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Case report

Use of polymerase chain reaction in the diagnosis of Whipple's disease

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ABSTRACT

Whipple's disease, a systemic, chronic infectious disease caused by *Tropheryma whipplei*, is extremely rare in Asian populations. A correct diagnosis is necessary due to its high mortality rate. Unfortunately, patients are apt to be misdiagnosed with connective tissue diseases since they typically present with arthritis or arthralgia. There are three diagnostic tools for Whipple's disease using intestinal tissues: 1) periodic acid-Schiff (PAS)-positive macrophages; 2) electron microscopic observation; and 3) polymerase chain reaction (PCR). It is challenging to diagnose this disease in the absence of histological findings, especially in Japan, where the clinical protocol currently used to make the diagnosis needs improvement, although symptomology and PCR results may be sufficient. Herein, we investigated a 24-year-old Japanese woman who had suffered from intermittent fever, migratory arthralgia, and watery diarrhea for several months. Her biopsied intestinal tissue was negative for foamy macrophages and PAS-positive cells, and electron microscopy did not provide diagnostic insight. PCR amplification of the specimens, however, successfully revealed *T. whipplei*. Whipple's disease was diagnosed based on a positive PCR result and strong clinical suspicion. The patient was treated parenterally with ceftriaxone (2 g daily) for two weeks, followed by oral treatment with 160 mg trimethoprim and 800 mg sulfamethoxazole twice per day. After one month of treatment, her symptoms disappeared and inflammatory markers returned to normal levels. This case illustrates the practicality and effectiveness of a PCR-based diagnostic test in combination with clinical suspicion to correctly diagnose Whipple's disease, especially in cases when a histological examination does not provide insight.

1. Introduction

Whipple's disease is a rare systemic, chronic infectious disease caused by *Tropheryma whipplei*, a Gram-positive bacillus. Early treatment is necessary because the mortality rate is high in the absence of treatment [1]. Since most patients with Whipple's disease present with intermittent, recurrent, and migratory arthritis or arthralgia during the early phase of the disease [2], they are apt to be misdiagnosed with rheumatoid arthritis [3] or other connective tissue diseases such as adult-onset Still's disease [4]. In both cases, biological immunosuppressive agents were prescribed instead of the antibiotics necessary to treat Whipple's disease [3,4]. This misdiagnosis can lead to the delay of an exact diagnosis, resulting in a higher chance of morbidity. Periodic acid-Schiff (PAS)-positive macrophages and electron microscopic observation of digestive tract tissues (or tissues from other potentially affected organs) are the current diagnostic tools for Whipple's disease. Successful culture from feces or tissue samples is limited to specialized laboratories and is not useful as a routine diagnostic approach because of the need for a specific axenic medium for cell-free culture and the amount of time required, which may be several months [5]. However, polymerase chain reaction (PCR) can be utilized when there is an absence of histological findings by electron microscopy [6]. In Japan, the clinical protocol currently used to make the diagnosis of Whipple's disease needs improvement, due to its low incidence rate and subsequent failure of clinicians to recognize the disease. In the absence of histological findings, it is difficult to make an accurate diagnosis; consequently, PCR may prove to be a powerful diagnostic tool. In this study, we report the case of a Japanese woman who presented with intermittent fever, watery diarrhea,

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and migratory arthralgia who was successfully diagnosed with Whipple's disease via PCR using biopsied tissue from her ileum.

2. Case report

A previously healthy 24-year-old Japanese woman who had experienced 5 months of intermittent fever and general fatigue, 3 months of watery diarrhea, and 2 weeks of lower back pain as well as migratory arthralgia arrived at our hospital. During the night, her fever exceeded 38.0 °C and she experienced chills as well as drenching sweat. The patient passed watery diarrhea (absent of melena) five to six times a day. Although her back pain was not alleviated, it was not further aggravated upon exertion. Migratory arthralgia emerged on the patient's shoulders, elbows, wrists, and knees. She claimed that she did not have any weight loss, appetite loss, nausea, or vomiting. Her medical history was unremarkable other than a case of endometriosis. She was not a smoker, did not drink alcohol, had no notable travel history within the previous year, and no notable family history of disease.

On admission, the patient's body temperature was 37.4 °C, her blood pressure was 125/84 mmHg, and her pulse was 109/min. A physical examination of the patient revealed pale conjunctivae, no signs of oral aphthae, and normal lymph nodes. Her breathing rate and heart rate were normal. Her abdomen was soft and flat, with slight tenderness upon palpation and percussion, and no signs of a rash or ulceration. Although she had migratory arthralgia, the patient did not have swollen or tender joints and she did not feel tenderness on percussion of her vertebrae. A laboratory analysis showed anemia (hemoglobin, 9.8 g/dl) and elevated inflammatory markers, including a C-reactive protein (CRP) of 11.6 mg/dl and an erythrocyte sedimentation rate of 109 mm/h. The patient's levels of anti-nuclear, proteinase 3-antineutrophil cytoplasmic, myeloperoxidase-antineutrophil cytoplasmic, and anti-cyclic citrullinated peptide antibodies were normal, as well as that of rheumatoid factor. Tests for rapid plasma regain, *Treponema pallidum* hemagglutination, hepatitis B surface antigen, and anti-hepatitis C virus antibody were all negative. She also tested negative for HLA-B27 antigen. Aerobic and anaerobic blood cultures were both negative. No bacteria, protozoans, or parasites were detected from the feces or biopsied samples that could explain the patient's diarrhea or loose feces (e.g., *Campylobacter jejuni*, *Clostridium difficile*, *Salmonella enterica*, *Entamoeba histolytica*, *Giardia lamblia*, or *Cryptosporidium*). Electrocardiography and cardiac ultrasonography produced normal results. Using contrast-enhanced computed tomography, we detected non-specific thickening of the small and large intestinal walls (Fig. 1). Fluorodeoxyglucose positron emission tomography indicated glucose uptake in the ileocecum (SUV_{max}, 8.57). Endoscopic examination of the upper gastrointestinal tract revealed a hiatal hernia. Colonoscopy did not indicate abnormal tissue. Capsule endoscopy revealed swollen and edematous villi in the small intestine. Double balloon endoscopy was utilized to biopsy the ileum and jejunum. The macroscopic findings from this technique mimicked the results obtained by capsule endoscopy (Fig. 2). Histological analysis of the tissue samples was negative for the presence of foamy macrophages and PAS-positive cells, both of which are indicators of Whipple's disease [1,2]. In addition, electron microscopy did not reveal any specific information. Despite the lack of weight loss, we suspected this disease because the patient suffered from diarrhea, arthralgia, and a fever of unknown origin (FUO), as well as elevated inflammatory markers without any evidence of other underlying diseases (e.g., connective tissue disease, inflammatory bowel disease [IBD], intestinal tuberculosis, or malignancy). Initially, Whipple's disease is sometimes recognized as FUO [7]. DNA was extracted from the biopsied tissue sample from the ileum and subjected to PCR. We utilized primers TW221U and TW394L to amplify a 192-bp fragment of TW113, a Whipplei surface protein, as demonstrated by Dreier et al. [8] (Fig. 3). After PCR amplification, the 192-bp fragments were excised from gels, purified for sequencing, and sequencing analysis was performed using the basic local alignment search tool (BLAST). The sequence of the *TW113* gene shared 99% identity with that of *T. whipplei* (accession number BX251410). Therefore, we identified the isolate as *T. whipplei*. This TW113 nucleotide sequence has been deposited into the DNA Data Bank of Japan (DDBJ) under accession number LC066216.

A diagnosis of Whipple's disease was made based on a positive PCR result and clinical suspicion of the disease, despite the absence

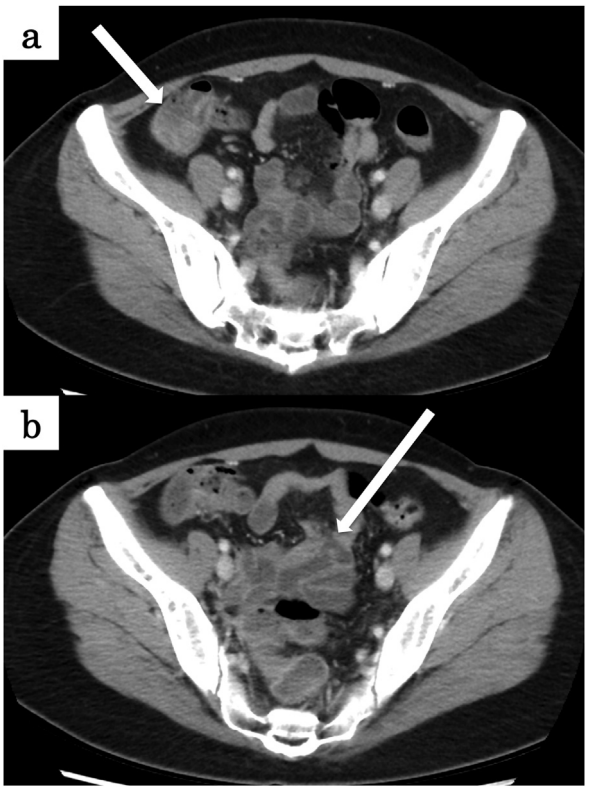

Fig. 1. Contrast-enhanced computed tomography of the abdomen revealed non-specific inflammation of the large intestine (a) and small intestine (b) (white arrow).

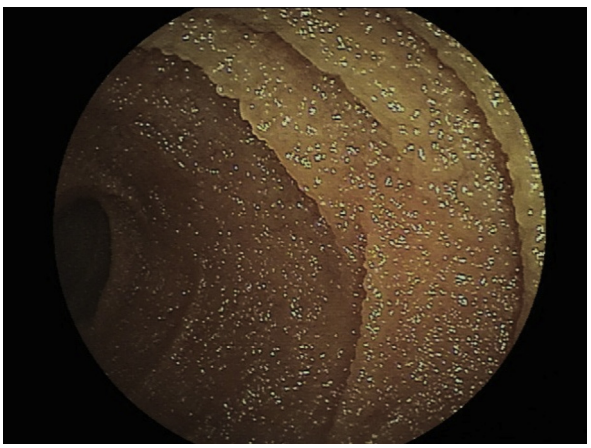

Fig. 2. Double balloon endoscopy revealed swollen and edematous villi within the jejunum.

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