Journal of Pakistan Association of Dermatologists. 2023;**33(4)**:1446-1453.

Original Article

Association of Human Herpesvirus Type 8 (HHV-8) expression in patients with Kaposi's sarcoma: A clinico-immunohistochemical study

Adil A. Noaimi[1], Ameer D. Hameedi[2], Mohammed M. Abdullah[3], Ghassan A. Ibrahim[3], Hassanien G. Hassan[4]

[1] Department of Dermatology, College of Medicine, University of Baghdad, Iraq.
[2] Department of Pathology, College of Medicine, University of Baghdad, Iraq.
[3] Dermatology and Venereology Center, Medical City, Baghdad, Iraq.
[4] Department of Pathology, Ibn Al-Nafees Teaching Hospital, Baghdad, Iraq.

Abstract

Objective To assess the association of latent nuclear antigen-1(LANA-1) of HHV-8 among KS patients with clinicopathological parameters and to evaluate if this procedure is valuable for diagnosing this disease through the first immunohistochemical study in Iraq.

Methods This is a clinico-immunohistochemical descriptive study conducted at the Dermatology Center/Medical City, Baghdad, Iraq. Thirty-two KS cases diagnosed by clinical and histopathological means in the Dermatology/Pathology Departments /Medical City and three Private Medical Laboratories were studied from the first of January 2016 to the first of October 2022. Retrospectively, 20 KS cases with clinical and histopathological data were extracted from a patient's registry while the remaining 12 cases were collected prospectively. All clinical and sociodemographic data were recorded then immuno-histopathological evaluations were done for them.

Results The most common type of KS was classical 27(84%) of cases followed by iatrogenic 4(13%) and HIV-associated 1(3%) case. Histomorphologically, 15(46.9%) of the cases were in the plaque stage, 11(34.3%) nodular stage and 6(18.8%) patch stage. The overall HHV-8 expression was detected in 27(84.4%) of the cases. The total histoscore was calculated by combining the staining intensity score and positive cell percentage score and shows a significant correlation with the stage of progression (P=0.02). No significant associations between HHV-8 expression and age, sex, disease recurrence, site of biopsy, and clinical types while the association with the disease duration was significant (P=0.032).

Conclusion Immunohistochemistry for HHV-8 is a sensitive and specific diagnostic method for KS. The majority of cases that did not express HHV-8 staining were in the early patch stages, with a relatively lower median duration than that of HHV-8 positive cases. Negative immunostaining for HHV-8 does not necessarily exclude KS in an appropriate clinicopathological setting.

Key words
Kaposi's Sarcoma (KS); Immunohistochemistry (IHC); Human Herpesvirus type 8 (HHV-8).

Introduction

Kaposi's Sarcoma (KS) is a low-grade angioproliferative neoplastic disorder of lymphatic endothelium-derived spindle cells infected with HHV-8.[1] The worldwide age-standardized incidence of KS ranges from <1 per 100,000 in countries of Europe and America to >22 per 100,000 in Africa.[2] The Global

Address for correspondence
Dr. Mohammed M. Abdullah
Dermatology and Venereology Center,
Medical City, Baghdad, Iraq.
Email: mohm772299@gmail.com

Cancer Observatory data 2020, the incidence of KS in Iraq was 0.16 % and the five years prevalence was 0.33 per 100,000.[3] HHV-8 is an oncogenic, large double-strand DNA virus. The seroprevalence of HHV-8 varies greatly and is classified as low seroprevalence (<5%) in America and Europe; intermediate (5% - 20%), including the Middle East; and high (>50%) in regions of Africa.[4] HHV-8 is the etiologic agent in all epidemiological forms of KS.[2] In reviewing of literature, HHV-8 is found in about 70-90 % of KS using immunohistochemistry (IHC).[5,6] In Iraq, despite many clinical and pathological studies being done for KS, none of them confirm this association by IHC, for that reason, the present work was arranged to assess the association of HHV-8 among KS patients and to evaluate that this maneuver is a valuable tool for confirming the diagnosis of this disease.

Patients and Methods

Study Design This is an observational, descriptive, clinico-immunohistochemical study carried out at the Dermatology Center/Medical City, Baghdad, Iraq. Thirty-two KS cases diagnosed by clinical and histopathological means in the Dermatology /Pathology Departments /Medical City and three Private Medical Laboratories were enrolled in this work.

Retrospectively, from the first of January 2016 to the first of November 2020, 20 paraffin-embedded tissue blocks (PETB) of KS cases were extracted. The data regarding age, sex, disease duration, lesion distribution, biopsy site, immunosuppression, and HIV status were collected from a patient's registry. Prospectively, 12 cases of KS attending the Dermatology Center/ Medical City, were collected from the first of November 2020 till the first of October 2022. Formal consent was taken from each patient after a full explanation of the goals of the study, the nature of the disease, and the prognosis that needs photographs, biopsy, and IHC. After a complete history and clinical assessment, data were recorded including age, sex, disease duration, biopsy site, immunosuppression, and HIV status. The patient was photographed by Realme phone, 64-megapixel rear camera, and a biopsy was taken.

Ethical approval was obtained from the Scientific Committee of the Scientific Council of Dermatology and Venereology of Arab Board for Health Specializations.

Immuno-histopathological analysis For all PETB of KS cases, new 4 µm thickness sections were stained with H&E, and the diagnosis was reviewed by a pathologist, then IHC was done for cases with typical clinicopathological features of KS.

Immunostaining was performed using the prediluted, HHV8-specific mouse monoclonal antibody against the LANA-1 of HHV-8 (Clone 13B10, BIO SB, Santa Barba, USA). A 4 µm thickness section was placed on positively charged slides and mounted vertically. The slides were sequentially soaked in fresh-Xylenes and absolute ethanols for deparaffinization/ rehydration. Pretreatment with heat-mediated antigen retrieving and washing the slides with a phosphate-buffered solution. Endogenous peroxidase blocking was followed by the addition of HHV-8 antibody and accomplished after Harris-hematoxylin (Dako) was added to the stained slides. One or two drops of distyrene, plasticizer, and xylene (DPX) mounting medium were added to the wet xylene parts and carefully covered with coverslips to eliminate any excess and air bubbles before drying.

Cells were classified as positive for HHV-8 when there was staining of spindle cells and/or

Figure 1 Fifty-three years old age male with multiple violaceous plaques on the extensor surfaces of both hand and feet for a 12-months duration.

Figure 2 Sixty years old age female with violaceous patches and edema of the lower limbs for 3 months duration.

endothelial cells of the vessel wall. The intensity of staining was scored from 1 to 3 (1, weak; 2, moderate; 3, strong) and the percentage of positive cells was scored from 1 to 4 (1, 1%-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%) as proposed by Chek *et al*.[7] A total semiquantitative histoscore was counted for each case by combining the two individual scores. The results of immune-histopathology were analyzed and photographed by using LEICA DM750/ICC50E module, digital microscope, Switzerland.

Statistical Analysis Analysis was performed with IBM SPSS (Statistical Package for Social Science) program (IBM SPSS, Inc., Chicago, IL) version 26. Qualitative data were presented as frequency and percentage. Quantitative data were presented as mean and standard deviation or median and range. The quantitative data were examined by Kolmogrov–Smirnov test for normality of data. Associations between categorical variables were assessed via cross-tabulation and chi-square. Mann-Whitney test and Kruskal–Wallis test were used to compare medians of continuous variables. Exact tests were used to calculate the P-value. In all statistical analyses, a P value equal to or less than 0.05 was considered significant.

Results

A total of 32 KS cases were studied. Their ages ranged from 27 to 85 years with a mean ±SD of 64.38±12.86 years. Among these cases 25(78%) were male and 7(22%) were female with a male-to-female ratio of 3.5:1.The disease duration ranged from 3 to 72 months with a median±SD 8.50 ±13.50 months. The most common clinical type of KS was classical 27(84%) followed by iatrogenic 4(13%) and HIV-associated type in only 1 (3%) case.

All cases showed the typical clinico-histopathological findings of KS in different stages of evolution, the patch stage **(Figure 2, 4A)**, plaque stage **(Figure 1, 5A)**, and nodular stage **(Figure 3, 6A)**.

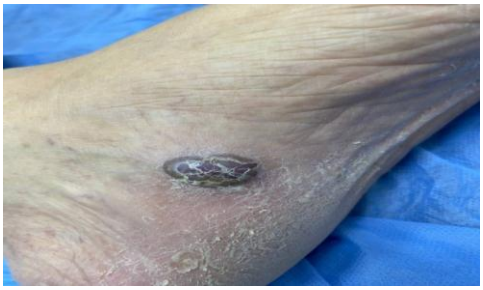

Figure 3 Seventy years old age male with a single pigmented nodule on the medial arch of the left foot for the 6-months duration.

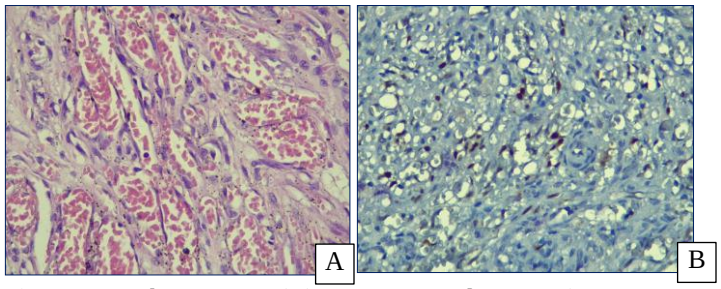

Figure 4 Patch stage KS, (A) H & E stained section (x100 magnification), (B) HHV-8 stained section, positive nuclear staining, strong intensity (x100 magnification).

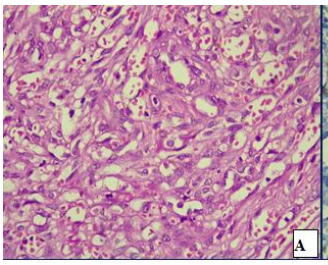

Figure 5 Plaque stage KS, (A) H & E stained section (x100 magnification), (B) HHV-8 stained section, positive staining, strong intensity (x100 magnification).

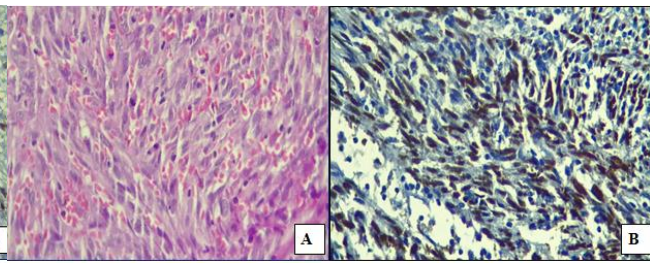

Figure 6 Nodular stage KS, (A) H & E stained section (x100 magnification),(B) HHV-8 stained section, positive staining, strong intensity (x 100 magnification).

Table 1 The total semiquantitative staining score of HHV-8 immunostaining among KS.

	Total staining score								*P value*
	0	*2*	*3*	*4*	*5*	*6*	*7*	*Total*	
Patch	4 66.7%	0 0.0%	1 16.7%	1 16.7%	0 0.0%	0 0.0%	0 0.0%	6 100.0%	0.02*
Plaque	1 6.7%	0 0.0%	1 6.7%	5 33.3%	4 26.7%	4 26.7%	0 0.0%	15 100.0%	
Nodule	0 0.0%	1 9.1%	2 18.2%	2 18.2%	2 18.2%	1 9.1%	3 27.3%	11 100.0%	
Total	5 15.6%	1 3.1%	4 12.5%	8 25.0%	6 18.8%	5 15.6%	3 9.4%	32 100.0%	

Table 2 HHV-8 expression in different stages of KS progression.

Stage	*HHV-8 Expression*		*Total*
	Negative	*Positive*	
Patch	4 66.7%	2 33.3%	6 100.0%
Plaque	1 6.7%	14 93.3%	15 100.0%
Nodule	0 0.0%	11 100.0%	11 100.0%
Total	5 15.6%	27 84.4%	32 100.0%

Histomorphologically, 6 (18.8%) of the cases were in the patch stage, 15 (46.9%) plaque, and 11 (34.3%) nodular stage. The overall positive HHV-8 expression was detected in 27(84.4%) cases while only 5 (15.6%) of the cases were negative.

There was a significant correlation between the total staining score and the stages of progression of KS **(Table 1)**. The comparison of (the patch vs. plaque and patch vs. nodule) were significant while (plaque vs. nodule) show no significant correlation **(Table 3)**.

No significant associations between HHV-8 positivity and different clinicopathological parameters **(Tables 4, 5)**. Regarding the HIV-associated type, a single case was recruited in this study which showed a negative HHV-8 staining **(Figure 7A, B)**.

Table 3 Comparison of HHV-8 total staining score with the stages of KS.

	Patch Median = 0.00	Plaque Median = 5.00	Nodule Median = 5.00	(H)/(df)	***P-value***
No.	6	15	11		
Total staining score	Mean Rank = 5.83	Mean Rank = 18.53	Mean Rank = 19.55	9.937 (2)	0.004*
Comparsion			*z* Score		*P-value*
Patch vs.		Plaque	-2.848		0.013*
Patch vs.		Nodule	-2.927		0.01*
Plaque vs.		Nodule	-0.276		1.0 (N.S)

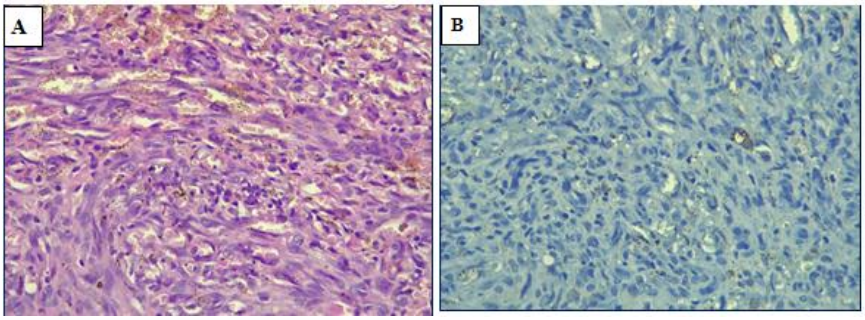

Figure 7 Plaque stage HIV-associated KS, (A) H & E stained section of (x100 magnification).& (B) HHV-8 stained section shows negative staining (x100 magnification).

Table 4 Association of HHV-8 expression with clinicopathological parameters.

Parameters		HHV-8 expression (IHC)		Total	*P value*
		Negative	Positive		
Sex	Male	4	21	25	1.0(N.S)
		16.0%	84.0%	100,0%	
	Female	1	6	7	
		14.3%	85.7%	100.0%	
Lesion number	Single	0	2	2	1.0(N.S)
		0.0%	100.0%	100.0%	
	Multiple	5	25	30	
		16.7%	83.3%	100.0%	
Disease recurrence	Present	5	24	29	1.0(N.S)
		17.2%	82.8%	100.0%	
	Absent	0	3	3	
		0.0%	100.0%	100.0%	
Classical KS	Absent	1	4	5	1.0(N.S)
		20.0%	80.0%	100.0%	
	Present	4	23	27	
		14.8%	85.2%	100.0%	
Iatrogenic KS	Absent	5	23	28	1.0 (N.S)
		17.9%	82.1%	100.0%	
	Present	0	4	4	
		0.0%	100.0%	100.0%	

No significant association between the median age and HHV-8 expression, while the association with disease duration was significant **(Table 6)**.

Discussion

The variable histological spectrum of KS and wide differential diagnosis ranges from inflammatory dermatosis to benign and malignant tumors that simulate KS, presenting a diagnostic pitfall that necessitates the need for immunohistochemistry for confirming the diagnosis.[8] To the best of our knowledge, this is the first immunohistochemical study in Iraq that use a monoclonal anti-HHV-8 antibody as a confirmatory diagnostic tool among KS cases in correlation with clinicopathological parameters.

In this study, patient's ages ranged between 27 to 85 years with a mean age of 64 years which is similar to that observed in the Sharquie *et al.* study (mean age=64 years),[9] while in the Al-Waiz *et al.* study,[10] the mean age was 54 years. Aging-related reduction in lymphocyte counts is an important risk factor, and classic KS typically develops in elderly men.[11] The classical type, which occurs in the elderly still the most common type of KS in Iraq, in this work, 84%

of cases were classical type, which is similar to that of the Sharquie *et al.* study.[9]

The male-to-female ratio was (3.5:1) which is corresponding to that of the Sharquie *et al.* study (3.1:1)[9] and is comparable with that of the Al-Waiz *et al.* study (2:1).[10] Sex-based differences emerge after puberty which might suggest a role of sex hormones in the pathogenesis. However, the lack of sex hormone receptors on spindle cells of KS is against the direct effect of sex hormones and suggests that other unknown factors may be involved in this difference.[12]

The mean disease duration was 11.5 months which is slightly higher than that of the Sharquie *et al.* study (mean 9 months),[9] and lower than that of the Al-Waiz *et al.* study (mean 19 months).[10] In the current study, there was a significant association between the median disease duration and HHV-8 expression. Unfortunately, by reviewing the literature no study or report documented the significance of this association, which was mentioned for the first time in this study.

In the present study, the overall positive HHV-8 expression was 84.4%, this finding is comparable to that of the Hong *et al.* study,[6] and Safaei *et al.* study,[5] where 78% and 90% of cases show positive HHV-8 expression, respectively. Most spindle cells of KS lesions are in the latent phase and expressing LANA-1 while only a small proportion (< 5%) of the infected cells are in the lytic phase of replication. Thus, most cells of KS lesions will accordingly be LANA-1 immunoreactive.[13]

There was a significant correlation between the stage of progression and the total staining score. This finding is similar to that of Van Bogaert *et al.* study,[14] and Cheuk *et al.* study.[7] Additionally, the comparison of the total staining score of (the patch vs. plaque and patch vs. nodule) was statistically significant, while (the plaque vs. nodule) show no significant correlation, which is similar to Van Bogaert *et al.* study.[14] Regarding the overall HHV-8 negative staining, only five cases show HHV-8 negative staining, four of these cases were in the early patch stage and only one case of HIV-associated type was in the plaque stage. Additionally, the majority of cases that did not express HHV-8 staining, have a relatively lower median duration than that of HHV-8 positive cases.

Table 5 Association of HHV-8 expression with the biopsy site of skin lesions.

		HHV-8 expression		*Total*	*P-value*
		Negative	*Positive*		
Biopsy site	Left foot	2	4	6	0.89 (N.S)
		33.3%	66.7%	100.0%	
	Right foot	1	6	7	
		14.3%	85.7%	100.0%	
	Left leg	1	7	8	
		12.5%	87.5%	100.0%	
	Right leg	1	6	7	
		14.3%	85.7%	100.0%	
	Trunk	0	1	1	
		0.0%	100.0%	100.0%	
	Right knee	0	1	1	
		0.0%	100.0%	100.0%	
	Right forearm	0	2	2	
		0.0%	100.0%	100.0%	
Total		5	27	32	
		15.6%	84.4%	100.0%	

Table 6 Association of HHV-8 expression with the median age and disease duration.

Parameters	*HHV-8 negative expression N = 5*		*HHV-8 positive expression N = 27*		*Mann-Whitney U*	*Z Score*	*P value*
	Median	*Mean Ranks*	*Median*	*Mean Ranks*			
Age (Years)	66.0	17.90	65.0	16.24	60.50	- 0.364	0.73 (N.S)
Disease duration (Months)	4.0	8.40	9.0	18.00	27.00	-2.118	0.032*

It's interesting to note that the single case of HIV-associated type with typical histopathological features of plaque stage KS; showed negative immunostaining for HHV-8. A literature search revealed similar findings in two cases with typical histopathological features of HIV- associated KS and negative HHV-8 immunostaining reported by Manraj *et al.* study,[15] and Kumar *et al.* study.[16] Additionally, Marti *et al.* study,[17] reports a case of HIV-associated anaplastic KS, that firstly showed positive HHV-8 expression but later on with disease recurrence and progression show negative HHV-8 staining. In the present study, we speculate that the negative immunostaining in the HIV-associated type might be related to the low viral load of HHV-8 within the tumoral cells of the KS lesion. This study revealed no significant associations between HHV-8 expression and different parameters including age, sex, lesion number, disease recurrence, clinical type of KS, and site of biopsy. These results were similar to that reported by the Hong *et al.* study.[6]

Conclusion

Immunohistochemistry for HHV-8 is a sensitive and specific method in the diagnosis of KS. The majority of cases that did not express HHV-8 staining were in the early patch stage, with a relatively lower median duration than that of HHV-8 positive cases. Negative immunostaining for HHV-8 does not necessarily exclude KS in appropriate clinicopathological settings, especially in the early patch stage and in the HIV-associated type. No significant difference between HHV-8 expression and age, sex, lesion number, disease recurrence, clinical types of KS, and site of biopsy.

References

1. Lebbe C, Garbe C, Stratigos AJ, *et al*. Diagnosis and treatment of Kaposi's sarcoma: European consensus-based interdisciplinary guideline (EDF/EADO/EORTC). European Journal of Cancer. 2019;114:117-127.
2. Kenneth Y. Tasi. Kaposi's Sarcoma. In: Christophoer. Griffiths, Jonathan Barker, Bleiker, Chalmers, Creamer. Rook's Textbook of Dermatology 9th ed. John Wiley & Sons; 2016;(139):1-6.
3. Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2021;71(3):209–49.
4. Dow DE, Cunningham CK, Buchanan AM. A review of human herpesvirus 8, the Kaposi's sarcoma-associated herpesvirus, in the pediatric population. Journal of the Pediatric Infectious Diseases Society. 2014;3(1):66–76.
5. Safaei NZ, Saberi S, Gransar A *et al*. Immunohistochemical Detection of the Human Herpes Virus 8 (HHV8) Latent Nuclear Antigen-1 in Kapasi Sarcoma Cases. Iranian Journal of Pathology. 2011;6(2):73-8.
6. Hong A, Davies S, Lee CS. Immunohistochemical detection of the human herpes virus 8 (HHV8) latent nuclear antigen-1 in Kaposi's sarcoma. Pathology. 2003;35(5):448-450.

7. Cheuk W, Wong KO, Wong CS, *et al*. Immunostaining for human herpesvirus 8 latent nuclear antigen-1 helps distinguish Kaposi sarcoma from its mimickers. American Journal of Clinical Pathology. 2004;121(3):335-342.
8. Pereira PF, Cuzzi T, Galhardo MCG. Immunohistochemical detection of the latent nuclear antigen-1 of the human herpesvirus type 8 to differentiate cutaneous epidemic Kaposi's Sarcoma and its histological simulators. Anais Brasileiros de Dermatologia. 2013;88:243–6.
9. Sharquie KE, Noaimi AA, Taha SF. Kaposi's Sarcoma is Increasing over Years. American Journal of Dermatology and Venereology. 2019;8(5):84–7.
10. Al-Waiz M, Sharquie KE, Al-Hamdani GA. An upsurge of new cases of Kaposi's sarcoma in Iraqi patients. Saudi Medical Journal. 2003;24(2):224–5.
11. Bhutani M, Polizzotto MN, Uldrick TS, *et al*. Kaposi's Sarcoma –associated herpesvirus-associated malignancies: epidemiology, pathogenesis, and advances in treatment. In: Seminars in oncology. WB Saunders, 2015; 223-246.
12. Radu O, Pantanowitz L. Kaposi's Sarcoma. Archives of Pathology & Laboratory Medicine. 2013;137(2):289–94.
13. Pantanowitz L, Caponetti G, Dezube BJ. The immunohistochemistry of Kaposi's sarcoma. Methods of Cancer Diagnosis, Therapy and Prognosis. 2010;405–31.
14. Van Bogaert L. Immunostaining by Human Herpes Virus 8 Latent Nuclear Antigen-1 of Kaposi's sarcoma: A Potential Biomarker of Severity of Disease. J Mol Biomark Diagn. 2013;5:002.
15. Manuraj S, Steve M, Heinz K, *et al*. Negative HHV-8 immunoreactivity in HIV associated Kaposi's sarcoma. Journal of Cutaneous Pathology. 2016;43(7):626-628.
16. Kumar P. Human herpes virus-8-negative kaposiform sarcoma in a HIV-positive male: Novel variant. Journal of Cancer Research and Therapeutics. 2020;16(1):177.
17. Martí N, Monteagudo C, Pinazo I, *et al*. Negative herpesvirus-8 immunoreactivity does not exclude a diagnosis of Kaposi sarcoma. British Journal of Dermatology. 2011;164(1):209-211.