

Is the Evaluation of *Entamoeba histolytica* Infection in HIV-Positive Patients of any Clinical Significance?

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Abstract- Amoebiasis caused by *Entamoeba histolytica* (*E. histolytica*) is one of the most problematic parasitic infections worldwide. Data regarding the effect of HIV-induced immunodeficiency on the status of *E. histolytica* infection are sparse in Iran. This study aimed to assess the seroprevalence of anti-*E. histolytica* IgG among Iranian HIV patients. Further, it determined whether the advancement of immunodeficiency accompanies an increased risk of amoebiasis. A total of 91 HIV-infected patients and 91 controls were enrolled in this case-control study. Controls were matched to cases with respect to age, gender, and where possible socioeconomic status. Patients with a history of treatment for intestinal parasitism within last two weeks were not included in the study. Blood samples were obtained from all participants. Serum IgG against *E. histolytica* measured using a commercial enzyme-linked immunosorbent assay (ELISA). The mean serum anti-*E. histolytica* IgG was significantly higher in HIV patients than controls (9.34 ± 4.18 vs. 2.07 ± 0.60 , $P < 0.001$). HIV-infected patients showed a significantly higher positive serology for *E. histolytica* IgG comparing healthy controls (30.8% vs. 0% $P < 0.001$). There was no statistical difference in the serology of *E. histolytica* among AIDS stage and non-AIDS HIV patients. This study demonstrated that HIV is significantly associated with higher prevalence of *E. histolytica* infection. Early evaluation and treatment of *E. histolytica* in this population is recommended to prevent and control this infection.

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Introduction

Amoebiasis caused by *Entamoeba histolytica* (*E. histolytica*) is one of the most problematic parasitic infections around the world particularly in developing countries and where contamination of food and water is high (1-3). Invasive amoebiasis is reported as the second most common cause of mortality from parasitic infections worldwide, accounting for about 100,000 deaths annually (1,3-5).

Although the high degree of diversity exists among regional *E. histolytica* strains, no major differences have been detected between regional genotypes (3). The reasons for this geographic variation are unclear, but it is likely linked to the higher background prevalence of *E. histolytica* infections in some areas especially in the Asia-pacific region (6).

Members of all age groups and both sexes are infected. The risk of infection increases with inadequate sanitary conditions (1,2,7). An increased prevalence of amoebiasis is reported among people, who have an increased risk of exposure in the agricultural occupations.

The life cycles of *E. histolytica* consist of an infective stage (cysts) and a multiplying trophozoite stage (2). The cysts excreted in the stool, are sturdy and resistant to adverse environmental conditions (1,2,6). After ingestion by a susceptible host, the cyst divides serially through three cycles in a small intestine and produces eight motile trophozoites (1,6,7). The motile trophozoites invade the tissues of the large intestine and may erode them so extensively that they gain entrance into the blood stream. Extra intestinal amoebiasis can afflict any organ or tissue (1,8).

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Clinical presentation of *amoebiasis* ranges from asymptomatic colonization to amoebic colitis including dysentery or diarrhea, and invasive extra-intestinal *amoebiasis* which is commonly seen in the form of liver abscess (1,2,9). Although patients may experience a wide range of manifestations, the majority of infected individuals are free of symptoms. Approximately 90% of patients with asymptomatic *E. histolytica* infection may excrete the infective stages of the organism (cysts) and get clear from the infection within 12 months. This highlights the importance of accurate diagnosis particularly in developing countries with poor hygienic measures (1,2).

Infection may be diagnosed by microscopic stool examination (Iron Haematoxylin Method, Merthiolat Iod Formol Concentration), or based on serological methods (CF, CIE, ELISA) (7).

Impaired host immunity is known to be associated with increased pathogenicity of invasive *amoebiasis*. Recent investigations caution about increased risk of invasive *amoebiasis* among HIV patients (1,7). Recent studies also indicate a higher prevalence of *amoebiasis* among HIV-infected men who have sex with men (10-13).

The prevalence of HIV infection has been rapidly increasing in developing countries during the past decades. Opportunistic infections and invasive parasitism associated with higher morbidity among this population, and emerged as a major health concern. Regarding limited data on the effect of HIV-induced immunodeficiency on the prevalence of *amoebiasis* in Iran; this study aimed to assess the seroprevalence of anti-*E. histolytica* IgG among Iranian HIV patients. Further, it determined whether the advancement of immunodeficiency accompanies an increased risk of *amoebiasis*.

Materials and Methods

Participants

A total of 91 consecutive patients with documented history of non-hemophilic HIV infection, referring to Imam medical center, Tehran, Iran, affiliated to Tehran University of medical science (TUMS) were recruited between Feb. 2012 and Feb. 2013. Additionally, ninety-one HIV negative individuals attending our laboratory for routine checkup were considered as controls. Controls were individually matched to cases with respect to age, sex, and where possible residential area. Diagnosis of HIV infection confirmed with serology, PCR or western-blot following the recommendation of

national AIDS control organization (NACO 2007).

Individuals with pregnancy, autoimmune diseases, malignancy or hematological disorders were not included in the study. Participants with a history of treatment for intestinal parasitism within last two weeks were also excluded.

Demographic characteristics including age, gender, residence area, and educational status were recorded from subjects' medical records. The study protocol was approved by the ethics committee of TUMS according to the declaration of Helsinki, and the written informed consent was taken from all participants.

Blood sampling

Blood samples were obtained from all participants and stored within ethylene diamine tetra acetate (EDTA) containers and stored at -70 c until tested. Serum IgG against *E. histolytica* was measured with Commercial enzyme-linked immunosorbent assay (ELISA, IBL international GMBH, Hamburg, Germany kit) with an intra-assay coefficient variants (CV) of 2.1%, and inter-assay CV of 3.9%. The laboratory kits' diagnostic sensitivity and specificity were both > 95%.

Based on the laboratory kit's instruction, the cut-off value of ≥ 11 (U) representing seropositive subjects.

Complete blood count was performed on blood samples anticoagulated with EDTA (Sysmex-K21, Japan). CD4+ and CD8+ T lymphocytes were counted by flow cytometry device (FCM, PARTEC, Japan). HIV patients with CD4 count below 200 cells/ml or specific clinical conditions suggestive of advanced immunodeficiency infection were categorized as AIDS stage (14).

Statistical analysis

Statistical analysis was performed using Stata software version 11 for Windows (Stata Corporation, College station, Texas, United States). The results were expressed as mean \pm SD or proportion (%), were appropriate. The statistical differences between proportions were determined by *Chi-square* test. Independent sample t-test was used to determine if there were any differences in continuous variables between two groups. The distribution of *E. histolytica* IgG among HIV patients and controls were shown using dot plot graph. *P*-value < 0.05 was considered as statistical significant.

Results

Table 1 represents the demographics and clinical

E. histolytica in HIV patients

characteristics of HIV patients and controls. There were no significant difference regarding the age, gender,

residence, and educational status between HIV patients and controls.

Table1. Demographic and Laboratory Characteristics of HIV Patients and Controls

	HIV Patients (n=91)	Controls (n=91)	P-value
Age (years)	37.40± 10.50	36.24± 9.81	0.562
Male (%)	70 (76.92%)	63 (69.23%)	0.158
Residence (%)			
Urban	68 (74.7%)	64 (70.3%)	0.309
Rural	23 (25.3%)	27 (29.7%)	
Educational status (%)			
Illiterate	3 (3.3%)	4 (4.4%)	0.499
Up to high school	45 (49.5%)	35 (38.5%)	
Diploma	28 (30.8%)	32 (35.5%)	
Higher levels	15 (16.5%)	20 (22%)	
BMI (kg/m ²)	24.70± 4.19	26.29± 4.71	0.013
CD4 count (/μl)	298.24± 200.21	1013.6± 271.76	<0.001
CD8 count (/μl)	725.73± 379.30	873.32± 209.54	0.001
Anti <i>E. histolytica</i> IgG (U)	9.34 ± 4.18	2.07 ± 0.60	<0.001
<i>E. histolytica</i> sero-positive rate (%)	28 (30.8%)	0 (0%)	<0.001

The mean value of serum anti-*E. histolytica* IgG was significantly higher in HIV patients than control individuals ($P<0.001$). Figure 1 show the distribution of serum anti-*E. histolytica* IgG in HIV Patients and Controls. Twenty (30.8%) out of 91 HIV patients were

seropositive for anti-*E. histolytica* infection. None of the control individuals were seropositive for this antibody ($P<0.001$).

Horizontal line indicates the cut-off value for detecting seropositive subjects.

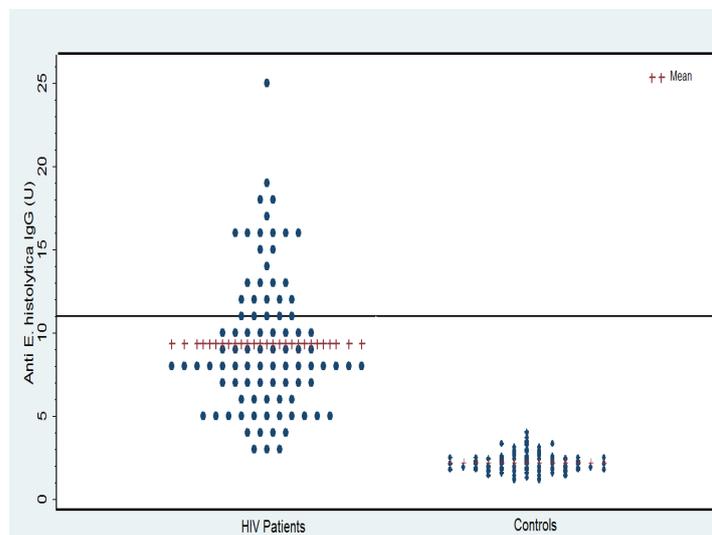


Figure 1. Serum Anti *E. histolytica* IgG Distribution in HIV Patients and Controls

Age, gender, residential area, educational level, and CD4+ cell count did not show a statistical association with serum *E. histolytica* IgG in both HIV and control groups.

Among 91 HIV patients, 65 were in AIDS stage.

There were no statistical difference in the positive serology and the mean anti-*E. histolytica* IgG between AIDS stage comparing non-AIDS HIV patients (29.2% vs. 34.6%, $P=0.62$ and 9.06 ± 3.85 vs. 9.81 ± 4.74 , $P=0.41$).

Discussion

Parasitic infections remain an important cause of morbidity and mortality in developing countries especially among immunodeficiency patients including HIV-infected individuals (2,8). Although HIV infection is not highly frequent in Middle East, it is rapidly spreading in these countries. Following the distribution of HIV/AIDS around the world, numerous studies reported that intestinal parasites such as *Cryptosporidium sp*, *microsporidia sp*, *Isospora belli*, and *Cyclosporacayetenesis* were frequently associated with episodes of severe diarrhea in both developing and developed countries (15-17). Whether HIV infection has an impact on the occurrence of *amoebiasis* remained controversial. Recent studies have revealed an increased prevalence of *E. histolytica* infection among HIV-infected patients in countries such as Taiwan, Japan, Mexico, China, South Africa, and Ethiopia (11,17-22).

Increased risk of *amoebiasis* is also reported in HIV-infected patients particularly among men who have sex with men in countries like Taiwan, Japan, South Korea, Australia, and China (10-13,23).

According to a molecular-based study, *E. histolytica* infection is rare in Iran. In the present study, all the controls were seronegative. Although *E. histolytica* infection is extremely rare in healthy individuals, it has a high sero-prevalence in HIV-infected patients (30.8%). As the prevalence of *amoebiasis* depends on the socioeconomic status of the population, in this study we tried to match the cases and controls regarding gender, age, residence, and educational level status.

Present study also revealed a higher seroprevalence for *E. histolytica* in HIV patients than the reports in Taiwan (7.1%) (22), China (12.1%) (21), and Australia (5.13%) (13).

Present study also categorized HIV patients into AIDS and non-AIDS stages based on the CD4 cell count (14), and found no significant difference in serum anti-*E. histolytica* IgG between two groups. Study performed in Mexico City (18), report a higher prevalence of *E. histolytica* in AIDS patients compared to control HIV group (25.5% vs. 18.4%) which failed to reveal statistical significance. In contrast to these results, higher seropositivity in HIV-infected patients with CD4 cell < 200 / μ l were reported in some studies in China, Ethiopia (17, 21)

This study did not find any considerable association between gender and *E. histolytica* IgG, which was in accordance with previous studies performed by Chen *et al.* (21) and Zali *et al.* (15).

Prevalence of *E. histolytica* shows variations according to different methods of parasite detection. Among multiple serological methods, ELISA is a reliable, easy to perform and rapid method for the diagnosis of *E. histolytica* infection. An ELISA to detect antibody to *E. histolytica* has been shown to be 97.9% sensitive and 94.8% specific for detection of *E. histolytica* antibody in *amoebic* liver abscess patients in a non-endemic country (24). As anti *E. histolytica* IgG is more prevalent in the invasive forms of *amoebiasis* than non-invasive intestinal forms and healthy carriers, high seroprevalence of this infection in the HIV-infected patients suggests that *amoebiasis* in these patients is more invasive.

Study performed by Moran P *et al.*, (18) was concluded that both AIDS and HIV groups who were infected with *E. histolytica* were asymptomatic cyst passers and suggested that the prevalent *E. histolytica* strains in their community are of low pathogenic potential. Present study did not assess the clinical symptoms of the participants. Small size of the study population is another limitation that could affect the results.

This study indicated that the positive *E. histolytica* serology is significantly more common in HIV-infected patients. Early evaluation and treatment of *E. histolytica* in this population is recommended to prevent and control this infection.

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