Bioengineering Research

Molecular and Immunohistochemical Analysis of Kaposi's Sarcoma Patients

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Abstract

Introduction: Kaposi's sarcoma (KS) is a neoplasm involving blood and lymphatic vessels that has been characterized in four different clinical and epidemiological forms (classic, endemic, iatrogenic and epidemic). KS incidence is reported low in the general population of Iran while it is reported high among Iranian renal transplant recipients.

Objective: There are only some studies regarding the epidemiological and clinical features of KS in Iranian renal transplant recipients, but no reports are available on classic KS.

Materials and Methods: Present study intended to assess retrospectively the demographic and clinical features of 44 KS patients referred to the Razi Hospital in Tehran from 2000 to 2009. It also intended to detect human herpesvirus 8 to confirm the KS histological diagnosis.

Results: In these series of patients, 77.3% were male and 22.7% were female. There was a male predominance with a ratio of 3.4/1. Classic, epidemic and iatrogenic KS are found in 40 (90.9%), 2 (4.55%) and 2 (4.55%) of patients, respectively. The mean age was 62.87 among 40 patients with classic KS. In most cases (93.2%), KS was limited to the skin, without mucosal, lymph node or visceral manifestations. KS lesions mainly localized to the skin of extremities, particularly lower extremities. HHV-8 antigen and genome was detected in all samples.

Conclusion: This study showed that the picture of KS is similar to previous reports in our region. Also, our results confirmed the pathological diagnosis of KS cases in our study group.

Keyword: Kaposi's sarcoma; Immunohistochemistry; PCR; Human herpesvirus 8.

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1. Introduction

Kaposi's sarcoma (KS) is a neoplasm involving blood and lymphatic vessels that was first described by Moriz Kaposi in 1872 [1]. Four different clinical and epidemiological forms of KS has been characterized [2]; 1) Classic KS, commonly occur in elderly men of Mediterranean or Eastern European origin [3-5]; 2) African-endemic KS [5,6]; 3) Iatrogenic KS, developing in solid organ transplant recipients [7,8] and 4) Epidemic or AIDS-associated KS [2,5,9].

The histological spectrum of KS can be divided in three stages as patch, plaque and nodule. In patch stage there is an irregular dermal proliferation of jagged thin walled vascular channels. In plaque stage of KS presence of cellular fascicles with a mostly

spindle cell is noted. In the most advanced nodular stage a mass of fusiform neoplastic cell which form interlacing bundles is apparent and slit-like spaces are spread throughout the tumor, yielding a sieve-like appearance [10]. The most classic characteristic of KS is the presence of spindle cells forming slits containing red blood cells [2].

In 1994 Chang et al. identified fragments of the human herpesvirus 8 (HHV-8) or Kaposi's sarcoma-associated herpesvirus (KSHV) genome in epidemic KS tissues. HHV-8 is considered to be the etiological agent of all forms of KS [11,12].

Distinguishing KS from other vascular tumors and other nonvascular spindle cell soft-tissue neoplasms may occasionally be complicated. Therefore, detection of HHV-8 using LANA immunohistochemistry (IHC) or/and polymerase chain reaction (PCR) would be valuable for discrimination of KS from other lesions with which it may be confused [13,14].

KS was reported as a rare cancer among Iranian population by the National Cancer Registry [15]. The annual age-standardized incidence rate (ASR) was reported to be from 0.10 to 0.17 per 100.000 in males and from 0.06 to 0.08 per 100.000 in females [15]. While in the general Iranian population KS incidence is low, among Iranian renal transplant recipients KS is more prevalent [16]. There are only some studies regarding the epidemiological and clinical features of KS in Iranian renal transplant recipients, but no reports are available on classic KS in Iranian patients. The present study aimed to assess retrospectively the demographic and clinical features of 44 KS patients referred to the Razi Hospital in Tehran from 2000 to 2009. Also, it intended to detect HHV-8 by IHC for latent nuclear antigen-1 (LANA-1) and PCR for ORF26 locus to confirm the KS histological diagnosis and distinguish it from non-KS vascular tumors.

2. Materials and Methods

2.1. Patients and samples

The records of 44 KS patients followed at the years of 2000-2009 in the Pathology Laboratory of Razi hospital in Tehran were reviewed retrospectively. The criteria for KS were histological confirmation of a clinically definitive diagnosis. The clinical and demographic data for all patients were obtained from the clinical files. The patient's age, gender, the region they lived in, the year of diagnosis, history of some risk factors, clinical stage, site and number of lesions were recorded. Also formalin-fixed paraffin-embedded tissue samples, obtained from 44 mucocutaneous biopsies of these patients, were retrieved from the archive of the Pathology Laboratory of Dermatology Department in Razi hospital to detection of HHV-8.

2.2. Detection of HHV-8 LANA-1 antigen in KS lesions

From each paraffin block 4-µm section were sliced, mounted on 5-aminopropyltriethoxylin (AAS) coated slides and allowed to dry overnight. The sections were deparaffinized by xylene and treated with microwave heating at 60oC 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 buffer saline (PBS), and non-specific binding of secondary antibody was blocked with normal serum. Routine streptavidin- biotin-peroxidase immunostaining with diaminobenzidin was achieved to the sections followed by overnight incubation with a murine monoclonal antibody directed against the C-terminal of the LANA-1 antigen of HHV 8 clone 13B10; novocastra of 1:50 dilution. Cells were considered as positive for HHV-8 when there was a strong, diffuse nuclear staining in >10% of tumor cells.

2.3. Detection of HHV-8 DNA in KS lesions

10-µm sections of each paraffin block were sliced and collected in sterile Eppendorf tubes for PCR analysis. Each tissue biopsy was extracted twice with 1 ml of xylene, for paraffin removal, and twice with 500 µl of 100% ethanol, for organic solvents removal. Tissue samples were digested with 100 µl of lysis buffer (10 mM Tris–HCl pH 7.6, 5 mM EDTA, 150 mM NaCl, 1% SDS) containing 200 µg per ml Proteinase K (37°C, overnight), followed by DNA purification by phenol and phenol–chloroform–isoamyl alcohol (25:24:1) extraction and ethanol precipitation in 0.3 M sodium acetate (pH 4.6). DNA quality test was evaluated on all samples by PCR using primers

GH20/PCO4 that amplify a 268-bp product from the human β-globin gene [17]. Following DNA quality assays all 44 KS samples were suitable for HHV-8 analysis.

HHV-8 ORF26 locus was amplified with specific primers (KS26233A: 5'-AGC CGA AAG GAT TCC ACC ATT-3' and KS26233B 5'-TCC GTG TTG TCT ACG TCC AGA -3') as a 233 bp product [11]. 5µl of the DNA template, 50 mmol/L KCl, 2.5 mmol/L MgCl2, 100 mmol/L Tris–HCl pH 8.3, 2.5 mM MgCl2, 200 µM dNTP, 1.5U Hot Start Taq DNA polymerase (QIAGEN), 20 pmol of each primers were added to the final volume of 50 µl. The PCR cycling conditions were set at 94◦C for 5 min, followed by 40 cycles of 94◦C for 45s, 56◦C for 45s and 72◦C for 60s, with a 10 min extension at 72◦C. Positive and negative controls were included in each set of PCRs.

3. Results

The demographic and clinical characteristics of 44 KS patients had summarized in Table 1. In our study group, 34 out of 44 KS cases (77.3%) were male and 10 out of 44 of them (22.7%) were female. There was a male predominance with a ratio of 3.4/1. Twelve (27.3%) of patients (9 men and 3 women) were ≤ 50 years and 32 (72.7%) of them (25 men and 9 women) were > 50 years. The age-adjusted male to female ratio was 3 for ≤ 50 years and 3.57 for above it.

Most of patients were not available to screen HIV seroprevalence. However, when they refer to pathology laboratory, the HIV status asked from patients and recorded in their medical documents. Based on their medical documents, most of them are HIV negative and only two patients were positive as considered epidemic KS. Also two patients were renal transplant recipients. Therefore, classic, epidemic and iatrogenic KS were seen in 40 (90.9%), 2 (4.55%) and 2 (4.55%) of patients, respectively. Among the 40 patients with classic KS (mean age ± SD = 62.87±17), 32 were men and 8 were women. Two iatrogenic KS patients were one man (43 years) and one woman (62 years). Two epidemic KS patients were one man (37 years) and one woman (42 years).

The geographical distribution of the patients was known to 33 patients. 22 out of 33 (66.7%) cases were from Tehran and 11 out of 33 (33.3%) patients were from the other cities of Iran that referred to Razi hospital in Tehran.

The lesions were patch, plaque, nodule and mix of two or three clinical forms in 9 (20.45%), 12 (27.3%), 14 (31.8%) and 9 (20.45%) of patients, respectively. Mix forms were as followed: 2 cases were patch and plaque, 5 patients were plaque and nodule and 2 cases were mix of patch, plaque and nodule. Lower and upper extremities were involved in 26 (59.1%) and 5 (11.4%) of subjects, respectively and lesions were confined to this area. The lesions were developed on both lower and upper extremities in 3 (6.8%) of subjects. Four subjects (9.1%) were developed lesions on trunk. In three cases (6.8%) lesions were on whole body including face, trunk and extremities. The mucus of genital tract was involved in 3 (6.8%) of patients. In one case, it was detected in a pregnant woman that was confined to this area, but it was disseminated in the other two patients. Limited and disseminated distribution were detected at 37 (84.1%) and 7 (15.9%) of cases, respectively. The number of lesions was presented on their body was single and multiple in 24 (54.5%) and 20 (45.5%) of subjects, respectively.

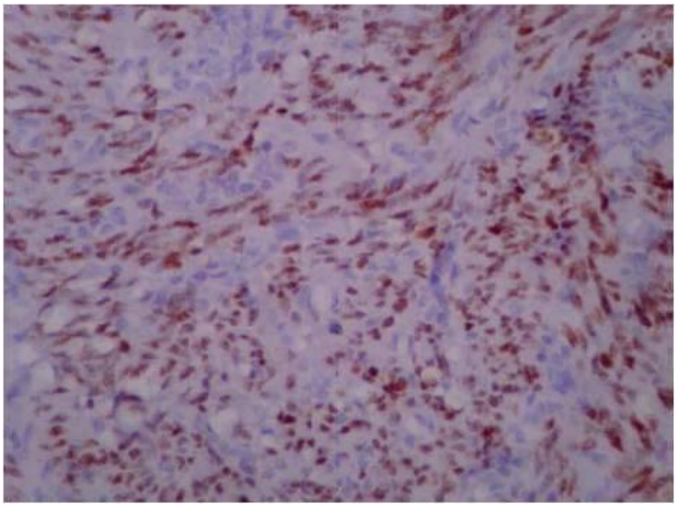

Figure 1. Immunohistochemical staining of spindle cell nuclei with HHV-8 expressing LANA-1×40 objectives.

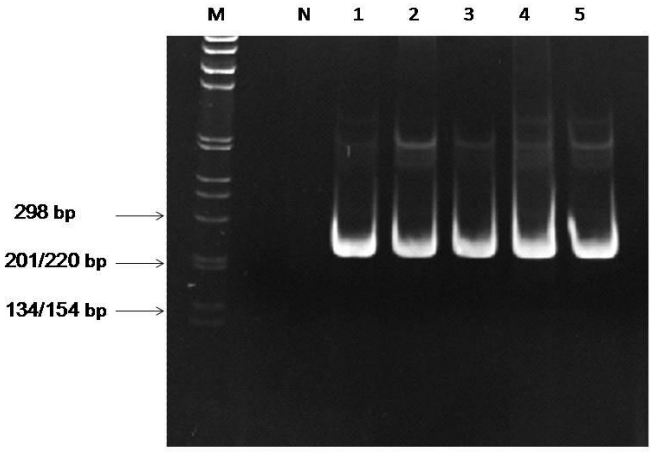

Figure 2. HHV-8 ORF26 PCR results. M: DNA Marker; N: negative control.

Among 32 males with classic KS one gave a history of chemotherapy to CML from 3 months before onset of KS and one was diabetic. Also the only woman with epidemic KS was intravenous drug user.

LANA-1 antigen of HHV-8 was detected in the nuclei of spindle cells and endothelial cells of the vascular channels of KS lesions by immunohistochemistry (Figure 1). 43 out of 44 KS cases revealed a strong, nuclear staining for HHV-8 (97.7%). One case had a week positivity that was early stage of KS. Nodular lesions commonly had more positive spindle cells in comparison to patch and plaque stage lesions.

The 233 bp fragment of the ORF26 locus of HHV-8 was amplified in all KS samples (Figure 2).

4. Discussion

Regarding data of the National Cancer Registry of Iran, KS is a rare cancer among Iranian [15]. Only 155 KS cases were recorded in National Cancer Registry from 2004 to 2006 [15,18,19], and 101 cases were registered in Tehran Population Based Cancer Registry from 1998 to 2002 [15,19]. Reports from some Middle Eastern countries have shown that the incidence of KS is relatively high in Israel and low in Bahrain and Kuwait [20].

In present study, KS was prominent in male that is consistent to previous studies reporting a male predominance in most studies in the world [21-23]. The higher incidence rate of KS among men compared with women suggests that gender is a common risk factor for KS in most populations [3]. The ages of patients ranged between 25-87 years with predilection for middle aged and elderly. In Iran the peak incidence of KS previously has been reported at ages of 50-79 for classic KS and below 50 years in renal transplant recipients [15,16,24]. Therefore, the elderly might be a common associated factor for development of classic KS in Iran [15]. Previous studies showed that classic KS occur commonly in individuals during their fifth and sixth decades of life with a lower rate of incidence at other ages and only sporadic cases occurring before the age of 30 years [3,25,26].

The distribution of lesions was as expected, with involvement of the extremities, particularly lower extremities which is characteristic of the classical form of KS [27,28]. In 41 out of 44 patients, KS was limited to the skin, without mucosal or lymph node involvement. Extracutaneous involvement including mucus of genital tract and lymph node was presented only in three cases (6.8%). Previous studies have been shown that involvement of internal organs is uncommon in classic KS and occurs in less than 10% of cases [3,21,22,27,29]. However, previous studies in Iran on renal transplanted KS patients showed more involvement of mucus or viscera [16,30-34]. The association between the aggressive clinical course of KS and immunosuppression has previously been confirmed [29]. The prognosis of KS in Iran is good [15]. According to National Death Survey reports, no death registered due to KS in 2001, 2003 and 2005 [15].

This study showed almost similar clinical findings to those reported previously from Turkey and Iraq in our region [21,26]. However, most KS reports from other countries in our region are in organ transplant recipients, and only few reports are available in classic KS patients [35-38].

Present work revealed that HHV-8 can be detected in almost all of KS lesions by IHC. The plaque and nodule stage have significant higher percentage of

Table 1. Demographic and clinical characteristics of KS patients

	Classic KS (n = 40)	Iatrogenic KS (n = 2)	Epidemic KS (n = 2)	Total (N = 44)
Gender				
Male	32	1	1	34
Female	8	1	1	10
Age (yr)				
≤ 50	9	1	2	12
> 50	31	1	0	32
KS site				
Lower extremities	24	1	1	26
Upper extremities	5	0	0	5
Lower and upper extremities	3	0	0	3
Whole body	1	1	1	3
Trunk	4	0	0	4
Genital	3	0	0	3
No. of lesions				
Single	22	1	1	24
Multiple	18	1	1	20
Distribution at presentation				
Limited	35	1	1	37
Disseminated	5	1	1	7
Clinical stage				
Patch	7	0	2	9
Plaque	12	0	0	12
Nodule	13	1	0	14
Mix	8	1	0	9

immunohistochemical staining for HHV-8 than patch stage. Also, HHV-8 genome detected in all KS lesions. Detection of LANA-1 antigen by IHC or HHV-8 genome by PCR has been proposed as techniques for the discrimination of KS from other vascular lesions with which it may be confused, such as cutaneous angiosarcoma, dermatofibrosarcoma protuberans, spindle cell hemangioma, pilar leiomyoma, vascular transformation of lymph nodes, pyogenic granuloma, stasis dermatitis, and spindled melanoma among others [13,14]. The high sensitivity and specificity of IHC for HHV-8 detection in KS lesions make it a reliable and cost-effective means of differentiating KS from other vascular and nonvascular spindle cell lesions [13]. Previous studies have been shown that PCR is extremely sensitive, but specificity is less than IHC because of possible contamination, which can give rise to false positives. Stringent laboratory protocols to avoid contamination and proper utilization of positive and negative controls can overcome to this problem [39].

In conclusion, it seems that the picture of clinical features of KS in Iran is similar to previous reports in our region. Also, these data confirmed that in Iran classic KS occurred predominantly in elderly patients with predilection in males. Moreover, our results confirmed the pathological diagnosis of KS cases in our study group.

Conflict of interests

The authors declare that they have no conflict of interests.

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