International Journal of Biomedical And Advance Research

ISSN: 2229-3809 (Online)
Journal DOI:10.7439/ijbar
CODEN:IJBABN

Research Article

Immunohistochemistry and antigenic expression of five proteins in Kaposi Sarcoma

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Abstract

Introduction: Kaposi sarcoma poses problems in histological diagnosis because of its broad morphologic variants and similarity to many vasoproliferative lesions. Establishing diagnosis on the basis of tumour marker alone (especially a single result) is fraught with associated pitfalls because of the problem of non- specificity.
Method: Fifteen (15) Kaposi sarcoma biopsies from seven (7) men and eight (8) women were used to find out the gene expression of vimentin, desmin, smooth muscle actin (SMA), S-100 and neuron specific enolase (NSE) in Kaposi sarcoma, Haematoxylin and eosin (H&E) method was used to confirm the diagnosis of Kaposi sarcoma in archival processed biopsies. Subsequently, Immunohistochemistry (IHC) was carried out to find out the expression of the antibodies listed.
Results: Vimentin and SMA were found to be most reactive. Neuron specific enolase was mildly reactive while the rest were non-reactive.
Conclusion: Only vimentin and SMA offer significant reactivity in the diagnosis of Kaposi sarcoma and are useful in undifferentiated neoplasms.
Keywords: *Girardinia heterophylla,* β-sitosterol, γ-sitosterol, ursolic acid

1. Introduction

In some areas of Africa, Kaposi Sarcoma is among the most commonly reported malignant skin tumor and was endemic in Africa even before the advent of HIV[1,2]. It is histologically characterized by infiltration of mononuclear inflammatory cells, formation of atypical small blood vessels and vascular slits (angiogenesis), extra vacation of erythrocytes and increased appearance of so-called spindle cells regarded as the tumor cells[3]. The histiogenesis of Kaposi remain controversial but several immunohistochemical studies favour an endothelial origin of the spindle cells. It is not clear whether these cells are of vascular or lymphatic origin[4,5,6]. Tan and Zander[7], suggest that a battery of immunohistochemical markers is required for differential diagnosis.

Vimentin is the major intermediate filament protein in mesenchymal cells, and it is frequently used as developmental marker of cells and tissues. Vimentin is normally expressed in fibroblast, chondroblast, smooth muscle cells, mesothelium, pericytes, melanocytes, and endothelial cells[8]. Vimentin is expressed in most of the sarcomas. It is frequently included in the so-called primary panel (together with CD45, cytokeratin, and S-100 protein): Intense staining reaction for vimentin without coexpression of other intermediate filament proteins is strongly suggestive of a mesenchymal tumour or a malignant melanoma.

Neuron Specific Enolase (NSE) is a glycolytic isoenzyme which is located in central and peripheral neurons and neuroendocrine cells[9]. NSE is frequently used clinically as a sensitive and useful marker of neuronal damage in several neurological disorders including stroke, hypoxic brain damage, status epilepticus, Creutzfeldt- Jakob disease, and herpetic encephalitis as well as a marker of tumors of neuroendocrine origin and small cell lung carcinoma (SCLC)[10]. It is here included to observe the reactivity of the peripheral neurons in kaposi sarcoma.

S100 protein is widely distributed in central and peripheral nervous systems. S100 protein is readily demonstrable in astrocytes, oligodendrocytes, schwann cells, folliculostellate cells of adenohypophysis, satellite cells of adrenal medulla, chondrocytes, adipocytes, myoepithelial cells, and various histiocytes which include Langerhans cells of epidermis and interdigitating reticulum cells of the lymph nodes. It is not present in perineurial cells[11]. S100 is positive in neurilemomas and neurofibromas, although the intensity and percentage of positive cells are far less in neurofibromas than in neurilemomas. This suggests that neurilemomas are composed of uniform population of schwann cells, whereas neurofibromas contain an admixture of fibroblast and perineural cells. S100 protien is specifically targeted at antigens on the sheath of connective tissue that covers a bundle of nerve fibres.

Desmin is a smooth-muscle type intermediate filament protein, expressed by smooth muscle cells, but also found expressed in fibrotic tissue in wound healing and in tumor 'desmoplastic' stroma, yet the origin of the cell type expressing desmin has been controversial[12]. Desmin is a type III intermediate filament found near the Z line in sarcomeres. It is a 52 kD protein that is a subunit of intermediate filaments in skeletal muscle tissue, smooth muscle tissue, and cardiac muscle tissue. Its presence in vascular smooth muscle is variable[13]. The muscle cell matures only if desmin is present. Vimentin is present in higher amounts during embryogenesis while desmin is present in higher amounts after differentiation. This suggests that there may be some interaction between the two in determining muscle cell differentiation[13]. Desmin is detected in rhabdomyosarcoma of all subtypes, benign smooth muscle tumours, benign skeletal muscle tumours and leiomyosarcoma (50%) [14]. Desmin shows slight immunoreactivity to various spindle cell lesions which are not traditionally considered of smooth muscle origin, which has been interpreted as evidence of focal myofibroblastic differentiation in tumours like fibromatosis, angiomatoid malignant fibrous histiocytoma, and myofibroblastoma[15].

Smooth muscle actins are thin filaments that form part of the contractile machinery in smooth muscles[16]. α-Actin is normally restricted to smooth muscle cells[17]. Antibodies directed against alpha smooth muscle actin show positivity for smooth muscle cells and myofibroblasts, but not cardiac and skeletal muscle. Actin plays an important role in carcinogenesis. The cell transformation is accompanied by a loss of actin filaments. Alterations of actin polymerization or actin remodeling played a pivotal role in regulating the morphologic and phenotypic events of a malignant cell[18].The actin antibody (HHF-35) can be used for immunostaining of myofibroblastic cells within granulation tissue, scar tissue, nodular fasciitis, and fibromatosis. Muscle specific actin antibody can express rhabdomyosarcoma, and the intensity of staining depends on the differentiation of the tumour[19]. Most of the leiomyosarcomas express muscle specific actin. Nodular fascitis and rhabdomyoma also show the expression of muscle specific actin. Bello et al. analyzed the expression of α-smooth muscle actin in ameloblastic carcinoma and found positive expression of smooth muscle actin in the stroma and concluded that smooth muscle actin could play an important role in tumour progression[20]. This study therefore used an Immunohistochemistry panel of 5 antibodies namely: Vimentin, Desmin, S100, Neuron specific enolase and smooth muscle actin. To determine the immunoreactivity in Kaposi sarcoma.

2. Method

Fifteen (15) archived paraffin embedded tissue samples from patients with Kaposi sarcoma were sectioned at thickness of 5U. One section from each sample was stained by the Haematoxylin and eosin [21] method to confirm diagnosis of Kaposi sarcoma. Subsequently 3U sections from each sample were stained by immunohistochemistry using the Streptavidin-biotin method[22] using the following antibodies Vimentin, Desmin, Smooth muscle actin(SMA), S-100 and Neuron specific enolase(NSE) (Zymet Antibody producst). All sections were dewaxed, hydrated and moistened with Phosphate buffer saline (pH 7.4). They were subsequently heated at 121^0C for 10minutes in 10mmol/L Citrate buffer (pH 6.0) and the antibodies applied for 30minutes after passing through peroxidise and protein blocks. Link, label, DAB and

haematoxylin counter stain were subsequently applied in sequence. Appropriate positive and negative controls for each antibody were setup alongside the test slides. Reactivity was observed as brown coloration and reported as mild if the brown coloration is faint and observed in not more than 10% of cells; moderate if brown coloration is darker and observed in 10-50% of cells and strong if colour is intense and observed in more than 50% of pleomorphic cells. Photomicrographs of reactive sections were taken.

The necessary institutional ethical clearance was sought and obtained from Meena Histopathology laboratory for access and use of the skin biopsies in this study.

3. Results

Table 1: **Frequency and degree of immunoreactivity for each antibody in Kaposi sarcoma**

Degree of Immunoreactivity	Nos (%) of samples reactive to Antibodies				
	Vimentin (%)	Desmin (%)	SMA (%)	S-100 (%)	NSE (%)
Negative	0	15(100)	0	15(100)	13(87)
Mild	0	0	0	0	2(13)
Moderate	1(6.67)	0	5(33)	0	0
Strong	14(93.3)	0	10(67)	0	0

N=15

Key: SMA - α smooth muscle actin; NSE - Neuron Specific Enolase.

Degree of immunoreactivity: Negative = Non reactive; Mild= less than 10% positivity; Moderate=10–50% positivity; Strong = more than 50% positivity.

Fig. 1:Histologic sections of Haematoxylin and eosin. (magnification X640)

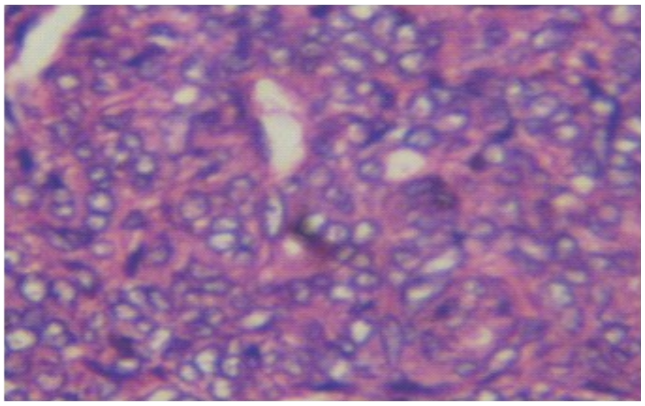


Fig. 2: Histologic sections of Vimentin-immunoreactive pleomorphic cells in Kaposi sarcoma.(magnification X640)

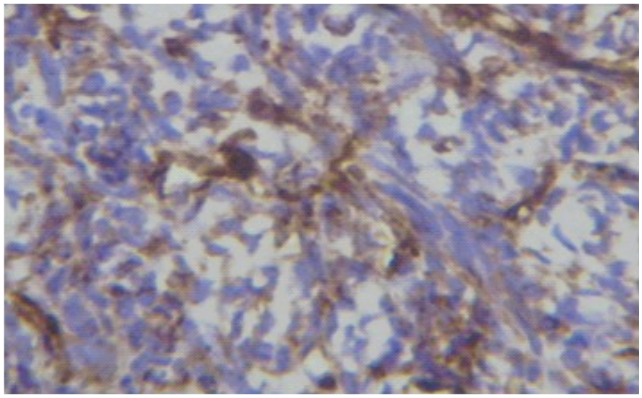
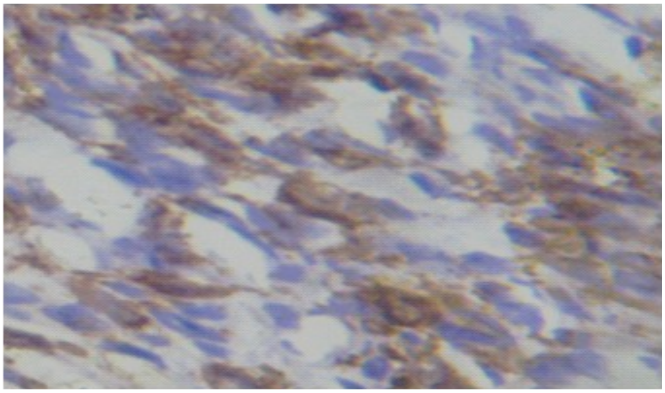


Fig. 3: Histologic Section of Smooth Muscle Actin Immunoreactive Pleomorphic Cells In Karposi Sarcoma.(magnification X 640)



4. Discussion

Each of the Haematoxylin and eosin stained sections revealed the classical tumor cells characterized by abnormal elongated spindle cells and heamorrhagic appearance (Fig.1). The immunoreactivity of vimentin, desmin, SMA, S-100 and NSE are summarized in the Table 1 above. 93.3% of samples are strongly reactive and 6.7% moderately reactive to vimentin; while 67% of the samples were strongly reactive with desmin and 33% moderately reactive. With the same antibody. This suggests that both antibodies are specific for antigens in Kaposi sarcoma but vimentin shows greater sensitivity. Intense staining reaction for vimentin without coexpression of other intermediate filament proteins is usually suggestive of a mesenchymal tumour or a malignant melanoma. The muscle cell matures only if desmin is present and its amount is higher after differentiation. Vimentin is expressed in most of the sarcomas. [23] and is present in higher amounts during embryogenesis. This suggests that there may be some interaction between the two in determining muscle cell differentiation[13]. This also suggests that 10(67%) of the samples were obtained from relatively advanced sarcoma lesions. The remaining antibodies were either non reactive or only mildly reactive to SMA, S100 protien and NSE.

Vimentin immunoreactivity was strong and diffuse as indicated by the dark brown coloration (Fig1[B]). Reactivity was observed in all of samples to various degrees and this is consistent with previous findings[24] where it was reported that they may be considered a monotypic proliferation of mesenchyme derived cells which lack the markers of full endothelial cell differentiation. It was however concluded that these mesenchyma cells are at an intermediate stage of maturity in vascular differentiation. Andrew *et al*[25] therefore deduced that vimentin is of value in the differential diagnosis of undifferentiated neoplasms, especially in sarcomas.

SMA reactivity was also strong in 67% and moderate in 33% of the samples used. Reactivity is observed to be lower in frequency and intensity when compared to vimentin. SMA reactivity has also been reported[26]. The smooth muscle isoform of SMA is routinely expressed by pilar and vascular smooth muscle reactive myofibroblasts, a subset of pericytes and myoepithelial cells. SMA is a highly sensitive marker of smooth muscle and myofibroblastic tumor in the skin[25]. The combined use of vimentin and SMA would be beneficial in highlighting the origin and staging of Kaposi sarcoma.

No reaction was observed with desmin as reported in Table1. This is consistent with the report of previous authors[25]. They reported that desmin expression is uncommon in myofibroblast. Others however, reported slight immunoreactivity to various spindle cell lesions which are not considered of smooth muscle origin and this has been interpreted as evidence of focal myofibroblastic differentiation in tumors like fibtomatosis, angiomatoid malignant fibrous histiocytoma and myofibroblastoma[15]. Though it's expression is almost always present in pilar smooth muscle tumors, it may be absent in smooth muscle tumor of vascular smooth muscle origin. When the differential diagnosis includes true smooth muscle and myofibroblastic tumor, strong desmin expression supports true smooth muscle differentiation.

S-100 was unreactive in all of the samples (Table1). However it can be weakly expressed in smooth muscle tumor[25]. S-100 is however present in 100% of normal melanocytes and nevi and in approximately 97% to 98% melanomas.

Neuron specific enolase was observed to be unreactive in 87% of the samples and mildly reactive in 13%. This cannot be a marker for Kaposi sarcoma as it has also been observed that it cannot be a marker for several other cancer types[27]. This finding also indicates that NSE cannot be used for neuronal involvement in kaposi sarcoma The organ or tissue specificity is still a subject of research. Only vimentin and SMA offer significant reactivity in the diagnosis of Kaposi sarcoma.

5. Conclusion

In conclusion, the study of the gene expression of vimentin, desmin, smooth muscle actin (SMA), S-100 and neuron specific enolase (NSE) reveals that only vimentin and SMA offer significant reactivity in the diagnosis of undifferentiated neoplasms and Kaposi sarcoma in particular.

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