# Non-Neoplastic Lymph Node Diseases.A 10 year review with the use of Immunohistochemical and special stains.

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**Abstract**

For long, emphasis had been put on neoplastic lymphoid lesions whereas the non-neoplastic, benign lesions had been treated with neglect despite the fact that non-neoplastic nodal lesions are more common than neoplastic ones. The non-neoplastic nodal lesions can be very difficult to differenciate from their neoplastic counterpart in some instances without the use of immunohistochemical and special stains.

A retrospective review of 232 lymph nodes diagnosed as non-neoplastic lymph node diseases, 9 cases were excluded after immunohistochemical and special stains because they turn out to be follicular lymphoma( four cases), CLL/SLL(two cases) and metastatic deposits (three cases) leaving a total of 223 lymph nodes. A combination of twelve immunohistochemical stains namely CD 1a, CD5, CD10, CD20, CD23, CD30, CD45,CD68, S-100, HMB 45, EMA, Bcl-2 and nine special stains ,Giemsa, tissue gram stain, Warthin stain, Congo red, Von Kossa, Zeiehl-Neelson, Periodic Acid Schiff, Masson Fontana and Grocotts Methylamine Silver were used.

The immunophenotype was typical for cases of Rosai- Dorfman disease which was positive for CD 68, S-100 and negative for CD1a. Castleman disease express strong staining for dendritic marker CD 23, it was also typical for a case of dermatopathic lymphadenitis (S-100 and CD68) positive. Some lymph nodes that were diagnosed as follicular hyperplasia stain positive with germinal center marker Bcl-2. Many lymph nodes were positive for the tubercle bacilli after Ziehl-Neelson stains.

Keywords: Lymphadenopathy, non-neoplastic, special stains, immunohistochemistry



## Introduction

The various components of the lymph node react to various known and unknown stimuli by undergoing reactive morphological changes [1]. Following their initial development from precursors in the bone marrow (B cells) and the thymus (T- cells), lymphocytes under the influence of specific cytokines and chemokines home to lymph nodes which constitute the peripheral lymphoid tissue [2]. Within the lymphoid follicles, several days of antigenic stimulation, the primary follicles enlarge and are transformed into pale staining germinal centers, where B cell acquire the capacity to make high-affinity antibodies against specific antigens [3]. The degree and pattern of the morphologic changes are dependent on the inciting stimulus and the intensity of the host response [4].

Infections and inflammatory stimuli often elicit regional or systemic immune reactions within lymph nodes [5]. In granulomatous inflammation, this is an organized immune response of the body to contain an offending agent that is difficult to eradicate [6]. Tuberculosis is the prototype of a granulomatous inflammation, but leprosy, sarcoidosis, cat scratch disease, lymphogranuloma inguinale, brucellosis, syphilis, mycotic infections, berylliosis and some autoimmune diseases can cause granulomatous reactions [7]. A granuloma is a focus of chronic inflammation consisting of a microscopic aggregation of macrophages that are transformed into epithelium-like cells, surrounded by collar of mononuclear leukocytes principally lymphocytes and occasionally plasma cells. The initiation of granulomatous reaction is centered on the interplay between the immune particularly macrophages, endothelial cells, stroma and the inciting agent/material. Macrophages are primary in the initiation of granuloma formation. Upon exposure to an antigen, previously activated T-cells recognize the antigen displayed by Antigen Presenting Cells (APC) and respond to the antigenic challenge [7]. $T_H1$ secretes cytokines mainly INF-**γ,** which are responsible for many of the manifestation of delayed-type

hypersensitivity. INF-γ activated macrophages are altered having now the ability to phagocytose and kill microorganisms and express more class II Major Histocompatibility Molecules on their cell surface, thus facilitating further antigen presentation. They secrete TNF, IL-2 and chemokines which promotes inflammation. They also produce more IL-12, thereby amplifying the $T_H1$ response [7]. Thus, activated macrophages serve to eliminate the offending antigen, if the activation is continued, inflammation and tissue injury result. Most infections in the lymph node, however, cause stereotypical patterns of lymph node reaction designated acute and chronic non-specific lymphadenitis.

Generally, the deviation from the normal architecture can present in several forms. Patterns of lymphoid hyperplasia will include follicular, parafollicular, sinus, diffuse and mixed patterns. Others include mantle zone hyperplasia, T-cell hyperplasia, marginal zone (monocytoid B-cell hyperplasia). Follicular hyperplasia is caused by stimuli that activate humoral immune responses [7]. It is defined by the presence of large oblong germinal centers (secondary follicles), which are surrounded by naïve B cells (mantle zone). Germinal centers are polarized into two distinct regions, a dark zone containing proliferating blast like B cells (centroblasts) and a light zone composed of B cells with irregular or cleaved nuclear contours (centrocytes). Follicular hyperplasia is seen in HIV/AIDS, syphilis, rheumatoid arthritis, toxoplasmosis and Castleman disease. Paracortical hyperplasia is caused by stimuli that trigger T cell mediated immune responses, such as acute viral infections e.g. infectious mononucleosis. The T cell regions typically contain immunoblasts, activated T cells three to four times the size of resting lymphocytes that have round nuclei, open chromatin, several prominent nucleoli, and moderate amount of pale cytoplasm. Sinus histiocytosis refers to an increase in the number and size of the cells that lined the lymphatic sinusoids, although nonspecific, this pattern is seen in Rosai-

Dorfman disease, Whipple disease, Langerhans cell histocytosis and many other conditions [7]. Diffuse pattern of lymphoid hyperplasia is seen in toxoplasmosis, dermatopathic lymphadenitis, Systemic Lupus Erythematosus, Kikuchi-Fujimoto lymphadenitis and Kawasaki's disease [7].

Occasionally, the anatomic site of the lymphadenopathy provides a clue to the etiology for example, Epstein – Bar virus infection and toxoplasmosis are characteristically associated with enlargement of the posterior cervical lymph nodes. The hilar lymph nodes are often involved in non-neoplastic disease such as sarcoidosis, tuberculosis, histoplasmosis as well as malignant lesions such as metastatic lung cancer. Since lymph node may be the site of a primary or secondary malignancy especially lymphoma, carcinoma or melanoma, the malignant cells occasionally are difficult to distinguish from reactive cellular background.



Immimohstochemistry is very useful to evaluate reactive changes from lymphoma mainly follicular lymphoma, lymphocyte-rich classical Hodgkin lymphoma, malignant melanoma and metastatic carcinoma. It is also useful in differentiating Langerhan cell histiocytosis from Rosai-Dorfman disease. In this report, we use imminohistochemical stains for reactive causes of lymph node enlargement and special stains for infective causes.

## Methodology

It was a 10 year retrospective review of all lymph nodes biopsied from 1st January 2004 to31st December 2013 and diagnosed as non – neoplastic. Paraffin embedded tissue blocks and slides were retrieved from the departmental achieve using patients laboratory number and request cards with attention to local guidelines for human research and ethical approval obtained.The infective causes of non-neoplastic lymphadenopathy undergo special stains to identify the etiologic agent. Special stains were use to stain for calcium in idiopathic calcinosis, to exclude amyloidosis of the lymph node and to identify melanin pigments in dermatopathic lymphadenitis. The special stains used include Giemsa, tissue gram stain, Warthin starry, Congo red, Von Kossa, Ziehl-Neelsen, Periodic acid Schiff and Grocott's methylamine silver and Masson Fontana. Immunohstochemistry was used on lymph nodes diagnosed as follicular hyperplasia to ascertain if it is neoplastic. It was also used for the rare causes of lymphadenopathy like Rosai Dorfman disease, Castleman disease and dermatopathic lymphadenitis to see the staining pattern. Twelve panels were used i.e, CD1a, CD10, CD20, CD23, CD30, CD45, CD68, HMB45, S-100, BCL-2, EMA and CD5. Genemed biotechnology immunohistochemical protocol was adapted in the study, data was analysis using statistical program for social science (SPSS) version 20. Student t and f tests was use for continuous variables and chi-square test for categorical variables with the Level of significant set at P=0.05.



## Results

A total of 232 cases of non-neoplastic lymph node diseases were reviewed, 9 cases were excluded after immunohistochemical Stains, leaving a total of 223 lymph nodes. Of the 223 lymph node biopsied 133 (59.6%) cases were males, while 90 (40.4%) cases were females with a male to female ratio of 1.5:1. The age range of patients was 0.5-75 years. The peak age of nodal biopsy for females was the third decade of life white that for males was in the first and second decade. The mean age of females 24.8 (146) was not significantly greater than that of males 23.3(16.6) t-0.713 df=221 p=0.335. The commonest site of biopsy was cervical region with 103 (46.2%) case followed by mesenteric group with 35 (15.7%) cases then axillary with 30(13.5%) cases 14(6.3%) cases had no regional site indicated( tables I and II).

The commonest lymph node diagnosis was tuberculosis accounting for 116 (52.0%) cases followed by follicular hyperplasia with 64 (28.7%) cases. Both male and females had tuberculosis as the most common diagnosis with 62 (27.8%) and 54 (24.2%) cases respectively (table III and figure 1). Disseminated tuberculosis from the lungs involving 4 (1.7%) lymph nodes were seen while co-infection with HIV was seen in 9(3.9%) cases. Three (1.3%) lymph nodes had paracortical hyperplasia. Two (0.9%) lymph nodes had suppurative inflammation and show gram positive cocci after tissue gram stain.

The cervical group of lymph node had the highest number of lymph node diagnosis with tuberculosis 67 (30.0%) cases and also has the highest number of lymph node diagnosed with follicular hyperplasia 24 (10.8%) the mesenteric group of lymph nodes had the highest number diagnosed as mixed follicular hyperplasia i.e. follicular hyperplasia and sinus histiocytosis. The variance for site and diagnosis was found to be statistically significant $x^2$= 121.92 df =70, p=0.0009.

Of the 64 (28.7%) cases of follicular hyperplasia 4 (1.7%) lymph nodes, turned out to be follicular lymphoma after immunohistochemical stains (bcl-2, CD20, CD10 and CD5 Positive) 2(0.9%) lymph nodes previously diagnosed as follicular hyperplasia came out to be CLL/SLL after immunohistochemical stains (CD5,CD20,CD10 bcl-2 Positive and CD10 negative). Three (1.3%) cases of follicular hyperplasia also had metastatic deposit from invasive ductal carcinoma nos of the breast (EMA positive) as shown in figure 4.

A single case of histoplasmosis which was previously diagnosed as chronic granulomatous inflammation was seen in the axillary lymph node of a 13 year old patient after staining with periodic acid schiff. Also seen is a case of Schistosoma haematobium involving the mesenteric lymph node of an 8 year old boy (figure 2).



Castleman disease hyaline vascular type was positive for the dendritic marker CD23 while all the two lymph nodes diagnosed as Rosai-Dorfman disease show both emperipolesis, CD68, S-100 Positive and CD1a negative as indicated in figure 3. A case of dermatopathic lymphadenitis from a 35 year old patient with mycoses fungoides was seen with lesional cells positive for CD68 and S-100 and stained positive with melanin special stain marker Masson Fontana. A case of idiopathic calcinosis was seen which was positive for Von Kossa special stain.

Table I: Percentage distribution of patients with lymph node biopsies based on age group

| Age Group | Male (%) | Female (%) | Total |
|---|---|---|---|
| 0-10 | 34 (15.2%) | 17 (7.6%) | 51 |
| 11-20 | 34 (15.2%) | 20 (8.9%) | 54 |
| 21-30 | 31 (13.9%) | 26 (11.7%) | 57 |
| 31-40 | 18 (8.1%) | 12 (5.4%) | 30 |
| 41-50 | 7 (3.1%) | 9 (4.0%) | 16 |
| 51-60 | 3 (1.3%) | 6 (2.7%) | 9 |
| 61-70 | 5 (2.2%) | 0 (0%) | 5 |
| 71-80 | 1 (0.4%) | 0 (0%) | 1 |
| **Total** | **133(59.6%)** | **90 (40.4%)** | **223** |

Table II: Site and sex distribution of patients with lymph node biopsies

| **Site** | **Female (%)** | **Male (%)** | **Total** | **Ratio M/F** |
|---|---|---|---|---|
| Axillary | 17(7.6%) | 13(5.8%) | 30 | 0.8:1 |
| Cervical | 44(19.7%) | 59(26.5%) | 103 | 1.3:1 |
| Inguinal | 5(2.2%) | 9(4.0%) | 14 | 1.8:1 |
| Mesenteric | 9(4.0%) | 26(11.7%) | 35 | 2.9:1 |
| NS | 6(2.7%) | 8(3.6%) | 14 | 4:3 |
| Posterior Auricular | 2(0.9%) | 2(0.9%) | 4 | 1:1 |
| Submandibular | 4(1.8%) | 8(3.6%) | 12 | 2:1 |
| Submental | 2(0.9%) | 2(0.9%) | 4 | 1:1 |
| Supraclavicular | 1(0.4%) | 6(2.7%) | 7 | 6:1 |
| **Total** | **90(40.4%)** | **133(59.6)** | **223** | **1.5:1** |

NS- No Site

Table III: Sex and mean age distribution of patients in relation to histological diagnosis

| **Disease** | **Female (%)** | **Male (%)** | **Mean Age(SD)** | **Total** |
|---|---|---|---|---|
| Castleman | 0(0%) | 1(0.4%) | 25 | 1 |
| Dermatopathic lymphadenitis | 1(0.4%) | 0(0%) | 35 | 1 |
| Follicular hyperplasia | 22(9.9%) | 42(18.8%) | 21.8(18.6) | 64 |
| Histoplasmosis | 1(0.4%) | 0(0%) | 13 | 1 |
| Idiopathic calcinosis | 0(0%) | 1(0.4%) | 18 | 1 |
| Mixed | 7(3.1%) | 24(10.8%) | 23(16.2) | 31 |
| Paracortical hyperplasia | 2(0.9%) | 1(0.4%) | 10.3(11.3) | 3 |
| RDD | 1(0.4%) | 1(0.4%) | 23(24) | 2 |
| Schistosomiasis | 0(0%) | 1(0.4%) | 8 | 1 |
| Suppurative inflammation | 2(0.9%) | 0(0%) | 1(0.35) | 2 |
| Tuberculosis | 54(24.2%) | 62(27.8%) | 26.2(13.8) | 116 |
| **Total** | **90(40.4%)** | **133(59.6%)** | **23.9(15.8)** | **223** |

**RDD**- Rosai Dorfman Disease

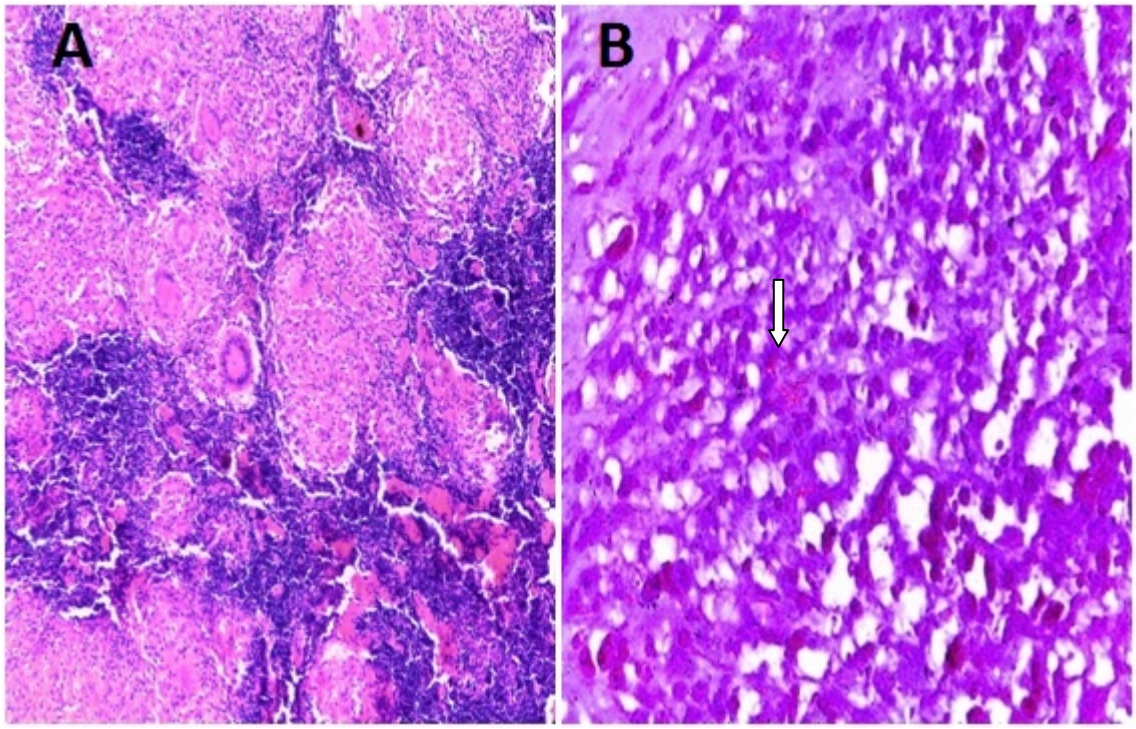




Figure 1: Photomicrographs showing caseating granulomatous inflammation from the cervical region of a 35 year old patient

A. Haematoxylin and eosin stain slides showing granulomata with langerhans type giant cells(X100)
B. Ziehl-Neelson stain slide showing tubercle bacilli shown by white arrow(X100)

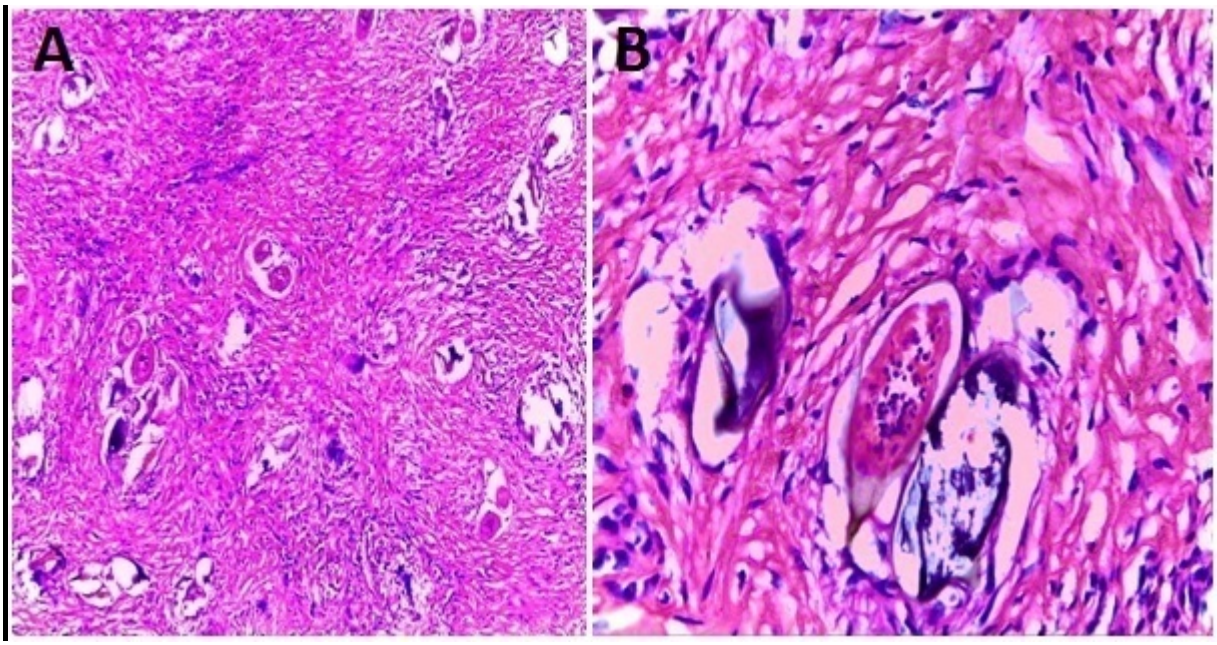


Figure 2: Photomicrographs showing Schistosoma haematobium ova in the mesenteric lymph node of a 5 year old boy.

A. Haematoxylin and eosin stain slide showing granulomata containing Schistosoma ova(X40)
B. Haematoxylin and eosin stained slide showing two calcified and one viable Schistosoma ova with terminal spine(X100)

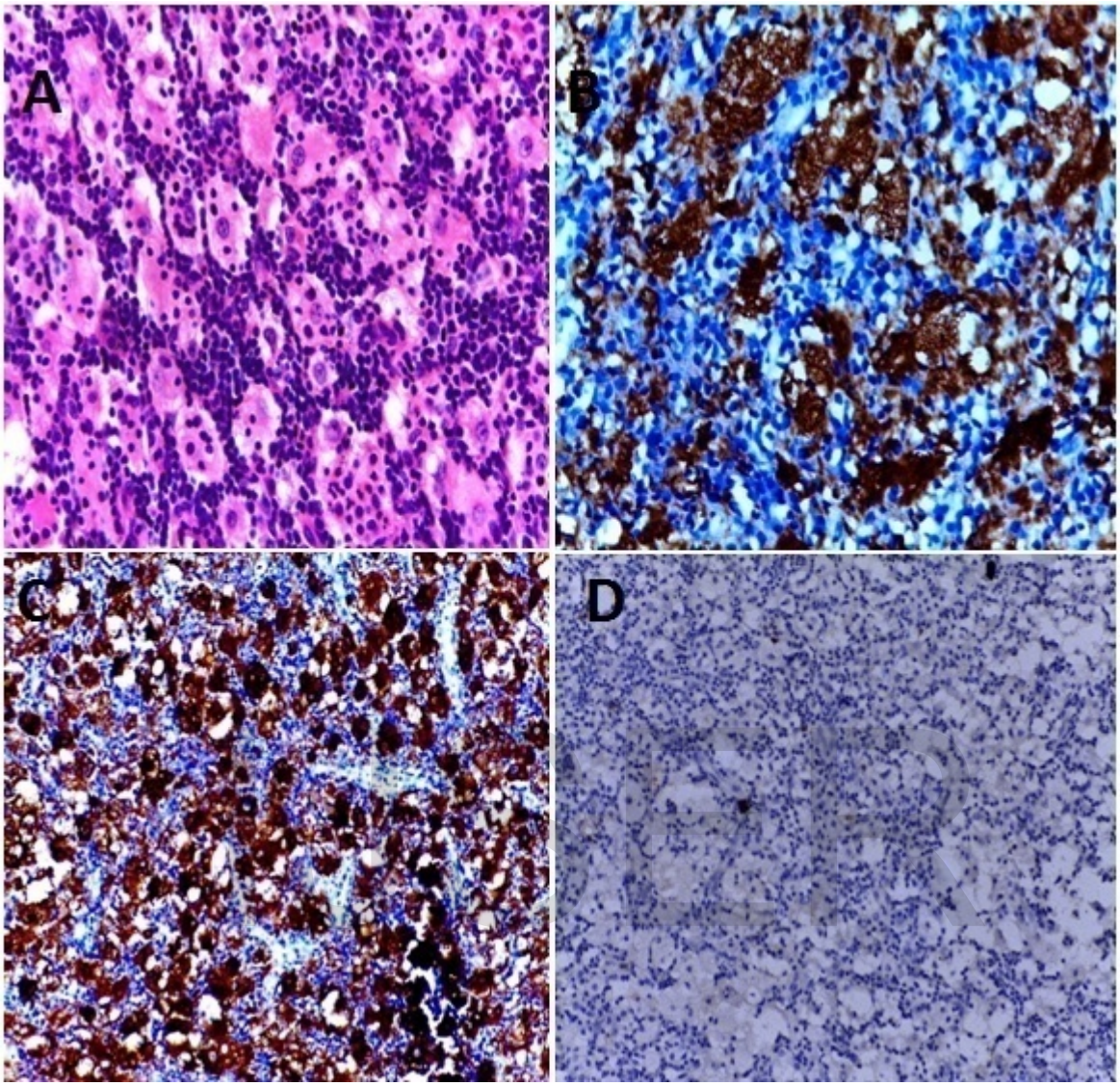


Figure 3: Photomicrographs showing a case of Rosai- Dorfman disease in the submandibular lymph node of a 6 year old female patient.

A. Haematoxylin and eosin stain slide showing emperipolesis(X100)
B. S-100 stain slide stain showing strong cytoplasmic stain by the macrophages(X100)
C. CD68 stain slide showing strong cytoplasmic stain by the macrophages(X100)
D. CD1a stain slide showing negative staining(X40)

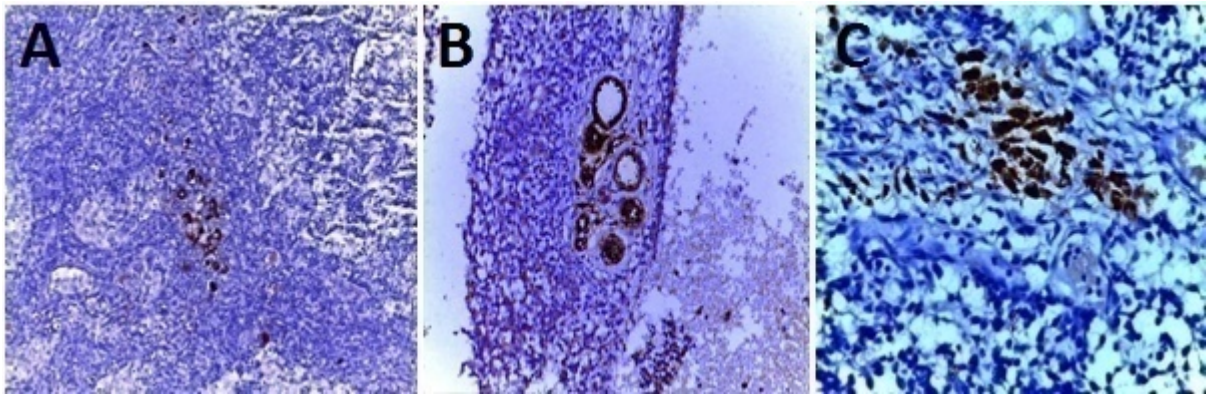


Figure 4: Photomicrographs showing three lymph nodes with metastatic deposits from the axillary lymph node of 36, 39, and 58 year old patients previously diagnosed as follicular hyperplasia.

A. A & B EMA stained slide showing strong membranous and cytoplasmic staining of epithelial cells in lymph node of patients with invasive ductal carcinoma (X40)

C.EMA stained slide showing strong membranous and cytoplasmic stain of epithelial cells in lymph node of patient with squamous cell carcinoma of the tongue(X100)

## Discussion

The commonest infections lymph node disease was tuberculosis in our environment this is supported by earlier studies by Oluwale et al (51.7%) Ochicha et al (46%) [8] .It affects the cervical group of lymph node commonly (46.2%). The reasons why cervical lymph node were most often biopsied is because probably they are often enlarged, very superficial and easily palpable and frequently involved in tuberculosis [9]. These findings are in tandem with that of Adelusola et al in western part of Nigeria, moreover most patients that present in our centers one farmers and with repeated injury and infection of their foot, the inguinal lymph node is unsuitable for biopsy because of fibrosis [10]. World Health Organisation(WHO) has indicated that tuberculosis is a common infections disease worldwide with emergence of MDR-TB and XDR-TB [11],[12],[13]**.** World Health Organisation (WHO) in its 2016 "Global Tuberculosis Report" which ranked Nigeria fourth behind India, Indonesia and China as one of the six countries which accounted for 60 per cent of the total TB burden. Some 1.8 million people died from TB in 2015, of whom 0.4 million were co-infected with HIV [14]. The WHO 10-year strategy (2006-2015) to cut down the burden of TB in the world worked elsewhere as it reportedly saved some 37 million lives while some countries halved the prevalence of the disease. But in Nigeria, the reverse is the case. According to the National TB and Leprosy Control Programme (NTBLCP), over 600,000 new cases of tuberculosis occurred in Nigeria from a global report conducted in 2014. Global strategies need to be strengthened to reduce transmission of TB. It requires commitment of stakeholders, donor groups and NGO's [14].

The immunophenotype was classical for Rosai Dofman disease CD68 positive, S-100 positive, CD1a negative and for Dermatopathic lymphadenitis S-100 and CD 68 positive, follicular hyperplasia also show classical negativity for the follicular center cells [15],[16],[17]. Castleman

disease also shows strong immunoreactions to CD23 [18],[19],[20]. What was worrisome in this study was not only the level of high tuberculosis but also the number of follicular hyperplasia that turned out to be lymphomas and metastatic deposits. Mimics of follicular hyperplasia can easily be distinguished from follicular lymphoma in that on H&E benign follicles tend to be mostly in the cortex and that they are of varying sizes, they show zonation of the germinal center and have abundant tingible body macrophages. But the most important thing to keep in mind is that neoplastic follicular center cells have lost vital growth regulatory mechanisms hence they express bcl-2 protien in 95% of cases. The two lymph nodes diagnosed as follicular hyperplasia instead of CLL/SLL had on H&E (Haematoxylin and Eosin) monomorphic appearance of the B lymphocytes with pseudo follicles in areas and stain positive for bcl-2, CD5, CD10, CD20, CD23 [21],[22],[23],[24],[25],[26].



**Conclusion**

This study has shows that occasionally lymph node from breast cancer patient needs to undergo immunohistochemical stain will either EMA or cytokeratin to ascertain if there is no metastatic deposit. This study highlights the need for optimal treatment of patients with non-neoplastic and neoplastic lesions based on accurate diagnosis. With new advances in the treatment of non-neoplastic and neoplastic diseases, clinicians now demand accurate characterization of most lesions. To the pathologist especially those practicing in the third world country like Nigeria; immunohistochemistry is the most important tool that has made this a reality.

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