Human herpesvirus 8 (HHV-8) detected in two patients with Kaposi’s sarcoma-like pyogenic granuloma

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Aims: To report the finding of human herpesvirus 8 (HHV-8) in two patients with Kaposi’s sarcoma (KS)-like pyogenic granuloma. This form of pyogenic granuloma closely resembles KS histologically and it has been reported that immunohistochemistry in such lesions may be positive for smooth muscle actin and factor VIII related antigen, which are typically negative in KS. In both patients the lesions were positive for CD31, CD34, smooth muscle actin, and factor VIII related antigen, a profile typical of KS-like pyogenic granuloma. The lesions were tested for the presence of HHV-8 DNA, which to date has been consistently found in all types of KS.

Methods: The lesions were tested for the presence of HHV-8 DNA using the polymerase chain reaction (PCR). A known HHV-8 positive KS specimen was used as the positive control. Six samples of non-KS vascular skin lesions were used as negative controls for the PCR reaction.

Results: Both lesions were positive on PCR for HHV-8 and the specificity of product was confirmed by direct sequencing. None of the six control vascular skin lesions was positive for HHV-8. These results strongly indicate KS as the true diagnosis and are supported by the reported clinical course in both cases.

Conclusions: Techniques targeting HHV-8 DNA for detection to confirm a diagnosis of KS are both sensitive and specific. In cases where the differential diagnosis includes KS-like pyogenic granuloma, caution should be taken not to diagnose solely on the basis of immunohistochemistry phenotype. In such cases, PCR targeting HHV-8 DNA sequences is a better diagnostic tool.

The increase in incidence of Kaposi’s sarcoma (KS), previously uncommon in Europe outside of the Mediterranean, has mirrored the rise in the incidence of human immunodeficiency virus (HIV) infection. The recent description of DNA sequences of a new herpes virus in KS lesions in patients with AIDS led to the demonstration of the virus in all forms of KS.

Previously reported studies have shown that PCR based detection of HHV-8 DNA or RNA in suspected skin lesions is indicative of Kaposi’s sarcoma and is rare in other skin lesions.

CASE HISTORIES

Case 1

South Infirmary: in 1994 a 72 year old man presented with a painful pigmented nodule on his left ankle. Initially, the biopsy was reported as KS and treatment was with intralesional vinblastine and cryotherapy. He returned in 1996 with two similar lesions behind his left knee, which were also reported as KS, and were also treated with intralesional vinblastine and cryotherapy. The following year he developed several small lesions on his left leg, which were successfully treated with electron beam radiotherapy, but recurred six months later, requiring more radiotherapy, after which the patient failed to attend follow up. HIV testing was negative before surgery for a rectal tumour in 1998.

Case 2

Cork University Hospital: in 2000 a 75 year old man presented with a well circumscribed, darkly pigmented, 0.6 cm diameter...
nodule in the first web space of the left foot. On examination, similar smaller lesions were present on the fourth toe of the left foot and two were seen on the left thigh. At surgery he underwent excision biopsy of all four lesions. Initially, the histopathology report was one of KS, but with the subsequent immunohistochemistry report this was modified to KS-like pyogenic granuloma. The patient has gone on to develop several similar lesions on both forearms and right foot and these have been treated with electron beam radiotherapy.

METHODS

Samples
Archival, formalin fixed, paraffin wax embedded histological samples from the two cases in question were obtained from the department of histopathology, Mercy Hospital, Cork, Ireland. Positive controls were from a known KS biopsy (kindly supplied by Dr J Coyne, Manchester, UK). Negative control tissue samples were from six similarly treated samples of non-KS cutaneous vascular lesions (table 1). Sections of

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**Table 1** Results of PCR of 186 bp HHV-8 DNA fragment in positive control Kaposi’s sarcoma, negative control cutaneous vascular lesions, and two cases of Kaposi’s sarcoma-like pyogenic granuloma

<table>
<thead>
<tr>
<th>Sample</th>
<th>Histological diagnosis</th>
<th>HHV-8 positive PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>HIV associated Kaposi’s sarcoma</td>
<td>4/4</td>
</tr>
<tr>
<td>10994</td>
<td>Case 1: Kaposi’s sarcoma-like pyogenic granuloma</td>
<td>4/4</td>
</tr>
<tr>
<td>663400</td>
<td>Case 2: Kaposi’s sarcoma-like pyogenic granuloma</td>
<td>4/4</td>
</tr>
<tr>
<td>528900</td>
<td>Epithelioid haemangiendothelioma</td>
<td>0/4</td>
</tr>
<tr>
<td>697400</td>
<td>Benign venous haemangiendothelioma</td>
<td>0/4</td>
</tr>
<tr>
<td>527898</td>
<td>Epithelioid angiosarcoma</td>
<td>0/4</td>
</tr>
<tr>
<td>708600</td>
<td>Capillary haemangiendothelioma</td>
<td>0/4</td>
</tr>
<tr>
<td>534400</td>
<td>Benign dermatofibroma</td>
<td>0/4</td>
</tr>
<tr>
<td>527800</td>
<td>Pyogenic granuloma</td>
<td>0/4</td>
</tr>
</tbody>
</table>

HHV-8, human herpesvirus 8; HIV, human immunodeficiency virus.
5 µm thickness were cut and duplicate sections were examined from each block.

DNA isolation
DNA was obtained from all samples using DNeasy® tissue kits (Qiagen Ltd, Crawley, UK) according to the manufacturer's instructions, resulting in a total volume of 100 µl/sample.

PCR
All reactions were carried out on an Eppendorf Mastercycler® gradient thermocycler (Eppendorf, Hamburg, Germany), and using HotStart Taq Master Mix® kit reagents (Qiagen). All PCR products were run on a 1 x Tris acetate EDTA, 2% agarose gel using SYBR® Green 1 nucleic acid stain (Amresco® Inc, Ohio, USA), according to the manufacturer's instructions, and visualised at 302 nm with a transilluminator. Confirmation of the effective isolation of DNA from the tissue sections was achieved by PCR amplification of a normal gene, namely a 133 bp fragment of the adenomatous polyposis coli (APC) gene. The primer pair was 5′-GGA CTA CAG GCC ATT GCA GAA-3′ and 5′-GGC TAC ATC TCC AAA AGT CAA-3′. The reaction mix contained 1 µl primer mix, 15 µl Taq mix, 9 µl sterile distilled H₂O, and 5 µl of DNA isolate. The reaction conditions were 95°C for 15 minutes, followed by 40 cycles of 94°C for one minute, 55°C for one minute, 72°C for one minute, and finally two minutes at 72°C.

A second round of PCR was then performed under the same conditions using 1 µl of the first PCR reaction solution in a reaction mix containing 1 µmol of primer mix, 15 µl Taq mix and 13 µl sterile distilled H₂O.

Methods used to counteract contamination
The PCR reagents were mixed in a separate area from that where the DNA was added. Before the addition of DNA the reaction mix was first placed in UV light of wavelength 254 nm and intensity 30 000 µJ/cm² for 2 x 2 minutes to crosslink any contaminating DNA. Negative controls containing no DNA were used in every PCR reaction. Filter tips were used throughout the entire process.

DNA sequencing
Where a PCR product was detected on a gel its specificity was confirmed by direct sequencing by standard methods. Briefly, PCR products were purified on centrifuge columns (Qiagen) and then dried by alcohol precipitation. The dried PCR product was then sent together with a sample of each primer for direct sequencing to MWG AG Biotech, Ebersberg, Germany according to their instructions.

RESULTS
Two 5 µm tissue sections were cut from each tissue sample examined, DNA was isolated from each section, and each DNA isolate was subjected to PCR analysis twice to give a total of four results for each histology sample. Amplification of the 133 bp fragment of the APC gene was successful in all samples (fig 2). The 186 bp HHV-8 DNA fragment was repeatedly detected in the positive control KS sample and both test cases, but not in the other cutaneous vascular lesions (fig 3). Thus, the specificity of the reaction was not affected by the high cycle number, as reflected in the lack of amplification of viral DNA in the negative control samples. The results of the DNA sequencing as reported by MWG AG Biotech were identical to
the predicted HHV-8 target sequence in product amplified from both test samples and the positive control.

DISCUSSION

The association between HHV-8 and all forms of KS is now well established and the detection of HHV-8 in suspected KS lesions has been proposed as a strong diagnostic indicator. In cases where diagnostic doubt exists, such as with cases of KS-like pyogenic granuloma, a recent report suggested immunohistochemical phenotyping as a useful tool in distinguishing KS from KS-like pyogenic granuloma, the former being negative and the latter positive for smooth muscle actin and factor VIII related antigen. Here, we report two cases of multiple KS-like pyogenic granulomas where the differential diagnosis includes KS. Although they are uncommon, similar cases have been reported previously. The clinical picture seen on follow up of both cases was typical of classic KS, which often runs a benign, indolent course for 10 years or more with gradual development of additional lesions. However the reported immunohistochemical profile supported a diagnosis of KS-like pyogenic granuloma, with lesions from both cases being positive for smooth muscle actin and factor VIII related antigen.

“The implications of this finding are to highlight the value of PCR as a diagnostic tool where diagnostic doubt exists in suspected cases of Kaposi’s sarcoma-like pyogenic granuloma”

We used PCR to show the presence of HHV-8 in both cases. The PCR system used was more sensitive and more specific than a nested system, which we also used to amplify first a 233 bp and then a 186 bp internal fragment of HHV-8 (data not shown). This is in agreement with previously published data that the degree of false positivity is increased with the nested PCR system. The specificity of product amplified was confirmed by direct sequencing.

The implications of this finding are to highlight the value of PCR as a diagnostic tool where diagnostic doubt exists in suspected cases of Kaposi’s sarcoma-like pyogenic granuloma. The pathological diagnosis of KS has important clinical consequences for patients presenting with hyperpigmented skin lesions, and in particular for those who have not been tested for HIV, although the clinical presentation of KS in the context of HIV infection is different to that seen in classic KS.

Given the benign clinical course, and that both patients in this report were immunocompetent, we imagine that the same lesions would behave more aggressively in the immunocompromised patient. It is already known that infection with the virus alone is not sufficient for the development of KS and that additional cofactors are required. The hypothesis now gaining acceptance is that all forms of KS (classic, African, immunosuppressive treatment related, and epidemic) have a common aetiology in HHV-8 and that the virus does not just opportunistically colonise lesions.

In conclusion, for cases where the differential diagnosis includes KS-like pyogenic granuloma caution should be taken not to base diagnosis solely on the immunohistochemical phenotype. In such cases, PCR techniques targeting HHV-8 DNA would appear to represent the better diagnostic tool.

REFERENCES