Role of Imprint Cytology in the Intraoperative Diagnosis of Soft Tissue Tumors

Lakshmy M R, a Krishna Balachandran b

a. Department of Pathology, Employees’ State Insurance Corporation Medical College Hospital, Parippally, Kerala, India; b. Department of Pathology, Government Medical College, Thiruvananthapuram, Kerala, India

Abstract

Tumors of the soft tissue constitute a heterogeneous group of neoplasms in terms of clinical presentation, morphology and behavior. Intraoperative diagnosis of soft tissue tumors is indicated not only for making a diagnosis, but also for evaluating margin status, determining tumor extent/spread, and obtaining an adequate sample for ancillary diagnostic tests. This study attempted to bring out the values and limitations of imprint cytology in providing a rapid and accurate diagnosis of soft tissue tumors and in particular to explore the potential application of cytology techniques in the intraoperative setting. The accuracy of this method was assessed by comparing the imprint diagnosis with paraffin section diagnosis. It was also aimed to describe the morphology of cells seen in the imprint smears of the different tumor types as well as to correlate the cytologic features with gross pathologic features like tumor size, location, depth, circumscription, encapsulation and presence of necrosis. This study included 87 patients admitted with a clinical diagnosis of soft tissue tumor in Government Medical College, Thiruvananthapuram from February 2007 to December 2008. Imprint and scrape smears were obtained intraoperatively from the tumors, fixed, stained and studied under a microscope. Imprint cytology and histopathology slides were compared. Of the 87 cases, 50 were malignant; 25 benign and 7 were of intermediate grade. Five cases turned out to be other soft tissue lesions. 18 benign tumors and 37 malignant tumors could be accurately categorized by imprint cytology. Weighted kappa statistics showed substantial agreement between imprint cytology and histopathology (0.78). Imprint smears were seen to provide very good cytologic and architectural details. Gross examination of specimens served as an adjunct to enhance the diagnostic accuracy. This study concludes that imprint cytology serves as an invaluable tool in the intraoperative evaluation of soft tissue tumors, the accuracy of which can be enhanced by correlating with clinical and radiological features. Imprints prepared from fresh specimens give excellent cytological details. However, intraoperative diagnosis of benign soft tissue tumors by imprint cytology is preliminary and warrants confirmation.

Key Words: Imprint Cytology, Intraoperative Diagnosis, Soft Tissue Tumors

Introduction

Soft tissue is defined as the supportive tissue of various organs and the non-epithelial, extra-skeletal structures exclusive of lymphohematopoietic tissues. Soft tissue tumors constitute a heterogeneous group of neoplasms that clinically range from totally benign to highly malignant neoplasms. Benign soft-tissue tumors are about 100 times more common than malignant soft-tissue tumors. 1

Although soft tissues constitute much of the human body, the incidence of soft tissue sarcomas is low and accounts for 30 per million per annum. Although fewer than 1% of the malignant tumors in adults are soft tissue sarcomas, they are nevertheless responsible for 2% of all deaths caused by cancer. Poorly differentiated soft tissue sarcomas in particular carry a poor prognosis that can be attributed to frequent hematogenous metastases (30-40%).

The clinical symptoms accompanying the diagnosis of soft tissue tumors are nonspecific. The most common finding at presentation is painless and gradually enlarging mass. Most soft tissue sarcomas arise de novo, but a small percentage originate in injured tissues such as scars or radiation-exposed areas.

More than 50 subtypes of proliferative soft tissue lesions are presently defined. The classification of soft tissue tumors is regularly updated. As per the 2002 WHO classification, the soft tissue tumors are divided into 4 categories - benign, intermediate (locally aggressive), intermediate (rarely metastasizing) and malignant.

Very few neoplasms challenge the diagnostic skills of pathologists more than soft tissue tumors. An accurate histopathological diagnosis is of utmost importance in the management of soft tissue tumors and is quite a challenging task demanding good clinical and radiological correlation.

Early tissue diagnosis is one of the most important components of multimodality treatment of soft tissue tumors. Imprint cytology is a valuable tool for rapid intraoperative diagnosis of soft tissue tumors. The method is rapid, easy, reliable and does not require special equipments.

Smears prepared from fresh specimens give excellent cytological details. Scrape cytology, a modification of imprint
cytology, has better diagnostic accuracy than imprint cytology or frozen sections alone. Despite its merits like speed, simplicity and excellent cellular detail, many centers do not utilize this technique routinely.

**Objectives**

1. To assess the diagnostic accuracy of imprint cytology in soft tissue tumors by correlating with histopathology.
2. To observe whether the correlation of the cytologic features with gross pathologic features such as tumor size, location, depth, circumscription, encapsulation, and presence of necrosis improves diagnostic accuracy.

**Methodology**

Patients admitted with clinical and/or radiological diagnosis of soft tissue tumor in the Department of General Surgery, Government Medical College, Thiruvananthapuram from February 2007 to December 2008 were included in this study. From this group, 87 cases were selected in whom malignancy was suspected clinically.

Fresh specimens of soft tissue tumors obtained at the time of surgery were cut and direct imprint taken by pressing a glass slide gently on the fresh cut surface. After this, tumor tissue from the cut surface was scraped off using a clean glass slide. This scraped tissue was spread on another clean glass slide.

The imprint and scrape smears were immediately fixed in 95% ethyl alcohol for 5-6 min. Rapid hematoxylin and eosin staining was done, and smears were studied under a microscope. A minimum of three imprint smear slides and two scrape smear slides were studied per case.

The tumor tissue was then fixed in 10% formalin, processed, stained with hematoxylin and eosin and studied under a microscope. Whenever possible, histopathological findings were correlated with clinical and radiological features and immunohistochemistry. Imprint cytology and histopathology slides were compared (Table 1).

Diagnostic accuracy of imprint cytology was determined using sensitivity, specificity, and Kappa value. Ethical Committee approval was obtained from Government Medical College, Thiruvananthapuram before starting the study.

**Observations**

Intraoperative imprint cytology was performed on 87 soft tissue tumors, which were clinically suspected as malignant. Histopathological confirmation was obtained in all cases (Table 2).

Of the 87 cases, 50 were malignant; 25 benign and 7 were of intermediate grade. 5 cases turned out to be other soft tissue lesions. Soft tissue tumors in this study occurred mostly between 31 and 60 years of age (52.4%). Malignant tumors predominated between fifth and eighth decades (64%) with a peak incidence from 41 to 50 years (22%). Among the 87 cases studied, 52 were males and 35 females.

The tumors ranged in size from 1.5 cm to 33 cm. Most of them (64.4%) were ≤10 cm in size. 71% of the tumors above 10 cm in size, 80% of the tumors above 15 cm in size and all the tumors above 20 cm in size were malignant.

In this study, soft tissue tumors were most frequently encountered in the extremities (57.5%) - lower extremities (40.2%) more than upper extremities (17.2%) - followed by the trunk (21.8%) and the retroperitoneum (18.4%). The head and neck was the least common site (2.3%). Most of the retroperitoneal tumors (81.2%) and the lower extremity tumors (68.6%) were malignant.

Most of the soft tissue tumors were located in the subcutaneous plane (52.4%) followed by the muscular plane (28%). Benign tumors occurred most frequently in the subcutis (84%). Malignant tumors showed more predilection to involve the muscle plane (36%), followed by the subcutis (34%). Of note, most of the tumors located in the muscular plane (72%) and in the body cavity (88%) were malignant.

About 80% of the benign tumors were well-circumscribed. 33 out of the 50 malignant tumors (66%) and 6 out of the 7 tumors of intermediate grade (85.7%) were not circumcised. 73.3% of the poorly circumscribed tumors were malignant. Encapsulation was noted in 8 sarcomas and 6 benign tumors. 40% of the malignant soft tissue tumors showed necrosis. Necrosis was not observed in any of the benign or intermediate-grade tumors. Only 3 of the 87 cases were recurrent tumors, all of which were high-grade sarcomas.

Based on the imprint and scrape cytology smears, the 87 cases under study were broadly classified into the following 5 groups:

1. Benign
2. Atypical
3. Suspicious of malignancy
4. Malignant
5. Inconclusive

Among the 25 benign tumors, 18 could be identified as benign by cytology. Two schwannomas showing cystic change and one cavernous hemangioma could not be diagnosed by cytology as the cytological details were obscured by inflammatory cells, blood and debris. Imprint smears of one granular cell tumor, one traumatic neuroma, and one case of nodular fascitis were also inconclusive due to scanty cellularity. One case of ancient schwannoma showed moderate nuclear pleomorphism and was hence categorized as atypical.

Among the 7 soft tissue tumors of intermediate grade, one case of hemangioendothelioma and one case of fibromatosis...
were categorized as benign tumors by cytology due to the low cellularity and lack of pleomorphism in the imprint smears. One case of hemangiopericytoma and two cases of fibromatosis were categorized as atypical on account of increased cellularity and mild nuclear pleomorphism. The study also included one case of solitary fibrous tumor and one case of inflammatory pseudotumor in which the imprint smears were inadequate for a definite categorization.

Among the 50 soft tissue sarcomas, 37 could be diagnosed as malignant by cytology. Among the remaining 13 cases, five liposarcomas, three rhabdomyosarcomas, two fibromyxoid sarcomas and one fibrosarcoma showed only mild to moderate cellular pleomorphism in smears and were categorized as suspicious of malignancy. Two cases of liposarcomas showed clusters of mature adipocytes only and were incorrectly categorized as benign tumors by cytology.

In the analytical part, for the comparison between imprint cytology and histopathology, weighted kappa was calculated as a measurement of agreement between the two diagnostic measures. Diagnostic accuracy was determined using sensitivity, specificity, positive and negative predictive values, after excluding the 8 soft tissue tumors that showed inconclusive diagnosis in imprint smears, as well as the 5 non-mesenchymal lesions.

**Discussion**

Intraoperative diagnosis is an invaluable aid in the management of soft tissue tumors, especially when clinical and radiological findings are unequivocal. On table, a broad categorization into benign and malignant tumors is possible, thus assisting the surgeon to decide on the extent of resection. This study employed imprint cytology from fresh specimens for intraoperative evaluation of soft tissue tumors and found this to be a simple, rapid and economical method.

An accurate intraoperative diagnosis requires a multimodal interdisciplinary approach correlating clinical, radiological and pathologic information including gross examination, cytological and histological examination and ancillary techniques, if facilities are available. The current study correlated the cytologic features with clinical parameters like age and sex of the patient as well as gross pathologic features such as tumor size, location, depth, circumscription, encapsulation and presence of necrosis.

In the present study, though malignant soft tissue tumors were found in all age groups, most of them (64%) occurred above 40 years of age. This is comparable to a study by Fang et al on 1118 cases of primary soft tissue sarcomas, where 63.06% occurred above 40 years of age. The male to female ratio for malignant soft tissue tumors was 2:1 in this study as in the study by Bhurgri et al on 96 sarcomas, while Fang et al found the ratio to be 1.4:1. However, the sex of the patient was not helpful in separating tumors into benign and malignant.

The tumor size was more useful in this respect. In the present study, 71% of the tumors above 10 cm in size and 80% of the tumors above 15 cm in size were malignant. Assessment of the plane of the tumor was found to be a useful parameter in deciding the benign and malignant behavior of the tumors.

Most of the sarcomas occurred in deeper planes - muscle plane (36%), followed by the subcutis (34%), whereas, 84% of the benign tumors involved the subcutaneous plane. By comparing clinical data for benign tumors and sarcomas, Rydholm A et al also found that a tumor 5 cm or larger in a deep tumor is relatively more likely to be a sarcoma.

The lower extremity (48%) was the most favored site for sarcomas. 81.2% of the retroperitoneal soft tissue tumors were malignant. Most of the malignant soft tissue tumors in this study were ill circumscribed (66%) and non-encapsulated (84%). Tumor necrosis was observed only in sarcomas, not in benign tumors. Gustafson P propose tumor necrosis as a strong and reliable factor that can be used to improve prognostic accuracy.

All the 87 cases selected in the present study were those clinically suspected to be malignant soft tissue tumors. Among them, 25 turned out to be benign tumors. There were 50 malignant soft tissue tumors consisting predominantly of liposarcomas, pleomorphic sarcomas, and malignant peripheral nerve sheath tumors.

Benign spindle cell tumors formed the largest category of the non-malignant tumors (21 cases). Among them, 12 were neural tumors. Correlating with the gross features, a possible neural origin could be suggested in 6 cases, but subtyping of the spindle cell lesions by cytology alone was difficult. This has been well recognized as a limiting factor in other studies such as Rekhi et al.

All the pleomorphic, round cell and myxoid type of tumors in this study were sarcomas. This is in accordance with the study on fine-needle aspiration cytology of soft tissue tumors by Rekhi et al.

Of the 82 soft tissue tumors studied, 55 (18 benign and 37 malignant tumors) were correctly categorized by imprint cytology as benign/malignant. A definitive categorization into benign or malignant was not possible in 13 cases. These were classified into 3 groups - atypical, suspicious of malignancy and inconclusive (Table 4).

Weighted kappa statistics showed substantial agreement between imprint cytology and histopathology (0.78) (Table 3). Imprint cytology showed a high sensitivity (92.7%) and specificity (94.7%) (Table 5). In comparison, imprint method was reported to have an accuracy of 95.5% by Sakai and Lauslahti, 93.8% by K C Suen et al and 98.4% by Shidham VB et al.
malignant as in the case of a large ill-circumscribed tumor with areas of necrosis and hemorrhage, imprint cytology confirms the pathologist’s gross impression.

On the other hand, 2 sarcomas and 2 intermediate grade tumors in the present study were wrongly categorized as benign by cytology. A negative imprint, therefore, does not necessarily exclude malignancy. Imprint smears should always be reported after careful correlation with gross pathological findings. If imprint smears of a grossly malignant appearing lesion are negative, situations that can result in a false-negative report should be carefully considered and ruled out before issuing a negative cytological report.

False-negative reports can occur in cytologically well-differentiated tumors, for example in well differentiated liposarcomas as in the present study, where the morphological changes of the neoplastic cells are often subtle. Also, in tumors with a dense fibrous stroma, large areas of necrosis, hemorrhage and/or cystic change, the cell-yield in imprint smears is often inadequate to provide an accurate diagnosis. In such situations, an erroneous interpretation can be avoided by careful correlation with clinical and radiological features as well as gross pathologic features such as size, location, depth and circumscription of the tumor and presence of necrosis.

The present study is in agreement with Marilyn M Bui et al in that cytology alone can accurately diagnose certain entities. Marilyn M Bui et al states that imprint smears are better than

Table 1: Cytohistological Correlation

<table>
<thead>
<tr>
<th>Tumor type by histopathology</th>
<th>Benign</th>
<th>Atypical</th>
<th>Suspicious</th>
<th>Malignant</th>
<th>Inconclusive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Malignant</td>
<td>2</td>
<td>0</td>
<td>11</td>
<td>37</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>4</td>
<td>11</td>
<td>37</td>
<td>8</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 2: Tumor types by histopathology

<table>
<thead>
<tr>
<th>Benign</th>
<th>Intermediate</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurofibroma (6)</td>
<td>Haemangioendothelioma (1)</td>
<td>Liposarcoma (12)</td>
</tr>
<tr>
<td>Schwannoma (5)</td>
<td>Fibromatosis (3)</td>
<td>Pleomorphic sarcoma (9)</td>
</tr>
<tr>
<td>Leiomyoma (3)</td>
<td>Inflammatory pseudotumor (1)</td>
<td>Malignant peripheral nerve sheath tumor (8)</td>
</tr>
<tr>
<td>Benign fibrous histiocytoma (3)</td>
<td>Solitary fibrous tumor (1)</td>
<td>Myxofibrosarcoma (5)</td>
</tr>
<tr>
<td>Granular cell tumor (2)</td>
<td>Haemangiopericytoma (1)</td>
<td>Rhabdomyosarcoma (4)</td>
</tr>
<tr>
<td>Nodular fascitis (1)</td>
<td>Synovial sarcoma (3)</td>
<td></td>
</tr>
<tr>
<td>Cellular myxoma (1)</td>
<td>Alveolar soft part sarcoma (2)</td>
<td></td>
</tr>
<tr>
<td>Traumatic neuroma (1)</td>
<td>Ewing’s sarcoma (2)</td>
<td></td>
</tr>
<tr>
<td>Lipoma (1)</td>
<td>Leiomyosarcoma (2)</td>
<td></td>
</tr>
<tr>
<td>Intramuscular lipoma (1)</td>
<td>Fibromyxoid sarcoma (2)</td>
<td></td>
</tr>
<tr>
<td>Cavernous haemangioma (1)</td>
<td>Fibrosarcoma (1)</td>
<td></td>
</tr>
<tr>
<td>Total: 25</td>
<td>Total: 7</td>
<td>Total: 50</td>
</tr>
</tbody>
</table>

Number of cases given in brackets

Table 3: Analysis of imprint cytology

<table>
<thead>
<tr>
<th>Imprint cytology</th>
<th>Histopathology</th>
<th>Benign</th>
<th>Intermediate</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Atypical/suspicious/inconclusive</td>
<td>7</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td></td>
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</table>

Weighted Kappa = 0.78 (There is 78% agreement between imprint cytology and histopathology)

Table 4: Statistical Analysis

<table>
<thead>
<tr>
<th>Imprint cytology</th>
<th>Histopathology</th>
<th>Malignant &amp; intermediate</th>
<th>Benign</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (malignant/suspicious/atypical)</td>
<td>51</td>
<td>1</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Negative (benign)</td>
<td>4</td>
<td>18</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>19</td>
<td>74</td>
<td></td>
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</table>

Table 5: Diagnostic accuracy of imprint cytology

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92.7%</td>
<td>94.7%</td>
<td>98.1%</td>
<td>81.8%</td>
</tr>
</tbody>
</table>

The low false-positive rate observed in this study indicates the reliability of imprint smears in diagnosing malignant soft tissue tumors accurately. In instances when a lesion is grossly
the frozen section for myxoid and adipocytic tumors. However, imprint cytology may not be as resourceful in diagnosing tumors having decreased cellularity or increased fibrosis. Therefore, the accuracy of intraoperative diagnosis can be enhanced by employing imprint cytology and frozen section together.20,28

Imprint cytology can also be utilized to provide samples for ancillary studies and also to ensure the adequacy of a biopsy sample.

Conclusions

Imprint cytology serves as an invaluable tool in the intraoperative evaluation of soft tissue tumors. There is substantial agreement (weighted kappa = 0.78) between imprint cytology and histopathology.

A diagnosis of malignant soft tissue tumor by the imprint smear method is highly reliable. However, intraoperative diagnosis of soft tissue tumors by imprint cytology is preliminary and warrants confirmation.

A negative diagnosis by imprint cytology does not necessarily exclude malignancy.

An interdisciplinary approach by correlating clinical, radiological, and pathological information can enhance the accuracy of diagnosis by imprint cytology.

End Note

Author Information

1 Dr. Lakshmy M R, Assistant Professor, Department of Pathology, Employees’ State Insurance Corporation Medical College Hospital, Parippally, Kerala, India. Email: lakshmymr1980@gmail.com

2 Dr. Krishna Balachandran, Professor, Department of Pathology, Government Medical College, Thiruvananthapuram, Kerala, India

Conflict of Interest

None declared.

References


