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Concurrent Infection of Homosexual Men with Human Immunodeficiency Virus and *Entamoeba histolytica* in Japan: Serodiagnosis of Amebic Infection

JUN-ICHI SANUKI[1], EIICHI OKUZAWA[1], EIKO IMAI[1], SEIKI KOBAYASHI[1], GOHTA MASUDA[2] AND TSUTOMU TAKEUCHI[1]

[1]*Department of Tropical Medicine and Parasitology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160, Japan.*
[2]*Department of Infectious Diseases, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo 177, Japan.*
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Abstract

We found eleven human immunodeficiency virus (HIV)-positive practicing homosexual men with amebic infection in Japan. Clinical symptoms compatible with invasive amebiasis were demonstrated in ten of them. Actively motile trophozoites of ameba with ingested erythrocytes, identified as *Entamoeba histolytica*, were detected in the stool or liver abscess pus from five of the symptomatic cases, whereas in another symptomatic case ameba, also identified as *E. histolytica*, was histopathologically found from a biopsied specimen of the colon. Anti-*E. histolytica* antibody was not detected in one of these six cases irrespective of the serologic methods employed, and gel diffusion precipitin test (GDP) was negative in another one of them. Among the remaining five individuals, two were negative by GDP, and one of the two also by enzyme-linked immunosorbent assay; however, these 2 GDP-negative cases were judged positive by polymerase chain reaction using *E. histolytica*-specific primers. These findings suggest that serologic tests for invasive amebiasis, in particular GDP, sometimes show misleading reactions in the case concurrently infected with HIV and *E. histolytica*. In contrast to western countries, invasive amebiasis by *E. histolytica* appears to be associated with acquired immunodeficiency syndrome (AIDS) in Japan.

Key words: *Entamoeba histolytica*; Protozoa, parasitic; sexually transmitted amebiasis; acquired immunodeficiency syndrome (AIDS); serology.

Introduction

Recently the concurrent infection with human immunodeficiency virus (HIV) and ameba has been found in male homosexual population of western countries (Allason-Jones *et al.*, 1988). This could be expected, since homosexual males, who were at the risk of HIV infection, had a high prevalence of amebic infection in these countries (Druckman and Quinn, 1988). However, such homosexual men infected with ameba were generally seronegative for *Entamoeba histolytica* infection and scarcely developed the clinical symptoms of invasive amebiasis despite the concurrent infection with HIV (Allason-Jones *et al.*, 1988). Indeed, acquired immunodeficiency syndrome (AIDS) cases with invasive amebiasis have been rarely found in western countries (Reed *et al.*, 1991).

On the other hand, our previous studies (Takeuchi *et al.*, 1987) on sexually transmitted amebiasis showed that we had many Japanese homosexual males with invasive amebiasis. Subsequently, Kobayashi *et al.* (1992) indicated that *E. histolytica* was frequently detected among Japanese homosexual men as examined by serologic procedures, isoenzyme analysis by Sargeaunt's method (Sargeaunt and Williams, 1978) and reactivity to the *E. histolytica*-specific monoclonal antibody, 4G6, produced by Tachibana *et al.* (1990). These findings led us to conceive that we might soon have Japanese male homosexual AIDS cases infected with *E. histolytica* which is pathogenic to man.

Correspondence: Tsutomu Takeuchi
佐貫潤一[1]，奥沢英一[1]，今井栄子[1]，小林正規，増田剛太[2]，竹内　勤[1]([1]慶応義塾大学医学部熱帯医学・寄生虫学教室，[2]東京都立駒込病院感染症科)

During subsequent studies, we found practicing homosexual men with positive anti-HIV antibody titers, who seemed to be also infected with *E. histolytica*. The present communication primarily deals with the serodiagnostic aspects of amebiasis in these homosexual males.

Materials and Methods

Sera of anti-HIV antibody-positive individuals were selected from those submitted to our laboratory for the serodiagnosis of amebic infection. Their clinical data including the presence or absence of anti-HIV antibody, results of morphological identification of ameba in their specimens and the status of sexual practice were obtained by questionnaire to the clinical organizations which sent the sera to us. According to the replies, anti-HIV antibody was evaluated initially by enzyme-linked immunosorbent assay (ELISA), passive agglutination test and/or indirect immunofluorescent antibody test, and finally confirmed by Western blotting (WB) in virtually all of the present cases. Serologic tests to detect anti-*E. histolytica* antibody in the HIV-positive individuals were conducted by the gel diffusion precipitin test (GDP) (Takeuchi and Kobayashi, 1983), WB (Joyce and Ravdin, 1988) and indirect hemagglutination test (IHA) utilizing a commercial kit (Japan Freeze-Thawing Inc., Tokyo, Japan) which was previously evaluated in our laboratory (Okuzawa *et al.*, 1993) and ELISA (Takeuchi *et al.*, 1988).

Results

As shown in Table 1, we found ten Japanese practicing homosexual males of 34 to 66 of age and one 25 years old Chinese homosexual man residing in Japan, all of whom seemed to be infected with

Table 1 Serologic and clinical data of homosexual men concurrently infected with HIV and ameba in Japan

Case No.	Age (yrs)	CD4 (/μl)	Clinical findings	Serologic data for amebic infection			
				IHA	GDP	ELISA	WB
1*	41	23	Liver abscess Persistent diarrhea	X80	+	+	+
2†	42	592	Dysentery Liver abscess	X320	++	+	+
3†	34	unknown	Dysentery Liver abscess	X160	+	+	+
4*	45	13	Dysentery	X640	++	+	+
5‡	25	15	Dysentery Liver abscess	–	–	–	–
6†	35	44	Diarrhea Liver abscess	X80	–	+	+
7	51	277	Dysentery	X320	–	–	+
8	36	24	Dysentery	X640	++	+	+
9	66	296	Liver abscess	X640	++	+	+
10	38	69	Asymptomatic	X640	+	+	+
11	39	420	Liver abscess	X160	–	+	+

Only clinical findings which seemed to be attributable to *Entamoeba histolytica* infection are listed. Criteria for judgement of these serologic tests for amebiasis are given in each literature.
* Detected *E. histolytica* trophozoites from the diarrheal stool.
† Detected *E. histolytica* trophozoites from the aspirated pus of liver abscess.
‡ Detected *E. histolytica* trophozoites from the tissue specimen obtained by colon biopsy.
IHA: indirect hemagglutination test, GDP: gel diffusion precipitin test, ELISA: enzyme-linked immunosorbent assay, WB: western blot analysis.

ameba judging from their clinical data and/or results of preliminary serologic evaluation by ELISA as well as of morphological detection of ameba. The number of CD4 lymphocyte ranged from 13 to 592/μl. In five of these cases, actively motile trophozoites of ameba ingesting host erythrocytes, identified as *E. histolytica*, were detected in the diarrheal stools or the aspirated pus from liver abscess. Moreover, trophozoite of ameba, also identified as *E. histolytica*, was detected in another case by histopathological examination of the biopsied tissue specimen of colon; most frequently in the submucosal tissue of cecum. These findings were later confirmed in our laboratory. Neither cyst nor trophozoite of ameba was detected in the other cases including an asymptomatic individual. Clinical symptoms, which were compatible with those of invasive amebiasis, were distinct in ten of the eleven individuals (Table 1). This appears to be consistent with the view that the amebae detected morphologically in the six cases mentioned above were *E. histolytica* but not *Entamoeba dispar* which is nonpathogenic to man. From these cases, we tried to isolate amebae utilizing Sargeaunt's methods (Sargeaunt and Williams, 1978) to further confirm the pathogenicity of amebae by isoenzyme analysis; however, all of such trials were unsuccessful.

Serologic tests were conducted for the sera from all of these cases, after informed consent was given by every case. Their serologic data did not seem to be completely compatible with the morphological observations. For instance, the case with trophozoites of *E. histolytica* in the colon tissue specimen was negative by virtually all of the procedures employed. GDP was negative in another case with *E. histolytica* in the liver pus. Moreover, also in the five individuals without morphological evidences on amebic infection, GDP was negative in two of them and ELISA in one of the two GDP-negative cases, though they had distinct clinical symptoms of invasive amebiasis. However, these two GPD-negative individuals were judged positive by polymerase chain reaction (PCR) utilizing *E. histolytica*-specific primers designed on the basis of nucleotide sequence of the gene coding a 30-kDa nuclear antigen according to Tachibana *et al.* (1992), suggesting that these two cases were also infected with *E. histolytica* (data not shown).

These serologic responses did not correlate well with the number of CD4 lymphocytes. For instance, though the CD4 lymphocyte of the case No 4 was extremely low i.e., 13/μl, the serum antibody against *E. histolytica* was detectable by all of these procedures.

None of these cases exhibited the absence of other concurrent infections. However, the fact that virtually all of these cases were successfully treated with metronidazole at the conventional doses (data not shown) also suggests that their gastrointestinal symptoms like mucobloody diarrhea and liver abscess were primarily due to *E. histolytica* infection. Other pathogens detected were *Treponema pallidum*, Herpes simplex virus and Hepatitis B virus. Moreover, three of the cases had subcutaneous abscess by methicillin-resistant *Staphylococcus aureus* (MRSA), Kaposi's sarcoma, pure red cell aplasia and/or hepatocellular carcinoma. Details of these will be presented elsewhere.

Discussion

Male homosexual population has the risk of concurrent infection with HIV and ameba in Japan as well as in western countries. However, as shown in our previous reports (Takeuchi *et al.*, 1987; Kobayashi *et al.*, 1992), circulation of pathogenic *E. histolytica* among homosexual men in Japan was expected to make a distinct contrast to the epidemiology and clinical course of the male homosexual AIDS cases infected with ameba in western countries, where *E. histolytica* was scarcely detected in the male homosexual population (Druckman and Quinn, 1988). The presence of such a difference between Japan and western countries was confirmed by the observation of Allason-Jones *et al.* (1988) that they did not find any evidences of invasive amebiasis in numerous male homosexuals including HIV-infected individuals in the United Kingdom. Moreover, Reed *et al.* (1991) showed that the AIDS cases with amebic infection they examined in the United States did not have detectable antibodies to *E. histolytica* and found no significant difference in the gastrointestinal symptoms between AIDS cases with and without amebic infection. Consequently, Allason-Jones *et al.* (1988) and Reed *et al.* (1991) attributed the amebic infection of virtually

all of these homosexual males and AIDS cases they examined to *E. dispar*. In contrast, our present study clearly supports the occurrence of invasive amebiasis by *E. histolytica* in Japanese homosexual men with HIV infection, which appears to be ascribed to the widespread distribution of *E. histolytica* among Japanese male homosexual population. In accordance with our present findings, Ohnishi *et al.* (1994) reported a Japanese homosexual AIDS case with invasive amebiasis. This patient was also judged negative by five serologic tests for *E. histolytica* infection including GDP, IHA and ELISA.

Although serologic tests, in particular GDP, are useful for diagnosis of invasive amebiasis by *E. histolytica* (Patterson *et al.*, 1980), our present study suggests that such procedures might give misleading results in the cases concurrently infected with HIV. It is not known at present whether decrease in CD4 lymphocyte number and/or many other concomitant opportunistic infections resulted in the low reliability, mostly due to false negative reaction of GDP and ELISA, of the serologic tests for invasive amebiasis. Ohnishi *et al.* (1994) suggested that the deficiency in T-cell and B-cell functions may cause such abnormal serologic data. Obviously, other sensitive methods to detect the parasite or its nucleic acid like PCR would be useful for such cases as those reported in this communication.

On the basis of the present findings, it is stressed that *E. histolytica* should be regarded as one of opportunistic pathogens associated with AIDS in Japan in contrast to western countries, where *E. dispar* has been primarily found in AIDS cases. In addition, the ameba circulating among male homosexual population needs to be identified in the areas where such an investigation has not been conducted. In particular, this should be done against the homosexual males in the endemic regions of invasive amebiasis, because previous studies (Masuda *et al.*, 1986) showed that such immunocompromised conditions as those by pregnancy or steroid administration affected the clinical course of invasive amebiasis, and sometimes resulted in the fatal amebic infection. In the present study, indeed, we found that one of the cases had surgical resection of the intestinal lesions because of the presence of extensive colon abscess (data not shown). He had a low CD4 lymphocyte number, which may suggest that the immunodeficiency caused by concurrent infection with HIV affects the clinical course of invasive amebiasis.

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