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Research Article

# Usefulness of fine needle aspiration cytology in diagnosis of soft tissue tumors and its correlation with histopathological features

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## ABSTRACT

**Objective:** Fine needle aspiration cytology (FNAC) is a standard diagnostic tool and has several advantages which are specific to soft tissue tumors (STT). STT arises from non-epithelial and extra-skeletal tissue of the body, excluding the reticuloendothelial system, glia, and supporting tissues of organs. Primary STTs are very rare and encompass a wide range of different tumor types; therefore, the classification and diagnosis remain complex. If the tumor is diagnosed as benign, surgery may not be necessary. However, in the case of malignancy, a cytological diagnosis allows for the initiation of palliative treatment. This study was conducted to evaluate the role of FNAC in the diagnosis of STT by sub classifying them based on their cytology features and finally correlating their FNAC findings with histopathological results with special reference to immunohistochemistry (IHC) in diagnostic challenging cases.

**Material and Methods:** It was a hospital-based retrospective study conducted in a tertiary care center, over a period of 2 years, i.e., from July 2016 to May 2018. After obtaining approval from the ethics committee of the institution, the study was carried out. All STT cases subjected to FNAC followed by incisional, excisional, or core needle biopsy or complete resection for histopathological examination were studied. The slides of these patients were retrieved and reviewed to establish a proper diagnosis. IHC was carried out in most cases whenever there was a diagnostic dilemma.

**Results:** In our study, among 50 patients who presented with STT, 40 were benign, and 10 were malignant. The median age of incidence was 41, and male-to-female ratio was 0.85:1. The most common site was the trunk. There were four discordant cases, and the final evaluation of all results showed sensitivity, specificity, positive predictive value of tests, and negative predictive value of tests of FNAC as 100%, 76.92%, 92.5%, and 100%, respectively.

**Conclusion:** In STT, most were benign tumors, with lipomas being the most common among them. While benign and malignant were easy to diagnose, intermediate spindle cell tumors were often missed on cytology. These intermediate tumors represent the diagnostic "grey zone." With increasing experience, STT can be diagnosed more accurately. FNAC is therefore a standard pre-operative diagnostic tool and a reliable alternative to open biopsy for diagnosing STT.

**Keywords:** Fine needle aspiration cytology, Immunohistochemistry, Negative predictive value of tests, Positive predictive value of tests, Soft tissue tumors.

## INTRODUCTION

Fine needle aspiration cytology (FNAC) is a standard first-line investigative tool and has a number of advantages in soft tissue tumors (STT), such as specimen adequacy, tissue sampling, and availability of material for various auxiliary techniques. All of which can be assessed in a single setting evaluation. Since it is an outpatient procedure, no patient preparation or general anesthesia is required. The procedure is safe, painless, and economical.<sup>[1]</sup>

However, FNAC does possess several disadvantages, some of which are specific to soft tissue tumors (STTs), like the absence of recognizable diagnostic tissue patterns and characterize soft tissue lesions into the different histological entities. The most important role of FNAC in STT is in distinguishing between malignant and benign.<sup>[2]</sup> Although FNAC cannot characterize soft tissue lesions into different histological entities, it will be able to classify malignant tumors into six categories on the basis of predominant cytological appearance, namely myxoid, spindle cell, pleomorphic cell, polygonal cell, round cell, and miscellaneous sarcomas.<sup>[3]</sup>

In this study, the utility of FNAC in diagnosing STT was evaluated by sub-classifying them according to their cytological features in these general categories, then correlating FNAC and histopathologic results. Immunohistochemistry (IHC) was carried out in most cases whenever there was a diagnostic dilemma.

## MATERIAL AND METHODS

This is a hospital-based retrospective study conducted over a period of 2 years, among 50 patients who presented to a tertiary care center with STT, i.e., from July 2016 to May 2018. This study was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki.<sup>[4]</sup> After obtaining approval from the Institutional Ethics Committee (Approval Number: SDMIEC:0869:2016 dated October 28, 2016), this study was conducted. Written informed consent was taken from the patients as a routine protocol in the institution for doing the procedure and publishing the pictures with the information obtained. If the study participant was below 18 years, consent was taken from their guardian or parent. The study posed no additional risks and did not adversely affect the welfare of the subjects involved. The patient's confidentiality was safeguarded at all stages of the study. Patients of all age groups who presented with STT and who underwent FNAC followed by excision biopsy were included in the study. Already diagnosed STT cases and those who were undergoing treatment were excluded from the study. Inadequate smears on FNAC were also excluded from the study. First and foremost, detailed patient details and clinical history were retrieved from the register and patient

file. Then, slides were retrieved and studied for cytological and histopathological features.

### Requirement of fine needle aspiration procedure

- Cameco FNAC gun
- Disposable needles size 22-24 G and length 1-1.5 inches (Precision Glide™ Needle- Medaxo, India - REF- 302804).
- Disposable 5 mL and 10 mL syringes (BD Discardit™ II - Imported and exported by Becton Dickinson India, Ltd.).
- Clean glass slide (7.5 × 2.5 cms) with FNAC number on them (Frosted - microscope slides, Blue star company, Mumbai).
- 95% alcohol was used as fixative (ALPHA-CHEM Company, Ambala, Haryana- Cat no - 5122).

### Technique used for aspiration and preparation of smears in cytology

The swelling should be immobilized using the left hand while the right hand manipulates the syringe to which the needle is attached. The overlying skin is cleaned with an antiseptic solution. Then, the needle is introduced into the swelling, and the syringe plunger is retracted to create negative pressure inside the syringe. Once the needle is inside the tumor, it is moved to and fro under constant suction to aspirate tissue fragments from various regions of the lesion. After sufficient material is collected, the plunger is released to eliminate negative pressure. The needle is then withdrawn from the tumor, and pressure is applied to the puncture site with a sterile cotton swab. Then, the needle containing aspirated material is detached from the syringe, the plunger is retracted to allow air entry into the syringe, and the needle is reattached. Finally, the contents of the needle are expelled onto the microscope glass slides by gently pushing the plunger, ensuring that only a single drop is deposited at the end of each slide. The aspirate deposited on the slide is first inspected with the naked eye. If the aspirate is semi-solid, it is then spread evenly using a spreader. For each patient, a minimum of 3-5 slides is prepared like this. Some of these slides are immediately immersed in 95% alcohol for wet fixation for 15 minutes, while the remaining slides are left to air dry. The alcohol used for wet fixation is supplied by ALPHA-CHEM Company, Ambala, Haryana (CAT no - 5122). The alcohol-fixed slides are subsequently stained using Papanicolaou prepared in-house from Qualigens - Thermo Fisher Scientific India Pvt. Ltd., Mumbai (OG6 - DK2D721735 and Eosin - DH4D741150) and conventional Hematoxylin and Eosin (H&E) stains, both provided by ALPHA-CHEM Company, Ambala, Haryana (Model No - H31001 and P3080). The air-dried smears are stained using Leishman stain prepared in-house from the Qualigens - Thermo Fisher Scientific India Pvt. Ltd,

Mumbai (DC3D721600). Finally, all slides are appropriately labeled and mounted with dibutylphthalate polystyrene xylene (DPX), mounting medium, also sourced from ALPHA-CHEM Company, Ambala, Haryana (DPX model no- 080415).

#### Cytology slides were reviewed for the following cytomorphology features, and cytopathology diagnosis was made

- Cellularity
- Cell morphology
- Nuclear features
- Cytoplasmic features
- Background
- Mitotic figures.

#### Steps involved in histopathology processing and reporting

Specimen is grossed and processed according to standard fixation and embedding procedures followed in our institution. The sections are cut from paraffin blocks using a rotary microtome from the Leica company and routinely stained with Hematoxylin and Eosin. Whenever a diagnostic dilemma arises, IHC was performed on the tissue blocks to reach the final diagnosis.

#### IHC steps and reporting

The prepared slides were first deparaffinized using frosted microscope slides obtained from PathinSitu Biotechnology, Nacharam, Hyderabad. Antigen retrieval was then carried out using a pressure cooker (PathinSitu MERS, Nacharam, Hyderabad- Model no- MERS-1). This was followed by three washes with phosphate-buffered saline (PBS) sourced from PathinSitu Biotechnologies-A10009OA. To block endogenous peroxidase activity, the slides were treated with 3% hydrogen peroxide for 10 min. Subsequently, the primary antibody was applied and incubated at room temperature for 30–40 min using the polyexcel hydrogen peroxide reducing protein and diaaminobenzidine (HRP/DAB) detection system (PathinSitu Biotechnologies, Nacharam, Hyderabad - D!0002TA). After another three PBS washes, the secondary antibody was added (PathinSitu Biotechnologies - R11160LA) and left at room temperature for 30 min. Following three additional PBS washes, the slides were immersed in a 3,3-Diaminobenzidine (DAB - D12002RA1) solution for 10-20 min. Finally, the slides were counterstained with hematoxylin for 2 min, cleared using xylene, and mounted in DPX (ALPHA-CHEM Company, Ambala, Haryana -Xylene Cat No- 5122 and DPX model no- 080415). Slides are then examined in a Nikon microscope (Nikon Corporation Company Ltd., Tokyo) for immunoreactivity/positivity, which is specific for all tumors, and the common markers of tumors are given

below [Table 1].<sup>[2]</sup> These markers helped us to render the final diagnosis. Then, later, microscopic pictures were taken using an Olympus Microscope with tri-head and an attached Camera for documentation (Olympus Corporation Ltd., Tokyo).

#### Statistical analysis

Finally, sensitivity, specificity, positive predictive value of test, negative predictive value of test, and the accuracy of FNAC in diagnosing STT were calculated using the Statistical Package for the Social Sciences software (2.0) and the following formula.

- Sensitivity = True Positive / (True Positive + False Negative)
- Specificity = True Negative / (True Negative + False Positive)
- Positive predictive value of test = True Positive / (True Positive + False Positive)
- Negative predictive value of test = True Negative / (True Negative + False Negative)
- Accuracy = (True Positive + True Negative) / (True Positive + False Positive + False Negative + True Negative)

## RESULTS

The mean age of incidence for STTs was 41 years, with a range spanning from 7 to 100 years. Benign tumors had a mean age of 40 years, while malignant tumors showed a higher mean age of 47 years. The highest prevalence was observed in the 21-40 year age group, where the majority of cases were benign. Females were more commonly affected in this study, with a male-to-female ratio of 0.85:1 [Table 2].

STTs were most commonly located in the trunk ( $n = 12$ ; 34%), followed by the upper extremities (26%). Among the various cytological patterns observed, tumors exhibiting a clear cell or adipocytic pattern (predominantly lipomas) were more commonly encountered. In contrast, round cell, polygonal, and pleomorphic patterns were primarily associated with malignant tumors. Spindle cell patterns, which ranged from benign to malignant lesions, constituted the diagnostic grey zone of STT [Table 3].

Lipomatous tumors ( $n = 27$ ; 54%) were found to be most common on histopathological diagnosis, followed by peripheral nerve sheath tumors, namely schwannoma and neurofibroma ( $n = 5$ ; 10%) [Table 4]. Schwannoma tumor is diagnosed due to the presence of verocay bodies, i.e., palisading arrangement of spindle cells, and on IHC, spindle cells (Schwann cells) show immunoreactivity to S100 [Figure 1]. Neurofibromas have spindle cells with wavy nucleus, and they show cytoplasmic positivity to neuron-specific enolase (NSE) and S100 as they originate from neurons [Figure 2].

**Table 1:** Common immunohistochemical markers and their associated tumors.

Marker (antibody)	Commonly detected in
Cytokeratin (Pan-Ck)	Carcinomas, epithelioid sarcomas, synovial sarcomas, some angiosarcomas, leiomyosarcomas, mesotheliomas, and rhabdoid tumors. <sup>[2]</sup>
Vimentin	Most sarcomas, melanomas, lymphomas, and a few carcinomas. <sup>[2]</sup>
Desmin	Both benign and malignant tumors originate from smooth and skeletal muscle. <sup>[2]</sup>
Neurofilament proteins	Tumors derived from neural tissue and neuroblastic origins. <sup>[2]</sup>
Smooth muscle actins	Smooth and skeletal muscle neoplasms, including myofibroblastic tumors and pseudotumors. <sup>[2]</sup>
Myogenic regulatory factors (MyoD1, Myogenin)	Typically present in rhabdomyosarcomas. <sup>[2]</sup>
S-100 protein	Melanomas, peripheral nerve sheath tumors (both benign and malignant), cartilage-derived tumors, Langerhans cells, adipose tissue, and others. <sup>[2]</sup>
Epithelial membrane antigen	Carcinomas, epithelioid sarcomas, synovial sarcomas, perineuriomas, meningiomas, and anaplastic large cell lymphomas. <sup>[2]</sup>
CD31	Vascular tumors of both benign and malignant nature. <sup>[2]</sup>
von Willebrand factor	Vascular neoplasms, including both benign and malignant types. <sup>[2]</sup>
CD34	Found in vascular tumors, solitary fibrous tumors, epithelioid sarcomas, dermatofibrosarcoma protuberans, and GISTs. <sup>[2]</sup>
CD99	Common in Ewing sarcoma family tumors, certain rhabdomyosarcomas, synovial sarcomas, lymphoblastic lymphomas, mesenchymal chondrosarcomas, small cell osteosarcomas, among others. <sup>[2]</sup>
CD68	Expressed in macrophages and tumors with fibrohistiocytic, granular cell, or sarcomatous origins, as well as some melanomas and carcinomas. <sup>[2]</sup>
Melanosomal antigens (HMB-45, Melan-A, gp100)	Associated with melanomas, PEComas, clear cell sarcomas, and melanotic schwannomas. <sup>[2]</sup>
MDM2 and CDK4	Atypical lipomatous tumors and dedifferentiated liposarcomas. <sup>[2]</sup>
Glut-1	Seen in perineuriomas and infantile hemangiomas. <sup>[2]</sup>
SMARCB1 (INI1)	Loss of expression is seen in extrarenal rhabdoid tumors, epithelioid sarcomas, some malignant peripheral nerve sheath tumors, and extraskeletal myxoid chondrosarcomas. <sup>[2]</sup>
Protein Kinase C-θ and DOG1	Diagnostic markers for GISTs. <sup>[2]</sup>
Bcl-2	Expressed in synovial sarcomas, solitary fibrous tumors, and various other spindle cell neoplasms. <sup>[2]</sup>
TLE1	Marker seen in synovial sarcomas. <sup>[2]</sup>

GISTs: Gastrointestinal stromal tumors, HMB-45: Human melanoma black-45, MDM2: Murine double minute2, CDK4: Cyclin-dependent kinase4, SMARCB1:SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1, DOG1:Discovered on GIST-1, Bcl-2:B cell lymphoma2, TLE1:Transducin-like enhancer of split 1

**Table 2:** Age and sex distribution of tumors.

Age (years)	Benign	Malignant	Total
0-20	6	2	8
21-40	18	2	20
41-60	12	1	13
61-80	4	4	8
81-100	0	1	1
Sex	Number of cases		
Male	23		
Female	27		
Total	50		

**Table 3:** Cytological diagnosis of soft tissue tumors.

Cytological patterns	No. of cases	%
Spindle cell pattern	12	24
Spindle cells with a myxoid background	3	6
Clear cell pattern	24	48
Round cell pattern	3	6
Polygonal pattern	2	4
Pleomorphic pattern	1	2
Others	5	10
Total	50	100

**Table 4:** Histopathology diagnosis of soft tissue tumors.

Type of tumor	No. of cases	%
Adipocytic tumors	27	54
Fibroblastic tumors	2	4
Fibrohistiocytic tumors	2	4
Smooth muscle tumors	2	4
Skeletal muscle tumors	1	2
Nerve sheath tumors	5	10
Perivascular tumors	2	4
Vascular tumors	2	4
Chondro-osseous tumors	1	2
Tumors of uncertain differentiation	3	6
Undifferentiated sarcomas	1	2
Others	2	4
Total	50	100

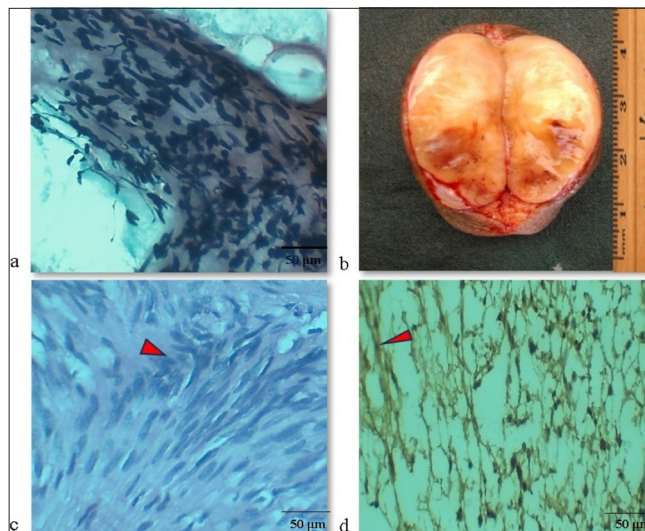
Four tumors were found to be discordant in this study. One tumor which was diagnosed as granulation tissue on cytology due to the presence of endothelial cells only. On histopathology, tissue sections showed smooth muscle cells proliferating around the capillaries lined by endothelial cells, which is a hallmark for angioleiomyoma. This Diagnosis was confirmed on IHC as endothelial cells showed CD34 positivity and smooth muscles showed vimentin and smooth muscle actin (SMA) positivity [Figure 3].

Another case diagnosed as lipoma on cytology due to the presence of benign-looking mature adipocytes in clusters turned out to be well-differentiated liposarcoma on histopathology after IHC [Figure 4], as these benign-looking lipoblasts showed MDM2 positivity. The rest of the discordant cases are like malignant glomus tumor, which was the histopathological diagnosis for cytological interpretation of angiomatosis, due to sparse cellularity, and dermatofibrosarcoma protuberans was the histopathological diagnosis for a benign spindle cell lesion on cytology.

Among 50 cases in the study, 74% ( $n = 37$ ) were benign tumors, 4% ( $n = 2$ ) were intermediate, and 22% ( $n = 11$ ) were malignant [Table 5].

Lipomatous tumors showed correlation in 96.3% of cases ( $n = 26$ ). Peripheral nerve sheath tumors showed complete correlation, i.e., 100% of cases ( $n = 5$ ). Fibrohistiocytic tumors also showed complete correlation, i.e., 100% of cases ( $n = 2$ ). Benign tumors showed correlation in 97.3% of cases ( $n = 36$ ). All intermediate tumors were missed on cytology and were diagnosed on histopathology ( $n = 2$ ). Correlation was found in 90.9% of malignant tumors ( $n = 11$ ) [Table 6].

Sensitivity of 100%, specificity of 76.92%, the positive predictive value of test was 92.5%, and negative predictive



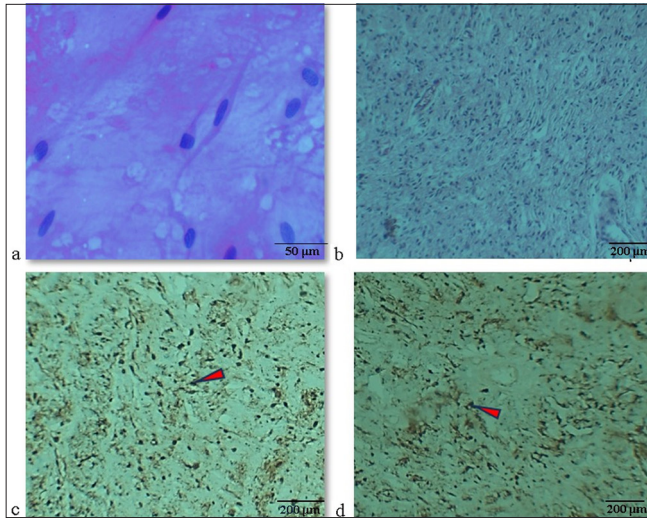
**Figure 1:** Schwannoma. (a) Fine needle aspiration cytology smear shows sheets of benign spindle cells (hematoxylin and eosin [H&E] stain,  $\times 400$  magnification, Scale bar = 50  $\mu\text{m}$ ). (b) Gross specimen with well-circumscribed gray white mass and hemorrhagic areas. (c) Histopathology section from the same case showed palisading arrangement (red arrow head) of benign spindle cells (H&E,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (d) Immunohistochemistry stain shows strong S100 positive in nucleus and cytoplasm of these spindle cells (red arrow head) (S100,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ).

value of test was 100%, as determined through statistical analysis of the correlation between cytopathology and histopathological results. These values were calculated using the formula provided under the subheading of statistical analysis [Table 7].

### Cytomorphology features of tumors with their cytology and histopathological diagnosis

#### Spindle cell pattern

In our study, 15 cases showed a spindle cell pattern, of which 9 were benign and 6 were malignant. In benign spindle cell lesions, the predominant lesions in this study were benign nerve sheath tumors, including 3 schwannomas and 2 neurofibromas [Figures 1 and 2]. Pitfalls in diagnosing spindle cell lesions were observed in 1 case of dermatofibrosarcoma protuberans, which was an intermediate spindle cell lesion, but was identified as a benign spindle cell lesion on cytology. Intermediate spindle cell lesions are mostly missed in cytology, and it is very difficult to differentiate between benign and intermediate spindle cell lesions on cytology. The other two cases were leiomyoma and vascular tumors, which also showed spindle cells and were diagnosed as spindle cell lesions of benign nature and confirmed on biopsy. Among the 5 malignant spindle cell lesions, 2 were synovial sarcomas, 1 was sarcomatoid metastatic deposits, 1 was



**Figure 2:** Diffuse neurofibroma. (a) Fine needle aspiration cytology smear shows sparse cellularity with few benign spindle cells in singles (hematoxylin and eosin [H&E], ×400 magnification, Scale bar = 50 μm), (b) Histopathology showed fascicular bundles of benign spindle cells with wavy nucleus (H&E, ×100, Scale bar = 200 μm). (c) Immunohistochemistry (IHC) showed S100 positivity in the nucleus and cytoplasm of these spindle cells (red arrow head) (S100, ×100, Scale bar = 200 μm). (d) IHC showed NSE positivity in the cytoplasm of these cells (red arrow head) (NSE, ×100, Scale bar = 200 μm).

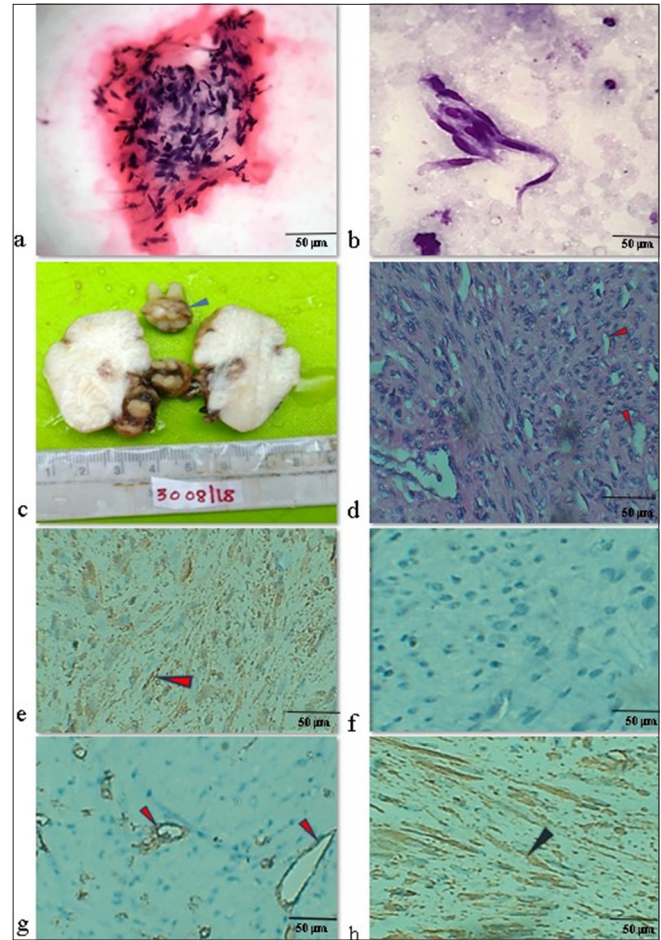
an undifferentiated sarcoma, and 1 was a dedifferentiated liposarcoma; all of them were confirmed on histology. Synovial sarcoma cases showed monomorphic spindle cells in sheets, which is typical for monophasic synovial sarcoma, and these monomorphic spindle cells showed cytoplasmic immunoreactivity to BCL2 and membrane positivity for CD99, which is very specific for synovial sarcoma [Figure 5].

#### ***Clear cell pattern***

In this study, 25 cases were identified as lipoma on cytology and had a clear cell pattern with vacuolated clear cytoplasm and an eccentric nucleus. One case that was reported as lipoma on cytology had clinical suspicion of liposarcoma, as it was a huge neck mass that was infiltrating into the mediastinum. Atypical hyperchromatic cells were seen on histology investigation, and well-differentiated liposarcoma was further given as the final diagnosis, after IHC, due to MDM2-positive atypical cells [Figure 4].

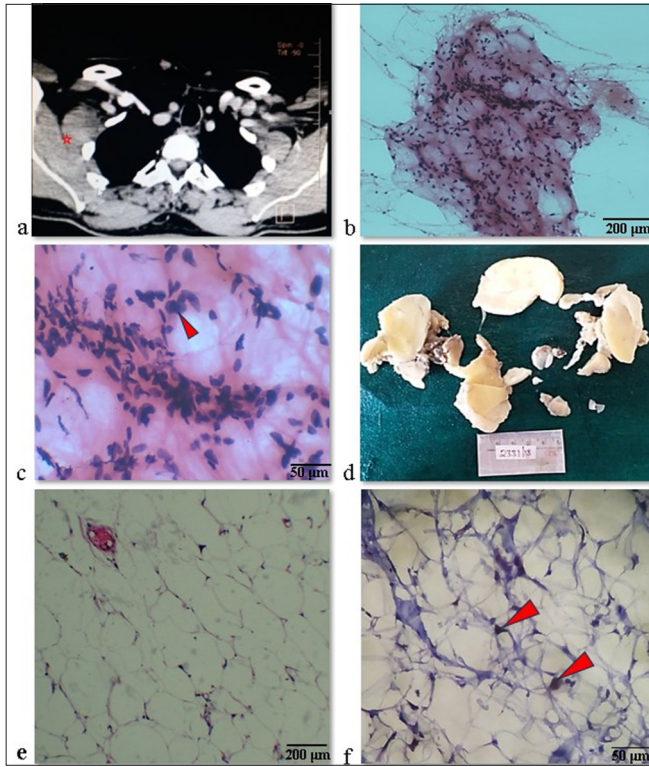
#### ***Pleomorphic pattern***

In our study, there was only one patient who presented with left forearm swelling and on FNAC, which showed cellular smears comprising pleomorphic cells in clusters, sheets, and singles against a dense matrix with multinucleated bizarre



**Figure 3:** Angioleiomyoma. (a) Fine needle aspiration cytology (FNAC) showed clusters of spindle cells (hematoxylin and eosin [H&E], ×400, Scale bar = 50 μm). (b) FNAC with clusters of spindle cells (Leishman's stain, ×400, Scale bar = 50 μm). (c) Cut surface of mass showing grey white areas with 2 molar teeth (blue arrowhead). (d) Histopathology section shows spindle cells in fascicles and blood vessels amidst (red arrow heads) (H&E, ×400, Scale bar = 50 μm). Immunohistochemistry staining: (e-h) Vimentin shows spindle cells displaying cytoplasmic positivity, anaplastic lymphoma kinase (ALK) is negative in these spindle cells, CD34 highlights the endothelial cells of blood vessels within the tumor, and SMA shows cytoplasmic positivity in the spindle cells (marked with red arrow heads) (×400, Scale bar = 50 μm).

cells, hemorrhage, and mitotic figures. The cytological diagnosis was pleomorphic sarcoma, and an excision biopsy was performed. The histopathology slide showed areas of differentiation consisting of large pleomorphic spindle cells arranged in a storiform pattern. The final diagnosis was given as dedifferentiated liposarcoma, and on IHC, tumor cells showed immunoreactivity to vimentin, focal immunoreactivity for MDM2, and negative for S100 and desmin, hence confirming the diagnosis of dedifferentiated liposarcoma [Figure 6].

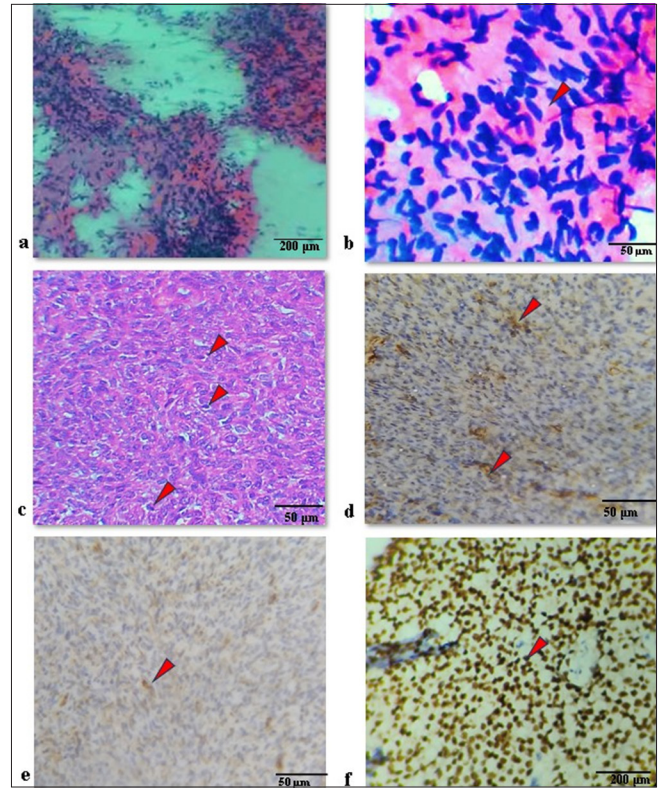


**Figure 4:** Well-differentiated liposarcoma. (a) Computed tomography scan shows an ill-defined lobulated fat density lesion in the right side of the neck, which is displacing the sternocleidomastoid muscle (red star). (b) Fine needle aspiration cytology smear shows a cluster of adipocytes with nuclear atypia (hematoxylin and eosin [H&E],  $\times 100$ , Scale bar = 200  $\mu\text{m}$ ). (c) Smear shows clusters of adipocytes with nuclear atypia (red arrowhead) (H&E,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ), (d) Gross specimen showing fibro-fatty tissue with homogenous yellow areas. (e) Histopathology section shows lobules of adipocytes with few atypical cells with irregular hyperchromatic nucleus (H&E,  $\times 100$ , Scale bar = 200  $\mu\text{m}$ ). (f) Immunohistochemistry stain highlights MDM2 positivity in the nucleus of the atypical cells (red arrow marks) (MDM2,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ).

### Small round cell pattern

In our study, one case of malignant glomus tumor was missed on cytology and misdiagnosed as angiomatosis due to scant cellularity. Histology slides of the same patient showed pseudopapillary-like dilated blood vessels surrounded by sheets of small and round cells. Occasional large pleomorphic nuclei and frequent mitotic figures were seen, so the final diagnosis was given as a malignant glomus tumor. However, one case of rhabdomyosarcoma and one Ewing's sarcoma/primitive neuro-ectodermal tumor (PNET) were accurately diagnosed as malignant round cell tumors.

A rhabdomyosarcoma patient presented with a tumor, pain in the right calf region, and right iliac lymphadenopathy. Aspiration from the right inguinal lymph node showed



**Figure 5:** Synovial sarcoma. (a) Fine needle aspiration cytology shows a highly cellular smear consisting of spindle cells in sheets with hyperchromatic nuclei (hematoxylin and eosin [H&E],  $\times 100$ , Scale bar = 200  $\mu\text{m}$ ). (b) Cellular smear with spindle cells displaying hyperchromatic nuclei (red arrow head) (H&E,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (c) Histopathology section shows sheets of monomorphic spindle cells with 6-8/high power field -400X (HPF) mitotic figures (red arrow heads) (H&E,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). Immunohistochemistry stains (d-f) BCL2 expression is localized to the cytoplasm of the tumor cells, CD99 is positive in the membrane (red arrow heads) (BCL-2, CD99,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ), and TLE-1 shows strong nuclear positivity (TLE-1,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ).

small, blue, round cells. A cell block was done, followed by IHC on the cell block, which showed small round cells that were immunoreactive for desmin. This was followed by excision and histopathological examination of the calf muscle excisional biopsy, which also showed small round rhabdomyoblast immunoreactive to desmin, hence confirming the diagnosis of rhabdomyosarcoma [Figure 7].

An 8-year-old boy came with a complaint of a right inguinal region mass, which was firm on palpation and subcutaneous in location. On aspiration, discohesive sheets contained small, round blue cells. On cytological diagnosis, a small round cell tumor was suspected of being malignant, and histological confirmation was advised. H&E sections showed small, round atypical cells arranged in sheets along with frequent mitosis, at places, there were pseudo-rosettes and

**Table 5:** Comparison of cytopathology and histopathology.

Type of tumors	Cytopathology			Histopathology		
	Benign	Intermediate	Malignant	Benign	Intermediate	Malignant
Adipocytic tumors	25	0	2	24	1	2
Fibroblastic tumors	2	0	0	1	1	0
Fibrohistiocytic tumors	2	0	0	2	0	0
Smooth muscle tumors	2	0	0	2	0	0
Skeletal muscle tumors	0	0	1	0	0	1
Nerve sheath tumors	5	0	0	5	0	0
Perivascular tumors	2	0	0	1	0	1
Vascular tumors	2	0	0	2	0	0
Chondro-osseous tumors	0	0	1	0	0	1
Tumors of uncertain differentiation	0	0	3	0	0	3
Undifferentiated sarcomas	0	0	1	0	0	1
Others	0	0	2	0	0	2
Total	40	0	10	37	2	11

**Table 6:** Cytology and histopathology correlation.

Type of tumors	Cases	Correlated	Not correlated
Adipocytic tumors	27	26	1
Fibroblastic tumors	2	1	1
Fibrohistiocytic tumors	2	2	0
Smooth muscle tumors	2	2	0
Skeletal muscle tumors	1	1	0
Nerve sheath tumors	5	5	0
Perivascular tumors	2	0	2
Vascular tumors	2	2	0
Chondro-osseous tumors	1	1	0
Tumors of uncertain differentiation	3	3	0
Undifferentiated sarcomas	1	1	0
Others	2	2	0
Total	50	46	4

**Table 7:** Statistical analysis of cyto-histopathological correlation.

Cytology diagnosis	Histopathology diagnosis	
	Benign	Malignant+Intermediate
Benign	37 (True positive)	3 (False positive)
Malignant+Intermediate	0 (False negative)	10 (True negative)

a large area of necrosis. The final diagnosis was given as Ewing's sarcoma, or PNET, which was confirmed by IHC, which showed positivity for CD99 and NSE [Figure 8].

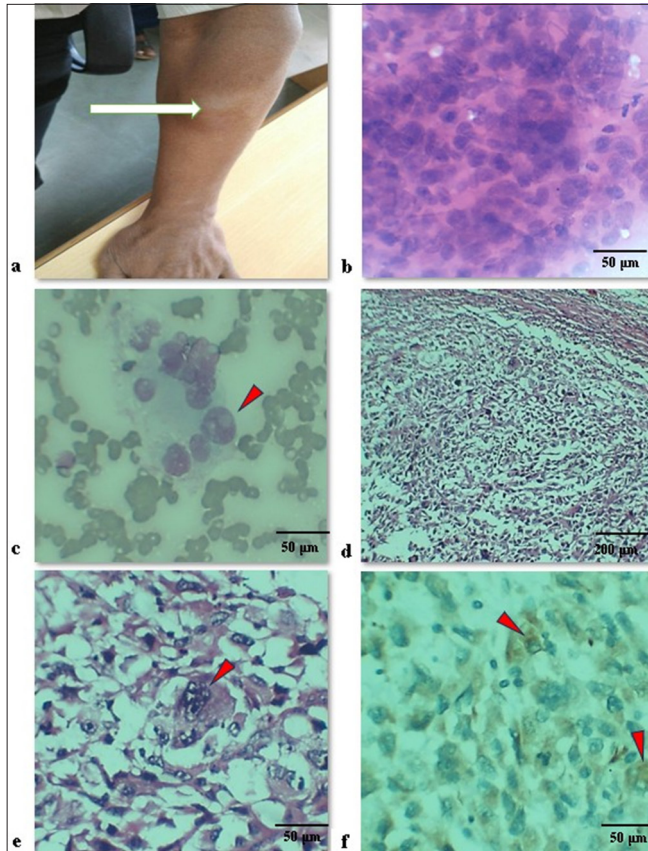
### *Epithelioid (polygonal) cell pattern*

In this study, there were two cases that showed polygonal cells on cytology. Both cases showed highly cellular smears comprising polygonal cells having a polygonal shape and strikingly pleomorphic nucleus with prominent nucleoli and mitotic figures. One of them showed rhabdomyoblast, i.e., cells with abundant eosinophilic cytoplasm and eccentrically placed nucleus; hence, the cytological diagnosis was given as epithelioid sarcoma (probably rhabdomyosarcoma). Histopathology sections showed cells with a polygonal shape arranged in sheets, and there was lacy osteoid formation amidst them. On IHC, the tumor cells showed immunoreactivity to desmin and myogenin [Figure 9]. The final diagnosis was given as extraskeletal osteosarcoma after correlating age, clinical findings, and IHC findings.

Tumors with a pure myxoid pattern were absent in our study. Tumors such as angioleiomyoma and vascular tumors could not be placed under a specific cytological category due to hemorrhagic aspirate and scanty endothelial and spindle cells. An incisional biopsy showed plump spindle cells in fascicles and bundles swirling away from vessels. Vimentin and SMA positivity were seen in spindle cells. CD34 showed positivity in endothelial cells. Hence, the final diagnosis was rendered as angioleiomyoma [Figure 3].

## DISCUSSION

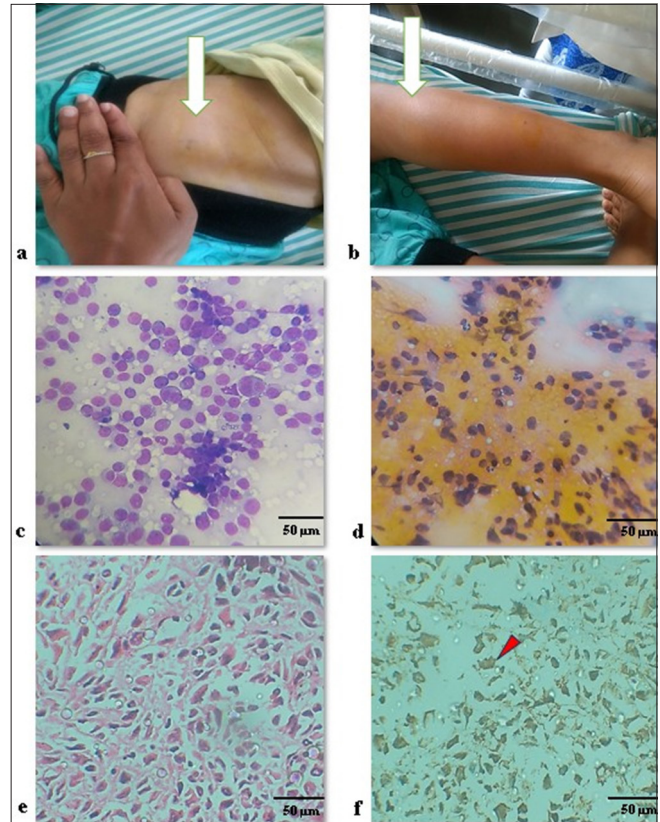
In our study, the mean age of incidence of STTs was 41 years. The mean age of incidence of benign tumors was 40 years, and 47 years for malignant tumors. Prevalence was highest in the age group of 21-40 years, followed by 41-60 years, with



**Figure 6:** Dedifferentiated liposarcoma. (a) Lesion in the left forearm region (arrow mark). (b) Fine needle aspiration cytology shows a highly cellular smear comprising pleomorphic cells in sheets with prominent nucleoli (hematoxylin and eosin [H&E], ×400 objective). Scale bar = 50 μm). (c) Smear comprises pleomorphic cells in clusters with prominent nucleoli (red arrow head) (H&E, ×400, Scale bar = 50 μm). (d) Histopathology (HP) shows pleomorphic spindle cells in storiform pattern, sheets, and lobules (H&E, ×100 objective). Scale bar = 200 μm). (e) HP shows pleomorphic spindle cells in storiform pattern, sheets, and lobules (red arrow head) (H&E, ×400, Scale bar = 50 μm). (f) Immunohistochemistry showing diffuse nuclear positivity of MDM2 (red arrow heads) (MDM2, ×400, Scale bar = 50 μm).

the majority of the lesions being benign, and lowest in the age group of 61-80 years. Chatura *et al.* found the mean age of incidence to be 42 years; Ogun found it to be 41 years, and Gonzalez Campora *et al.* found it to be 46 years in their studies.<sup>[5-7]</sup> In studies by Chatura *et al.*, Ogun, and Tailor *et al.*, the most affected age groups were 31-40 years, 30-50 years, and 50-70 years, respectively.<sup>[5,6,8]</sup>

As reported in studies carried out by Chatura *et al.*, Ogun, Tailor *et al.*, and Roy *et al.*, the ratio of male to female was 1.25:1, 1:1, 1.3:1, and 1.29:1, respectively.<sup>[5,6,8,9]</sup> In our study, the male-to-female ratio was 0.85:1. Roy *et al.* showed the most affected site as the trunk (40.95%), similar to the present study, whereas the upper extremity was the most common

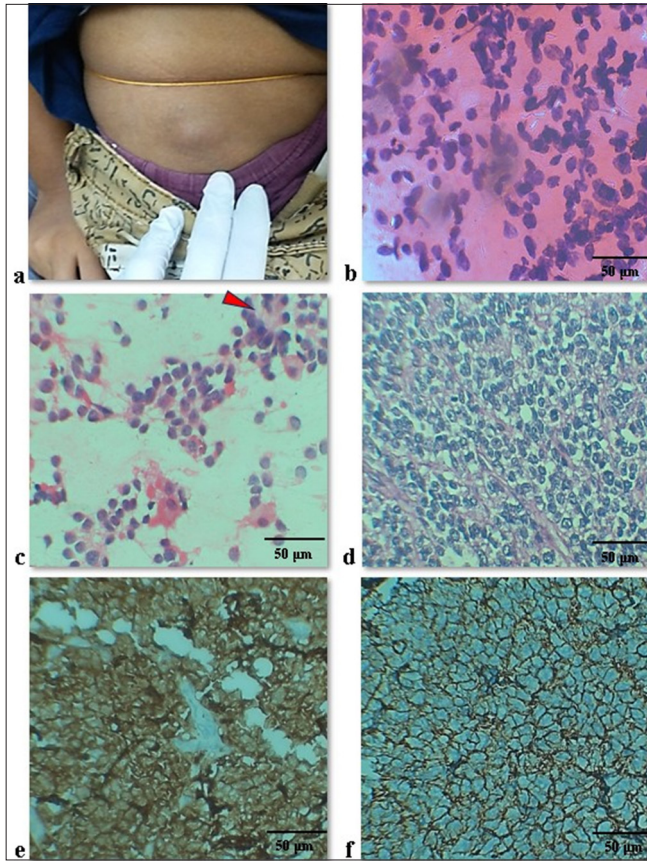


**Figure 7:** Rhabdomyosarcoma. (a) Photograph of the right iliac region with lymph node enlargement. (b) Lesion over the right calf leg calf region (white arrow). (c) Fine needle aspiration cytology (FNAC) shows a highly cellular smear composed of round cells with eccentric nucleus in the background of lymphoid cells (Leishman's stain, ×400, Scale bar = 50 μm). (d) FNAC shows highly cellular smears of round cells with eccentric nucleus in the background of lymphoid cells (Pap stain, ×400, Scale bar = 50 μm). (e) Histopathology shows atypical cells in sheets and lobules with rhabdomyoblast (hematoxylin and eosin, ×400, Scale bar = 50 μm). (f) Immunohistochemistry shows desmin positivity in the cytoplasm of these rhabdomyoblasts (red arrowhead) (Desmin, ×400, Scale bar = 50 μm).

site for STT in the study led by Tailor *et al.*<sup>[8,9]</sup> Beg S *et al.* observed the head and neck as the most common location affected in their study.<sup>[10]</sup>

In our study, benign cases outnumbered malignant cases, similar to the research undertaken by Chatura *et al.*,<sup>[5]</sup> who found 91.5% of cases to be benign, and Tailor *et al.*,<sup>[8]</sup> who found 93.58% to be benign. Roy *et al.*<sup>[9]</sup> found 61.9% of total cases to be benign, and Ogun<sup>[6]</sup> found 52.3% to be benign in their studies.

Akerman and Domanski classified the STTs into five groups based on the principal cytological pattern observed in their study.<sup>[11]</sup> Clear cell or lipomatous pattern was the predominant cell pattern based on studies performed by Beg



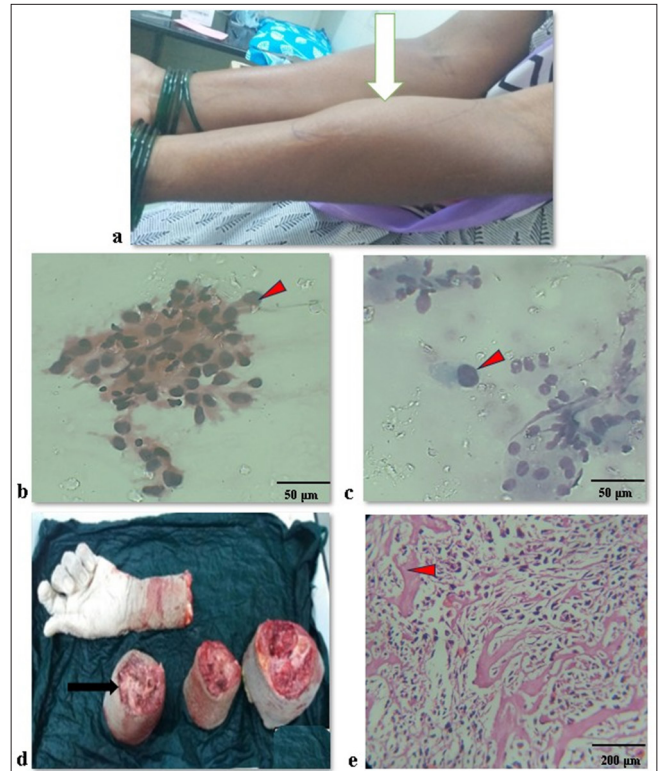
**Figure 8:** Ewing's sarcoma/primitive neuro-ectodermal tumor. (a) Lesion over the right iliac region. (b) Fine needle aspiration cytology (FNAC) shows a highly cellular smear composed of round cells (hematoxylin and eosin [H&E],  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (c) FNAC smear shows round cells forming rosettes (red arrowhead) (H&E,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (d) Histopathology section shows solid sheets of small round cells (H&E,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). Immunohistochemistry stains. (e and f) Diffuse and strong membranous positivity of CD99 highlighted in tumor cells (CD99,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ) and NSE with strong cytoplasmic positivity in the tumor cells (NSE,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ).

S *et al.*<sup>[10]</sup> and Arul and Masilamani,<sup>[12]</sup> which are consistent with our results. According to a study carried out by Rekhi *et al.* at a tertiary care facility where all patients with malignant tumors are referred, the spindle cell pattern was the predominant cell type.<sup>[1]</sup>

### Discussion on each cytological pattern

#### Spindle cell pattern

Spindle cell tumors form a grey zone in STT. There is a diagnostic challenge for the pathologist to differentiate the dizzy array of spindle cell lesions, which have overlapping features, due to the presence of many pseudo-sarcomatous lesions mimicking malignant lesions under



**Figure 9:** Extraskeletal osteosarcoma. (a) Lesion over the left forearm anterior aspect (white arrow). (b) Fine needle aspiration cytology (FNAC) shows highly cellular smear comprising pleomorphic polygonal cells with eccentric nuclei (red arrowhead) in sheets and clusters (Pap,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (c) FNAC shows highly cellular smears comprising pleomorphic polygonal cells with eccentric nuclei in sheets and clusters (red arrowhead) (Leishman's,  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (d) Specimen of above elbow amputation showing grey white fleshy tumor replacing skeletal muscle (hematoxylin and eosin [H&E],  $\times 400$ , Scale bar = 50  $\mu\text{m}$ ). (e) Histopathology section shows polygonal pleomorphic cells in patternless sheets with trabeculae of osteoid seams (red arrowhead) amidst them (H&E,  $\times 100$ , Scale bar = 200  $\mu\text{m}$ ).

this category.<sup>[13,14]</sup> It is very difficult to differentiate well-differentiated fibrosarcoma from nodular fasciitis, fibromatosis, dermatofibrosarcoma protuberans, and synovial sarcoma. According to Kilpatrick *et al.*,<sup>[15]</sup> malignant spindle cell tumors have moderate to highly cellular smears comprising pleomorphic cells with hyperchromatic nuclei with coarse chromatin and irregular margins in discohesive sheets or individually dispersed tumor cells. Frequent mitotic figures and necrosis suggest malignancy, but on the contrary, spindle lesions like synovial sarcoma have uniform, bland spindle cells with slight pleomorphism and a variable mitotic figure.<sup>[15]</sup>

In research conducted by Costa *et al.*, FNAC demonstrated a high rate of false-positive rates in patients with a known diagnosis of sarcoma, as clinical history favors based

interpretation towards positive diagnosis and may make the interpretation of atypical spindle cell proliferations with a prior history of sarcoma particularly challenging.<sup>[16]</sup> Hence, when cytological diagnosis is not clearly conclusive, surgical biopsy becomes essential. Powers *et al.* also emphasize that while evaluation of FNAC smears rich in spindle cells, cellularity, individual cells, cell patterns, and background stromal features, when considered along with precise clinical history, can help to narrow down the differential diagnosis, particularly in distinguishing benign and malignant. However, they advise caution in the precise classification of spindle-cell tumors based on FNAB, as this misclassification can significantly affect patients' management.<sup>[17]</sup>

#### **Clear cell pattern**

In 2000, Pranab Dey described two cases of well-differentiated liposarcoma and concluded that the diagnosis of well-differentiated liposarcoma on cytology should be made cautiously, with careful correlation of lesions' anatomical location and radiographic findings.<sup>[18]</sup> According to Enzinger and Weiss, well-differentiated liposarcomas are frequently misdiagnosed as lipomas on cytology, which is the case in our study because the smears are moderately cellular and primarily comprise mature-appearing fat cells in fragments.<sup>[2]</sup> In such cases with clinical suspicion, MDM2 IHC should be done to come to the diagnosis, as we did in our study.

#### **Pleomorphic pattern**

In the case report led by Al-Maghraby *et al.* and Al-Rohil M *et al.*, cytology smears of dedifferentiated liposarcoma revealed multinucleated pleomorphic giant cells with abundant cytoplasm with smaller clusters of cells with a high nuclear/cytoplasmic ratio, and elongated nuclear features in dense matrix material, which is similar to our study's findings, i.e., pleomorphic cells and giant cells in dense matrix material.<sup>[19,20]</sup> According to Aleixo PB *et al.*, the MDM2 and CDK4 markers are supportive methods for establishing the diagnosis of dedifferentiated liposarcoma and well-differentiated liposarcoma.<sup>[21]</sup> In our study, we also have a similar report where dedifferentiated liposarcoma and well-differentiated liposarcoma show MDM2 positivity in tumor cells.

#### **Small round cell pattern**

The tumors that fall under this group are glomus tumors, rhabdomyosarcomas, Ewing's sarcoma, PNET, and lymphomas, which can be confused with sarcomas.<sup>[1]</sup> In this study, we had a case of rhabdomyosarcoma and Ewing's sarcoma, which was diagnosed right as round cell sarcoma on cytology, but we missed to diagnose a glomus tumor due

to low cellularity. This can be avoided by repeated aspiration and USG-guided aspiration from solid areas.

#### **Epithelioid (polygonal) cell pattern**

These are tumors primarily composed of polygonal cells or epithelioid cells. These tumors have cells with an epithelioid-like pattern. These cells are rounded to polygonal cells with distinct cytoplasm and usually prominent nucleoli. The differential diagnosis for these tumors includes various carcinomas, melanomas, and lymphomas.<sup>[2,13]</sup> In this study, 2 cases showed epithelioid pattern; one was diagnosed as extraskelatal osteosarcoma on histopathology, and the other one was a tumor of uncertain origin.

#### **Myxoid pattern**

In benign lesions, it includes spindle cells and tumors with a predominantly myxoid background, such as diffuse neurofibroma, palmar fibroma, and leiomyoma with myxoid degeneration. Malignant myxoid tumors may originate from fibroblastic, myoblastic, lipoblastic, chondroblastic, neurogenic, and various other cell types. A characteristic feature of these tumors is a copious amount of mucinous material. Myxoid sarcomas have a more favorable outcome than their non-myxoid counterparts. Malignant and non-malignant myxoid tumors are impossible to separate by cytology alone. In this study, there were no tumors that were placed in this pattern.

#### **Accuracy**

The main aim of this study was to determine the utility of FNAC in diagnosing STTs by cytomorphologically subclassifying them and then correlating them with their histological counterpart, and then calculating sensitivity, specificity, predictive value of positive test, and predictive value of negative test. This study showed sensitivity of 100%, specificity of 76.9%, positive predictive value of test as 92.5%, and negative predictive value of test as 100% [Table 8].

All other variables correlated with studies done by Chatura *et al.*,<sup>[5]</sup> Rekhi *et al.*,<sup>[1]</sup> Ogun<sup>[6]</sup> Chaithanya and Dinesh,<sup>[22]</sup> Beg *et al.*,<sup>[11]</sup> and Khalbuss *et al.*,<sup>[23]</sup> except for specificity, which was low compared to other studies. Specificity was low due to scanty material in a few samples, which can be alleviated by more sampling of 3-4 aspirations [Table 8].

After final calculation, the FNAC demonstrated high reliability in diagnosing STT in our study, with a diagnostic accuracy of 92% and the inconsistency rate was 6%.

The accuracy rate of our study correlated with the studies carried out by Wakely and Kneisl,<sup>[24]</sup> Chaithanya and Dinesh,<sup>[22]</sup> Ogun,<sup>[6]</sup> Kilpatrick SE *et al.*,<sup>[15]</sup> Roy *et al.*,<sup>[9]</sup> and Akerman *et al.*,<sup>[25]</sup> but it was not as high as Rekhi

**Table 8:** Sensitivity, specificity, Positive predictive value of test (PPV), and negative predictive value of test of various studies (NPV).

Study	Sensitivity (%)	Specificity (%)	Positive predictive value of test (%)	Negative predictive value of test (%)
Chatura <i>et al.</i> <sup>[5]</sup>	84.61	85.71	84.61	85.6
Rekhi <i>et al.</i> <sup>[11]</sup>	100	83.3	98	100
Ogun <sup>[6]</sup>	95	100	100	95
Chaithanya and Dinesh <sup>[22]</sup>	100	97.93	100	97.7
Beg S <i>et al.</i> <sup>[10]</sup>	98	96.7	97.2	-
Khalbuss <i>et al.</i> <sup>[23]</sup>	96	98	99	92
Present study	100	76.9	92.5	100

PPV: Positive predictive value of the test, NPV: Negative predictive value of the test.

**Table 9:** Diagnostic accuracy of FNAC in various studies.

Study	Percentage of accuracy
Rekhi <i>et al.</i> <sup>[11]</sup>	98
Wakely and Kneisl <sup>[24]</sup>	98.3
Chaithanya and Dinesh <sup>[22]</sup>	97
Ogun <sup>[6]</sup>	97.5
Kilpatrick SE <i>et al.</i> <sup>[15]</sup>	95
Roy <i>et al.</i> <sup>[9]</sup>	90
Akerman <i>et al.</i> <sup>[25]</sup>	85
Parajuli and Lakhey <sup>[26]</sup>	80
Present study	92

*et al.*<sup>[11]</sup> study, nor as low as Parajuli and Lakhey's study [Table 9].<sup>[26]</sup>

Nonetheless, the potential of cytology in evaluating soft tissue lesions can be enhanced through further studies exploring the use of ancillary techniques such as IHC and cytogenetics, an approach we have incorporated in our study. Studies on the utility of FNAC in the diagnosis of STT are increasing, namely, Rakheja *et al.* and Pandey G *et al.* Our study is different from these studies because in all the cases we have correlated the cytology with histopathology diagnosis, and we also used ancillary techniques like IHC for confirmation of the final diagnosis on histopathology in most of the cases, whenever there was a diagnostic dilemma regarding cell of origin.<sup>[27,28]</sup> Rakheja *et al.* study was a retrospective study, and in their study, cytological features were correlated with histological findings wherever available, not for all. In Pandey G *et al.* study, they have not used the ancillary techniques; they have merely done subcategorizations based on morphology and correlated with histopathology findings.<sup>[28]</sup> Incorporating ancillary techniques like Immunocytochemistry can help in improving the reliability of FNAC and histopathology diagnosis. We have used IHC for most of the cases when faced with a diagnostic dilemma on histopathology. Hence, all these

points make our study different, more reliable, and more accurate as compared to other studies done.

## CONCLUSION

In STT, most were benign tumors, with lipomas being the most common among them. The mean age affected was the fourth decade, with the most common site being the trunk. While benign and malignant tumors were easy to diagnose, intermediate spindle cell tumors were often missed on cytology. These intermediate tumors represent the diagnostic "grey zone." With increasing experience, STT can be diagnosed more accurately as benign or malignant. FNAC has many advantages that outweigh the disadvantages when it comes to STT, such as low morbidity, high compliance, and acceptable accuracy. FNAC is therefore a standard pre-operative diagnostic tool and a reliable alternative to open biopsy for diagnosing STT. Cytological categorization of sarcomas especially helps in the early formulation of an effective management protocol.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the study are of the first and corresponding author, which she obtained from the institute during the thesis study as a part of her postgraduation program, as already mentioned and are available from the corresponding author upon reasonable request.

## ABBREVIATIONS

FNAC: Fine needle aspiration cytology  
 IHC: Immunohistochemistry  
 NPV: Negative predictive value of tests  
 PPV: Positive predictive value of tests  
 STT: Soft Tissue Tumor  
 DPX: Dibutylphthalate polystyrene Xylene  
 HRP/DAB: Hydrogen Peroxide reducing protein and Diaaminobenzidine  
 GISTs: Gastrointestinal stromal tumors

HMB-45: Human Melanoma Black-45  
MDM2: Murine Double Minute2  
CDK4: Cyclin –dependent kinase4  
SMARCB1: SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1  
DOG1: Discovered on GIST-1  
Bcl-2: B cell lymphoma2  
TLE1: Transducin-like enhancer of split 1  
NSE: Neuron-specific Enolase  
SMA: Smooth Muscle Actin

## AUTHOR CONTRIBUTIONS

MD: Contributed to the conception and design of the study, data acquisition, analysis, and interpretation, as well as drafting and critically revising the manuscript, and provided final approval for publication; PKS: Contributed to data acquisition, data analysis and interpretation, critical review of the manuscript, and final approval; USD: Contributed to the study conception and design, data analysis and interpretation, critical review, and final approval; SV, APJ: Contributed to the critical review and final approval. All authors accept collective responsibility for the accuracy and integrity of the work. All authors meet the authorship status of ICMJE.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research/study was approved by the Institutional Review Board at SDM College of Medical Sciences and Hospital, Sattur Dharwad, Karnataka, India, number SDMIEC:0869:2016, dated 28-10-2016 and has obtained all appropriate patient consent. This study was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki. The authors certify that they have obtained all the appropriate patient consent forms.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## EDITORIAL/PEER REVIEW STATEMENT

To ensure the integrity and highest quality of Cytojournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through an automatic online system.

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