BRIEF COMMUNICATION

Herpes simplex virus type II infection of ileum mesothelium: A case report and review of the literature

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Disseminated herpes simplex virus (HSV) infection usually manifests in the immunocompromised. However, anecdotal examples of visceral HSV disease and viremia have complicated type I diabetes. A case of a 53-year-old type I diabetic patient with bowel obstruction one week subsequent to bronchitis is reported. At laparotomy, a perforated segment of ileum was associated with an adhesive peritoneal band. HSV cytopathic atypia and HSV immunohistochemical staining were confined to fibrocytes and mesothelial cells without involvement of the epithelium. Dissemination of symptomatic HSV pneumonia was verified by histology, immunohistochemistry, in situ hybridization, polymerase chain reaction and direct fluorescence antibody. Intravenous acyclovir resolved symptoms.

This is a novel documentation of HSV complicating ileal adhesive band disease. Furthermore, this case indicates that the HSV cytopathic effect is not unique to the epithelium. Disseminated infection can manifest in myofibrocytes and mesothelium, distinguishing it from standard epithelial atypia of localized HSV infection.

Key Words: Adhesive band disease; HSV; Mesothelium

Gastrointestinal herpes simplex virus (HSV) infection is predominantly a disease of the esophagus in immunodeficient patients. Patients are usually asymptomatic but may experience odynophagia, retrosternal chest pain or fever (1). Reported cases of disseminated HSV infection in immunocompetent patients are commonly complications of pregnancy (2). However, rare anecdotal case reports describe HSV viremia, proctocolitis and hemorrhagic cystitis in patients with diabetes mellitus (3-5).

The standard gastrointestinal pathology literature describes HSV infection as a localized phenomenon confined to the epithelial tissue circumscribing ulcers (6). However, few studies describe typical findings of disseminated HSV and its tropism within the gastrointestinal tract.

We report a case of disseminated HSV infection of the lung that also complicated ischemic adhesive band disease of the ileum. This patient was predisposed to disseminated HSV infection due to several autoimmune illnesses: type I diabetes, hypothyroidism and celiac disease. HSV cytopathic effect was identified histologically in the ileum mucosal ulcer bed and serosal cellular exudates. Immunohistochemistry (IHC), in situ hybridization (ISH) and conventional and real-time polymerase chain reaction (PCR) confirmed a HSV type II infection.

HSV cytopathic atypia and HSV IHC staining was confined to myofibrocytes and mesothelial cells without involvement of epithelium. HSV was isolated in bronchial secretions post-operatively by direct fluorescence antibodies. To our knowledge, this is the first documentation of the distribution of HSV cytopathic changes in the ileum of a patient with symptomatic disseminated HSV infection complicating type I diabetes. Our case portends that disseminated HSV infection can have a viral cytotropism differing from that of a localized epithelial HSV gastrointestinal tract infection.

CASE PRESENTATION

A 53-year-old Caucasian woman with chronic type I insulin-dependent diabetes, hypothyroidism and hypertension developed clinical and radiological features of a small bowel obstruction. A diagnosis of celiac disease was established on endoscopic biopsy five months before her latest hospital admission. She responded rapidly to a gluten-free diet and upon admission denied symptoms that could be attributed to celiac disease. She had been diagnosed with hypothyroidism several years previously and was asymptomatic on adequate thyroid replacement therapy. She had a history of oral cold sores and human papilloma virus cervical dysplasia. Medications included clopidogrel 75 mg per day,
insulin (neutral protamine Hagedorn: 16 U every morning and 14 U every night; Humulin-R [Eli Lilly Canada Inc]: 8 U to 15 U every morning and 8 U to 14 U every night) and losartan 25 mg per day. Her past surgical history was significant for resection of an ovarian fibroma, an iliac vein thrombectomy, caesarian section and appendectomy.

One week before admission, she was diagnosed with bronchitis and treated with an oral antibiotic. She subsequently developed nausea, vomiting, bloating, abdominal distension and tenderness. She was admitted to the surgical unit for a small bowel obstruction that was confirmed radiologically.

Laparotomy identified right lower quadrant adhesive band disease. The ileum had perforated at a congested and stenulated segment that was tightly applied to peritoneal adhesive bands. An end-to-end primary anastomosis was performed.

She remained febrile postoperatively despite receiving piperacillin and Tazocin (Wyeth, Canada) 3.375 g intravenously every 6 h. Hypoxic respiratory failure developed on day 2 postsurgery. She was transferred to the intensive care unit with presumptive adult respiratory distress syndrome, intubated and ventilated. Confirmation of HSV overgrowth at the bowel perforation site (by histology, IHC, ISH and PCR) and in bronchial secretions (direct fluorescence antibody) prompted intravenous acyclovir therapy. She defervesced two days later with improvement of hypoxia and the radiological pulmonary infiltrates. At no time were mucocutaneous vesicles identified in oral, genital or perianal sites. A negative lumbar puncture excluded encephalitis. Serology was negative for HIV and immunoglobulin subsets were normal. She had a complicated course and care was withdrawn 23 days after admission.

**METHODS**

**IHC**

Antibodies studied were polyclonal rabbit anticalretinin (PADDC8, Zymed Lab Inc, USA; 1:100 dilution); rabbit anti-HSV type II (1:100); rabbit anti-HSV type I (1:500); monoclonal mouse anticytomegalovirus (CMV) clone CCH2 (1:500); monoclonal mouse antihuman CD68 clone KP1 (Dako, Denmark A/S; 1:400); monoclonal mouse anti-CD31 JC/70A (Cell Marque, USA; 1:1000); and monoclonal mouse antileukocyte common antigen clone MEM28 (Zymed; 1:800). Immunostaining employed the Envision antimouse and antirabbit antibody and the antigen clone MEM28 (Zymed; 1:800). Immunostaining employing the Envision antimouse and antirabbit antibody and the diaminobenzidine-Plus kit (Dako). Positive controls were run in parallel with test material.

**In situ hybridization**

Dewaxed and dehydrated sections were digested with 100 µL of pepsin (Carezyme II, Biocare Medical, USA) at 37°C for 5 min. Slides were rinsed in Tris-buffered saline, dehydrated in ethanol and air dried. Ten microlitres to 20 µL of biotin-labelled HSV type I, HSV type II, CMV and adenovirus probes (ENZO Life Sciences Inc, USA) were placed on slides and cover slipped. The sections with probes were denatured at 95°C for 5 min and the slides incubated at 37°C overnight. After hybridization, slides were washed in Tris-buffered saline until the cover slips detached. Digoxigenin-labelled hybrids were then identified by the ENZO detection kit (ENZO Life Sciences Inc). The slides were then counterstained with hematoxylin and cover slipped.

**PCR**

DNA was extracted from histological slide bowel tissue and amplified by 40 cycles (approximately 2.5 h) according to a standard protocol (7). Amplified DNA was analyzed by gel electrophoresis for expected size products. Appropriate DNA was then exposed to dot blot testing to confirm the correct DNA sequencing. Material was tested against CMV primers (HSV [91]-P1 [5] and HSV [91]-P2 [7]), and varicella zoster virus (VZV) primers (VZV-P1 [5] and VZV-P2 [7]) (7). Positive HSV sequences are 474 base pairs versus 241 base pairs for VZV (7). All specimens were also analyzed for genes p27 and p21 to quantitate the DNA and screen for PCR inhibitors.

Real-time (light cycler) PCR used DNA extracted from paraffin blocks according to the QIAGEN DNA Mini Kit (Qiagen, Canada) recommendations. Extracted DNA was tested on the LightCycler employing the LightCycler HSV ½ Detection Kit (Roche Diagnostics, Canada) and its recommended parameters. Melting curve data were generated and analyzed by the LightCycler software.

**RESULTS**

The laboratory received an 11.2 cm bowel segment fixed in a “U-shape” due to serosal adhesions. A central stenosed region had a transverse 2.4 cm defect with exudates coating adjacent serosa. Mucosal tissue surrounding the defect was congested. The impression was one of infarction and perforation of an externally compressed segment of small bowel.

Histological assessment revealed a nondescript mucosal ulcer with a granulation tissue base and overlying cellular exudates. Ulcer borders were sharply delineated by intact mucosa. The ulceration extended into the muscularis propria and its focal attenuation was the perforation site. Acute serositis was demonstrated by prominent purulent exudates coating reactive mesothelium.

High power examination of the submucosal ulcer bed delineated rare spindle shaped cells with a ground glass nuclear appearance, circumscribed by a rim of dense chromatin. These cells also had irregular nuclear membranes and were occasionally multinucleated. Similar cytopathic atypia was in the vicinity of the serosa beneath the ulcer. No viral atypia was detected in endothelial cells or mucosal epithelium.

Cells with viral cytopathic effect in the ulcer bed had a HSV type I, HSV type II, vimentin and a focally actin-positive IHC phenotype. Such cells were negative for CMV, CD1a, CD68, CD45, CD20, CD31, S-100 and PGP9.5. Serosal cells with viral inclusions were HSV type I, HSV type II- and calretinin-positive. These cells were also negative for those antigens that ulcer bed cells failed to react with.

ISH and PCR detected HSV DNA with a melting point characteristic of HSV type II (Figure 1). No CMV, adenovirus or VZV genetic material was detected.

**DISCUSSION**

HSV infection of adulthood is predominantly a disease of the immunocompromised (8). In the bone marrow transplant population, 70% of seropositive patients will reactivate, usually within the first month (8). These patients commonly have oral/genital mucositis with or without esophagitis (9). The rare patient develops HSV dissemination, primarily manifesting as encephalitis, pneumonia or hepatitis (8).

Individuals with substandard immunity (pregnancy and newborns) have a greater propensity for HSV-related illnesses (10-12). Anecdotal cases of HSV viremia, proctocolitis and hemorrhagic cystitis have also been described in patients with diabetes mellitus (3-5).
HSV infection in immunocompetent patients is usually a localized mucocutaneous or neural ganglia disease (13). However, data suggest that subclinical dissemination may be common. Acute HSV infection of genitalia is associated with a mild elevation of aminotransferases in 14% of otherwise healthy adults (14). HSV DNA has been isolated in the serum of 20% of healthy adults experiencing recurrent herpes labialis (15).

The origin of disseminated HSV infection is presumably an extension of oral/genital mucositis or reactivation of neural ganglia latent virus (3). It is postulated that isolated visceral HSV disease may be related to: a large viral load at the time of primary exposure; subtle host T cell/macrophage deficiencies; reinfection by a second strain; and/or existence of viscerotropic strains (16).

Gastrointestinal HSV infection is primarily an esophagitis, but proctitis is common as a sexually transmitted illness (17-19). HSV infection is usually restricted to the anorectum in the homosexual population, but rare examples of fulminating left-sided colitis have been identified (20,21). Of the 10 reported cases of HSV enteritis/colitis diagnosed antemortem, seven subjects were immunocompromised or on immunosuppressive therapy (9,21-26). One of the remaining three had type IIb diabetes mellitus as a predisposing condition (4). One patient had confirmed Epstein-Barr virus pharyngitis and another had undergone colon carcinoma resection two months previously (20,27). Our patient is the second reported case of herpes enteritis/colitis complicating diabetes mellitus (Table 1).

### TABLE 1
Cases of antemortem herpes simplex virus (HSV) enteritis/colitis published in the English literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age / sex</th>
<th>Predisposition</th>
<th>Signs and symptoms</th>
<th>Other viral manifestations</th>
<th>Site of HSV</th>
<th>Gross appearance of GI tissue</th>
<th>HSV cytopathic features</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wasselle et al (23)</td>
<td>77 M</td>
<td>Pure red cell aplasia, corticosteroids</td>
<td>Abdominal pain, diarrhea</td>
<td>None</td>
<td>Jejunum</td>
<td>15 punctuate ulcers (0.2 cm to 0.4 cm in diameter)</td>
<td>Histology- and IHC-positive HSV type I and type II in serosa exudate</td>
<td>Perforation, acyclovir, resolution</td>
</tr>
<tr>
<td>Kingreen et al (9)</td>
<td>27 F</td>
<td>45 days post-BMT, GVHD, prednisone (3 mg/kg)</td>
<td>Bloody diarrhea, Skin lesions</td>
<td>Jejunum</td>
<td>Massively inflamed, friable</td>
<td></td>
<td>Histology-negative, IHC-positive HSV type I and type II in ulcer</td>
<td>Death 7 days postoperatively</td>
</tr>
<tr>
<td>Adler et al (25)</td>
<td>53 M</td>
<td>10 months post-kidney transplant, azathoprine (150 mg) prednisone (10 mg)</td>
<td>Abdominal pain, diarrhea, fever, Oral, perianal lesions</td>
<td>Colon</td>
<td>Erythematous, friable, large symmetrical ulcers</td>
<td></td>
<td>Histology-negative, electron microscopy-positive, stool culture- and HSV type I- positive</td>
<td>Acyclovir, resolution</td>
</tr>
<tr>
<td>Colemont et al (20)</td>
<td>78 F</td>
<td>2 months post-colon cancer resection, no medications</td>
<td>Abdominal pain, bloody diarrhea</td>
<td>None</td>
<td>Friable cecum, aphthous ulcers, pseudopolyps, necrotic ulcers in transverse and left colon</td>
<td></td>
<td>Histology-negative, HSV type I- and HSV type II- in ulcer</td>
<td>Acyclovir, resolution</td>
</tr>
<tr>
<td>Guttman et al (21)</td>
<td>36 M</td>
<td>HIV</td>
<td>Abdominal pain, diarrhea, Perianal vesicles</td>
<td>Colon</td>
<td>Multiple necrotic ulcers throughout colon</td>
<td></td>
<td>Viral effect in endothelial/stromal cells, rectum and skin biopsy culture HSV-positive</td>
<td>Toxic megacolon, antibiotics, resolution</td>
</tr>
<tr>
<td>Naik and Chandrasekar (26)</td>
<td>45 M</td>
<td>BMT, GVHD, Clostridium difficile infection</td>
<td>Abdominal pain, diarrhea, Penile ulcers ileum colon</td>
<td>Fribale ileum, small ulcers in ileocecum patchy erythema of entire colon</td>
<td></td>
<td></td>
<td>Histology-negative; necrotic ulcer biopsy culture- HSV type II- and CMV-positive</td>
<td>Gancyclovir resolution</td>
</tr>
</tbody>
</table>

BMT Bone marrow transplantation; CMV Cytomegalovirus; F Female; GI Gastrointestinal; GVHD Graft-versus-host disease; IHC Immunohistochemistry; M Male
HSV enterocolitis manifests as abdominal pain with diarrhea (20,21,23,25,26). Bloody diarrhea and an acute abdomen are less common symptoms (9,20,27). Bowel perforation was identified in three of the 10 reported cases (23,24,27). A slight majority of patients had associated oral/genital mucositis (six of 10) and their symptoms resolved with acyclovir or gancyclovir therapy (six of 10) (9,20-27). Our case of HSV pneumonia complicated by HSV-infected ileal adhesive band disease similarly resolved after surgery and intravenous acyclovir therapy. However, our patient is unique in that bowel symptoms were those of obstruction, indicating that HSV enteritis was a secondary infection of ischemic bowel.

HSV colon infection is classically a diffuse colitis with confluent ulceration (6). Ulcers may be diamond-shaped and most numerous in the sigmoid (6). Five of the 10 reported ante-mortem cases had a pancolitis (20-22,25,26). However, in one case, ulcers were described as symmetric and possibly diamond shaped. Other descriptions included aphthoid, punctate, strictureting and indurated ulceration in one case each (4,20,22,23,25). Three of 10 patients had perforating ulcers (23,24,27).

Classical histology of HSV infection is multinucleated or single cells having Cowdry type A inclusions and ground glass nuclear changes. Cowdry type A bodies are red intranuclear inclusions with a clear halo. Chromatin is relegated to the periphery as a rim of clumped nuclear material. It is commonly regarded that HSV cytopathic atypia is restricted to epithelia, specifically, those cells circumscribing mucosal ulcers (6). This viral effect is typical of primary cutaneous and esophageal lesions.

Of the 10 previously reported ante-mortem HSV enterocolitis cases, six (60%) had viral cytopathic effect identifiable on routine histological sections (4,21-23,25,27). Specific cells harbouring nuclear atypia included epithelium, endothelium and stromal cells and not otherwise specified (21,25,27). In our case, rare actin- and vimentin-positive myofibrocytes of the ulcer bed had viral cytopathic atypia (Figure 2). Numerous calretinin-positive mesothelial cells also had HSV cytopathic atypia and reacted with HSV IHC antibodies (Figure 3).

To our knowledge, this was the first documentation of HSV type II infecting the mesothelium. This finding was not surprising because other herpes virus subtypes are known to infect mesothelium. Human herpes virus-8 was identified in mesothelial cells of three of five patients with pleural effusions and Kaposi's sarcoma or Castleman's disease in a recent publication (28).

CONCLUSIONS
This was a unique case of disseminated HSV type II complicating adhesive band disease. Although HSV type II can disseminate systemically in insulin-dependent diabetes mellitus, this was the first documentation of viral cytopathic atypia in an ileal ulcer bed of such a patient. Previous studies have suggested that HSV-induced cytopathic atypia is generally confined to the intestinal epithelial cells at the perimeter of an ulcer. We report that disseminated HSV type II infects various intestinal cell lineages including myofibrocytes and mesothelium about an ulcer bed, in the absence of intestinal epithelial cell involvement.

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