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ALPHA- SMOOTH MUSCLE ACTIN, C-KIT AND DESMIN IMMUNOHISTOCHEMICAL EXPRESSION FEATURES OF COMMONLY DIAGNOSED SARCOMAS IN NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL NNEWI

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ABSTRACT

Sarcoma is a group of complex heterogeneous malignant tumours of soft tissues and bones with mesenchymal origin with lots of diagnostic challenges unconnected with their heterogeneous nature, varied histological types and subtypes with considerable morphological overlaps between the different diagnostic entities, hence the need for immunohistochemical diagnosis and classification for specific management strategies and prognosis. The present study was aimed at evaluating α- smooth muscle actin, c-kit and desmin immunohistochemical expression features of commonly diagnosed sarcomas in Nnamdi Azikiwe University Teaching Hospital Nnewi. Twenty four (24) archived paraffin wax processed sarcoma samples were sourced from the histopathology laboratory and museums of the hospital while necessary data were obtained from records. Tissue blocks were re-embedded with fresh paraffin wax and 3µ thick sections cut with the aid of a rotary microtome. Haematoxylin and Eosin staining method was employed to confirm diagnosis before proceeding to immunohistochemistry. Monoclonal antibodies for α- smooth muscle actin, c-kit and desmin were employed for immunohistochemistry while exposed mouse and rabbit specific horseradish peroxidase/diaminobenzidine detection IHC kit was employed for immunostaining. Mean age of patients was 26 years, 14 (58.3%) females and 10 (41.7%) males. There were 9 commonly diagnosed sarcoma types α-smooth muscle actin and desmin were positive for 12 and samples respectively while as c-kit was negative for all samples. SMA and desmin co-expressed in metastatic liposarcoma and low grade leiomyosarcoma. Alpha-SMA, desmin and c-kit showed consistent expression features in many sarcoma types and could be explored further for sarcoma characterization in Nigeria.

KEYWORDS: Sarcoma, immunohistochemistry, α- smooth muscle actin, c-kit, desmin.

INTRODUCTION

Sarcoma is a group of complex heterogeneous malignant tumours of soft tissues and bones with mesenchymal origin [1]. They develop in connective tissues such as skeletal muscle, fat, tendons, fibrous tissue, smooth muscle and the neurovascular elements that support these components and in bone. They are believed to account for less than 1% of all malignant tumours but have distinctive biological characteristics, which include a high incidence of aggressive local behavior and a predilection for metastasis.[1] An earlier study by Mandong et al, [2] reported that out of 22,353 confirmed cancers between 1994 and 2003, 11.3% (266) were sarcomas. A similar but latter study by Dauda et al [3] reported 81(39%) soft tissue sarcomas out of 210 confirmed childhood malignancies. Children and young adults are more vulnerable to soft tissue tumours and sarcomas in particular.[4] Commonly diagnosed sarcoma cases in Nigeria include but not limited to dermatofibrosarcoma protuberans, malignant peripheral nerve sheath tumour, osteoblastic osteosarcoma, fibromyxoma, malignant mesenchymal tumour, fibromyxoma, alveolar rhabdomyosarcoma, metastatic liposarcoma, synovial sarcoma and low grade leiomyosarcoma.[2,3,4]

Sarcomas pose lots of diagnostic problems and this may not be unconnected with their heterogeneous nature, with greater than100 histological types and subtypes with considerable morphological overlaps between the different diagnostic entities.[5] According to (Anderson and Hornik,[6] a combination and integration of clinical,

histomorphological and molecular features are necessary for accurate and definitive diagnosis of sarcomas. Definitive diagnosis and classification of sarcomas engender effective management strategies and prognosis for the different types. The application of immunohistochemistry and molecular diagnostic methods has helped in no small measure in classification of these cancers.[5] In a review study by Fisher.[7] the objective of immunohistochemistry is to identify differentiation in the neoplastic cells which according to the authors becomes more evident in sarcomas, each type with specific chromosomal translocations representing novel lineages. The authors further stated that many soft tissue neoplasms, show many variants, which include but not limited to spindle cell, epithelioid cell, and small round cell or pleomorphic morphology on initial examination and therefore, require further characterization by Immunohistochemistry. Rehg and Ward[8] further established the role of immunohistochemistry in the morphological characterization of sarcomas in a study involving genetically engineered sarcomas in animal model. Anderson and Hornik[6] corroborated the role of immunohistochemistry in diagnosis and management of sarcomas, which according to the authors has expanded, owing to exploitations of genomic alterations in the development of biomarkers which serve as surrogates for sarcoma detection and management. The need for continuous study toward gaining more insights into the cellular differentiation and pathogenesis of sarcomas as well as potential diagnostic biomarkers and therapeutic targets has long been opined by Kristin et al.[1]

The significance of alpha- smooth muscle actin (α-SMA) c-kit and desmin in sarcoma biology, pathogenesis and diagnosis has been reported. Alpha-smooth muscle actin (α-SMA) was shown to predominate the vascular smooth muscle cells and plays an active important role in fibrogenesis.[9] The presence of the protein, α-SMA was recently shown to in mouse subcutaneous tissue fibroblasts in the absence of tissue injury were also reported by Shen et al.[9] Myofibroblasts, metabolically and morphologically distinctive fibroblasts, expressed α-SMA with their activation playing a key role in development of the fibrosis and activated cease to proliferate but rather begin to synthesize large amounts of extracellular component proteins, the expression of α-SMA correlating with the activation of myofibroblasts.[9] Smooth muscle actin (α-SMA) stains smooth muscle fibers, fibroblasts and myofibroblasts and is over expressed in some mesenchymal tumors such as leiomyoma, leimyosarcoma, myofibroblastoma, inflammatory myofi-broblastic tumor, and gastrointestinal stromal tumors with myogenic differentiation and therefore is used as marker for differential diagnosis of sarcomas.[10] C-Kit (CD117) on other hand is a cytokine receptor, which binds to stem cell factor, that causes certain types of cells to grow, expressed on hematopoietic stem cells surface and as well as other cell types. Altered forms of this cytokine receptor may be associated with some types of cancer. Immunoreactivity of c-kit has been utilized to identify cancer patients who can be treated with tyrosine kinase inhibitors. Furthermore, desmin is a component of the cytoskeleton of striated muscles which is located at the periphery of Z-disks where it is arranged in a honey comb-like structure within the Z plane of the myofibers where it is involved in the connection of neighboring Z-disks.[11] According to the authors, desmin which usually co-exist with vimentin play a relevant role in maintaining sarcomere cytoarchitecture and the expression in humans affected by different neuromuscular and myopathic disorders have been investigated. Soglia et al[11] reported that over expression of desmin undoubtedly is an evidence of muscular dystrophy and should be considered a biomarker for the regenerative processes taking place within the muscles.

Medinger et al,[121] noted that out of 20 different sarcoma samples evaluated for c-kit immunoreactivity, 7(35%) were positive. This comprises of chondrosarcomas (2), leiomyosarcomas (2), myofibroblastic (1), sarcoma (1), histiocytoma of the parotid gland (1) and breast angiosarcoma (1). Alpha-SMA and c-kit immunoreactivity for classical Kaposi sarcoma was 60% and 40% respectively, with higher c-kit expression associated with lower α-SMA expression [10]. In a much earlier study, Hasegawa et al[13] reported strong immunoreactivity for SMA in 100% of leiomyosarcoma samples, most cases showing up to 50% cell immunostaining. Rhabdomyosarcoma tumour cells are usually positive for intermediate filaments and other proteins typical of differentiated muscle cells, such as desmin, vimentin, myoglobin, actin, and transcription factor myoD.[14,15] The aim of the present study is to evaluate the α- smooth muscle actin, c-kit and desmin immunohistochemical expression features of commonly diagnosed sarcomas which is the first towards developing diagnosis and prognosis algorithm of this rare but important group of cancers.

MATERIALS AND METHOD

Study area
This study was carried out at the Nnamdi Azikiwe University Teaching Hospital, Nnewi and National hospital Abuja.

Study design
This is a cross sectional study involving 24 archived samples of 10 different already diagnosed sarcoma.

Ethical Clearance
Ethical clearance to carry out this study was obtained from the Ethical Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi.

Sample collection
Twenty four (24) archived paraffin wax processed tissue block sarcoma samples were sourced from the histopathology Laboratory and museums of the Nnamdi

Azikiwe University Teaching Hospital (NAUTH) Nnewi. Necessary data were obtained from clinical records, operation notes and histopathology reports of the patients.

Tissue preparation

The tissue blocks were re-embedded with fresh paraffin wax and 3µ thick sections cut with the aid of a rotary microtome. Cut sections were floated out on a lukewarm Leica water bath, mounted on slides previously coated with poly-l-lysine, drained, labelled and placed on Leica hot plate in order to dry and affix the tissue onto the slides.

Staining

Haematoxylin & Eosin (H&E) Staining Method

Sections were stained by Haematoxylin and Eosin (H&E) method and morphological diagnosis of each sample confirmed before proceeding to immunohistochemistry.

Immunohistochemical Staining (IHC)

IHC of formalin processed sarcoma materials and controls were carried according to a method by (Nishio et al.[16] Monoclonal antibodies for α- smooth muscle actin, c-kit and desmin were employed. Exposed Mouse and Rabbit Specific horseradish peroxidase/ diaminobenzidine (HRP/DAB) detection IHC kit was employed for immunostaining while detection of immunoreactivity was performed according to manufacturer's instruction. Both antibodies and detection kits were procured from Abcam Plc Cambridge UK.

Immunoreactivity Scoring

The immunohistochemical staining was semi-quantitatively scored according to Zlobec et al [17]. This was based on percentage of cells (area) that will stain positive and the intensity of the staining (strong, moderate, weak). A score of 5+ was assigned to 80% or more of epithelial and/ or stromal cells that stained positive with strong intensity, 4+ was assigned to 50% or more (but less than 80%) of epithelial cells and/ stromal cells with strong intensity or 80% of cells or more with moderate to weak intensity; 3+ was assigned to 30% or more of epithelial and/ or stromal cells with strong intensity or 50% or more (but less than 80%) of positive cells with moderate to weak intensity; 2+ was assigned to 10% or more cells that stained positive with strong intensity or 30% or more (but less than 50%) that stained moderate to weak and 1+ was assigned to 10% or more cells (less than 30% of positive cells) that stained positive with moderate to weak intensity; 0 was assigned to less than 10% of positivity irrespective of the intensity of staining.

Data Analyses

All numerical data will be summarized using mean and standard deviation, whereas categorical data will be presented using frequency and proportion. Immunoreactivity pattern was expressed as percentages.

RESULTS AND DISCUSSION

The histopathological features and α- smooth muscle actin, c-kit and desmin immunoreactivity pattern are shown in Table 1. The patients' ages ranges from 11 to 48 years with a mean age of 26 years. Fourteen (14) (58.3%) samples were from females while 10 (41.7%) were from males. The sarcoma types included 4 dermatofibrosarcoma protuberans, 4 malignant peripheral nerve sheath tumour, 4 fibromyxoma and 2 each of osteoblastic osteosarcoma, malignant mesenchymal tumour, alveolar rhabdomyosarcoma, metastatic liposarcoma, synovial sarcoma and low grade leiomyosarcoma. Alpha-smooth muscle actin had positive immunoreactivity for 12 samples (dermatofibrosarcoma protuberans (4), fibromyxoma (2), alveolar rhabdomyosarcoma (2), metastatic liposarcoma (2) and low grade leiomyosarcoma (2)), desmin showed positive immunoreactivity for 4 samples (metastatic liposarcoma (2) and low grade leiomyosarcoma (2)) where as c-kit expressed negative immunoreactivity for all samples. Strong α-SMA immunoreactivity (≥4+) was recorded for 10 samples while 2 samples were moderately positive (3+). Desmin similarly expressed strong positivity 2 samples and moderate positivity for 2 samples. Co-expression of SMA and desmin in metastatic liposarcoma and low grade leiomyosarcoma were also observed. Photomicrographs of immunohistochemical staining pattern of anti α- smooth muscle actin, c-kit and desmin for all sarcoma samples are as shown in (Figure 1).

Table 1: Histopathological features and α- smooth muscle actin, c-kit and desmin immunoreactivity.

Expression/ Immunoreactivity pattern				
Tumour type	**No of samples**	**α-SMA**	**c-Kit**	**Desmin**
Dermatofibrosarcoma protuberans	4	4(100%) 3+(2), 4+(2)	0(0%)	0(0%)
Malignant peripheral nerve sheath tumour	4	0(0%)	0(0%)	0(0%)
Osteoblastic osteosarcoma	2	0(0%)	0(0%)	0(0%)
Fibromyxoma	4	2(50%)4+(2)	0(0%)	0(0%)
Malignant mesenchymal tumour	2	0(0%)	0(0%)	0(0%)
Alveolar rhabdomyosarcoma	2	2(100%)5+(2)	0(0%)	0(0%)
Metastatic liposarcoma	2	2(100%)4+(2)	0(0%)	2(100%) 3+(2)
Synovial sarcoma	2	0(0%)	0(0%)	0(0%)
Low grade leiomyosarcoma	2	2(100%)5+(2)	0(0%)	2(100%) 5+(2)

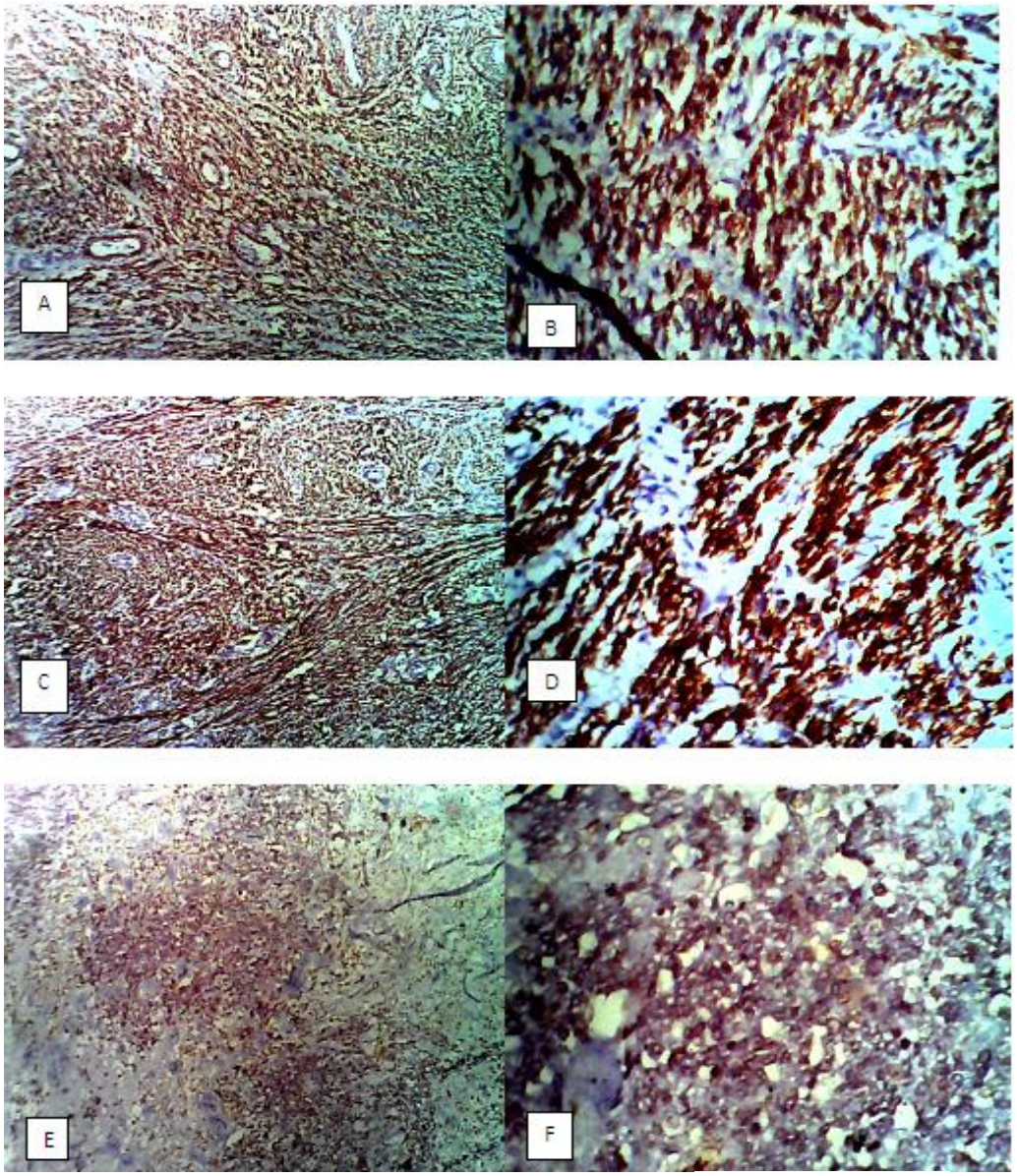

Figure 1: Immunohistochemical staining pattern of monoclonal anti α- smooth muscle actin and desmin. A: Strongly positive α- SMA staining for low grade leiomyosarcoma (X100), B: Strongly positive α- SMA staining for low grade leiomyosarcoma (X400), C: Strongly positive desmin staining for low grade leiomyosarcoma (X100), D: Strongly positive desmin staining for low grade leiomyosarcoma (X400), E: Strongly positive α- SMA staining for Alveolar rhabdomyosarcoma (X100), F: Strongly positive α- SMA staining for Alveolar rhabdomyosarcoma (X400).

DISCUSSION

The significance and expression features of alpha-smooth muscle actin (α-SMA) c-kit and desmin in cancers as well as in sarcoma biology, pathogenesis and diagnosis have been reported.[6,9,10,11,12] The present study reported an age range of 11 to 48 years, with an average age of 26 years and a male to female ratio of 1:1.4. This agrees with the report of Ikeri et al [4] who reported a median age of 33 years and a male to female ratio of 1:1.3 in an earlier study on the histological types of soft-tissue sarcomas at the Lagos University Teaching Hospital but however, differs from those of Dauda et al [3] and Mandong et al.[2] who reported male to female ratio of 2:1 is similar but separate studies. The duo however, corroborated the most commonly affected age ranges and the most commonly diagnosed sarcomas in Nigeria. The deviation from male to female ratio though not very significant could be a factor of time, facility and sample size.

Positive immonoreactivity was reported for dermatofibrosarcoma protuberans, fibromyxoma, alveolar rhabdomyosarcoma, metastatic liposarcoma and low grade leiomyosarcoma while malignant peripheral nerve sheath tumour, osteoblastic osteosarcoma, malignant mesenchymal tumour and Synovial sarcoma were all negative. α –SMA was reported positive for 5 tumours, desmin and positive for 2 and c-kit negative for all tumours studied. Smooth muscle actin expression agrees with Hasby et al[10] who reported 40% focal positivity in Kaposi sarcoma spindle cells. The expression of α –SMA in sarcomas, according to the authors, may suggest involvement of differentiation of myofibroblast-like cells which supports mesenchymal to endothelia transition and not vice versa. Hasby et al [10] observed that the difference in expression pattern of α –SMA in Kaposi sarcoma tumours is not unconnected to their differences in genotypic and phenotypic heterogeneity of different tumours and accounts for differences in response to antiangio-genic therapy. This is not particular to Kaposi sarcoma but applies to all tumours in general and sarcomas in particular, hence the need to determine protein expression pattern, not only for diagnosis but for classification towards determining best management options. A much earlier study by Hasegawa et al[13] reported expression of smooth muscle markers, SMA and desmin in leimyosarcoma samples especially those with myofibroblastic features. The report not only agreed with the finding of the current study but emphasizes the need to classify all sarcomas based on the protein expression features and the degree of expression.

The current study observed a strong positivity and co-expression of SMA and desmin in metastatic liposarcoma and low grade leiomyosarcoma. This is in line with earlier findings (Soglia et al[11] and Hasby et al.[10] This could be explained by a mutation involving Fibroblastic/myofibroblastic differentiations. Al-Daraji et al[18] emphasized the need for classification and distinction of Fibroblastic/myofibroblastic sarcoma which according to them is a controversial group of low grade sarcomas with a prominent myofibroblastic differentiation. The authors further reported that these lesions express variable immunophenotypes but are consistently positive for at least one myogenic marker. The application of SMA and desmin besides other myogenic markers aids their diagnosis and management. This was the view of Parham[19] when he reported the imperative of IHC and its potential applications, not just for diagnosis and prognostication, but for personalized therapy decisions. The strong positivity and co-expression of SMA and desmin in metastatic liposarcoma and low grade leiomyosarcoma also agreed with the study of Rehg and Ward.[8] The authors in a study titled morphological and immunohistochemical characterization of sarcomatous tumours in wild-type and genetically engineered mice also reported strong positivity and co-expression in rhabdomyosarcoma. C-Kit negative immnoraectivity which was observed in all the sarcoma samples considered in the present study was all so the same with findings of Rehg and Ward.[8]

CONCLUSION

The role of immunohistochemistry, using appropriate panel of biomarkers, in diagnosis and characterization of sarcomas for specific management regime and prognostication has further been x-rayed in this study. Alpha-SMA, desmin and c-kit have shown consistent expression features in many sarcoma types and could be explored further, standardized and in the diagnostic regimen of sarcoma in all facilities in Nigeria.

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