DIAGNOSIS OF GIARDIASIS BY SPECIFIC IgM ANTIBODY ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary
An enzyme-linked immunosorbent assay (ELISA) was used to detect serum IgM and IgG antibodies against Giardia in fifty-two patients with diarrhoea from the United Kingdom and south India. Serum anti-Giardia IgM responses occurred in patients with giardiasis; in two of the three patients studied longitudinally IgM levels had fallen to normal 2–3 weeks after treatment. At serum dilutions of 1:200 to 1:400 there was almost complete separation of ELISA readings between patients with Giardia and Giardia-free controls; the sensitivity and specificity of the ELISA were both 96%. Serum anti-Giardia IgG was detected in patients and controls but was unable to distinguish current from previous infection.

Introduction

Available methods for routine diagnosis of giardiasis rely on microscopic demonstration of the parasite in faeces or duodenal juice. Steal microscopy is laborious and inaccurate, and several samples must be examined owing to the variable pattern of parasite excretion. Examination of duodenal juice, endoscopic brush cytology of the duodenal mucosa, and histology of jejunal biopsy samples improve diagnostic precision, but they are invasive and not readily available everywhere. Detection of Giardia antigen in faeces by enzyme-linked immunosorbent assay (ELISA) is a promising approach, but it is still being evaluated and is not yet available to routine diagnostic laboratories.

A rapid and specific serological test would be a useful alternative. Serum IgG responses occur in giardiasis, but because anti-Giardia IgG persists after the primary infection, IgG levels are unable to distinguish current infection from previous exposure to the pathogen. Specific serum IgM responses occur early in many acute infections and decline rapidly, usually within 2–3 weeks. We have therefore evaluated the diagnostic value of measuring specific serum anti-Giardia IgM responses in patients undergoing investigation of diarrhoea both in the United Kingdom and in an area endemic for giardiasis in India.

Patients and Methods

Serum was taken from fifty-two patients (twenty-six from the United Kingdom and twenty-six from south India) undergoing investigation of diarrhoea of less than 6 weeks' duration. Twenty-two patients were shown to have giardiasis by microscopic identification of Giardia forms in faeces, duodenal juice, or jejunal biopsy specimens. Other intestinal parasites were found in eight Indian patients in addition to Giardia (three Trichuris hominis, four Entamoeba coli, and one both hookworm and Strongyloides stercoralis). All thirty control patients were Giardia-free but in six Indian control subjects other intestinal parasites were identified (two T. hominis, two E. coli, and two both hookworm and S. stercoralis). We took serum samples from three patients during convalescence 2–3 weeks after treatment.

Toxoplasmas of Giardia lamblia (Pentand 1 strain) were harvested from axenic culture, washed in phosphate-buffered saline, and stored at −20°C. They were thawed at room temperature and appropriate dilutions made in phosphate-buffered saline, pH 7.2. Test serum samples were also stored at −20°C, thawed at room temperature, and diluted in phosphate-buffered saline. As the second antibody we used affinity-purified antibody against human IgG or IgM conjugated to peroxidase (Sigma Chemical Company, Poole). Peroxidase substrate was 3,3',5,5'-tetramethylbenzidine in dimethyl sulfoxide. Optimum conditions for use of this substrate have been described previously.13
IgG and IgM anti-Giardia Antibody ELISA

Giardia trophozoites (about 2 × 10^6 in 50 μl phosphate-buffered saline, pH 8.0) were added to polyvinyl microtitre wells (Dynatech Laboratories, UK) and adsorbed for 16-18 h at 4°C. Plates were washed three times with phosphate-buffered saline, pH 7.2 containing 0.05% Tween 20 (0.01% by volume) by means of Dynatech Skanwash 1. Doubling dilutions of test serum samples (final volume 50 μl) were made in phosphate-buffered saline containing 2% bovine serum albumin and incubated with antigen for 1 h at 37°C. After another wash, 50 μl anti-IgG or anti-IgM peroxidase conjugate diluted 1/100 in phosphate-buffered saline with bovine serum albumin was added to each well. After 1 h incubation and a final wash, 150 μl tetramethylbenzidine substrate (0.1 g/l in 0.1 mol/l citric acid/0.05 mol/l sodium acetate buffer, pH 6.0) containing 1.3 mmol/l hydrogen peroxide was added to each well. After a 30 min incubation at room temperature, the reaction was stopped by the addition of 25 μl 2.5 mol/l sulphuric acid. Optical density was read at 450 nm ('Microtest 12', Dynatech Laboratories). Each plate held at least two positive and two negative serum samples. To test the specificity of the anti-Giardia IgM ELISA, six positive samples diluted 1/200 in phosphate-buffered saline with bovine serum albumin were absorbed at room temperature with live Giardia trophozoites (2 × 10^6) and the ELISA optical density readings compared with values before absorption. Non-specific cross-reactivity with chymotrypsinogen factor was tested for in all serum samples by the 'RAHA' test (Fujirebio Inc, Tokyo, Japan) and 'RAPIDTEX' RF (Behringwerke AG, Marburg, West Germany).

Results

In giardiasis patients there was a specific anti-Giardia serum IgM response (fig 1). In addition, at serum dilutions of 1/100 to 1/400 there was almost complete separation in optical density readings between patients with Giardia and those without, a finding equally true for Indian (some of whom had other parasites) and for UK patients. One false-positive and one false-negative result occurred, thus the sensitivity and specificity of the anti-Giardia IgM ELISA were both 96%. Absorption of IgM-positive test serum samples with Giardia trophozoites caused optical density readings to fall by 52±3% confirming the specificity of the test. Rhumatoid factor was present in only one UK serum sample (1/320 titre); this patient did not have giardiasis or anti-Giardia IgM.

Fig 2 shows serum anti-Giardia IgG optical density readings. IgG optical density readings in two of three patients for whom they were measured fell within the normal range within 3 weeks (fig 3).

Discussion

We have developed a simple, specific serum anti-Giardia IgM ELISA which confirms that an IgM response occurs in giardiasis and that it is short-lived. This study also suggests that serum IgM responses can distinguish acute giardiasis from previous exposure to the pathogen. Although anti-Giardia IgM has been detected previously, this is the first time that measurement of serum levels of specific anti-Giardia IgM has been evaluated as a diagnostic test for giardiasis.

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**Fig 1**—Serum anti-Giardia IgM responses in patients with giardiasis (°) and in Giardia-free controls (O). Horizontal line is upper limit of normal optical density reading (mean plus two standard deviations) for Giardia-free controls.

**Fig 2**—Serum anti-Giardia IgG responses in patients with giardiasis (°) and in Giardia-free controls (O).
Fig 3—Serum anti-Giardia IgM responses in 3 patients with giardiasis on presentation (solid lines) and 2–3 weeks later after clearance of the parasite (broken lines).

The role of IgM in the development of protective immunity against Giardia is interesting. There have been several reports that IgM-containing cells predominate in the intestinal mucosa in human beings with Giardia infection.14–16 IgM-containing cells also predominate in nodular lymphoid hyperplasia of the small intestine,16 a disorder that occurs in association with chronic giardiasis.

The anti-Giardia serum IgG responses in our giardiasis patients accord with previous results, confirming that anti-Giardia IgG persists for some time after initial infection and is therefore unhelpful in deciding whether a patient has a current infection.16 The vast majority of the patients from Veliko had anti-Giardia IgG.

The serum anti-Giardia IgM ELISA is simple to carry out and seems to be useful in identifying patients with current infection. Whether serum IgM remains high in patients with chronic giardiasis persisting for many months remains to be established, but it seems likely that this may be a diagnostic subgroup in whom the test will prove to be unhelpful.

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REFERENCES

References continued at foot of next column.