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Research Article

Vimentin and Cytokeratin Immunostaining: The Role in Basic Diagnosis and Prognosis of Sarcomas

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ABSTRACT

Introduction: Specific diagnosis which provides diagnostic, prognostic, and therapeutic information to guide patient care defines the primary goal of sarcoma management and care. The role of immunohistochemistry, using vimentin (mesenchymal lineage marker) and cytokeratin (epithelial lineage marker) as basic markers for diagnosis and classification of sarcomas for specific management strategies and prognosis is elucidated in the present study.

Materials and Methods: Twenty four (24) archived paraffin wax processed tissue block sarcoma samples were randomly selected from the histopathology Laboratories and museums of the Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and National hospital Abuja and necessary data obtained from records. 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. Antibodies for vimentin and pancytokeratin were employed for immunohistochemistry while exposed mouse and rabbit specific horseradish peroxidase/diaminobenzidine detection IHC kit was employed for immunostaining.

Results: The mean age of patients was 26 years while the ages range from 11 to 48 years with 14 (58.3%) females and 10 (41.7%) males. Vimentin had strong positive immunoreactivity for all sarcoma samples whereas cytokeratin had positive immunoreactions for synovial sarcoma only, which also showed co-expression of both genes.

Conclusion: Vimentin and cytokeratin may play vital role as basic biomarkers not only for diagnosis and characterization of sarcomas but for specific management regime and prognostication. However, IHC must be performed at high standard using appropriate antibodies, samples and reagents.

Keywords: Sarcoma, immunohistochemistry, vimentin, cytokeratin, biomarker.

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1.0 INTRODUCTION

Vimentin is a gene which encodes for type III intermediate filament protein. Intermediate filaments, along with microtubules and actin microfilaments constitute the cytoskeleton. It has a role in neuritogenesis and cholesterol transport and functions as an organizer of a number of other critical proteins involved in cell attachment, migration, and signaling with its mutations associated with congenital cataracts in human patients. Vimentin expressed in certain types of carcinomas (renal cell carcinoma, spindle cell carcinoma), as well as lymphomas and melanomas. It is also

expressed in mesenchymal cells though, not specific for them ¹. Due the crucial role of vimentin in maintaining muscle cyto-architecture, it is considered an essential marker for muscle regeneration ². Cytokeratins are members of the keratin gene family and consists of basic or neutral proteins arranged in pairs of heterotypic keratin chains and co-expressed during differentiation of simple and stratified epithelial tissues. According to Wei et al ¹, they are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue. They are specifically expressed in the simple epithelia lining the cavities of the internal organs and in the gland ducts and

blood vessels and therefore, are basic makers for carcinomas. Due to the expression pattern of vimentin and cytokeratins in cancers, their immuno-reactivity pattern play a vital role in basic characterization of neoplasms into carcinomas and sarcomas^{2, 3, 4, 5}. It is noteworthy however, that both genes have their pitfalls as biomarkers of characterization of sarcomas and carcinomas. Wei et al¹ in a review report on soft tissue immunohistochemistry update noted that vimentin may be expressed in certain types of carcinomas and may not be expressed in some mesenchymal tumours. The authors however, opined that if the mesenchymal tissue is negative for vimentin, it may indicate that the tissue is not of a mesenchymal differentiation. Cytokeratin, similarly may be expressed in some sarcomas especially epitheloid sarcomas^{2, 4}. Vimentin, though, cannot solely be used to differentiate mesenchymal from non mesenchymal neoplasms, the role as screening marker for neoplasms of mesenchymal lineage cannot be emphasized⁴. The authors further noted frequent co-expression of vimentin and cytokeratin in some carcinomas which may suggest certain types of epithelial tumors as possible primary sites in the evaluation of metastatic tumors.

Sarcomas are connective tissues malignant neoplasms usually found in the skeletal muscle, fat, tendons, fibrous tissue, smooth muscle and the neurovascular elements that support these components and in bone with diverse and varied histogenesis⁶. Though, believed to account for 1% of all human malignancies, the growing incidence, the very aggressive and invasive nature and the propensity of most sarcomas to metastasize has brought it to limelight as a major health challenge⁶. Sarcomas have predilections for children and young adults with a male to female ratio of 2:1^{7, 8}. Dermatofibrosarcoma protuberans, malignant peripheral nerve sheath tumour, osteoblastic osteosarcoma, fibromyxoma, malignant mesenchymal tumour, fibromyxoma, alveolar rhabdomyosarcoma, metastatic liposarcoma, synovial sarcoma, low grade leiomyosarcoma to mention but a few are examples of most commonly diagnosed sarcoma cases in Nigeria⁸. The wide heterogeneous nature of sarcomas, with greater than 100 histological types and subtypes with considerable morphological overlaps between the different diagnostic entities⁵ makes it very challenging in establishing definitive diagnosis of most sarcoma types. Furthermore, sarcomas assume poorly differentiated, undifferentiated with no obvious line of differentiation or may even form as secondary implant (metastatic tumours) in distant sites, thereby increasing the challenge of definitive diagnosis. According to Wei et al¹ the general approach to reaching a definitive differential diagnosis of soft tissue tumors is to first consider clinicoradiologic, histomorphologic, and cytomorphologic features of the tumour. Tumours with obvious line of differentiation such as smooth-muscle, skeletal-muscle, vascular, neural, and Chondro-osseous lineages may be diagnosed with morphological features. Contrariwise however, immunostaining, using appropriate marker will be required to actualize diagnosis.

In order actualize definitive and specific diagnosis that provides diagnostic, prognostic, and therapeutic information to guide patient care, immunohistochemistry play a crucial role¹. The role of immunohistochemical expressions of vimentin and cytokeratin, as afore stated, in the basic classification of sarcomas cannot be overemphasized. The present study aimed at evaluating the immunohistochemical expression pattern of vimentin and cytokeratin using selected samples of commonly diagnosed sarcomas in our facility. The expression pattern among the implications will highlight the role of the genes in basic characterization of

sarcomas which is a basic a critical step towards patients' care and management especially in a middle income country like Nigeria.

2.0 MATERIALS AND METHOD

2.1 Study area/ Study design

This was a cross sectional study using 24 archived samples of already diagnosed sarcoma which was carried out at the Nnamdi Azikiwe University Teaching Hospital, Nnewi and National hospital Abuja.

2.2 Ethical approval

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

2.3 Sample collection

Twenty four (24) archived paraffin wax processed tissue block sarcoma samples were randomly selected from the histopathology Laboratories and museums of the Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and National hospital Abuja. Necessary data were obtained from clinical records, operation notes and histopathology reports of the patients.

2.4 Tissue preparation

Tissue blocks were re-embedded in fresh paraffin wax, 3µ thick sections cut with the aid of a rotary microtome, cut sections 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.

2.5 Staining

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

2.6 Immunohistochemical Staining (IHC)

IHC of test materials and positive controls were carried according to a method by Nishio et al⁹. Antibodies for vimentin and pancytokeratin were employed for immunohistochemistry. 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.

2.7 Immunoreactivity Scoring

Immunoreactivity was semi-quantitatively scored according to Zlobec et al¹⁰. 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.

2.8 Data Analyses

Numerical data were summarized using mean and standard deviation, whereas categorical data were being presented using frequency and proportion. Immunoreactivity pattern was expressed as percentages.

3.0 RESULTS

The mean age of patients was 26 years while the ages range from 11 to 48 years with 14 (58.3%) females and 10 (41.7%) males. The breakdown of sarcoma samples were as follows: 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. Histopathological and immunoreactivity features for vimentin and cytokeratin were as shown in Table 1. Vimentin had strong positive immunoreactivity for all sarcoma samples where as cytokeratin had positive immunoreactions for synovial sarcoma only, which also showed co-expression of both genes Photomicrographs of immunohistochemical staining pattern of anti vimentin and cytokeratin sarcoma samples are as shown in (Figure 1).

Table 1: Histopathological and immunoreactivity features for vimentin and cytokeratin

Tumour type	No of samples	Vimentin	Cytokeratin
Dermatofibrosarcoma protuberans	4	4(100%) 4+(2), 5+(2)	0(0%)
Malignant peripheral nerve sheath tumour	4	4(100%) 5+(4)	0(0%)
Osteoblastic osteosarcoma	2	4(100%) 4+(2)	0(0%)
Fibromyxoma	4	4(100%) 5+(4)	0(0%)
Malignant mesenchymal tumour	2	2(100%) 5+(2)	0(0%)
Alveolar rhabdomyosarcoma	2	2(100%) 5+(2)	0(0%)
Metastatic liposarcoma	2	2(100%) 5+(2)	0(0%)
Synovial sarcoma	2	2(100%) 5+(2)	0(0%)
Low grade leiomyosarcoma	2	2(100%) 5+(2)	2(100%) 3+(2)

Figure 1: Immunohistochemical staining pattern of anti vimentin and cytokeratin

A: Strongly positive vimentin staining for low synovial sarcoma (X400), B: Strongly positive vimentin staining for low grade leiomyosarcoma (X400), C: Strongly positive vimentin staining for fibromyxoma (X400), D: Focally positive cytokeratin staining for synovial sarcoma (X400).

4.0 DISCUSSION

The significance role of IHC expression features of vimentin and cytokeratin in characterization of sarcoma have been reported ^{1, 3, 4, 5}. The present study reported 26 years as the mean age of patients, while the ages range from 11 to 48 years with a male to female ratio of 1:1.4, thus agreeing with the finding of Ikeri et al ¹¹ 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 differs from that of Dauda et al ⁸ and Mandong et al ⁷ who reported male to female ratio of 2:1 in a similar but separate studies. The authors however, corroborated the present study on the most commonly affected age ranges and the most commonly diagnosed sarcomas in Nigeria. The slight deviation from male to female ratio though not very significant could be a function of time and sample size.

Strongly positive vimentin immunoreactivity was reported for all sarcoma samples and negative cytokeratin expression except for synovial sarcoma. This agrees with earlier studies. Bashyal et al ³ reported positive vimentin immunoreactivity and negative cytokeratin IHC staining for most round cell malignant sarcomas, which led them to conclude that IHC provide important tool for clear distinction between tumours. This finding not only highlights the need for inclusion of IHC as an important ancillary diagnostic tool but strongly support the application of vimentin and keratin IHC for both diagnosis and characterization of sarcomas. Suffice it to note once again that sarcomas with their characteristic divergent histogenesis with the attendant morphological presentations most times cannot be granted definitive diagnosis using only morphological appearance, hence the need for IHC. Vimentin and keratin which are known markers of mesenchymal and epithelial lineage respectively present an indispensable tool in characterization of sarcomas especially when the lineage is not obvious. Despite the fact that Wei et al ¹ opined that vimentin positivity has a limited value in the diagnosis of soft tissue tumors, the authors however, accepted its role in establishing that a tumour is of mesenchymal differentiation and not of epithelial differentiation. The co-expression of vimentin and keratin in synovial sarcoma could be explained by the nature of the tumour in question. According to Morgan ¹, synovial sarcoma may either be biphasic (with both mesenchymal and epithelial components present) or monophasic (with either mesenchymal or epithelial morphology) histologically. Histologically biphasic tumour show positive immunoreactivity for both vimentin and cytokeratin while monophasic type show immunoreactivity for either of the genes depending on their lineage. This fact further established the role of the markers not only for diagnosis and characterization but for determination specific therapy/management options towards better patients' care and treatment. Besides the application of vimentin and cytokeratin IHC in diagnosis and characterization of sarcomas it aids their management and prognosis. This was the view of Parham ⁵ in his reported on the imperative of IHC and its potential applications, not just for diagnosis and prognostication, but for personalized therapy decisions. The strong positivity of vimentin in all sarcoma samples studied not only agreed with previous studies but validates its presence in mesenchymal tumours.

5.0 CONCLUSION

It could be concluded from the finding of the present study that vimentin and cytokeratin may play vital role as basic biomarkers not only for diagnosis and characterization of sarcomas but for specific management regime and prognostication. However, IHC must be performed at high standard using appropriate antibodies, samples and reagents.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest

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