Asymptomatic Intestinal Spirochetosis Found Incidentally in Endoscopic Specimens

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Abstract

Intestinal spirochetosis is a zoonotic infection caused by gram-negative bacteria of the genus Brachyspira. In Japan, several cases have been reported since Nakamura et al. first reported the disease in 1998, and although it has been recognized by pathologists, there have only been a handful of reports on the disease. In this study, we investigated an incidence of intestinal spirochetosis in samples obtained through colonoscopic biopsy, polypectomy, and EMR. Ten patients (6 men and 4 women) were diagnosed with intestinal spirochetosis and had been infected with Brachyspira aalborgi among 537 patients (318 men and 219 women). Ages ranged from 49 to 73 years. The overall incidence was 1.9%, and the incidence in men (1.88%) and women (1.82%) was almost equal. In past reports, intestinal spirochetes was more common among men, although only one report examined the sex-specific incidence of this disease. Our results suggested that a similar number of infections occur in women, but the infection route is not clear.

Key word

Intestinal spirochetosis

Introduction

Intestinal spirochetosis (IS) is a zoonotic infection caused by gram-negative spiral bacteria of the genus Brachyspira. Currently, there are 7 known species of Brachyspira, and both Brachyspira aalborgi (BA) and Brachyspira pilosicoli (BP) have been known to infect humans. BA was first isolated and identified by Hovind-Hougen et al.¹ in Denmark, and infection was confirmed in human and macaque monkeys who are also primates like humans. Besides humans, BP can infect various animals such as dogs, pigs, and waterfowl²,³, and cause diarrhea in pigs and birds. As such, in the livestock and poultry industry, bacteria have been shown to cause diarrhea and enteritis, leading to decreased growth rate and productivity.⁴ BP also causes diarrhea in household pets, which may be transmitted from animals to humans.⁵ BA and BP are both anaerobic bacteria but are relatively resistant to oxygen and do not die even when exposed to oxygen for a short period of time. The biggest difference between these two species is their culture periods. While BP takes 5 to 7 days to culture, BA takes 14 to 21 days, making the latter difficult to culture. Among the bacteria of the genus Brachyspira that do not infect humans, B. hyodesenteriae infects the intestinal tract of pigs, causing swine dysentery, primarily characterized by mucus and bloody stool. Swine dysentery is also considered a communicable disease to be reported under the Japanese Domestic Animal Infectious Diseases Control Law. While the mortality rate of swine dysentery is only about 5%, it causes a huge economic loss because of delayed growth and reduced feed efficiency.⁶

BA is common in developed countries, while BP is common in developing countries. Generally, BA is non-pathogenic or has low pathogenicity.⁷ In humans, BP is often transmitted in developing countries, but has also been observed in gay men living with HIV in western countries.⁸ In an immunodefi-
cient state, BP may become an opportunistic pathogen causing diarrhea and enteritis. Although BP has a low incidence in developed countries, it may cause opportunistic infections or aggravate the patient’s condition.

In Japan, intestinal spirochetosis was first reported by Nakamura in 1998, and since then, a number of reports have been published. Despite this, due to the limited number of patients and unclear incidence, pathogenicity, and clinicopathological features, the disease has not garnered sufficient recognition by pathologists and clinicians. In this study, we investigated the incidence and clinicopathological features of IS using results from colon biopsy, endoscopic mucosal resection (EMR), and polypectomy at Kawasaki Municipal Tama Hospital.

Materials and Methods

This study examined samples from taken from colonoscopic biopsies polypectomies, and EMR conducted at Kawasaki Municipal Tama Hospital from April 1, 2019 to September 30, 2019. There were 537 patients — 318 men (21–95 years old) and 219 women (20 to 91 years old), with a median age of 70 years for both men and women. This study was approved by the Bioethics Committee of St. Marianna University School of Medicine (No. 4675).

A hematoxylin-eosin (HE)-stained specimen prepared from a formalin-fixed paraffin-embedded tissue block of the target specimen was microscopically examined by a pathologist using an optical microscope. After regular pathological diagnosis, the presence or absence of IS was determined. IS-positive cases had a fuzzy basophilic band or a purple band with a thickness of 2 to 3 μm, called a pseudo brush border found on the surface of the colonic mucosal epithelium. These patients were histologically diagnosed as IS. The incidence and clinicopathological features of IS were examined.

DNA extraction to identify the bacterial species

DNA was collected by slicing three 10-μm sections from the paraffin block. Using the method of Mikosza et al., two species were identified from the electrophoresis pattern for each 16s ribosomal RNA: BA and BP. Table 1 shows the sequence of primers and the sizes of the PCR products.

Results

IS was noted in 10 (1.9%) of the 537 patients. Table 2 shows the clinicopathological characteristics of these 10 patients. IS was noted in 6 (1.88%) of 318 men and 4 (1.82%) of 219 women. IS was noted in patients whose ages ranged from 49 to 73 years, with a median age of 69.5 years. The median age was 71 years (range, 49 to 72 years) in men and 63.5 years in women (range, 56 to 73 years). There was no significant difference in terms of the sex-specific incidence of IS infection. In terms of the purpose of colonoscopy, 8 patients were asymptomatic, which included 2 patients (20%) for examination due to a positive fecal occult blood test, 4 patients (40%) for adenoma...
EMR, and 2 patients (20%) for screening. Two patients had clinical abdominal symptoms, 1 patient (10%) experienced hematochezia and abdominal pain, and 1 patient (10%) had abdominal pain. During colonoscopy, adenomas were found in all patients. In all patients, IS was noted in the non-adenoma area rather than in the adenoma area (Figure 2a and b). Adenomas were found in 2 patients who underwent examination for clinical abdominal symptoms. Endoscopy did not reveal findings such as enteritis as the cause of abdominal pain. The cause of hematochezia and abdominal pain in Patient 7 was diverticular bleeding, and the cause of abdominal pain in Patient 8 was diverticulitis. Both patients are currently under observation.

Underlying diseases included the following: hypertension (5 patients), hyperlipidemia (3 patients), diabetes mellitus (4 patients), hyperuricemia (1 patient), myocardial infarction (1 patient), cerebral infarction (1 patient), hyperthyroidism (1 patient), IPMN (1 patient), and postoperative gastric cancer (2 patients). Only 1 patient did not have any underlying diseases. It is unclear whether the patient had a history of raising pets, HIV infection, or evident immunodeficiency.

The results of the genetic analysis revealed that all 10 patients of IS were caused by BA and BP was not present in any of the patients (Figure 3).

Table 1. Sequence of Primers to Detect the Gene for 16S rRNA of Brachyspira aalborgi and Brachyspira pilosicoli

| Primer         | Sequence                                      | Size of products(bp) |
|----------------|-----------------------------------------------|----------------------|
| B. aalborgi 16S rRNA | F: TACCGCATATACTCTGTTGAC R: CCTCAAAATATCCAAGAACCC | 472                  |
| B. pilosicoli 16S rRNA | F: AGAGGAAAGTTTTCGCTTTCC R: GTGCCTCATCAGACTTT | 196                  |

F;Forward. R;Reverse

Table 2. Clinicopathological Feature of Intestinal Spirochetosis

| Patient | Age | Sex | Purpose of CS | Abdominal Symptoms | Pathological diagnosis | Infection area | Location | Finding of CS | Treatment of IS | Others | Result of PCR | Comorbidities/ Past history | Animal breeding history | HIV infection |
|---------|-----|-----|---------------|--------------------|-----------------------|-------------------|----------|--------------|----------------|---------|---------------|--------------------------|------------------------|--------------|
| 1       | 72  | M   | FOB           | Tubular adenoma    | Non-adenoma area | T                  | None     | None         | None           | BA      | H/T, H/L     | Unknown Unknown          |                        |              |
| 2       | 73  | F   | screening     | Tubular adenoma    | Non-adenoma area | S                  | None     | None         | None           | BA      | None         | Unknown Unknown          |                        |              |
| 3       | 71  | M   | screening     | Tubular adenoma    | Non-adenoma area | A                  | None     | None         | None           | BA      | Hyperuricemia | Unknown Unknown          |                        |              |
| 4       | 56  | F   | EMR of the adenoma | Tubular adenoma | Non-adenoma area | A                  | None     | None         | None           | BA      | None         | Unknown Unknown          |                        |              |
| 5       | 68  | M   | EMR of the adenoma | Tubular adenoma | Non-adenoma area | R                  | None     | None         | None           | BA      | H/T, H/L     | Unknown Unknown          |                        |              |
| 6       | 49  | M   | EMR of the adenoma | Tubular adenoma | Non-adenoma area | T                  | None     | None         | None           | BA      | DM, H/T      | Unknown Unknown          |                        |              |
| 7       | 59  | F   | EMR of the adenoma | Tubular adenoma | Non-adenoma area | C                  | None     | None         | None           | BA      | Hyperuricemia | Unknown Unknown          |                        |              |
| 8       | 71  | M   | Examination of the abdominal pain | Tubular adenoma | Non-adenoma area | C                  | None     | None         | None           | BA      | DM, H/T, H/L | Unknown Unknown          |                        |              |
| 9       | 68  | F   | FOB           | Tubular adenoma    | Non-adenoma area | T                  | None     | None         | None           | BA      | None         | Unknown Unknown          |                        |              |
| 10      | 71  | M   | EMR of the adenoma | Tubular adenoma | Non-adenoma area | A                  | None     | None         | None           | BA      | H/T, H/L     | Unknown Unknown          |                        |              |

C:Cecum, A:ascending colon, T:transverse colon, D:desending colon, S:sigmoid colon, R:rectum, FOB:Fecal occult blood, H/T:Hypertension, H/L:Hyperlipidemia
※1: Cause of abdominal pain and hematochezia was diverticulum hemorrhage
※2: Cause of abdominal pain was diverticulum
※3: Past history of the gastric cancer

Discussion

In this study, BA was detected in all 10 patients of IS, but BP was not detected. These results are consistent with previous reports stating that most patients of IS in Japan are caused by BA. Furthermore, in this study, 8 patients were asymptomatic, and 2 patients were symptomatic. The abdominal symptoms in the 2 patients were caused by diverticulitis and diverticular bleeding. Enteritis or similar symptoms were not noted; thus, all BA patients in this study were considered asymptomatic. Our results support previous findings that BA is non-pathogenic or has low pathogenicity. The results also indicate that IS patients caused by BA are mostly asymptomatic.

Primarily, IS is orally transmitted through the ingestion of water and food contaminated feces. IS infection patterns include those in developing countries where infection is seen in groups within families and tribes, and opportunistic infection patterns reported in immunocompromised patients such as AIDS in Europe and the United States. Essentially, IS is a zoonotic disease, but the cause of infection remains unclear. In this study, many patients were complicated by various underlying diseases such as diabetes, hypertension, and post-carcinectomy. However, it was unclear whether these patients were immunodeficient. Furthermore, it was also unclear whether...
these patients had pets or were living with HIV. All patients had unknown transmission routes; thus, moving forward, we believe it is important to elucidate the transmission route of IS.

In developed countries, the incidence of IS has been reported to be 1.1% to 1.9%, and BA has been shown to have a higher infection rate than BP. Conversely, in developing countries, IS has been shown to have a much higher incidence (11%–64%). In developed countries, HIV-positive patients and gay people have a higher incidence (20–62.5%). In previous reports in Japan, the incidence of IS was reported to be 0.4% by Tanahashi et al., 4.1% by Shiozawa, 0.6% by Tanabe, 1.7% by Tateishi, and 0.2% by Ichimata. In Sweden, Thorell et al. reported an incidence of 2.3%. In this study, the incidence was 1.9%, which is similar to the incidence in other developed countries; therefore, we believe that the mode of infection and opportunistic infections that can be seen in developing countries were not involved in our patients.

Past reports concerning IS have noted sex-specific differences, stating that IS was predominant among men. The male-to-female ratio was 17:3 as reported by Tanahashi et al., 36:6 as reported by Shiozawa et al., 28:6 as reported by Tanabe et al., and 18:1 as reported by Tateishi et al. However, Ichimata reported only one involving a woman. In contrast, Thorell reported 9 cases in men and 8 cases in women. In this study, the cases involved 6 men and 4 women. Although there were more men, they were not significantly predominant. These results suggest that a certain number of infections also occur in women. From the viewpoint of the number of patients by sex, 6 out of 318 males (1.88%) and 4 out of 219 females (1.82%) were infected, indicating an almost equal incidence in men and women. Among the previous reports, only Tateishi et al. recorded the sex-specific incidence of IS. In that report, the incidence was significantly higher in men (2.3%) than in women (1.1%). However, there are no other reports examining the sex-specific incidence of IS. We believe it is important to continue investigating whether IS infection is found predominantly among men or if it also occurs at a certain percentage among women. To date, one of the reasons why IS is considered more common in men is the high IS rate among gay men and people living with HIV. However, Shiozawa explained that sexual orientation could not possibly be the only reason for the predominance among men because the incidence of intestinal spirochetes in Japan, which has fewer people living with HIV, is the same as in Western countries. In this study, data on sexual orientation and HIV status of all patients were not available. However, since people living with HIV tend to be relatively young, it is unlikely that any of these patients who were older people, were living with HIV. It has also been reported that 26.7% of healthy people have IS in Oman, which is an Islamic state that prohibits homosexuality. This suggests that sexual orientation and HIV status are not the reasons for the observed male pre-
dominance in IS, leading us to believe that a different transmission route is involved.

There is no consensus on treatment strategies for IS. Most people with IS are asymptomatic and do not require treatment. However, based on reports of severe cases, treatment is required in immunocompromised patients and in cases where patients experience clinical symptoms with no other plausible cause aside from IS. Furthermore, there may be a risk of spreading the infection because of chronic infection. We believe that follow-up without treatment would suffice for asymptomatic patients; however, treatment should be actively administered to symptomatic patients.

**Conclusion**

IS may be difficult to detect unless shown by a pathologist, because the causative bacteria require a long time to culture. All cases of IS in this study were all asymptomatic BA infections. The incidence of IS in this study was similar to that previously reported in developed countries. Furthermore, the sex-specific incidence was almost the same for both men and women. The infection route is not clear, and further studies are needed in the future.

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**Conflicts of Interest**

The authors have nothing to disclose.

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