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Original Research Article

Evaluation of the Role of Imprint Cytology and Frozen Section in Soft Tissue Lesions with Histopathological Correlation

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Abstract

Introduction: Frozen section and imprint cytology are important intraoperative diagnostic procedures. Performance of frozen section is useful in determining the type of neoplasm, degree of malignancy and the adequacy of surgical margins. Imprint cytology has also been shown to be quite reliable in diagnosis of various soft tissue tumours. The tissue architecture seen in frozen section is close to the sections made in histopathology while imprint cytology provides a better cellular detail of tissue. Both the procedures show high degree of accuracy, in this study we have planned to compare the accuracy of imprint cytology and frozen section using histopathology sections as gold standard for soft tissue lesions.

Materials and Methods: This was a prospective study of 60 cases of soft tissue lesions which were submitted to department of Pathology at a tertiary care teaching hospital for a period of 18 months. Frozen section (FS) and Imprint smears (I) were performed on fresh, unfixed specimens along with macroscopic evaluation. The results of frozen section and imprint cytology were analyzed by comparing to the results of gold standard histopathological section results.

Results: Out of 60 cases, 59 were benign and only 1 case was of intermediate grade**.** Among all the lesions, lipoma was the commonest of all the soft tissues lesions (61.7%). The male: female ratio was found to be 1.5:1. Frozen section and imprint smears were performed on all the soft tissue lesions and showed a diagnostic accuracy of 98.33% and 91.67% respectively. When both these procedures were used in combination, the overall diagnostic accuracy was 98.33%. Kappa statistics calculated was 0.964 which showed an almost perfect agreement between imprint, frozen section and histopathology diagnoses on lipoma lesions.

Conclusion: In our study, it was observed that diagnosis of soft tissue tumors can be best appreciated on imprint smears and frozen sections when performed in conjunction.

Keywords: Soft Tissue Lesions, Frozen Section, Imprint Smears, Diagnostic Accuracy.

Introduction

Soft tissue is represented by fibrous tissue, adipose tissue, skeletal muscle, blood vessels, lymphatic vessels and peripheral nervous system. Many disease processes can involve soft tissue such as infective process, tumour and tumour like conditions. Soft tissue lesions arise from various components. So the morphology will be highly variable. To complicate this further many tumour like conditions are also identified in the soft tissues which can clinically and pathologically simulate a neoplasm.

Frozen section and imprint cytology are important intraoperative diagnostic procedures. Performance of frozen section is useful in determining the type of neoplasm, degree of malignancy and the adequacy of surgical margins.[1]

Imprint cytology has also been shown to be quite reliable in diagnosis of various soft tissue tumours. The tissue architecture seen in frozen section is close to the sections made in histopathology while imprint cytology provides a better cellular detail of tissue. Both the procedures show high degree of accuracy, however there are limitations for frozen section like proper grading cannot be given. From the studies reported bone and soft tissue lesions constitute a small percentage of the total lesions.[2,3]

In this study we tried to compare the diagnostic accuracy of frozen section and imprint cytology results in conjunction with histopathology diagnosis on routine paraffin section which is the gold standard for soft tissue lesions.

Materials and Methods

This was a prospective study of 60 cases of soft tissue lesions which were submitted to department of Pathology at a tertiary care teaching hospital for a period of 18 months. A total number of 60 cases were included in the study after obtaining ethical clearance. All the surgical specimens excised from soft tissue location were included in the study with the exclusion of true cystic lesions with epithelial lining.

The specimens were taken in an unfixed state immediately after surgery. The gross evaluation with macroscopic appearance was documented and imprint smears were taken from the cut section. These smears were stained with Toluidine blue, May-Grunwald-Giemsa and Papanicolaou stains. The cytologic diagnosis was given based on these smears.

A fresh slice of tissue was given for frozen section (FS). The tissue submitted for FS analysis was sectioned, embedded and frozen immediately within a cryostat and cut into 8 microns sections and these sections were stained with Hematoxylin and Eosin and diagnosis was given. Oil red O special stain was performed on the frozen sections of adipocytic tumors.

Remaining tissue was processed to get paraffin blocks and these sections were stained with Hematoxylin and Eosin and examined under a microscope. The findings of imprint and frozen section were compared with the routine histopathological sections which is gold standard.

The frequency of occurrence of parameters was represented in percentage, descriptive statistics was done to describe the study data and diagnostic accuracy of frozen section and imprint smears in comparison to histopathological sections was calculated using Chi-Square test. SPSS version 24 was used.

Results

Out of 60 cases, 59 were benign and only 1 case was of intermediate grade. Frozen section was performed on the cases received following which the imprint smear was taken, before exposing the specimen to fixation and its cytology was analyzed.

Among all the lesions, lipoma was found to be the commonest lesions (61.7%) followed by neurofibroma (15.00%) and schwannoma (6.67%). The other lesions encountered were cases of fibrous histiocytoma, lobular capillary haemangioma, fibroma, aggressive angiomyxoma, hypertrophic scar and giant cell tumour of tendon sheath.

Frozen section of these lesions and imprint smears were analysed and their diagnosis correlated with the histopathology which was gold standard, exceptions being 2 benign cases of lobular capillary haemangioma which was diagnosed as inconclusive in imprint smear diagnosis in respect to the scant cellularity and 1 intermediate case of angiomatoid fibrous histiocytoma which was diagnosed as benign spindle cell tumour in imprint diagnosis and as fibrous tumour in frozen section diagnosis.

In the present study soft tissue lesions were found to be the most common in the 4^{th,} 5th and 6th decades of life (71.7 %). There was a single case of intermediate grade (angiomatoid fibrous histiocytoma) in the age of 5th decade of life.

In our study, the overall soft tissue lesions encountered were in the 30-65 years followed by age group of 18-30 years. Lipomas were found occurring in the age group of 30-65 years followed by occurrence after 65 years as well as 18-30 years.

Neurofibroma was seen most common in the age group of 30 to 65 years of life followed by occurrence in the age group of 18-30 years. It is the 2nd most common soft tissue lesion after lipoma in the present study.

The soft tissue lesions were deep seated (78.33%) followed by superficial location (21.7%) in the present study.

The secondary changes seen in the frozen section of soft tissue lesions most commonly are nil in this study. The secondary changes next most commonly found were presence of red blood cells and fibrous septae which are encountered in cases of lipoma and of fibrolipoma. The presence of only red blood cells was found in lipoma, neurofibroma, fibrous histiocytoma and angiolipoma. The giant cell finding was preserved well in the frozen section of giant cell tumour of tendon sheath.

The secondary changes encountered in lipoma on frozen section were nil (50.00%). The other secondary changes found in lipoma were presence of RBC and fibrous septae (25.00%). Apart from identifying the morphology, the demonstration of fat in the adipocytes was also possible on frozen sections.

The other findings on imprint cellularity were evaluated for all soft tissue lesions in the present study. Lipoma which constituted the majority of soft tissue lesions showed nil findings most commonly (92.86%) followed by presence of RBC (3.57%) and blood vessels (3.57%). Verocay bodies were found in all the schwannoma imprints which were not seen in any other soft tissue lesions.

There was presence of mast cells and its combination with nerve twigs in neurofibromas imprints in this study which constituted 77.78% of the overall other findings. Mast cells alone was encountered in cases of neurofibroma which made up 22.22% of overall other findings. These findings were not observed in lesions other than neurofibroma.

The most common architecture found in frozen section is diffuse type. The other architectures found are fascicles, lobules and storiform types on frozen section. The diffuse type of architecture was found most commonly in lipomas (35) followed by neurofibromas (8). The fascicles architecture on frozen section was found most commonly in schwannomas(4) followed by neurofibromas (1) and Giant cell tumour of tendon sheath (1). The lobular architecture was commonly found in lobular capillary haemangiomas (2) followed by lipomas (1).

High cellularity is found in the majority of the imprint smears (51.67%) followed by moderate cellularity (40.00%) and scant cellularity (8.33%). High cellularity on imprint smears was found in lipomas (14) followed by neurofibromas (6) and fibrolipomas (5). Moderate cellularity was shown in 13 cases on imprint smears of lipoma and fibrolipoma (2) and neurofibroma (2) and fibrous histiocytoma (2). There was scant cellularity in imprint smear of giant cell tumour of tendon sheath. Among the benign vascular tumours, lobular capillary haemangiomas showed scant cellularity and aggressive angiomyxoma showed moderate cellularity.

There were various patterns found in the present study wherein the most common pattern observed was tight clusters which was common in lipomas (28). There were 8 cases of neurofibromas seen with tight clusters pattern on imprint smears. The next common pattern observed on imprint smears was branching clusters which was seen in peripheral nerve sheath originating tumours predominantly in schwannomas (4) and neurofibromas (1). The remaining imprint patterns seen were combinations of clusters with scattered singles seen in lobular capillary haemangioma (2).

Results of Imprint cytology in all soft tissue cases

Imprint cytology yielded a diagnostic accuracy of 91.67% in the study of all the cases. The total errors shown were 8.33% in all cases. Among 37 cases of lipoma on imprint were concordant with 37 cases of lipoma on histopathologic evaluation.

1 case on imprint (lipoma) was not concordant with its histopathologic diagnosis (neurofibroma). Kappa value is 0.964(almost perfect agreement between Imprint and Histopathology diagnoses). p value is less than 0.001 which is significant. Frozen section yielded a diagnostic accuracy of 98.33% in the study of all the cases. The total errors shown are 1.67%. in all cases.

When the frozen section method and imprint cytology are used in combination, the diagnostic accuracy is 98.33% in our study because one intermediate grade lesion was missed on both the imprints and frozen sections and misdiagnosed as benign spindle cell lesion on imprint and benign fibrous histiocytoma on frozen section. All the frozen sections on lipoma cases were concordant with histopathologic evaluations. Kappa value is 0.964 (almost perfect agreement between frozen section and histopathology). The p value is less than 0.999.

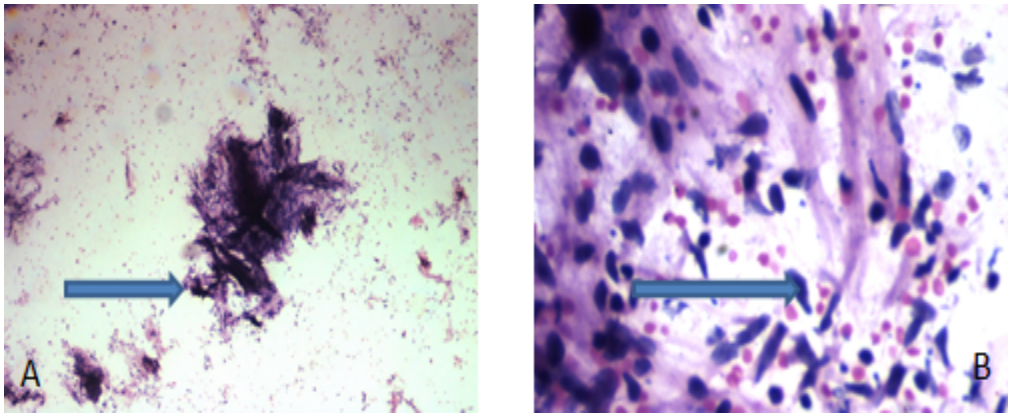

Figure 1: Imprint cytology of scwannoma

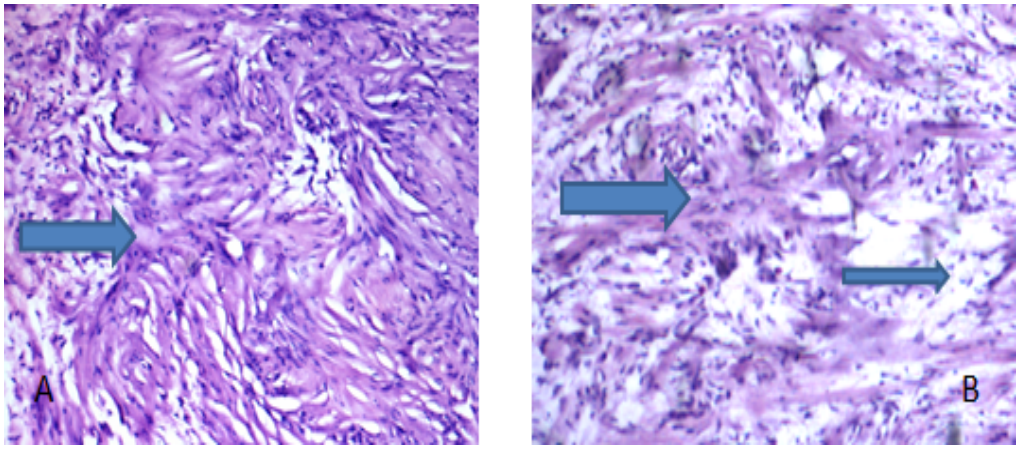

Figure 2: Frozen section of schwannoma

Discussion

Evaluation of gross and microscopic features of soft tissue neoplasms is one part of pathologic assessment for management of the patient. During the intraoperative consultation for on table diagnosis, the frozen section of tissue specimen along with gross findings and touch imprints enables the surgeon to know the diagnosis rapidly, assess if the surgical margins are free from tumour, see the extension of invasion and adequacy of specimen.[4] In this context, review of literature and present study validates the role of frozen section and imprint smear in this regard.

Among the 60 soft tissue lesions received, our results showed a vast majority (59 cases) of these to be benign and the remaining minority to be intermediate tumours (1). Studies conducted by Kransdorf *et al*.[5] and Batra *et al*.[6] have categorised the soft tissue tumours as a whole on the basis of gender, age distribution and distribution of benign and malignant lesions.

In our study, only benign lesions were observed in females. Even in male patients except for one intermediate soft tissue lesion, other lesions were benign. Total 28 soft tissue lesions were encountered in females and the remaining 32 soft tissue lesions were encountered in males. Male: Female ratio in our study was found to be 1.5:1 which shows a higher male preponderance compatible with the study by Kransdorf *et al*.[5] and Batra *et al*.[6]

Coming to distribution of soft tissue lesions our observations slightly varied due to different sample size. Lipoma was the most common lesion of all soft tissue lesions in our study followed by neurofibroma which was compatible with the study by Batra *et al*.[6]

Suen *et al*[7] had an overall diagnostic accuracy rate of 93.6% in imprint procedures. The accuracy rate of frozen sections alone was not evaluated due to the imprint findings given priorly. The combination of both methods had given an overall diagnostic accuracy of 98.3% with 1.7% false negatives and no false positives. The diagnostic accuracy yielded when used in combination was higher when compared to the accuracy given when imprint is used alone. Thus the author recommends using both the methods in conjunction.

Mair *et al*.[8] reported accuracy with each method (frozen section and imprint) as 97.6% with no significant difference between the methods results. They found that quality of cytological preparation was superior to the frozen section made. However, after combining both the techniques found that the diagnostic accuracy was increased to 100%. Through their study, it was shown that cytologic and frozen section analysis are

complementary to each other rather than competitive techniques, thus to be used as adjuncts to each other for offering a correct diagnosis.

In our study, the diagnostic accuracy achieved on imprints when performed alone for overall soft tissue lesions, yielded 91.67% accuracy which is correlating with the study conducted by Tabei *et al*[9] and Suen *et al*[7] wherein lesser number of cases were taken for their study. Thus there are variations in the observations made in our study because of differing sample sizes.

The diagnostic accuracy found when frozen section is performed alone for overall soft tissue lesions, gave 98.33% accuracy which is similar to the study done by Guarda *et al* [10] and Mair *et al*[8].

In our study, frozen section had shown technical errors like freezing artefacts which was encountered in 2 cases of neurofibroma and 2 cases of fibrous histiocytoma, thus making the interpretation difficult. These artifacts can be avoided by combining the usage of imprint cytologic evaluation in order to yield a higher diagnostic accuracy. Frozen section had an added advantage of sub catgeorisation of lipomas and also subtyping the benign fibrous and neural tumours which was not possible on imprint smears.

Imprint cytology, however, lacks the preservation of architecture which is well maintained on frozen sections. This was observed in our study when imprint evaluation could not assess the lobular architectures of capillary hemangioma which was appreciated on frozen section. Due to the presence of hemorrhagic background and scant cellularity, they were non diagnosable on imprints but were diagnosed on frozen section and confirmed by histopathology evaluation. Thus this shows that a negative imprint does not necessarily exclude a diagnosis.

Also from our observations it was found that the presence of mast cells, nerve twigs and tight clusters with fibrillary background was more in favour of neurofibroma whereas verocay bodies and branching clusters in a fibrillary background were in favour of schwannoma on imprint smears.

Limitations

Various limitations have been encountered during the course of our study like shrinkage of tissue, not sampling the representative areas and limited clinical details. While performing the touch imprint of giant cell tumour of tendon sheath, it was difficult because of its firm to hard consistency nature, due to which getting adequate material was taken with the help of scraping which is a modified method of imprint cytology. The tissue was folded on frozen section of giant cell tumour of tendon sheath which is another limitation observed during our study.

Conclusion

Through our study it was observed that diagnosis of soft tissue tumours can be best appreciated on imprint smears and frozen sections when done in conjunction. The cellular details and background were better observed on imprint smears. Frozen section yields better results with respect to the architecture and preservation of morphology when compared to imprint smears.

Both techniques have limitations in diagnosis of intermediate grade tumours and they complement each other wherever difficulty in interpretation was encountered. When both the procedures were used together diagnostic accuracy of 98.33% could be achieved as per our study.

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