Comparison of Stool Microscopy and Serology (Enzyme Linked Immunosorbent Assay) in Epidemiology of Amebiasis

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Abstract

Stools from 634 individuals from Varanasi were examined for *Entamoeba histolytica* (EH). Serology was done in these subjects by enzyme linked immunosorbent assay (ELISA) employing filter paper technique. Stools were positive for EH in 16.9% and serology in 15.9%. Both the tests were positive in only 5.2%. In 72.4% both the tests were negative. In 11.7% of stool positive cases, serology was negative, and in 10.7% with positive serology stool examination did not reveal EH. A majority (92.5%) of stool positive subjects had only cysts. Additional parasites were detected in 15.3%.

Key words: Amebiasis, epidemiology, enzyme linked immunosorbent assay, stool microscopy

Introduction

Amebiasis, the infection caused by *Entamoeba histolytica* (EH), has a worldwide distribution. However, a highly variable incidence (5% to 58%) has been reported in the Indian population using stool microscopy. Similarly variable figures (2% to 41%) have been reported in seroepidemiological surveys. Epidemiological studies correlating stool microscopy and serology in the same subjects are lacking. We carried out both these tests (stool microscopy and serology using ELISA) in subjects from different population groups to determine their correlation.

Material and Methods

Collection of blood and stool samples from 936 subjects residing in different localities in and around Varanasi was planned. In addition 43 food handlers from the hostel mess of Banaras Hindu University, Varanasi were also included in the study. From these 979 individuals, only 634 stool samples (compliance 65%) and 931 blood samples (compliance 93%) were collected. Among the 634 subjects, a single stool sample and blood sample were obtained on the same or next day. Stool was examined by light microscope after formalin ether concentration and staining with iodine.

Blood was collected by finger prick on Whatman No. 3 filter paper to a size of 10 mm circular spot (0.05 ml of blood). This was dried, cut and transferred to screw capped bottles containing 0.4 ml PBS (pH 7.2) and kept overnight. Serum was eluted and a dilution of 1:16 was obtained. Lyophilised axenial EH (NIH-200) was used for serological purpose. Micro-ELISA was performed as described by Voller et al. Microtitre ELISA plates (Dynatech), antigen concentration 20 µg/ml for coating of wells and a single serum dilution of 1:20, was used as described earlier and optical density at 492 nm was recorded. Optimum dilution of different reagents used was obtained by checker board titration done during standardisation of the test. Optical density > 0.153 (mean + 2SD of healthy subjects) was taken as ELISA positive.

Results

Serology was positive in 16.2% of the 931 samples collected. In the 596 subjects from the general population in whom both stools and blood samples were collected the stool positivity was 16.1% and ELISA positivity 15.3%. As compared to general population, the 38 food handlers had significantly higher stool positivity (28.9%; p < 0.05). Seropositivity was also higher (26.3%) but the difference was not statistically significant. A majority (92.5%) of stool positive subjects had only cysts (81.8% in food handlers and 93.7% in the general population). A high titre ELISA positivity (> mean + 2SD of healthy subjects) was seen in only 3.8%.

Among 634 individuals, both ELISA and stool were positive in 5.2%; both were negative in 72.4%. Stool alone and ELISA alone were positive in 11.7% and 10.7% respectively. A history of clinical amebiasis in the preceding one year was available in 18 (2.8%), in the form of amebic liver abscesses (2) and intestinal amebiasis (16). The relationship between stool positivity, seropositivity and clinical amebiasis is shown in the Table. Additional parasites were detected in the stools in 15.3%; these included *Ascaris lumbricoides* (6.8%), *Ancylostoma duodenale* (6.1%), *Trichuris trichiura* (1.1%), *Enterocephalus vermicularis* (0.8%) and *Giardia lamblia* (0.3%).
Discussion
Serology as well as stool microscopy revealed evidence of amebiasis in one sixth of the general population. However, agreement between the two tests was poor: in only 5.2% were both these tests positive, whereas serology and stool tests alone were positive in 10.7% and 11.7% respectively. This discrepancy appears to be related to the nature of amebiasis. A majority (92.5%) of stool positive subjects were cyst passers and most of these (96%) had no evidence of clinical disease. Thus most stool positive subjects (91.6%) had asymptomatic luminal or noninvasive amebiasis, where serology was positive infrequently (20.5%). Among the remaining four (4%) cyst passers with clinical amebiasis serology had high positivity (3 of 4). Likewise in subjects having stool positive for trophozoites, both ELISA positivity and clinical disease were high (87.5% and 62.5% respectively). Low incidence of invasive disease and seropositivity in asymptomatic cyst passers have also been reported earlier. In an earlier study, we had found only one third of cyst passers were ELISA positive, that too in low titre. Another reason for the high stool positivity and the discrepancy with serology might be occasional misdiagnosis of E. hartmanni as E. histolytica, as ocular micrometer was not used in this study.

The absence of E. histolytica in 67.3% of ELISA positive subjects might be due to E. histolytica infection being in the recent past, with no parasite in the colon at the time of sample collection. Moreover a single stool examination may not be sufficient to pick up the organism due to intermittent fecal excretion. Similar poor correlation between stool microscopy and seropositivity was found in studies from Venezuela and the United States, and it was concluded that E. histolytica was a commensal.

The highest number of clinical cases was recorded in subjects in whom both these tests were positive, and none when both were negative, which suggests a supplementary role for these two tests. Serology determines the overall prevalence of invasive amebiasis and stool microscopy reflects the point prevalence of intestinal infection.

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<tr>
<th>Table: Relationship of stool and seropositivity</th>
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<td><strong>Stool microscopy</strong></td>
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<tr>
<td>EH trophozoites</td>
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<td>EH cyst</td>
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<td>EH negative</td>
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<td>Total</td>
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* One case in each group had amebic liver abscess.

References