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Original Research Article.

Cytohistopathological and Immunohistochemical Correlation of Soft Tissue Lesions

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ABSTRACT

Introduction: In the present era, FNAC has proved to be an essential primary diagnostic procedure for soft tissue lesions. To correlate its efficacy and to further subtype, histopathological examination is done aided by IHC if required. The present study aimed to evaluate the epidemiological distribution of soft tissue lesions with reference to age, sex and site and to assess the utility of FNAC in terms of sensitivity, specificity, positive and negative predictive values, and overall histological correlation percentage of cytology in diagnosing various types of soft tissue lesions.

Materials and Methods: Prospective study was carried out during the period of Sept 2017 to May 2019 for FNA examination of soft tissue lesions. Cytopathological and histopathological examination was carried out in all cases with immunostaining done in few cases.

Results: Of 463 soft tissue lesions that could be successfully followed up, 347 were benign lesions and rest were malignant. Most common age group affected were 31-40 years with slight male preponderance (M:F=1.37:1). Most common site being Lower extremities. Lipomas were the most common soft tissue lesions (169 cases) and spindle cell sarcomas were the most common malignant lesions. The cytological and histopathological diagnosis correlated well in almost all cases except discordance was seen in 5 cases. The sensitivity and specificity of the procedure were 97.4% and 99.4% respectively.

Conclusion: FNAC was found to be a highly specific and sensitive tool in diagnosing soft tissue lesions and can be fairly implemented as it is well tolerated and cost effective for patients.

Keywords: FNAC, Soft Tissue Lesions, Histopathology, Immunostaining.

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INTRODUCTION

Soft tissue is defined as the non-skeletal connective tissue of the body, excluding supporting tissue of the internal organs,glia and hematopoietic tissue. Soft tissue lesions arise nearly everywhere in the body, the most important locations being the extremities, trunk and abdominal cavity; rarely may arise in internal organs.[1]

In the present era, Fine Needle Aspiration Cytology (FNAC) has proved to be an important diagnostic tool for several pathologic lesions. It is useful for screening both superficial and deep-seated masses. It played a very important role for initial diagnosis of various soft tissue tumours.[2]

In the age of growing popularity of Fine Needle Aspirations (FNAs), Core Needle Biopsy(CNB) and Immunohistochemistry (IHC) soft tissue lesions can be diagnosed more successfully.

Most of the soft tissue lesions (Neoplastic and Non-neoplastic) present in an accessible anatomical site of the body as cutaneous or subcutaneous masses except few malignant lesions.[3]

Centers/Laboratories where the triad of facilities (Cytopathology, Histopathology and Immunohistochemistry) are available categorizing soft tissue lesions with more sensitivity and specificity is ever rewarding.[4]

Since Cytohistological correlation of soft tissue lesions with minimal immunohistochemical markers is growing area of research, the present study has both academic and clinical importance. It needs a combined effort and collaboration of Pathologists, Radiologists and surgeons for proper evaluation to reach at a definite diagnosis.[5]

AIMS AND OBJECTIVES

- To study the epidemiological distribution of soft tissue lesions with reference to age, sex and site.
- To assess the utility of fine needle aspiration cytology in terms of sensitivity, specificity, positive and negative predictive values, and overall histological correlation percentage of fine needle aspiration cytology in diagnosing various types of soft tissue lesions.

MATERIALS AND METHODS

This study was carried out in the Department of pathology of MKCG Medical College & Hospital during the period of Sept 2017 to May 2019. It was a Prospective study. The subjects of study were the cases of soft tissue lesions sent from the surgical departments for FNA examination. All the palpable lesions suspected of soft tissue lesions sent for FNA to the department of pathology were included in the study. The lesions which were non-palpable, deep seated lesions requiring USG/CT guided aspiration were excluded from the study. Similarly, swellings presented as inflammatory lesions, with scanty or no cells on aspiration, patients who failed to follow-up and who denied for a valid consent were excluded from the study. Out of total recruited 497 patients, 463 patients were selected according to strict inclusion and exclusion criteria. 22-24 gauge disposable needles and 10 and 5 cc disposable syringes depending upon the size of the lesions were used for aspiration. Franzen pistol handle was used in 27 cases to aid in aspirating thick and scanty materials. The smears drawn were air dried and wet fixed in 95% alcohol. The air dried smears were stained with Diff-Quik (DQ) and May-Grunwald-Giemsa (MGG) stains. Whereas, the wet fixed smears were stained with Haematoxylin- Eosin (H&E) and Pap stain. The slides were then subjected to detailed microscopical study under low and high magnification. The patients were followed up with their histopathological reports. Immuno-hitstochemical (IHC) study was performed in 97 cases to increase the diagnostic accuracy using vimentin, Desmin, Smooth Muscle Actin (SMA), S-100, Cytokeratin (CK). Histopathological and IHC reports were correlated to FNAC reports to assess the diagnostic accuracy, expressed as percentage.

All the data were collected and analysis was carried out using SPSS (version-19) software. Frequency and Percentage was used for descriptive analysis. Sensitivity, Specificity, Positive and Negative predictive values were calculated by respective formulae. Various cytological categories were compared by chi-square test. P value < 0.05 was considered to be significant.

Table 1: Age Wise Distribution

Age (Yrs)	Benign Lesions	Malignant lesions	Total
0-10	8	0	8
11-20	38	4	42
21-30	83	8	91
31-40	113	14	127
41-50	51	34	85
51-60	32	24	56
61-70	12	21	33
71-80	10	11	21
Total	347	116	463

Soft tissue lesions are more common in 30-40 years age group, median age-34.93 years

Table 2: Site Wise Distribution

Site	Benign Lesions	Malignant lesions	Total
Lower Extremities	164	33	187
Upper Extremities	105	18	123
Trunk	36	53	89
Head & Neck	42	22	64
Total	347	116	463

Most of the Soft tissue lesions occurred in lower extremities (40.38%).

Table 3: Sex Wise Distribution

Sex	Benign Lesions	Malignant lesions	Total
Male	191	77	268
Female	156	39	195
Total	347	116	463

Male : Female ratio of Soft tissue lesion = 1.37 : 1

RESULTS

3590 patients attended the cytology department for FNAC of which 497 cases were of soft tissue lesions (13.8%). Only 463 cases were recorded with complete follow-up, following strict exclusion and inclusion criteria of the study. Most cases were referred for primary diagnosis (92%), few presented with recurrent lesions. The 463 soft tissue lesions were broadly categorised into 347(74.9%) benign and 116(25.1%) malignant lesions on cytology. Soft tissue lesions were more prevalent in the age group 31-40 yrs (27.43%)(Table-1), the median age being 34.93 years, with a slight male predominance of (M:F=1.37:1)(Table-2). Most lesions occurred in lower extremities (40.38%) with malignant lesions mostly occurring in trunk (Table-3).

Of the 347 cases labeled as benign soft tissue lesions included most common entity were lipomas 169 cases(48.7%), Benign nerve sheath tumours (neurofibromas, neurilemmomas) 90 cases(25.9%),Giant Cell Tumour of Tendon Sheath 15 cases(4.3%),Ganglion cyst 20 cases(5.7%),nodular fasciitis 8 cases, benign fibrous histiocytoma 12 cases, hemangiomas 6 cases, tuberous xanthoma 3 cases, tumoral calcinosis 2 cases and benign mesenchymal tumours, in which subtyping was not possible ,7 cases. (Table-4) 116 cases were labeled as soft tissue sarcomas in FNAC into categories based on cytomorphological appearance- Myxoid sarcomas-44 cases (37.9%), spindle cell sarcomas-55cases (47.4%), pleomorphic sarcomas-10 cases, round cell sarcomas-6cases, epitheliod /polygonal cell sarcoma 1 case which were further subtyped and confirmed by biopsy and IHC in few cases.(Table-5)

Cases where there were mixed components, subtyping was done based on the predominant morphological pattern.

The commonest occuring soft tissue lesions were Lipomas 169 of 463 cases(36.5%),most of which were conventional lipomas showing strands of mature adipocytes and scattered cells with peripherally situated nucleus on a fatty background.1 case was of Giant lipoma,a 45year old male presented with a huge pedunculated swelling on the anterior abdominal wall .2 cases were of pleomorphic lipoma with characteristic multinucleated floret like giant cells with hyperchromatic nuclei and spindle cells ,2 cases of Angiolipoma that on biopsy showed blood vessel proliferations with fibrin microthrombi. Another case of 8 month male child presented with swelling on thigh, which on biopsy was found to be intramuscular lipoma.3 case were of fibrolipoma.

The benign nerve sheath tumours included most cases of neurilemmomas (67cases), 20 cases of neurofibromas, and 3 cases of neuromas-Traumatic (2) and Morton (1) confirmed and subtyped using biopsy study. Neurilemmomas were diagnosed by their high cellularity of spindled cells, fish hook appearance of cells with myxoid stroma at places and characteristic Antoni A, Antoni B areas and classic Verocay bodies on biopsy. Neurofibromas are grossly without capsule and relatively less cellular than neurilemmomas with some showing mucoid material on cytosmear, confirmed on histopathology by characteristic shredded carrot type collagen, mast cells, axons.

Giant cell tumour of tendon sheath was diagnosed in 15 cases due to the presence of spindled mononuclear cells in loose clusters and dispersedly with scattered osteoclast like multinucleated giant cells. Hemangiomas on FNAC yielded bloody aspirates mostly and their diagnosis was confirmed on biopsy. Ganglion cysts yielded jelly like material with few mononuclear cells on proteinaceous background.

Table 4: Benign Soft Tissue Lesions

Serial No.	Diagnosis	No of cases
1	**Lipoma**	169
	a. Conventional lipoma	163
	b. Spindle cell/ pleomorphic lipoma	2
	c. Angiolipoma	2
	d. Intramuscular lipoma	1
	e. Giant lipoma	1
2	**Benign Nerve seath tumour**	90
	a. Neurofibromas	20
	b. Neurilemmomas	67
	c. Neuromas (Morton,Traumatic)	3
3	**GCT of Tendon seaths**	15
4	**Ganglion cysts**	20
5	**Nodular fascitis**	8
6	**Haemangiomas**	6
7	**Benign Fibrohistiocytic lesions**	12
	a. Benign Fibrous Histiocytoma	11
	b. Lipidized BFH	1
8	**Tuberous Xanthoma**	3
9	**Tumoral Calcinosis**	2
10	**Solitary Fibrous tumour**	3
11	**Keloid**	4

347 cases of benign soft tissue lesion out of 463 (74.9%) , Most common lesion is lipoma (48.7%).

Table 5: Malignant Soft Tissue Lesions

Serial No.	Diagnosis	No of cases
1	Spindle cell Sarcoma	55
	a. Dermatofibrosarcoma protruberans	26
	b. Fibrosarcoma	19
	c. Synovial sarcoma	6
	d. Cutaneous leiomyosarcoma	2
	e. Malignant peripheral nerve seath tumour	2
2	Myxoid sarcoma	44
	a. Myxoid Liposarcoma	33
	b. Extraskelital myxoid chondrosarcoma	2
	c. Myxoid malignant fibrous histiocytoma	9
3	Round cell sarcoma	10
	a. Extraskeletal Ewing's sarcoma	4
	b. Round cell liposarcoma	5
	c. Alveolar Rhabdomyosarcoma	1
4	Pleomorphic sarcoma	10
	a. Pleomorphic liposrcoma	8
	b. Soft tissue melanoma	1
	c. Pleomorphic rhabdomyosarcoma	1
5	Polygonal/ Epitheliod sarcoma	1

116 cases of malignant soft tissue lesions out of 463 (25.1%) , Most common lesion is spindle cell sarcoma (47.4%).

Table 6: Cytodiagnosis & Histological Diagnosis Correlation

Types of soft tissue lesions	Cytodiagnosis	Histological diagnosis	
		Concordant	Discordant
Lipomatous	169	167	2
Spindle cell	148	145	3
Pleomorphic	12	12	0
Polygonal	1	1	0
Round	10	10	0
Other Benign lesion	123	123	0
Total	463	458	5

Table 7: Final Result

FNAC	BIOPSY		Total
	Benign	Malignant	
Benign	344 (True Negative)	3 (False Negative)	347
Malignant	2 (False Positive)	114 (True Positive)	116
Total	346	117	463

A 37 year old female presented with multiple nodular swellings on both upper and lower extremities, yielded plenty of histiocytes along with spindled fibroblast cells, was diagnosed to be Tuberous xanthoma, and 2 cases of Tumoral calcinosis yielded chalky white material on aspiration and confirmed on biopsy.

Most common sarcoma encountered was spindle cell sarcomas of which commonest was Dermatofibrosarcoma protruberans followed by Fibrosarcoma, synovial sarcoma, Malignant peripheral nerve sheath tumour diagnosed on biopsy and IHC correlation. A case of cutaneous leiomyosarcoma was diagnosed in a 42 year old lady with swelling on right arm, the lesion yielded fragments of spindled cells ,cigar shaped nuclei, cellular atypia, mitotic figures, which was confirmed on biopsy and IHC using Smooth Muscle Actin (SMA) and Desmin indicating smooth muscle origin of the tumour.

Under the myxoid sarcoma category myxoid liposarcoma was commonly found with 3 cases of myxoid malignant fibrous histiocytoma and 1 case of Extraskeletal Myxoid Chondrosarcoma. The aspirates were myxoid in nature, a clue to the diagnosis. Lipoblasts in cytosmears and characteristic

arborizing capillaries suggested diagnosis of myxoid liposarcoma which was confirmed on biopsy.

Pleomorphic sarcomas (10 cases) included 8 cases of pleomorphic liposarcoma along with 2 cases each of pleomorphic rhabdomyosarcoma. Smears from pleomorphic liposarcoma showed lipoblasts, tumor giant cells on a fatty background.

Round cell sarcomas (10 cases) included Extraskeletal Ewing's sarcoma 4 cases and round cell liposarcoma 5 cases along with one case of alveolar rhabdomyosarcoma of vulval swelling of 42 year lady. The smears showed typical round cell morphology with hyperchromatic nucleus so was advised biopsy. IHC was done in few case for vimentin and Desmin for confirmation.

One case of Epitheloid sarcoma was diagnosed due to presence of polygonal cells and pleomorphic cells which was proved on biopsy and IHC for CK and Desmin.

Of all the cases the cytologic and histologic diagnoses were in concordance except for 5 cases. In terms of malignancy there were 3 false negatives and 2 false positives. 2 cases were diagnosed as lipoma which came out to be low grade liposarcoma on histopathology, 2 cases were labeled as malignant spindle cell sarcoma which came out to be ancient neurilemmoma, further confirmed by IHC for S-100 and one case of benign spindle cell lesion on FNAC came out to be Fibrosarcoma. (Table-6)

The diagnostic accuracy overall is therefore 98.9% and for benign soft tissue lesions is 99.4% and 97.4% for malignant soft tissue tumours. In terms of malignancy the sensitivity of the procedure was found to be 97.4%, specificity 99.4%, Positive Predictive Value 98.2% and Negative Predictive Value 99.1%, indicating FNAC to be a very effective diagnostic tool for soft tissue lesions.(Table-7)

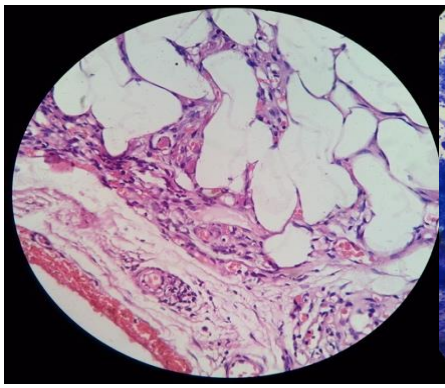

Fig 1: Histosection of Angiolipoma (H&Ex400)

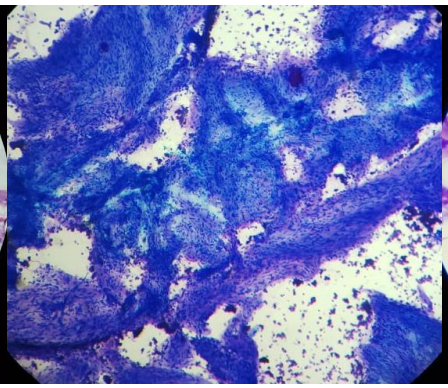

Fig 2a: Cytosmear of neurilemmoma from Antoni A area (DQx100)

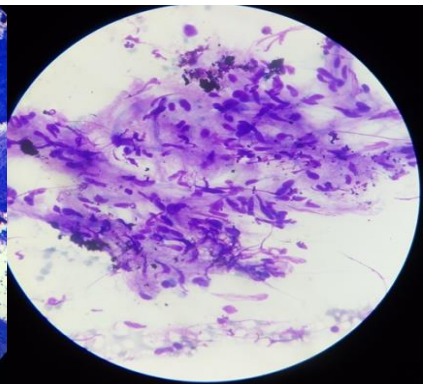

Fig 2b: Cytosmear of neurilemmoma from antoni B area (DQ x100)

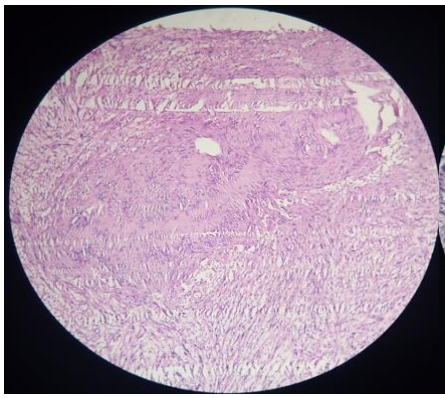

Fig 2c: Histosection of neurilemmoma (H&Ex100)

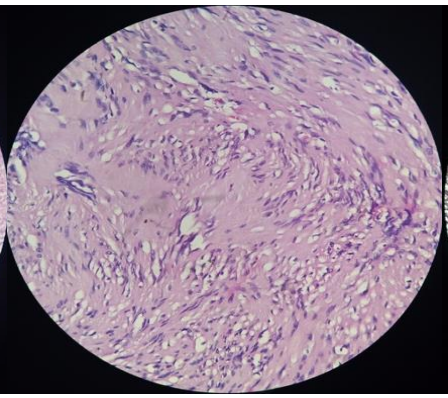

Fig 2d: Histosection of neurilemmoma showing Verocay bodies (H&Ex400)

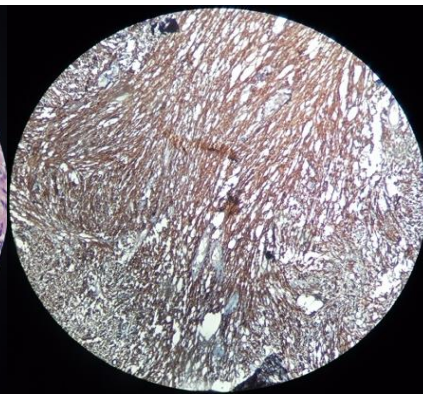

Fig 2e: S-100 intense positivity in neurilemmoma (x100)

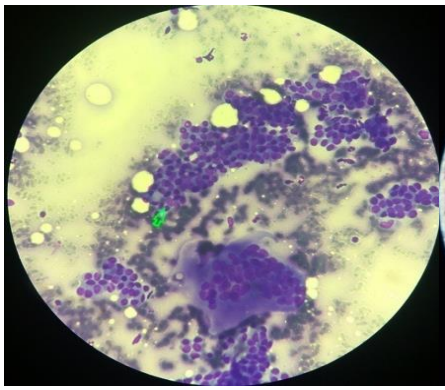

Fig 3a: Cytosmear of GCT of Tendon sheath (DQx400)

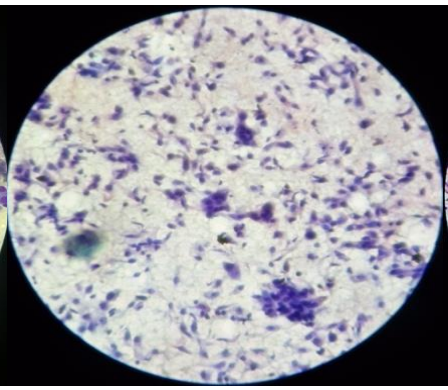

Fig 3b: Cytosmear of GCT tendon sheath (PAPx100)

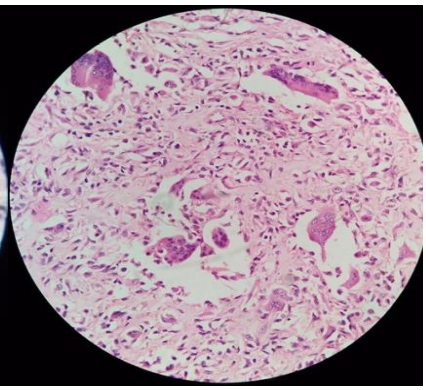

Fig 3c: Histosection of GCT tendon sheath (H&E x400)

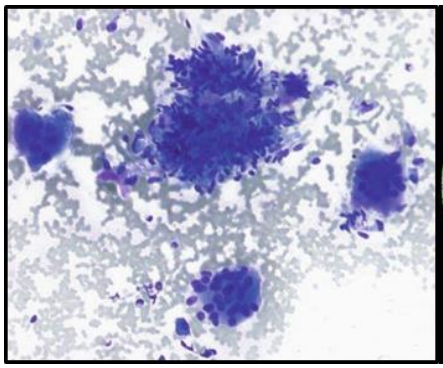

Fig 4a: Cytosmear of cutaneous leiomyosarcoma (MGGx400)

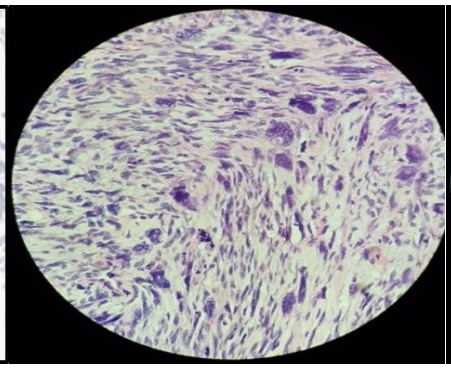

Fig 4b: Histosection of cutaneous leiomyosarcoma (H&Ex400)

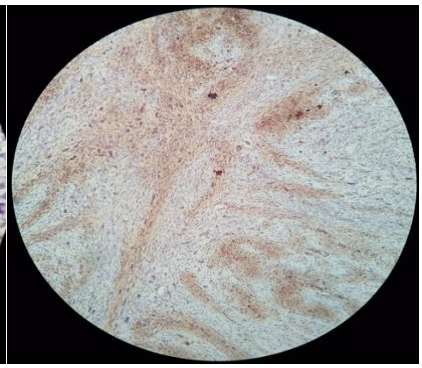

Fig 4c: Desmin positivity positivity in cutaneous leiomyosarcoma (x50)

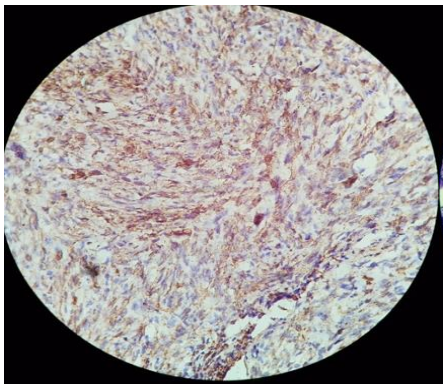

Fig 4d: SMA positivity in cutaneous leiomyosarcoma (x100)

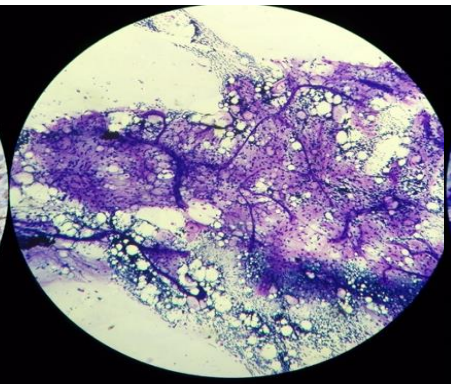

Fig 5a: Cytosmear of myxoid liposarcoma showing branching capillaries (MGGx100)

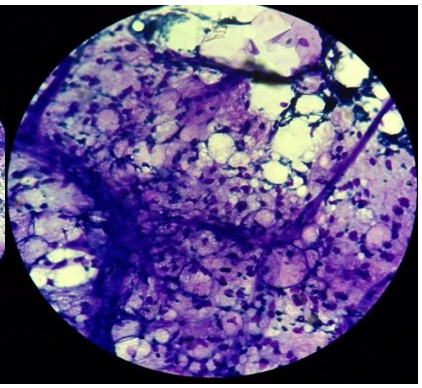

Fig 5b: Cytosmear of myxoid liposarcoma showing lipoblasts (MGGx400)

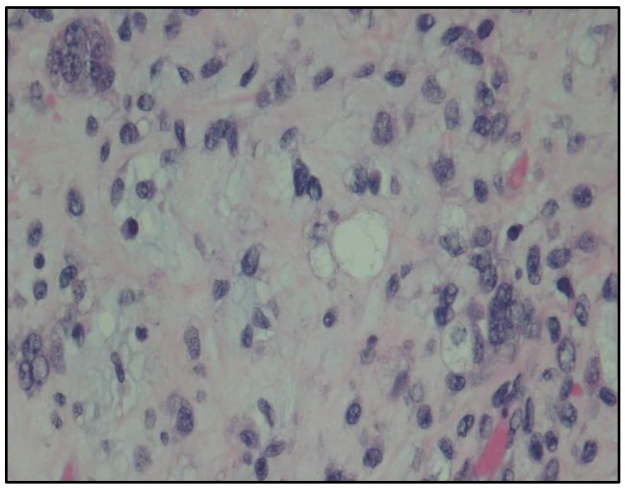

Fig 5c: Histosection of myxoid liposarcoma (H&Ex400)

DISCUSSION

In our study most of the soft tissue lesions were found to occur in the age group 31-40 years, with median age being 34.93years, which was in consistent to Talati et al.[6], Hajdu et al.[7] as well as Ezinger[2] who stated soft tissue lesions are more common in middle aged to elderly patients. The sex wise distribution in our study revealed relatively high incidence in males, the M:F ratio being 1.37:1 which correlated well with study done by Chandralekha J et al.[8] according to them it was 1.76:1. Most of the soft tissue lesions occurred in lower extremities (40.38%) with trunk being the most common site for malignant soft tissue lesions (11.44%) which was in concordance to studies by Ezinger[2], Campora et al.[9], Chandralekha J et al.[8] who showed 45%, 40%, 46.25% respectively.

The occurrence of benign and malignant soft tissue lesions were 347 cases (74.9%) and 116 cases (25.1%) of total 463 soft tissue lesions presented to our department. According to Nagiraet al.[10] it were 61.43% and 38.57% respectively but our's was in contrasting to Rekhiet al.,[11] where malignant lesions formed a greater proportion because of high number of referral cases at their centre. The most common soft tissue entity was lipoma in our study (169 out of 463 cases) followed by nerve sheath tumours (90 cases), both being benign lesions. In malignant lesions spindle cell sarcomas predominated (55 cases) which correlated well with studies of Akerman et al.[12], Campora et al.[9], Ezinger et al.[2] and Layfield et al.[13] who found 78% lipomas in their study.

We had 6 cases of Hemangiomas (1.7% of benign soft tissue lesions) while Nagiraet al.[10] cited 20% Hemangiomas of soft tissue tumours in his study. As aspirates were mostly bloody with few spindled cells he stated biopsy to be confirmatory in those cases. 15 cases of Giant Cell Tumour of Tendon sheath were observed with 100% diagnostic accuracy in concordance with Keiko Nagiraet al.[10] and Adil et al.[14] Tumours with spindle cell morphology were difficult to diagnose with 100% accuracy based on cellularity, stromal fragments, nuclear characteristics. Mostly smears with high cellularity, hyperchromatic nuclei, irregular stromal fragments, mitotic figures were sarcomas in consistent to studies done by Kilpatrik SE et al.[15] and Costa MJ et al.[16] who also included necrosis to be considered. In our study 3 spindle cell lesions were diagnosed incorrectly based on the biopsy reports that followed; 2 cases were labeled as malignant spindle cell sarcoma which came out to be ancient neurilemmoma, further confirmed by IHC for S-100, which was diffuse and intense; and one case of benign spindle cell lesion on FNAC came out to be Fibrosarcoma. 2 cases diagnosed as Lipoma on FNAC came out

to be low grade Liposarcomas. It is easy to miss the diagnosis of low grade liposarcomas on cytology as a keen search of lipoblasts on appropriate background is required. Enzinger[2] too mentioned it and Kilpatrik SE et al.[15] too faced the similar problem in his study.

There were 20 cases of Ganglion cysts, which yielded characteristic jelly like aspirate and can be easily distinguished from other site specific soft tissue lesions like intramuscular myxoma and juxtaarticular myxoid lesions which correlated well with study done by Wakely JR PE et al.[17]

Our study showed a diagnostic accuracy of 98.9% and for benign soft tissue lesions is 99.4% and 97.4% for malignant soft tissue tumours. The diagnostic accuracy were compared to studies done by Akerman et al. (85%)[12], Nagira K et al.(88%)[10], Palmer et al. (90%)[18], Kulkarni et al. (93.33%)[19], Sapi et al. (95%)[20], Wakely PE (98.3%)[17], Chandralekha J et al.(96.88%)[8] In terms of malignancy the sensitivity of the procedure was found to be 97.4%, specificity 99.4%, Positive Predictive Value 98.2% and Negative Predictive Value 99.1%, indicating FNAC to be a very effective diagnostic tool for soft tissue lesions. The values were compared to studies of Layfieldet al.[21] who had sensitivity and specificity of 95% respectively for soft tissue lesions diagnosis. The correlation with other studies is well known (Table-8).

Table 8: Sensitivity and Specificity Correlation with Existing Studies

Author	Sensitivity	Specificity
Rekhi et al[11]	100%	87%
Akerman et al[12]	86.66%	93.49%
Benzabih[23]	88.5%	81.5%
Nagira et al[10]	92%	97%
DeMay et al[22]	95%	95%
Layfield et al[13]	95%	95%
Kilpatrick et al[15]	95%	95%
Wakely PE[17]	100%	88%
Sapi et al[20]	100%	94.4%
Palmer et al[18]	100%	95.12%
Chandralekha J et al[8]	95.08%	97.98%
Our study	97.4%	99.4%

CONCLUSION

Our study was carried out to highlight importance of Fine Needle Aspiration Cytology in diagnosing Soft tissue lesions and can be fairly implemented as the procedure is well-tolerated by patients and risk of complications is minimal, multiple site sampling can be done as opposed to a single small core biopsy or open biopsy. The cytologic categorisation of soft tissue tumours was highly sensitive, specific and accurate though subtyping and confirmation of some cases mostly sarcomas require biopsy interpretation and help of Immunohistochemical staining.

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