

Case Report

CORRELATIVE STUDIES ON IMPRINT SMEAR FINDING WITH HISTOPATHOLOGICAL FINDING IN VARIOUS LYMPHADENOPATHIES

***Mamta Jain¹, Manju Raghav² and Abhishek Jain³**

¹*Department of Pathology, Government Medical College, Kota*

²*Department of Pathology, Metro Mass Hospital, Jaipur*

³*Department of E.N.T., Jain ENT Hospital, Kota*

**Author for Correspondence*

ABSTRACT

Imprint cytology is proved to be rapid and inexpensive tool in diagnosis of various lymph node lesions. The present study was conducted to correlate diagnostic accuracy, sensitivity, specificity of imprint cytology of various lymph node lesions with histopathological diagnosis. 50 cases were included, imprint smear were taken, stained with H&E and geimsa and then compared with histopathological diagnosis. When compared with histopathology imprint smears showed overall sensitivity of 88.15%, specificity 97.52%, diagnostic accuracy 96%, positive & Negative predictive value 95.65% & 96.23% respectively. The imprint smear technique is simple, rapid and did not require sophisticated instruments. The smears showed almost perfect agreement in majority of the lymph node lesions and hence, can be used routinely as an adjunct to histopathology.

Keywords: *Imprint Cytology, Lymph Node, Tubercular, Reactive Lymphnode, Metastatic Lymph Node*

INTRODUCTION

Lymph node enlargement is a common clinical problem which frequently requires a biopsy to establish a diagnosis. Lymphadenopathy may be primary or secondary manifestation of numerous disorders. The causes of lymphadenopathy include infection (both local and systemic), autoimmune and neoplastic disorders (either primary or metastatic).

The imprint cytology has been used as a rapid and standard method for diagnosing lymphadenopathies since several years.

Since the past imprint cytology was mainly used as an adjunct to histopathological diagnosis. Imprint cytology is a special variation of applied cytology that can be used for various purposes (Khalid and Haque, 2004). The cytological study of lymph node lesions is best achieved by examining imprint smear and contribute much to understanding and implicitly diagnosing them.

Lymph node touch imprints are also suitable for analysis of immunophenotype, morphometry and DNA ploidy. The advantages of the imprint technique include ease of preparation and preservation of cellular morphology, although, it is said to introduce selective bias in favour of cells which are more adhesive to a glass slide.

As a diagnostic method its position lies between cytological smear technique and routine histopathological section (Iochin and Ratech, 2002).

Imprint cytology is important tool for diagnosis of various lesions as it is rapid, inexpensive and very useful while waiting for histopathological report especially in cases of malignancies where diagnosis is urgently needed. Lymphnode lesion is important because of large number of lesions both infective and malignancies are associated with it.

Fine needle aspiration cytology is widely used modality nowadays but chance of failure is also noted mainly due to inexperience hand, further it is noted that, there is difficulty in aspirating deep lymph node. Final diagnosis also cannot be made on FNAC, especially, in cases of malignancies where excisional biopsies are needed in all such cases. Thus, an imprint smear can be useful in such cases where excisional

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biopsy was taken and patient is waiting for histopathology report which usually takes 3 – 5 days in good setup (Arif *et al.*, 2011).

Imprint cytology is a technique that complements the tissue sections and is too often neglected in the examination of touch preparation from the cut surface of the fresh lymph node stained with Geimsa or Wright solution. This is particularly useful in the evaluation of lymphoma & leukemia & in the initial triage of specimen (such as sending tissue for culture if granuloma are seen). For instance, granulocytic leukemia can closely simulate large cell lymphoma in a H&E stained section, but an imprint will readily distinguish the two conditions (Rosai and Acherman, 2011).

Touch imprints are easily prepared from unfixed lymph nodes received in the laboratory for frozen section or as soon as possible after excision. Dignostic imprints in some cases can preclude the need for frozen sections, thereby saving tissue for routine diagnosis, ancilliary studies, or tissue banking (Iochin and Ratech, 2002).

The Usefulness of TIC is not limited to simple differentiation between benign and malignant lesions. TIC has been found quite reliable and useful in determination of surgical resection margins, sentinel lymph nodes, adenomatous goiter and confirmation of parathyroid tissue.

TIC reveals crisper cytological details. TIC has further advantage of being inexpensive, simple and quicker than frozen section (Khalid and Haque, 2004).

Because imprint smears sample the entire surface of the specimen, is less time consuming and avoids the issues of specimen loss and freezing artifacts, it is gaining popularity as a technique for intra operative evaluation of margin status.

Intra operative pathologic evaluation of margin status may be useful to reduce the need for re excision. Intra operative imprint smear are the key tools to assess the margin status during surgery. Re excision of the margin can be performed during same surgery if imprint smear are positive, thus, avoiding a second surgery.

Intra-operative assessment of the sentinel lymph nodes allows immediate and therapeutic completion of axillary dissection during the same anaesthetic session if nodes are found to be tumour-positive. In addition, delayed axillary clearance after sentinel lymph node biopsy can be technically challenging with the potential risk of increased morbidity (particularly infection and seroma) and lower overall nodal yield. Intra-operative touch imprint cytology (TIC) will help by avoiding the economic and psychological costs of a second operation and prevents further delay in the commencement of adjuvant systemic treatment, chemotherapy and radiotherapy for high-risk patients with confirmed node involvement (Jahromi *et al.*, 2009).

Despite its simplicity, speed, and excellent cellular detail, we believe many centres are still not utilizing the technique to its fullest extent. The lack of quantitative analysis of large series of imprint materials in the literature prompts us to report our experience with this method.

MATERIALS AND METHODS

This study was conducted in Department of Pathology, Mahatma Gandhi Medical College & Hospital, Jaipur, Rajasthan from May 2011 to August 2012. In this study 50 patients with Lymphadenopathy were selected and all of them were subjected to Imprint smear cytology and Histopathology.

Before putting the specimen into 10% formaline, it was sliced into two halves. The excess of blood or serum from the cut surface is blotted gently. The sliced half is held gently either with forceps or with one hand so that the flat cut surface faces upwards; with the other hand a grease free clean glass slide was smoothly rolled at constant speed without pulling, over the cut surface. Thus, two to three imprint smears were prepared. The lymph node is then fixed in neutral formalin and processed for histopathological examination.

The imprint smears were prepared from 50 freshly excised lymph nodes. Smears were fixed in 95% alcohol and stained with Hematoxylin & Eosin and dry smear were stained with MGG. Then, compared

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with the histopathological sections and results were statistical analyzed in terms of Sensitivity, Specificity, Diagnostic Accuracy, Positive Predictive Value & Negative Predictive Value.

RESULTS AND DISCUSSION

The observations laid during the course of the study were as follows:

Depending on the cytomorphological features in the imprint smear the lesions were grouped into four main types like: tubercular lymphadenitis, Chronic Non Specific lymphadenitis, Metastatic lymphadenopathy & Lymphoma (Table 1).

Table 1: Incidence of Lymphadenopathy

Disease	No. of Patients	% Age of Patients
TB	19	38
Chronic Non Specific	17	34
Meta Adeno Ca	6	12
Metastatic	4	8
NHL	3	6
Lymphoma	1	2

In our study 72% cases were of inflammatory lesion which includes tubercular lymphadenitis in 38% cases (Figure 1), chronic non specific lymphadenitis in 34% cases, followed by metastatic tumor 20%, which include 12% cases of adenocarcinoma and 6% cases of squamous cell carcinoma (Figure 2). Primary tumors which include both Hodgkin lymphoma and Non Hodgkin Lymphoma account for 8% cases.

Age group ranged between 4 years to 70 years. Metastatic lesions were seen in older age group where as in others there was no age predilection.

The male to female ratio in this study was 1.38:1. Cervical lymph node was the most common site of presentation comprised of about 52% of cases.

In the present study results of imprint smear corrected quite well with histopathology reports (Table 2). 18 cases were diagnosed as TB on touch imprint smear, but this turned out to be 19 cases on histopathology. One case which was misdiagnosed as TB was reported as chronic non specific lymphadenitis.

Out of 17 cases of chronic non specific lymphadenitis on touch imprint smear 15 cases were confirmed on histopathological evaluation; one case was diagnosed as lymphoma and other one as TB.

All the 10 cases of metastatic carcinoma and 3 cases of lymphoma were correctly diagnosed on imprint smear. Two inconclusive smears were reported as TB & chronic non specific lymphadenitis (one each).

Table 2: Correlation of Imprint Cytology and Histopathology

S. No	Imprint Diagnosis	Smear	No. of Cases by Imprint	Histopathology Diagnosis				
				TB	Chronic Non Specific	Metastasis	Lymphoma	
1	TB		18	17	1	-	-	
2	Chronic non specific		17	1	15	-	1	
3	Metastasis		10	-	-	10	-	
4	Lymphoma		3	-	-	-	3	
5	Inconclusive		2	1	1			
	Total		50	19	17	10	4	

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Statistical Analysis

Statistical Analysis in terms of Sensitivity, Specificity, Diagnostic Accuracy, Positive Predictive Value & Negative Predictive Value has been shown in table 3.

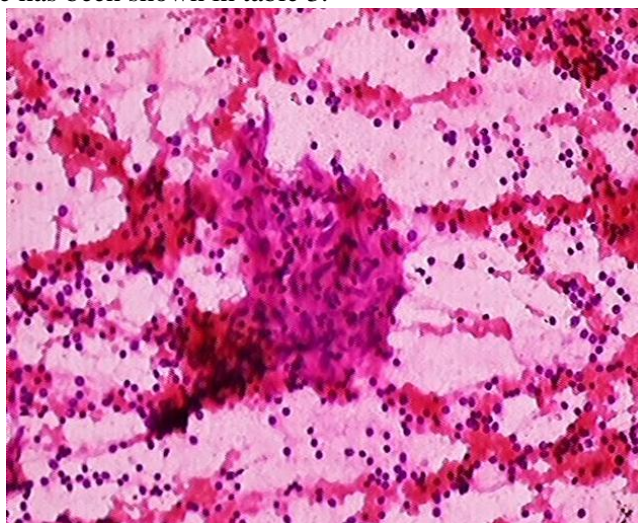


Figure 1: Imprint Smear Revealing Formation of Granuloma- Tubercular Lymph Node

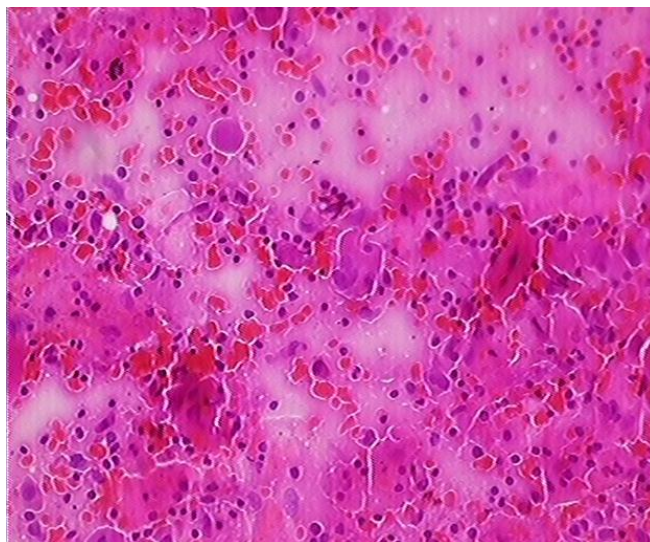


Figure 2: Imprint Smear Shows Keratinized Squamous Cells- Metastatic Squamous Cell Carcinoma

Table 3: Statistical Analysis

S. No.	Diagnosis	Sensitivity	Specificity	Diagnostic Accuracy	Positive Predictive Value	Negative Predictive Value
1	TB	89.4	96.7	94	94.4	93.75
2	Chronic non Specific	88.2	93.4	92	88.2	93.4
3	Metastasis	100	100	100	100	100
4	Lymphoma	75	100	98	100	97.8
	Total	88.15	97.52	96	95.65	96.23

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The overall percentage accuracy of 96 was observed. The percentage accuracy obtained for Metastatic nodes and for NHL was 100, where as for tubercular lymphadenitis was 94, for chronic non specific lymphadenitis was 92.

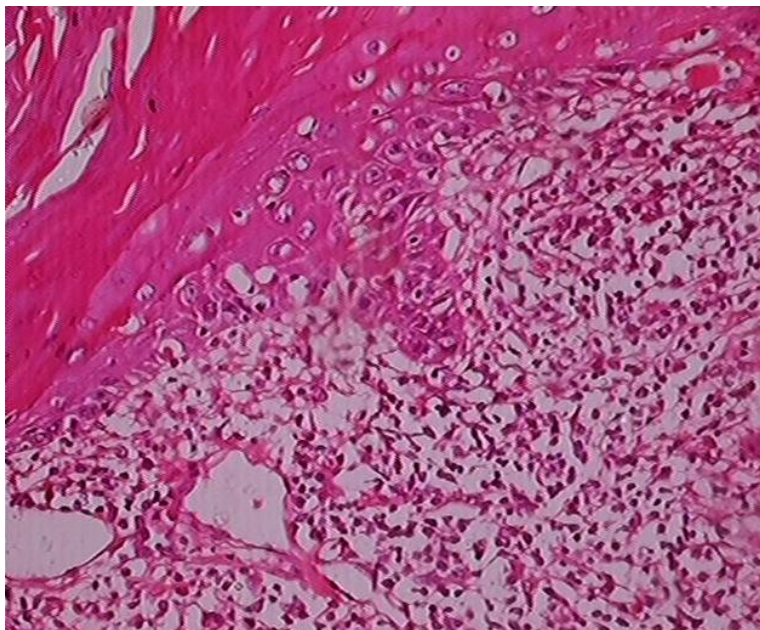


Figure 3: Squamous Cell Carcinoma (H & E Stain)

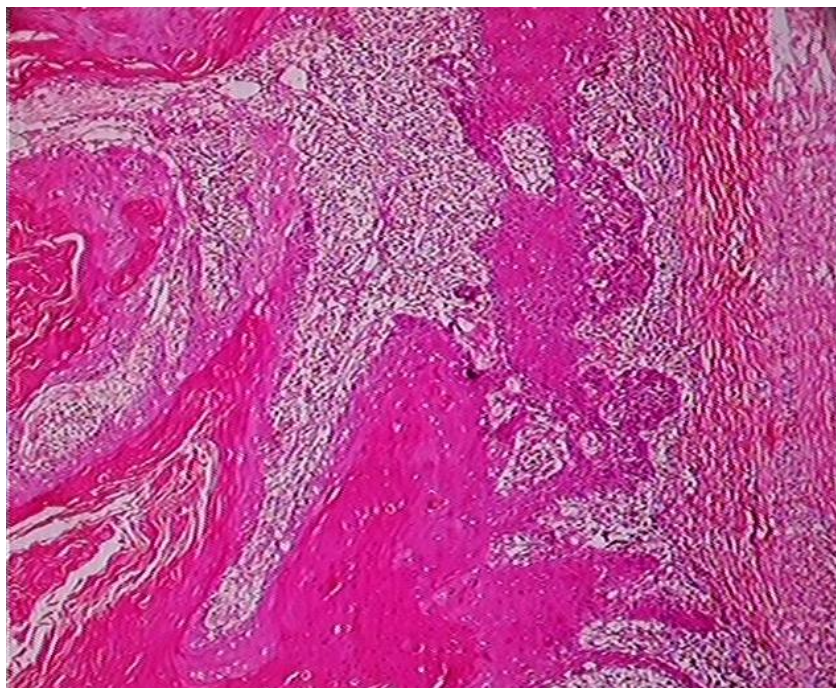


Figure 4: Squamous Cell Carcinoma (H & E Stain)

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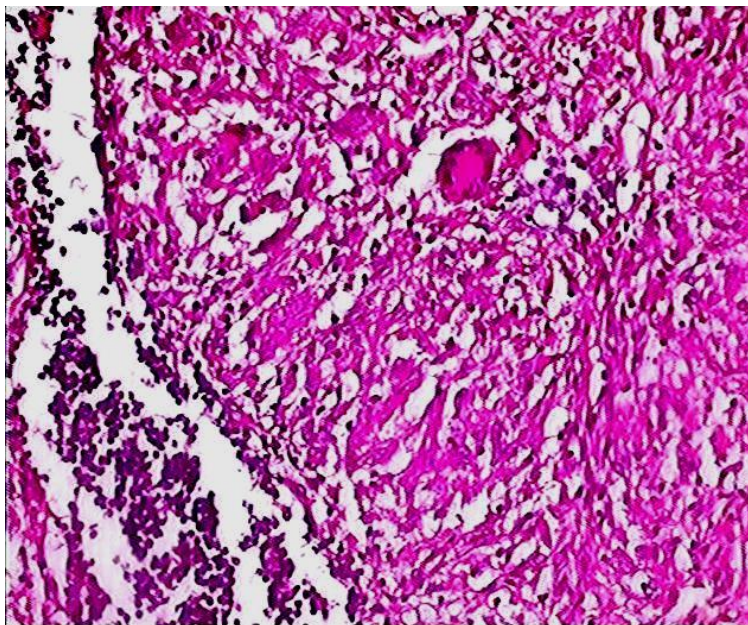


Figure 5: Tuberculosis (H& E Stain)

Discussion

Imprint cytology is a type of applied cytology. The imprint smears reveal very subtle diagnostic changes in the various lymphoid cells and make it possible to arrive at a diagnosis in shorter period of time. Imprint smear study is simple, speedy and provides excellent cytomorphological details.

Forkner (1927) introduced the imprint cytology technique of cytodiagnosis for various lesions of excised lymph nodes.

In our study highest incidence was of tuberculosis followed by reactive lymphnoditis which is comparable to the incidence found in other studies Hussain *et al.*, (2008).

The maximum number of patients was in the age group 11 to 20 years which is in accordance with the study conducted by Arif *et al.*, (2011).

Males predominated in the present study with the male to female ratio in this study was 1.381. The study done by Patra *et al.*, (2003) showed a male population of 61% and female population of 39%. Arif *et al.*, (2011) found male female ratio 1.1:1.

In present study, we found that cervical chain of lymph nodes were involved most commonly in primary tumors and inflammatory lesions whereas axillary group of lymph nodes were often secondary to breast carcinoma which is in accordance with study done by Arif *et al.*, (2011).

The overall accuracy rate among 50 cases was 96%. The range for overall accuracy rate in literature varied from 66% to 100%. Our result is comparable with those of Patra *et al.*, (2003), Nagpal *et al.*, (1982) and Arif *et al.*, (2011), where the accuracy ranged from 92 to 98%.

The overall specificity and sensitivity in present study is 97.52% and 88.15% respectively which is in accordance with Clark *et al.*, (1994).

One case of tuberculous lymphadenitis missed by imprint was diagnosed as reactive. The corresponding histological section of the same case showed fibrosis and a very few noncaseating granulomas. Ademiluyi *et al.*, (1986) came across difficulty in diagnosing tuberculosis by imprints. He observed an accuracy of 31%. The main reason attributed was possibility of disruption of the granuloma during the process of imprinting.

One case of tuberculous lymphadenitis was found to be inconclusive as smear had necrosis only and AFB staining was also negative. The histopathology also had marked areas of necrosis. The lymph nodes which had marked necrosis were likely to limit the transfer of cells from cut surface of the lymph node on to the slide Molyneux *et al.*, (1997).

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One case was misdiagnosed as TB as we misinterpreted histiocytes as epithelioid cell, on histology showed sinus histiocytosis.

The accuracy rate in NHL was 100% in our study as we could able to diagnose all the three cases correctly. This is comparable with different studies, Agrawal *et al.*, (1997), Nagpal *et al.*, (1982), Al – Mulhim *et al.*, (2004). In our study all the three cases were large cell lymphoma. Further categorization was not done as it needs immunohistochemistry.

We could not diagnose a case of HL on imprint cytology. We misdiagnose it as chronic non specific lymphadenitis because of presence of monomorphic picture on smear.

Diagnostic accuracy, sensitivity and specificity in metastatic tumor came out to be 100% which is well compared with Bhabra *et al.*, (1989), Ogawa *et al.*, (1992) & Arif *et al.*, (2011). The identification of metastasis lesions in lymph nodes by cytological examination was relatively easy. As the abnormal cancer cells were invariably present in the smears which were absolutely foreign to the lymphoid elements the detection did not cause any difficulty.

Two cases were found to be inconclusive on imprint smear. This was seen in the initial phase of our study. This could be due to the improper technique.

As said by Ulmann *et al.*, (1958) the successful use of imprints was greatly influenced by the good technique.

Conclusion

The imprint smear technique is simple, rapid and did not require sophisticated instruments. The smears showed almost perfect agreement in majority of the lymph node lesions and hence, can be used routinely as an adjunct to histopathology. The method was useful in the rapid intra operative evaluation of the draining lymph node status, thereby helped to decide about the nodal clearance in a single surgical set up. It is important tool in diagnosis of various lymph node diseases while awaiting histopathology report. This is especially useful in malignancies where diagnosis is urgently needed. This procedure has also been considered as an alternative procedure to frozen section to avoid cumbersome procedure.

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