Manifestations of Three HHV-8-Related Diseases in an HIV-Negative Patient: Immunoblastic Variant Multicentric Castleman’s Disease, Primary Effusion Lymphoma, and Kaposi’s Sarcoma

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INTRODUCTION

Human herpesvirus-8 (HHV-8, also called Kaposi’s sarcoma-associated herpesvirus) is a γ herpesvirus that was discovered by representational difference analysis from a Kaposi’s sarcoma (KS) biopsy of a patient with acquired immune deficiency syndrome (AIDS) [1]. Since its discovery, HHV-8 has been found in all forms of KS, suggesting an etiologic role [2–4].

Subsequent studies have found HHV-8 to be associated specifically with two other rare disorders: primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD) [5–7]. Two groups initially recognized the unique aspects of some effusion-based lymphomas of B-cell origin in patients with AIDS, predominantly in advanced stages of immunosuppression [8,9]. Rarely, PEL is diagnosed in HIV-negative patients [10–12].

Patients with MCD are at an increased risk to develop KS and B cell lymphomas. HHV-8 was described in all MCD cases from HIV-positive patients, but only in 40% of HIV-negative, MCD patients [6]. Subsequently, HHV-8 was found to be present in immunoblastic or plasmablastic cells in MCD, and HHV-8 is associated specifically with an immunoblastic variant of MCD [7].

Occasionally, patients may present with a combination of two HHV-8-related diseases, most commonly KS with PEL or KS with MCD. We describe an HIV seronegative patient presenting with symptomatic hypoglycemia and who was subsequently diagnosed as suffering from all three HHV-8 associated diseases: KS, PEL, and MCD. This is the first report of a patient suffering from three HHV-8 tumors and the first report of severe hypoglycemia as a presenting symptom of these diseases.

CASE REPORT

A 73-year-old Jewish female patient of Ashkenazi origin was referred to the Soroka Medical Center, Beer Sheva, Israel, because of complaints of visual hallucinations and paranoid delusions of 2-week duration. The patient immigrated to Israel from Russia 2 years before her referral, and her medical history had been uneventful apart from a single past admission for nephrolithiasis.

On examination, the patient looked pale and diaphoretic with a temperature of 36°C, pulse 76/min, and blood pressure 150/70 mmHg. Physical examination of her lungs, heart, and abdomen was unremarkable. Neurolog-

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cal examination showed no abnormalities. Blood glucose level taken on admission was 46 mg%. After administration of 50 ml of 20% dextrose solution, her symptoms resolved.

Further biochemical studies showed normal renal and liver function tests. Hematological studies revealed a hemoglobin of 11.7 g/dl, a white cell count of $9.0 \times 10^9 /l$ with a normal differential count, and a platelet count of $152 \times 10^9 /l$.

Following 18 hr of fasting, the glucose level dropped from 97 to 30 mg% at which time her neuropsychiatric symptoms appeared with visual hallucinations, diaphoresis, tachycardia, and anxiety. These symptoms resolved after the administration of intravenous glucose (the Whipple triad for hypoglycemia).

Insulin and C-peptide serum levels at different serum glucose levels were consistently within lower-normal range. Additional studies including serum cortisol, thyroid-stimulating hormone, insulin-like growth factor 1 (IGF-1), and insulin-like growth factor 2 (IGF-2) were not contributory; no studies of IGF binding proteins or subtypes were performed. Serum tumor markers including: carcinoembryonic antigen (CEA), CA-125, CA15-3, and α-fetoprotein were all normal.

Radiological studies including chest X-ray and CT scans of the abdomen and brain were normal. Because no apparent etiology for her hypoglycemia was found, the patient was advised to keep a complex carbohydrate-rich diet. However, she continued to experience recurrent episodes of fasting hypoglycemia.

One month later, the patient presented with left axillary lymphadenopathy. Lymph node biopsy was consistent with the diagnosis of Castleman’s disease of the mixed, hyaline-vascular, and plasma cell type (Fig. 1A). Immunohistochemistry for HHV-8 by staining with an antibody against the latent nuclear antigen 1 (LNA-1) [13] showed that HHV-8 positive immunoblastic cells were localized in the mantle zone of the follicle, with up to 30% of the cells positive for LNA-1 (Fig. 1B).

The patient was followed for another 11 months, during which time ascites, generalized lymphadenopathy, and purplish lesions on her right third toe developed. Apart from a serum glucose level of 45 mg/dl, all other routine biochemistry and hematology results were normal. A high titer of anti-HHV-8 antibodies (1:25,600) was detected in a serum sample and in a sample from the ascitic fluid by an indirect immunofluorescent antibody assay (IFA) [14]. ELISA serologies for HIV, hepatitis B surface antigen, and hepatitis C were negative.

A CT scan of the abdomen confirmed the presence of ascites, showing also para-aortic lymphadenopathy. The liver and the spleen were of normal size. A CT scan of the chest showed mediastinal lymphadenopathy. Analysis of the ascitic fluid showed an excess of atypical lymphoid cells (>1,000/µl) and an albumin level of 1.9 g/dl (concurrent serum albumin level 2.7 g/dl). Cytological analysis of ascitic fluid showed lymphocytes of anaplastic morphology (Fig. 2). Cells prepared from formalin-fixed, paraffin-embedded cytospin block stained positive for CD30 and epithelial membrane antigen (EMA) but lacked reactivity for CD3, CD79a, the ALK protein (ALK1), and the EBV latent-membrane antigen (LMP-1). The morphology and immunocytochemistry of the ascitic cells were therefore consistent with primary effusion lymphoma [5,10,15]. The patient refused a CT-guided biopsy of para-aortic nodes. Biopsy of the purplish lesion on the right third toe showed plaque-type KS (Fig. 3A). Staining of the KS lesion for HHV-8 LNA-1
showed that over 90% of the spindle cells were positive with the characteristic stippling pattern of LNA-1 reactivity (Fig. 3B).

Polymerase chain reaction (PCR) for HHV-8 DNA sequences was positive in the KS lesion, lymph node with MCD, and cells from peritoneal fluid (Fig. 4).

Cytotoxic therapy with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) was started. After 4 cycles of treatment there was no response in the size of the lymph nodes or the amount of the ascites. Therapy was then stopped. Subsequently, she developed generalized lymphadenopathy, progressive KS lesions on both legs, and frequently required tapping of her ascites. KS progressed along both lower limbs. During her stay in hospital, recurrent unexplained episodes of hypoglycemia continued. She died 4 months following the diagnosis of PEL. Permission for an autopsy was not given.

METHODS

Cytological Studies

Cytological studies were performed by (i) Wright-Giemsa staining of cytospin preparations, (ii) hematoxylin–eosin staining of formalin-fixed, paraffin-embedded cytospin block, and (iii) immunoperoxidase studies on formalin-fixed, paraffin-embedded cytospin block, and antibodies to CD30 (Immunotech, Marseille, France), CD3 (Novocastra, Newcastle, U.K), CD79а, epithelial membrane antigen, Epstein-Barr virus latent membrane protein 1 (EBV-LMP-1), and ALK protein (ALK1) (all from Dako, Carpinteria, CA).

Pathologic Studies

Pathologic studies were performed on biopsies from (i) axillary lymph node and (ii) KS lesion (paraffin-embedded with hematoxylin–eosin staining).

Serology for HHV-8

Serology for HHV-8 was studied against the HHV-8 latent nuclear antigen (LNA-1) by the indirect immunofluorescence assay (IFA) as previously described [2]. In summary, the HHV-8 positive, Epstein-Barr negative cell line, BCP-1, was used for an IFA to detect human IgG antibodies against HHV-8 antigen.

Expression of HHV-8-LNA-1

Three to 5 μm paraffin-embedded sections were cut onto sialin-coated slides. Sections were deparaffinized with xylene and 100% ethanol and microwaved in 10 mmol/l citrate buffer, pH 6.0, at 780 W for 6 min. After treatment with 20% acetic acid, sections were incubated with a monoclonal antibody against the latent nuclear
antigen-1 (LNA-1) or HHV-8 named LN53 (dilution 1 in 500 in phosphate buffer saline, PBS) for 1 hr, at 22°C [13,16]. Slides were then washed twice with 0.1% Tween in PBS. Incubation of the primary antibody was followed by a streptavidin–biotin complex alkaline phosphatase system (Vector Laboratories, Burlingame, U.K.), and the sections were counterstained with hematoxylin.

PCR for Human Herpesvirus 8 (HHV-8)

A 50-μg amount of DNA was extracted from paraffin-embedded material taken from (i) KS lesion, (ii) lymph node with MCD, and (iii) cytospin from peritoneal fluid. DNA was tested for HHV-8 ORF 26, as previously described [12].

DISCUSSION

We present the first case of an HHV-8 infected patient who presented initially with multicentric Castleman’s disease manifested as hypoglycemia and 11 months later developed multiple KS lesions and PEL. The patient was an elderly, Jewish female of Ashkenazi origin, who immigrated to Israel from Eastern Europe. She was HIV-seronegative and had never required blood transfusions or received immunosuppressive treatment before. Yet, she developed three aggressive diseases, each known to be associated with HHV-8 and states of immunodeficiency [17].

HHV-8 appears to be a rare infection in blood donors in Northwestern Europe and North America [18,19] but is more common in countries where classic and endemic KS are found, e.g., Mediterranean Europe and Africa [14,20,21]. HHV-8 is also relatively common among all the Jewish ethnic groups in Israel [22].

In contrast to homosexual men, where HHV-8 is transmitted mainly during sex [23], HHV-8 is transmitted in Mediterranean Europe and Africa also from mother to child [22,24].

The first HHV-8-related disease diagnosed in our patient was MCD. Two distinct histopathological variants with different clinical characteristics have been described in MCD: the hyaline-vascular type and the plasma cell variant. The more common hyaline-vascular type usually presents with a solitary mass in the mediastinum or the retroperitoneum, and is frequently, surgically curable. The rarer, plasma cell variant typically presents with generalized lymphadenopathy and systemic symptoms, designated MCD. HHV-8 is almost universally present in HIV-positive individuals with MCD [6]. The association between MCD and interleukin-6 (IL-6) is noticeable: IL-6 is present at high levels in MCD biopsies. Also peripheral blood mononuclear cells of patients with MCD were shown to secrete high levels of IL-6 [25]. The diagnosis of MCD in this patient was inferred through the pathologic diagnosis of MCD in a lymph node and the later development of generalized lymphadenopathy. HHV-8 staining confirmed the presence of HHV-8 in plasmablastic cells in the mantle zone (Fig. 1B) [7].

The second HHV-8-related disease diagnosed in our patient was PEL. This unusual lymphoma possesses a unique constellation of features [10]. Patients with PEL usually present with malignant effusions in the pleural, pericardial, or peritoneal cavities without significant tumor mass or lymphadenopathy. PEL occurs predominantly in HIV-positive individuals with advanced stages of immunosuppression [26] but is occasionally diagnosed in HIV-negative patients [10–12]. In contrast to HIV-positive cases, which are usually also EBV-positive, almost all HIV-negative cases with PEL are EBV-negative, as was our patient. The reason for these virologic differences is unknown. The existence of EBV-negative cases of PEL, clearly illustrates that this disease can develop in the absence of EBV and, in turn suggests that HHV-8 alone is important in the etiopathogenesis of PEL. The finding of HHV-8 by PCR, in lymphocytes separated from the ascitic fluid, finally verified the diagnosis of PEL in this case (Fig. 4). Interestingly, we also detected high titers of anti-HHV-8 antibodies in the patient’s ascitic fluid.

KS was the third HHV-8-related disease, diagnosed concomitantly with PEL. The immunosuppressive effects of the chemotherapy given for PEL probably worsened the KS, which rapidly progressed over both legs in a manner reminiscent of its behavior in immunocompromised hosts. HHV-8 was demonstrated in spindle cells of KS by immunocytochemistry (Fig. 3B), in addition to HHV-8 DNA detection by PCR (Fig. 4).
The mechanism responsible for HHV-8 activation, resulting in the evolution of all three aggressive diseases, as well as the cause of the hypoglycemia remain unknown in this patient. Iatrogenic factors were eliminated from the beginning, and extensive studies to find a hormonal abnormality were inconclusive. The temporal association of hypoglycemia with MCD indicates that the mechanism of hypoglycemia was probably linked directly to MCD or to infection with HHV-8.

Primary effusion lymphoma and KS in an immunosuppressed cardiac transplant recipient and MCD and PEL in acquired and iatrogenic immunosuppression have previously been described [27]. All HHV-8-related diseases are more common in populations with a higher prevalence of HHV-8 infection. There was no obvious indicator of immunosuppression in our patient to explain the outgrowth of HHV-8 infected PEL cells, immunoblasts in MCD and KS spindle cells.

In summary, we described the clinical manifestations of three known, HHV-8-related diseases occurring in an elderly, female patient with no previous history suggesting impaired immunity. Just as intriguing remains the pathogenesis of hypoglycemia in this case.

REFERENCES


