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Original Research Article

Immunohistochemical Approach to Diagnosis of Soft Tissue Tumors of Lower Extremities with its Possibilities and Limitations

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ABSTRACT

Introduction: Soft tissue tumors have wide range of morphological differentiation. In many cases soft tissue tumors can be diagnosed by histopathological examination alone. In certain cases the confusing morphology, inspite of all efforts, leaves the pathologist uncertain about the exact nature of the neoplasm. For that reason we need the help of ancillary techniques like immunohistochemistry (IHC). Thus the purpose of our study was to evaluate usefulness of IHC in diagnosing soft tissue tumors with its limitations and we took only those cases which involved lower extremity as lower extremity is the commonest site of all soft tissue sarcomas of body.

Method: Specific morphological patterns of soft tissue tumors were identified by hematoxylin and eosin (H&E) staining. IHC was done by different panels of antibodies according to the morphological patterns. Then comparison was made between H&E diagnosis and immunohistochemistry aided diagnostic method.

Result: Out of 42 cases 28 cases were diagnosed by H&E and 14 cases were doubtful. IHC was helpful in diagnosing 38 cases with a z score 0f 2.69 and P value less than 0.01.

Conclusion: The accurate diagnosis by immunohistochemistry depends on thorough clinical evaluation, precise histomorphological analysis followed by selection of proper antibody panel. There are few limitations of this ancillary technique which should not be over looked. A regular audit of whole of the procedures of IHC may help to identify the problem areas.

Keywords: Soft Tissue Tumor, Lower Extremity, Immunohistochemistry.

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INTRODUCTION

Soft tissue tumors are mesenchymal proliferations that occur in extraskeletal, nonepithelial tissues of the body, exclusive of the viscera, coverings of the brain and lympho-reticular system.[1] Soft tissue tumors show wide range of morphological differentiation inspite of clinically being indistinctive.[2] In many cases soft tissue tumors can be diagnosed by histopathological examination alone. In certain cases the confusing morphology, inspite of all efforts, leaves the pathologist uncertain about the exact nature of the neoplasm. It causes the introduction of several ancillary techniques which generally play a supportive role and help to reach a particular diagnosis with confidence. One of such ancillary procedures, immunohistochemistry (IHC) has become a powerful tool to assist the pathologist in diagnosing soft tissue tumors. It has emerged as the most valuable adjunct to hematoxylin and eosin staining in diagnostic pathology.[3] IHC is a method for localizing specific antigens in tissues or cells based on antigen-antibody reaction.[4] To find out the possibilities and limitations of IHC in diagnosing soft tissue tumors we took only those cases which involved lower extremity as lower extremity is the commonest site of all soft tissue sarcomas of body. Approximately 45% of soft tissue sarcomas occur in the lower extremity.[5]

MATERIALS AND METHODS

The present cross-sectional and observational study was done in Burdwan Medical College and Hospital over a period of one year after clearance of ethical committee and included 42 cases of soft tissue tumors. At first different morphological patterns were identified in Hematoxylin and eosin stained sections and

differential diagnoses were made. The corresponding unstained sections were taken for IHC with different panel of antibodies according to their or morphological pattern observed in H&E stained sections.

Following panels of antibodies were used for each category:

Spindle Cell Tumors: CK, Desmin, SMA, S100, CD31, CD34.

Round cell Tumors: LCA, HMB45, Desmin, myogenin

Pleomorphic Soft Tissue Tumors: S100, HMB45, Desmin, Myogenin

Epithelioid Soft Tissue Tumors: CK, Vimentin, SMA, Desmin, CD31, CD68

RESULT & ANALYSIS

Out of 42 cases maximum cases were seen in the 4th decade (26.19%). The mean age for benign tumors was 27.95 ± 9.02 years, for intermediate tumors it was 45.75 ± 5.68 years, and for malignant tumors it was 49.59 ± 18.52 years.

The overall male to female ratio for total soft tissue tumors was 4:3. The ratio of male to female in benign tumor was 1.33:1, in intermediate soft tissue tumors there was equal sex ratio, and in malignant form the male: female ratio was 1.43:1.

The most cases of benign soft tissue tumors (42.86%) occurred in the leg and the most common site for malignant (76.47%) and intermediate (100%) soft tissue tumors of lower extremity was thigh. Among 42 cases 32 cases (76.19%) presented with only swelling and 10 cases (23.81%) presented with pain along with swelling.

Out of 42 soft tumor cases the tissue of origin of 28 cases could be diagnosed confidently by H&E examination and rest 14 cases were doubtful between two differential diagnoses or specific diagnosis could not be made from a broad category. Among the 28 cases maximum number of cases was tumors of fibrohistiocytic lineage, and tumors originated from neural tissue each comprising of 7 cases (16.67%), followed by adipose tissue comprising of 6 cases (14.29%), then 3 cases of fibrous origin (7.14%), 2 tumors (4.76%) originated from smooth muscle, followed by 1 case each (2.38%) from the category of skeletal muscle, vascular and melanocytic origin. (Table 1)

Immunohistochemical aid confirmed the diagnosis of 38 cases including following ten cases which were doubtful morphologically -leiomyosarcoma, MPNST, hibernoma, neurofibroma, glomus tumor, angiosarcoma, malignant melanoma of soft part, malignant fibrous histiocytoma, pleomorphic rhabdomyosarcoma and myxofibrosarcoma. Efficacy of IHC aided diagnosis was compared with diagnosis by histomorphology alone and Z score was found 2.659 and P-value was <0.01. (Table 2)

Immunohistochemistry did not aid in the diagnosis in 3 cases and misleading in 1 case indicating its limitations.

Table 1: Distribution of Cases According To the Tissue of Origin on H&E Examination

TISSUE OF ORIGIN	NO. OF CASES (%)
Definitive	**28 (66.67%)**
Adipocytic (lipoma-2; lipoblastoma-1; liposarcoma-2; myxoid liposarcoma-1)	6 (14.29%)
Fibrous (Nodular fascitis-1; Dermatofibrosarcoma protuberans-2)	3 (7.14%)
Fibrohistiocytic (benign fibrous histiocytoma-3; malignant fibrous histiocytoma-3; tendon sheath giant cell tumor-1)	7 (16.67%)
Smooth muscle (Angioleiomyoma-2)	2 (4.76%)
Skeletal muscle (Alveolar Rhabdomyo sarcoma-1)	1 (2.38%)
Vascular (Capillary Hemangioma-1)	1 (2.38%)
Neural sheath (Schwannoma-4; Neurofibroma-2; malignant peripheral nerve sheath tumor-1)	7 (16.67%)
Miscellaneous (Malignant melanoma of soft part-1)	1 (2.38%)
Doubtful	**14 (33.33%)**
Spindle cell tumor	1 (2.38%)
Melanocytic/Neural sheath	1 (2.38%)
Neural sheath/Fibrous	2 (4.76%)
Adipocytic/Sk. Muscle	1 (2.38%)
Round cell/Metastatic RCC	1 (2.38%)
Adipocytic/Fibrous	1 (2.38%)
Sm. Muscle/fibrous	1 (2.38%)
Myxoid Neoplasm	1 (2.38%)
Pericytic/small round cell	1 (2.38%)
Vascular/Melanocytic	1 (2.38%)
Pleomorphic	3 (7.14%)
Total	42 (100%)

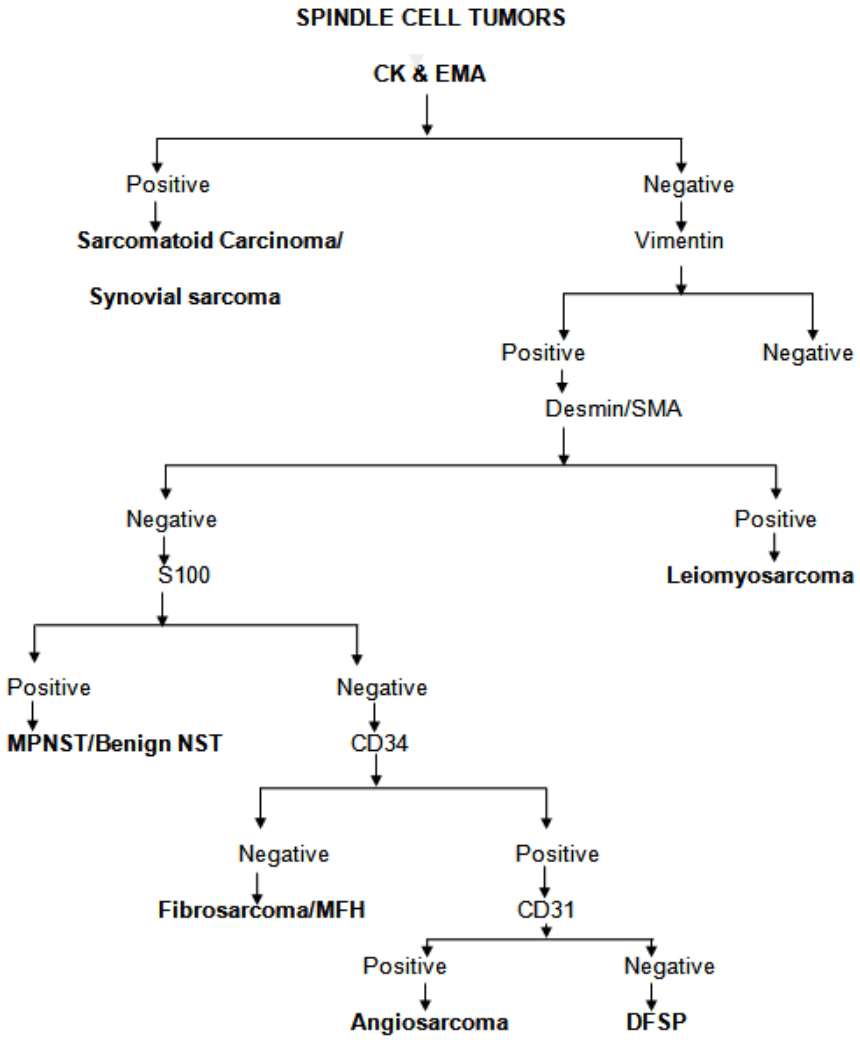
Table 2: Efficacy of Histo-Morphological and Immunohistochemical Aided Diagnosis in Soft Tissue Tumor Cases

Final Diagnosis	H&E Based	IHC Aided
Confirmatory	28 (66.67%)	38 (90.48%)
Doubtful	14 (33.33%)	4 (9.52%)
Total Cases	42 (100%)	42 (100%)

DISCUSSION

Soft tissue tumors are highly heterogeneous group of neoplasms and IHC has immense role in diagnosing those tumors. Before going to discuss about immunological reagents for specific tissue lineages we have to emphasize on the critical factors like the acquisition, handling, fixation, specimen delivery to the laboratory and antigen retrieval. The specimen fixed in formalin, followed by its paraffin inclusion are the internationally most used histological techniques. According to Webster JD, et al[6] (2009) some antibodies like, CK-7, high-molecular weight cytokeratin, and laminin is diminished by prolonged formalin fixation. Paraffin impregnation was done under adequate temperature control in our study, because paraffin inclusion in high temperature (>60^{0}C) may compromise the specimen antigenicity.[7] In the present study, all sections were of 4-5µm in thickness and poly-lysine coated slides were used for adhering the sections over slides. According to Yaziji H et al.,[7] (2006) the block sections must preferentially range between 3 to 7 µm and must be deposited on slides previously coated with an adhesive, most commonly poly-lysine. For antigen retrieval, microwave method was used in our study with strict temperature and time control.

We used monoclonal antibodies (except S-100) to maintain the maximum level of specificity. The selection of antibody panels were tried to be done judiciously and precisely for optimal applicability of immunohistochemistry and unnecessary cost crisis. Two studies from Jensen HE et al,[8] (1997) and Jensen ML et al,[9] (1997) established that in immunohistochemistry the selection of antibody panel and the interpretation of the reaction patterns of each case were the most important factors for the final diagnosis. In the present study negative and positive controls were included in each panel for proper validation of findings. Following scheme was followed to use minimum but appropriate number of antibodies



Tumors of Adipose Tissue

No specific markers are available for the diagnosis of adipocytic tumors. In most of the cases histomorphology is sufficient for diagnosis of fatty tumors. Normal fat cells and some neoplastic adipocytes show positive S100 immunostaining and according to Dei Tos AP et al,[10] (1998) S100 can be useful in the diagnosis of round cell liposarcoma from poorly differentiated tumors.

1 case of hibernoma was confused with rhabdomyoma on H&E tissue section; but it was confirmed by immunohistochemistry with S-100 staining.

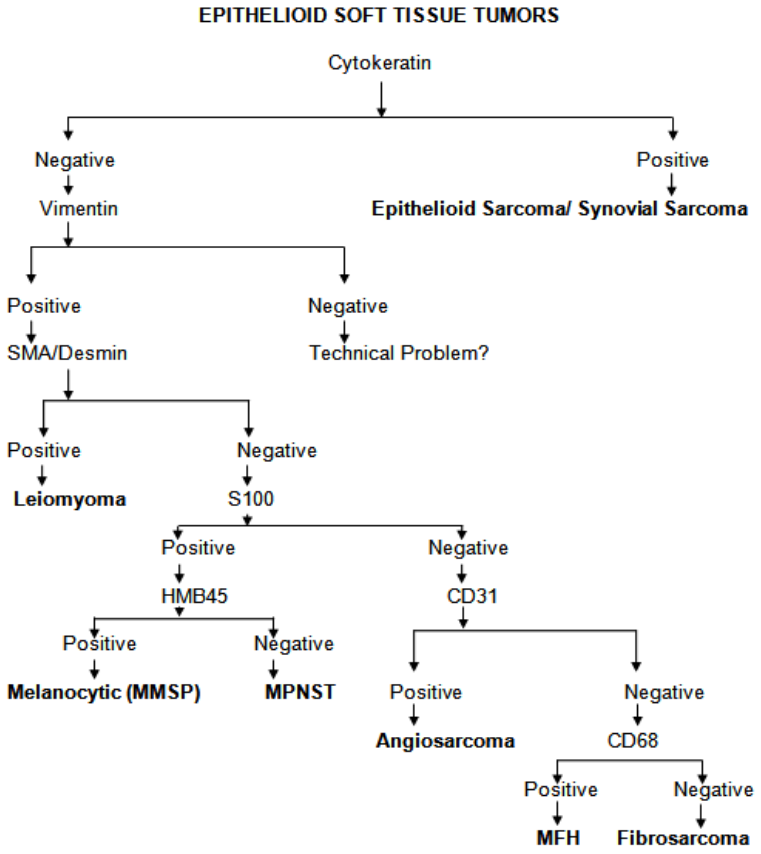
Fibroblastic/Myofibroblastic Tumors

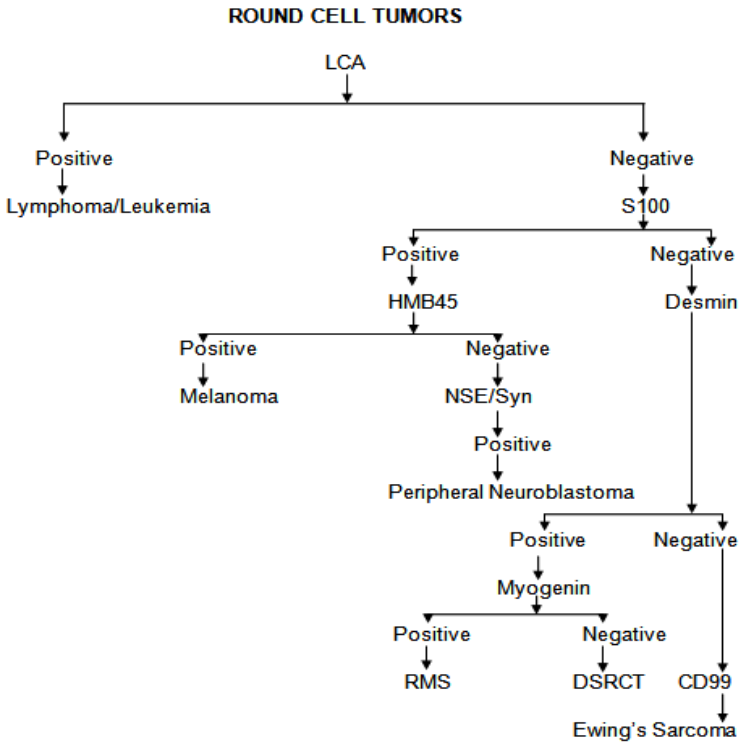
There are no universally applicable markers for fibroblastic lineage. Dermatofibrosarcoma protuberans (DFSP) is strongly positive for CD34 (Cohen PR et al, 1994).[11] In the present study 2

cases of DFSP were diagnosed by H&E tissue section and confirmed by immunohistochemistry with positive vimentin and CD 34 immunostaining.

Nodular fasciitis and some myofibroblastic lesions are characterised by expression of smooth muscle actin and muscle specific actin but lack expression of desmin (Franquemont DW, 1993).[12] In our study 1 case of nodular fasciitis was diagnosed by histomorphology and it was confirmed by immunostaining like the above study.

In the present study, 1 case of myxoid fibrosarcoma was confused with myxoid liposarcoma; but confirmed by IHC as myxoid fibrosarcoma due to S100 negativity.





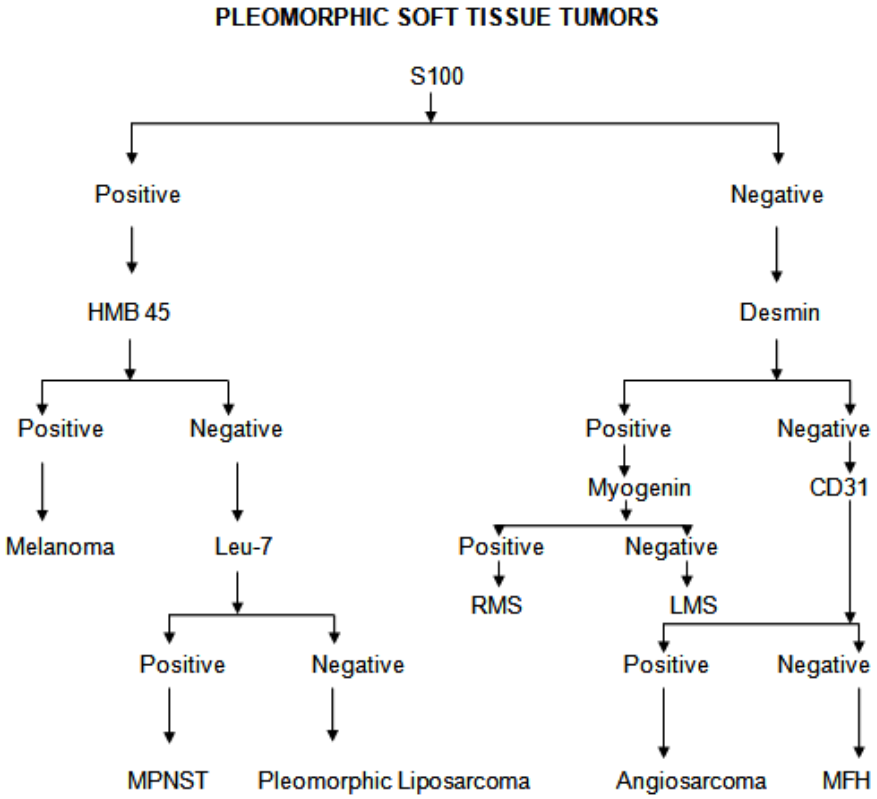
[RMS- Rhabdomyosarcoma; DSRCT- Desmoplastic small round cell tumor; NSE-Neuron Specific Enolase; Syn- Synaptophysin.]

So-Called Fibrohistiocytic Tumors

Malignant fibrous histiocytoma (MFH) is a designation used for poorly differentiated sarcomas that do not show any specific differentiation. This diagnosis is, therefore, made by exclusion of other specific diagnoses. However, MFH cells often express CD68; but it should not be used as evidence of histiocytic lineage inspite of being expressed in approximately 50% of MFH cases[13] (Weiss LM et al., 1994). In the present study, 3 cases of MFH and 1 pleomorphic sarcoma were positive for vimentin and CD68 (focal), and diagnosed as MFH. The result of our study partially support the above study, probably MFHs of lower extremities contain higher numbers of reactive histiocytes than other sarcomas, and thus in our study all cases of MFH were focally positive for CD68. Benign fibrous histiocytoma (BFH) is often confused with DFSP in H&E tissue section, and by immunohistochemistry it is differentiated by CD34 staining[14] (Kutzner H, 1993). BFH is CD34 negative and in our study 3 cases of BFH were diagnosed in H&E section and confirmed with immunostaining (vimentin+, CD34).

Smooth Muscle Tumors

In present study 2 cases of angioleiomyoma were diagnosed by histomorphology and they were positive for smooth muscle actin (SMA). One case of leiomyosarcoma was confused with fibrosarcoma by H&E section due to presence of herring-bone pattern. After immunohistochemistry, it was positive for vimentin, SMA and desmin. Smooth muscle actin is expressed only in smooth muscle neoplasms and in non-smooth muscle lesions with myoid differentiation such as nodular fasciitis and myofibroblastic lesions.[12] (Franquemont DW, 1993).



[RMS- Rhabdomyosarcoma; LMS- Leiomyosarcoma;
MPNST- Malignant Peripheral Nerve Sheath Tumor]

Tumors of Skeletal Muscles

Most rhabdomyosarcomas and rhabdomyoblastic components in other tumors are positive for desmin and muscle actins (HHF-35), but are typically negative for alpha-SMA (Azumi N et al,[15] 1988) and (Schmidt RA et al,[16] 1988). Myogenin is extremely specific for rhabdomyoblastic differentiation.[17] In the present study 1 case of alveolar rhabdomyosarcoma was positive for desmin and myogenin and negative for SMA. 1 case of pleomorphic sarcoma, diagnosed by histomorphology was found to be positive for desmin, focally positive for myogenin and negative for SMA and it was diagnosed as pleomorphic rhabdomyosarcoma finally.

Vascular Tumors

In the present study CD31 was taken as a marker to determine the vascular lineage. 1 case of epithelioid haemangioma was diagnosed by H&E tissue section and it was positive for CD31. 1 case of cutaneous angiosarcoma was confused with malignant melanoma. On immunostaining it was negative for S100and HMB45 and positive for CD31. Finally it was diagnosed as angiosarcoma. 100% of angiosarcomas are CD31 positive regardless of grade or subtype.[18] (De Young BR et al., 1993).

Schwann Cell Tumors (Nerve Sheath Tumors)

1 case of neurofibroma was confused with fibroma of tendon sheath and it was confirmed as neurofibroma by S100 positivity. 1 case of MPNST was confused with fibrosarcoma and it was also confirmed as MPNST by S100 immunostaining. Schwannoma took the most distinct and strong nuclear and cytoplasmic stain among the 3 different neural tumors. According to Kahn HJ et al.[19]

(1983), main use of S100 is in the evaluation of peripheral nerve sheath and melanocytic tumors. Schwannoma and low grade MPNSTs showed a more diffuse S100 staining compared to high grade MPNST, which had a patchy and weak staining pattern.[20]

Pericytic Tumors

In our study 1 case of glomus tumor initially confused with small round cell tumor like Ewing's sarcoma due to its histologic appearance as well as its location. After IHC it was positive for SMA and negative for CD99. Glomus tumors are considered to be arising from modified smooth muscle cells and therefore they show a smooth muscle-like phenotype by their consistent muscle actin (HHF-35) and alpha-SMA positivity; but they are consistently negative for CD31.[21]

Miscellaneous Tumors

1. **Juxta Articular Myxoma (JAM):** The tumor develops adjacent to large joints, especially the knee. This tumor is negative for S100 just like cellular myxoma and its histologic features overlap with low grade myxofibrosarcoma (LGMFS).[2] In our study immunohistochemistry could not help in distinguishing between JAM and LFMFS.
2. **Synovial Sarcoma:** On application of IHC synovial sarcoma generally expresses low and high molecular weight cytokeratins(CK) and EMA, even in monophasic fibrous form, where histological examination does not reveal any evident epithelial components[22] (Corson JM et al, 1984). In our study 1 case of monophasic synovial sarcoma was confused with fibrosarcoma and after immunostaining it was EMA positive but CK negative. Van De Rijn M et al,[23] (1999) stated that EMA may be a more sensitive marker than keratins for monophasic and poorly differentiated synovial sarcomas, and most cases show patchy or streaky reactivity. So, our study is partially supported by above studies.
3. **Malignant Melanoma of Soft Part/ Clear Cell Sarcoma (MMSP/CSS):** This tumor shows melanocytic differentiation. Clear cell sarcoma is typically and variably positive for S100 protein and usually strongly positive for HMB-45, while negative for keratin and muscle cell markers[24] (Kaufmann O et al, 1998). In the present study one case was diagnosed as MMSP/CSS and confirmed by IHC with both S100 and HMB45 positivity. 1 case was confused with epithelioid MPNST and after IHC it was strongly positive for both S100 and HMB45 and confirmed as MMSP.
4. **Alveolar Soft Part Sarcoma (ASPS):** Specific marker for ASPS is still under research. It sometimes shows positivity for desmin as observed in our case. In per iodic Schiff (PAS) staining presence of PAS+ diastase resistant intracytoplasmic granules and crystalline rods are diagnostic hallmark of this lesion; but no more than 50% shows this.[2]

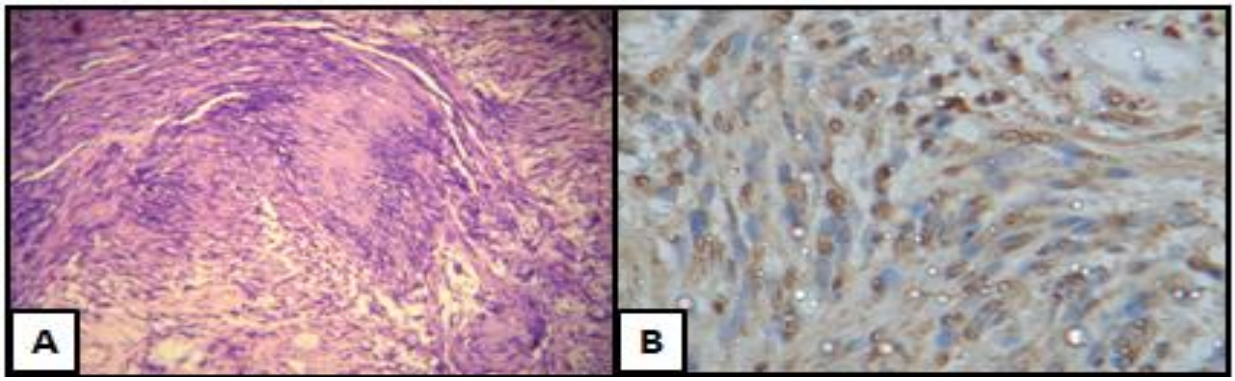


Figure 1: (A) Microphotograph showing Verocay body in schwannoma (H &E; 10X)
(B) nuclear and cytoplasmic S100 positivity (40X)

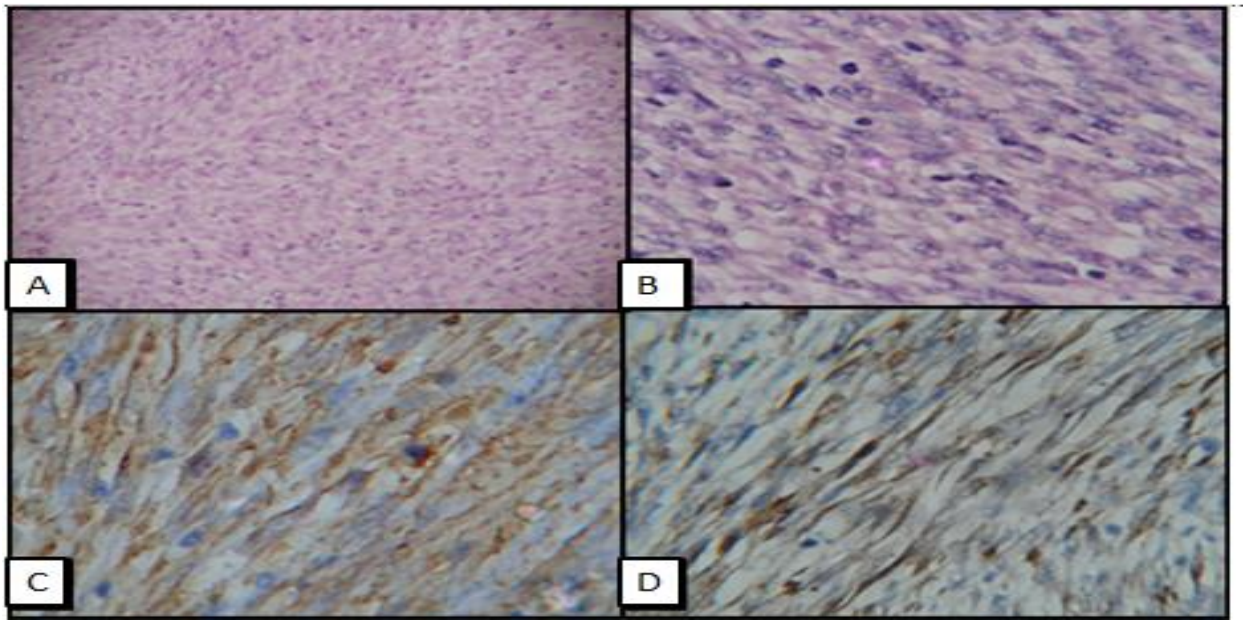


Figure 2: (A) Microphotograph showing Leiomyosarcoma (H &E 10X); (B) Microphotograph showing leiomyosarcoma with numerous mitotic figures (H &E 40X); (C) Cytoplasmic Smooth muscle actin positivity (40X); (D) Cytoplasmic Desmin positivity (40X)

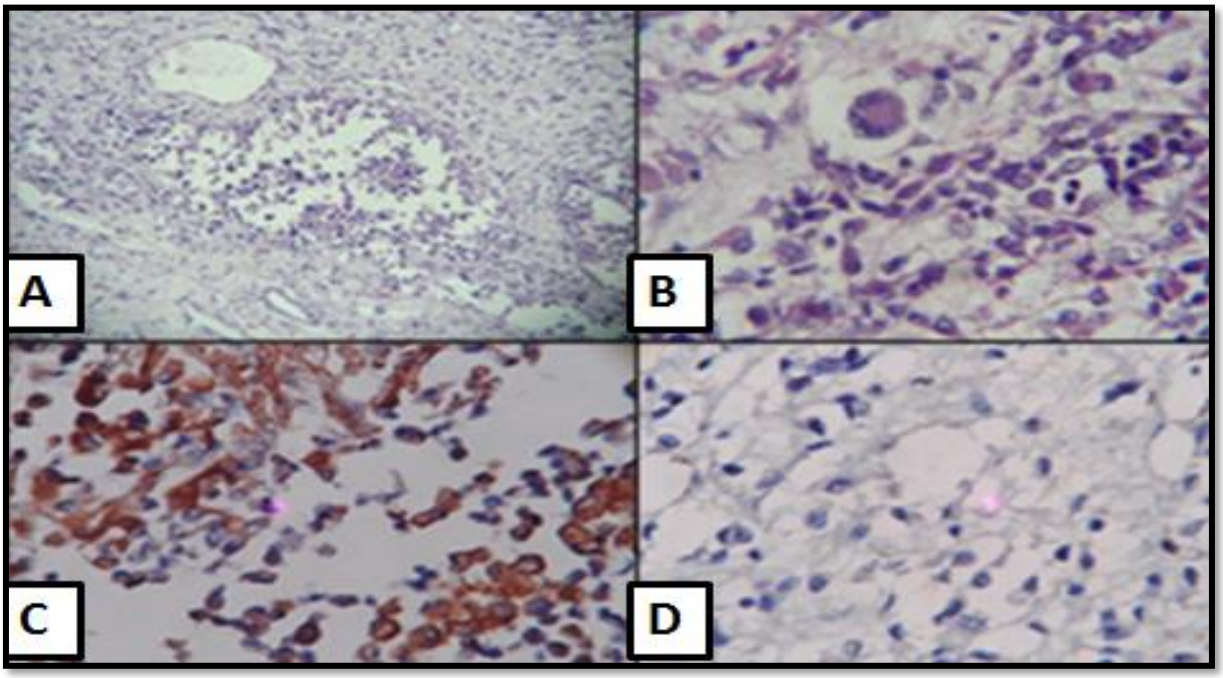


Figure 3: (A) Microphotograph showing Alveolar Rhabdomyosarcoma (H&E, 10X); (B) Giant cell and rhabdomyoblasts (40X); (C) Cytoplasmic Desmin positivity (40X); (D) Smooth muscle actin negativity (40X)

DIAGNOSTIC ACCURACY OF IMMUNOHISTOCHEMISTRY

In the present study immunohistochemistry confirmed the diagnosis of 38 cases among 42 cases (90.48%) in comparison to histomorphology which only confirmed 28 cases (66.67%) among 42 cases (Z score is 2.659; P-value <0.01). Werner and colleagues[25] (2005) also evaluated the role of immunohistochemistry in a study where IHC aided the diagnosis of neoplasms and pseudo-neoplastic lesions, and concluded that IHC is a helpful complementary diagnostic method in 95% of cases and with low cost and high benefit it contribute toward surgical and therapeutic conducts.

LIMITATONS OF IMMUNOHISTOCHEMISTRY

In the present study immunohistochemistry did not aid in the diagnosis in 4 cases.

- 1 case of benign tumor, juxta articular myxoma could not be differentiated from malignant tumor, low grade fibromyxoid tumor as both were S100 negative. Thus IHC in some tumors indicate the tissue lineage rather than indicating its neoplastic nature.
- 1 case of alveolar soft part sarcoma could not be diagnosed confirmly by IHC because there is no such specific IHC marker for this tumor. However CK negativity and positive vimentin and desmin helped to differentiate from Renal cell carcinoma metastasis.
- 1 case of pleomorphic sarcoma could not be categorised as all the useful markers SMA, desmin, S100 and CD68 were negative.
- 1 case of synovial sarcoma was negative for cytokeratin, thus misleading the diagnosis.

Thus, few limitations of immunohistochemistry should not be overlooked. According to Swanson,[26] in 1997 no method should be universally acceptable; rather the choice should be based on the technique that, in the experience of the laboratory or of the school followed by researchers, best solves the diagnostic question.

CONCLUSION

This is to be concluded from our study that soft tissue tumors can present with a wide range of morphological variations, frequently confusing and overlapping and an accurate diagnosis can be made in majority of the cases by using immunohistochemistry as an adjunct to histomorphological evaluation. The accurate diagnosis by immunohistochemistry depends on thorough clinical evaluation, precise histomorphological analysis followed by selection of proper antibody panel. Immunohistochemical application in the present study justifies the cost and benefit of the patients; and the scientific, diagnostic and prognostic applications of this methodology must be explored.

There are few limitations of this ancillary technique which should not be over looked. A regular audit of whole of the procedures of IHC may help to identify the problem areas. Newer discoveries and inventions should be added to IHC so that it can tackle the limitations and drawbacks and emerges as a miraculous investigative tool.

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