Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection

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Objectives: To describe the incidence and presentations of invasive amoebiasis (IA) in patients with HIV infection in an area endemic for amoebic infection and to assess the role of the indirect haemagglutination (IHA) assay in the diagnosis of IA in HIV-infected patients.

Design: Retrospective study of 18 cases of IA and HIV infection.

Setting: A university hospital, the largest centre for management of HIV-associated complications in Taiwan.

Methods: Medical, microbiological and histopathological records of 296 HIV-infected patients and serological data of IHA assay of 126 HIV-infected patients were reviewed to identify cases of IA from 23 June 1994 to 31 March 1999. An IHA titre $\geq 1 : 128$ was considered positive. Clinical characteristics of HIV-infected patients with IA and without IA were compared.

Results: Eighteen of the 296 patients (6.1%) with HIV infection were diagnosed with IA: 12 patients were diagnosed with definite IA and six with probable IA. The clinical manifestations included amoebic colitis (13 patients), amoebic liver abscess (nine), both colitis and abscess (four), and pleural effusion (two). IA was the initial presentation of HIV infection in nine patients. Co-infection with other enteric pathogens was diagnosed in six patients with IA. Compared with the 161 patients without IA who were newly diagnosed with HIV infection, the nine patients with IA had a higher median CD4⁺ lymphocyte count ($202 \times 10^6/l$ versus $33 \times 10^6/l$; $P = 0.0017$), were less likely to be diagnosed with AIDS (55.6% versus 85.4%; $P = 0.039$), and had fewer concurrent AIDS-defining illnesses (median number 0 versus 2; $P = 0.003$). Estimated mean survival duration was not significantly different between the two groups (597 days versus 611 days). Fourteen out of 126 patients (11.1%) had an IHA titre $\geq 1 : 128$. Of the 18 patients diagnosed with IA, 13 had a titre $\geq 1 : 128$. The sensitivity of IHA assay in the diagnosis of IA was 72.2% (13 out of 18) and the specificity was 99.1% (107 out of 108). The positive predictive value of IHA test for IA of this patient population was 92.9% (13 out of 14) whereas the negative predictive value was 95.5% (107 out of 112).

Conclusion: IA is an increasingly important parasitic disease among patients with HIV infection in Taiwan. IHA assay has a good specificity and high negative predictive value in diagnosis of IA.

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Introduction

Invasive amoebiasis (IA) is an important parasitic disease associated with significant morbidity and mortality world-wide in persons residing in or travelling to endemic areas [1,2]. It has been estimated that 40 million people develop disabling amoebic colitis or extra-intestinal abscesses world-wide, resulting in 40 000 deaths annually [2]. However, despite the fact that *Entamoeba histolytica* can be identified in 20–30% of homosexual men who practice oral/anal sex, it has long been regarded as a gastrointestinal commensal in this population [3–7]. Invasive disease may be rare in homosexual men because isolates of *E. histolytica* most often display non-pathogenic zymodemes [4,8,9]. Infected homosexual men do not develop antibodies reactive with *E. histolytica* [8] and spontaneous eradication of the protozoa may occur, whether the patient is HIV-infected or not [5].

Similarly, cases of IA are only rarely or never reported in HIV-infected patients, either from developed

[10–15] or developing countries [16–20]. The reason remains unclear why IA is rare in HIV-infected patients despite their declining T-cell immunity, the major immunity against *E. histolytica* infection [2,21].

IA is a reportable disease in Taiwan. Over the past 10 years, a total of 639 cases have been diagnosed (Fig. 1) [22]. Outbreaks of amoebic dysentery occurred in 1993 and 1994. Since 1994, we have identified several incident cases of HIV-infected patients who developed IA. In some of these patients, IA was the initial presentation of HIV infection. In order to understand better the incidence and clinical manifestations of IA in HIV-infected patients from an area endemic for amoebic infection, we embarked on this retrospective study and an investigation of the diagnostic value of indirect haemagglutination (IHA) assay in this patient population.

Materials and methods

Patient population

National Taiwan University Hospital is a major referral centre for HIV/AIDS care in Taiwan where 1959 native cases of HIV infection had been diagnosed from 1 January 1986 to 31 December 1998. From 23 June 1994 to 31 March 1999, a total of 296 non-haemophiliac HIV-infected patients were seen. Two-hundred and nine of the 258 patients (81.0%) with available CD4+ lymphocyte counts had counts $< 200 \times 10^6/l$ at presentation and 83.4% of the 296 patients were diagnosed with AIDS. The mean initial CD4+ count was $115 \times 10^6/l$ (median, $33 \times 10^6/l$). Of the 296 patients, 126 (42.6%) underwent IHA testing, which formed the study population to test the role of the assay.

Investigations

Since June 1994, a standardized protocol was followed to investigate the etiologic diagnosis for any presenting symptoms. For patients with diarrhoea, at least two stool specimens were obtained for bacterial cultures for *Salmonella* species, *Shigella* species, *Campylobacter* species, *Yersinia* species, *Clostridium difficile*, and mycobacteria. Concentrated wet preparations from each stool specimen were examined by direct microscopy for ova, cysts, and parasites. Faecal smears were stained with modified acid fast stain to look for *Cryptosporidium* species and *Isospora* species. Examinations to detect microsporidia were not performed during the study period. If routine examinations and cultures were negative, colonoscopy was performed for biopsy of any colonic ulcers or random biopsy of the terminal ileum and rectum. Biopsy specimens were fixed in formalin and processed in paraffin. Sections were stained with haematoxylin and eosin, periodic acid–Schiff, Ziehl–Neelson, and Gomori–methenamine stains. Sections were also stained by immunohistochemical stain for cytomegalovirus (CMV).

Fig. 1. Reported case number of invasive amoebiasis (IA) in Taiwan, 1989–1998. Cases of HIV infection with IA were from 23 June 1994 to 31 March 1999 are shown.

Sonography was performed for patients who had abnormal liver function tests, pleural effusion, or prolonged fevers. Sonography-guided percutaneous biopsy or aspiration of the lung or liver was performed if a space-occupying lesion of the liver or pleural effusion was detected. An aspirate was submitted for IHA assay and wet mount and biopsy specimens were submitted for histopathology and cultures for viruses, bacteria, fungi, and mycobacteria.

IHA assay (Cellognostics, Boehringer Diagnostics GmbH, Marburg, Germany) was routinely performed following the manufacturer's instructions to detect serum antibodies on patients admitted to the hospital, regardless of gastrointestinal symptoms.

Demographics, clinical presentations, and outcome of all 296 HIV-infected patients were recorded on a standardized case record form. Clinical characteristics and survival were determined for newly diagnosed HIV-infected patients with IA (nine patients) compared to patients without IA (161).

Definitions

Definite IA was diagnosed when erythrophagocytic trophozoites were identified in clinical specimens; probable IA was defined when the aspirates showed a high titre of IHA assay but microbiological cultures or histopathological examination of aspirate and biopsy specimens were negative, and patients responded to metronidazole monotherapy. Results of the IHA assay were considered positive if the titre was $\geq 1:128$. CMV colitis was diagnosed if intracytoplasmic or intranuclear inclusion bodies and positive immunohistochemical stain were identified by histopathologic examination of the biopsy specimens.

Treatment

All patients with definite and probable IA were treated with metronidazole at a dose of 500 mg every 6 hours for 10 days, followed by a 21-day course of iodoquinol, 650 mg three times a day. Antiretroviral therapy was started when diarrhoea had resolved. Protease inhibitors were not available until April 1997, after which time highly active antiretroviral therapy (HAART) was freely provided to all Taiwanese patients with HIV infection at any stage.

Statistical analysis

Statistical analysis was performed with SPSS version 8.0 (SPSS, Chicago, Illinois, USA). Categorical variables were compared using the χ^2 test or Fisher's exact test; continuous variables were compared using Wilcoxon's rank sum test. The survival curve was plotted by the Kaplan–Meier method and comparison of survival between the two groups was performed using the log-rank test. A *P* value < 0.05 was considered significant.

Results

Characteristics of patients with IA

Eighteen patients (6.1%) were diagnosed with IA over the 57 months of the study: 12 patients with definite IA and six with probable IA. The clinical characteristics of the 18 patients with IA are summarized in Table 1: all were male with a mean age of 34 years; 13 patients (72.2%) acquired HIV infection by having sex with men; the mean CD4+ lymphocyte count of the 18 patients when IA was diagnosed was $205 \times 10^6/l$ (range, $7\text{--}805 \times 10^6/l$); 11 patients had a CD4+ lymphocyte count $< 200 \times 10^6/l$. Concurrent AIDS-defining illnesses were also diagnosed in 10 patients; gastrointestinal CMV disease was diagnosed in five patients: four with colitis and one with oesophagitis. With the exception of patient 7, patients with CMV colitis had definite amoebic colitis. The probable amoebic colitis of patient 7 was diagnosed by a high IHA titre (1024) and clinical response to subsequent metronidazole therapy when diarrhoea continued despite 2 weeks treatment with ganciclovir.

Clinical manifestations and treatment outcome

Nine patients had amoebic liver abscess and 13 patients had amoebic colitis. Four were diagnosed with both conditions. Amoebic colitis was diagnosed definitively in 10 patients by colonoscopy and in two patients by colectomy after presenting with colonic perforation and peritonitis. *E. histolytica* trophozoites and cysts were detected in the stool specimens of two patients. The main presenting symptoms of IA were fever (13), chronic diarrhoea (12), and abdominal pain (five). In two patients with fever and chronic cough, IA was complicated by pleural effusion in addition to liver abscess; the initial diagnosis was bacterial empyema and liver abscess was found incidentally by chest sonography. The titres of IHA assay of the aspirate of pleural effusion of the two patients were 4096 and 1024; both patients responded to metronidazole and iodoquinol therapy.

Of the 18 patients, nine were concurrently diagnosed with HIV infection and IA: eight with liver abscess and five with colitis. In the nine others, IA developed after HIV infection had been diagnosed. Concomitant opportunistic infections were detected with IA in 10 patients. Oral candidiasis (eight patients) and gastrointestinal CMV disease (five) were the most common concurrent opportunistic infections. In one patient (patient 13), IA developed concurrently with acute retroviral syndrome when he presented with prolonged fever, chronic diarrhoea, and subsequent colonic perforation; he was found to seroconvert during the treatment course of IA.

All of the patients were treated successfully with metronidazole followed by iodoquinol in combination with medications for other opportunistic infections,

Table 1. Characteristics of 18 HIV-infected patients with invasive amoebiasis (IA).

Patient	Age / sex	Route of HIV transmission	CD4+ lymphocyte count ($\times 10^6/l$)	Presenting symptoms	Involvement of IA	IHA value (blood/ aspirate)	Concurrent diseases	Outcome
1	49/M	Heterosexual	64	Chronic cough; fever	Liver abscess; pneumonia with empyema	4096/4096 (liver; PE)	Nil	Survived
2	29/M	Homosexual	763	Chronic diarrhoea	Liver abscess; colitis	64/NA	Nil	Lost to follow-up
3	44/M	Homosexual	147	Chronic diarrhoea; fever	Colitis	2048	CMV colitis; oral candidiasis	Survived
4	35/M	Homosexual	805	Chronic diarrhoea	Colitis	1024	Nil	Lost to follow-up
5	43/M	Heterosexual	186	Chronic cough; fever	Liver abscess; pneumonia with empyema	13684/1024 (PE)	Nil	Survived
6	31/M	Homosexual	137	Chronic diarrhoea; fever	Colitis	1024	CMV oesophagitis; pulmonary tuberculosis	Lost to follow-up
7	35/M	Bisexual	NA	Chronic diarrhoea; fever	Colitis	1024	CMV colitis and polypneuropathy; oral candidiasis	Died
8	49/M	Heterosexual	202	Abdominal pain	Liver abscess	256/128 (liver aspirate)	Liver cirrhosis	Died
9	32/M	Homosexual	10	Chronic diarrhoea; fever	Colitis	128	CMV colitis; oral candidiasis; cryptococcal meningitis	Died
10	39/M	Homosexual	17	Chronic diarrhoea; fever	Liver abscess; colitis	256/128 (liver aspirate)	Oral candidiasis	Survived
11	31/M	Heterosexual	7	Chronic diarrhoea; fever	Colitis	512	Oral candidiasis; pulmonary tuberculosis	Lost to follow-up
12	27/M	Homosexual	10	Chronic diarrhoea; fever	Colitis	0	CMV colitis	Survived
13	31/M	Homosexual	252	Chronic diarrhoea; fever; abdominal pain	Liver abscess; peritonitis; colitis	256/256 (liver aspirate)	Acute antiretroviral syndrome	Survived
14	45/M	Homosexual	254	Chronic diarrhoea; fever	Liver abscess; colitis	0/0	Nil	Survived
15	26/M	Homosexual	57	Abdominal pain; fever	Liver abscess	512/512 (liver aspirate)	Oral candidiasis	Survived
16	30/M	Homosexual	163	Abdominal pain; fever	Liver abscess	1024/128 (liver aspirate)	Oral candidiasis	Survived
17	23/M	Heterosexual	279	Abdominal pain	Peritonitis; colitis	0	Oral candidiasis	Survived
18	20/M	Homosexual	135	Chronic diarrhoea	Colitis	0	Aeromonas enteritis	Survived

CMV, Cytomegalovirus; NA, not available; PE, pleural effusion.

including ganciclovir for CMV colitis. After a mean observation of 515 days (range, 37–1590 days), four patients were lost to follow-up and three patients died of diseases other than IA. Eleven patients continued to receive HAART and no relapse of IA was observed.

In order to understand the differences of clinical characteristics and survival, the first comparison was made between all of the HIV-infected patients with and without IA. Compared with the 278 patients without IA, the 18 patients with IA had a significantly higher median CD4+ lymphocyte count, were exclusively men, and more likely to be bisexual or homosexual (data not shown). The second comparison was limited to the 170 patients who were newly diagnosed with HIV infection during the study period (Table 2): nine patients with IA and 161 without IA. IA appeared to occur in the earlier stage of HIV infection: patients presenting with IA while HIV infection was diagnosed had a higher median CD4+ lymphocyte count ($202 \times 10^6/l$ versus $33 \times 10^6/l$; $P = 0.017$) and fewer concurrent AIDS-defining illnesses (median number, 0 versus 2; $P = 0.003$). Despite the differences, the mean survival was similar between these two groups (Fig. 2).

The diagnostic role of IHA assay for IA in HIV-infected patients

One-hundred and twenty-six unselected patients, including the 18 patients with IA, were tested by IHA assay. The following clinical characteristics were not significantly different between patients who either did or did not undergo IHA testing: age, sex, route of transmission, mean or median CD4+ lymphocyte count at entry, patient number in each stratum of CD4+ lymphocyte count (data not shown). However, patients who underwent IHA testing were more likely to be men having sex with men or bisexual (45.6% versus 36.8%; $P = 0.017$).

Fourteen of the 126 patients (11.1%) had a titre $\geq 1 : 128$ and 13 of the 18 patients diagnosed with IA had a titre $\geq 1 : 128$ (range, 128–13 684). Five others had no reaction or a low titre (1 : 64). The four patients without antibody response had a mean CD4+ lymphocyte count of $133 \times 10^6/l$ (10 – $254 \times 10^6/l$). Repeat determinations of IHA of the four patients without antibody response remained negative after a mean duration of 6 months.

One patient had a titre of 1 : 256 but had no symptoms consistent with invasive amoebiasis. Colonoscopy and abdominal sonography were negative. Therefore, the sensitivity of IHA assay in the diagnosis of IA was 72.2% (13 out of 18). The specificity of the assay was 99.1% (107 out of 108). The positive predictive value of the assay in this patient population was 92.9% (13 out of 14) whereas the negative predictive value was 95.5% (107 out of 112).

Discussion

To our knowledge, this is the largest case series of IA in HIV-infected patients and our findings suggest that IA may be the initial presentation of HIV infection. By performing the IHA assay and other intensive investigations, we found that the IHA assay had a good specificity and negative predictive value in diagnosing IA in HIV-infected patients. However, its sensitivity and positive predictive value were only moderate.

Despite the high rates of *E. histolytica* infection in a population at risk for HIV infection, invasive infection is rare [3–8]. Even in patients from endemic areas for both amoebiasis and HIV infection, IA is only infrequently described or is not documented by extensive investigations [16–20]. The reason why IA is rare may be that most of the *E. histolytica* isolates identified in the stool specimens of homosexual men with or without

Table 2. Comparison of patients with invasive amoebiasis (IA) and without IA who are newly diagnosed with HIV infection.

Characteristics	Patients with IA (n = 9)	Patients without IA (n = 161)	<i>P</i> ^a
Mean age (SD) (years)	37 (9.7)	38 (10.4)	0.68
Sex (ratio male : female)	9/0	143/18	0.60
Median CD4+ lymphocyte count at presentation (range) ($\times 10^6/l$)	202 (57–763)	33 (0–805)	0.0017
Number (%) of patients with CD4+ lymphocyte count of			
< $50 \times 10^6/l$	0 (0%)	89 (60.1%)	< 0.001
$50 \times 10^6/l \leq \text{CD4+} < 100 \times 10^6/l$	2 (22.2%)	23 (15.5%)	
$100 \times 10^6/l \leq \text{CD4+} < 200 \times 10^6/l$	2 (22.2%)	10 (6.8%)	
$\text{CD4+} > 200 \times 10^6/l$	5 (55.6%)	26 (17.6%)	
Patients with AIDS at presentation	5 (55.6%)	135 (85.4%)	0.039
Median number of concurrent AIDS-defining illness (range)	0 (0–2)	2 (0–5)	0.003
Estimated mean survival time (SE) (days)	597 (209)	611 (167)	0.7795

^a*P*-value calculated from Fisher's exact test.

Fig. 2. Kaplan-Meier survival curve of newly diagnosed HIV-infected patients with IA (161 patients) and without IA (nine patients). Mean survival duration for patients with IA and without IA was 597 days and 611 days, respectively ($P = 0.7795$).

HIV infection belong to non-pathogenic groups by zymodeme analysis [4,5,9]. However, geographic differences may exist as to the zymodeme patterns and pathogenicity of *E. histolytica*.

In our report, although zymodeme study was not performed, 18 patients with IA were diagnosed: 13 with amoebic colitis by histopathology and clinical response and nine with liver abscess by sonography-guided aspiration biopsy. Two patients even presented with colonic perforation and peritonitis. More than 11% of the 126 HIV-infected patients developed antibodies reactive with *E. histolytica*, which is suggestive of invasive infection. In other studies of homosexual men conducted in Japan, Italy, and the United States of America prevalence of seropositivity for *E. histolytica* infection ranges from 4.1 to 21% [7,23–25]. Fifty cases of invasive amoebiasis were reported in Japanese homosexual men and pathogenic zymodemes were identified in the *E. histolytica* isolates [25].

Eleven other cases of HIV-infected patients with IA from whom detailed clinical history could be obtained have been described in the literature (Table 3) [10–15]. Combined with our 18 patients, the majority of the patients were homosexual or bisexual men and had AIDS although CD4+ lymphocyte counts were not known in most reported cases. IA often co-existed with other enteric infections, with CMV colitis being the most common. Co-infection might also contribute to the clinical manifestations in these patients [10–15]. In the report by Fatkenheuer *et al.* [15] two cases of concurrent amoebic colitis and CMV colitis were diagnosed and they were successfully treated only with metronidazole and ganciclovir [15].

E. histolytica appears to be more virulent than other opportunistic pathogens as it can cause severe infections both in immunocompetent and immunodeficient hosts [2]. In this report, of note is that nine of the 18 patients had their HIV infection diagnosed concurrently with IA, without any other preceding opportunistic infections. Compared with patients newly diagnosed with HIV infection, they had a significantly higher median CD4+ lymphocyte count ($202 \times 10^6/l$) and were less likely to present with other concurrent opportunistic infections than those without IA. In contrast with other opportunistic pathogens which cause relapse in HIV-infected patients if suppressive therapy is not continued, *E. histolytica* does not often cause relapse or reinfections [2]: there were no relapses in our patients following treatment with metronidazole and iodoquinol and HAART. In patients without HIV infection, studies have shown that patients may develop immunity after IA which may confer protection against re-infection [26–29]. Thirteen of our 18 patients produced antibodies against *E. histolytica*. Whether this immunity occurs even more frequently among HIV-infected patients with IA in the HAART era remains to be studied.

IHA assay depends on the appearance of antibody against *E. histolytica* and the sensitivity may vary with the time when blood is obtained after IA [30]. By using a cut-off titre of 1 : 128, a seroprevalence of 11.1% was detected in our HIV-infected patients. Whereas the sensitivity of the IHA assay ranges from 72.4 to 86.9% in non-HIV-infected patient population [30], the sensitivity of IHA assay among HIV-infected patients is unknown. In our HIV-infected patients, the sensitivity of IHA assay was 72.2% and the specificity was 99.1%; the positive predictive value was 92.9% and the negative predictive value was 95.5%. Four patients with

Table 3. Summary of reported invasive amoebiasis in HIV-infected patients in the literature.

References	Age/sex	Risk factor	CD4+ lymphocyte count ($\times 10^6/l$)	Concurrent enteric pathogens	Clinical presentations	Methods of diagnosis	Serology tests
[10]	NA/M	Homosexual	NA	CMV <i>Cryptosporidium</i> <i>Dientamoeba fragilis</i> <i>Salmonella flexneri</i>	Watery diarrhoea	Stool examination; endoscopy	NA
[10]	NA/M	Homosexual	NA		Watery explosive diarrhoea	Stool examination; endoscopy	NA
[10]	NA/M	Homosexual	NA	Nil	Watery diarrhoea	Stool examination; endoscopy	NA
[10]	NA/M	Homosexual	NA	CMV <i>Campylobacter jejuni</i> <i>Salmonella typhimurium</i> <i>Mycobacterium avium complex</i> <i>Campylobacter jejuni</i>	Loose-watery diarrhoea	Stool examination; endoscopy	NA
[10]	NA/M	Homosexual	NA		Watery diarrhoea	Stool examination; endoscopy	NA
[11]	39/M	Homosexual	18	Nil	Bloody diarrhoea, liver abscess	NA	NA
[12]	44/M	Haemophilia	220	Nil	Bloody diarrhoea	Stool examination, response to treatment	NA
[13]	43/M	Homosexual	122	Nil	Bloody diarrhoea, intestinal perforation, peritonitis	Endoscopy	NA
[14]	30/M	Homosexual	19.5	NA	Bloody diarrhoea	Stool examination	(-)
[15]	35/M	Bisexual	NA	CMV	Recurrent bloody diarrhoea	Endoscopy	(-)
[15]	42/M	Homosexual	NA	CMV	Recurrent diarrhoea	Endoscopy	NA

NA, Not available; CMV, cytomegalovirus. (-) denotes a negative result.

definite IA did not produce antibody. The lower sensitivity may be because anti-amoebic antibody may not be produced in patients with advanced HIV infection (81.0% of our HIV-infected patients had a CD4+ lymphocyte count $< 200 \times 10^6/l$). However, in the 11 patients with a CD4+ lymphocyte count $< 200 \times 10^6/l$, nine (81.8%) developed anti-amoebic antibodies. Therefore, many HIV-infected patients who have depleted CD4+ lymphocyte count can still develop reactive antibodies against *E. histolytica* and IHA assay may be considered a helpful diagnostic tool in those patients presenting with gastrointestinal symptoms. If IHA assay gives a negative result in patients with continuing diarrhoea, further diagnostic investigation, such as colonoscopy, should be performed.

In conclusion, our report suggests that IA is an emerging parasitic infection in HIV-infected patients and may be the initial presentation of HIV infection in areas endemic for amoebic infection. Although the case number is small, our report should alert clinicians in endemic areas to consider HIV infection in patients diagnosed with IA if they have risk factors for HIV infection, even though they may be free of other concomitant immunocompromising diseases.

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