Clinical Significance of High Anti-Entamoeba histolytica Antibody Titer in Asymptomatic HIV-1-infected Individuals

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**Background.** Anti-Entamoeba histolytica antibody (anti-E. histolytica) is widely used in seroprevalence studies though its clinical significance has not been assessed previously.

**Methods.** Anti-E. histolytica titer was measured at first visit to our clinic (baseline) in 1303 patients infected with human immunodeficiency virus type 1 (HIV-1). The time to diagnosis of invasive amebiasis was assessed by Kaplan-Meier method and risk factors for the development of invasive amebiasis were assessed by Cox proportional-hazards regression analysis. For patients who developed invasive amebiasis, anti-E. histolytica titers at onset were compared with those at baseline and after treatment.

**Results.** The anti-E. histolytica seroprevalence in the study population was 21.3% (277/1303). Eighteen patients developed invasive amebiasis during the treatment-free period among 1207 patients who had no history of previous treatment with nitroimidazole. Patients with high anti-E. histolytica titer at baseline developed invasive amebiasis more frequently than those with low anti-E. histolytica titer. Most cases of invasive amebiasis who had high anti-E. histolytica titer at baseline developed within 1 year. High anti-E. histolytica titer was the only independent predictor of future invasive amebiasis. Anti-E. histolytica titer was elevated at the onset of invasive amebiasis in patients with low anti-E. histolytica titer at baseline.

**Conclusions.** Asymptomatic HIV-1-infected individuals with high anti-E. histolytica titer are at risk of invasive amebiasis probably due to exacerbation of subclinical amebiasis.

**Keywords.** seroprevalence; Entamoeba histolytica; HIV-1; anti-E. histolytica antibody; amebiasis.

Invasive amebiasis caused by Entamoeba histolytica is the second most common cause of parasite infection-related mortality worldwide, accounting for 40 000–100 000 deaths annually [1]. Recently, it was reported that invasive amebiasis is prevalent not only in developing countries where food or water is contaminated with stool, but also in East Asian developed countries (Korea, China, Taiwan and Japan) and Australia as a sexually transmitted infection (STI) [2–4]. On the other hand, the annual incidence of human immunodeficiency virus type 1 (HIV-1) infection is also on the rise among men who have sex with men (MSM) in these countries [5–8], with resultant growing concern regarding invasive amebiasis in HIV-1-infected MSM [9–14].

Serum anti-E. histolytica antibody (anti-E. Histolytica) is widely used as an index marker for the presence of amebiasis. It is used not only in developing countries [15–22] but also in developed countries where amebiasis is spreading as an STI [3, 9, 23–26]. Furthermore, the seroprevalence of anti-E. histolytica antibody in HIV-1-infected individuals is generally higher than in HIV-1 negative ones [3, 9, 15, 24]. However, only limited information is available on the seroprevalence of amebiasis in Japan [25, 26] despite the increasing number of invasive amebiasis among HIV-1-infected individuals reported lately [27, 28].
Serum anti-\textit{E. histolytica} antibody is also widely used for the diagnosis of invasive amebiasis based on the high sensitivity and good differentiation ability from other amoeba species, such as \textit{Entamoeba dispar} and \textit{Entamoeba moshkovskii} [29]. However, the primary disadvantage of this method is that it cannot distinguish current infection from past infection. Moreover, anti-\textit{E. histolytica} antibody titer can be elevated even in asymptomatic infected individuals, and seroconversion of anti-\textit{E. histolytica} was reported in the absence of any symptoms in longitudinal follow-up in endemic areas [14]. At present, the pathogenesis of amebiasis in asymptomatic anti-\textit{E. histolytica} -positive individuals remains poorly understood.

In the present study, we found high seroprevalence of anti-\textit{E. histolytica} antibody in HIV-1-infected adult Japanese. Retrospective analysis of these seropositive individuals indicated that those with high anti-\textit{E. histolytica} titer are prone to future invasive amebiasis. These findings highlight the clinical significance of anti-\textit{E. histolytica} positivity and enhance our understanding of the pathogenesis of invasive amebiasis.

**MATERIALS AND METHODS**

**Ethics Statement**
This study was approved by the Human Research Ethics Committee of our hospital, the National Center for Global Health and Medicine, Tokyo. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

**Study Design and Population**
The present study was a single-center retrospective cohort study. Our facility is one of the largest core hospitals for patients with HIV-1 infection in Japan, with >3000 registered patients. The study population was HIV-1-infected patients who were referred to our hospital for management of HIV-1 infection for the first time between January 2006 and April 2012.

**Anti-\textit{E. histolytica} Antibody Testing**
Indirect fluorescent-antibody (IFA) assay was used for the detection of anti-\textit{E. histolytica} antibody in serum by using a slide precoated with fixed \textit{E. histolytica}. This method can distinguish amebiasis caused by \textit{E. histolytica} from that caused by other amoeba species, such as \textit{E. dispar} and \textit{E. moshkovskii}. The sensitivity and specificity of this method for the detection of \textit{E. histolytica} infection are comparable with other methods, such as counterimmunoelectrophoresis and indirect hemagglutination amebic serology [29, 30]. The commercial kit, Amoeba-Spot IF (bioMerieux SA), is currently approved for the diagnosis of \textit{E. histolytica} infection in Japan. Based on the instructions enclosed with the kit, the biological samples were initially diluted at 1:100 with phosphate-buffered saline (PBS) and then incubated for 30 minutes at room temperature on slides precoated with fixed \textit{E. histolytica}. Then, the slides were washed with PBS twice, treated with the fluorescent-labeled anti-human antibodies, and incubated for another 30 minutes at room temperature. The slides were washed again, and cover slips with buffered glycerol were placed over the slides. Fluorescence in each slide was examined with fluorescence microscope and compared with negative control slides. Seropositivity was defined as positive response in serum sample diluted at 1:100, and anti-\textit{E. histolytica} titer was determined by the highest dilution for the positive response.

**Development of Invasive Amebiasis in Patients Without History of Nitroimidazole Treatment**
Newly registered HIV-1-infected individuals who underwent anti-\textit{E. histolytica} testing at first visit were included in this analysis. Patients were excluded from the follow-up study (1) if they had been treated previously with nitroimidazole (metronidazole or tinidazole) or (2) if they were treated with nitroimidazole at first visit to the clinic. The clinical characteristics and results of serological tests for other STIs, such as syphilis and hepatitis B and C viruses (HBV and HCV), were collected from the medical records. The follow-up period spanned from the time of the first visit to May 2012, unless patients died from other causes during this period, dropped out, or were referred to other facilities.

The diagnosis of invasive amebiasis was based on the medical records of 3 different clinicians and satisfied one of the following 2 criteria, as described elsewhere [12–14]: (1) identification of erythrophagocytic trophozoites in biological specimens (stool or biopsy sample) of HIV-1-infected patients with symptoms of invasive amebiasis, such as fever, tenesmus, and diarrhea, (2) identification of liver abscess by imaging studies in seropositive (titer ≥100) patients with symptoms related to invasive amebiasis who showed clinical improvement after nitroimidazole monotherapy. For patients who developed invasive amebiasis during follow-up, we compared anti-\textit{E. histolytica} titer at the time of onset of invasive amebiasis with those at first visit (baseline) and after nitroimidazole therapy.

**Statistical Analysis**
The patients’ characteristics and results of serological tests on STIs were compared using \(\chi^2\) test or Student \(t\) test for qualitative or quantitative variables, respectively. The time to the diagnosis of invasive amebiasis was calculated from the date of the first visit of our hospital to the date of diagnosis of invasive amebiasis. Censored cases represented those who died, dropped out, or were referred to other facilities during the follow-up. The time from first visit to the diagnosis of invasive amebiasis was calculated by the Kaplan-Meier method followed by log-rank test to determine the statistical significance. The Cox proportional-hazards regression analysis was used to estimate the impact of anti-\textit{E. histolytica} titer at baseline on the incidence of invasive amebiasis. The impact of basic clinical characteristics,
such as sexuality and serology status of other STIs, was estimated with univariate Cox proportional hazards regression. We also conducted multivariate Cox hazards regression analysis using variables identified in univariate analysis with P values of < .20. In all analyses, statistical significance was defined as 2-sided P value of < .05. We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the impact of each variable on the development of invasive amebiasis. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

RESULTS

Clinical Characteristics of Asymptomatic Anti- \textit{E. histolytica}-positive HIV-1-infected Patients

A total of 1519 patients were referred to our hospital during the study period. Anti- \textit{E. histolytica} testing was conducted in 1303 patients at first visit, including 73 with history of invasive amebiasis, and anti- \textit{E. histolytica} was positive in 277 of these (21.3%). Among the anti- \textit{E. histolytica}-positive individuals, the rates of MSM (88.4%) and those with previous exposure to syphilis (TPHA test positive) (54.9%) and HBV (68.8%) were higher than those of anti- \textit{E. histolytica}-negatives individuals, indicating that sexually active MSM are prone to \textit{E. histolytica} infection among HIV-1-infected individuals in Japan (Table 1).

Eight patients were diagnosed with invasive amebiasis at first visit, including 7 cases of amebic colitis and 1 case of amebic liver abscess, and they were treated immediately with metronidazole.

Incidence of Invasive Amebiasis During Follow-up of HIV-1 Infected Individuals

To assess the frequency of development of invasive amebiasis in patients free of symptomatic invasive amebiasis and who had not previously received nitroimidazole therapy, we excluded 96 patients from the analysis, including 73 patients because they had been treated previously for invasive amebiasis, and 23 patients (7 cases of amebic colitis, 1 case of amebic liver abscess, and 15 asymptomatic but anti- \textit{E. histolytica}-positive cases treated preemptively) because they were treated with nitroimidazole at first visit (Figure 1). The remaining 1207 patients, including 195 anti- \textit{E. histolytica}-positive patients (16.2%), were followed-up for median period of 25.3 months (interquartile range: 7.0–47.2). During the follow-up period, 18 patients developed invasive amebiasis (median time to onset: 9.1 months), including amebic appendicitis in 1 patient.

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<th>Table 1. Characteristics of All Patients Who Underwent Anti- \textit{E. histolytica} Testing (n = 1303)</th>
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<td>Age, years (range)</td>
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<td>Japanese nationality, no. (%)</td>
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<td>Male sex, no. (%)</td>
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<td>MSM, no. (%)</td>
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<td>TPHA test positive, no. (%)</td>
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<td>HBV exposure, no. (%)</td>
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<td>HCV Ab positive, no. (%)</td>
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<td>Past history of IA, no. (%)</td>
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<td>Diagnosis of IA at first visit, no. (%)</td>
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Abbreviations: Ab, antibody; Anti- \textit{E. histolytica}, anti- \textit{Entamoeba histolytica} antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; MSM, men who have sex with men; TPHA, \textit{Treponema pallidum} hemagglutination.

a HBV exposure: HBsAg-positive or HBsAb-positive, and/or HbcAb positive.

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cases include one case of appendicitis and 6 cases of liver abscess.

P. histolytica titers at baseline, compared with negative anti-
P. histolytica evidence in patients with ×100 (P≤ .001), ×800 (×200 at baseline) than in the low anti-
P. histolytica titer group (patients with anti-
P. histolytica ≥×400 at baseline) or in the high anti-
P. histolytica titer group (patients with anti-
P. histolytica ≥×400 at baseline). Moreover, most patients of the high anti-
P. histolytica (confirmed by identification of erythrophagocytic trophozoites in surgically removed specimen), amebic liver abscess in 6, and amebic colitis in 11 (confirmed by identification of erythrophagocytic trophozoites in stool samples). The median anti- 
P. histolytica titer at baseline was significantly higher among patients who developed invasive amebiasis than that among those who did not, but the other clinical and laboratory parameters were not different between the 2 groups (Table 2). Although no significant differences in the frequency of invasive amebiasis were evident in patients with ×100 (P = .77) and ×200 (P = .18) anti-
P. histolytica titers at baseline, compared with negative anti-
P. histolytica patients (<×100), the frequency was higher in patients with ×400 (P < .001), ×800 (P = .025), and ≥×1600 (P < .001) anti-
P. histolytica titers at baseline, compared with negative anti-
P. histolytica patients. Univariate and multivariate analyses also showed that future development of invasive amebiasis correlated only with high titer of anti-
P. histolytica antibody at baseline (≥×400: Univariate, HR: 20.985, 95% confidence interval [CI], 8.085–54.467; multivariate, HR: 22.079, 95% CI, 7.964–61.215) (Table 3). Furthermore, the risk of development of invasive amebiasis was significantly higher in the high anti-
P. histolytica titer group (patients with anti-
P. histolytica titer ≥×400 at baseline) than in the low anti-
P. histolytica titer group (patients with anti-
P. histolytica titer ≤×200 at baseline; log-rank test: χ² = 80.203, P < .001, Kaplan-Meier estimate, Figure 2). Moreover, most patients of the high anti-
P. histolytica

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<th>Table 2. Comparison of Clinical Characteristics of Patients With and Without Invasive Amebiasis</th>
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<td>Age (years), average (SD)</td>
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<td>TPHA test-positive, no. (%)</td>
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<td>HBV exposure, a no. (%)</td>
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<td>HCVAb-positive, no. (%)</td>
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<td>Anti-P. histolytica at baseline, median (IQR)</td>
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<td>Anti-P. histolytica at the onset of IA, median (IQR)</td>
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<td>Follow-up period, median months (IQR)</td>
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Data were compared using χ² test, Student t test, or Mann–Whitney U test for qualitative or quantitative variables, respectively.

Abbreviations: Ab, antibody; Anti-P. histolytica, anti Entamoeba histolytica antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; IA, invasive amebiasis; IQR, interquartile range; MSM, men who have sex with men; SD, standard deviation; TPHA, Treponema pallidum hemagglutination.

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<th>Table 3. Risk Analysis for Development of Invasive Amebiasis by Cox Proportional Hazard Regression Model</th>
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<td>HCVAb-positive</td>
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<td>Anti-P. histolytica titer ≥×400</td>
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The Cox proportional-hazards regression analysis was used to estimate the impact of anti-P. histolytica titer at baseline on the incidence of invasive amebiasis. The impact of basic clinical characteristics, such as sexuality and serology status of other STIs, was estimated with univariate Cox proportional hazards regression. Multivariate Cox hazards regression analysis using variables identified in univariate analysis with P values of < .20. In all analyses, statistical significance was defined as P value of < .05.

Abbreviations: Ab, antibody; Anti-P. histolytica, anti Entamoeba histolytica antibody; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; IA, invasive amebiasis; IQR, interquartile range; MSM, men who have sex with men; TPHA, Treponema pallidum hemagglutination.
Titer group developed invasive amebiasis during the first year of follow-up, whereas those of the low anti-E. histolytica titer group developed this complication more lately and new cases of invasive amebiasis were diagnosed throughout the follow-up period.

**DISCUSSION**

In the present study, the seroprevalence of anti-*E. histolytica* antibody among HIV-1-infected patients was 21.3%, which was much higher than those reported in other developed countries where amebiasis is considered as an STI [3, 9, 23, 24]. In addition, our results showed that sexually active MSM tend to be seropositive for *E. histolytica* infection, in agreement with previous studies from our group [27, 28].

The pathogenesis of amebiasis, such as incubation period after cyst ingestion and the mechanism of spontaneous remission, remains unclear. Although previous study showed anti-*E. histolytica*-positive children were more susceptible to *E. histolytica* infection than their seronegative counterparts [31], the clinical significance of anti-*E. histolytica* seropositivity and its titer in asymptomatic individuals had not been fully assessed. We measured serum anti-*E. histolytica* immunoglobulin M (IgM) levels in 18 patients at the onset of invasive amebiasis [32], but the level was detectable only in 3 patients with amebic colitis and 1 patient with liver abscess. The present study demonstrated that patients with high anti-*E. histolytica* titer (≥x400) at first visit developed invasive amebiasis much more frequently than those with low anti-*E. histolytica* titer (≤x200). The cumulative risk for invasive amebiasis among patients with high anti-*E. histolytica* titer at baseline rapidly increased during the first one year of follow-up but plateaued thereafter, suggesting that exacerbation of subclinical amebiasis occurs frequently within one year in these patients. On the other hand, the cumulative risk for invasive amebiasis among patients with low anti-*E. histolytica* titer at baseline increased more slowly and
developed at the same pace throughout the follow-up period, suggesting that the invasive amebiasis in these patients represented new infection rather than exacerbation of subclinical infection. The median anti-\textit{E. histolytica} titer at the onset of invasive amebiasis in patients of high anti-\textit{E. histolytica} titer group was not higher than that at first visit, whereas the titer increased at the onset compared with that at baseline in low anti-\textit{E. histolytica} titer group. In addition, uni- and multivariate analyses identified high titer of anti-\textit{E. histolytica} antibody at baseline as the only significant risk factor for future development of invasive amebiasis; seropositivity to other STIs was not a significant factor. These results add support to the aforementioned hypothesis regarding the difference in the pathology of invasive amebiasis between the high and low anti-\textit{E. histolytica} groups. In this study, 15 asymptomatic but anti-\textit{E. histolytica}-positive patients were treated with metronidazole at first visit (excluded from the follow-up analysis study), and none of them developed invasive amebiasis (median follow-up period, 11.7 months), suggesting the potential effectiveness of preemptive therapy for asymptomatic individuals with high anti-\textit{E. histolytica} titer.

In conclusion, our results showed a relatively high prevalence of amebiasis in HIV-1-infected individuals in Japan, and that subclinical amebiasis is common among these individuals. The results emphasize the difficulty of disease control in not only individual patients with amebiasis but also in epidemiological control of this condition due to the long duration of subclinical infection of \textit{E. histolytica}. Anti-\textit{E. histolytica} testing for high-risk individuals could be helpful in early diagnosis of subclinical amebiasis, and early treatment of patients with such infection could prevent the development of invasive amebiasis and the transmission to others in the same community. Further studies to clarify the pathogenesis of invasive amebiasis are warranted.

Notes

Acknowledgments. We thank all clinical staff at the AIDS Clinical Center for their help in the completion of this study.

Financial support. This work was supported by a grant from the Ministry of Health, Labor, and Welfare of Japan (H25-promotion-general-014).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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