Invasive Amebiasis as an Emerging Parasitic Disease in Patients With Human Immunodeficiency Virus Type 1 Infection in Taiwan

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Background: Whether risk of invasive amebiasis due to *Entamoeba histolytica* is higher among human immunodeficiency virus (HIV)–infected persons than uninfected persons remains unclear, although intestinal colonization by *Entamoeba dispar* is common among men who have sex with men. Our objective was to determine the prevalence of invasive amebiasis and intestinal colonization by *E histolytica* and *E dispar* in HIV-infected persons and uninfected controls.

Methods: We assessed the prevalence of invasive amebiasis by case review of 951 HIV-infected persons and by serologic studies of 634 of the 951 HIV-infected persons, 429 uninfected controls with gastrointestinal symptoms, and 178 uninfected healthy controls using indirect hemagglutination antibody assay. We assessed the rate of intestinal colonization by *E histolytica* and *E dispar* by fecal antigen and polymerase chain reaction tests in 332 asymptomatic HIV-infected persons and 144 of the 178 uninfected healthy controls.

Results: Forty-nine (5.2%) of 951 HIV-infected persons had 51 episodes of invasive amebiasis. A high indirect hemagglutination antibody titer was detected in 39 (6.2%) of 634 HIV-infected persons compared with 10 (2.3%) of 429 uninfected controls with gastrointestinal symptoms and 0 of 178 uninfected healthy controls ($P<.001$). Stool specimens from 40 (12.1%) of 332 HIV-infected persons and 2 (1.4%) of 144 uninfected healthy controls were positive for *E histolytica* or *E dispar* antigen ($P<.001$). Ten (25.0%) of the 40 antigen-positive stool specimens from HIV-infected persons contained *E histolytica*.

Conclusion: Persons infected with HIV in Taiwan are at increased risk for invasive amebiasis and exhibit a relatively high frequency of elevated antibody titers and intestinal colonization with *E histolytica*.

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IT IS ESTIMATED THAT 20% TO 30% of homosexual men are colonized with *Entamoeba dispar* in Western countries.[1-5] This is attributed to oral-anal sex practices.[2,3] Despite the high rates of colonization with *E dispar*, invasive amebiasis due to *Entamoeba histolytica*, with amebic colitis or liver abscess, has rarely been reported in homosexual men or persons infected with human immunodeficiency virus (HIV) in Western countries. The discrepancy between colonization and diseases is because the isolates of *E dispar* are nonpathogenic[2,4-9] and tend to spontaneously disappear.[8] The HIV-infected persons with diarrheal disease who are colonized with *E dispar* are commonly coinfected with other intestinal pathogens, such as cytomegalovirus, *Giardia lamblia*, *Cryptosporidium parvum*, microsporidia, and *Shigella* species. These microorganisms could readily account for the diarrhea.[2,9-11]

There seem to be major geographic differences in the prevalence of invasive amebiasis in homosexual men and HIV-infected persons. Investigators in Japan and Italy found a higher prevalence of antibody to *E histolytica* in homosexual men with or without HIV infection.[12-15] Case reports[16-22] from various countries describe invasive amebiasis in homosexual men with or without HIV infection. Recent reports from Taiwan[23] and Korea[24] describe cases of invasive amebiasis in homosexual men. In many of these men, invasive amebiasis was the initial illness for HIV infection.[23] A likely explanation for differences between the Asia-Pacific region and Western countries is the higher prevalence of *E histolytica* infection among people living in the Asia-Pacific region. To better understand the reason for the increase in invasive amebiasis among HIV-infected persons in Taiwan, we conducted a 9-year retrospective case review

of HIV-infected persons and 2-year prospective serologic and parasitologic investigations among HIV-infected persons and uninfected controls. We show that invasive amebiasis, high titers of indirect hemagglutination antibody (IHA), and fecal colonization with *E histolytica* are relatively common in homosexual men infected with HIV in Taiwan.

METHODS

RETROSPECTIVE CASE REVIEW AND SEROPREVALENCE STUDIES

The medical records of 951 consecutive, nonhemophilic, HIV-infected persons 15 years or older seen at the National Taiwan University Hospital between June 1, 1994, and December 31, 2003, were reviewed. A standardized form was used to record demographic information, HIV status, laboratory data, and the presence of invasive amebiasis. The comparison groups consisted of 429 HIV-uninfected persons who sought medical attention because of gastrointestinal symptoms and 178 HIV-uninfected healthy controls. These groups were enrolled between November 1, 2001, and December 31, 2003. The former controls were identified because their blood specimens had been submitted to the parasitology laboratory for *E histolytica* IHA testing and included 268 males and 161 females with a median age of 56 years (range, 15-93 years). The latter control group included 87 men and 91 women, with a median age of 24 years (range, 20-69 years).

A standardized protocol was used to investigate the etiologic diagnosis of gastrointestinal symptoms among cases.[25] In brief, at least 2 stool specimens were obtained for bacterial cultures for patients with diarrhea. Concentrated wet mount preparations of stool were examined by direct microscopy. Fecal smears were stained with modified acid-fast stain. Colonoscopy and biopsy for histopathologic examination were performed when routine examinations of the stool specimens did not yield positive results. The IHA assays (Cellognostics; Boehringer Diagnostics GmbH, Marburg, Germany) were performed according to the manufacturer's instructions. Abdominal sonography was performed for patients with abnormal liver function test results. Sonography-guided aspiration biopsy was performed if a space-occupying lesion of the liver was detected. The aspirate of the liver was submitted for histopathologic examination, culture, and IHA assay.

A patient was considered to have definite invasive amebiasis when erythrophagocytic trophozoites or a positive polymerase chain reaction (PCR) for *E histolytica* was identified in clinical specimens from patients with colitis and liver abscess.[22] A patient with consistent clinical findings was considered to have probable invasive amebiasis when the aspirates or blood specimens showed high IHA titers and when findings from microbiologic cultures or histopathologic examination of aspirates and biopsy specimens were negative and the patient responded to metronidazole monotherapy. The IHA assay result was considered positive if the titer was 1:128 or greater. AIDS was defined according to the 1993 revised classification system for HIV infection and the expanded surveillance case definition for AIDS among adolescents and adults.[26] Highly active antiretroviral therapy was defined as antiretroviral therapy containing 2 nucleoside reverse transcriptase inhibitors and a protease inhibitor(s) or a nonnucleoside reverse transcriptase inhibitor.

PARASITOLOGIC INVESTIGATIONS

Stool specimens were prospectively collected from 332 asymptomatic HIV-infected persons (310 men; median age, 37 years; age range, 17-80 years) and 144 of the 178 uninfected healthy controls (83 men; median age, 27 years; age range, 20-69 years). The study was conducted between November 1, 2001, and December 31, 2003. The *E histolytica* and *E dispar* antigens were detected using the *Entamoeba* test (TechLab, Blacksburg, Va) according to the manufacturer's instructions.

Stool specimens with positive test results for *E histolytica* or *E dispar* antigen were examined by PCR to detect *E histolytica*. The protocol was adapted from Boom et al[27] and Walsh et al[28] with modifications. Total DNA was isolated from the stool samples using diatom beads in the presence of guanidine thiocyanate. Approximately 0.5 g of fresh stool was mixed into 2.5 mL of 5.3M guanidine thiocyanate. The tube was vigorously agitated for 10 minutes in a cell disruptor (FastPrep FP120 and BIO 101; Qbiogene Inc, Irvine, Calif) at 5.5 m/s. After vortexing, the sample solution was clarified by centrifugation for 5 minutes at 20000*g*. A total of 450 µL of the supernatant was aliquoted and incubated in 50 µL of 10% Nonidet P-40 (Calbiochem-Novabiochem Corp, San Diego, Calif) for 10 minutes at room temperature. The mixture can be stored at –20°C, or DNA can be extracted directly. To the mixture (500 µL) was added 50 µL of diatom suspension, which is made of 10 g of Celite 545 (Merck KGaA, Darmstadt, Germany) in 50 mL of water and 0.5 mL of 32% hydrochloride. The mixture was incubated for 10 minutes, with continuous shaking and mixing, at room temperature. The diatom pellets were collected by centrifugation for 2 minutes at 14000*g* and were washed with 1 mL of 9.3M guanidine thiocyanate solution once, 1 mL of 70% ethanol twice, and 0.2 mL of acetone once. The diatom with bound DNA was dried for 10 minutes in dry bath at 60°C. The DNA was then washed off by incubating the diatom in 200 µL of preheated (60°C) TE buffer (10mM Tris hydrochloride, 1mM EDTA, pH 8.0) for 10 minutes. The supernatant containing DNA was recovered after centrifugation for 3 minutes at 20000*g*. To the supernatant was added 100 µL of 10% Chelex 100 resin (Bio-Rad Laboratories, Inc, Hercules, Calif) in TE buffer. The mixture was vortexed briefly and centrifuged for 2 minutes at 14000*g*. The supernatant was transferred into other tubes and was used for PCR reaction directly or was stored at –20°C.

The primer sets for a multiplex nested PCR were based on the variable regions between 16S-like ribosomal DNAs of *E histolytica* (GenBank accession No. X56991) and *E dispar* (GenBank accession No. Z49256). Oligonucleotide pair Outer1-Outer1R directed the first PCR for the amplification of an 823–base pair product for *E histolytica* and *E dispar*. The primer set UidA1-UidA2, specific for the *Escherichia coli* β-glucuronidase gene, was also included for the internal control PCR reaction. The reaction mixture (50 µL) includes 5 µL of DNA template, 0.5µM Outer1-Outer1R and UidA1-UidA2 primer sets, 10mM Tris hydrochloride, pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, 200µM dNTP, 2% (weight per volume) sucrose, 0.1mM cresol red, bovine serum albumin (0.1 µg/µL), and 2.5 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, Calif). The reaction was initiated by heating for 2 minutes at 94°C, followed by amplification for 35 cycles of denaturation for 15 seconds at 94°C, annealing for 15 seconds at 47°C, and extension for 1 minute at 72°C. The final reaction cycle was extended for 6 minutes at 72°C. The second step of PCR involved the amplification of different gene fragments with sizes of 447 and 603 base pairs for *E histolytica* and *E dispar,* respectively, using Eh1-Eh2 and Ed1-Ed2 as primer sets (**Table 1**). The reaction mixture (25 µL) includes 5 µL of DNA template, 0.5µM Eh1-Eh2 or Ed1-Ed2 primer sets, 10mM Tris hydrochloride, pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, 200µM dNTP, 2% (weight per volume) sucrose, 0.1mM cresol red, bovine serum albumin (0.1 µg/µL), and 1.25 U of AmpliTaq

DNA polymerase. After heating for 2 minutes at 94°C, amplification was performed by 35 cycles of denaturation for 15 seconds at 94°C, annealing for 15 seconds at 52°C, and extension for 40 seconds at 72°C, followed by final extension for 6 minutes at 72°C. The PCR products were fractionated by electrophoresis on a 3% agarose gel (Nusieve 3:1 Agarose; Cambrex Corp, East Rutherford, NJ), stained by ethidium bromide, and visualized under UV illumination.

STATISTICAL ANALYSIS

All statistical analyses were performed using a statistical software program (SAS version 8.1; SAS Institute Inc, Cary, NC). Categorical variables were compared using the χ^2 or Fisher exact test. Noncategorical variables were compared using the Wilcoxon rank sum test. A multivariate analysis was performed to identify risk factors associated with invasive amebiasis or the presence of high IHA titers. The survival probabilities were estimated using the Kaplan-Meier method. The Cox proportional hazards model was used to assess the impact of the presence of invasive amebiasis on mortality rates, with adjustment for age, sex, year of invasive amebiasis diagnosis or year of enrollment, baseline CD4 lymphocyte count, the presence of concurrent AIDS-defining opportunistic illnesses[25] at diagnosis of invasive amebiasis or at enrollment, and the use of highly active antiretroviral therapy. Hazard ratios and 95% confidence intervals (95% CIs) were also calculated. All tests were 2-tailed. $P<.05$ was considered statistically significant.

The mortality rate for each group was calculated as the number of deaths per 100 person-years of observation. Exact 95% CIs for mortality rates were calculated on the basis of the Poisson distribution. The survival duration of patients without invasive amebiasis was estimated from the date of enrollment to death, loss to follow-up at this hospital and other designated hospitals in Taiwan, or the end of the study. To better define the mortality rate, survival duration, and HIV progression, the registry of the Taiwan Center for Disease Control of the Taiwan Department of Health was searched to identify deaths among patients who might not be regularly followed at this hospital. The institutional review board of the hospital approved the study protocol.

RESULTS

RETROSPECTIVE CASE REVIEW

The characteristics of the 951 nonhemophilic HIV-infected persons enrolled during the 9-year period according to the presence or absence of invasive amebiasis are given in **Table 2**. Seventy percent of these patients were diagnosed as having AIDS at the time of enrollment. Most of the persons acquired HIV through sex routes: 60.5% were men having sex with men and 33.0% were heterosexual. By the end of the study, 712 HIV-infected persons (74.9%) had received highly active antiretroviral therapy.

Forty-nine (5.2%) of the 951 HIV-infected persons were diagnosed as having 51 episodes of invasive amebiasis, including 30 persons with 31 episodes of amebic liver abscess, 31 persons with 32 episodes of amebic colitis, and 12 persons with amebic colitis and liver abscess. The estimated incidence of invasive amebiasis was 2.0 per 100 person-years. The median interval between enrollment and the development of invasive amebiasis was 1 day (range, 1-1179 days); 77.6% of the invasive amebiasis episodes were diagnosed within 1 month of enrollment. Patients with invasive amebiasis were more likely to be men having sex with men and to have high IHA titers and baseline CD4 counts (Table 2). The disease severity of invasive amebiasis was not related to CD4 count; the CD4 counts at the diagnosis of amebic colitis only, amebic liver abscess only, and amebic colitis and liver abscess were 147×10^6/L (range, 6-805$\times10^6$/L), 223×10^6/L (range, 57-763$\times10^6$/L), and 247×10^6/L (range, 14-737$\times10^6$/L), respectively ($P=.18$) (data not shown).

Table 1. Primers for the Differentiation of *Entamoeba histolytica* and *Entamoeba dispar*

Category	Primer Sets (Forward and Reverse)	PCR Product Size, base pair
First PCR	Outer1: 5′-GAA ATT CAG ATG TAC AAA GA-3′	823
	Outer1R: 5′-CAG AAT CCT AGA ATT TCA C-3′	
Second PCR	Eh1: 5′-AAG CAT TGT TTC TAG ATC TG-3′	447
	Eh2: 5′-CAC GTT AAA AGA GGT CTA AC-3′	
	Ed1: 5′-AAA CAT TGT TTC TAA ATC CA-3′	603
	Ed2: 5′-ACC ACT TAC TAT CCC TAC C-3′	
Internal control*	UidA1: 5′-AGA TAT TCG TAA TTA TGT GG-3′	320
	UidA2: 5′-AGA AAT CAT GGA AGT AAG AC-3′	

Abbreviation: PCR, polymerase chain reaction.
*Primers for the *Escherichia coli UIDA* gene.

In multivariate analysis, only the homosexual and bisexual routes of HIV transmission were statistically significantly associated with the development of invasive amebiasis. The adjusted odds ratio was 4.16 (95% CI, 1.37-12.61; $P=.01$) compared with other risk factors for HIV transmission. The persons were followed for a median of 774 days (range, 2-3478 days). The total duration of observation was 2541 person-years. Although the overall mortality rate was significantly lower among patients with invasive amebiasis ($P<.05$), the survival of the 2 groups is similar after adjustment for age, sex, baseline CD4 lymphocyte count, the presence of AIDS-defining opportunistic illnesses, and the use of highly active antiretroviral therapy. The adjusted hazard ratio was 0.66 (95% CI, 0.29-1.50; $P=.32$).

PARASITOLOGIC INVESTIGATIONS

Serologic Investigations

During the 9-year study, 634 (66.7%) of the 951 HIV-infected persons underwent an IHA assay. The characteristics of the persons who did and did not undergo an IHA assay were similar (data not shown). A high IHA titer (≥128) was detected in 39 (6.2%) of 634 HIV-infected persons compared with 10 (2.3%) of 429 HIV-uninfected controls with gastrointestinal symptoms and 0 of 178 HIV-uninfected healthy controls ($P<.001$). The HIV-infected persons with high IHA titers were more likely to be men having sex with men and to develop invasive amebiasis (**Table 3**). In multivariate analysis, only ho-

Table 2. Clinical Characteristics of HIV-Infected Patients With and Without Invasive Amebiasis (IA)

Characteristic	Patients With IA (n = 49)	Patients Without IA (n = 902)	All Patients (N = 951)	*P* Value
Age, median (range), y	32 (20-57)	34 (15-83)	34 (15-83)	.28
Males, No. (%)	49 (100)	833 (92.4)	882 (92.7)	.04
Route of HIV transmission, No. (%)				
MSM	41 (83.7)	534 (59.2)	575 (60.5)	
Heterosexual	8 (16.3)	306 (33.9)	314 (33.0)	
IDU	0	19 (2.1)	19 (2.0)	.006
Other	0	43 (4.8)	43 (4.5)	
Antiretroviral naive, No. (%)	38 (77.6)	676 (74.9)	714 (75.1)	.24
AIDS at enrollment, No. (%)	29 (59.2)	619 (68.6)	648 (68.1)	.09
AIDS-OI at enrollment or at IA diagnosis, No. (%)	23 (46.9)	512 (56.8)	535 (56.3)	.18
IHA titer, median (range)	256 (0-13 684)	0 (0-2048)	0 (0-13 864)	<.001
IHA titer ≥128, No. (%)	31 (63.3)	8 (0.9)	39 (4.1)	<.001
Baseline CD4 count, median (range), $\times 10^6$/L	209 (6-805)	63 (0-1202)	71 (0-1202)	<.001
Patients with baseline CD4 counts, No. (%)				
$<200 \times 10^6$/L	23 (47.0)	586 (69.4)	609 (68.2)	
200-349 $\times 10^6$/L	17 (35.4)	104 (12.3)	121 (13.6)	ND
$\geq 350 \times 10^6$/L	8 (16.7)	155 (18.3)	163 (18.3)	
CD4 count at IA diagnosis, median (range), $\times 10^6$/L	218 (6-805)	NA	NA	ND
Patients with CD4 counts at IA diagnosis, No. (%)				
$<200 \times 10^6$/L	19 (45.2)	NA	NA	
200-349 $\times 10^6$/L	16 (38.1)	NA	NA	ND
$\geq 350 \times 10^6$/L	7 (16.7)	NA	NA	
Baseline PVL (range), $\log_{10}$ copies/mL	5.48 (3.64-5.88)	5.19 (2.60-5.88)	5.19 (2.60-5.88)	ND
Patients with PVL, No. (%)	25 (51.0)	553 (61.3)	578 (60.8)	
PVL ≥5 $\log_{10}$ copies/mL	17 (68.0)	317 (57.3)	334 (57.8)	ND
PVL <5 $\log_{10}$ copies/mL	8 (32.0)	236 (42.7)	244 (42.2)	
IA diagnosed within 1 mo of enrollment, No. (%)	38 (77.6)	NA	ND	ND
HAART ever initiated, No. (%)	41 (83.7)	671 (74.4)	712 (74.9)	.14
Observation duration, median (range), d	1165 (48-3456)	753 (2-3478)	774 (2-3478)	.01
Incidence of IA (95% CI), per 100 person-years	ND	ND	2.01 (1.95-2.06)	ND
Outcome, No. (%)				
Survived	42 (85.7)	685 (75.9)	727 (76.4)	
Lost to follow-up	0	7 (0.8)	7 (0.7)	.27
Died	7 (14.3)	210 (23.3)	217 (28.4)	
Mortality rate (95% CI), per 100 person-years	4.23 (3.93-4.55)	8.84 (8.72-8.96)	8.54 (8.43-8.65)	.05

Abbreviations: AIDS-OI, AIDS-defining opportunistic illness; CI, confidence interval; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IDU, intravenous drug use; IHA, indirect hemagglutination antibody; MSM, men having sex with men; NA, not applicable; ND, not determined; PVL, plasma HIV RNA load.

mosexual and bisexual routes of HIV transmission were significantly associated with the presence of high IHA titers. The adjusted odds ratio for developing a high IHA titer among HIV-infected men having sex with men compared with other risk factors for HIV transmission was 4.46 (95% CI, 1.61-12.39; $P=.004$).

When the analysis was restricted to men aged 20 to 49 years, 39 (7.2%) of 541 HIV-infected patients and 5 (4.5%) of 111 HIV-uninfected controls with gastrointestinal symptoms had high IHA titers ($P=.06$). The rate of high IHA titer was 9.1% (34/374) for HIV-infected homosexual men aged 20 to 49 years. The adjusted odds ratio for higher IHA titers of HIV-infected persons aged 20 to 49 years was 2.37 (95% CI, 0.88-6.41) compared with HIV-uninfected controls with gastrointestinal symptoms of the same age group.

Amebic Antigen Testing and PCR of the Stool Specimens

To investigate whether the risk of amebic infection was higher in HIV-infected persons, we tested stool specimens for amebic antigen. Forty (12.1%) of 332 HIV-infected persons and 2 (1.4%) of 144 uninfected healthy volunteers had positive test results ($P<.001$). The clinical characteristics of HIV-infected persons with intestinal amebic colonization are given in **Table 4**. These persons were more likely to be men having sex with men. In multivariate analysis, the adjusted odds ratio for intestinal colonization with *E histolytica* or *E dispar* of HIV-infected men having sex with men compared with other risk factors for HIV transmission was 1.849 (95% CI, 0.80-4.29; $P=.15$).

Ten (25.0%) of the 40 stool specimens of HIV-infected patients contained *E histolytica*, which was identified by PCR using primers specific for *E histolytica*; 9 of the 10 isolates were from homosexual men. The 2 isolates from HIV-uninfected persons were *E dispar*. *Entamoeba histolytica* infection was associated with a high IHA titer; 2 of the 10 HIV-infected persons colonized with *E histolytica* exhibited high IHA titers compared with 0 of the 30 HIV-infected persons colonized with *E dispar* ($P=.01$) and 16 of the 292 HIV-infected persons without amebic infection ($P=.06$) (Table 4).

Table 3. Clinical Characteristics of HIV-Infected Patients With and Without Indirect Hemagglutination Antibody (IHA) Titers of 128 or Greater*

Characteristic	Patients With IHA ≥128 (n = 39)	Patients With IHA of 0 (n = 578)	*P* Value
IHA titer, median (range)	512 (128-13 684)	0	ND
Age, median (range), y	34 (20-49)	35 (15-81)	.49
Males, No. (%)	39 (100)	537 (92.9)	.09
Route of HIV transmission, No. (%)			
MSM	34 (87.2)	363 (62.8)	
Heterosexual	4 (10.3)	198 (34.3)	.02
IDU	1 (2.6)	11 (1.9)	
Other	0	6 (1.0)	
Antiretroviral naive, No. (%)	31 (79.5)	449 (77.7)	.38
AIDS at enrollment, No. (%)	28 (71.8)	427 (73.9)	.71
AIDS-OI at enrollment, No. (%)	23 (59.0)	360 (62.3)	.68
Invasive amebiasis, No. (%)	31 (79.5)	14 (2.4)	<.001
Baseline CD4 count, median (range), $\times 10^6$/L	178 (1-805)	56 (0-1202)	.008
Patients with CD4 count, No. (%)	38	559	
$<200 \times 10^6$/L	23 (60.5)	409 (73.2)	
200-349 $\times 10^6$/L	12 (31.6)	70 (12.5)	.004
$\geq 350 \times 10^6$/L	3 (7.9)	80 (14.3)	
Baseline PVL, median (range), $\log_{10}$ copies/mL	5.42 (3.64-5.88)	5.26 (2.60-5.88)	.25
Patients with PVL, No (%)	23	369	
PVL ≥5 $\log_{10}$ copies/mL	16 (69.6)	228 (61.8)	.46
PVL <5 $\log_{10}$ copies/mL	7 (30.4)	141 (38.2)	
HAART ever initiated, No. (%)	30 (76.9)	499 (86.3)	.10
Observation duration, median (range), d	937 (3-3242)	875.5 (2-3478)	.72
Incidence of IA (95% CI), per 100 person-years	27.75 (26.82-28.71)	0.84 (0.80-0.89)	<.001
Outcome, No. (%)			
Survived	31 (79.5)	455 (78.7)	
Lost to follow-up	1 (2.6)	5 (0.9)	.55
Died	7 (17.9)	118 (20.4)	
Mortality rate (95% CI), per 100 person-years	5.89 (5.47-6.33)	7.10 (6.97-7.23)	.63

Abbreviations: AIDS-OI, AIDS-defining opportunistic illness; CI, confidence interval; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IA, invasive amebiasis; IDU, intravenous drug use; MSM, men having sex with men; ND, not determined; PVL, plasma HIV RNA load.

*Seventeen other HIV-infected persons had IHA titers ranging from 8 to 64 and were not included in the analysis.

COMMENT

The major findings of this large retrospective case study of persons with HIV infection in Taiwan are that invasive amebiasis occurs much more frequently than reported in Western countries and is a relatively common parasitic disease at a time when CD4 counts are relatively high. Findings from serologic studies indicate that there is a high frequency of previous exposure to *E histolytica* and that it is more common among homosexual men. The concentration of cases among homosexual men seems to be attributable to the practice of anal-oral sex.[2,3] The key microbiologic finding was our ability to identify *E histolytica* in 25% of the colonizing strains in the stool. The higher rate of invasive disease in Taiwan and other areas in the Asia-Pacific region compared with Western countries is best explained by the higher endemicity of intestinal colonization with *E histolytica*.[23]

The prospective comparison study demonstrated that serologic evidence of past infection with *E histolytica* is more common in HIV-infected homosexual men than in HIV-uninfected persons with or without gastrointestinal symptoms. Although the comparison groups were not perfectly matched to the HIV-infected persons, we believe that this is not a critical issue because the study was conducted in a single medical center in an endemic region for amebiasis.[23]

There are only a few scattered case reports[16-22] of invasive amebiasis among HIV-infected persons in Western countries. Lowther and associates[11] conducted a large retrospective review of the medical diagnoses of more than 34 000 HIV-infected persons. Only 111 (0.3%) were diagnosed as having *E histolytica* or *E dispar* infection, and only 2 of these had extraintestinal invasive amebiasis. The estimated incidence of *E histolytica* and *E dispar* infections was 13.5 cases per 10 000 person-years.[11] Although some of the HIV-infected persons developed colitis or diarrhea, concomitant infection with other pathogens was a common confounding factor.[11] No attempt was made to differentiate between *E histolytica* and *E dispar* by molecular methods, and serologic studies were not performed. Thus, it was difficult to provide an accurate prevalence rate of invasive amebiasis.

Longitudinal follow-up studies are needed to assess the duration of colonization, the risk for development of invasive amebiasis, and the necessity of antimicrobial therapy. Spontaneous loss of or reinfection or persistent infection with the parasite may occur in untreated homosexual men. Treatment of asymptomatic homosexual men who pass cysts is not warranted because of

Table 4. Clinical Characteristics of HIV-Infected Patients With or Without Intestinal Colonization With *Entamoeba histolytica* and *Entamoeba dispar*

Characteristic	Patients With *E histolytica* (n = 10)	Patients With *E dispar* (n = 30)	Patients Without Amebic Infection (n = 292)	All Patients (N = 332)	*P* Value*
Age, median (range), y	35.5 (30-53)	38.5 (26-63)	37 (17-80)	37 (17-80)	.44/.45/.94
Males, No. (%)	10 (100)	30 (100)	270 (92.5)	310 (93.4)	NA/.24/.37
Route of HIV transmission, No. (%)					
MSM	9 (90.0)	20 (66.7)	177 (60.6)	206 (62.1)	.53
Heterosexual	1 (10.0)	7 (23.3)	109 (37.3)	117 (35.2)	.05
IDU	0	1 (3.3)	2 (0.7)	3 (0.9)	.32
Other	0	2 (6.7)	4 (1.4)	6 (1.8)	
Patients ever having IHA titer ≥128, No. (%)	2 (20.0)	0	16 (5.5)	18 (5.4)	.01/.38/.06
Ever diagnosed as having IA, No. (%)	1 (10.0)	1 (3.3)	18 (6.4)	20 (6.0)	.44/.50/.50
AIDS-OI within 1 mo of stool examination, No. (%)	4 (40.0)	20 (66.7)	190 (65.1)	214 (64.5)	.16/.86/.18
CD4 count within 3 mo of first stool collection, median (range), $\times 10^6$/L	242 (44-1021)	200 (2-760)	269 (1-1230)	265 (1-1230)	.33/.13/.72
Patients with CD4 count within 3 mo of first stool collection, No. (%)					
$<200 \times 10^6$/L	4 (40.0)	12 (48.0)	104 (39.1)	120 (39.9)	.46
200-349 $\times 10^6$/L	2 (20.0)	8 (32.0)	62 (23.3)	72 (23.9)	.21
$\geq 350 \times 10^6$/L	4 (40.0)	5 (20.0)	100 (37.6)	109 (36.2)	.97
PVL within 3 mo of stool collection, median (range), $\log_{10}$ copies/mL	2.60 (2.60-5.65)	2.95 (2.60-5.65)	2.60 (2.60-5.88)	2.60 (2.60-5.88)	.68/.22/.84
PVL <400 copies/mL, No. (%)	6 (60.0)	14 (46.7)	186 (65.6)	206 (62.0)	.47/.05/.75
Receiving HAART, No. (%)	9 (90.0)	28 (93.3)	264 (90.4)	301 (90.7)	.73/.60/.97

Abbreviations: AIDS-OI, AIDS-defining opportunistic illness; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IDU, intravenous drug use; IHA, indirect hemagglutination antibody; MSM, men having sex with men; NA, not available; PVL, plasma HIV RNA load.

*Patients with *E histolytica* vs patients with *E dispar*/patients with *E dispar* vs patients without amebic infection/patients with *E histolytica* vs patients without amebic infection.

the nonpathogenicity of *E dispar*.[4] However, a substantial proportion of the asymptomatic persons in the present study with intestinal amebic infection were colonized with *E histolytica*. The spread of *E histolytica* in the community is a major concern and may pose a threat to public health.[3,21] Therefore, eradication with luminal agents, such as iodoquinol or paromomycin sulfate, may be considered in patients colonized with *E histolytica*, although the long-term efficacy remains to be established. In conclusion, this study demonstrates that HIV-infected persons compared with uninfected persons in Taiwan are at increased risk for invasive amebiasis and exhibit a relatively high frequency of elevated antibody titers and intestinal colonization with *E histolytica*.

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