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Immunohistochemistry expression of Human Herpes Virus-8 in cutaneous vascular lesions

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Abstract

Backgrounds: Kaposi sarcoma is a low-grade vascular tumor with the uniform expression of latent nuclear antigen-1 of the human herpes virus 8 (HHV-8). Differentiation of Kaposi sarcoma from other benign and malignant vascular or non-vascular spindle cell lesions is sometimes a manner of challenge. Thus, the expression of human herpes virus 8 in a fixed specimen would be diagnostically useful. This study aimed at immunohistochemical detection of human herpes virus-8 in cutaneous vascular lesions.

Methods: This cross-sectional study was conducted on 46 cases of cutaneous vascular lesions including six cases of Kaposi Sarcoma, twenty-five cases of Pyogenic Granuloma, four cases of Angiolymphoid Hyperplasia with Eosinophilia, three cases of Masson Tumor, two cases of Arteriovenous Hemangioma, two cases of Sclerosing Hemangioma, three cases of Angiofibroma, and one case of Lymphangioma. After histologic confirmation, immunohistochemistry was done on sections of paraffin-embedded tissue with mouse anti-HHV-8 antibody.

Results: Of 46 patients, 27 (58.7%) were male and 19 (41.3%) were female, and the mean age was 46.36±17.48 years. All 6 Kaposi sarcoma cases showed strong, nuclear staining for HHV-8 (100%). All 6 patients with HHV-8 positive results were older than 60 years. Sarcoma cases included four (66.7%) resection specimens from the soft tissues of the leg and two (33.3%) resection specimens from the soft tissues of the hand.

Conclusions: The high sensitivity and specificity of the immunohistochemical method for detecting HHV-8 in skin lesions, especially Kaposi sarcoma, makes it a reliable and cost-effective tool to differentiate Kaposi sarcoma from other vascular and non-vascular spindle cell lesions.

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1. Introduction

Cutaneous vascular lesions are divided into two categories: vascular tumors and vascular abnormalities. The most common cutaneous vascular tumors are hemangiomas (1). Epithelioid hemangioma (EH) as a benign vascular proliferation was first described by Wells and Whimster as angiolymphoid hyperplasia with eosinophilia. In immunohistochemical studies, EH is usually stained with endothelial differentiation markers including ERG, CD31, and CD34 (2).

Kaposi sarcoma (KS) is a low-grade malignant tumor of vessel origin that was first introduced by Mortis Kaposi as Idiopathic multiple-pigmented sarcoma of the skin in 1872 (3). KS is a nodular lesion consisting of

spindle-shaped endothelial cells, extravasated erythrocytes along with mononuclear inflammatory cells, hemosiderin, and fibrosis, which is initially formed in the skin but can also develop in lymph nodes, viscera, and the oral cavity. KS is the most common malignancy encountered in human immunodeficiency virus (HIV)-infected patients. These lesions have three stages: patch, plaque, and nodule, in the order of increasing the number of spindle-shaped cells (4).

Four clinical subtypes of KS are described: classic KS mainly in men of Mediterranean or eastern European origin, African (endemic) type in young and children in central Africa, epidemic (HIV-associated) type in immunosuppressed patients with HIV infection and KS-associated with immunosuppressive therapy in transplanted patients (5,6).

Chang and colleagues found a herpes-like virus in the KS cells of a patient with AIDS (7). The virus is now known as Kaposi's sarcoma-associated herpes virus (KSHV) or human herpes virus 8 (HHV-8) (8). HHV-8 causes KS via different pathways. The virus has oncogenic potential. It affects cell cycle regulation and inhibits apoptosis (6). Then the researchers found that, it is present in all cases of KS and other lesions including primary effusion lymphoma, some cases of multicentric Castleman's disease, reactive angioendotheliomatosis, and plasmablastic lymphoproliferative disorders (9-12).

Differentiation between KS and other benign or malignant vascular neoplasms and other nonvascular spindle cell soft-tissue neoplasms may be a manner of challenge in histopathology. The differential diagnosis may include angiosarcoma, spindle cell hemangioma, lobular capillary hemangioma, dermatofibrosarcoma protuberans, leiomyoma, stasis dermatitis, and spindle cell melanoma. Thus, immunohistochemical expression of HHV-8 in fixed specimens may be useful for differentiation between KS and these entities.

To identify HHV-8, conventional molecular methods such as polymerase chain reaction (PCR) were traditionally used which are extremely laborious and time-consuming and also require the skills of the laboratory personnel (13). Immunohistochemical studies of HHV-8 in fixed tissues, in such that 4-micron sections, are prepared from paraffin blocks of samples for H&E and immunohistochemistry (IHC) staining and compared with positive and negative controls, can be useful in terms of diagnosis.

Therefore, this study aimed at immunohistochemistry detection of HHV-8 in cutaneous vascular lesions.

2. Materials and methods

2.1. Setting and Design

This cross-sectional study was performed on patients with skin vascular lesions who were referred to the pathology laboratory of Mahdieh clinic in Kermanshah University of Medical Sciences (KUMS), Kermanshah, Iran.

2.2. Data collection

In total, 46 cases of cutaneous vascular lesions including six cases of Kaposi Sarcoma, twenty-five cases of Pyogenic Granuloma, four cases of Angiolymphoid Hyperplasia with Eosinophilia, three cases of Masson Tumor, two cases of Arteriovenous Hemangioma, two cases of Sclerosing Hemangioma, three cases of Angiofibroma, and one case of Lymphangioma were studied (**Figure 1**).

2.3. Immunohistochemical detection

Four-micron cuts were prepared from paraffin blocks and IHC staining was performed with an HHV-8 marker. Mouse Anti-Human Herpes Virus 8 (HHV-8) Antibody (Clone 13B10) of Vitro master diagnostica (Spain) was used. IHC positive control was a confirmed case of KS and visualization was nuclear according to the manufacturer's brochure. For diagnosis, it was enough to distinguish positive from negative cases, and the intensity of staining was not considered. The H&E sections of each case were examined and the diagnoses were confirmed by a dermatopathologist. The previous diagnosis of one case changed from pyogenic granuloma to Kaposi sarcoma by the dermatopathologist.

2.4. Statistical analysis

Descriptive statistics were applied consist of mean ± standard deviation (SD), median, frequencies, and percentages for analysis. Also, we used an independent t-test for continuous and normally-distributed variables and a chi-square test (or Fisher's exact test) for categorical variables for subgroup analysis. All analyses were done using Stata software (version 14.1) (Stata Corp, College Station, TX, USA).

2.5. Ethics

Ethics committee approved the study (Ethics Code: IR.KUMS.REC.1399.1087). Information on the patients was kept confidential.

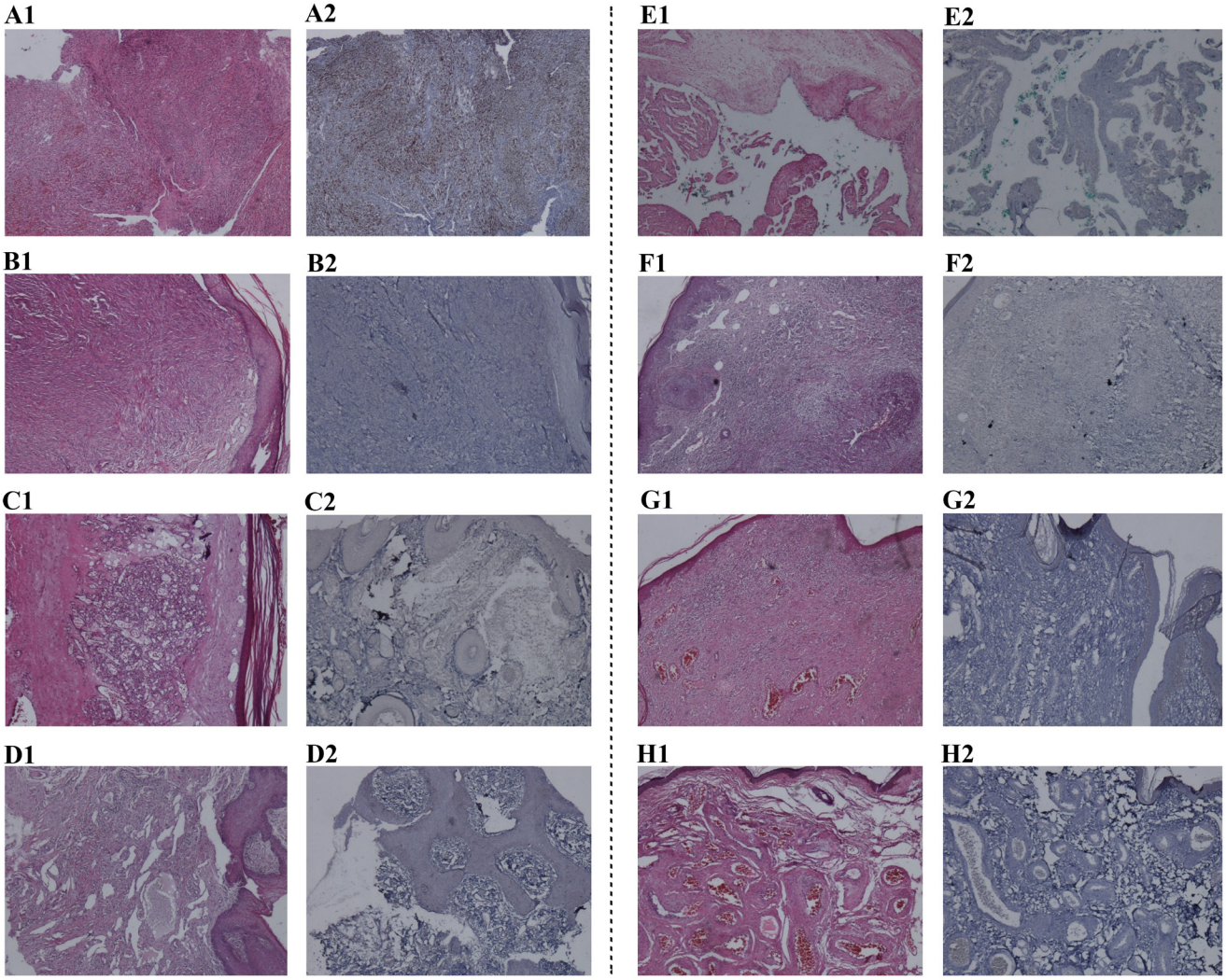

Figure 1. **Representative view of the cases.** A) Kaposi sarcoma, B) Sclerosing hemangioma, C)Pyogenic granuloma, D)Lymphangioma, E)Masson hemangioma, F)Angiolymphoid Hyperplasia with Eosinophilia (ALHE), G)Angiofibroma, H)Arteriovenous hemangioma, (1-Hematoxylin-Eosin stain 2-Immunohistochemistry stain for HHV-8, x40 Magnifications). Only Kaposi sarcoma shows positivity for HHV-8.

3. Results

Of 46 patients, 27 (58.7%) were male and 19 (41.3%) were female, and the mean age was 46.36±17.48 years. The results of the immunohistochemical analysis are shown in **Table 1**.

All 6 Kaposi sarcoma cases showed strong, nuclear staining for HHV-8 (100%). There was no statistically significant difference between males and females based on the results of the immunohistochemical analysis (p=0.213). All 6 patients with HHV-8 positive results were older than 60 years.

Kaposi sarcoma cases included four (66.7%) resection specimens from the soft tissues of the leg and two (33.3%) resection specimens from the soft tissues of the hand (**Table 2**).

4. Discussion

This study showed that the relative frequency of confirmed antibodies in the studied patients was 13.04% (6 out of 46 patients). The prevalence of HHV-8 expression in different groups is different according to the diagnostic method used and the geographical area studied. In a study, Hoffman and his colleagues investigated the prevalence of HHV-8 in apparently healthy blood donors. The results of their study showed that 5.11% of apparently healthy American blood donors had positive results (14). Some studies also examined the expression of HHV-8 in the community: prevalence of 4% in the general population of India, the prevalence of 4.4% in the general population of Malaysia, the prevalence of 2.4% in the general

Table 1. Immunohistochemistry staining results.

Tumor type	HHV-8 positive	HHV-8 negative	Total	Percent
Kaposi Sarcoma	6	0	6	13.04
Pyogenic Granuloma	0	25	25	54.35
Angiolymphoid Hyperplasia with Eosinophilia	0	4	4	8.70
Masson Tumor	0	3	3	6.52
Arteriovenous Hemangioma	0	2	2	4.35
Sclerosing Hemangioma	0	2	2	4.35
Angiofibroma	0	3	3	6.52
Lymphangioma	0	1	1	2.17

Table 2. Frequency of HHV-8 marker according to lesion location.

Location of the lesion	HHV-8 positive	HHV-8 negative
Face	0	9(22.5)
Head	0	7(17.5)
Tongue	0	3(7.5)
Leg	4 (66.7)	7(17.5)
Vulva	0	1(2.5)
Hand	2 (33.3)	9(22.5)
Abdominal wall	0	1(2.5)
Lips	0	3(7.5)

population of Czechoslovakia, the prevalence of 3% in the general population of Taiwan, the prevalence of 2.8% in the general population of Brazil, and a prevalence of 4% has been reported in the general population of Argentina (15-18). We demonstrated that the expression of the HHV-8 marker was not statistically significant between men and women. In line with our results, Naraghi et al. (19), Tappero et al. (20), Beral et al. (21), and Montagnino et al. (22) reported that there was no important association between HHV-8 marker expression and gender. Masia and his colleagues reported that the level of HHV-8 infection in two genders or different age groups does not differ from each other (23). In a study, Patel et al investigated the expression status of HHV-8 immunohistochemical markers in cutaneous vascular lesions. Their results showed that all cases of Kaposi's sarcoma (100%) were positive for HHV-8, while the remaining cases included spindle cell hemangioma (9 cases), cutaneous angiosarcoma (5 cases), dermatofibrosarcoma protuberans (5 cases), Pilar leiomyoma (4 cases), stasis dermatitis (4 cases), pyogenic granuloma (4 cases), and spindle melanoma (3 cases) were negative for this antibody (3). Schulz et al reported that HHV-8 is not found in non-sarcoma tissues of KS patients and is not present in other vascular tumors such as angioma and angiosarcoma and other skin tumors such as squamous cell carcinoma and melanoma (24). In a study, Shah Siah and his colleagues investigated the prevalence of HHV-8 genotypes in KS. The results of their study showed that 35 of the 45 patients studied had positive PCR results (77.8%) (25). In another study conducted in China by Zhang and his colleagues on KS lesions, 84.48% had positive PCR results (7). Holger et al reported that none of the angiosarcoma cases and none of the spindle cell hemangioma specimens showed positive immunohistochemical staining. In contrast, HHV-8 was identified in all cases of KS (5). Angela et al identified HHV-8 in 78% of 37 cases of KS by immunohistochemical method (26).

In the present study we showed that the KS cases included four (66.7%) resection specimens from the soft tissues of the leg and two (33.3%) resection specimens from the soft tissues of the hand. In the study of Higgins and his colleagues in Afghanistan, the location of the lesion was reported in the lower limb (legs) and then the upper limb (hands) in most patients (27). In the study of Koldarova and his colleagues, the focus of lesions on the lateral surfaces of the legs, feet,

and hands was also reported (28).

Although Naraghi and her colleagues used the same clone of HHV-8 as us for IHC in KS patients, they found positivity in about 88-90% of cases. They found a higher percentage of positivity in higher stages including plaques and nodules, but no difference for age, gender, and immune status (19).

Immunohistochemistry for the detection of HHV-8 in skin lesions mainly KS, has high sensitivity and specificity, so it can be used as a reliable and cost-effective marker for differentiation between KS and other vascular and non-vascular spindle cell lesions. In addition, since it does not rely on nucleic acid amplification and permits nuclear localization in light microscopy, false positive results may be lower. We believe that this will be a very useful marker for the pathologist who encounters a lesion in which KS is suspected.

5. Conclusion

In general, the frequency of confirmed antibodies in the studied patients was 13.04%, all of whom were over 60 years old. HHV-8 marker expression was not statistically significantly different between men and women. The expression of the HHV-8 marker was reported only in KS lesions, where the location of the lesion was the foot in 4 patients and the hand in 2 patients. One of the limitations of this study is the lack of access to the complete clinical information of the patients, including AIDS.

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Conflict of Interest

The authors declare no conflicts of interest.

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