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TOTAL SERUM IgE AND PARASITE - SPECIFIC IgG AND IgA ANTIBODIES IN HUMAN STRONGYLOIDIASIS

Cláudio L. ROSSI (1), Emília E. H. TAKAHASHI (1), Cláudia D. PARTEL (1), Lívia G.V.L. TEODORO (1) & Luiz J. da SILVA (2)

SUMMARY

Total serum IgE, and **Strongyloides** - specific IgG and IgA antibodies were studied in 27 patients with parasitologically proven strongyloidiasis. Clinical manifestations in this case series were investigated by a restrospective study of the patient's records. Total serum IgE levels were elevated (greater than 250 IU/ml) in 59% of the patients (mean concentration = 1364 IU/ml). Parasite - specific IgG and IgA antibodies were detected by ELISA in the serum of 23 (85.2%) and 21 (77.8%) patients, respectively.

Elevated serum IgE and clinical manifestations were not useful indexes of the presence of strongyloidiasis. On the other hand, our results support the view that serologic tests, particularly ELISA for detecting **Strongyloides**— specific IgG antibodies, can be usefully exploited for diagnostic purposes in strongyloidiasis.

KEY WORDS: Strongyloides stercoralis; Strongyloidiasis; Immunodiagnosis.

INTRODUCTION

Strongyloidiasis is a nematodiasis of worldwide distribution having a higher prevalence in tropical countries[9]. In some areas of Brazil, prevalence rates as high as 23% have been found in some studies[6].

In immunocompetent individuals **S.stercoralis** infection is usually asymptomatic or is associated with mild nonspecific clinical symptoms[4,18]. On the other hand, massive as well as disseminated infections accompanied by a high mortality rate may occur in malnourished or immunosuppressed individuals[1,4,16].

The diagnosis of strongyloidiasis has traditionally been made by parasitological methods based on microscopical identification of **S.stercoralis** larvae in stools of infected persons. However, detection of the parasite in stools may be extremely difficult because parasitic larvae are frequently absent from examined specimens or are present in very small numbers[14,19].

Serum IgE levels and blood eosinophilia have been used as non-specific indicators of parasitic infections. The usefulness of such markers in strongyloidiasis is controversial. Some authors have reported normal IgE levels in almost all individuals with strongyloidiasis[24] whereas others have detected increased levels in 50 to 84% of the cases[2,5]. Similarly, a large variation in the percentage of **S.stercoralis** carriers with blood eosinophilia has been observed among the published case series[9,12,13,24].

Several studies support the view that detection of parasite-specific antibodies in individuals with **S.stercoralis** infection may be a useful complement to the traditional diagnosis of strongyloidiasis by parasitological methods[3,7,8,11,12,17,20,24]. So far, most of studies have concentrated on detection of specific IgG antibodies directed against antigens of **S.stercoralis** larvae[3,7,8,20,24]. The life cycle of **S.stercoralis** suggests that the parasite can elicit local and systemic IgA responses. Previous studies indicate that **Strongyloides** - specific IgA antibod-

(1) Departamento de Patologia Clínica da Faculdade de Ciências Médicas da Universidade Estatual de Campinas, São Paulo, Brasil.
(2) Departamento de Clínica Médica da Faculdade de Ciências Médicas da Universidade Estadual de Campinas, São Paulo, Brasil.
Address for correspondence: Dr. Cláudio L. Rossi, Departamento de Patologia Clínica, Faculdade de Ciências Médicas UNICAMP. Caixa Postal 6111, 13081 - 970 Campinas, SP, Brazil.

ies may be detected in the majority of individuals with strongyloidiasis[11,12]. However, few published data are available regarding the simultaneous detection of parasite - specific IgA and IgG antibodies in **S.stercoralis** carriers. In this paper we present results regarding total serum IgE and parasite - specific IgG and IgA antibodies in a group of 27 patients with parasitologically proven strongyloidiasis. Finally, the frequency of clinical manifestations in these individuals is reported.

MATERIALS AND METHODS

Study group

The patients included in this study were seen at the Universitary Hospital of the State University of Campinas, São Paulo, Brazil. This hospital is a 400 - bed acute care facility that serves as a principal referral for an area of approximately 3 million inhabitants. Twenty-seven patients, seen between November 1991, and March 1992, with parasitologically proven strongyloidiasis were studied. In all cases, no concurrent infection with other gastrointestinal parasites was observed in at least three stool examinations. Parasitological examinations are routinely made in our laboratory by a sedimentation-concentration method[15], and by a modification of the Baermann method[22].

Patient's records were available for all the 27 cases. Clinical manifestations at time of parasitological diagnosis of strongyloidiasis in this case series were investigated by a retrospective study of patient's records.

Chemicals and reagents

Unless specified otherwise, all chemicals and reagents were obtained from Sigma Chemical Company, Saint Louis, MO. Thirty percent hydrogen peroxide (H_2O_2) was obtained from Aldrich Co., Milwaukee, WI.

Preparation of S.stercoralis antigen

S.stercoralis antigen was prepared according to GAM, NEVA & KROTOSKI[7]. Briefly, larvae from **S.stercoralis** were recovered from faecal - charcoal cultures by a modification of Baermann method[22], and washed several times by centrifugation. After sonication, larval antigens were extracted in phosphate - buffered saline (PBS) pH 7.2 for 16 hr. The material was centrifuged at 32,000 x g, for 30 min., and the supernatant fluid was aliquoted and frozen for storage at - 70°C.

Antibody assays

IgA and IgG specific antibodies to **S.stercoralis** were measured by ELISA. The techniques were adapted from methods previously described[10,12]. Briefly, polystyrene flat-bottomed plates (Corning, New York) were used, and the reaction volume of each reagent at each step was 0.2 ml/well. Wells were sensitized for 3 hr at 37°C with antigen diluted at 4 μg/ml in 0.1 M carbonate-bicarbonate buffer pH 9.6. Serum samples were diluted in PBS containing 0.1% Tween 20 and 1% bovine serum albumin. Each serum was tested at single dilution of 1:8, both in a well with antigen and in a well without antigen. In the ELISA for IgA, a preabsorption of the sera with Sepharose 4B - protein G was performed, in order to remove IgG antibodies. The optimal concentrations of the conjugates, goat anti - human IgG labelled with peroxidase and goat anti - human IgA labelled with peroxidase, were determined on preliminary conjugate titration experiments using, respectively, human IgG or IgA -coated microtiter plates. The substrate solution used contained 0.42 mM 3, 3', 5,5' tetramethylbenzidine and a final concentration of 0.004% (vol/vol) H_2O_2. Thirty minutes after addition of the substrate system, the reactions were stopped by adding 0.05 ml of 4N H_2SO_4, and the absorbancies of the wells were measured at 450 nm with a microtiter plate spectrophotometer. The final optical density (OD) for each serum was determined by subtracting the absorbance of the well without antigen from that of the sensitized well. A result was considered positive when the final OD was 2 SD above the mean of a group of negative sera obtained from 12 control subjects tested in the same plate[7].

Total IgE determination

Total serum IgE was measured by a quantitative ELISA (Abbott Laboratories Diagnostic Division, North Chicago, Illinois, USA). Values of 250 IU/ml or less were considered normal.

RESULTS

Clinical findings

Thirteen (48.15%) of the 27 patients with strongyloidiasis had some form of digestive - system related manifestation at time of parasitological diagnosis of strongyloidiasis. The clinical symptoms referred by the 27 patients are listed in table 1. Abdominal discomfort, either identified as epigastric pain or as abdominal pain was referred in 11 occasions, being the most frequent symptom.

TABLE 1
Frequency of symptoms in 27 patients with strongyloidiasis

Symptoms*	Nº	%
	Digestive system - related	
epigastric pain	7	25.9
abdominal pain	4	14.8
constipation	3	11.1
vomiting	2	7.4
pyrosis	2	7.4
diarrhoea	1	3.7
nausea	1	3.7
	Other	
weight loss	5	18.5
headache	5	18.5
fever	5	18.5
edema	4	14.8
cough	4	14.8
general fatigue	3	11.1
loss of apetite	1	3.7
discomfort in throat	1	3.7

* Symptoms are listed as referred by patients

Underlying diseases or conditions were present in 15 of 27 patients with **S.stercoralis** infection. Two patients had pyelonephritis, and one of each had hypothyroidism, liver cirrhosis, esophageal cancer, ischemic heart disease, non-Hodgkin's lymphoma, hemoglobinopathy, pulmonary chronic obstructive disease, iron-deficiency anemia, chronic arterial obstructive disease, liver failure, pleural inflammatory disease, heart failure, and consumptive syndrome.

Immunologic studies

Total serum IgE and parasitic-specific IgG and IgA antibodies against **S.stercoralis** antigens were measured in sera from 27 patients with strongyloidiasis. Total IgE levels were elevated (greater than 250 IU/ml) in 16 (59.26%) of the 27 patients, the mean concentration being 1364 IU/ml. Specific IgG and IgA antibodies were detected, respectively, in 23 (85.2%) and 21 (77.8%) of the 27 patients.

DISCUSSION

The majority of **S.stercoralis** carriers are asymptomatic or exhibit symptoms that can not be clearly attributable to the presence of the parasite[4,9,18]. Since the pathogenetic effects of **S.stercoralis** are usually more prominent in the digestive tract, digestive - system related symptoms are commonly reported in symptomatic patients[4,9,12,21]. However, in most cases such symptomatology is not sufficiently distinctive to suggest the diagnosis of strongyloidiasis. Furthermore, other associated parasitic and non-parasitic chronic or debilitating medical illness can bepresent in several individuals with strongyloidiasis[5,25]. In this study, almost fifty percent of the patients with **S. stercoralis** infection had some form of digestive-system related manifestation at time of parasitological diagnosis of strongyloidiasis. Although infections with other intestinal parasites in our study group had been excluded by parasitological examinations, many of the individuals in this series had the concurrent presence of other underlying diseases or conditions that may have gastrointestinal manifestations. As the clinical information was obtained from patient's records, it was impossible to identify symptoms as due to strongyloidiasis or to underlying disease or condition.

Several studies report that the sensitivity of the ELISA for detection of specific IgG antibodies against **S.stercoralis** antigens in individuals with proven strongyloidiasis is between 82 and 90 percent[3,5,7,12,20,24]. **Strongyloides** - specific IgA antibodies have been detected by ELISA in 87.5 percent of presumably immunocompetent individuals with chronic uncomplicated strongyloidiasis and in 73 percent of immunocompromised individuals with **S.stercoralis** infection[11]. Data resulting from simultaneous detection of parasite - specific IgG and IgA antibodies in strongyloidiasis are scant. In one previous report, specific IgG and IgA antibodies against **S.stercoralis** antigens were detected by ELISA in 83.3 percent and 89.5

percent, respectively, of the individuals with parasitologically proven strongyloidiasis[12]. In this study, we detect specific IgG and IgA antibodies in 23 (85.2%) and 21 (77.8%), respectively, of 27 patients with proven strongyloidiasis. None of the patients with negative serology for IgG or IgA specific antibodies was on corticosteroid or immunosuppressive therapy. Concentrations of total serum IgG in patients with undetectable **Strongyloides** - specific IgG antibodies were all within normal limits. Of 6 patients with undetectable parasite - specific IgA antibodies, 4 had total IgA within normal limits, 1 had IgA moderately elevated, and 1 had IgA slightly reduced (results not shown).

Differences in the percentage of **S.stercoralis** carriers with detectable **Strongyloides** - specific IgA in this and other series may be due to the heterogeneity of the patients studied. Immunoblot analysis have revealed a considerable variation in the reactivity of sera from individuals with strongyloidiasis to the larval antigens of the parasite[23]. Furthermore, antigen recognition patterns detected by immunoblotting have shown that **Strongyloides** - specific IgG and IgA antibodies can recognize different **S.stercoralis** antigens[12].

The clinical significance of raised serum IgE in helminth infections is not known. Fifty-nine percent of the individuals in this series had elevated serum IgE levels. Correlations between IgE levels and clinical findings were not observed in this and in other series[2]. Compared to strongyloidiasis' serology results obtained in this study, the practical value of IgE determination was negligible.

S.stercoralis has potential for propagating within the human host, through an internal autoinfectious cycle. This peculiar property of the parasite could explain both the long - term persistence of infection in the absence of continued exposure and the frequently fatal hyperinfection in immunocompromised hosts. In this situation screening for strongyloidiasis is very important in candidates for immunosuppression, particularly in areas where the prevalence of **S.stercoralis** is high. Our results support the view that serologic tests for the detection of specific antibodies to **S.stercoralis** antigens, particularly ELISA for detecting parasite - specific IgG antibodies, can be usefully exploited for diagnostic purposes in strongyloidiasis.

RESUMO

IgE sérica total e anticorpos IgG e IgA anti - *S.stercoralis* na estrongiloidíase humana.

Níveis de IgE sérica total e anticorpos específicos IgG e IgA anti - **Strongyloides stercoralis** foram pesquisados, através de técnicas imunoenzimáticas, em 27 pacientes com estrongiloidíase comprovada por métodos parasitológicos. Manifestações clínicas nesta série de casos foram investigadas pelo estudo retrospectivo dos prontuarios dos pacientes. Os níveis de IgE sérica foram elevados (maior do que 250 UI/ml) em 59% dos pacientes (concentração média = 1364 UI/ml). Anticorpos específicos IgG e IgA anti - **S.stercoralis** foram detectados, respectivamente, em 23 (85.2%) e 21 (77.8%) pacientes.

No presente estudo, níveis elevados de IgE sérica e manifestações clínicas apresentadas pelos pacientes não contribuiram significativamente para o diagnóstico de estrongiloidíase. Por outro lado, os resultados deste trabalho corroboram o ponto de vista que as técnicas sorológicas para a detecção de anticorpos específicos anti-**S.stercoralis**, particularmente as técnicas imunoenzimáticas para a detecção de anticorpos específicos da classe IgG, podem ser de grande utilidade para o diagnóstico da estrongiloidíase.

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