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Original Article

Pathology Section

Histocytopathological Evaluation of Soft Tissue Tumours: A Retrospective Study

ALAKA SAHU[1], CHITTA RANJAN PRASAD[2], ANUPA TOPPO[3]

ABSTRACT

Introduction: Soft Tissue Tumours (STTs) are a heterogeneous group of lesions arising from non-epithelial extraskeletal tissue of the body. The use of Fine Needle Aspiration Cytology (FNAC) in the evaluation of STTs is debatable because of their extremely varied morphology.

Aim: To assess the efficacy of FNAC in the diagnosis of STTs as a routine procedure.

Materials and Methods: The present study was a retrospective study conducted at VSS Institute of Medical Science and Research, Burla, Sambalpur, Odisha, India, from December 2016 to November 2018. A total of 186 cases of STT were correlated with histopathological studies. Fine needle aspirate air-dried smears were stained with Diff-Quik and 95% alcohol fixed smears were stained with Papanicolaou (PAP) stain. Corresponding histopathological sections were stained with Haematoxylin and Eosin (H&E) stain. Immunostaining was performed as and when required to confirm histological diagnosis. Results were analysed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics 22.0.

Results: Out of 186 cases, 162 (87.09%) were diagnosed as benign and 24 (12.91%) as malignant. Three false negative and two false positive cases were reported. The sensitivity, specificity, Positive Predictive Values (PPV), Negative Predictive Values (NPV) and overall diagnostic accuracy were 87.5%, 98.7%, 91.3%, 98.1% and 97.3%, respectively. There was statistically significant difference between the cytological diagnosis and the final histological diagnosis (χ^2=35.5, p<0.05).

Conclusion: Fine Needle Aspiration (FNA) is very important for initial diagnostic work-up, while histopathology with the help of immunostain provides a final diagnosis.

Keywords: Benign, Immunostain, Malignant

INTRODUCTION

Soft Tissue Sarcomas (STSs) account for <1% of all adult cancers and nearly 15% of all paediatric malignancies [1]. Soft tissue is loosely defined as the complex of non-epithelial extraskeletal structures of the body such as tendon, muscle, skin, fascia exclusive of the supportive tissue of the various organs and the haematopoietic/lymphoid tissue. It is composed of fibrous (connective) tissue, adipose tissue, skeletal muscle, blood and lymph vessels, and peripheral nervous system [2]. STTs have been diagnosed with 'Time-honoured histopathology' which is recognised as the gold standard for their assessment [3]. However, as a preliminary procedure, FNA is relatively a non-traumatic and outpatient sampling procedure for both superficial and deep seated masses [4]. Their role in recurrent and malignant tumours has mostly been noted [5-7]. In the present study, there was an attempt to assess the efficacy of FNA in the diagnosis and histological correlation of STTs. Authors have highlighted the need of immunostain in rare difficult cases. Routine light microscopic analysis is often not enough to diagnose these tumours because of its varied morphology and lack of proper tissue architecture and lack of familiarity since they are very rare.

As the facility of immunohistochemistry and immunocytochemistry is not available in all the centres, so diagnosis of STT from H&E staining has always become a challenge and this study has been undertaken to find out the efficacy of diagnosing these group of tumours using H&E stained slides.

MATERIALS AND METHODS

The present study was a retrospective study conducted in the pathology department at Veer Surendra Sai Institute of Medical Science and Research, Burla, Sambalpur, Odisha, India between December 2016 and November 2018 and the findings were analysed in 2019.

Inclusion and Exclusion criteria: Patients with STTs attending Surgery outpatient department and cytology/histopathology sections of Department of Pathology were included in this study. Non-palpable, deep seated lesions which required Ultrasound/ Computed Tomography (USG/CT) guided aspiration; FNA findings with inflammatory lesions, and patients who failed to follow-up without biopsy were excluded from the study.

FNA was done using a 22-24 gauge needle with a 10 mL syringe. Standard PAP stain was used for wet fixed smears and Diff-Quik stain was used for air dried smear [8]. A detailed cytomorphological study was done by pathologists. The cases were kept in track for histopathological examination. The tissues were grossed and processed in an automatic tissue processor in the laboratory. Routine H&E staining was performed. The histological findings of the corresponding biopsies performed were analysed by a different group of pathologists. Immunohistochemical stains like Human Melanin Black (HMB) (antibodies manufactured by Biogenex mouse monoclonal antibody) and S-100 (Cell Marque mouse monoclonal antihuman antibody; manufactured by Dako Rabbit polyclonal antihuman antibody) antibodies were done to confirm the diagnosis in selected cases by immunoperoxidase procedure.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics 22.0 software. Cytological findings were compared with histological findings by calculating sensitivity, specificity, PPV, NPV. The significance of cytological categories like benign and malignant was analysed using Chi-square test. The p-value <0.05 was considered as significant.

RESULTS

Out of total 210 cases aspirated, only 186 cases were obtained for histological examination as the remaining 24 cases were untraceable after FNAC examination. Out of 186 cases, 162 (87.09%) were diagnosed as benign STTs, while 24 (12.91%) were diagnosed as malignant STTs on FNAC examination [Table/Fig-1].

Cytodiagnosis	No.	Histopathology	No.
Benign			
Lipoma	128	Lipoma	114
		Fibrolipoma	11
		Lipomatosis	2
		Angiolipoma	1
Spindle cell tumour	12	Fibroma	5
		Schwannoma	2
		Neurofibroma	2
		Desmoid	1
		DFSP	1
		Leiomyoma	1
Low-grade fibrosarcoma	1	Fibromatosis	1
MPNST	1	Ancient schwannoma	1
Inconclusive	1	DFSP	1
Fibrohistiocytic tumour	8	Benign fibrous histiocytoma	8
Vascular tumour	6	Haemangioma	5
		Lymphangioma	1
Inflammatory pseudotumour	2	Inflammatory pseudotumour	2
Round cell tumour	1	Neuroblastoma	1
Giant cell tumour of the tendon sheath	1	Giant cell tumour of the tendon sheath	1
Calcinosis cutis	1	Calcinosis cutis	1
Total cases	162	Total cases	162
Malignant			
Fibrohistiocytic tumour	8	Malignant fibrous histiocytoma	8
Spindle cell tumour	5	Leiomyosarcoma	2
		Myxofibrosarcoma	2
		Synovial sarcoma	1
Spindle cell tumour (fibroma)	1	Fibrosarcoma	1
Pleomorphic sarcoma	6	Undifferentiated Sarcoma	2
		Leiomyosarcoma	2
		Pleomorphic liposarcoma	1
		MPNST	1
Round cell tumour	4	Extraskeletal Ewing sarcoma	1
		Rhabdomyosarcoma	1
		Liposarcoma	1
		Malignant melanoma of soft part	1
Total cases	24		24

[Table/Fig-1]: Lesion wise cytohistopathological diagnosis of 186 Soft Tissue Tumours (STT). (Benign=162, malignant=24).
DFSP: Dermatofibrosarcoma protuberance; MPNST: Malignant peripheral nerve sheath tumour

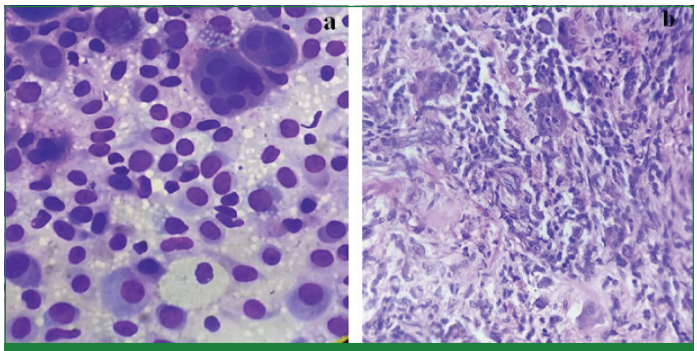
Most benign tumours were found in the age group of 21-40 years. The age range of patients with benign tumour varied from 2 years to 65 years, with a mean age of 33.8 years. On the other hand, the maximum number of cases of malignant STTs was found in the age group 61-80 years. The age ranged from 10 to 78 years, with an average age of 40.3 years [Table/Fig-2]. Male patients outnumbered female patients in both benign and malignant tumours with a male to female ratio of 1.32:1 and 1.15:1, respectively.

Age (years)	Benign	Malignant	Total
0-20	35	2	37
21-40	74	3	77
41-60	42	7	49
61-80	11	12	23
Total	162	24	186

[Table/Fig-2]: Age wise distribution of Soft Tissue Tumours (STT).

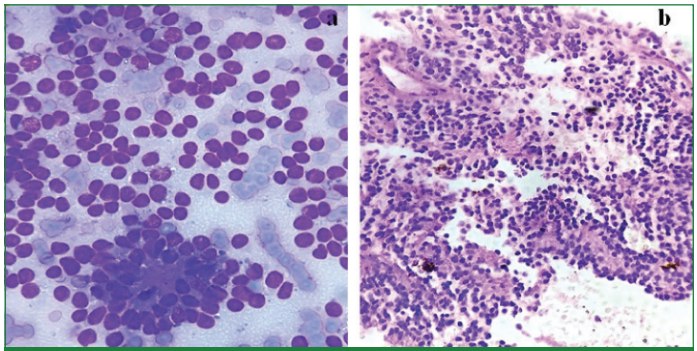
Benign tumours were most frequently seen in the head and neck regions 60 cases (32.25%) followed by trunk 48 cases (25.80%), lower extremity 35 cases (18.81%) and upper extremity 26 cases (13.97%). Lower extremity was the most common site of malignancies in 12 cases (6.45%) and head and neck were the least common site 5 cases (2.68%).

The FNAC smears were categorised as benign and malignant tumours with subtyping whenever possible. Lipoma was the most common benign tumour with 128 cases (68.81%) followed by benign spindle cell tumour 12 cases (6.45%), benign fibrohistiocytic tumour 8 cases (4.30%), vascular tumour 6 cases (3.22%), inflammatory pseudotumour 2 cases (1.07%), 1 case (0.53%) each of neuroblastoma, giant cell tumour of the tendon sheath [Table/Fig-3], schwannoma, fibromatosis and calcinosis cutis. One case that was inconclusive in cytology was later diagnosed as dermatofibrosarcoma protuberance in histopathology [Table/Fig-1].

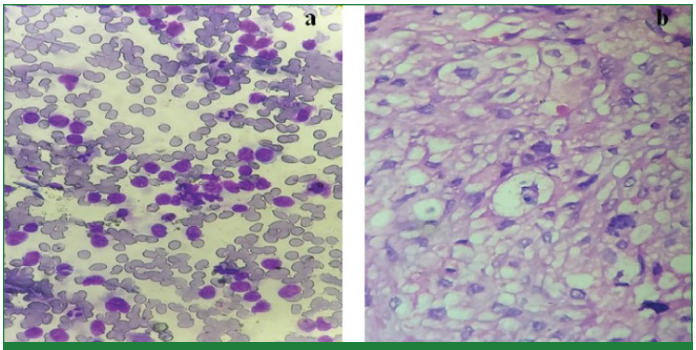


[Table/Fig-3]: Giant cell tumour of tendon sheath: a) Cytosmear shows plump oval cells with bland nuclei and multinucleated giant cells (Diff Quick, 400X); b) Microphotograph shows histiocyte like cells, multinucleated giant cells traversed by fibrous bands (H&E, 400X).

The most common malignant STT in this study was malignant fibrous histiocytoma which constituted 8 cases (4.30%). Out of 6 cases (3.22%) of malignant spindle cell tumour, two cases each of leiomyosarcoma, myxofibrosarcoma, and one case each of synovial sarcoma and fibrosarcoma was diagnosed histologically. Cytologically diagnosed 6 cases (3.22%) of pleomorphic sarcoma, subsequently on biopsy were proved to be two cases of leiomyosarcoma, two cases of undifferentiated sarcoma, one case of liposarcoma, and Malignant Peripheral Nerve Sheath Tumour (MPNST). Out of 4 cases (2.15%), diagnosed as round cell tumour in cytology came out as ewing sarcoma, rhabdomyosarcoma, liposarcoma and malignant melanoma of soft part on histopathology. Aspirate of ewing sarcoma yielded high cellularity with numerous solitary mononucleated cells with a high nuclear/cytoplasmic ratio and pseudorossette formation [Table/Fig-4]. The nuclei appeared round with evenly dispersed fine chromatin and inconspicuous nucleoli. Absence of lymphoglandular bodies helped in the differentiation from lymphoma. One case of round cell liposarcoma showed cellular smear with round nuclei. Atypical lipoblast could not be identified and a diagnosis of round cell tumour was rendered. A subsequent biopsy revealed numerous atypical lipoblasts [Table/Fig-5].

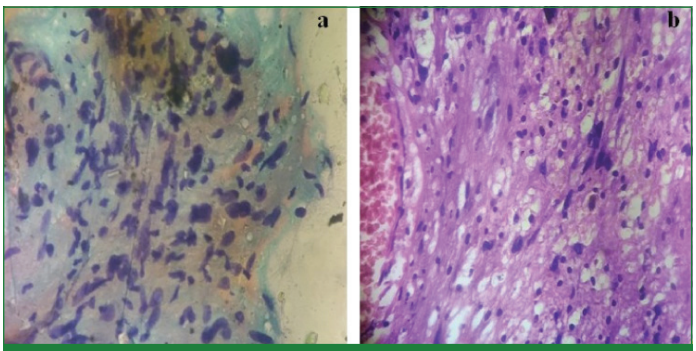


[Table/Fig-4]: Extraskeletal ewing sarcoma: a) Cytological smear shows uniform round cells forming rosette (Diff Quik, 400X); b) Microsection shows round cells surrounding a cental fibrillary core (H&E, 400X).



[Table/Fig-5]: Round cell liposarcoma: a) Cytological smear shows numerous round cells dispersed on a haemorrhagic background (Diff Quik, 400X); b) Microsection shows diagnostic multivacuolated lipoblasts (H&E, 400X).

It was found that there were two false positive and three false negative cases. One case of low-grade fibrosarcoma was later diagnosed as fibromatosis in histopathology. One case of malignant peripheral nerve sheath tumour in cytology was diagnosed as ancient schwannoma in histology [Table/Fig-6]. There was significant nuclear atypia, nuclear hyperchromasia, and coarse chromatin pattern leading to misdiagnosis. One case of fibroma in FNA diagnosis was later proved to be well-differentiated fibrosarcoma in the biopsy. Purely based on cytomorphologic features, it was difficult to distinguish between the aspirations of well-differentiated fibrosarcoma and fibromatosis. Aspirates of synovial sarcoma were dominated by spindle cells, which on histology showed a biphasic pattern of both spindle cell and epithelial cell differentiation.



[Table/Fig-6]: Ancient schwannoma- a) Cytological smear shows spindle cells with significant nuclear atypia (Papanicolaou stain, 400X); b) Microsection shows degenerative atypia, cyst formation and no mitotic figures (H&E, 400X).

Poorly preserved or degenerated clear cell sarcomas having shrivelled cells that cling to the fibrous bands were easily mistaken for round cell tumour in cytosmears. Subsequently, microsections of the biopsy revealed nests of round cells with prominent nucleoli with clear cytoplasm. Immunohistochemistry in this case, showed S-100 protein and HMB-45 positivity, thus expressing an antigen associated with melanin synthesis, and established a diagnosis of malignant melanoma of soft parts (clear cell sarcoma) [Table/Fig-7].

The results were evaluated as follows [Table/Fig-8]:

Sensitivity=(True positive/True positive+False negative)×100=(21/21+3)×100=87.5%.

Specificity=(True negative/True negative+False positive)×100=(160/160+2)×100=98.7%.

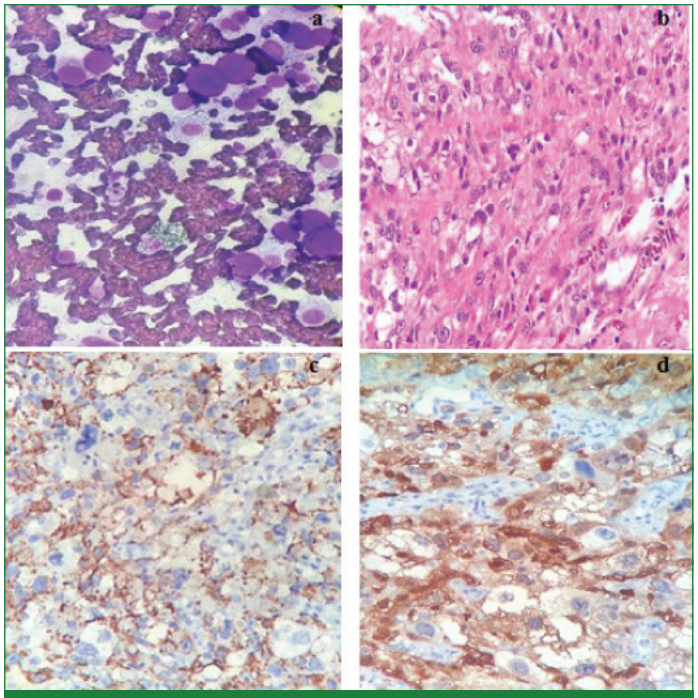
Positive predictive value=True positive/True positive+False positive)×100=(21/21+2)×100=91.3%.

Negative predictive value=(True negative/True negative+False negative)×100=(160/160+3)×100=98.1%.

False positivity=(False positive/Total no.)×100=2/186×100=1.07%.

False negative=(False negative/Total no.)×100=3/186×100=1.6%.

Diagnostic accuracy=(True positive+True negative/Total no.)×100=(21+160/186)×100=181/186×100=97.3%. In this study a sensitivity of 87.5%, specificity of 98.7% and diagnostic accuracy of 97.3% could be achieved.



[Table/Fig-7]: Malignant melanoma of soft part: a) Cytological smear showing large round cells (Diff Quik, 400X); b) Microsection showing nests of clear cells separated by dense fibrous septae (H&E, 400X); c) HMB45 positivity (IHC, 400X); d) Diffusely expressed S100 (IHC, 400X).

Cytological (FNAC) diagnosis	Final histological diagnosis		Total
	Benign	Malignant	
Benign	160 (True negative)	3 (False negative)	163
Malignant	2 (False positive)	21 (True positive)	23
Total	162	24	186

[Table/Fig-8]: Evaluation of results of Soft Tissue Tumours (STT).

DISCUSSION

The application of FNAC has contributed to a reasonably accurate diagnosis of various types of STTs in different parts of the body. Complex heterogeneity is a challenging factor in the diagnosis of STTs. However, Maitra A et al., have shown that FNAC leads to an accurate diagnosis of many types of tumours in various parts of the body [8]. FNAC has a low risk of complications such as bleeding, infection and the risk of tumour spread is negligible [9].

In this study, there was an attempt to assess the accuracy of FNA diagnosis in comparison to an open biopsy. In this analysis, 186 cases of cytohistological evaluations were available. Benign cases outnumbered malignant cases, which can be compared to studies conducted by Vijayabharathi I et al., Soni PB et al., and Beg S et al., Tailor HJ et al., in which benign: malignant tumour ratio was 83.3%: 16.6%, 95.3%: 3.34%, and 83.3%: 16.7%, 93.58:6.42, respectively [9-12]. In present study benign: malignant tumour ratio was 87.09%: 12.91% [Table/Fig-9].

Author name, Year of publication	Benign %	Malignant %
Beg S et al., [11], 2012	83.3	16.7
Tailor HJ et al., [12], 2013	93.58	6.42
Soni PB et al., [10], 2014	95.3	3.34
Vijayabharathi I et al., [9], 2015	83.3	16.6
Present study, 2021	87.09	12.91

[Table/Fig-9]: Benign: malignant tumour ratio in several studies [9-12].

Tumours with spindle cell pattern posed diagnostic difficulties. It was difficult to differentiate between benign tumours with high cellularity, such as fibrosarcoma, fibromatosis, DFSP, ancient schwannoma and synovial sarcoma. As described by Kilpatrick SE et al., the criteria for malignancy was that FNA smear depicted as a sarcoma when it showed moderate to highly cellular areas, nuclear hyperchromasia in almost all the sampled cells and ill-defined edges of the neoplastic fragments [4].

The very concept of fibrohistiocytic differentiation in most cases exhibits the histiocytic component is non-neoplastic. In giant cell tumour, the histiocytic component contributes remarkably to the development of the lesion. Malignant Fibro Histiocytic tumor (MFH), a group of lesions that until the early 2000 accounted for approximately 50% of sarcoma diagnosis [13], round cell sarcoma of soft tissue and bone also represents a major step forward of the 2020 World Health Organisation (WHO) classification [Table/Fig-10]. This new section the prototypical round cell sarcoma named ewing sarcoma as well as three distinct subsets that differs from ewing sarcoma clinically, pathologically and molecularly [14,15].

Ewing sarcoma
Round cell sarcoma with EWSR1-non-ETS fusions
CIC-rearranged sarcomas
Sarcoma with BCOR genetic alterations

[Table/Fig-10]: 2020 WHO classification [13].
EWSR1 (EWS RNA binding protein1) a protein coding gene; CIC (Capicua transcriptional repressor); BCOR (BCL6 Corepressor)

Described by Enzinger in 1965, the clear cell sarcoma is rare melanin producing STT. While, it is commonly often referred to as malignant melanoma of soft parts. Despite certain similar histologic findings it is clinically, biologically, and genetically distinct from cutaneous melanoma. Clear cell sarcoma invariably arises in the deep soft tissue of the distal extremities, and balanced translocation t(12;22) (q13;q12) is consistently seen in 70% of cases that is not found in cutaneous melanoma and is believed to be an early, if not primary, event in tumourigenesis [16].

The sensitivity, specificity, PPV, and NPV in this study were 87.5%, 98.7%, 91.3%, and 98.1%, respectively. The overall diagnostic accuracy of FNA in STT was 97.3%. There was statistically significant difference between the cytological diagnosis and the final histological diagnosis (χ^2=35.5, p<0.05). Rekhi B et al., reported the diagnostic accuracy of FNA in STT as 98%, with a PPV of 98% in malignant cases and a NPV of 100% in benign cases [3]. Roy S et al., observed the diagnostic accuracy of FNA of STT for benign and malignant tumours as 90.6% and 91.3%, respectively, with an overall accuracy rate of 90.8% [17]. Vijayabharathi I et al., found diagnostic accuracy of FNA for STTs 95.37%, sensitivity 84.2% and specificity 97.75% [9]. Dey P et al., observed sensitivity, specificity, and positive predictive value of FNAC for STT as 91.5%, 92.5%, and 95.5%, respectively [18]. Parajuli S and Lakhey M noted that the overall diagnostic accuracy of FNA in STT was 80% and the sensitivity and specificity of benign STTs were 97.36% and 66.67%, respectively and of malignant STTs 66.67% and 97.36%, respectively [19]. Arul P and Masilamani S, could achieve a sensitivity of 91.7% and specificity of 97.7% [20]. The results were well correlated with the present study.

Limitation(s)

FNAC of STT has important limitations. In densely collagenised or sclerotic or vascular lesions, FNAC may yield sparce cellularity making diagnosis difficult. But, still it can be concluded that FNA in STT has several advantages that outweigh disadvantages.

CONCLUSION(S)

Diagnosis of STT is difficult because of the heterogeneity of the tumours and also due to rarity of occurrence. Incidence of STT is difficult to assess because not all benign STTs get biopsied. FNAC is a simple, useful, safe, rapid and cost-effective tool for the preliminary diagnosis of both superficial and deep STTs. Specific diagnosis is almost always made on histomorphology with the help of immunomarkers that lead to proper management.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India.
2. Associate Professor, Department of Transfusion Medicine, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India.
3. Assistant Professor, Department of Pathology, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Chitta Ranjan Prasad,
Qr. No-3r/44, Doctors Colony, Burla, Sambalpur-768017, Odisha, India.
E-mail: drchitta.in@gmail.com

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