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Bartonella henselae is associated with heartburn, abdominal pain, skin rash, mesenteric adenitis, gastritis and duodenitis in children and adolescents.

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Abstract

Ten patients between the ages of 7 and 16 years presented with a history of cat scratches and or tic bites, chronic abdominal pain, esophageal heartburn, a purpuric skin rash, mesenteric adenitis and a positive Immunoglobulin G titer for *Bartonella henselae (B. henselae).* Endoscopy assessed the gastrointestinal (GI) mucosa for inflammation and biopsies were examined for *Helicobacter pylori* by microscopy and for *B. henselae* by polymerase chain reaction (PCR). Biopsies were PCR positive for *B. henselae* DNA in all patients. Chronic gastritis and or chronic duodenitis was found in all and associated with the detection of *B. henselae* DNA in the GI tract at the site of the inflammation.

Introduction

Cat scratch disease is a syndrome that is characterized by regional lymphadenopathy after a cat scratch or bite distal to the involved lymph node. In addition, many patients have atypical presentations other than regional adenopathy such as neurological syndromes, self-limited granulomatous hepatitis, splenitis, osteitis, atypical pneumonitis, endocarditis and a syndrome of prolonged fever of unknown origin in children.[1] Gastrointestinal presentations of cat scratch disease have included hepatosplenic abscess presenting as abdominal pain[2] and posterior pancreatic duodenal lymphadenitis presenting as abdominal pain.[3] We describe a new atypical clinical presentation of cat scratch disease in which pediatric patients presented with heartburn, abdominal pain, mesenteric adenitis and a purpuric skin rash following a cat scratch or tic bite.

Methods

All patients included had no prior history of GI complaints. They were referred to the Pediatric Gastroenterology and Nutrition service of Jersey Shore Medical Center for evaluation of chronic abdominal pain and heartburn pain which radiated from the sternum up the esophagus and was associated with a purpuric skin rash. Ten consecutive patients satisfying the above clinical criteria who had not been on any steroid medications were evaluated from July 2001 through February 2002 (mean age 12.5 $\pm$ 3.0 years, range 7-16). Each case included a history in which two consecutive months of heartburn and esophageal pain were not affected by diet, position or time of day. The pain was not relieved by histamine blocking medications, antacids or proton pump inhibiting medication. There was a documented history of a tic bite or cat scratch one month prior to evaluation and a positive blood IgG titre for *Bartonella henselae* (greater than or equal to 1:64) at the time of endoscopy. A complete blood cell count, liver function tests, esophagoduodenoscopy(EGD) were performed on all patients. As an additional inclusion criteria, prior to endoscopy, CT scan of the abdomen confirmed mesenteric adenitis (lymph nodes measured 1cm or larger in diameter in all of the patients) without the presence of hepatosplenic granulomas. Stool samples were examined for white blood cells, occult blood, *Salmonella, Shigella, Yersinia, Campylobacter*, ova and parasites and *Clostridium difficile* toxin A and B. GI biopsies assessed the mucosa by microscopy and were evaluated for the presence of *Helicobacter pylori(H. pylori)*. Biopsy specimens were taken from areas of the GI tract that looked inflammed during EGD. Biopses were reported as acutely inflammed when polymorphonuclear cells were present in the mucosa and chronically inflammed if six or more plasma cells and lymphocytes were

present in the gastric mucosa without polymorphonuclear cells. Chronic duodenitis was diagnosed when greater than six intraepithelial lymphocytes per 100 surface absorptive cells were present in tissue biopsies in conjunction with a distortion in glandular architecture.

Polymerase chain reaction for DNA to *B. henselae* was performed on all biopsies by Medical Diagnostic Laboratories, Mount Laurel, New Jersey as described below.

DNA EXTRACTION

The lymphocytes were dissolved in 470μL of tris-EDTA buffer (10mM tris-hydrochloride [pH8.0] and 1 mM EDTA), 25 μL of 10% sodium dodecyl sulfate and 12μL of freshly prepared deoxynuclease-free proteinase K(10mg/ml). The mixture was incubated at 55^{o}C for 2 hours; DNA was extracted by phenolchloroform extraction and ethanol precipitation. The purified DNA was dissolved in pyrogen-free, double-distilled water and quantified using a Genesys-5 spectrophotometer (Spectronics Instruments, Rochester, NY). The purified, quantitated DNA was used as a template for *Bartonella henselae* PCR analysis.

PRIMERS

The PCR primers for the identification of *B henselae* have been described.[4] The primers were syntesized by Research Genetics (Huntsville, Ala) and purified by high-performance liquid chromatography. Their sequences are given in table 1 and previously reported by Eskow, Rao and Mordechai.[4]

POLYMERASE CHAIN REACTION

The PCR mixtures (50μL) contained extracted DNA (5μL, 0.2μg/μL), P24E and P12B primers (50nM), 10mM tris-hydrochloride (pH,8.3), 50mM potassium chloride, 3mM magnesium chloride, 0.001% (wt/vol) gelatin, the nucleotides dATP, dCTP, dGTP, and

dTTP (each at a concentration of 200mmol/L) and 2.5U of Taq DNA polymerase (Perkin-Elmer, Foster City, CA). The PCR was carried out in 0.2 ml tubes. The thermocycler was a Perkin-Elmer Gene AMP PCR system 2400. The PCR program ran for 3 minutes at 94°C, followed by 40, one-minute cycles at 94°C, 1 minute at 56°C and 1.5 minutes at 72°C. The program finished with an additional 10-minute extension step at 72°C. A 30-μL sample of the the final reaction product was run on a 1% agarose gel containing 0.5μg of ethidium bromide per milliliter and the gel was photographed under UV light. For optimization of the PCR conditions for clinical specimens, normal blood was artificially spiked with in vitro-cultivated *B henselae.* A controlled number of *B henselae* (American Type Culture Collection 49882, ATCC, Rockville, MD), ranging from 10^1 to 10^5 pathogens was added to 5ml of whole blood. These spiked samples were treated as described above.

HISTONE PCR

Aliquots (5μL) of the newly extracted DNA were mixed in a 50μL PCR reaction mixture containing 10X PCR buffer (Perkin Elmer), 3 mM magnesium chloride, 200mM dNTP, 2.5μL of Taq DNA polymerase (5U/μL) and 1μL (8pmol) OF 5'-and 3' histone amplifier primer set. The histone primers are complementary to the DNA of a constitutively expressed human histone gene H3.3 as decribed.[15] The amplification process was subjected to 30 cycles of PCR (each cycle at 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 90 seconds) in a 2400 Perkin-Elmer DNA thermocyler. The histone primers served as internal controls for the sample's DNA integrity, presence of inhibitors and intersample equivalency of total amount of DNA analyzed.

PRECAUTIONS AGAINST CONTAMINATION

The extraction of DNA and PCR were performed under sterile conditions and in separate rooms. All positive samples were confirmed by reextraction from the original sample, followed by amplification in triplicate. *Bartonella henselae* DNA- positive status was defined as samples that were positive initially and in at least one of the replicates after reextraction. Pyrogen free water was used in the isolation of DNA from the biopsy specimens. The Eppendorff microcentrifuge tubes and the PCR tubes were sterilized in an autoclave and UV irradiated. New Finn pipettes were used solely with the filter tips for PCR. Disposable plastic trays were used to prepare PCRs in a UV irradiated PCR biohood. The laboratory performing the PCR analysis was blinded to the diagnosis of all specimens they received. Blood and CSF samples (n=10 of CSF and n=5 of blood) from individuals with no evidence of cat scratch disease were used in the PCR assays as negative controls.

Results

The skin rash was a purpuric, serpinginous, nodular, papular rash that did not blanche upon applying pressure to the affected area. In patient 3, the rash presented in the right armpit as three distinct 10cm long vertical lines. In patient 4, it presented in the left groin as three 5cm long serpinginous lines that ran horizontally. The rash did not appear on any other body parts. In patients 7 and 8, the rash appeared only on the lower back in a christmas tree pattern that faded away from the spine and spanned a vertical distance of 15cm(Photo1). In patient 9, the rash was found on his left breast only (Photo 2). There were lines eminating from the areola in a centrifugal pattern. In patient 10, the rash was on the posterior aspect of the right knee only and no other lesions were visualized on the body. (Photo 3).

B. henselae DNA was detected in either the stomach (8 of 10) or the duodenum (8 of 10) of all of the patients. The IgG titer for *B. henselae* was positive in all patients and the IgM titer was negative in all of them. All of the patients with a positive PCR DNA for *B. henselae* had an associated biopsy proven gastritis and/or duodenitis at the site of the infection(Table 2). All biopsies were negative by microscopy for *H. pylori* . There was no evidence of acute inflammation, granulomas or ulcers on EGD. White blood cells, ova and parasites, occult blood in the stool, *Salmonella, Shigella, Yersinia, Campylobacter* and *Clostridium difficile* toxins A and B were not detected in any of the stool samples. CT of the abdomen did not reveal any gallstones, biliary tract disease, pancreatitis or thickening of the distal ileum.

Discussion

Contrary to most patients with typical uncomplicated CSD, our patients were most disturbed by persistent heartburn and abdominal pain. The absence of peripheral lymphadenopathy makes the diagnosis of CSD difficult eventhough hepatosplenic CSD and abdominal pain has already been reported.[2] Due to the insidious and nonspecific nature of the abdominal pain of CSD, diagnosis may be delayed until a history of cat exposure or tic bite prompts CT abdominal imaging-which demonstates multiple mesenteric lymph nodes. Eskow et al implicated *B. henselae* as a potential human- tick borne pathogen in patients with a history of neuroborreliosis who have incomplete resolution of symptoms. A clinical clue to a possible infectious etiology of the heartburn and abdominal pain in this report was the presence of a purpuric skin rash in patients who had not been on steroid medications and who had not had a sudden or abrupt recent weight gain.

Except for a primary inoculation papule found in 60% to 90% of cases, skin lesions are rare, having been reported in about 5% of CSD patients. These rare skin manifestations include maculopapular and urticarial eruptions, granuloma annulare, erythema nodosum, erythema marginatum, thrombocytopenic purpura, leukoclastic vasculitis, multiple granulomatous lesions and erythyma annulare.[1,5] The rash in our patients resembled the striae or "stretch marks" in patients on steroids or those with recent, abrupt, weight gain yet the purpuric nature and nodularity felt on palpation of the rash is more suggestive of a vasculitis as previously reported with *Bartonella henselae* infections.[5]

The constellation of the rash, mesenteric adenitis on CT scan and the presence of IgG antibodies to *B. henselae* suggests a prior infection. The additional detection of *B. henselae* DNA in the stomach and duodenum further supports the presence of *B.*

henselae in the GI tract.[6] The detection of *B. henselae* in the GI tract and the presence of GI inflammation was not proven to be cause and effect. However, inflammation in the GI tract can be attributed to circulating interleukin (IL)-2, IL-6 and IL-10 which are significantly higher in patients with *B. henselae* than in control subjects.[7] IL-6, a multipotent cytokine that may be elicited by *B. henselae,* induces inflammation which histopathology confirmed in the stomach and duodenum of PCR biopsy positive patients.

Further studies, including culture of organisms and a rising sequential IgG titer to *B. henselae* are necessary to further attribute an association of GI inflammation to *B. henselae*. Punch biopsies of the rash will provide a histopathology of the rash and further delineate between striae distensae and the purpuric rash of leukoclastic vasculitis seen in patients with *B. henselae*. Additional biopsies of the rash, the gastrointestinal tract and the mesenteric lymph nodes for culture of organisms or in situ hybridization might help to strengthen the association between *B. henselae* and rash, gastritis, duodenitis, mesenteric adenitis, heartburn and abdominal pain.

Figure Legends and Illustrations

Table 1. Sequences and Positions of Oligonucleotide Primers Used for *Bartonella henselae* Polymerase Chain Reaction Amplification.

*Oligo indicates oligonucleotide primer; rRNA, ribosomal RNA; and rDNA, ribosomal DNA. Arrows indicate the direction of the primer.

Table 2. Biopsy results in Ten Children with Abdominal pain, heartburn, rash and mesenteric adenitis.

Photo 1 Rash appearing only on the lower back of patient 7 who also presented with abdominal pain, heartburn, mesenteric adenitis and chronic gastritis, *B. henselae* IgG titer of 1:256 was positive and the detection of *B. henselae* DNA by PCR of the gastric biopsy was confirmed.

Photo 2 This rash was unilaterally found only on the left breast of patient 9 who presented with heartburn, abdominal pain and mesenteric adenitis. Gastric biopsy revealed chronic gastritis with the detection of *B. henselae* DNA by PCR.

Photo 3 In patient 10, a rash which only appeared on the posterior aspect of the right knee was visualized in a 16 year old patient who presented with persistent heartburn, and abdominal pain and mesenteric adenitis. Gastric biopsy revealed chronic gastritis with the detection of *B. henselae* DNA by PCR.

Abbreviations

Ig-Immunoglobulin

IL-Interleukin

GI- gastrointestinal

PCR-polymerase chain reaction

DNA-deoxyribonucleic acid

rRNA-ribosomal ribonucleic acid

EGD-esophagogastroduodenoscopy

H. pylori- Helicobacter pylori

B. henselae- Bartonella henselae

CSD- cat scratch disease

Table 1

*Oligo Name	Oligo Sequence	Target Gene	Equivalent Nucleotide	Position
P24E	GGA ATT CCC TCC TTC AGT TAG GCT GG	16S r RNA Bartonella henselae	964-990	--->
P12B	CGG GAT CCC GAG ATG GCT TTT GGA GAT TA		1243-1214	<---

Table 2

Patient number	Age (yrs)	GASTRIC BIOPSY	DUODENAL BIOPSY
1	11	gastritis, B. henselae by PCR	duodenitis, B. henselae by PCR
2	16	no pathology	duodenitis, B. henselae by PCR
3	14	gastritis, B. henselae by PCR	duodenitis, B. henselae by PCR
4	13	gastritis, B. henselae by PCR	duodenitis, B. henselae by PCR
5	7	gastritis, B. henselae by PCR	duodenitis, B. henselae by PCR
6	15	gastritis, B. henselae by PCR	duodenitis, B. henselae by PCR
7	15	gastritis, B. henselae by PCR	no pathology
8	13	gastritis, B. henselae by PCR	duodenitis, B. henselae by PCR
9	7	gastritis, B. henselae by PCR	no pathology
10	9	no pathology	duodenitis, B. henselae by PCR

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	Age (years)	Presentation Abdominal Pain plus:	Blood Titer	Gastric Biopsy	Gastric PCR	Duodenal Biopsy	DuodenalPCR
1	11	heartburn, rash, mesenteric adenitis	B. henselae IgG 1:64	gastritis	B. henselae DNA	duodenitis	B. henselae DNA
2	15	heartburn, eosinophilia, mesenteric adenitis	-	-	B. henselae DNA	duodenitis	B. henselae DNA
3	14	heartburn, rash	B. henselae IgG 1:64	gastritis	B. henselae DNA	duodenitis	B. henselae DNA
4	13	rash, eosinophilia, mesenteric adenitis	-	-	B. henselae DNA	*H. pylori*	B. henselae DNA
5	7	rash, eosinophilia	-	-	B. henselae DNA	duodenitis	B. henselae DNA
6	15	heartburn, rash	B. henselae IgG 1:256	-	B. henselae DNA	-	-
7	15	heartburn,, eosinophilia	-	-	B. henselae DNA	duodenitis	B. henselae DNA
8	15	heartburn, rash, eosinophilia	B. henselae IgG 1:256	gastritis	B. henselae DNA	duodenitis	-
9	13	rash, eosinophilia, mesenteric adenitis	B. henselae IgG 1:128	gastritis	-	duodenitis	B. henselae DNA
10	7	rash, eosinophilia	B. henselae IgG 1:128	gastritis	B. henselae DNA	duodenitis	-
11	9	rash, eosinophilia, mesenteric adenitis	B. henselae IgG 1:64	gastritis	B. henselae DNA	duodenitis	-
12	17	heartburn, rash, mesenteric adenitis	-	gastritis	B. henselae DNA	duodenitis	B. henselae DNA
13	16	heartburn, eosinophilia, mesenteric adenitis	B. henselae IgG 1:64	gastritis	B. henselae DNA	duodenitis	B. henselae DNA
14	11	heartburn, rash	-	gastritis	B. henselae DNA	duodenitis	B. henselae DNA
15	14	heartburn, eosinophilia	B. henselae IgG 1:256	gastritis	-	-	B. henselae DNA
16	13	heartburn, rash	B. henselae IgG 1:256	gastritis	B. henselae DNA	-	B. henselae DNA
17	20	heartburn, rash, mesenteric adenitis	-	gastritis	B. henselae DNA	duodenitis	-
18	11	heartburn, eosinophilia	B. henselae IgG 1:256	gastritis	B. henselae DNA	duodenitis	-