Evaluation of c-kit expression in classic Kaposi’s sarcoma in a cohort of Egyptian patients

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Kaposi’s sarcoma; Classic Kaposi’s sarcoma; C-kit; HHV8; Imatinib; Immunohistochemistry

Abstract Background: Kaposi’s sarcoma (KS) is an angioproliferative disorder associated with human herpesvirus 8 infection. Classic KS is the most prevalent type of KS in countries of the Mediterranean basin including Egypt. Several in vitro studies have detected c-kit expression in AIDS related-KS however, only a few studies addressed this issue in the classic type with no data on the ethnicity of studied cases. The prospect of installing targeted anti-c-kit treatment to KS patients presents a promising avenue in KS therapeutics.

Aim: To elucidate the expression of c-kit in classic KS cases and study possible relations with expression of HHV8 latency-associated nuclear antigen-1 (LANA-1) and other clinicopathological parameters.

Methods: Twenty four cases of classic KS of the plaque and nodular stages in the lower limb were studied. Immunohistochemical detection of HHV8-LANA-1 and c-kit was carried out on archival paraffin embedded tissue, possession of the Pathology and Dermatology Departments, Alexandria School Of Medicine, Egypt. Statistical analysis of possible relations between both antigens and clinicopathological parameters (patient’s age and gender and histological stage) was performed.
Introduction

Kaposi’s sarcoma (KS), first described in 1872, is an angioproliferative disorder associated with infection with human herpesvirus 8 (HHV8) [1]. It is classified into four clinico-epidemiological types: classic, endemic (African), iatrogenic (post-transplantational) and AIDS-associated (epidemic) [2,3].

Classic KS typically presents in middle or old aged men of Mediterranean, Eastern European and Jewish descent in whom lesions usually occur on the distal extremities [2,4,5].

Clinically, classic KS presents with purplish skin patches, plaques, and nodules which may ulcerate and bleed. Skin lesions vary in size from minute to several centimeters in diameter and can remain static for years or pursue a rapid course within a few weeks [6].

Histopathologically, the earliest lesion, the ‘patch’ stage features thin-walled vascular spaces in the upper dermis with few mononuclear inflammatory cells [3]. Next, the ‘plaque’ stage presents increasing vascular spaces surrounded by spindle-cell bundles. In the late ‘nodular’ stage, the tumor becomes more solid with large fascicles of spindled cells and fewer vascular spaces, inflammatory cells and entrapped extravasated erythrocytes and macrophages [4,7].

In 1994, a novel human herpesvirus (HHV8), Kaposi’s Sarcoma-associated Herpesvirus (KSHV), was found to be present in almost 100% of KS lesions of all epidemiological types, in multicentric Castleman’s disease and primary effusion lymphoma [8,9].

The HHV8 viral genome comprises about a 140-kb long unique region flanked by multiple terminal repeat sequences [10]. The virus is difficult to cultivate, therefore, diagnosis of infection rests on demonstration of antibodies to the virus or detecting viral nucleic acid in clinical specimens. Proteins expressed by the virus depend on the stage of infection whether latent or lytic [3,10,11].

During latency, three genes are expressed from the viral episome: orf72, K13 (vFLIP) and orf73 (Latency-Associated Nuclear Antigen, or LANA-1) [13]. LANA-1 is a nuclear phosphoprotein essential for HHV8 episomal maintenance and segregation [10], and is a highly sensitive and specific marker of Kaposi’s sarcoma [12]. The antigen has been shown to bind and inactivate the tumor suppressor functions of the pRb and p53 proteins and to immortalize endothelial cells [3,10].

In experimental studies involving HHV8-infected endothelial cells, one of the most consistent virus-induced genes was c-kit [13,14] which was found to be important in the transformation of endothelial cells [3] and imply that inhibition of the c-kit receptors may represent a promising target for pharmacological intervention [15].

C-kit is a transmembrane glycoprotein receptor (defined by the CD117 antigen) of the tyrosine kinase family encoded by the c-kit proto-oncogene. C-kit is normally expressed on hematopoietic cells, endothelial cells, germ cells and melanocytes [16]. Through its interactions with its ligand; stem cell factor, C-kit mediates crucial cellular functions as cell proliferation, intercellular adhesion and cellular spindling [14,16,17].

In the current work we employed several scoring systems to fully explore c-kit expression in the classic variant of KS, the type most prevalent in the Mediterranean city of Alexandria; in relation to expression of HHV8 LANA antigen and other clinicopathological parameters.

Material and methods

Patients and specimens

This retrospective study was approved by the Ethics Committee of Alexandria Faculty of Medicine. It consisted of 24 consecutive, clinically and histologically proven cases of classic, cutaneous Kaposi’s sarcoma. Patients were diagnosed and treated at the Alexandria Main University Hospital, Alexandria, Egypt, between January 2002 and May 2010. All tissue samples were formalin-fixed and paraffin-embedded.

Cases with history of immunosuppressive therapy, organ transplantation or HIV-1 infection were excluded.

Histopathological and immunohistochemical staining

Five micron-thick sections cut from archival paraffin blocks were H&E stained and examined microscopically to determine the histological stage.

Immunohistochemical studies were performed using the streptavidin–biotin peroxidase system with Diaminobenzidine as a chromogen. Heat-induced epitope retrieval was performed by microwaving the samples for 30 min for HHV8 and 20 min for c-kit at full power in 10 mmol/L sodium citrate buffer (pH 6.0).

Immunostaining for HHV8: was performed using the primary rat monoclonal antibody; HHV8 (LN53) (Santa Cruz Biotechnology, Inc., CA, USA, Catalogue Number sc-73588) at a 1:50 dilution incubated with tissue sections overnight at 4 °C. The rat ABC Staining System detection kit (Santa Cruz Biotechnology, Inc., CA, USA, Catalogue number sc-2019) was used to develop the signal. The positive control consisted of a lymph node involved by Castleman’s disease [18]. Labeling for HHV8 was considered positive if the staining was granular and exclusively nuclear within spindle and endothelial cells.

Results: HHV8 expression was detected in 100% of cases while c-kit immunoreactivity was found in 54.2% of cases. There was no correlation between c-kit and HHV8 immunoreactivity or any of the studied clinicopathological parameters.

Conclusions: This is the first report of c-kit expression in classic KS in an ethnically homogeneous cohort of Arabs of the Mediterranean region. We detected c-kit expression in about half the cases with no relationship to HHV8 LANA expression or clinicopathological parameters.

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Semiquantitative scoring of HHV8 immunostaining entailed assigning a score 0: when no or <1% positive cells were perceived, +1: ≤10% positive cells; +2, 10–50% positive cells and +3: >50% positive cells [13].

Immunostaining for c-kit: was carried out using the primary rabbit monoclonal anti-c-kit (CD117) antibody (prediluted PATHWAY C-KIT (CD117), clone 9.7, Ventana Medical Systems, Inc., Tucson, AZ, USA, Catalogue Number 790-2951) optimized for use on a Ventana automated slide stainer (The BenchMark XT, Part Number N750-BMKXT-FS) whereby tissue sections were incubated with the primary antibody for 32 min at 42 °C, according to the manufacturer’s protocol.

Built in positive control was embodied in native mast cells as well as basal keratinocytes [16,17].

Cytoplasmic and/or membranous staining reaction in >1% of tumour cells was considered positive. Detailed assessment of c-kit immunoreactivity entailed evaluation of c-kit percentage, intensity and pattern of staining. The same scoring system applied for assessing HHV8 immunoreactivity was employed for evaluation of c-kit percentage [13]. C-kit staining intensity was scored as 1+ (weak), 2+ (moderate) and 3+ (strong) [13]. In addition, c-kit staining pattern was assessed whether diffuse, patchy (for clusters of cells) or focal (for individual cells) [17].

Negative control slides consisted of sections to which no primary antibody was added.

Statistical analysis

Statistical analysis was done using SPSS for Windows software (version 13.0; SPSS Inc., Chicago, IL, USA). Fisher exact test, t test and Spearman rank correlation coefficient were used for statistical analysis. Significance level was set at the 5% level.

Results

In the current study, 24 cases were included. Fourteen were males (58.3%) with a male: female ratio = 1.4:1. The mean age (SD) was 59.79 (4.67) years ranging from 45 to 68 years. All lesions were located in the lower limbs.

Histopathologically, there were no cases in the patch stage while 8 (33.3%) were plaque stage and 16 (66.7%) were nodular stage tumours (Fig. 1A and B).

Immunostaining showed that positive, granular HHV8–LANA-1 staining was detected in all cases in nuclei of spindle cells and endothelial cells. A score 3+ staining reaction was detected in 13 cases (54.2%) (Fig. 1C) while a score 2+ reaction (Fig. 1D) was seen in 11 cases (45.8%). Reactivity was confined to lesional areas only.

C-kit immunoreactivity was detected in 13 cases (54.2%). C-kit percentage was 3+ in 4 cases (30.8% of positive cases) and 2+ in 9 cases (69.2% of positive cases). Intensity of the staining reaction was 1+ in 5 cases (38.5% of positive cases) and 2+ in 8 cases (61.5% of positive cases). C-kit staining pattern was diffuse (Fig. 2A) in 8 cases (61.5% of positive cases) and focal (Fig. 2C) in 5 cases (38.5% of positive cases).

Positive staining was seen in mast cells (Fig. 2B) and basal keratinocytes (Fig. 2D) infiltrating KS lesions in all cases.

There was no significant difference in the correlation between age distribution and immunoreactivity for HHV8 (Spearman rho = 0.29, P = 0.17). The same observation was encountered with c-kit whereby there was no significant correlation between age distribution and overall c-kit immunoreactivity (r = 1.07, P = 0.30), staining percentage (Spearman rho: −0.20, P = 0.51), intensity (r = 0.02, P = 0.99) or pattern of expression (r = 0.42, P = 0.68).

![Figure 1](image-url) Plaque and nodular stages of KS and HHV8 immune expression. (A) Plaque stage-KS (H&E; X200), (B) Nodular-stage KS (H&E, X100), (C) Score +3, (D) Score +2 (C&D: Anti-HHV8 LANA-1, X200).
Similarly, no significant relation was found between gender and either of HHV8 expression scores ($P = 0.70$) or c-kit immunoreactivity whether overall positivity ($P = 0.67$), staining percentage ($P = 1.00$), intensity ($P = 0.27$) or pattern of expression ($P = 0.10$).

There was no relation between HHV8 expression score and histological stage of the tumour ($P = 0.39$) (Table 1). No statistically significant relation existed between stage and any of the assessed c-kit parameters including overall reactivity, expression percentage or intensity of staining ($P = 1.00$, $0.23$ and $1.00$, respectively). A statistically significant relation was found, however, between c-kit expression pattern and histological stage, whereby diffuse immunoreactivity (in contrast to focal pattern) was solely seen in the nodular stage ($P = 0.007$) (Table 1).

There was no statistically significant relation between expression of HHV8 and c-kit including overall reactivity, percent, intensity or pattern ($P = 0.44$, $1.00$, $0.27$ and $1.00$, respectively) and other parameters (Table 2).

Discussion

In Egypt, where the classic type of KS clearly dominates over all other epidemiological types, we studied the immune expression of c-kit in classic KS in relation to HHV8 expression and some clinicopathological parameters. We employed several scoring systems aiming at fully exploring this putative relation.

The invariable immunoreactivity for HHV8 [3,19] detected in the present study was previously reported in KS [12,13,20]. Other authors [20,21] extrapolated that HHV8 LANA-1 detection could be used to distinguish Kaposi’s sarcoma from other vascular and nonvascular spindle cell lesions. However, lower figures of antigen detection (78%) were reported [22] which can be ascribed to the efficiency of epitope retrieval procedures employed [21] and the multiplicity of epidemiological types included in that study with their inherent differences in antigen expression. This was detailed in the study by Pantanowitz et al. [17] where HHV8 LANA-1 was detected in all cases of classic KS, in 91% of AIDS-related and 60% of endemic cases.

In this work, as in others’ [22] no significant difference was found between HHV8 immunoreactivity scores and patients’ age or gender.

There was no correlation between HHV8 expression scores and histological stage ($P = 0.39$) suggesting that progression was not dependent on virus load.

In the present work, 54.2% of cases expressed c-kit. Although a number of studies in the literature have scrutinized c-kit expression in KS cases, their results exhibited notable controversies [13,15,17,23,24]. Whereas some authors found no expression [25], others detected the protein in 15.3% [24], 25.6% [26] or even in 43% of cases [17]. Kandemir et al. ascribe this stark contradiction to the fact the former two studies [24,25] did not specify the exact epidemiological type or histological stage under study [13]. When we followed this debate, it was clear that our figures were closest to those reported by Pantanowitz et al. (56%) [17] in their work conducted exclusively on classic KS. However, another research group [13] working on classic KS could report a higher figure of c-kit expression (62.9%) they explained the deviation in their results with respect to others by the variation in technical parameters, as length of storage and fixation of tissues, the variation in

Figure 2  C-kit cytoplasmic immune expression in KS. (A) Strong c-kit immunopositivity in dispersed mast cells (X400), (B) basal keratinocytes expressing c-kit (x200), (C) diffuse, weak c-kit expression in nodular KS (x100), (D) focal, strong c-kit immunopositivity in plaque-stage KS (x200).
immunohistochemical procedures [3] employed (dilutions and antibodies types) in addition to the possibility of sampling bias [17]. Comparing our results regarding c-kit positivity in the different histological stages, to others’, reactivity in the plaque cases (50%) was comparable to that obtained in the same stage by Pantanowitz et al. (53%) [17]. However, our rates in the nodular phase (56.2%) exceeded theirs (47%). It is worth noting, however, that the latter study was conducted on epidemiologically heterogeneous types of KS.

In the current study, c-kit staining pattern was diffuse in 61.5% and focal in 38.5% of positive cases in a finding that was reported before [17]. We can ascribe this to the fact that two thirds of our cases belonged to the nodular stage which proved to be significantly related to the diffuse pattern of c-kit immunopositivity.

No correlation was found between c-kit and HHV8 immunoreactivity. The same conclusion was reached previously [13,17] which lead Kandemir et al. [13] to infer that LANA expression is not directly under c-kit regulation.

### Table 1  Relation between tumor stage and each of HHV8 and c-kit immunostaining scores.

<table>
<thead>
<tr>
<th>HHV8 Score 2</th>
<th>Stage Plaque</th>
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<th>5</th>
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<tr>
<td></td>
<td>N</td>
<td>%</td>
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<th>HHV8 Score 3</th>
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<td></td>
<td>%</td>
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<td>Negative</td>
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<tr>
<td>Positive</td>
<td>%</td>
<td>37.6%</td>
<td>100.0%</td>
<td>1.00</td>
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<tr>
<th>C-kit percent</th>
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<td>Score 2+</td>
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<td>Score 3+</td>
<td>%</td>
<td>30.8%</td>
<td>100.0%</td>
<td>0.007*</td>
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* Statistically significant at $P < 0.05$.

### Table 2  Relation between HHV8 and c-kit expression.

<table>
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<th>HHV8 Score +2</th>
<th>Score +3</th>
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<tr>
<td>C-kit expression</td>
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<td>Total</td>
</tr>
<tr>
<td>Negative</td>
<td>%</td>
<td>45.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Positive</td>
<td>%</td>
<td>53.8%</td>
<td>100.0%</td>
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<th>C-kit percent</th>
<th>Nodule</th>
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<th>Total</th>
<th>P value of Fisher exact</th>
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<tr>
<td>Score 2+</td>
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<td>+2</td>
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<td>100.0%</td>
<td>0.27</td>
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<td>Focal</td>
<td>%</td>
<td>80.0%</td>
<td>100.0%</td>
<td>1.00</td>
</tr>
<tr>
<td>Diffuse</td>
<td>%</td>
<td>60.0%</td>
<td>100.0%</td>
<td>1.00</td>
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</tbody>
</table>

In the current study, c-kit staining pattern was diffuse in 61.5% and focal in 38.5% of positive cases in a finding that was reported before [17]. We can ascribe this to the fact that two thirds of our cases belonged to the nodular stage which proved to be significantly related to the diffuse pattern of c-kit immunopositivity.

No correlation was found between c-kit and HHV8 immunoreactivity. The same conclusion was reached previously [13,17] which lead Kandemir et al. [13] to infer that LANA expression is not directly under c-kit regulation.
Several research groups demonstrated that inhibitors of c-kit activation and signaling inhibited HHV8-associated proliferation in culture [14–16]. Imatinib mesylate (Gleevec), a selective inhibitor of tyrosine kinase used in the treatment of gastrointestinal stromal tumor (GIST) [15,27] showed clinical and histological regression in some cases of AIDS related KS [3,27,28].

The tempting prospect of installing anti-c-kit treatment to KS cases, that immunohistochemically express c-kit is now standing on firm grounds and awaits clinical assessment. Our work is the first report of c-kit expression in cases of classic KS in an Arab population in the Mediterranean basin. A study from Turkey [13] addressed the same issue, however in an ethnically different, non Arab cohort.

In conclusion, this is the first report of c-kit expression in classic KS in Egyptians; an ethnically homogeneous cohort of Arabs of the Mediterranean region. We demonstrated expression of c-kit immunoreactivity in about half the studied cases. No relationship was found between the immune expression of c-kit and HHV8 LANA-1 or clinicopathological parameters. Diffuse pattern of c-kit reactivity was significantly related to the nodular stage. Inhibiting c-kit activity might provide an important therapeutic strategy for patients with classic KS.

The authors hereby declare the total absence of conflicting or dual interests.

References


