

Original Article

Immunohistochemical Detection of the Human Herpes Virus 8 (HHV8) Latent Nuclear Antigen-1 in Kaposi Sarcoma Cases

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ABSTRACT

Background and Objectives: The molecular genetic of the human herpes virus 8 (HHV8) has now been characterized the pathogenesis of kaposi sarcoma (KS). This study attempted to determine the rate of HHU-8 infection in KS in an Iranian cross sectional study.

Method & material: In this cross-sectional study, we used paraffin-embedded specimens of 54 clinically well-characterized kaposi sarcoma cases. Routine streptavidin- biotin- peroxidase immunostaining with diaminobenzidine was performed on paraffin-embedded tissue of 54 KS cases using a monoclonal antibody directed against the c-terminum of the latent nuclear antigen-1 molecule of HHV-8 (colone 13B10; Novocastra) at 1:50 dilution.

Result: Positive HHV8 nuclear staining was detected in the nuclei of the spindle cells and endothelial cells of the vascular channels in about 88.9%. (48/54) of all cases and in 90.56% (48/53) of cases with presence of residual tumor in specimen (1 of 54 paraffin blockes has no residual tumor). HHV8 was detected in higher percentage in plaque and nodule stage rather than patch stage with $p=0.035$. HHV 2 was detected in lesser percentage in lesion of hand and trunk rather than other sites with $p=0.042$. There was no significant difference in HHV8 positivity between the presence of HHV8 and age, gender and history of immunosuppression.

Conclusion: Immunohistochemical detection of LANA HHV-8 is helpful in diagnosis of suspicious cases of KS, specially in lesion of plaque and nodule stage that are not located only in hand or trunk.

Keyword: Human Herpes virus 8, LNA-1 Antigen, Kaposi Sarcoma

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Introduction

Kaposi Sarcoma (KS) composed of vessels and spindle-shaped cell, was first described by Kaposi in 1872 as idiopathic multiple pigment-sarcoma of the skin (1). KS is an invasive vascular neoplasm characterized by proliferation of angular vessels in the form of vascular splits in dermis (2). It is classified as classic, African, Iatrogenic and AIDS related (3,4). In classic KS the disease manifests itself by multiple dermal blue plaques or nodules starting on the feet and leg. The histologic features of KS are identical to its clinical forms (5).

Microscopically three features are seen as early (patch), plaque and nodule stage; in early or patch stage an irregular dermal proliferation of jagged thin-walled vascular channels is noted, in plaque stage of KS presence of cellular fascicles with a largely spindle cell is seen, and in the nodular stage a mass of fusiform neoplastic cells which form interlacing bundles is evident and slit-like spaces are scattered throughout the tumor, yielding a sieve-like appearance (6). The most typical feature of KS is the presence of spindle cells forming slits containing red blood cells (7).

In 1994, Chang *et al.* identified DNA belonging to a novel virus in tissue affected by KS. This virus, human herpes virus type-8 (HHV-8) was originally known as KS-associated herpes virus (KSHV) (8). As with other cell-transforming DNA viruses, infection with HHV-8 alone is probably not sufficient for the development of KS, additional cofactors are probably required (9). Further, the explosive incidence of HIV-associated KS in early 1980s resulted from colliding epidemics of HIV and HHV-8 infection in homosexual and bisexual communities (10).

It is believed to be necessary but not sufficient to cause the disease, with other factors (such as immunosuppression) probably playing a major contributory role. Parenthetically, HHV-8 is also involved in the pathogenesis of multicentric Castleman's disease and primary effusion lymphoma (11-14). The most important recent development in this field has been the discovery that a new human

herpes virus (HHV-8) is present in almost 100% of KS lesions, whether HIV-related, classic, endemic, or iatrogenic (12).

The aim of this study was to assess the rate of HHV-8 infection in an Iranian series of KS cases using an immunohistochemical method and an available antibody against the HHV-8 latent nuclear antigen-1 (LANA-1).

Methods and Material

Material: Paraffin embedded specimens of 54 clinically well-characterized KS cases were studied.

Immunohistochemistry: Paraffin sections were cut of 4 μm , mounted on 5-aminopropyltriethoxysilane (AAS) coated slides and allowed to dry overnight. The sections were dewaxed in xylene and treated with microwave heating at 60°C for 20 min in a Citrate buffer (2.1 g/1000 ml; pH 6.01) for antigen retrieval after blocking of endogenous peroxidase, the sections were washed in phosphate-buffered saline (PBS), and non-specific binding of secondary antibody was blocked with normal serum. Routine streptavidin-biotin-peroxidase immunostaining with diaminobenzidine was applied to the sections followed by overnight incubation with a murine monoclonal antibody directed against the C-terminus of the latent nuclear antigen-1 molecules of HHV-8 clone 13B10; Novocastra of 1:50 dilution. Cells were classified as positive for HHV-8 when there was nuclear staining.

Statistical analysis

Correlation between HHV-8 immunostaining and clinicopathological features were analyzed using chi-squared (χ^2) contingency test and Fisher's exact test. Only *P* value of less than 0.05 was considered as significant.

Results

HHV-8 was detected in the nuclei of the spindle cells and endothelial cells of the vascular channels of infected KS lesions (Fig. 1,2). The lesions that are

positive show nuclear staining in at least 5% of the lesional cells. Positive HHV 8 nuclear staining was detected in about 88.9% (48/54) of all cases and in 90.56% (48/53) of cases in which the residual tumor was present (1 of 54 cases has no residual tumor). 14.8%(8/54) has history of renal transplantation and all of which showing positive result. The median age of positive and negative cases is 61.2 and 57.8 respectively.

There was significant difference between stage and site of the lesions and positive immunohistochemical staining for HHV 8.

HHV 8 was detected in 69.23% of patch stage, 95.24% of the plaque stage and 95% of the nodule

stage with significant higher percentage in plaque and nodule stage (P value= 0.035) (Table 1).

HHV 8 was detected in 66.67% of the lesions that present only in upper extremity , 94.44% of lesion that present only in lower extremity, 50% of lesions present only in trunk, 80 lesions that involve whole of body and 100% lesions that involve both upper and lower extremity, with significant lesser HHV 8 positive percentage in only upper extremity and only trunk lesions (P value= 0.042). However there was no significant difference in HHV 8 positivity between the presence of HHV and age, gender and history of immunosuppression ($P=$ 0.661), 0.574 and 0.279 respectively) (table 2).

Table 1: Immunohistochemical detection of HHV 8 in different stages

Pathological subtypes	No, of cases	HHV 8 immunodetection	
		positive	negative
Patch	13	9	4
Plaque	21	20	1
Nodule	20	19	1

Table 2: HHV8 expression in KS and correlation with clinical and pathological features

Clinicopathological parameters	No, of cases	HHV 8 immunodetection	
		positive	negative
Sex			
Male	41	37	4
Female	13	11	2
Site			
Upper extremity	3	2	1
Lower extremity	36	34	2
Trunk	4	2	2
Whole body	5	4	1
Upper or lower extremity	6	6	0
Immunosuppression	8	8	0

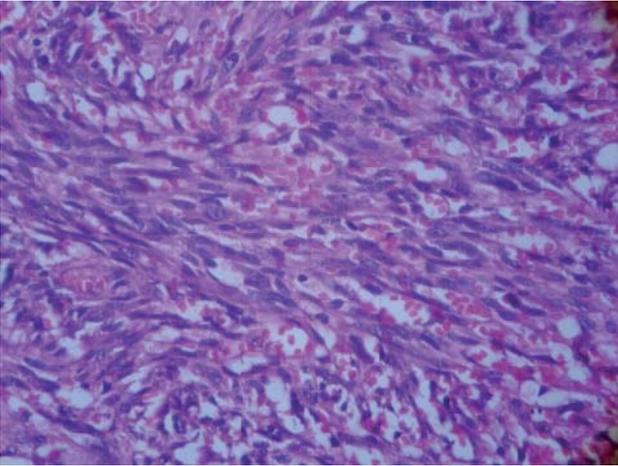


Fig. 1: H & E staining of kaposi sarcoma ×40 objective (nodular stage , mass of fusiform neoplastic cell forming interlacing Bundles)

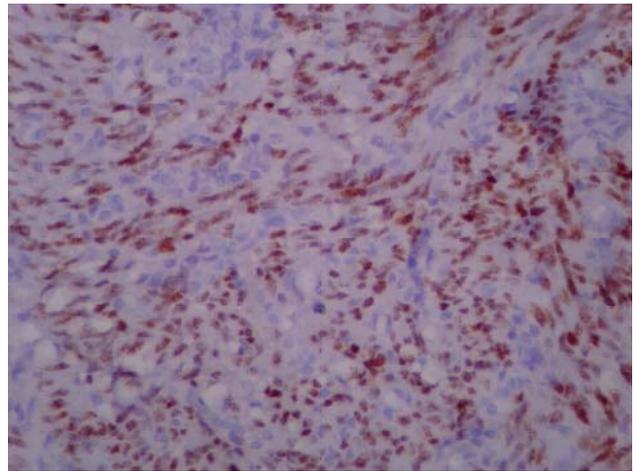


Fig. 2: Immunohistochemical staining of spindle cell nuclei with HHV 8- LANA-1 ×40 objective

Discussion

In this study we have shown that HHV-8 infection can be detected by immunohistochemistry in the majority of KS lesions as other studies (2-4), (Table 1).

The plaque and nodule stage have significant higher percentage of immunohistochemical staining for HHV-8 than patch stage, also lesions that are present only in hand or trunk has significant lesser percentage of positivity for HHV8 immunohistochemical staining than other sites (Table 2).

Because of lesser percentage of positivity in patch stage than plaque and nodule stage there is questionable sensitivity for this method to confirm the diagnosis of lesions in patch stage and the lesion involve only hand or trunk that does not show in other studies (5,6). Holger *et al.* reveal that none of the angiosarcoma cases and none of the negative control samples (juvenile haemangiomas) revealed positive immunohistochemical staining with the LANA-1 antibody. In contrast, HHV-8 LANA-1 was clearly detected in all analyzed cases of KS and multicentric Castleman's disease. These results were confirmed by PCR assay at the DNA level (5). Angela *et al.* detected HHV-8 in 78 percent of 37 cases of KS by immunohistochemical method (6). Both of these studies say that the immunohistochemical staining for LANA of HHV-8 is sensitive to confirm the diagnosis of KS in patch stage, and limits the study only to

cutaneous and extracutaneous site, but in our study this sensitivity about patch stage is in question and we limit the site of study to upper extremity, lower extremity, whole of body, trunk and upper and lower extremities and we show that the sensitivity of this method is in question in lesion that involve only trunk or hand.

We do not access to PCR method to confirm our negative result in patch stage about HHV-8 compared to Angela *et al.* and Holger *et al.* that use this method, that limits our study. There is no significant difference about the result of our study compare to other studies regarding other stage (except patch stage) and other site (except lesion involving only hand or trunk). There was no significant correlation between the presence of HHV8 and age, gender, and history of immunosuppression similar the results of other studies (15-17). We could not find any study that call in question the pathogenesis of HHV-8 in KS cases (18-20).

Conclusion

Our study showed that the immunohistochemical detection of latent nuclear antigen-1 of HHV-8 was helpful in confirm and diagnosis of suspicious cases of KS specially in lesion of plaque and nodule stage that are not located only in hand or trunk, and

the sensitivity of this IHC method is in question in patch stage lesion that are only located in hand or trunk.

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