of wolfberry fruit, ophiopogon root liquorice, boat-fruited sterculia seed and safflower, etc.

**Statistical analysis.** The positive rates were expressed as percentage, and t and χ² tests were used in statistical analysis. A probability value of less than 0.05 was considered statistically significant.

**RESULTS**

**Stool examination.** The positive rate of mites in stool samples in all the individuals was 17.01% (41/241), and was 15.86% (23/145) and 18.75% (18/96), respectively in samples from male and female subjects, without significant difference (χ²=0.34, P>0.05). The mites separated from stool samples were confirmed to be Acarus siro, Tyrophagus putrescentiae, Dermatophagoides farinae, D. pteronyssinus, Glycyphagus domesticus, G. ornatus, Carpglyphus lactis and Tarsonemus granaries. Among 41 cases with mites in stools, adult mites, larval mites, both adult and larval mites, adult mites and eggs, adult and larval mites and eggs, larval mites and eggs, and both hypopus and eggs were found in 15, 6, 11, 3, 2, 2 and 2 cases, and the constituent ratios of them were 36.59%, 14.63%, 26.83%, 7.32%, 4.88%, 4.88% and 4.88%, respectively. In addition, the statistics of this investigation showed that the number concentration of mites was more in negative control, it was regarded as positive.

**Skin prick test.** The percentages of cases with "++", "+", "±" and "-" were 9.13% (22/241), 7.47% (18/241), 5.81% (14/241), 4.98% (12/241) and 72.61% (175/241), respectively. The positive number of skin prick test was 54, of which, 41 subjects with mites in stools were all included. In other words, all the 22 subjects with "++" reaction were confirmed to be mite-positive, and 14 subjects with mites in stools were found in the 18 subjects with "++" reactions, 5 subjects with mites in stools were found in the 14 subjects with "+" reaction. However, all the 41 subjects with mites in stools were positive in skin prick test.

**Detection of total IgE and specific IgE.** The levels of total IgE and mite-specific IgE in 41 cases with mites were higher than those in individuals without mites (P<0.01) (Table 1).

**Relationship between diarrhea caused by acaroid mites and occupation.** Among the 241 patients with diarrhea, the positive rate of mites in experimental group was 26.74% (23/86), which was higher than that in control group (χ²=8.97, P<0.01) (Table 2).

**DISCUSSION.** In this study, Acarus siro, Tyrophagus putrescentiae, Dermatophagoides farinae, D. pteronyssinus, Glycyphagus domesticus, G. ornatus, Carpglyphus lactis and Tarsonemus granaries were separated from stool of patients with diarrhea. This confirmed that acaroid mites were able to parasitize in human intestines, which might play an important role in diarrhea. Like other intestinal parasites, the mites living in intestinal tract may stimulate mechanically and damage intestinal tissues with its gnathosoma, chelicera and feet[17-21]. Certainly they may also intrude into mucous and deep tissues, and cause inflammation and necrosis.

The results of this study support the idea that the patients with mites in stool samples are allergic to acaroid mites. Skin
prick test is one of the specific methods for clinical diagnosis of allergic disease. After superficial layer of skin is pierced by a special needle, interaction occurs between the test extract and mastocytes in the brain, which causes mastocytes degranulation and inflammation-media release like histamine that is able to increase capillary telangiectasia and permeability. Thereby wheal and flush appear on the surface of skin tested\(^{26,27}\). Among the 241 patients tested with skin prick test, 54 subjects had positive reactions. This demonstrated that some of the patients with diarrhea were allergic to acaroid mites. Moreover, the results of skin prick test on 41 patients with mites in their stools were all positive. It provided the evidence that acaroid mites in intestine might lead to the allergy of patients to acaroid mites. The reason why there were no mites found in stools taken from 13 patients with positive reaction of skin prick test is that mites live in other locus besides intestines, and that mites may be missed in detection with saturated saline flotation methods.

Although the serum level of total IgE is a marker of human sensitization to extrinsic allergen, the level of specific IgE is a sensitive index for allergic reaction to acaroid mites\(^{28,29}\). In this study, the levels of specific IgE in 41 patients with mites were higher than those without mites. This provides another evidence that acaroid mites can cause allergy. The dejecta, products of metabolism, and cleaved pieces of dead mites in intestine may stimulate lymphocytes and reticuloendothelial system, and produce specific antibodies, such as IgE.

This study confirms that the prevalence of diarrhea caused by acaroid mites was associated with the patients’ occupation. The positive rates of mites in stools of staffs working in traditional Chinese medicine storehouses and rice storehouses or mills were 29.79 % (14/47) and 23.08 % (9/39), respectively, which were higher than those of patients with other occupations. However, no significant association was observed between diarrhea caused by the organism with the gender of patients. The positive rates of mites in male and female patients were 15.86 % (23/145) and 18.75 % (18/96), respectively.

Eight species of acaroid mites separated from stool samples could be found in the house dust collected from traditional Chinese medicine storehouses, rice storehouses and flourmills, suggesting that the source of diarrhea caused by acaroid mites is mites in our living environment and stored food. Generally speaking, the path of the mites invading human is related to ingestion of stored food. However, some mites in dust or in air might invade intestine through mouth, or nasal cavity, gorge\(^{35,36}\). We have set up eight sampling sites in traditional Chinese medicine storehouses by dust sampler, and separated thirteen mites from dust samples collected from 640 L of air in work environment of the storehouses.

In conclusion, acaroid mites in our living and working environments may invade human intestines and cause diarrhea. The levels of total IgE, mite-specific IgE of the patients with diarrhea caused by acaroid mites increased, the prevalence was associated with the patient’s occupation rather than gender. It is suggested that separation of mites from stool samples, skin prick test and detection of total IgE and mite-specific IgE should be used in the diagnosis of diarrhea caused by acaroid mites.

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