

# TOUCH IMPRINT CYTOLOGY OF LYMPH NODES AND THEIR HISTOPATHOLOGICAL CORRELATION

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## Abstract

**Introduction:** Lymph nodes serve as an integral part of the immune system. Lymphadenopathy is one of the earliest manifestations of the underlying disease. It can be caused due to the infiltration or proliferation of either inflammatory or neoplastic cells into the node. Touch Imprint Cytology, a non-exfoliative cytology technique, is a useful procedure to reach a conclusive diagnosis in the aforementioned circumstances in a relatively short amount of time. The aim of our study is to perform a direct comparison between Touch Imprint Cytology and Biopsy on the same Lymph nodes, operated in a single center.

**Materials and Methods:** We conducted a prospective, diagnostic, cross-sectional study on the diagnosis of various lymph node lesions by using touch imprint cytology and to correlate it with the histopathological diagnosis. This study was conducted over the period of two years in our institute, at the Department of Pathology.

**Results:** We received 52 freshly excised samples of lymph nodes over the above mentioned period of time. The sensitivity for detecting neoplastic lesions was 60%, but it was 100% for detecting non-neoplastic lesions. The overall specificity and accuracy of our study was 100% and 92.31% respectively, with the "p-value" being less than 0.001.

**Conclusion:** As stated above, the "p-value" of our study was less than 0.001, indicating that touch imprint cytology was effective for identifying intra-operative lymph node lesions. Touch imprint cytology is therefore a simple, quick, and cost-effective way of tissue analysis.

**Keywords:** Lymph nodes, Imprint cytology, Histopathology

## Introduction

Lymph nodes are secondary lymphoid organs, located at anatomically constant sites, in clusters of small groups or chains, along the course of lymphatic vessels <sup>[1,2]</sup>. Peripheral lymph nodes are situated deep in the subcutaneous tissue. The human body houses approximately 600 lymph nodes and are normally less than one centimeter in size, but it may vary from site to site <sup>[3]</sup>. Lymph nodes are a part of peripheral lymphoid organs of the immune system <sup>[4]</sup> and their main function is filtration of lymph and processing of the antigens <sup>[1]</sup>.

Lymphadenopathy is termed when there is enlargement of size of lymph nodes due to either neoplastic or non-neoplastic causes <sup>[5]</sup>. Patients visiting the out-patient department come with lymphadenopathy as one of the most common complaints, which may be localized or generalized <sup>[6]</sup>. A commonly used mnemonic acronym for the various causes of lymph node enlargement is “MIAMI”, which is an abbreviation for Malignancies, Infections, Auto-immune disorders, Miscellaneous causes and Iatrogenic causes <sup>[1,5]</sup>.

The investigation of choice used to detect the cause of lymph node enlargement is fine needle aspiration cytology (FNAC), but it may fail to provide accurate diagnosis in few cases due to very deep-seated lymph nodes, inexperience of the clinician or inadequate sample, wherein core needle or excisional biopsy is performed to determine the cause of lymphadenopathy <sup>[7]</sup>. These biopsies are gold standard, but the whole process takes at least 5-7 days in a well-equipped set-up <sup>[5]</sup>.

In the above-mentioned cases, Touch Imprint Cytology (TIC) has proven to be of great help. Touch Imprint Cytology is a sort of non-exfoliative cytology which can be used to reach a definitive diagnosis in a short period of time <sup>[7,8]</sup>. It is a well-recognized, simple technique which is rapid and can be used intra-operatively for diagnosis, as it not only conserves the tissue, but also prevents freezing artefact <sup>[9]</sup>. A well-prepared lymph node impression smear shows a broad cellular monolayer, yielding enough architectural and cytological information <sup>[10]</sup>. The benefits of lymph node imprint cytology comprise typically dispersed and isolated cells with occasionally loose cohesive clusters created by germinal centres <sup>[4]</sup>.

Sentinel lymph nodes are considered to be the representative nodes for tumor infiltration. In early 1970s, sentinel lymph node biopsy (SLNB) was introduced to identify the patients at risk of regional and systemic spread and to rule out the need for lymphadenectomy <sup>[11]</sup>. TIC has been recommended by the College of American Pathologists for evaluation of sentinel lymph nodes intra-operatively <sup>[12]</sup>, as the imprint cytology shows dispersed and isolated cells, with occasional loose cohesive clusters formed due to germinal centres <sup>[4]</sup>.

## Materials and Methods

We conducted a diagnostic cross-sectional prospective study in the Department of Pathology at our institution. All fresh excisional lymph node biopsies which were received in a normal saline were included in the study. The gross findings were noted and imprint cytology was done immediately, so as to prevent any cytological artefacts induced by formalin. Then the lymph nodes were kept in 10% formaldehyde for further histopathological evaluation.

For the touch imprint cytology, the lymph nodes received were bisected and the cut surface of the sliced half was held face upwards and four imprints were made on grease free glass slides. The prepared smears were then air-dried or wet –fixed and stained by Papanicolaou, Leishman and Haematoxylin and Eosin. Special stains like Ziehl-Neelson were done when indicated.

The fresh lymph nodes were fixed in 10% formalin solution overnight and further histopathological processing was done. Three-to-five-micron paraffin embedded sections were taken and were then stained by Haematoxylin and Eosin stain. These slides were then studied along with the imprint cytology slides.

The touch imprint cytology smears were compared against the histopathological slides, as the latter is considered gold standard. The results of both procedures were then compared, to rule out any false positives or false negatives in the imprint cytology smears.

The college ethical committee clearance was acquired before the study began and patient consents were taken.

## Results:

We received 52 freshly excised lymph nodes in our department, over the course of two years, from September 2020 to August 2022. These were patients from four years to 86 years of age. The majority of lymph node specimens excised were from patients aged 31 to 40 years, with the fewest cases seen at both extremes of age. The median age of the patients included in our study came out to 47 years. (Table 1). Our study showed slight female predominance with Male: Female: 1:1.4. (Table 2).

Table 1: Age wise case distribution

Age (years)	Number of Cases	Percentage
1-10	01	1.92
11-20	04	7.70
21-30	06	11.54
31-40	11	21.15
41-50	09	17.31
51-60	08	15.38
61-70	08	15.38
71-80	03	5.77
81-90	02	3.85
<b>Total</b>	52	100

Mean Age – 45.87

Median Age - 47

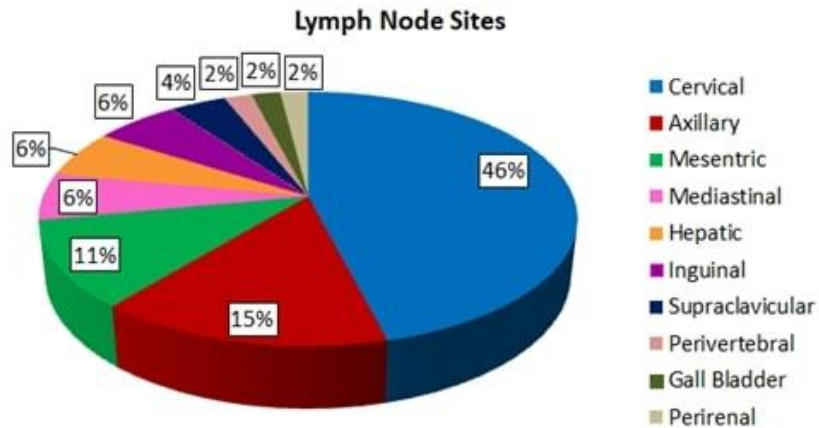
Table 2: Gender wise case distribution

Gender	Number of Cases	Percentage
Male	22	42
Female	30	58
<b>Total</b>	52	100

The majority of the lymph nodes we received for examination were from the cervical region (46%), followed by the axillary region (15%) and the mesenteric region (11%). Perivertebral, perirenal, and gall bladder were the least common locations, accounting for 2% among all cases. (Figure 1).

Figure 1: Chart showing distribution of cases according to the site of lymph node involved.

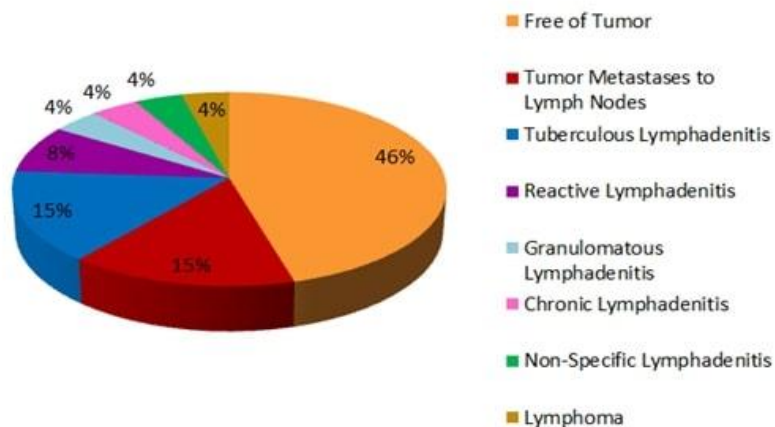
## Distribution of cases according to the site of Lymph node involved



We received both neoplastic as well as non-neoplastic lymph node lesions in the above-mentioned course of time. Most of the cases from the neoplastic category were suspected for tumor metastases, but were diagnosed to be free of tumor. Amongst the non-neoplastic lesions, tuberculosis was the most common diagnosis. (Figure 2).

Figure 2: Chart showing Neoplastic and Non-Neoplastic lesions of lymph node diagnosed on histopathological examination.

## Distribution of Neoplastic and Non-neoplastic lesions according to Histopathological diagnosis



24 of the 32 specimens we obtained for detection of tumor metastases were free of tumor, as determined by both imprint cytology and histology. Four of the remaining eight patients were diagnosed with tumour metastases on histopathological examination, while the others were wrongly labelled as tumor-free on imprint smears. Two cases of Non-Hodgkins Lymphoma were correctly diagnosed on both imprint smears and histopathology.

In the non-neoplastic category, we received a total of 18 lymph nodes, out of which tuberculous lymphadenitis was the most common lesion, followed by reactive lymphadenopathy. The least common lesions we encountered were of granulomatous lymphadenitis, non-specific lymphadenitis and chronic lymphadenitis.

## Discussion

Lymph nodes are a vital component of the immune system. These small bean-shaped structures are found in clusters or small chains at specific anatomical sites. They are specialised connective tissue formed by reticular cells and fibres that trap lymphocytes and belong to the lympho-reticular system's secondary lymphoid organs <sup>[13]</sup>.

Changes in lymph node size, consistency, and texture are frequently among the first symptoms of the underlying disease. Lymphadenopathy is defined as an abnormality in the size or nature of lymph nodes triggered by the influx or proliferation of either inflammatory or neoplastic cells into the node. It can be categorized as either localised or broad <sup>[5]</sup>.

Touch imprint cytology is a simple technique that uses excised tissue biopsies to preserve the complete specimen. These have proven to be quite beneficial in evaluating malignancies and hence, the imprint cytology has been commonly employed in the examination of sentinel lymph nodes in breast cancer cases <sup>[14]</sup>.

Our study included patients ranging in age from four years to 86 years, with the average age was 45.87 years. Our findings were similar to those of Memar et al <sup>[15]</sup> and Adhya et al <sup>[16]</sup>, who also had patients with mean ages of 47 and 48.4 years, respectively.

Most of the lymph node biopsies in our study were suspicious of tumor metastases from various sites, but instead were found to be tumor-free, followed by the detection of metastatic tumor deposits. The Non-Hodgkin Lymphoma was the least common neoplastic lesion which we encountered in our study.

Creager et al <sup>[17]</sup>, Deo et al <sup>[18]</sup>, Memar et al <sup>[15]</sup>, Bell et al <sup>[19]</sup>, Khanna et al <sup>[20]</sup>, Lumachi et al <sup>[14]</sup>, Ersoy et al <sup>[21]</sup>, Horvath et al <sup>[22]</sup>, Uno et al <sup>[23]</sup>, Petursson et al <sup>[24]</sup>, Chang et al <sup>[25]</sup>, and Hadalin et al <sup>[26]</sup> reported equivalent findings to ours when using touch imprint cytology to analyze sentinel lymph node metastases.

Usually, a direct correlation has been found between the tumor size and incidence of metastases, as the lymph nodes involved are bigger in size and may show features of hemorrhage and necrosis in advanced stages. However, that may not be always true. In our study, out of the 32 lymph nodes suspected for tumor metastases, eight tested positive on histological inspection, and we were able to detect tumor metastases in four of the eight on touch imprint cytology. The lymph nodes positive for tumor metastases were detected by the presence of pleomorphic malignant cells from the primary organ as opposed to the presence of polymorphous lymphoid population seen in the otherwise non-involved lymph nodes. (Figure 3). The lymph nodes which were free from tumor metastases resembled the reactive lymphadenitis in their histomorphological features. (Figure 4). Non-Hodgkin Lymphoma slides showed monotonous aggregates of lymphoid cells with increased nuclear to cytoplasmic ratio. (Figure 5).

Figure 3A: Photomicrograph of lymph node imprint smears showing positive tumor metastases, with pleomorphic cells (H&E stain, 400X).

Figure 3B: Photomicrograph of lymph node section showing multiple pleomorphic cells with high nuclear cytoplasmic ratio, indicating metastases (H&E stain, 400X).

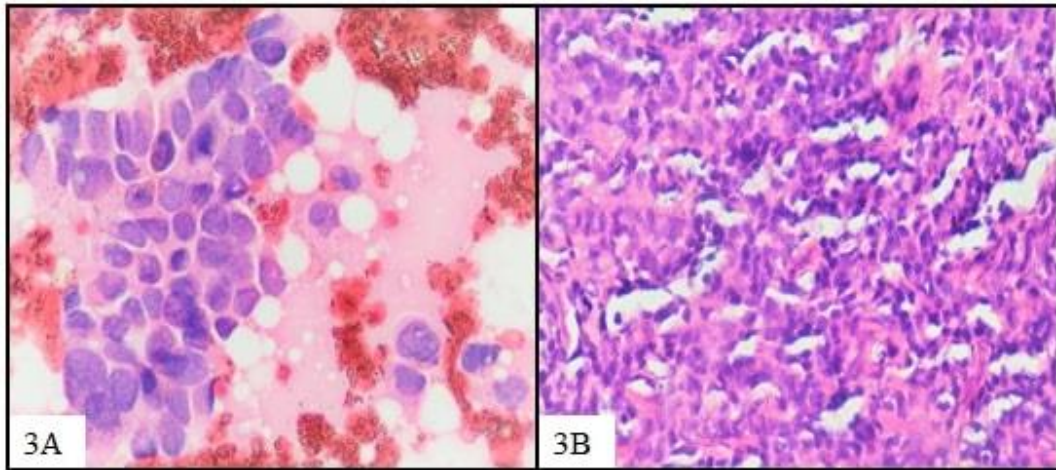


Figure 4A: Photomicrograph of lymph node imprint smears showing a polymorphous lymphoid cell population, along with tangible body macrophages (black arrow) against a haemorrhagic background (Leishman stain, 400X).

Figure 4B: Photomicrograph of lymph node section showing scattered lymphoid cells in various stages of maturation (H&E stain, 400X).

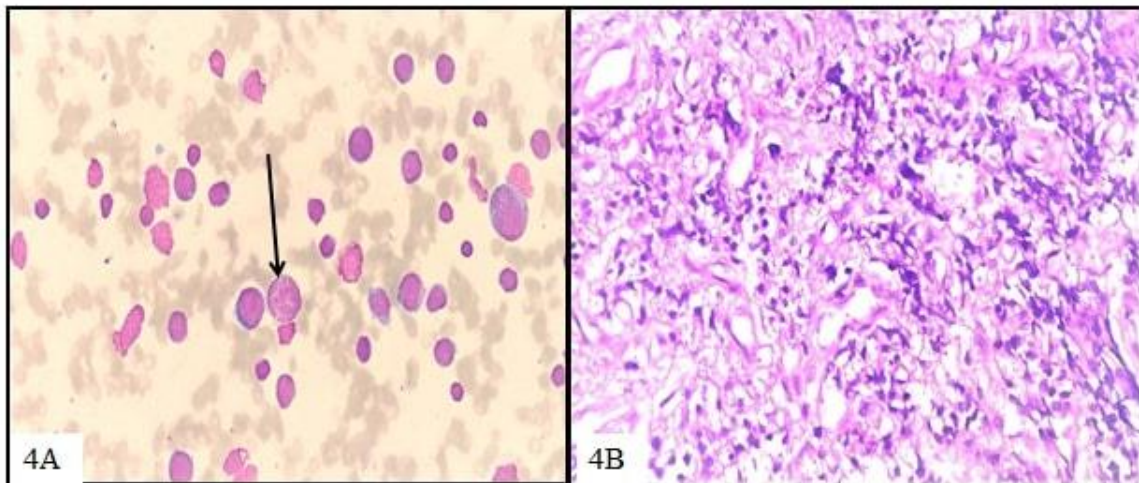
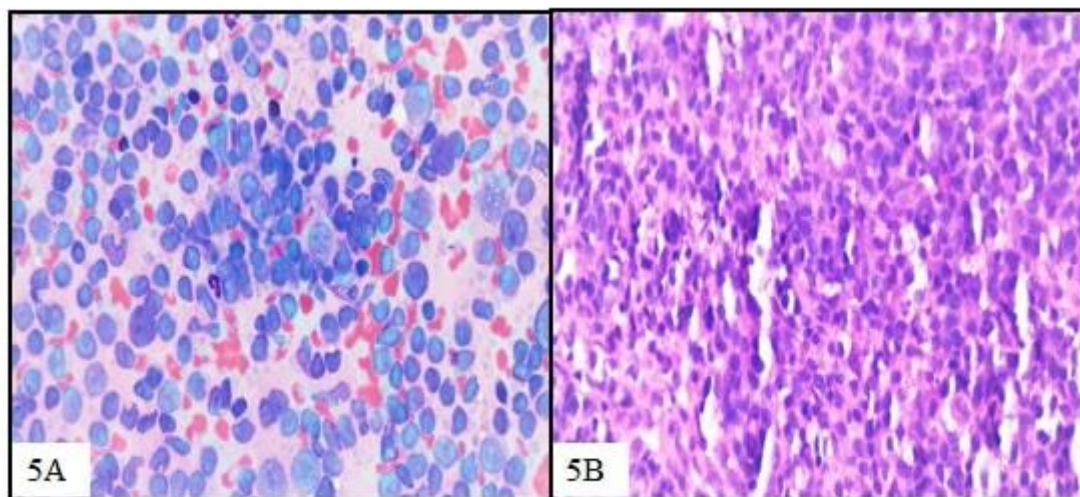


Figure 5A: Non-Hodgkin Imprint smears – Photomicrograph of lymph node smears showing monotonous population of lymphoid cells with an increased nuclear cytoplasmic ratio (Leishman stain, 400X).

Figure 5B: Photomicrograph of lymph node smears showing monomorphic population of lymphoid cells with high nuclear cytoplasmic ratio and hyperchromatism, indicating Non-Hodgkin Lymphoma (H&E stain, 400X).



In the diagnosis of malignant lesions, due to the presence of four false negatives in our study, we had a sensitivity of 60% and a specificity of 100%. (Table 3). This was similar to the study by Ademiluyi et al.<sup>[27]</sup>, which showed a sensitivity of 66%. Also, it was in agreement with the studies conducted by Hovarth et al.<sup>[22]</sup>, Petursson et al.<sup>[24]</sup> and Marano et al.<sup>[28]</sup>, for assessing sentinel lymph nodes in breast carcinoma. Hovarth et al.<sup>[22]</sup> achieved a sensitivity of 57.18%, a specificity of 99.63%, and a false positive rate of 0.85%, with no false negatives. Petursson et al.<sup>[24]</sup> had 68.6% sensitivity and 99.8% specificity. Marano et al.<sup>[28]</sup> had a sensitivity of 68.4%, specificity of 98.7% and a false negative rate of 12.8%.

Table 3: Sensitivity and Specificity

Malignancy		HPE		Total
		Positive	Negative	
Imprint	Positive	6	0	6
	Negative	4	42	46
Total		10	42	52

Positive predictive value= 100%, CI= 60.97-100%

Negative predictive value= 91.3%, CI= 79.68-96.57%

Accuracy= 92.31%, CI= 81.83-96.97%

In our study, non-neoplastic lesions comprised TB, granulomatous, reactive, chronic, and non-specific lymphadenitis. On both histological and touch imprint smears, the tuberculous lymphadenitis displayed epithelioid cell granulomas and localised caseous necrosis, as well as the appearance of acid fast bacilli indicated by Zeihl-Neelson stain. Langhan type of giant cells were also seen in few cases. (Figure 6). In the instance of granulomatous lymphadenitis, primarily epithelioid cell granulomas were seen on histological sections and imprint smears, along with few multinucleated giant cells. (Figure 7). On impression smears, reactive

lymphadenitis revealed a polymorphous lymphocytic population with only a few tingible body macrophages and plasma cells. The histological sections of the same had comparable results. (Figure 4). On both histopathological and imprint slides, chronic and non-specific lymphadenitis demonstrated a polymorphous population of lymphocytes with a modest lymphocytic predominance, as well as a few plasma cells and histiocytes.

Figure 6A: Photomicrograph of lymph node imprint smears showing extensive caseous necrosis (H&E stain, 400X).

Figure 6B: Photomicrograph of lymph node section showing a band of caseous necrosis. Inset shows Langhan type of giant cell (H&E stain, 400X).

Figure 6C: Photomicrograph of Lymph node imprint smears showing multiple positive Acid Fast Bacilli (ZN stain, 1000X).

Figure 6D: Photomicrograph of lymph node section showing scattered Acid Fast Bacilli (ZN stain, 1000X).

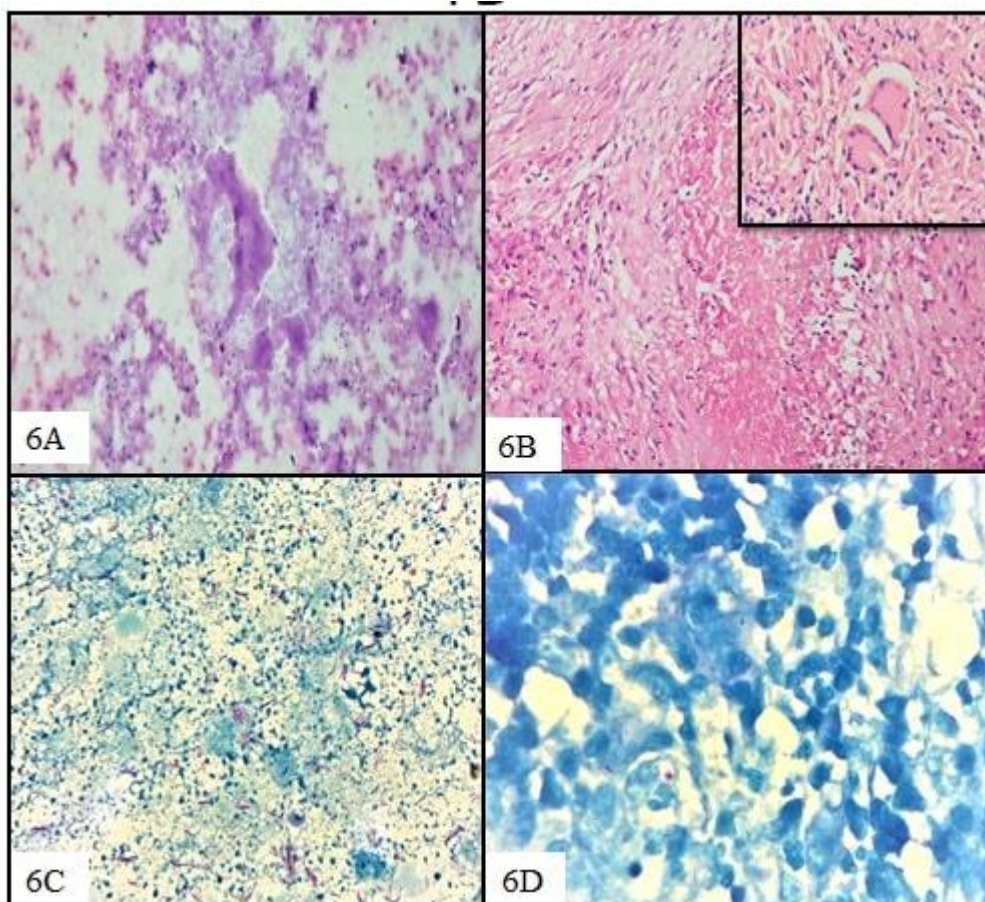
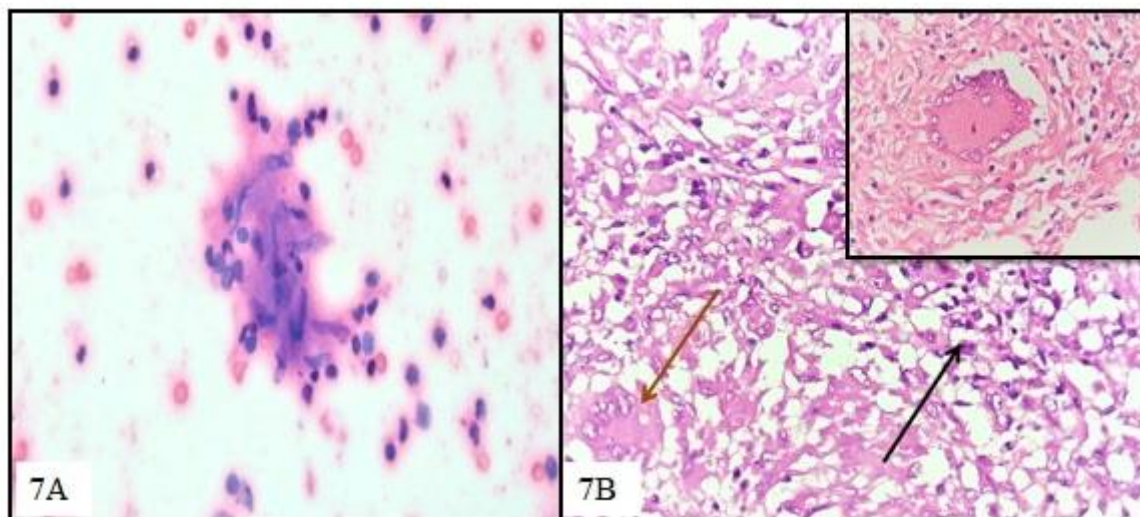


Figure 7A: Photomicrograph of lymph node imprint smear showing ill-formed epithelioid cell granuloma along with few lymphoid cells in the background (H&E stain, 400X).

Figure 7B: Photomicrograph of lymph node section showing multiple scattered epithelioid histiocytes (black arrow) and a single giant cell (brown arrow). Inset shows multinucleated giant cell (H&E stain, 400X).



Among the non-neoplastic lesions we received, tuberculous lymphadenitis was the most common. This was similar to the non-neoplastic lesions in the studies conducted by Ademiluyi et al [27], Arif et al [29] and Choudhary et al.[7]. The least common lesions we encountered were of granulomatous lymphadenitis, non-specific lymphadenitis and chronic lymphadenitis. Both Arif et al [29] and Choudhary et al.[7] analyses exhibited a sensitivity of 90.90% and a specificity of 98.57%. This was equivalent to our study in which we had 100% sensitivity and specificity in diagnosing the non-neoplastic lymph node lesions by touch imprint cytology.

Our study had a 100% positive predictive value, which was comparable to the studies conducted by Soo et al [30], Tamiolakis et al [31], Memar et al [15], Lumachi et al [14], and Gore et al [8]. While the negative predictive value of our study came out at 91.3%, which was comparable to research published by Soo et al [30], Tamiolakis et al [31], and Petursson et al [24].

Our study's overall accuracy, which was 92.31%, is most equivalent to the investigations of Lumachi et al.[14] and Khanna et al [20], which obtained overall accuracy results of 89.1% and 94%, respectively. Both have undertaken research to identify metastases in sentinel lymph nodes in breast cancer patients. Gore et al [8], Choudhary et al [7], Creager et al [17], Arif et al [29], Deo et al [18], Ersoy et al [21], Uno et al [23], Adhya et al [16] and Chang et al [25] all exhibited very identical rates of accuracy.

Our study's overall "p-value" of less than 0.001 demonstrates the value and parity of imprint cytology smears with histopathological diagnosis. With the "p-value" in all of the aforementioned studies being less than 0.001, this is very similar to one of the earlier studies conducted by Ademiluyi SA et al [27] and another study conducted by Gore et al [8]. The study conducted by Soo et al [30] had a "p-value" of less than 0.05, which was comparable to our study.

## Conclusion

Lymph nodes are an essential component of the immune system. Changes in the consistency and size of lymph nodes are frequently connected with underlying illness. Lymph nodes constitute one of the most important tissues that serve as ideal breeding grounds for a range of diseases. Lymphadenopathy, which can be either localized or widespread, is among the most common complaints that patients comes with to their doctors. With the discovery

of several new diseases and a large number of individuals seeking treatment at an advanced stage of their illness, early detection is crucial for disease management.

With all the problems described above in fine needle aspiration cytology and the lengthy wait for histopathology diagnosis, touch imprint cytology provides a simple, quick, and cost-effective method of tissue testing that is critical for patient care. It, in combination with frozen section analysis, can offer a nearly precise intra-operative diagnosis of removed tissues, assisting in patient care.

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