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Case Report

Human Intestinal Spirochetosis Accompanied by Human Immunodeficiency Virus Infection: A Case Report

Masaaki Higashiyama[a,b], Sho Ogata[a,c]*, Yoshikazu Adachi[d], Junichiro Nishiyama[a], Ichiyo Ohara[a], Meri Okamura[a], Koji Matsuzaki[a], Yasushi Okusa[a], Kimiya Sato[e], Ryota Hokari[b], and Soichiro Miura[b]

[a]Division of Medicine, Japan Self Defense Forces Hospital Yokosuka, Yokosuka, Kanagawa 237–0071, Japan, Departments of [b]Internal Medicine II and [c]Pathology and Laboratory Medicine, National Defense Medical College, Tokorozawa, Saitama 359–8513, Japan, [d]Animal Health Laboratory, School of Agriculture, Ibaraki University, Ami, Ibaraki 300–0331, Japan, and [e]Department of Pathology, Japan Self Defense Forces Central Hospital, Setagaya-ku, Tokyo 154–8532, Japan

We present a middle-aged, heterosexual Japanese man with mixed infections including human intestinal spirochetosis, which led us to the detection of human immunodeficiency virus (HIV) infection. The patient had syphilis without related physical or neurological findings. An examination for the serum antibody for HIV performed 9 years previously was negative. In a complete medical checkup at the present time, human intestinal spirochetosis and unspecified entamebic cysts were suggested by histological examination of colonic biopsy material and parasitic examination of the intestinal fluid, respectively. Moreover, a serological test for the antibody for HIV was positive. In specimens obtained by colonoscopy, *Brachyspira aalborgi* was diagnosed by ultrastructural study and the polymerase chain reaction method for bacterial 16S ribosomal deoxyribonucleic acid. Although HIV infection remains at low prevalence in Japan, we recommend examination for HIV infection in patients with human intestinal spirochetosis, especially when other co-infections are apparent.

Key words: *Brachyspira*, entameba, human immunodeficiency virus, human intestinal spirochetosis, syphilis

Human intestinal spirochetosis (HIS) is an infectious disease caused by a spiral microorganism (*Brachyspira* species) [1]. One member of the spirochete family is *Treponema pallidum* (the causative agent of syphilis, but genetically unrelated). In HIS, *Brachyspira* species attach to the colorectal surface epithelium, giving rise to the typical so-called fringe formation.

HIS patients often are asymptomatic, and HIS exists as a single infectious disease in many cases [2]. HIS has also been observed in critically ill patients [3] and in immunocompromised patients infected by human immunodeficiency virus (HIV) [4]. Here, we present a Japanese man who was found to have HIV infection during a check-up of his immune state (carried out when he was found to have mixed infections, including HIS). As a consequence, we recommend examination for HIV infection in patients with mixed infections that include HIS.

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*Corresponding author. Phone: +81–4–2995–1505; Fax: +81–4–2996–5192
E-mail: sogata@ndmc.ac.jp (S. Ogata)

Case Report

The patient was a middle-aged, heterosexual Japanese man with a past history of pneumonia (with the symptom of 3-month-long cough) 9 years ago. During therapy for his pneumonia, syphilis was diagnosed (without related symptoms). At that time, an examination for serum HIV antibody proved negative. He had been treated with penicillin, and his pneumonia had improved. Unfortunately, he had not received periodical medical examinations and was not followed up until the present admission.

This time, the subject was admitted to the Japan Self Defense Forces (JSDF) Hospital Yokosuka (Kanagawa, Japan) for a complete medical checkup. His syphilis remained not well treated: rapid plasma reagin was positive at 1 : 32 diluted plasma, and the *Treponema pallidum* hemagglutinin test was also positive at 1 : 2,480 diluted serum. He had no syphilis-related or other clinical symptoms, but he had soft stools that were occasional bloody. A fecal occult blood test was positive. On colonoscopic examination, a small polyp was found in the cecum, and histological examination of the polyp revealed a tubular adenoma with a so-called fringe formation on the luminal side of the epithelium, suggesting HIS (Fig. 1A–C). As described in a previous report [6], the fringe formation was observed not only on the non-neoplastic surface epithelium but also on the adenomatous epithelium. While he was awaiting a second colonoscopy for a definitive diagnosis of HIS, we advised the patient to have his immune state checked, including a serum HIV antibody test, and we received his consent. The results revealed that serum HIV antibody was positive

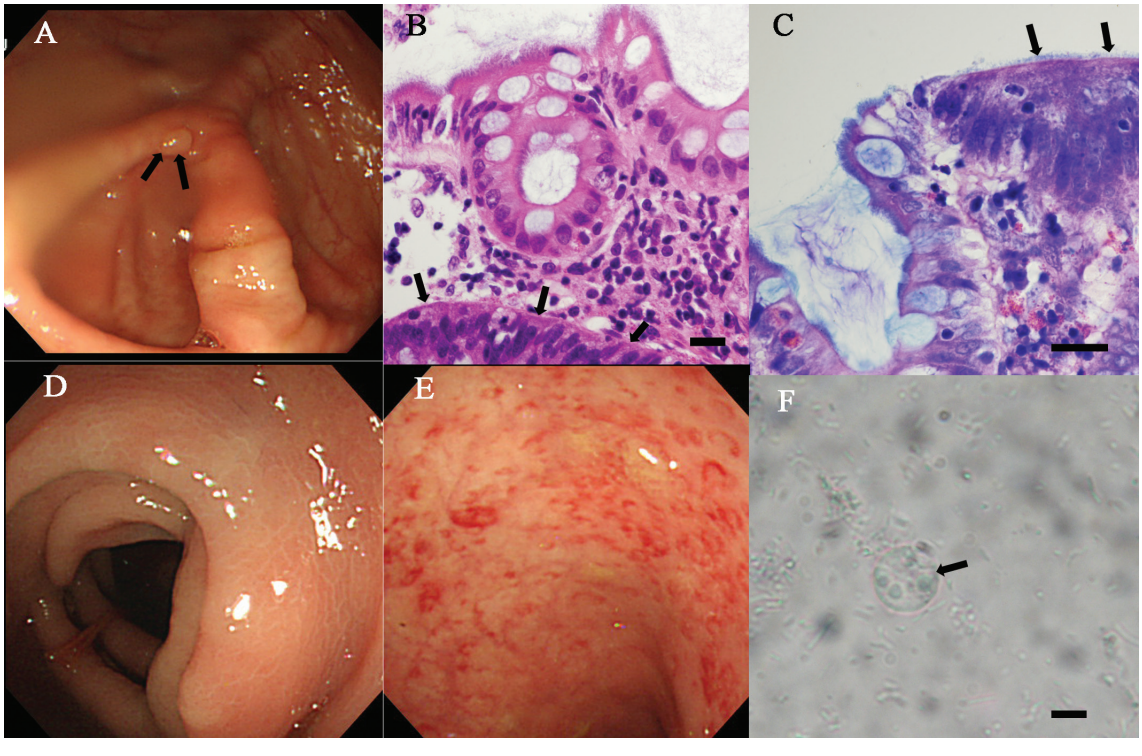

Fig. 1 Endoscopic view (**A**) and histology (**B**, **C**) from the first colonoscopy, and endoscopic views (**A**, **B**) and parasitic examination (**C**) from the second colonoscopy. **A**, A small colonic polyp (arrows) was revealed in the cecum. The background colonic mucosa appeared slightly edematous; **B**, The so-called fringe formation was revealed on the luminal side of the non-neoplastic, colonic surface epithelium. Tubular adenomatous glands (arrows) were also seen below the non-neoplastic epithelium (hematoxylin-eosin stain); **C**, With Giemsa staining, the so-called fringe formation was accentuated and eosinophilic infiltration was observed. Fringes were seen not only on the non-neoplastic surface epithelium but also on the neoplastic epithelium (arrows) (Giemsa stain); **D**, **E**, Slightly rough, edematous mucosa was seen in the ascending colon (**D**), and proctitis was observed (**E**); **F**, Direct microscopic examination of the intestinal fluid revealed an unspecified entamebic cyst (arrow) with 4 nuclei-like internal structures. Scale bars in **B**, **C**, and **F** indicate 10 μm each.

(by the Western blot method) at this time. Further tests showed that the ribonucleic acid titer for HIV in the peripheral blood was 5.3×10^3 copies per milliliter, and that the number of CD4-positive lymphocytes in the peripheral blood had decreased to the level of 300 cells per microliter.

The patient underwent the scheduled second colonoscopic examination for the definitive diagnosis of HIS by ultrastructural examination, culturing and isolation, and the polymerase chain reaction (PCR) method. Endoscopically, a slightly rough and edematous mucosal configuration in the ascending colon and scattered red spots in the rectum were observed. Parasitic examination of the aspirated intestinal fluid revealed oval materials with 4 nuclei-like internal structures (Fig. 1D–F). Although we thought that these might be entamebic cysts, moving trophozoites were not found, and permanent specimens for definitive diagnosis were unfortunately not preserved. Examination for the serum antibody for *Entameba histolytica* later proved to be negative. Histologic examination of stepped biopsy specimens from the cecum to the rectum revealed a typical so-called fringe formation on the surface mucosa, suggesting that HIS was distributed throughout the entire colorectum, without any other protozoan infection. The diagnosis of the amebic cysts remained "unspecified entameba".

In the ultrastructural study, glutaraldehyde-prefixed and osmium-postfixed biopsy specimens were sequentially dehydrated with propylene oxide and then embedded in epon resin. Specimens were cut ultrathin, stained with uranyl acetate and lead citrate, and finally observed using a transmission electron microscope (H-7500; acceleration voltage 80 kV; Hitachi, Tokyo, Japan). Spiral microorganisms were observed attached to the luminal side of the colonic surface epithelium without apparent invasion (Fig. 2A), an appearance typical of HIS.

Culturing and isolation for *Brachyspira* was performed as described elsewhere [7]. Briefly, biopsy specimens were incubated (in an anaerobic chamber at 37 degrees Celsius) with Trypticase soy agar containing 5% sheep blood with spectinomycin and other antibiotics. *Brachyspira* was successfully isolated after 3 weeks of culturing. Total deoxyribonucleic acid (DNA) was extracted from each of the isolated bacterial and biopsy specimens using InstaGene Matrix™ (Bio-Rad Laboratories, Hercules, CA, USA), and then amplified by the PCR method for Brachyspira 16S ribosomal DNA. The causative agent of the patient's HIS infection proved to be *Brachyspira aalborgi* (Fig. 2B).

The results of the examinations we performed revealed that this patient had mixed infections involving HIS, syphilis, unspecified entameba, and HIV. Treatment was started with amoxicillin trihydrate

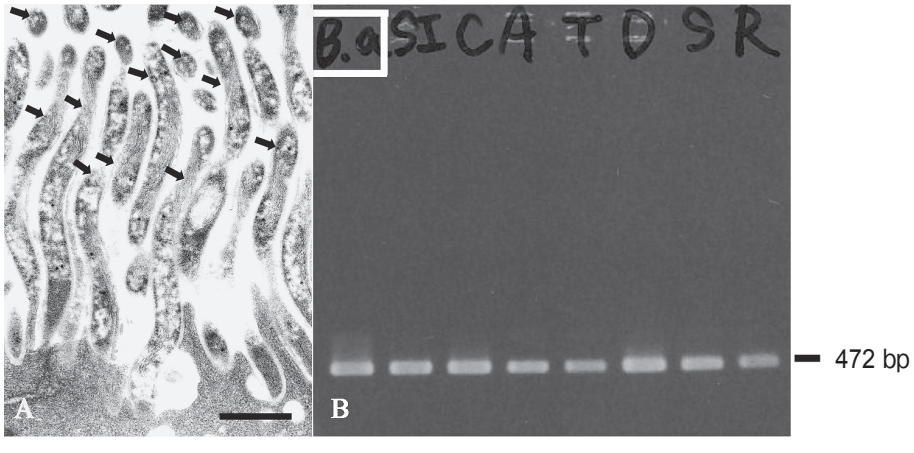

Fig. 2 Additional confirmation studies for spirochetes. **A**, Ultrastructurally, spiral organisms (arrows) are perpendicularly attached to the luminal surface of the colonic surface epithelium. The scale bar indicates 1 μm; **B**, Electrophoresis of PCR products from samples taken from 7 intestinal regions reveals a row of 472 base-pair bands compatible with amplified regions of the 16S ribosomal cDNA of *Brachyspira aalborgi* [7]. Label B. a indicates the control lane for *Brachyspira aalborgi* (ATCC 11492 (BA)). Lane labels SI, C, A, T, D, S, and R indicate samples from the small intestine, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum, respectively.

against the syphilis and HIS, and he was then transferred to another hospital to receive highly active antiretroviral therapy for his HIV infection. Unfortunately, he did not visit our hospital again, and so we could not perform further medical examinations to determine the status of his syphilis and HIS infections.

Discussion

HIS was first described in 1967 by Harland and Lee [8], although spirochetes in the stools had been observed as long ago as during Van Leeuwenhoek's era [1]. Although a large number of reports on HIS have been published, its clinical significance remains controversial, even in immunocompromised patients with HIV infection [2, 9–11]. Some authors have regarded HIS as harmless, while others have suggested that it might play a role in the appearance of abdominal symptoms, including diarrhea, abdominal pain, and bloody stools. In the present case, the patient had soft stools that were occasionally bloody, although he reported no abdominal complaints. Moreover, mixed infections, including HIS, were detected by colonoscopy. However, we could not determine whether a significant relationship existed between occasionally bloody soft stools and co-infection with entameba and HIS, because follow-up observations could not be made.

The prevalence of HIS infection is high (11.4–64.3%) in developing countries, but low (1.1–5%) in countries in which living standards are high [12]. However, a relatively high prevalence of HIS infection (12.1%) was recently reported from Italy [13], and moreover HIS is frequently observed (20.6–62.5%) in homosexual males [12]. Although little is known about the transmission of HIS to humans, 2 different populations may be supposed: one consists of healthy people who are exposed to contaminated water and/or contact with infected animals, and the other of homosexuals or immunocompromised patients exhibiting deterioration in their immune state [12]. In Japan, a single study of the prevalence of HIS recently suggested it to be as low as 0.4% [14]. With regard to this reported low prevalence of HIS in Japan, 3 speculative explanations may be put forward: i) the clinicopathologic features of HIS are not widely recognized, leading to failure in diagnosis, ii) exposure to contaminated water or infected animals (the supposed HIS infection route in developing countries) is rare, and iii) there is only a small population of homosexuals in Japan.

The possible relevance of the speculation that there is only a small population of homosexuals in Japan may be compounded by the apparently weak relationship between HIS and HIV among Japanese homosexuals. Concerning the relationship between HIV and HIS among homosexual men, Law and colleagues reported that among 54 cases with HIS in Australia, the percentage of cases with positive HIV antibody was 50% [4]. In contrast, only one HIV-positive case was detected among 20 HIS cases collected by a single hospital in Japan [14]. This lower HIV-positive percentage among Japanese HIS cases may support the idea that HIS infection routes are not commonly found among homosexuals in Japan. Be that as it may, HIV infection leads to profound immunocompromisation and is frequently accompanied by several other infections, for example, tuberculosis, syphilis, cytomegalovirus infection, candidiasis, cryptosporidiosis, and HIS [2, 15]. HIS may be an opportunistic infection among immunocompromised hosts, but unfortunately no report has been published concerning the prevalence of HIS in Japanese immunocompromised patients or in patients with therapeutic immunosuppression. Therefore, the inclusion of immunocompromised patients in future epidemiological studies will be necessary to estimate the true prevalence and uncover the main HIS infection route in Japan.

Although the prevalence of HIV infection in Japan was estimated to be below 0.1% in 2006, it is increasing year by year [5]. Thus, the potential risk of acquiring HIS may increase with the expected increase in HIV infection in the future. The present case may provide us with a clue about the better detection of HIV-positive Japanese patients. In Japanese social health systems, complete medical checkups and annual screening, including total colonoscopy, are widely performed. Provided diagnostic pathologists are familiar with HIS, it ought to be found with ease in routine colorectal biopsy specimens. Furthermore, the presence of HIS, especially with other infections, may suggest some immunological derangements or breakdown in the intestines, because HIS infection may be frequently found in patients with HIV infection, at least in some countries [4]. In patients with

HIS (especially those with co-infection), an immunological workup, especially examination for HIV, should be considered, since early detection of HIV infection would not only improve patients' prognoses but also reduce socioeconomic expenditure by public health and insurance organizations. In contrast, examinations targeted only toward HIS in immunocompromised patients (including HIV-positive patients) may be unwarranted, unless they have apparent gastrointestinal symptoms, because in most HIS cases the clinical manifestations are modest [4].

In conclusion, we present here a rare Japanese case of HIS with accompanying syphilis, unspecified entameba, and HIV infection. Co-infection including HIS may suggest a derangement in the immune state of the intestines and may be a pointer towards possible HIV infection. On that basis, it may be advantageous if HIS patients with co-infections are checked for HIV infection.

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