Serum anti-\textit{Giardia} IgA in human \textit{Giardia lamblia} infection

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Serum anti-\textit{Giardia} IgA titres were determined by enzyme-linked immunosorbent assay (ELISA) in 39 patients with giardiasis from the U.K. and India and in 39 \textit{Giardia}-free controls from the same locations. Anti-\textit{Giardia} IgA titres were increased in nine of 25 (36\%) of U.K. patients and four of 14 (29\%) of Indian patients. Anti-\textit{Giardia} IgA titres decreased in three patients within two to three weeks following eradication of the parasite. These findings suggest that the rise in anti-\textit{Giardia} IgA titres are relatively short-lived and thus like serum IgM titres, may be useful in the diagnosis of acute infection.

Keywords: \textit{Giardia lamblia}, giardiasis, serology, IgA.

Introduction

\textit{Giardia lamblia}, the most common human enteric protozoan pathogen, is found worldwide and is highly prevalent in warmer climates and areas of poor hygiene. \textit{Giardia} is one of the most common causes of epidemic waterborne diarrhoea in affluent societies\textsuperscript{1} although large numbers of asymptomatic carriers are found in tropical countries. Diagnosis of giardiasis generally relies on the detection of \textit{Giardia} cysts or trophozoites by microscopic examination of faeces and duodenal luminal fluid or smears prepared from duodenal biopsies. However, other approaches to diagnosis have been sought because faecal microscopy requires a skilled observer, is labour intensive and may be falsely negative despite examination of several stool specimens\textsuperscript{2}, and obtaining duodenal fluid or biopsies involve invasive procedures.

Immunodiagnostic techniques have been developed which involve either (i) detection of \textit{Giardia} antigen in faeces or (ii) measurement of serological responses to the parasite. Several groups have developed a faecal antigen ELISA with some success\textsuperscript{3,4} but as yet this is not available in routine diagnostic laboratories and remains a research tool. Serum anti-\textit{Giardia} IgG can be detected in the majority of patients with giardiasis but these cannot distinguish current from previous infection since antibody titres remain elevated for months or years after the initial infection\textsuperscript{5,6}.

We have shown, however, that serum anti-\textit{Giardia} IgM antibodies are present in

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individuals with acute giardiasis\(^2\), an observation which has recently been confirmed during experimental human *Giardia* infection\(^6\). Although serum anti-*Giardia* IgA responses have been found during human\(^1\) and experimental infection in mice\(^6\) their role in diagnosis has not been established. We have, therefore, measured serum anti-*Giardia* IgA titres in infected patients with *Giardia* infection and local controls from the U.K. and India, and made a preliminary assessment of their value in diagnosis.

**Subjects and methods**

Serum was collected from a total of 78 patients and controls. Fifty-four patients were from the UK, 25 of whom had giardiasis diagnosed by faecal or duodenal juice microscopy. The remaining 29 patients acted as controls and were patients with other gastrointestinal diseases (Crohn's disease, ulcerative colitis or coeliac disease) or were healthy hospital staff with no history of giardiasis. There were 24 patients from India with a variety of gastrointestinal disorders, 14 of whom had *Giardia* infection and 10 of whom were *Giardia* free as judged by microscopic examination of at least three faecal specimens and duodenal fluid. Serum samples were stored in aliquots at \(-20^\circ\text{C}\) until assayed.

**Anti-*Giardia* IgA ELISA**

Ninety-six well microtitre plates (Dynatech Laboratories) were sensitized with 50 \(\mu\)l suspension of approximately 20,000 *Giardia* trophozoites (Portland 1 strain) per well as described previously\(^3\). Control wells (the bottom row of each plate) were sensitized with 1% bovine serum albumin (BSA) in phosphate-buffered saline (BSA-PBS) pH 7.6. Plates were incubated at 4°C overnight (12–18 h) in a humid atmosphere. Plates were washed with PBS containing 0.5% Tween-20 (PBS-T), unreacted sites were blocked with BSA-PBS incubated at 37°C in a humid atmosphere for 60 min. BSA-PBS (50 \(\mu\)l) was pipetted into each well. Sera were thawed at room temperature, diluted 1:25 in 1% BSA, and 50 \(\mu\)l pipetted into the top and bottom wells of each row. Serial doubling dilutions were made from the top well to the seventh in each row. The sample in the bottom well was mixed and 50 \(\mu\)l removed and discarded. Each row, therefore, had serial dilutions of one serum from 1:50 to 1:3200, and an internal control well with 1:50 dilution. Each plate held a reference positive serum titrated out in the same way.

The plates were incubated at 37°C for 60 min, washed with PBS-T, and each well was filled with 50 \(\mu\)l of 1:1000 dilution of goat antihuman-IgA-peroxidase in BSA-PBS. After 60 min plates were washed then treated with substrate and tetramethylbenzidine; the reaction was stopped after 30 min with 25 \(\mu\)l sulphuric acid as described previously\(^1\). Absorbance was read with a through-the-plate ELISA reader (Microtis II, Dynatech Laboratories).

As each serum acted as its own control, the actual absorbance value (representing *Giardia*-specific antibody) was calculated as the reading for each dilution minus the reading from the well without *Giardia*, the internal control well. Results are expressed as titres, the reciprocal of the dilution with an absorbance value twice that of the control well.

**Results**

Anti-*Giardia* serum IgA titres for U.K. and Indian patients are shown in Figure 1. Serum
IgA titres in UK controls (median 0, range 0–100) were significantly lower than those of Indian controls (median 100, range 0–200; \( P < 0.01 \), Mann-Whitney U Test). Serum IgA titres were elevated in nine of 25 (36%) of U.K. patients and four of 14 (29%) of Indian patients. Of the four patients in whom pre- and post-treatment sera were available, three of the four with raised titres in the first serum samples had lower titres two to three weeks post therapy. The other patient had a 2-fold rise in titre between pre- and post-therapy levels (Figure 2).

**Discussion**

The present study shows that approximately one-third of patients with *G. lamblia* infection have serum anti-*Giardia* IgA antibodies. The prevalence of anti-*Giardia* IgA was similar in U.K. and Indian patients, although titres were lower in U.K. *Giardia*-free controls than Indian controls. These findings appear to concur at least superficially with those of Baveja and Warhurst who found that 48% of their patients with giardiasis had anti-*Giardia* IgA by an indirect immunofluorescent test. Specific IgA was also present in 43% of their *Giardia*-free controls. It might be argued that the *Giardia* IgA-positive controls in both studies had been infected previously and that serum IgA titres are, like
IgG titres to *Giardia*², long-lived. Since in three of four patients studied longitudinally there was a substantial decrease in anti-*Giardia* IgA titre after two to three weeks and in experimental human *Giardia lamblia* infection, six of ten volunteers had a serum anti-*Giardia* IgA response which was generally maximal at two to three weeks and decreased thereafter³, it is likely that serum anti-*Giardia* antibodies IgA in human giardiasis are relatively short-lived. The higher titres in the Indian controls may reflect recent exposure to *Giardia* antigens.

The presence of serum anti-*Giardia* IgA (titres > 100 for U.K. patients; titres > 200 for Indian patients) indicates current infection. These results suggest, therefore, that although detectable serum anti-*Giardia* IgA antibodies are found less commonly than IgM antibodies during natural human infection with *Giardia* they may be useful for diagnosis in a proportion of subjects when parasites cannot be detected microscopically in faeces.

Although there is considerable evidence both from experimental infections in animals and studies in humans that secretory immunity, particularly secretory IgA is important for both passive immunization of breast fed infants and for parasite clearance⁴ the relationship between serum IgA antibodies and secretory immunity has not been established. Nash *et al.*⁵, however, have clearly shown that five of the ten experimentally-

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Figure 2. Serum anti-*Giardia* IgA titres in four patients at presentation and 2-3 weeks following eradication of the parasite.
infected volunteers who developed significant rises of IgA antibodies in intestinal fluid also had specific serum IgA antibodies. These observations suggest that the presence of specific anti-Giardia IgA in the serum is a marker of the secretory responses to this parasite.

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References

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