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Original Article

Pathology Section

Immunohistochemical Evaluation of Myxoid Sarcomas- A Tertiary Care Hospital Experience

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ABSTRACT

Introduction: Myxoid sarcomas are a rare and heterogeneous group of tumours exhibiting overlapping histomorphological features with varied biological behaviour. Hence, additional ancillary techniques like Immunohistochemistry (IHC) are necessary for definite diagnosis and categorisation of the myxoid sarcomas.

Aim: To identify the distribution of myxoid sarcomas among patients and also to evaluate the utility of basic IHC in the diagnosis of myxoid sarcomas.

Materials and Methods: This was six years retrospective observational cross-sectional study carried out in the Department of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, Tamil Nadu, India, during the period of January 2008-December 2013. Relevant pathological data of all the myxoid sarcomas reported during the study period were retrieved from the medical records. Corresponding Haematoxylin and Eosin (H&E) stained slides were reviewed and IHC was done using a panel of markers for confirmation.

Results: Among the 57 myxoid sarcomas, 46% occurred in the age group of 41-60 years with a striking male preponderance (74%). Myxofibrosarcoma was the most common histological type (33.33%). All cases of myxofibrosarcoma were positive for vimentin while two cases showed focal Smooth Muscle Actin (SMA) positivity and one case showed focal CD34 positivity. Low grade fibromyxoid sarcomas were positive for only vimentin. Myxoid liposarcomas and extra-skeletal myxoid chondrosarcomas showed vimentin and S100 positivity. Myxoid Dermato Fibrosarcoma Protuberans (DFSP) was positive for vimentin and CD34 while synovial sarcoma with myxoid change was positive for vimentin and Pancytokeratin (Pan CK). Myxoid Malignant Peripheral Nerve Sheath Tumour (MPNST) showed 100% vimentin and S100 positivity while CD34 was positive in 12.5% of cases. Leiomyosarcoma with myxoid change was positive for vimentin, SMA, desmin and Pan CK.

Conclusion: The IHC is a valuable adjunct to light microscopy for the diagnosis of myxoid sarcomas and can provide as a judicious tool for diagnosis of this uncommon and challenging group of malignant soft tissue tumours.

Keywords: Glycosaminoglycans, Immunohistochemistry, Malignant, Soft tissue, Tumours

INTRODUCTION

Soft tissue sarcomas are uncommon and heterogeneous group of malignant tumours that show differentiation towards connective tissue elements like vessels, fat, fibrous tissue, peripheral nerves and tendons. Soft tissue sarcomas are relatively rare lesions accounting for less than 1% of all malignancies [1]. Among the soft tissue sarcomas, certain tumours are characterised by abundant extracellular myxoid matrix and are referred to as myxoid sarcomas [2]. The myxoid matrix in these subset of sarcomas is composed of sulphated and non sulphated Glycosaminoglycans (GAGs) [3]. The physical properties (increased viscosity and low compressibility) of GAGs favour the migration of tumour cells and the diffusion of metabolites thereby facilitating the growth of tumour cells [4,5]. Sulphated GAGs like chondroitin sulphate also modulate the survival of tumour cells by preventing apoptosis and promoting tumour cell proliferation. Myxoid matrix also possess high affinity for cell adhesion molecules and growth factors thereby facilitating cell to cell interaction and cell proliferation [6]. All these factors contribute to the highly malignant behaviour of sarcomas with GAGs rich Extracellular Matrix (ECM). Many of these sarcomas exhibit overlapping histological features thereby necessitating additional ancillary techniques like IHC for definite diagnosis and categorisation of the myxoid sarcomas [2]. IHC plays a vital role in the diagnosis of myxoid tumours of soft tissue and it is used as a complement to morphological diagnosis. It helps to rule out the non mesenchymal tumours and also for categorising the sarcomas into their specific lineage of differentiation [7]. Use of a single immunostain will lead to potential misdiagnosis due to the lack of specificity and frequent aberrant reactivity of the immunological markers [8]. Use of a panel of immunohistochemical markers based on the H&E differential diagnosis will lead to a correct diagnosis of this challenging group of tumours [9]. The antibodies most commonly employed in soft tissue tumour pathology are Vimentin, SMA, Muscle Specific Actin (MSA), S100, CD34, CD99, Desmin, Myogenin, Cytokeratin and Epithelial Membrane Antigen (EMA). There is very limited comprehensive data in literature regarding the immunohistochemical characteristics of this broad group of myxoid sarcomas, though there have been isolated studies on few individual tumours in this group. Hence, this study was intended to determine the basic immunohistochemical profile of myxoid sarcomas. The objectives of this study was to identify the distribution of myxoid sarcomas among patients admitted in Rajiv Gandhi Government General Hospital, Chennai, Tamil Nadu, India, and to evaluate the utility of basic IHC in the diagnosis of myxoid sarcomas.

MATERIALS AND METHODS

The present study was a six years retrospective observational cross-sectional study carried out in the Department of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, Tamil Nadu, India, during the period of January 2008-December 2013 the analysis of the study was done from January to March 2014 after approval by the Institutional Ethics Committee. (IEC Ref No: MMC/03032012). Informed consent was obtained from all the patients while receiving the biopsy samples in the Department of Pathology.

Sample size calculation: A total of 57 samples were collected using purposive sampling technique for selection of desired samples according to the inclusion criteria.

Inclusion criteria: All cases of myxoid sarcomas diagnosed by histopathological examination. All types of samples including wide local excision, resection and incisional biopsy samples. Patients of all age groups and gender were included in the study.

Exclusion criteria: Soft tissue sarcomas without myxoid matrix. Tumour-like soft tissue lesions with myxoid areas. Benign myxoid soft tissue tumours were excluded from the study.

Study Procedure

Relevant pathological data of all the myxoid sarcomas reported during the study period were retrieved from the medical records of the Department of Pathology, Madras Medical College, Chennai, Tamil Nadu, India. H&E stained sections of the paraffin tissue blocks of these formalin fixed specimens were reviewed. The immunohistochemical panel of markers was decided based upon the histological picture and all the myxoid sarcomas were subjected to immunohistochemical evaluation. The basic panel of IHC markers covering all the lineages of soft tissue tumours that are commonly employed are Vimentin, SMA, MSA, S100, CD34, CD99, Desmin, Myogenin, Cytokeratin and EMA [9]. Based on this the basic immunohistochemical markers used in this study were Vimentin, PanCK, CD34, Desmin, SMA, S100 and CD99.

- **Vimentin-** Positivity of vimentin indicates antigen preservation of the tissue and serves a control marker function [9].
- **Cytokeratin-** Among the soft tissue sarcomas, synovial sarcoma and epithelioid sarcoma characteristically display this antigen. Certain other sarcomas like leiomyosarcoma and MPNST show aberrant cytokeratin reactivity [9].
- **Desmin-** It is the specific marker of myogenic differentiation [9].
- **SMA** is a marker of smooth muscle differentiation. SMA reactivity is also seen in non muscle tissue with myoid phenotype like the various myofibroblastic lesions, myoepithelial lesions etc., [9].
- **S100** was first isolated from Central Nervous System (CNS) where it is localised in the cytoplasm and nucleus of astrocytes, oligodendrocytes and Schwann cells. A wide variety of other mesenchymal tissues like adipocytes and chondrocytes also express this antigen [9].
- **CD34-** Among the sarcomas, CD34 immunoreactivity is found in solitary fibrous tumour, extra-gastrointestinal stromal tumours, DFSP, spindle cell lipoma and few nerve sheath tumours in addition to vascular neoplasms [9].
- **CD99** is a cell surface glycoprotein uniformly expressed in Ewing sarcoma and Primitive Neuro Ectodermal Tumour (PNET). But it lacks specificity and is expressed in a number of soft tissue tumours including synovial sarcoma, rhabdomyosarcoma and desmoplastic small round cell tumour [9].

Immunohistochemical Evaluation

Immunohistochemical analysis was done in paraffin embedded tissue samples using the Next Generation Micro-Polymer Horse Radish Peroxidase (HRP) system based on non biotin polymeric technology provided by Thermo Scientific Ultravision Quanto detection system for IHC. 4µ thick sections from formalin fixed paraffin embedded tissue samples were transferred to gelatin coated slides. Heat induced antigen retrieval was done using microwave oven in appropriate temperature (480 watts, 640 watts, 800 watts and 800 watts for 5 minutes each) with appropriate buffer (Citrate buffer). The antigen was bound with monoclonal antibody. It was then detected by the addition of secondary antibody conjugated with HRP-polymer and diaminobenzidine substrate. The details of antibodies used for IHC is provided in [Table/Fig-1].

Interpretation and Scoring System

The immunohistochemically stained slides were analysed for the presence of reactivity, cellular localisation (nuclear/cytoplasmic/membranous), percentage of cells stained and intensity of reaction. In this study, IHC staining was graded by using semi-quantitative scale ranging from 0 (no immunoreactive cells) to 4+ (75-100% of the neoplastic cells are immunostained). The symbols 1+, 2+, and 3+ refer to the immunostaining of up to 25%, 25-50% and 50-75% of the neoplastic cells respectively [10].

Antigen	Vendor	Species (Clone)	Dilution	Positive control
Vimentin	BIOGENEX	Mouse	Ready to use	Uterus
Pan-cytokeratin (CK)	BIOGENEX	Mouse	Ready to use	Skin
Desmin	BIOGENEX	Mouse	Ready to use	Leiomyoma
Smooth Muscle Actin (SMA)	BIOGENEX	Mouse	Ready to use	Colon
CD34	BIOGENEX	Mouse	Ready to use	Vessels
S100	BIOGENEX	Mouse	Ready to use	Skin
CD99	BIOGENEX	Mouse	Ready to use	Tonsil

[Table/Fig-1]: Antibodies for Immunohistochemistry (IHC).

STATISTICAL ANALYSIS

The data is shown in tables and results are expressed in terms of frequency and percentages.

RESULTS

A total of 57 myxoid sarcomas were reported during the six years study period and majority (46%) of them occurred in the age group of 41-60 years of age. Around 35% of myxoid sarcomas occurred in patients less than 41 years of age while 19% of them were encountered in patients more than 60 years of age. In the present study, myxoid sarcomas showed a striking male preponderance with 74% of cases occurring in male patients. Among the myxoid sarcomas, eight different histological types were encountered in this study with myxofibrosarcoma being the most common histological type accounting for 33.33% of cases. The other histological types are mentioned in [Table/Fig-2].

S. No.	Histological type	Number of cases	Percentage
1	Myxofibrosarcoma	19	33.33%
2	Myxoid liposarcoma	15	26.32%
3	MPNST with myxoid change	8	14.04%
4	Low grade fibro myxoid sarcoma	4	7.02%
5	Dermato Fibrosarcoma Protuberans (DFSP) with myxoid change	4	7.02%
6	Extra-skeletal myxoid chondrosarcoma	3	5.26%
7	Synovial sarcoma with myxoid change	3	5.26%
8	Leiomyosarcoma with myxoid change	1	1.75%
	Total	57	100%

[Table/Fig-2]: Histomorphological distribution of myxoid sarcomas.

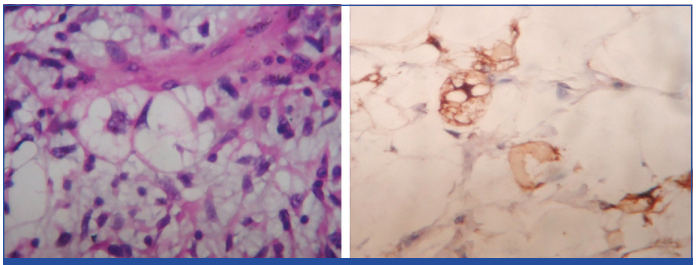
Immunohistochemical analysis was done for all the 57 cases of myxoidsarcomas using a panel of markers based on the light microscopic features of H&E stained sections. The Immunohistochemical markers employed were vimentin, PanCK, CD34, desmin, SMA, S100 and CD99.

Immunohistochemical results of myxoid sarcomas is shown in [Table/Fig-3]: Immunohistochemical analysis of 19 myxofibrosarcomas showed (4+) positivity for vimentin in all the cases while 2 cases (10.5%) showed focal (1+) SMA positivity and one case (5.3%) showed focal (1+) CD34 positivity. S100 and Desmin were negative in all the 19 cases.

A panel of Vimentin and S100 were used for immunohistochemical confirmation of myxoid liposarcomas. All the 15 cases of myxoid liposarcomas in this study showed consistent (4+) positivity for vimentin and S100 [Table/Fig 4,5].

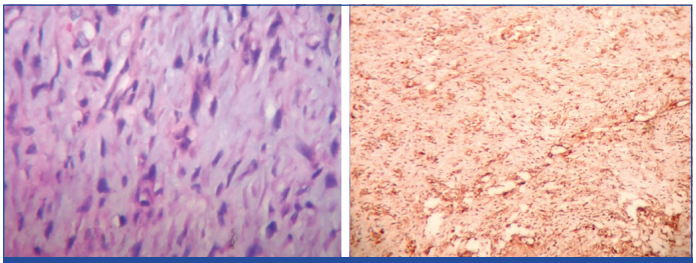
S. No.	Histological type	Vimentin	SMA	Desmin	S100	CD34	CD99	PanCK
1	Myxofibrosarcoma	4+(100%)	1+(10.5%)	Neg	Neg	1+(5.3%)	-	-
2	Myxoid liposarcoma	4+(100%)	-	-	4+(100%)	-	-	-
3	MPNST with myxoid change	4+(100%)	Neg	Neg	4+(100%)	1+(12.5%)	Neg	-
4	Low grade fibro myxoid sarcoma	4+(100%)	Neg	Neg	Neg	Neg	Neg	Neg
5	DFSP with myxoid change	4+(100%)	-	-	Neg	4+(100%)	-	-
6	Extra-skeletal myxoid chondrosarcoma	4+(100%)	-	-	4+(100%)	-	-	-
7	Synovial sarcoma with myxoid change	4+(100%)	Neg	-	Neg	Neg	Neg	4+(100%)
8	Leiomyosarcoma with myxoid change	4+(100%)	4+(100%)	4+(100%)	Neg	-	-	4+(100%)

[Table/Fig-3]: Immunohistochemical results of Myxoid Sarcomas.



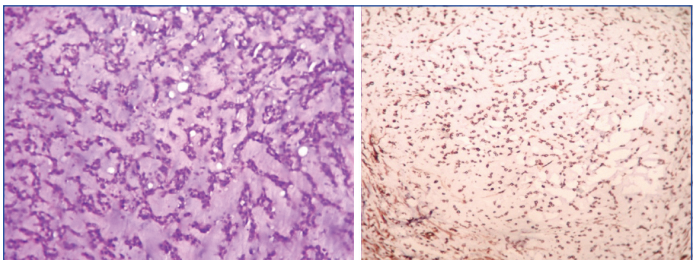
[Table/Fig-4,5]: Lipoblasts, spindle cells and branching capillaries in a myxoid matrix of myxoid liposarcoma 40X (H&E).
S100 positivity of lipoblasts in myxoid liposarcoma 40X (IHC). (Images from left to right)

Immunohistochemical evaluation of eight cases of MPNST with myxoid change was carried out using a panel of markers including vimentin, S100, CD34, SMA, desmin and CD99. All the eight cases showed (4+) positivity of tumour cells for vimentin and S100 while one case (12.5%) showed focal (1+) CD34 positivity. Desmin, SMA and CD99 were negative in all the cases [Table/Fig 6,7].



[Table/Fig-6,7]: Malignant spindle cells with wavy nuclei in a myxoid matrix of MPNST with myxoid change 40X (H&E). S100 positivity of tumour cells in MPNST with myxoid change 10X (IHC). (Images from left to right)

The IHC of the four cases of low grade fibromyxoid sarcomas in this study showed negativity for all markers except vimentin (4+). Immunohistochemical study of all the four cases of DFSP with myxoid change showed diffuse strong positivity (4+) of the tumour cells for vimentin and CD34 while they were negative for S100. All the three cases of synovial sarcoma with myxoid change showed (4+) positivity for vimentin and PanCK while the tumour cells were negative for SMA, S100, CD34 and CD99. IHC was done for all the three cases of extra-skeletal myxoid chondrosarcoma in this study and they showed (4+) positivity for vimentin and S100 [Table/Fig-8,9]. Immunohistochemical study of the one case of leiomyosarcoma with myxoid change in this study showed (4+) positivity of the tumour cells for vimentin, SMA, desmin and PanCK. S100 was negative.



[Table/Fig-8,9]: Cords of round to ovoid cells with dense eosinophilic cytoplasm in an abundant myxoid matrix of extra-skeletal myxoid chondrosarcoma 10X (H&E). S100 positivity of tumour cells in extra-skeletal myxoid chondrosarcoma 10X (IHC). (Images from left to right)

DISCUSSION

Myxoid change encountered in benign and malignant soft tissue tumours can pose serious diagnostic challenge owing to their overlapping histomorphological features. There is very limited comprehensive study and data in literature regarding the immunohistochemical characteristics of this group of myxoid sarcomas, though there have been isolated studies on few individual tumours in this group.

The median age for myxoid sarcomas was 65 years as per World Health Organisation (WHO) statistics [11] unlike the present study where majority of cases were encountered in the 41-60 years age group. Coindre JM et al., from France and Yücetürk G et al., from Turkey reported male predominance of myxoid sarcomas similar to the present study [12,13].

Out of the total 57 myxoid sarcomas, myxofibrosarcoma (33.33%) was the most common histological type followed by myxoid liposarcoma (26.32%) in this study which was in concordance with the studies of Coindre JM et al., from France and Yücetürk G et al., from Turkey [12,13].

All the 19 cases of myxofibrosarcomas in this study showed positivity for vimentin while 10.5% of cases showed patchy SMA positivity and 5.3% of them showed focal CD34 positivity. Tumour cells were negative for desmin and S100. These results were identical to that of Mentzel T et al., [14].

Immunohistochemical analysis of 15 cases of myxoid liposarcoma showed consistent positivity of vimentin and S100 in all the cases. Graadt van Roggen JF et al., reported vimentin positivity in all the cases while S100 was positive in 35-50% of cases [2].

All the eight cases of MPNST with myxoid change showed vimentin and S100 positivity. One case (12.5%) showed focal CD34 positivity. Other markers including SMA, desmin and CD99 were negative in all the cases. These findings were in concurrence with that of Yamaguchi U et al., [15].

Only vimentin was positive in all the four cases of low grade fibromyxoid sarcoma while they were negative for SMA, desmin, S100, CD34 and CD99. Graadt van Roggen JF et al., reported consistent vimentin positivity similar to the present study while occasional cells showed positivity for SMA, desminand CD34 unlike this study [2]. S100 was consistently negative in their study.

All the four cases of myxoid DFSP showed diffuse strong positivity for vimentinand CD34 while they were negative for S100. Reimann JDR and Fletcher CDM reported CD34 positivity in 95% of cases while all were S100 negative [16].

All the three cases of synovial sarcoma with myxoid change in this study were positive for vimentin and Pan CK while they were negative for SMA, S100, CD34 and CD99. These findings were similar to study done by Coli A et al., [17].

Three cases of extra-skeletal myxoid chondrosarcoma in this study showed positivity for vimentin and S100. Graadt van Roggen JF et al., claimed consistent positivity for vimentin while S100 was positive in 10-20% of cases [2].

The one case of leiomyosarcoma with myxoid change in this study showed vimentin, SMA, desmin and Pan CK positivity. S100 was negative in the tumour cells. Graadt van Roggen JF et al., reported consistent vimentin and SMA positivity, desmin positivity in 70% of cases while occasional Cytokeratin positivity. S100 was negative in all the cases [2].

Limitation(s)

Since, this is a rare group of malignancy, the sample size was less even though the study was carried out in a tertiary care referral hospital. Also, the prevalence of the individual histological subtypes of myxoid sarcomas was not uniform and was highly variable among population. Hence, future studies can be carried out by including more number of samples covering all the histological subtypes in adequate numbers by extending the study duration. This will provide a better picture of the pathological characteristics of all the individual histological subtypes of myxoid sarcomas.

CONCLUSION(S)

The IHC is a valuable adjunct but never a replacement for light microscopy, particularly in this challenging group of myxoid neoplasms with overlapping histomorphological features. Though there are certain specific markers in practice for some of these sarcomas like myxoid liposarcoma and synovial sarcoma, the feasibility of usage of such markers in all the institutions is limited due to cost-effectiveness. Hence, with careful light microscopic examination and judicious usage of the basic routine immunohistochemical markers, accurate diagnosis is always possible in this uncommon and challenging group of malignant soft tissue tumours.

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