

**Original Research Article****A Triple Correlation Study - Fine Needle Aspiration Cytology and Imprint Cytology of Lymph node with Histopathological Examination****Anbumozhi M.K.<sup>1</sup>, Bheema Rao<sup>2</sup>**

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**Abstract**

**Introduction:** Lymphadenopathy is a term meaning disease of the lymph nodes. It is, however, almost synonymously used with swollen or enlarged lymph nodes due to infection, auto-immune disease or malignancy. FNAC and imprint is a valuable adjunct in prior to the histological diagnosis of lymphadenopathy.

**Aim** of the Study is to evaluate the diagnostic accuracy and efficacy of fine needle aspiration cytology and imprint cytology study in correlation with histopathological study.

**Materials and Methods** was carried out in the department of Pathology, Sreebalaji medical college and hospital, Chrompet, Chennai. 120 Fine needle aspiration cytology (FNAC) and corresponding imprint cytology prior to histopathology were studied in 53 cases.

**Results:** A total of 120 FNAC for lymph node enlargement were done which revealed higher incidence of non neoplastic lesions 85% (102 cases) in comparison to neoplastic lesions 15% (18 cases), 62 cases were males and 58 cases were females. Out of 120 cases that underwent FNAC, excision biopsy was received in 53 cases, in which imprint was made from all 53 biopsies prior to HPE diagnosis which included 66.03% (35 cases) of benign lesions and 15% (18 cases) of malignant lesions.

**Conclusion:** FNAC cytology for suspicious palpable lymph nodes can be one of the first procedures performed during the patient's work up as it could differentiate the infectious process from the neoplastic one, for mainly avoiding unnecessary surgery. Imprint is a reliable, rapid, and inexpensive tool in diagnosis of various lymph node diseases while awaiting histopathology report.

**Keywords:** FNAC; LN; HP.

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**Introduction**

Lymphadenopathy, enlargement of lymph nodes may be due to infection, auto-immune disease or malignancy. Lymph nodes are small, bean-shaped glands throughout the body being part of the lymphatic system, carries fluid (lymph fluid), nutrients, and waste material between the body tissues and the bloodstream which plays a major

role in immune system, the body's defence [1]. Common sites of enlargement include the neck, groin and axilla. When lymph nodes swell in more areas of the body, it is called generalized lymphadenopathy [1].

Fine needle aspiration cytology (FNAC) is a simple, rapid, inexpensive outpatient technique used for the diagnosis of any palpable mass. It holds a unique place as a diagnostic

tool in the field of pathology, ever since its introduction in the 1920 by martin and ellis. FNAC plays a important role in the diagnosis of lymphadenopathies (Chhotray & Acharya, 1987) from reactive lymphadenopathy, recognition of primary lymphoid malignancy (lymphomas), tometastatic deposits. With recent advances in cytology, FNA smear can be subjected to immunoflowcytometry (IFC), cell block immunohistochemistry (IHC), and cell block also subjected to fluorescent in situ hybridisation for c-myc alteration and special stains like Ziehl–Neelsen stain (ZN), Giemastain (GMS) and Periodic acid–Schiff (PAS) [2].

Imprint cytology is a special variation of applied cytology, made by gently touching,(without smearing) the freshly cut surface of tissue by glass slides. This technique was favourably reported by Dudgeon and Patrick (1927) and Bamforth and Osborn (1958). In recent scientific research cytologic imprints are very apt for cytophotometric measurements of DNA contents in nuclei as well as for karyologic studies [3].

All these are therefore a valuable adjunct to the histological diagnosis of lymphadenopathy, but final diagnosis is by histopathological examination since loss of architecture of the lymph nodes and capsular invasion cannot be made out by a study of FNAC and imprints.

### Aims and Objectives

- To study the role of fine needle aspiration cytology in the evaluation of lymphadenopathies.
- To study the role of imprint cytology prior to submission of the tissues for histopathological study.
- To evaluate the diagnostic accuracy and efficacy of fine needle aspiration cytology and imprint cytology study in correlation with histopathological study.

### Method

This study of fine needle aspirationcytology, imprint cytology and histopathologicalstudy correlation of lymph node lesions was conducted in the department of

pathology, Sree Balaji Medical College And Hospital, Chennai. During the period fine needle aspiration was performed on 120 patients who presented with symptoms and signs of lymph node enlargement. Prior to FNAC the procedure was explained to the patients and consent was obtained for the same. When the open biopsy was performed on lymph node lesions, imprints were taken from the fresh tissue followed by routine processing for histopathological examination. For imprint cytology the lymph nodes were gently sectioned by holding between the index finger and the thumb, clean microscope slides were pressed gently but firmly on the freshly cut surface. Gliding, sliding and lateral movements were avoided (which tend to distort the shape of the cells). The imprints obtained were rapidly fixed using by using 99% isopropyl alcohol and stained with haematoxylin and eosin later followed by HPE exmamination. Special stains and immunohistochemical stains were carried in appropriate lesions after histopathological examination.

### Observation and Results

Study stated, cervical lymphnode was most commonly affected 75.8% (91 cases) followed by inguinal and axillary lymphnode. A total of 120 FNAC for lymphnode enlargement were done during this study period . FNAC study revealed higher incidence of non neoplastic lesions 85% (102 cases) in comparison to neoplastic lesions 15% (18 cases)-Table 1. FNAC for lymphnode enlargement was done which included acute lymphadenitis (13 cases),suppurative lymphadenitis (12 cases), chronic lymphadenitis (14 cases), TB lymphadenitis (42 cases), reactive hyperplasia(21 cases), lymphomas (7 cases) and metastatic deposits (11 cases). It played a major role in avoiding surgery for patients, with suppurative lymphadenitis and acute lymphadenitis. From the 120 cases, 52% (62 cases) were males and 48% (58 cases) were females. There is overall male preponderance with male: female ratio 1.5:1 Non neoplastic lesions were predominantly seen in female patients and neoplastic lesions were predominantly seen in male patients. The peak incidence of benign lymphnodelesios was noted in the age group 21-30 years (17.64%) and malignant lesions was noted in the age group of >50yrs. The most common

**Table 1:** Distribution of FNAC diagnosis of in lesions

FNAC Diagnosis	No	%
Acute Lymphadenites (AL)	13	10.8%
suppurativeLymphadenites (SL)	12	10.0%
Chronic Lymphadenites (CL)	14	11.6%
TB Lymph Node (TBLN)	42	35.1%
Reactive Hyperplasia (M)	21	17.5%
Hodgkin's Lymphoma (HL)	3	2.5%
Non-Hodgkin's Lymphoma (NHL)	4	3.3%
Secondary Deposits (SD)	11	9.2%
Total	120	100%

**Table 2:** Distribution of imprint cytology diagnosis of Inlesions

Imprint Cytology Diagnosis	N	%
Chronic lymphadenitis (CL)	9	16.98
TB Lymph Node (TBNL)	22	41.50
Reactive Hyperplasia (RH)	4	7.54
Hodgkin's Lymphoma (HL)	3	5.66
Non-Hodgkin's Lymphoma (NHL)	4	7.54
Malingency (M)	11	20.78
Total	53	100.0

**Table 3:** Distribution of FNAC and imprint along with HP indagnosis of Inlesions

Diagnosis	Histopathological Results	Imprint Results	FNAC Results
Chronic Lymphadenites (CL)	6	6	9
TB Lymph Node (TBLN)	25	25	22
Reactive Hyperplasia (RH)	4	4	4
Hodgkin's Lymphoma (HL)	3	3	3
Non-Hodgkin's Lymphoma (NHL)	4	4	4
Secondary Deposits (SD)	11	11	11
Total	53	53	53

benign lesion causing cervical lymphnode enlargement is reactive hyperplasia and malignant lesion is secondary deposits

Out of 120 cases that underwent FNAC, excision biopsy was received in 53 cases. In which imprint was made from all 53 biopsy -Table 2, prior to HPE diagnosis which included 66.03% (35cases) of benign lesions and 15% (18 cases) of

malignant lesions are chronic lymphadenitis (9 cases), TB lymphadenitis (22 cases), reactive hyperplasia (4 cases), lymphomas (7 cases) and metastatic deposits (11 cases).

We tabulated the resultsof FNAC and IMPRINT along with HP study- Table 3. Imprint shows 100% diagnostic accuracy when compared with histopathology study as we have taken touch preparation of freshly cut specimen and studied. In FNAC the positive diagnosis carries value as it is well correlated with histopathology diagnosis .But negative value cannot be taken assuch because the clinical and other investigation correlation may prompt for repeat FNAC .

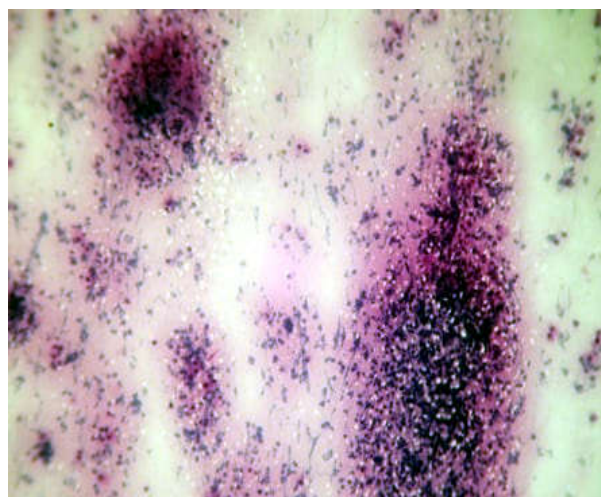
As for as neoplastic lesions were concerned a 100% correlation was seen between FNAC and HP. In non neoplastic lesions 25 cases of TB lymphadenitis diagnosed by HP study whereas 22 cases were reported in FNAC.



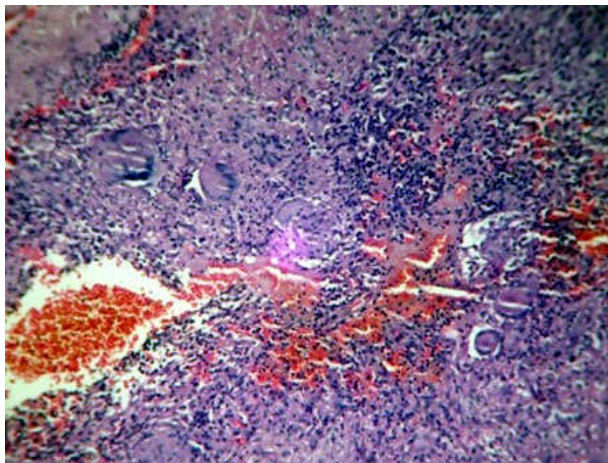
**Fig. 1:** 24 yr female left cervical lymphadenitis



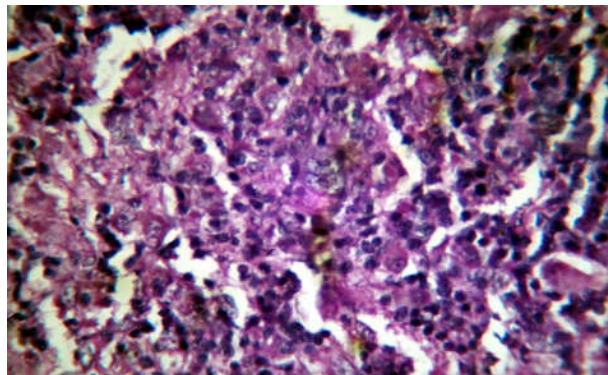
**Fig. 2:** Cut surface of lymph node



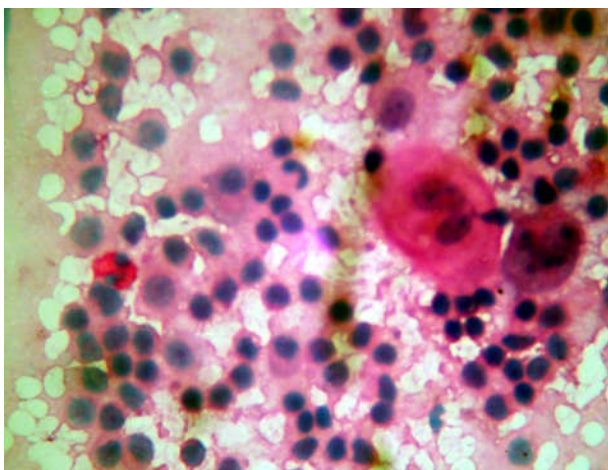
**Fig. 3:** Imprint smear shows plenty of lymphocytes in a background of caseous necrosis. (H&E 10X)



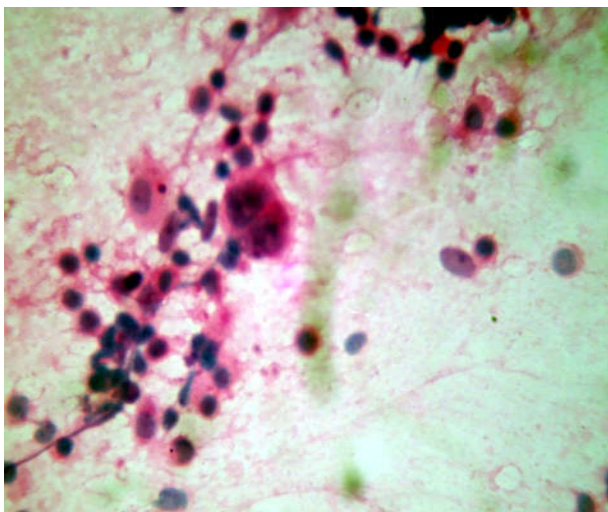
**Fig. 4:** H&E section showed multiple granulomata composed of caseous necrosis, epithelioid cells, lymphocytes and multinucleated langhans type giant cells (H&E 10X)



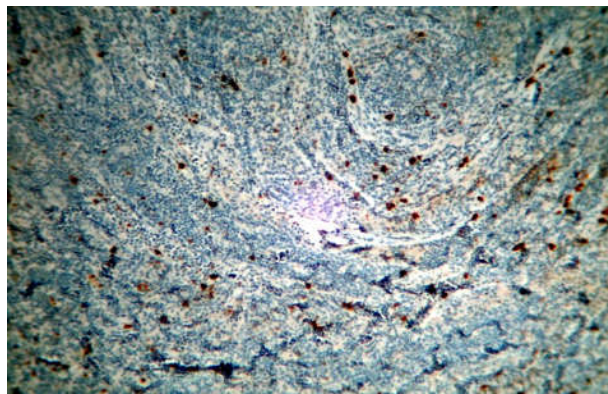
**Fig 5c:** H&E section shows Reed-Sternberg cells which were present in a 'characteristic background' of reactive inflammatory cells of various types(H&E 40X)



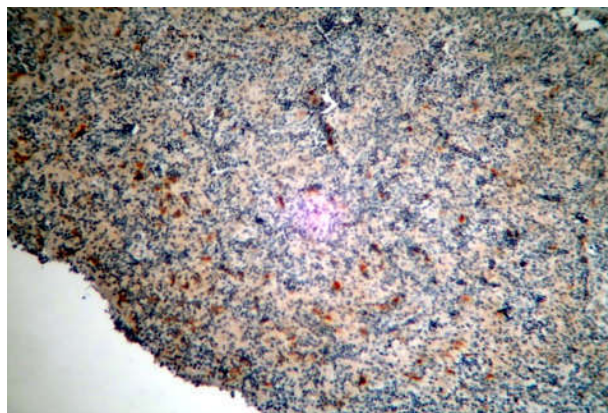
**Fig. 5a:** Hypercellular FNAC smear with small lymphocytes with variable numbers of plasma cells and classic Reed-Sternberg cell (H&E 40X)



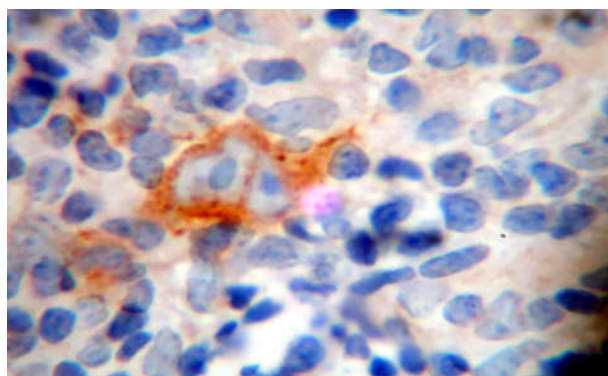
**Fig. 5b:** Imprint smear showed a characteristic Reed Sternberg giant cells with pale eosinophilic cytoplasm and mirror image type of nuclei and prominent nucleoli. (H&E 10X)



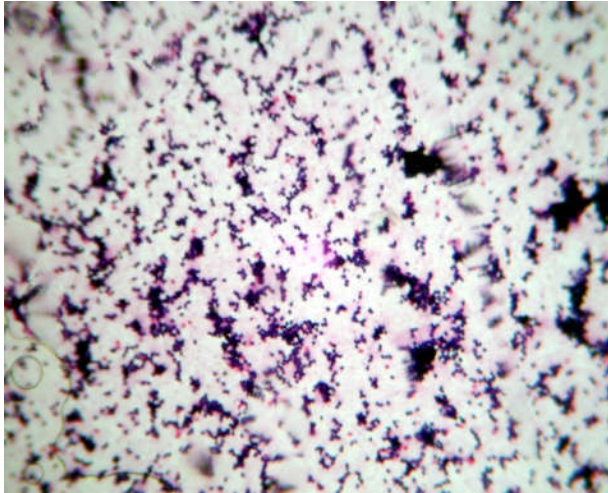
**Fig. 6a:** CD15



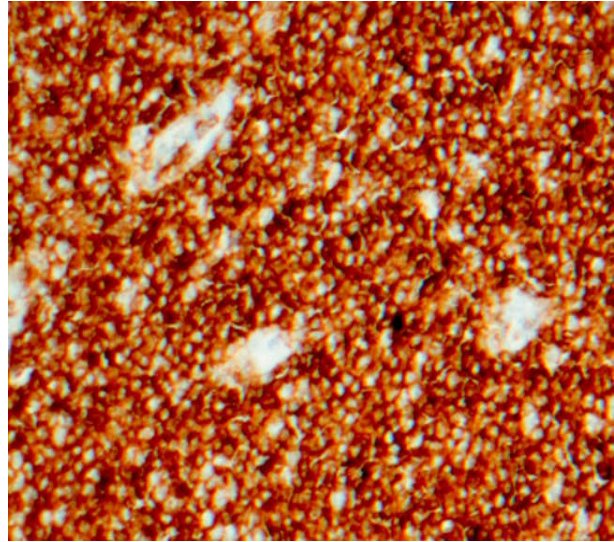
**Fig. 6b:** CD30



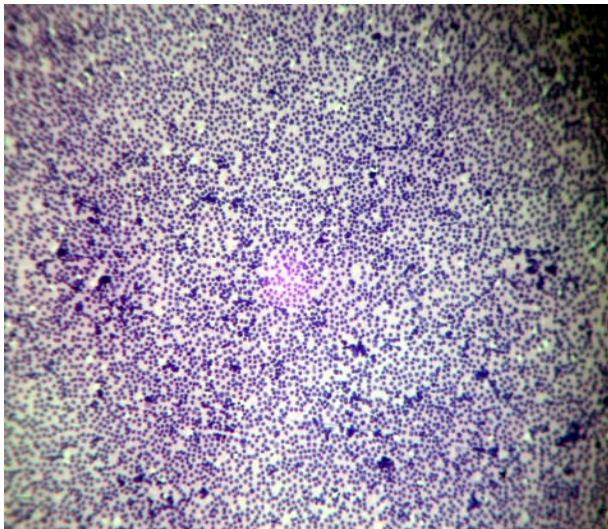
**Fig. 6c:** CD4Diffuse cytoplasmic POSITIVE



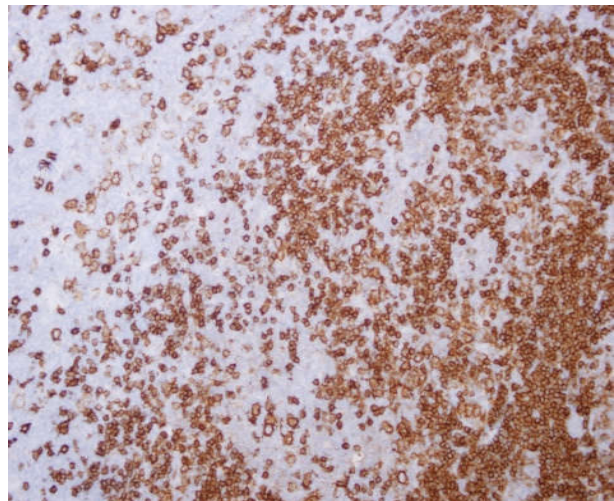
**Fig. 7a:** FNAC smear shows monotonous sheet of lymphoblasts. (H&E 10X)



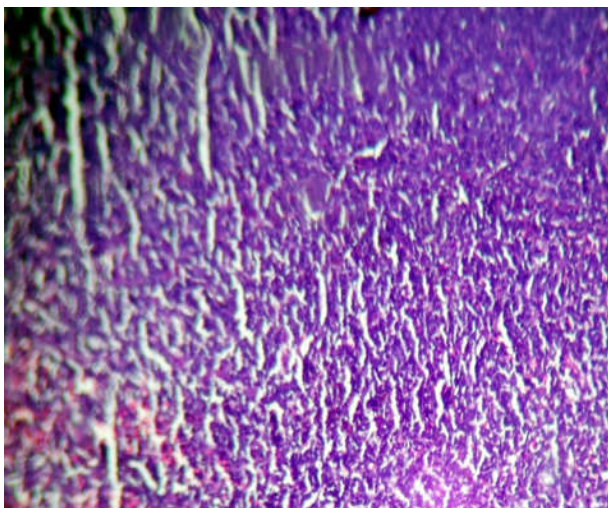
**Fig. 8a:** CD 19 +



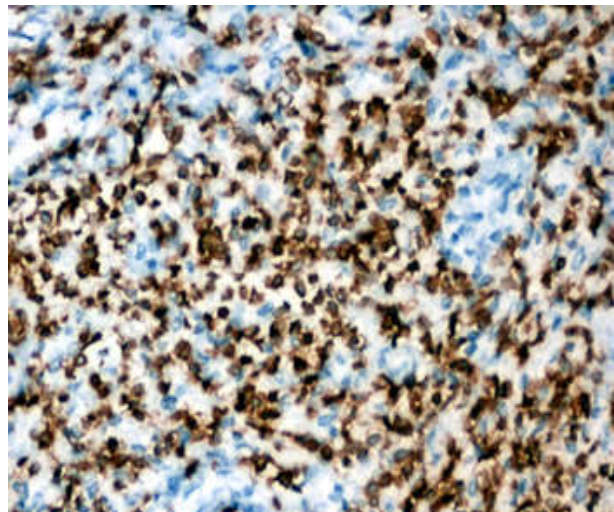
**Fig. 7b:** Imprint Smear showed hypercellularity with monomorphic population of predominantly neoplastic lymphocytes(H&E 10X)



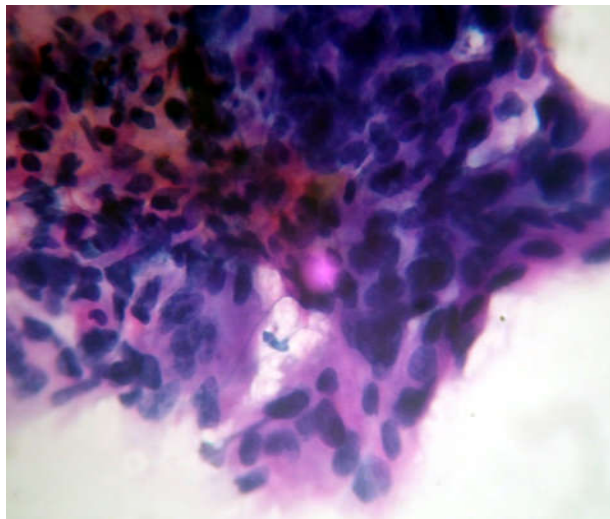
**Fig. 8b:** CD 20+



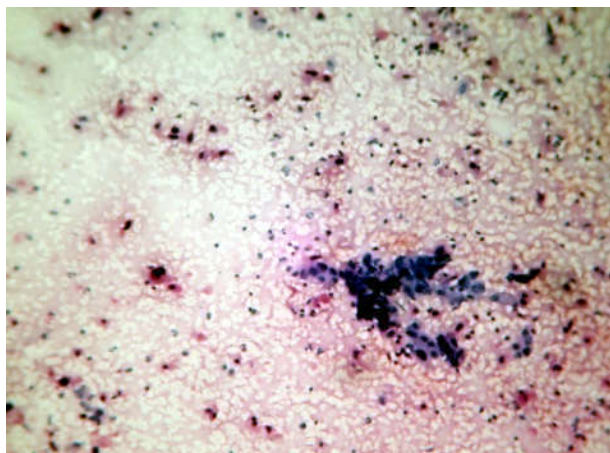
**Fig. 7c:** H&E section shows loss of normal architecture of lymphnode replaced by diffuse monotonous sheets of lymphoblasts. (H&E 40X)



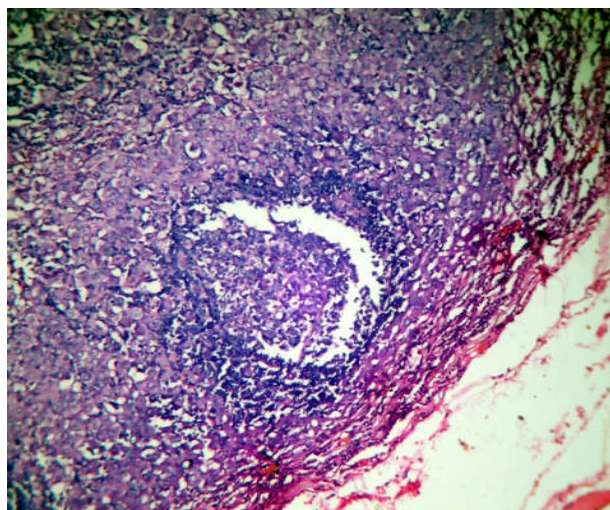
**Fig. 8c:** Angioimmunoblastic T-Cell Lymphoma-Immunostaining for CD3 highlights a large number of immunoblasts



**Fig. 9a:** FNAC smear shows Loosely cohesive cluster of tumor cells with large hyperchromatic nuclei, prominent nucleoli and high nuclear cytoplasmic ratio in a mist of scattered lymphocytes. (H&E 40X)



**Fig. 9b:** Imprint smear shows loose clusters of pleomorphic cells with hyperchromatic nuclei and lymphocytes(H&E 10X)



**Fig. 9c:** Metastatic ductal carcinoma breast in axillary lymph node showing nests of pleomorphic ductal cells (H&E 10X)

3 cases of specific diagnosis of tuberculosis was missed. It might be due to the needle ,which might not hit the granulomatous area.

### Discussion

Lymphadenopathy is considered as one of the most common clinical problems affecting different age groups involving any site of the body. The most frequent cause of lymphadenopathy is reaction to some symptomatic or asymptomatic inflammatory process followed by tumors either primary or metastasis.

Fine-needle Aspiration is accepted by most people as a non-invasive method for evaluating lymphadenopathy because this technique is proved to be reliable, rapid, and inexpensive OP procedures in diagnosis. They can differentiate well between nonneoplastic and neoplastic lesions. But cytological diagnosis should be followed by histological diagnosis for accurate classification and grading in cases of neoplastic lesion. Imprint cytology is a simple technique where the morphology of individual cells is seen from the touch preparation from freshly cut surface of lymph nodes as soon as surgical removal of the specimen. The diagnosis is made easy by studying the morphology of individual cells but however final diagnosis is by histopathological examination since loss of architecture of the lymph nodes and capsular invasion cannot be made out by FNAC and imprints. In 1977 [11] Bloustein et. al. and Padara K Agarwal et al found imprint as unique and quite reliable in diagnostic advantages with accuracy of 98%. The value of imprint was understood mainly in intraoperative procedures over frozen section. Fisher CJ et. al. (1993) [12] studied on Intraoperative assessment of nodal status in the selection of patients with breast cancer for axillary clearance by imprint cytology. The technique was useful in reducing the morbidity of breast cancer surgery without increasing the risk of local and regional or distant recurrence. Similar observation was seen by Cserni G in 2001 [13]. Stephen A Shiver et. al. in 2002 [14], Andrew J. Creager et. al. in 2004 [15], Wang YS et. al. in 2012 [16] and Al-Ramadhani S et. al. in 2013 [17].

Non neoplastic lesion reported by FNAC, acute lymphadenitis (13 cases) and (12 cases) suppurative lymphadenitis were managed medically and improved well hence risk of surgery was averted, Shakya G et. al. (2009) [18] and V Koo et. al. (2006) [19] had similar observation with opinion. The 14 cases of chronic lymphadenitis reported by FNAC in which 9 cases of chronic lymphadenitis did not improve well on medical treatment so biopsy were done, Out of which 6 were happen to be non specific lymphadenitis and 3 cases were TB lymphadenitis by imprint and HPE. This pitfall of FNAC might be due to the needle, which has not aspirated the granulomatous area. The diagnostic accuracy for chronic lymphadenitis

by FNAC was 94.33% and imprint was 100% when compared with histopathology, others similar studies are by Singh JP et. al. (1989) [20], Morrison et. al. (1952) [21] and by Ulmann et. al. (1958) [22].

Tuberculous lymphadenitis was the most common non neoplastic lesion, 22 cases of TB lymphadenitis were diagnosed by FNAC. These cases were mostly found to involve the cervical lymph nodes. Males were most commonly affected in 2- 4<sup>th</sup> decades of life. Ziehl-Neelsen staining for mycobacterium tuberculosis was carried out in all 22 cases which showed positivity. The diagnostic accuracy of FNAC was 94.33% and imprint cytology was 100% respectively. Abdulrahman S. Al-Mulhim (2004) [23] and Prasanta et al (2008) [24] had similar observation. Gupta SK et. al. (1993) [25] found that for bacilli to be demonstrated in smears, their number should be 10,000 to 1,00,000/ml of material. All these patients were treated with antituberculous chemotherapy.

Therefore, In granulomatous lymphadenitis, cytological diagnosis based upon the presence of lymphocytes, macrophages, histiocytes of epithelioid type forming cohesive clusters and multinucleated and Langhans, s giant cell (Figure 3). Histologically (Figure 4) presence of necrotic material and Langhans giant cells may suggest TB, but TB remain in the differential diagnosis whether necrosis is present or not and this diagnosis requires bacteriological confirmation - Al-Mulhim AS et. al. (2004) [23].

Cytology diagnosis of reactive hyperplasia, 4 cases in both techniques by fine needle aspiration and imprint cytology showed 100% accuracy as it revealed a mixed and abundant of small and large lymphocytes which accumulate around the histiocytes forming lymphohistiocytes aggregates. Diagnostic accuracy, particularly in certain cases of reactive lymphadenopathy depended on the representatives of the aspirate and the quality of the cytological preparations - Al-Mulhim AS et. al. (2004) [23] had similar observation

Neoplastic lesions reported (3 cases) of Hodgkin's disease (Figure 5a,b,c) the accuracy of both FNAC and Imprint cytology was 100%. This diagnosis based upon the presence of the characteristic Reed-Sternberg cells, lacunar cells, eosinophils, plasma cells and lymphocytes - Al-Mulhim AS et. al. (2004) [23] Adhikari P et. al. (2011) [26], Anne R. wilkinson et. al. (2013) [27], Morrison et. al. (1952)

[21] and Nagpal et. al. (1982) [28] had similar opinion with a diagnostic accuracy respectively. Das Dilip K et. al. (1990) [29] depicted scope and limitations of FNAC in the diagnosis of Hodgkin's lymphoma and its subtypes.

In 4 cases of Non Hodgkin's (Figures 7a,b,c) disease the accuracy of both FNAC and Imprint cytology was 100%. The diagnosis depending on the presence of monomorphic cellular infiltrate and absence of phagocytosis. Cytological sub typing of non-Hodgkin's lymphoma was difficult and requires extensive experience. Daskalopoulou Dimitra et. al. (1995) [30] described that, a relatively monotonous lymphoid cell population is the corner of the diagnosis of Non Hodgkin lymphoma by aspiration cytology and their results did not differ substantially from others in the overall diagnostic accuracy. Other studies are Adhikari P et. al. (2011) [26], Anne R. wilkinson et. al. (2013) [27], Morrison et. al. (1952) [21] and Ulmann et. al. (1958) [22]. All lymphomas was further studied with immunohistochemistry mainly to find, B cell or T cell in origin for the final confirmatory diagnosis and classification.

The identification of metastasis lesions in lymph nodes by cytological examination (FNAC, IC) was relatively easy with a diagnostic accuracy of 100%. The criteria for diagnosis were malignant cells not related to that origin - Al-Mulhim AS et. al. (2004) [23] had similar observation. Lee Robert E et. al. (1987) [31] used fine needle aspiration cytology of lymph nodes in patients having known or suspected malignancy, and described a good correlation between cytology and histopathology.

### Conclusion

The study revealed a diagnostic accuracy of FNAC and Imprint techniques when compared with histology as follows:

From this study it could be concluded that, FNAC cytology for suspicious palpable lymph nodes can be one of the first procedures performed during the patients' work up as it could differentiate the infectious process from the neoplastic one, for mainly avoiding unnecessary surgery when patients can be treated with medications (acute lymphadenitis and TB lymphadenitis). The technique is also useful to detect the presence of lymph node metastasis in a subject with establishing the diagnosis of carcinoma.

Histopathological diagnosis	Diagnostic accuracy of FNAC when compared with histopathology	Diagnostic accuracy by IMPRINT when compared with histopathology
Reactive lymphadenitis	100%	100%
Tuberculosis	94.33%	100%
Chronic lymphadenitis	100%	100%
Metastasis	100%	100%
Hodgking lymphoma	100%	100%
Non Hodgkins Lymphoma	100%	100%

Cytology diagnosis of lymphoma and metastasis should be followed by excisional biopsy for accurate classification and grading.

Imprint is an reliable, rapid, and inexpensive tool in diagnosis of various lymph node diseases while awaiting histopathology report. So it is useful in the intra operating period. This procedure has also been considered as an alternative procedure to frozen section to avoid cumbersome procedure.

So the diagnostic accuracy of imprint is superior over FNAC since the whole specimen is available and lesion is in direct contacts. The overall accuracy of Imprint cytology was 100%, higher than that of fine needle aspiration being 94.33%. It diagnosed all cases of TB lymphadenitis, reactive hyperplasia, Hodgkin's lymphoma, non-Hodgkin's lymphoma and metastatic deposits.

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