Three cases of HHV-8 DNA found in patients with iatrogenic Kaposi’s sarcoma

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Summary

Cases of Kaposi’s sarcoma (KS) have been previously reported in patients treated with immunosuppressive agents. We report here three cases of KS in immunosuppressed patients with nephrotic syndrome, erythema elevatum diutinum and bullous pemphigoid (BP). One patient died due to the rapid progression of KS and in two patients there was a complete regression or considerable improvement after reduction or withdrawal of corticosteroids and radiation treatment. Human herpes virus 8 (HHV-8) DNA was detected from the tumor specimen and peripheral blood mononuclear cells (PBMCs) in all three patients.

KEY WORDS: Kaposi’s sarcoma; HHV-8; iatrogenic.

Introduction

Kaposi’s sarcoma (KS) is a neoplasm of vascular endothelial cells caused by infection with HHV-8. It is classified into four subtypes: classic KS, endemic KS, acquired immunodeficiency syndrome (AIDS)-associated KS, and KS in iatrogenically immunosuppressed patients. Since 1979, there have been numerous cases of iatrogenic KS reported in patients treated with immunosuppressive agents for organ transplantations and those with autoimmune disorders or malignant neoplasms (1).

HHV-8 DNA was first found in tissues obtained from patients with AIDS but has since been identified in all four clinical forms of KS (2-4). HHV-8 DNA was detected in 100% of the lesional specimens and in 69.6% of the PBMCs in HIV seronegative patients with KS. Moreover, it was not always detectable in the PBMCs among the same patients at different times (4).

Here, we report three cases of KS that developed after patients underwent immunosuppressive therapies and had HHV-8 DNA both in their tumors and PBMCs. It is likely that the HHV-8 DNA became detectable when patients’ conditions with KS worsened, allowing the tumors to spread in the body. In this study, we discuss the relationship between immunosuppression induced by corticosteroids and proliferation of HHV-8.

Report of cases

Case 1

In April 2012, a 68-year-old Japanese man was diagnosed as having nephrotic syndrome and began receiving oral prednisolone (maximum dose 60 mg/day). Cyclosporin (maximum dose 150 mg/day) was added in June of that year. In September, rapidly evolving violaceous patches and nodules appeared on his extremities and abdomen. Because of the decrease in the patient’s blood pressure, as well as the aggravation of anasarca and exudation from the cutaneous lesions, he was transported to Sapporo Medical University Hospital by a doctor helicopter on October 22.

Upon admittance to our hospital, a physical examination revealed widespread purpura and edema on the trunk and extremities with multiple violaceous plaques and nodules over the trunk, extremities, right eyelids, upper lip and oral mucosa (Figure 1a, b).

A complete blood examination revealed lymphocytopenia (969/μl, well below the normal range of 1,500-4,000/μl) and the immunochemical fecal occult blood test was positive. The result of a serologic test for the human immunodeficiency virus (HIV)-1 was negative. Computed tomography (CT) of the chest revealed the storage of fluid in the thoracic cavity but there was no visceral involvement or swollen lymph nodes upon abdominal CT examination.

A histopathological examination of the purplish nodule showed proliferation of spindle-shaped cells that were positive for CD31, CD34 and HHV-8 with slit-like spaces and red blood cell extravasation in the dermis (Figure 2a, b, c).

Genomic DNA was extracted from the samples of a skin biopsy specimen and PBMCs and assayed for the presence of HHV-8 DNA using two kinds of primer sets as previously described (5). The PCR amplified 310 bp bands both from the tumor and the PBMCs (Figure 3). Sequence analysis revealed that the PCR products were almost identical (98%) to the HHV8 sequence. These findings confirmed that the patient was
suffering from iatrogenic KS induced by cyclosporin and corticosteroid therapy. After he entered the hospital, cyclosporin and corticosteroid were withdrawn and he was treated with fluid replacements, albumin products, blood transfusions and pressors. However, he died on the tenth day of hospitalization.

Case 2

An 82-year-old man who was born in North Korea and moved to Japan when he was 15 years old was diagnosed with erythema elevatum diutinum in 1999. Treatment consisting of the administration of oral prednisolone (maximum dose 20 mg/day) was started in August 2012. The multiple purplish to black-colored plaques and nodules on both lower thighs accompanied by edema and stasis change appeared in the end of November and rapidly increased in size and number (Figure 1c). Similar plaques and papules were also seen on his left hand. Laboratory studies showed a white blood cell and lymphocyte count of 5,400/mm³ and 637/μl, respectively. The results of biochemical analyses were normal, and the patient was seronegative for HIV. CT, an esophagogastroduodenoscopy and a colonoscopy found no KS lesions. A histopathological examination showed numerous irregular blood vessels and proliferation of spindle-shaped cells in the dermis. Immunohistochemical staining for CD31 and CD34 was positive in the endothelial cells and the spindle-shaped cells. In addition, nuclei of proliferative cells were stained with the HHV-8-specific antiserum (Figure 2d, e, f). PCR-amplified specific bands from the biopsied tumor sample and PBMCs were processed to sequencing analysis to find the presence of HHV-8 DNA (Figure 3). Based on these clinical, laboratory and histologic findings, he was diagnosed with iatrogenic KS induced by corticosteroid therapy.
After corticosteroid therapy was withdrawn, the edema in his legs was reduced and the nodules gradually decreased in size. However, the multiple skin nodules and purpura still existed on the lower thighs and the patient received topical radiotherapy. A dose of 36 Gy was administered using 3.6 Gy per fraction on his legs and a dose of 39 Gy was given at 3.0 Gy per fraction on his left hand. The skin nodules completely disappeared after four months. The patient remains under close observation and there has been no recurrence during 31 months post-treatment.

**Case 3**

In May 2013, an 89-year-old Japanese woman was diagnosed with BP, as confirmed by a serum anti-BP180 antibody examination indicating that serum levels were elevated to 942 (normal range <9), and began receiving oral prednisolone (maximum dose 25 mg/day). Four months after starting corticosteroid treatment, she presented with multiple bean-sized purple nodules on her thighs, feet, inguinal region and oral mucosa. The nodules on her feet increased in number quickly and were edematous and ulcerated (Figure 1d, e). Histological findings revealed numerous irregular blood vessels and proliferation of spindle-shaped cells with slit-like vascular channels filled with erythrocytes in the dermis (Figure 2g). Immunohistochemistry showed that the tumor cells were positive for CD31, CD34 and HHV-8 (Figure 2h, i). Laboratory findings were within normal limits except for increased levels of serum level of anti-BP180 antibody 20 and the presence of lymphocytopenia (lymphocyte
700/μl). The serum antibody test against HIV was negative. CT of the chest and abdomen was normal and an esophagogastroduodenoscopy and a colonoscopy found no involvement of KS. PCR was carried out on DNA from a tumor specimen and substrates in PBMCs to detect HHV-8-specific bands from both tissues (Figure 5). We concluded that the patient had been immunosuppressed with prednisolone for BP and subsequently developed KS. Because the disease condition of BP was brought under control, the steroid dose was gradually reduced to 6 mg/day. Only symptomatic tumors on the feet were treated by radiotherapy (30 Gy in 10 fractions). The KS lesions of the feet decreased in size and thickness three months after radiotherapy and the lesions of the body and oral mucosa also resolved spontaneously.

Discussion

We described the clinical presentations of KS in immunosuppressed patients with nephrotic syndrome, erythema elevatum diutinum and BP. The onset of KS in our cases was probably associated with the administration of corticosteroid therapy alone or combined with cyclosporin, as indicated by the development of the KS lesions during the use of these therapies and the spontaneous improvement seen after treatment dosages were reduced cases 2 or suspended cases 3. Treatment modalities for KS comprise of local therapies such as surgery, radiotherapy and local chemotherapy of vinca alkaloids, interferon and systemic chemotherapies and immunologic medications (6). Because a classification of the disease activity and standard therapeutic guidelines for KS do not exist, the therapeutic options have to be chosen depending on its subtype as well as the immune status of the patient. In patients with iatrogenic KS, immunosuppressive medication needs to be reduced depending on the host condition. Although the increase of the KS lesions was inhibited by withdrawing or reducing corticosteroid therapy, radiotherapy was additionally performed in cases 2 and 3 to achieve clinical remission against local complications that seriously impaired the patients’ quality of life. It is conceivable that because the reduction or cessation of immunosuppressive medication may lead to remission of KS, a decrease in tumor volume is an important purpose of treatment for some patients with iatrogenic KS, even if a cure is not the endpoint of therapy. The relationship between the appearance of KS and the duration and/or dosage of the corticosteroid is not immediately clear. However, the interval of the appearance varies from 22 days to more than 20 years (average: 22.6 months) (7, 8). In our study, in cases 1, 2 and 3, KS appeared five, four and four months after the beginning of immunosuppressive therapy, respectively. The reason for this early presentation is unclear. It may have occurred in the first patient due to the combination of corticosteroid and cyclosporin at relatively strong immunosuppressive dosages. For the second and third patients, old age may have been the cause. Moreover, lymphocytopenia was seen in all three cases. Fauci has previously described how corticosteroid therapy induces transient lymphocytopenia and especially in T lymphocytes (9). Guo et al. have also shown that corticosteroids stimulate the proliferation of KS tumor cells derived from AIDS patients (10, 11). Furthermore, they have shown that the corticosteroid receptors are expressed at high levels on KS tissues, both in the cytoplasm and the nucleus and can be up-regulated by corticosteroid stimulation. Although it has not been examined in non-AIDS KS, this suggests that both the immunosuppressive effects of corticosteroid therapy and proliferative effects of KS cells may induce iatrogenic KS. Thus, there is the possibility that the transient lymphocytopenia, immunosuppression and proliferative ability of KS cells caused by corticosteroids participated in the development of KS.

The seroprevalence of HHV-8 in the Japanese general population is 1.4-5% (12, 13). The main transmission routes of HHV-8 are considered to be a horizontal transmission among children in countries with endemic infections and a sexual transmission among homosexual men in countries with nonendemic infections. In our report, one patient was from North Korea and the others were Japanese nationals with no overseas or bisexual history. That the seroprevalence in homosexual Japanese men is 11.7% (13), a rate significantly higher than that in the control group, indicates that the transmission route in Japanese, including our patients, remains unclear. Through PCR and sequencing analysis, HHV-8 DNA was detected both in the KS lesions and PBMCs in our three cases. HHV-8 DNA is not always detectable in PBMCs as described previously (4, 5). Cattani et al. reported that HHV-8 DNA was detected in 100% of the lesional specimens in their study and in 69.6% of the PBMCs from HIV seronegative patients with KS (4). For this reason, although technical problems cannot be ignored, there is possibly a correlation between HHV-8 viremia and the clinical stage of KS. In our study, it may have been possible to detect HHV-8 DNA both in KS lesions and PBMCs because the samples were extracted soon after diagnosis without any treatment and, additionally, the lesions were not localized but rather spread to the extremities or almost the entire body. Corticosteroids are widely used, but at present it is impossible to predict the future development of KS. HHV-8 is considered essential but is not a sufficient factor for the development of all forms of KS, which must have a multistep process including genetics, genotypes, inflammatory cytokines and host immunity. Kanno et al. (14) investigated the genotypes of the K1 gene of HHV-8 in Japanese people by sequencing. Genotype A of the K1 gene was detected more frequently in AIDS-associated KS than non-AIDS-associated KS and genotype D was detected only in non-AIDS-associated KS. These data are one indication of the differences between AIDS- and non-AIDS-KS with regard to age of onset or gender in Japan. It is impor-
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tant that future molecular biologic and epidemiologic studies clarify the route of HHV-8 infection among non-HIV individuals and help lead to the development of a standard treatment guideline.

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Conflicts of interest

None declared.

References