A CORRELATIVE CYTOLOGICAL AND HISTOPATHOLOGICAL STUDY ON SUPERFICIAL LYMPHADENOPATHY

DISSERTATION SUBMITTED FOR

M.D. (BRANCH - III)
PATHOLOGY

MARCH 2007

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY

CHENNAI – TAMIL NADU

Department of Pathology **Madurai -20**

Madurai Medical College and **18.10.2006.**

Government Rajaji Hospital

Madurai.

CERTIFICATE

This is to certify that the dissertation entitled "**A CORRELATIVE CYTOLOGICAL AND HISTOPATHOLOGICAL STUDY ON SUPERFICIAL LYMPHADENOPATHY**" presented herewith by **Dr.P. VISALAKSHI** to the faculty of Pathology, The Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D. degree in Pathology is a bonafide work carried out by her during the period January 2004-December 2005 under my direct supervision and guidance.

Dr. D.GOMATHINAYAGAM, M.D.,
Professor and Head
Department of Pathology
Madurai Medical College
Madurai-625 020.

CONTENTS

INTRODUCTION

The lymph node is one of the major anatomic components of the immune system. Lymph nodes are the most widely distributed and easily accessible component of lymphoid tissue and hence they are frequently examined for diagnosis of lymphoreticular disorders.

Diagnosis of lymphadenopathy depends mainly on excision of a gland and histopathological examination. For this, general anaesthesia and hospitalisation are required. Fine needle aspiration cytology, on the other hand, is free from these disadvantages and can safely be used as an alternative or complementary investigative technique.

Anatomy:

Lymph nodes are organized collections of lymphoreticular tissue in the form of pink gray, kidney shaped, encapsulated organs. They are located at anatomically constant points along the course of lymphatic vessels. The common sites of distribution for clinical consideration are the cervical, axillary, supraclavicular and inguinal regions.

Histology:

The lymph node consists of lymphocyte aggregations, intermeshed with lymphatic sinuses supported by reticular fibre framework and surrounded by connective tissue capsule.

The lymph node has cortex, para cortex, and medulla.

The cortex is situated beneath the capsule and contains large number of follicles. The medulla close to the hilum is rich in lymph sinuses, arteries, and veins but contains

only a minor lymphocytic component. Both cortex and medulla represent 'B'zones. The cortex contains primary and secondary follicles, which are characterized by the presence of central pale area of germinal centers. The cells present in these formations are B-lymphocytes known as follicular center cells (centroblasts, centrocytes or small and large cleaved and noncleaved cells), macrophages, and follicular dentritic cells. A mantle of dark staining small B-lymphocytes surrounds the germinal centre. The para cortex is the zone situated between the cortex and medulla which contains the mobile pool of T lymphocytes[50]

Development:

In human embryo, the lymph sacs from which the lymph vessels are derived are six in number. 2 paired (jugular and the posterior lymph sacs) and two unpaired (the retroperitoneal and cysterna chyli). From the lymph sacs, the lymph vessels bud out along lines corresponding more or less closely with the course of embryonic blood vessels, most commonly veins.

All the lymph sacs except the cysterna chyli are at a later stage divided by a number of slender connective tissue bridges; subsequently they are invaded by lymphocytes and transformed into groups of lymph nodes.

Fine needle aspiration cytology of lymph node:

A normal lymph node is rarely palpable. Cytological characteristics of cells from a normal lymph node are essentially based on the morphology of individual cells as observed in the aspirate from a reactive lymph node. The lymphocytes constitute 87% to 99%, Plasma cells 0% to 5% and remainder cells (histiocytes, mast cells, eosinophils and neutrophils) 1% to3%.

AIM OF STUDY

- To statistically evaluate the incidence of lymph node lesions.
- To assess the usefulness of the cytological study in the diagnosis of lymph node lesions.
- To evaluate the accuracy of FNAC studies in correlation with histopathological study.
- To compare the sensitivity, specificity and accuracy with previous studies conducted at other centres.
- To determine and evaluate the causes for false positivity and negativity and to arrive at possible suggestions to minimize the percentage, in this regard.

REVIEW OF LITERATURE

Grieg and Gray[36] used aspiration of lymph nodes as early as in 1904 for the diagnosis of sleeping sickness by recognizing mobile trypanosomes.

Guthrie in 1921[42] compiled one of the earliest series on aspiration biopsy from cervical lymphadenopathy, describing cell specimens from lymphadenitis, metastatic carcinoma, and Hodgkin's disease. Forkner[32] in 1927 studied node punctures from 30 patients, and Martin and Ellis[80] in 1930 aspirated from lymph nodes in 23 cases.

FNA biopsy rarely interferes significantly with histological interpretation[5]. FNA biopsy can be useful to obtain material for culture, because a specific diagnosis can lead to specific therapy.[65]

Normal lymph node and reactions:

Ninety-five percent of the cells found in FNA biopsy specimens of normal lymph nodes are Lymphocytes in which small, mature lymphocytes normally compose about 67% to 90% of the cells, immature lymphocytes about 5% to 16%. Histiocytes and monocytes comprise less than 8%. Mast cells are sparse in the normal lymph node, about 0.1% of the cells. Neutrophils comprises 0% to 2.2% of cells, Plasma cells are 0% to 5%. Eosinophils are about 0% to 0.3%. Basophils comprise about 0% to 0.2% of the

cells.[73]

Cyto diagnosis of lymph node aspirate can be grouped as:[78]

1. Lymphadenitis and hyperplasia
2. Lymphomas and Leukemias
3. Metastatic neoplasms

Lymphadenitis and hyperplasia:

Depending on the type of stimulus, a node may react with one of three basic histological and cytological patterns:

1. Reactive hyperplasia
2. Suppurative lymphadenitis
3. Granulomatous lymphadenitis

Reactive hyperplasia

The causes of reactive lymphoid hyperplasia would make a long list of bacteria, viruses, chemical, environmental pollutants, drugs, altered tissue components, and numerous other substances acting as antigens or allergens. Most enlarged lymph nodes involved by reactive lymphoid hyperplasia or atypical lymphoid hyperplasia do not exhibit morphologic patterns indicative of specific agents, and therefore the cause of reactive lymphoid hyperplasia is listed as unknown.

Cytology:

High cell density, polymorphic cytological pattern, and tingible body macrophages (TBM) are the three important characteristics of follicular hyperplasia.[114]

Follicular Center Fragments represent intact or fragmented lymph node follicles,[87] the presence of all three components in the follicular center fragment (i.e., range of maturation, tingiblebody macrophages, lymphohistiocytic aggregates) generally indicates a benign, reactive lymphoid process.

Although similar fragments can be seen in lymphoma, they are usually missing at least one element, e.g., tingiblebody macrophages. In addition, the presence of follicular center fragments does not exclude Hodgkin's disease or metastasis.

However, any high-grade lymphoma can have tingiblebody macrophages. In addition, lymph nodes harboring metastatic tumor, Hodgkin's disease, or those only partially involved by lymphoma may have tingiblebody macrophages on FNA biopsy.

On the other hand, tingiblebody macrophages (TBM) may be rare in benign, indolent lymph nodes. Therefore, the mere presence of TBMs does not guarantee that a lymph node FNA biopsy is benign. Moreover, the absence of tingiblebody macrophages does not necessarily mean the aspirate is malignant.

Lymphohistiocytic aggregates are also a common feature of benign lymph node aspirates, found in up to three quarters of cases. Lymphohistiocytic aggregates are often indicating follicular center formation, but it can also be seen in sinus histiocytosis or medullary cord expansion.

Lymphohistiocytic aggregates are also common in aspirates of follicular hyperplasia and they are thought to be markers of follicle formation.[87]

However, a diagnosis of reactive hyperplasia, or chronic lymphadenitis, does not necessarily exclude the possibility of metastatic carcinoma, granuloma, or Hodgkin's disease, all of which can occur in a reactive background.[39]

In the absence of characteristic cells (malignant, epithelioid, or Reed-Sternberg), the cytologic diagnosis would be largely descriptive.

Histopathology:

Most benign, inflammatory hyperplasia are seen in youth.[67]

Lymphadenitis and lymphadenopathies tend to exhibit one of four characteristic histologic patterns: follicular, sinus, diffuse, or mixed.[26] These represent expansion of the normal follicular, paracortical, medullary and sinusoidal lymph node compartments.

The sinus pattern is particularly noticeable in sinus histiocytosis with massive lymphadenopathy and in lymph nodes draining malignant tumors.

In Diffuse Pattern, the entire lymph node parenchyma consists of sheets of lymphocytes admixed with immunoblasts and occasional macrophages. Diffuse immunoblastic, Para cortical hyperplasia is caused by certain viral infections, particularly infectious mononucleosis, vaccinations, drug reactions (classically Dilantin) and also seen in some autoimmune diseases, such as systemic lupus erythematosus.[107]

Important clues of benign lymphadenopathy include the presence of plasma cells,

in different stages of maturation and immunoblasts, together with other lymphoid cells and histiocytes, without obvious signs of anaplasia.[127]

The reactive follicles do not infiltrate the surrounding adipose tissues largely, even when highly stimulated.

Suppurative lymphadenitis:

In the initial phase, mixture of lymphocytes and neutrophils may be seen in the smear; in the florid state the smear contains both well-preserved as well as degenerated neutrophils and cell debris. With organization of the inflammatory exudates, especially in the antibiotic treated cases, the smear contains polymorphs, lymphocytes, plasma cells and histiocytes along with cell debris.

Granulomatous lymphadenitis:

Tuberculosis:

Cytology:

Nodular collections of epithelioid histiocytes with Langhans' giant cells and caseous necrosis are most characteristic in FNAC.[2] However, occasionaly, granulomas without necrosis or conversely only necrosis, without epithelioid or giant cells may be aspirated.[18]

Aspirated granulomas may form acellular, homogeneous, eosinophilic structures with irregular shapes and well-defined edges.[91]

Aspirated material can also be cultured to help make the specific diagnosis of tuberculous lymphadenitis.[99]

Mycobacteria cannot be seen in Diff-Quik, Papanicolaou, Gram stain and it is most likely to be demonstrable by using acid fast or auramine-rhodamine stains in the necrotic material.[38]

Lau S-K et al (1991)[64] stated that the combined use of tuberculin skin testing and FNA biopsy are complementary and efficient in the diagnosis of tuberculosis.

Histopathology:

The appearance ranges from multiple small epithelioid granulomas to huge caseous masses surrounded by Langhans' giant cells, epithelioid cells and lymphocytes.

Monocytoid B cells may play a role in recruitment of neutrophils and development of necrosis in Chronic Granulomatous Disease.[30]

Giant cells are not normally present in lymph nodes but it can be seen in a wide variety of benign and malignant conditions. Benign giant cells are associated with acute, chronic or granulomatous lymphadenitis. Malignant giant cells are associated with T-cell lymphoma, anaplastic large cell lymphoma (T or B), Hodgkin's disease and metastasis.[102]

The epithelioid histiocytes may proliferate to the point of mimicking a neoplasm (spindle cell pseudo tumor).[15]

Granulomas can be seen in Hodgkin's disease, T cell non-Hodgkin's lymphoma and some carcinomas, particularly with squamous differentiation.[90]

Martelli G (1989)[79] stressed that in a clinically suspected case, especially if the aspirate contains pus, a bacteriological examination should be tested for acid-fast bacilli and a culture made to improve the diagnostic accuracy.

FNAC is a reliable diagnostic tool in helping to avert the more invasive surgical procedures undertaken in the diagnosis of tuberculous adenitis.

The success result of FNAC in tuberculous lymphadenitis was 80% (Bloch 1967)[10] 87.18% (Patra et al 1983)[93]and 83.33% (Dandapat et al 1986)[17]

Bezabih M et al (2003)[9] studied 880 patients presenting with enlarged superficial lymph nodes and pointed that among the benign lesions the most frequent cause of lymph node enlargement was tuberculous lymphadenitis (66.3%), followed by reactive hyperplasia (19.2%).

FNA biopsy can play an important role in evaluation, including diagnosis of opportunistic infections, Kaposi's sarcoma and lymphoproliferative lesions such as persistent generalized lymphadenopathy and lymphomas.[11]

Lymphomas:

Lymphomas are classified broadly into Hodgkin’s Lymphoma and Non-Hodgkin’s Lymphoma. There are malignant proliferations of lymphoid cells, which are normally found in lymphoid organs such as lymph nodes, tonsils, spleen and thymus gland. In addition, lymphomas may arise in every other type of organ such as the brain or stomach and these are termed extra-nodal lymphomas.

Non-Hodgkin's Lymphoma:

Rappaport(1966)[101] published classification of Non-Hodgkin's Lymphoma according to tissue architectural pattern and cytologic findings.

In 1970s, Lukes and Collins[75, 76] deemphasized growth pattern and introduced the immunologic concepts of B and T cells.

The Kiel classification is based entirely on cytological criteria[68] hence, it can be directly applied to cytological biopsy material.[89]

Working Formulation was proposed in the 1980s, in an attempt to overcome the diagnostic chaos caused by so many different classification systems.[69, 83]

According to Working Formulation Non Hodgkin Lymphomas are classified into low, intermediate and high grade based only on morphologic appearance and not on immunology. Realizing the limitations of cytology in the diagnosis of histologic characteristics such as nodularity, the grouping of the various cytologic types of NHL has been slightly modified.

REAL system (1994) categorizes entities based on the neoplasm's cell of origin. The new WHO classification for lymphomas is similar to the REAL system with minor modification.

Half of the patients with NHL are in their fifties to sixties. NHL more frequently affects men than women.

Cytology:

FNA biopsy should be the first line of morphologic investigation of lymphadenopathy.[19]

The diagnosis of lymphoma can actually be easier with cytologic than with histological studies (single cell pattern, lympho glandular bodies, enhanced cytological detail).[127]

In addition in some cases, the cytologist rather than the histologist may be better able to sub classify malignant neoplasm of the lymphoreticular system.[10]

The diagnosis of lymphoma particularly non-Hodgkin's type, by FNAC should be usually confirmed before therapy. Methods of confirmation depend on the individual diagnosis and can include examination of the peripheral blood, bone marrow, serum and urine proteins.

Usually, a tissue biopsy is obtained for histologic classification and verification of a primary cytological diagnosis of lymphoma.[33]

The diagnosis of low-grade lymphomas can sometimes be difficult, particularly for those that have a conspicuous nonmalignant component.[115]

There may be a diagnostic gray zone between low and intermediate grade lymphomas, because architectural growth pattern usually cannot be assessed cytologically.[120]

Cytodiagnosis is particularly important in patients who are in poor surgical risks, in whom an abnormal superficial lymph node is unavailable for tissue biopsy[102] and if

the only accessible node is in a location that makes excisional biopsy hazardous, e.g., directly on an important nerve.[72]

Reactive lymphadenopathy is the most likely benign process to be interpreted as malignant.[100]

On the other hand, if a lymphoma is mistakenly considered to be a benign, reactive process it will not follow a typical, benign clinical course or significantly respond to medical therapy.[97]

False-negative results are usually as the result of inadequate sampling due to small or inaccessible nodes, fibrosis, necrosis, or small cell lymphomas, or sampling the wrong node.[105]

In some cases of lymphoma, the clinical signs and symptoms may be misleading, suggesting some other disease. FNA biopsy can help point to the correct diagnosis.[102]

A dispersed population of single cells and lymphoglandular bodies that can sometimes make the diagnosis of lymphoma is more obvious in cytologic than histologic studies.[98]

When confronted with a poorly differentiated neoplasm, the diagnosis of lymphoma can actually be easier with cytologic than with histologic studies.[127]

If the FNA biopsy is obtained to document recurrence or for staging of known disease, i.e., secondary diagnosis, then the cytologic diagnosis alone is generally sufficient.

Secondary diagnosis of lymphoma are usually easy, accurate and reliable and may obviate open biopsy.[98]

FNA biopsy can usually distinguish between low-grade and high-grade non-Hodgkin's lymphomas.[22]

Cytologic studies can be useful for documenting transformation to a higher-grade malignancy as may be encountered in cases like Richter syndrome.

FNA biopsy is also useful in obtaining diagnostic material for special studies such as flow cytometry, electron microscopy, immunocytochemistry, molecular cytogenetics, gene rearrangement, polymerase chain reaction or research.[119]

Berard et al [7] (1981) pointed that monoclonality usually indicates malignancy and polyclonality favors a benign lesion.

Epstein-Barr virus may also play a role in the development of non-Hodgkin's lymphomas in a proportion of HIV-infected patients. In HIV patients, most lymphomas are non-Hodgkin's and usually high grade such as small noncleaved or large cell immunoblastic.[37]

Low-grade, small cell, or mixed lymphomas and lymphomas confined to follicles or only partially involving a node are more difficult to recognize and subclassify with cytologic studies, often mimicking reactive lymphadenopathy.[126]

FNA biopsy can usually diagnose high-grade Non-Hodgkin's lymphomas and most cases of Hodgkin's disease. Diagnosis of low-grade lymphomas can sometimes be difficult, particularly for those that have a conspicuous nonmalignant component.[96]

Occasionally, vague dense aggregates of lymphoma cells, apparently corresponding to follicles, suggest follicular differentiation in FNA biopsy specimens.[89]

The FNA biopsy of *lymphoblastic lymphoma* consists, in essence of numerous blasts with frequent mitoses.[52]

The smear of *small lymphocytic lymphom*a is composed of monotonous population of lymphoid cells, the cells have scanty light blue cytoplasm and round nuclei with multiple coarse chromatin. Mitotic activity is rare.

FNA Biopsy of *Large cell lymphoma* shows predominantly noncleaved large cells.

The aspirate of *Lymphoplasmacytic lymphoma* is similar to ordinary small lymphocytic lymphoma, except that many lymphocytes are plasmacytoid.[20]

In *Small Cleaved cell Lymphoma* the most characteristic feature is the cleaved nucleus which is irregular and markedly indented with deep folds.

The nuclei of *Burkitt's lymphoma* are round to oval and occasionally multiple. The nuclear membrane is generally smooth (ie, noncleaved), but may be notched or indented.[21]

The FNA biopsy specimen of *Mycosis Fungoides* shows a variable mixture of atypical small and large lymphoid cells plus a reactive component of eosinophils, plasma cells, and nonneoplastic lymphocytes. The small cells have small, hyperchromatic nuclei and inconspicuous nucleoli. As the disease progresses, large lymphoid cells with pale, vesicular nuclei and prominent nucleoli become more

numerous.[130]

The aspirate of *T-cell lymphomas* are usually cellular, unless fibrosis is prominent and is characterized by cellular polymorphism.[53]

The FNA biopsy of *Anaplastic Large Cell Lymphoma* shows large pleomorphic cells, which range in appearance from immunoblast-like to bizarre and anaplastic.[120]

Histopathology:

The morphologic appearance of a lymphoma depends on the infiltration of normal structures, the host immune response and reactive changes that result from secretion of cytokines, all of which can vary from site to site, patient to patient and tumor to tumor.

Small lymphocytic lymphoma constitutes only 4% of NHL. It occurs in late adult life peaking in the sixth or seventh decade.

Up to 70% of patients with *small lymphocytic lymphoma* have leukemia (chronic lymphocytic leukemia, CLL) or an absolute lymphocytosis (>4,000/mm^3).[92]

In Small lymphocytic lymphoma, most of the cells (>80%) resemble ordinary small lymphocytes, except that they are often slightly larger than normal and the chromatin is not quite as dense.[103]

These cells are mixed with variable numbers of larger cells called prolymphocytes. In many areas prolymphocytes gather together focally to form proliferation centers.

In some cases of *small lymphocytic lymphoma* the chromatin is clotted (cellules grumelées, cells with little clots) and resembles exaggerated clock face chromatin of plasma cells.[113]

small lymphocytic lymphoma cells are small and the nuclear membranes are smooth except for the rare T-cell type.[71]

Immunohisto chemistry for Small Lymphocytic Lymphoma were B cell phenotype, Monoclonal surface Igs (low density), CD5+, CD10-, CD19+, CD20+, CD23+, FMC7-,bcl-1 protein-, bcl-2 protein +.

In the diffuse variant of Small Cleaved cell Lymphoma, the chromatin may be relatively delicate or blastoid and the cytoplasm are scant, but may be slightly more abundant than in a small lymphocyte, azurophilic granules can be seen in a few cases in Diff-Quik. Mitoses are usually rare.[60]

45% of adult lymphomas are *Follicular lymphoma,* it usually presents in middle age and afflicts males and females equally.

Follicular lymphoma tends to progress with time to a diffuse pattern and larger cell type.[44]

Dendritic reticulum cells or lymphohistiocytic aggregates are usually present and often abundant in *follicular lymphomas* whereas, these are only occasionally seen in diffuse lymphomas.[61]

The presence of follicular center fragments or dendritic reticulum cells, which apparently correspond to lymphohistiocytic aggregates are more commonly present and more abundant in follicular than diffuse NHL.[61]

Certain lymphomas, such as follicular center cell lymphomas and many T-cell lymphomas, including lymphoblastic can show marked pleomorphism.[127]

Approximately 60% to 70% of the cases of Follicular center lymphoma have bone marrow involvement at the time of diagnosis.

Follicular lymphoma is generally associated with a better prognosis than diffuse lymphoma of the same cell type.[131]

Immunohisto chemistry for Follicular lymphoma are CD5- CD10+, CD19+, CD23- ,CD43-,CD79a+,Monoclonal sIg+

The two main cell types of large cell lymphomas are centroblastic and immunoblastic as in the Kiel classification.[108]

The prognosis of both follicular and diffuse lymphomas correlates with the proportion of small and large cells. The more small cells have better prognosis, the more large cells have the worse prognosis.[101]

Lymphoplasmacytic lymphoma is morphologically and clinically similar to small lymphocytic lymphoma, except for the presence of plasmacytoid lymphocytes and the more common occurrence of a monoclonal gammopathy.

Intracytoplasmic (Russell) bodies and intranuclear (Dutcher) bodies, composed of immunoglobulin are highly characteristic of Lympho plasmacytic lymphoma if present, but they are not diagnostic features.[60]

Immunohisto chemistry for Lymphoplasmacytic lymphoma were CD5-CD10-CD19+, CD20+, CD23- ,Monoclonal surface IgM+.

The cells of Burkitt's lymphoma are cytologically indistinguishable from acute lymphoblastic leukemia, FAB-L3.[56]

Morphologic findings alone cannot reliably determine whether a lymphoma is of B or T cell origin,[102] but can differentiate Burkitt's and non-Burkitt's lymphomas.

Lennert's lymphoma is the occurrence of numerous epithelioid histiocytes but usually not well-formed, sarcoid-like granulomas.[94]

Mixed lymphoma is a "mixed bag" of diseases characterized by heterogeneous cell morphologies and cell types.[123]

The immunologic studies of Mixed Lymphoma have shown that most (~70%) cases are of B-cell origin, about 20% are T cell, 10% Null cell and less than 5% are true histiocytes.[132]

Most cases of lymphoblastic lymphoma show T cell phenotype, but a small proportion of cases expresses Precursor-B or B-cell phenotypes.[16]

Proliferative activity of cells also correlates with grade in general; the higher the mitotic count, the higher is the grade of the lymphoma[102]

Dividing the cells into small, medium, and large size groups correlates, to some extent with low, intermediate and high-grade lymphoma.[104]

In low-grade lymphomas, most cells (50%- 80%) are small with relatively few large cells.[109]

Intermediate lymphocytic lymphoma shares many features with small lymphocytic lymphoma and small cleaved lymphoma, the diseases are closely related.[117]

In Intermediate lymphocytic lymphoma, most of the cells are small to medium sized lymphocytes with slightly irregular nuclear contours, the chromatin is less coarse than that of normal lymphocytes and occasionally is fine. Nucleoli are distinct and like prolymphocytes, may be surrounded by a rim of chromatin .[60]

The demonstration of a dominant or abnormal T-cell phenotype in morphologically atypical cells suggests T-cell malignancy.[111]

A characteristic feature of Adult HTLV-Associated *T-Cell Lymphoma* is the presence of highly pleomorphic, lobulated (flower-like) lymphoid cells in the blood.[102] Elavathi et al (1989)[28] described multilobated nuclei in one variant of T-cell lymphomas.

In T-cell lymphomas, cytoplasm varies from scant to relatively abundant and may contain fine granules,[88] lymphoglandular bodies may be sparse or absent.

The cells of *Anaplastic Large Cell Lymphoma* are predominantly single, but occasional clustering may suggest an epithelial malignancy[4].

The nuclei of Anaplastic Large Cell Lymphoma are large, frequently indented or lobulated with granular chromatin and one or more conspicuous nucleoli.[88]

Anaplastic Large Cell (Ki-1, CD30) Lymphoma is usually of T-cell origin, although markers are heterogenous and include B cell, Monocytic, and Null cells.[95]

Immunophenotyping or genotyping is usually required to confirm T-cell origin, in

contrast with B-cell lymphomas, in which morphologic appearance alone is often sufficient.[59]

The correlation of cytologic with histologic studies, immunology, and cytometry is excellent.[3]

Composite lymphoma refers to the coexistence of two distinct types of Non-Hodgkin's lymphoma or rarely, NHL and Hodgkin's disease, in a single organ or tissue.[31]

Discordant lymphoma is the occurrence of two different types of lymphoma at separate anatomic sites.

The prognosis depends on the higher-grade lymphoma, even though the low-grade lymphoma may be disseminated.[55]

The incidence of bone marrow involvement is higher in low-grade lymphomas than in many of the high-grade lymphoma.[23]

Lopes Cardozo P (1980)[71] stated that in fresh cases of lymphoma surgical biopsies should always be done, whereas in relapses cytology alone will be sufficient.

Gupta et al (1977)[41] and Tripathi et al (1985)[121] quoted the diagnostic accuracy of lymphoma as 84% and 80% respectively.

Nasuti JF, et al (2000)[84] observed 387 cases of FNAC of lymph node over a 5-year period. In their study, they diagnosed successfully 61 cases of lymphoma via FNAC with no false positives.

Hodgkin's Lymphoma:

Classification:

In 1944 Jackson and Parker[47] divided Hodgkin's Lymphoma into three subtypes- paragranuloma, granuloma and sarcoma.

Lukes and Butler in 1966,[74] classified Hodgkin's Lymphoma in to six types, shortly afterward at the Rye conference this was simplified into four subtypes as Lymphocyte predominance, Nodular sclerosis, Mixed cellularity and Lymphocyte depletion.

In 1994 REAL classification and with further modification by WHO Hodgkin's Lymphoma is divided into two major entities, as lymphocyte predominance and classic. Classic type is further subdivided into Nodular sclerosis, Lymphocyte rich, Mixed cellularity and Lymphocyte depletion.

Cytology:

Hodgkin's Lymphoma accounts for approximately 20% of newly diagnosed lymphomas. FNAC has an important but limited role in the initial diagnosis of Hodgkin's Lymphoma.[133] However, FNAC is very useful in diagnosing recurrent disease.[19]

Reed Sternberg cells (RS) are essential in the primary diagnosis of Hodgkin Lymphoma. Lacunar cells are unusually large variants of RS cells with abundant cytoplasm, which are a characteristic feature of nodular sclerosis Hodgkin's disease.[74] However lacunar cells are seen in other types of HD particularly mixed cellularity.[14]

RS-like cells can be seen in other diseases, so the RS cells must be found in the characteristic background, i.e., in the proper milieu to diagnose Hodgkin's disease.[118]

Bizarre, Reed-Sternberg-like cells can occur occasionally, in CLL/SLL.[108]

In Lymphocyte-predominant Hodgkin's disease, the predominant cells are small, mature and activated (T) lymphocytes. Classic Reed-Sternberg (RS) cells are rare.[86]

Reed-Sternberg cells and variants are usually easy to find in Mixed Cellularity Hodgkin's Disease.

Pleomorphic R.S cells are relatively abundant and nonneoplastic lymphoid cells are sparse in Lymphocyte-depleted Hodgkin's disease.[102]

The ratio of neoplastic to reactive cells is of primary importance in classifying the subtype of Hodgkin's disease.[3]

Benign, reactive lymphoid cells, plasmacytoid cells, plasma cells, histiocytes, granulomas, metachromatic fibrillar material, eosinophils, neutrophils and necrosis may all be seen in the background of Hodgkin's disease and in cases of recurrent Hodgkin's disease, which progressed rapidly despite therapy.[34]

Rarely, neutrophils, histiocytes and necrosis mimicking suppurative lymphadenitis or an abscess dominates the background.[35]

Gupta et al (1977)[41] demonstrated the possibility of overlap among reactive hyperplasia, lymphocytic lymphoma and Hodgkin's disease in aspiration smears of lymphoma cases

The cytologic studies of Lymphomatoid Granulomatosis show a mixed inflammatory infiltrate, including atypical lymphocytes, some with Reed-Sternberg-like features, in a granulomatous background with numerous giant cells.[128] Granulomatous inflammation is a good prognostic sign in Hodgkin's disease.[106]

FNA biopsy is adequate to evaluate the stage of the disease, assess the therapeutic response and document recurrence in Hodgkin's disease.[24]

Histopathology:

Hodgkin's disease (HD) primarily involves lymph nodes. It arises in a single lymph node or group of nodes and characteristically tends to spread in contiguity from group to group.[51]

Hodgkin's disease has bimodal age distribution with first peak between 15 and 34 years and the second peak after 54 years.[77]

Men are somewhat more commonly affected than women (4:3) and also have a worse prognosis. Half of the patients are between the ages of 20 and 40. Less than 10% are younger than 10 years or older than 60 years.

Cervical lymph nodes are by far the most common primary site, followed by mediastinal and then axillary nodes.

Unlike Non-Hodgkin's lymphoma, HD virtually never presents in an extranodal site although later it can disseminate.[102]

Hodgkin's disease is curable, especially when low stage. The overall 3-year

survival rate is better than 80%.[54]

The ratio of neoplastic to reactive cells is of primary importance in classifying the subtype of HD[3]. When neoplastic cells, including RS cells and their variants are rare, as in lymphocyte predominant HD the prognosis is better.

When neoplastic cells are numerous, as in lymphocyte depleted HD the prognosis is worse. Mixed cellularity is intermediate.[3]

For the diagnosis of Nodular sclerosis Hodgkin's Lymphoma, a minimum of a single polarizable fibrous band is required.

The presence of a nodular sclerosing stromal reaction is associated with a favorable prognosis.

Two types of Lymphocyte depletion Hodgkin's Lymphoma are diffuse fibrosis type and reticular type.

Reactive lymphadenopathy due to viral infection, infectious mononucleosis, Toxoplasmosis and drug hypersensitivity reaction (eg, to hydantoin) are among the most common benign entities to be mistaken for Hodgkin's disease.[112]

Occasionally lacunar cells form syncytial clusters (syncytial variant), which may suggest large cell non-Hodgkin's lymphoma or poorly differentiated carcinoma, sarcoma, melanoma, thymoma or germ cell tumors[6]

The incidence of bone marrow involvement in Hodgkin's lymphoma is approximately 10% in classical Hodgkin's mixed cellularity type and 1% in lymphocyte predominant Hodgkin's and lymphocyte rich classical Hodgkin's disease. Nodular

sclerosis has an incidence of 3%.[82]

Singh MK et al (1988)[110] pointed out that in the diagnosis of lymphoma, false-negative cases tend to be more common and are generally based on sampling rather than diagnostic errors such as the absence of Reed-Sternberg cells which are important in the diagnosis of Hodgkin's disease.

Jimenez- Heffernan JA et al (2001)[49] evaluated cytodiagnosis of 170 cases of Hodgkin's Diseases with the final histopathological report. The sensitivity found in this series was 82.4%. The positive predictive value was 91.2%

Leukemia:

Cytology:

Lymphadenopathy is commonly present in patients with leukemia. Any category of leukemia including lymphoid, myeloid and monocytic can cause lymphadenopathy.

Enlargement of lymph nodes occur most commonly in the course of Acute leukaemias.

Lymphadenopathy is present in 50% patients with Acute myeloid leukemia, 50% of patients with Acute monocytic leukemia and 80% of patients with Acute lymphoblastic leukemia.

Dunphy (1989) pointed out that FNA biopsy is useful in the diagnosis of leukemic lymphadenopathy.[27]

In the FNA biopsy of lymphoblastic lymphoma, the cells are indistinguishable from the lymphoblasts and prolymphocytes that are characteristic of

acute lymphoblastic leukemia (ALL).

In cases of Acute myeloblastic leukaemia with lymphadenopathy, the FNA biopsy specimen is characterized by myeloid cells in various stages of maturation, the cytologic findings depend on the degree of differentiation. The presence of Auer rods supports the diagnosis.[63]

Cytochemical stains, eg, chloroacetate esterase, sudan black, and myeloperoxidase may be helpful in cases dominated by blasts.[129]

.

In FNAC of a leukemic infiltrate in bloody smears, the malignant cells could be contaminants from the blood.[102]

Histopathology:

Nodal architecture may be partially or completely effaced in myeloid leukemia.

Prominent infiltrates are seen in the medullary and paracortical areas of nodes. In some cases, eosinophilic myelocytes are indicators of the leukemic nature of the infiltrate.[85]

Some of the case show prominent megakaryocytic differentiation and may be confused with Hodgkin's disease.

In Lymphoid leukemia nodal architecture is usually effaced by infiltrates of primitive cells resembling those of T cell lymphoblastic lymphoma.

Metastatic Deposits:

Lymph nodes are commonly involved by metastatic cancer. Malignant melanoma, carcinomas and sarcomas exhibit a propensity to metastasize to lymph nodes.

Metastatic carcinoma becomes progressively more common with old age.

Cytology:

Berg (1961)[8] identified the following circumstances that facilitate the diagnosis of lymph node metastases by FNAC

1. For confirmation of a secondary lesion in cases of known or an occult primary
2. For diagnosis of a secondary lesion during follow up of treated primary malignancy.
3. Cases with clinical diagnoses other than metastatic lymphadenopathy.
4. For staging of malignancies.

FNA biopsy useful in the diagnosis of metastatic malignancy, the diagnosis is usually easy, accurate and safe.[29]

FNA biopsy has a lower risk of subsequent complications, including tumor recurrence, than histologic biopsy. FNA biopsy has also considerable value in staging the disease or documenting recurrence.[66]

Anucleated squames may also appear as spindle shaped and tadpole shaped cells arranged in a “cell within cell” pattern.[62]

Metastatic adenocarcinomas regardless of the site of origin, usually contain

tumour cells that are arranged singly or in cohesive groups of various sizes.[62]

The presence of aggregates of tumour cell with nuclear molding and extensive necrosis is characteristic of small cell carcinoma.[62]

Special studies such as electron microscopy or immunocytochemistry can be applied to FNA biopsies if necessary, to assist in the differential diagnosis of tumour cells.[25]

The highest diagnostic accuracy with FNA biopsy of lymphadenopathy is in the diagnosis of metastatic carcinoma.[58]

Histopathology:

The metastatic tumour often starts as isolated cellular clusters in the subcapsular sinuses, with gradual replacement of the nodal parenchyma. This may be accompanied by a desmoplastic reaction or inflammatory reaction including granuloma formation.

The number and level of lymph nodes involved, the size of nodal deposit and presence or absence of extra capsular extension are the most significant prognostic factors.

Epithelial elements may be found in lymph nodes mimicking metastatic carcinoma. Salivary gland inclusions are commonly found within intraparotid and cervical lymph nodes. Rarely, mammary inclusions occur within axillary lymph nodes. Thyroid follicle inclusions in cervical lymph nodes are very rare.[45]

Benign epithelial inclusions occurring in lymph nodes can be diagnosed by using

uniform microscopic size, lack of cytologic atypia and lack of stromal reaction.

Metastatic cancer may be bland in appearance and mistaken for developmental lesions. For example, cystic metastasis from squamous cell carcinoma of the head and neck region or papillary thyroid carcinoma may mimic branchial cyst.[13]

Some metastatic carcinomas, melanoma and germinoma are difficult to distinguish from lymphoma.

Special stains such as reticulin and mucin stains are sometimes valuable in differentiating the nature of a lymph node metastasis.

The reticulin fibrillar framework of lymphoid tissue becomes apparent and serves as a useful criterion to distinguish large cell lymphoma from metastatic undifferentiated carcinoma or melanoma.

A well developed, fine, branching, reticulin network with pericellular fibrils is characteristic of lymphoma, whereas thick reticulin fibers with an alveolar pattern surrounding nests and cords of cells are indicative of metastatic non-lymphoid tumours.

Immunohistochemical studies for cytokeratin, CD45 (LCA) and S-100 protein are helpful to distinguish epithelial malignant tumours from lymphoma. However, the occurrences of cytokeratin positive dentritic cells in lymph nodes are also possible.[14]

The accuracy of metastatic carcinoma reported by Gupta et al (1977)[41] and Tripathi et al (1985)[121] were 90 percent and 80.25 percent respectively.

Kline TS et al (1984)[57] reviewed the results of 376 superficial lymph nodes.

Diagnosis of metastatic carcinoma based on “alien” cells and melanoma was easily made with an accuracy of 95%

Haque MA et al[43] studied 117 cases of FNAC lymph nodes in a period between November 2001 and April 2002 and pointed out that in the malignancy of lymph node, sensitivity and specificity of FNAC were 82.76% and 97.92% respectively.

Ustun M et al (2002)[124] pointed out that the most challenging lesions to assess using FNAC were metastatic lymph nodes showing cystic change and they also suggested that FNAC should be repeated in cases of suspicious hypo cellular cystic aspirates, especially in patients with known malignancy.

Tseng FY et al (2002)[122] studied the cytological characteristics of metastatic papillary thyroid carcinoma in cervical lymph nodes. In their study, they pointed out that metastatic papillary thyroid carcinoma in cervical lymph nodes had a higher frequency of cystic degeneration than intra thyroidal lesions.

Cangiarella et al (2000)[12] described their experience with aspiration biopsy of metastatic melanoma in lymph nodes. Except for one false positive error caused by dermatopathic lymphadenopathy and two false negative results in 115 patients, the results of FNA were in concordance with histopathology and clinical follow up data.

Ramzay I et al (1985)[100] analyzed Fine needle aspiration biopsy of lymph nodes in 350 patients and modified biopsy technique used in same patients. Of the 350 patients, 209 aspirates were categorized as cytologically malignant, 102 as benign, 30 as suspicious for malignancy and 9 as unsatisfactory. Cervical Lymph nodes were most commonly sampled (58%), and supra clavicular nodes were most likely to be malignant (90%). The overall diagnostic accuracy achieved was 94%, one false positive and nine

(2%) false negative results were observed. In 3% of the 30 cases diagnosed as suspicious for malignancy, follow up open biopsy of the lymph nodes proved them benign. They said that there were some possible sources of error, particularly in the diagnosis of lymphomas.

Pilotti S et al (1993)[96] studied 285 patients with enlarged superficial lymphnode and demonstrated high rate of conclusive cytologic diagnoses in the assessment of metastatic malignancies, with an overall accuracy rate of 99.1% and a typing accuracy rate of 96.5%. They also pointed out that Immunocytochemistry appeared to be of limited value in the distinction between centroblastic-centrocytic follicular lymphomas and reactive follicular hyperplasia. In low grade NHL, morphologic and immunocytochemical methods need to be supplemented by molecular techniques in order to achieve conclusive diagnoses.

Lioe TF et al (1999)[70] studied aspirate from 157patients with enlarged superficial lymph nodes which were obtained over a 5 year period in a combined surgical/FNAC. A definitive diagnosis was achieved in over 77% of the cases; benign 52% malignant 25.1%; the diagnostic accuracy was 94.4%, sensitivity 85.4% and specificity 100%.

Hema Arora (2000-01)[46] studied 130 cases of superficial lymphadenopathy. In her study, maximum number of patients was in their 3rd and 4th decade. Involvement of cervical lymphnodes were maximum (75%) followed by axillary (13%), supraclavicular (7.6%) and inguinal (6.15%). In metastatic lesion of lymph nodes, squamous cell carcinoma was found to be maximum (71.42%) followed by adenocarcinoma (28.57%). FNAC had an overall diagnostic accuracy in 69.56% of cases.

Van de Schoot L et al (2001)[125] retrospectively studied FNACs of 73 peripheral lymph nodes and showed 92% sensitivity and 90% specificity respectively. FNAC

helped to avoid additional surgical diagnostic procedures in 86% of cases

Gupta RK et al (2003)[40] analyzed in 218 cases with enlarged lymph nodes by FNAC and histology. They pointed out that the overall sensitivity was 92.7% and specificity was 98.5 %.

Al-Mulhim AS (2004)[1] studied 94 patients with lymphadenopathy and concluded that overall accuracy of FNAC was 93%.

MATERIAL AND METHODS

The present study was carried out in the Department of Pathology, Madurai Medical College, Madurai, India for a period of 2 years from January 2004 to December 2005.

The cytological materials were obtained in the form of smears, which were fixed in 95% alcohol for PAP and H&E staining. The aspiration syringes used were 10-20ml and needle size between 22-23 gauges.

The specimens were received from Department of Surgery, Government Rajaji Hospital, Madurai. Details of the patients such as clinical and personal history were recorded including details of operative findings, macroscopic and microscopic features. The working proforma is appended in the annexure I.

The histopathology specimens were fixed in 10% buffered formal saline. After paraffin embedding 5 micron thick sections were made, stained with H&E[48] and PAP[48]. Special stains such as Reticulin were used as and when required. Immuno histochemical studies were done in relevant cases. (Annexure II)

Photographs of the specimen, photomicrographs of the smears and sections were taken wherever needed.

The results of the lymph node were divided in the following diagnostic categories:

Reactive Lymphadenitis,Tuberculous Lymphadenitis, Lymphomas and metastatic deposits.

A suspicious report was issued when there were only few abnormal cell or cells lacking the majority of criteria for malignancy. Specimens were considered unsatisfactory when there were sparse lymphoid elements, poor cell preservation or much blood.

Due to nonavailability of routine Immunological studies, Working Formulation has been followed for classification of Lymphomas. Conversion table for working formulation to WHO classification is shown in the annexure III.

The results of both procedures were compared.

The formulae for assessing sensitivity and specificity are as follows:

Screening test results	Diagnosis		Total
	Diseased	Not Diseased	
Positive	a(True Positive)	b(False Positive)	a+b
Negative	c(False negative)	d(True negative)	c+d
Total	a+c	b+d	a+b+c+d

The following measures are used to evaluate a screening:

Sensitivity= a/ (a+c) x100

Specificity=d/ (b+d) x100

Accuracy=a+d/ (a+b+c+d) x100

OBSERVATION AND RESULTS

In the two-year study period from Jan 2004 to Dec 2005, 10,506 general biopsy materials were received from Government Rajaji Hospital, Madurai. Of these, Lymph node lesions accounted for 634 cases. Hence, the overall incidence of lymph node lesion in this hospital was 6.03%

The total numbers of benign lymph node lesions were 499. Out of these, reactive lymphadenitis was 154 (24.3%) and tuberculous lymphadenitis was 342 (53.94%). Others were three in number (0.47%) (Kimura's disease-1, Dermatopathic Lymphadenitis-1 and Lepromatous Lymphadenitis-1). Thus over all incidence of benign lesion was 78.7%

The total numbers of malignant lesions were 135. Out of these, metastatic

deposits were 92 (14.15%), Lymphomas were 41 (6.46%) and Leukemia involving the Lymph node was two (0.32%). Out of Lymphomas, Non Hodgkin's lymphoma was 18 and Hodgkin's lymphoma was 23. Hence, overall incidence of malignant lesion was 21.3% (Diagram 1 & 2)

Age incidence:

Benign lymph node lesions had peak age incidence in the second decade.

The age incidence of lymphomas ranges from 4 to 80 years. In that, Hodgkin's lymphoma occurs in young (from 4 years) as well as in the old age (up to 79). Non Hodgkin's lymphoma commonly occurs in patients with age group of 40 to 80years. Secondary carcinomatous deposit was commonly encountered in persons of 40 to 70 years.

The distribution of lymph node lesions according to age is shown in diagram 3.

Sex incidence:

Out of the 499 benign lymph node lesions, 299 cases occurred in females (incidence 59.9%), 200 cases occurred in males (incidence 40.1%). Hence, male to female ratio in benign conditions were 1:1.5.

Out of 92 patients with secondary carcinomatous deposits, 33cases were females (35.9 %), 59 cases were males (64.1%) and out of 41 cases with lymphomas 12 cases were females (29.7%) 29 cases were males (70.3%)

Node Enlargement:

79.8 % of the patients had cervical node enlargement, 10.2% of patients had

axillary node enlargement and only 5.7% of patients had inguinal lymphadenopathy, followed by supraclavicular node enlargement, which was only 4.3 %.(Diagram 4)

Cytological evaluation of lymph node lesions:

During the study period, 388 smears were received for cytological examination. Out of these 87cases had post surgical follow-ups.

Out of the 87 smears studied, 21 cases (24.1%) were reactive lymphadenitis(Fig 1), 26 cases (29.9%) were Tuberculous lymphadenitis (Fig4), 6 cases (6.9%) were Non Hodgkin's Lymphoma (Fig7,Fig9,Fig10 &Fig12), 3 cases(3.4%) were Hodgkin's Lymphoma (Fig18), 30 cases (34.5%) were Metastatic deposits(Fig25,Fig26, Fig27,& Fig28) and one case was (1.2%) found to be unsatisfactory. (Table 1)

The commonest non-neoplastic lesion encountered was tuberculous lymphadenitis. The most common malignant lesion diagnosed was metastatic carcinomatous deposits.

Histopathological Diagnosis:

The histopathological diagnosis offered for the 87 cases is shown in table2. Out of the 87 cases 20 cases (23%) were reactive lymphadenitis(Fig2),29 cases (33.3%) were Tuberculous lymphadenitis (Fig5), 4 cases (4.6%) were Non Hodgkin's Lymphoma (Fig8&Fig11) , 4 cases (4.6%) were Hodgkin's Lymphoma (Fig19), one case (1.2%)

was Leukemia involving the lymph node (Fig22) and 29 cases (33.33%) were metastatic deposits.(Fig29&30)

The most common non-neoplastic lesion encountered was tuberculous lymphadenitis. Among the malignant lesion, most common was metastatic carcinomatous deposits.

Correlation between cytological and histopathlogical diagnosis:

Out of the 21 smears of reactive lymphadenitis, 18 were diagnosed as reactive lymphadenitis by histopathology, 2 were diagnosed as tuberculous lymphadenitis, and one case was diagnosed as Hodgkin's Lymphoma. All the 26 smears of tuberculous lymphadenitis were confirmed by histopathology.

Out of 6 smears of Non Hodgkin's Lymphoma, only 4 cases were diagnosed by histopathology, one case turned out to be a reactive lymphadenitis and one case was turned out to be a Leukemia involving the lymph node. All the 3 smears of Hodgkin's Lymphoma were confirmed by Hodgkin's Lymphoma by histopathology. Out of 30 smears of metastatic deposits, 29 were diagnosed as same by histopathology and one was diagnosed as reactive lymphadenitis.

There was an excellent correlation in cases of tuberculous lymphadenitis (100%) and Hodgkins Lymphoma (100%).

Table 3 illustrates the correlation obtained between cytological and histopathological diagnosis.

Sensitivity and Specificity:

Reactive Lymphadenitis:

Out of 21cases, which were diagnosed cytologically as reactive lymphadenitis, 18 cases turned out to be reactive process (True Positive-18) and 2 cases were tuberculous lymphadenitis and one case turned out to be a Hodgkin's Lymphoma. (False Positive-3) The sensitivity of FNAC in diagnosing reactive lymphadenitis was found to be 90%. One case, which was diagnosed as metastatic deposits and other case that was diagnosed cytologically as lymphoma turned out to be a reactive lymphadenitis histopathologically. Hence, specificity for this lesion is 95.6% and accuracy was 93.1%.

Tuberculous Lymphadenitis:

26 cases, which were diagnosed as tuberculous lymphadenitis cytologically were confirmed by histopathology also. (True Positive-26). Two cases, which were diagnosed as reactive lymphadenitis cytologically, turned out to be tuberculous in histopathology (False Negative-2). The sensitivity, specificity and accuracy rates were 92.8%, 100%, and 96.5% respectively.

Non-Hodgkin's Lymphoma:

Out of 6 cases diagnosed cytologically, only 4 were confirmed by histopathology (True Positive-4). One case turned out to be a Leukemia involving the lymph node and other turned out to be a reactive lymphadenitis. The sensitivity of Non-Hodgkin's

Lymphoma was 100%, the specificity was 97.5%, and accuracy was 97.7%.

3 out of 4 cases had bone marrow involvement.

Immunohistochemical staining showed positive for CD20 in three cases of Non Hodgkin's Lymphoma.(Fig16&Fig17)

Hodgkin's Lymphoma:

Only 3 cases were diagnosed cytologically as Hodgkin's Lymphoma, and all the three cases were confirmed histopathologically also (True Positive-3). However, one case which was diagnosed histopathologically as Hodgkin's was diagnosed cytologically as reactive lymphadenitis (False negative-1). Hence, the sensitivity of FNAC in Hodgkin's Lymphoma was 75%, whereas specificity was 100% and accuracy was 98.6%.

Immunohistochemical markers CD15 and CD30 were positive in a case of Hodgkin's Lymphoma mixed cellularity type.(Fig20&Fig21)

Metastatic Deposits:

Out of 30 cases diagnosed as metastatic deposits cytologically, 29 cases were confirmed histopathologically (True Positive-29), One case turned out to be reactive Lymphadenitis (False Positive-1). The sensitivity of metastatic deposits by FNAC is 100%, specificity was 98.2%, and accuracy was 98.8%.

Thus, overall sensitivity, specificity and accuracy of F.N.A.C in superficial lymphadenopathy were 91.6 %, 98.3%, 97 % respectively.

The details of sensitivity, specificity and accuracy of each lesion were illustrated in Table 4.

DISCUSSION

The recent trend in medical practice is toward adopting a diagnostic modality, which is both cost effective and minimally invasive. In this regard, FNAC is often used as a first line of investigation for screening cases with lymphadenopathy, since this method is easy to perform, rapid, and inexpensive. FNAC can help not only to differentiate among lymphoma, and metastasis, but also to identify nonspecific reactive lymphadenitis and specific infections such as tuberculous lymphadenitis.

Age Incidence:

In our study the age of the patients ranged between 3 and 70 years. (Diagram 2).

The peak age incidence of non-neoplastic lymph node lesions were found to be in the second decade.

For metastatic lesions, the age ranged from 40 to 80 years, whereas lymphomas had bimodal peak at second and seventh decade.

Sex Incidence:

In the present study the male to female ratio was1: 1.5

Site of Swelling:

Involvement of cervical lymph nodes were maximum (79.8 %) followed by axillary (10.2%), inguinal (5.7%), and supraclavicular node (4.3%)

A similar incidence had been quoted by Kline et al (1984)[57] and Hema Arora (2001)[46]. (Table 5)

Reactive Lymphadenitis:

The cytological features of reactive lymphadenitis revealed cellular specimens composed of small lymphocytes predominated with a few neutrophils, plasma cells, histiocytes elongated fibroblasts and phagocytes. (Fig 1)

Grossly the affected lymph nodes were enlarged and firm. Histo pathologically reactive lymphadenitis showed follicular, sinus, or mixed patterns.

In follicular hyperplasia, histopathological examination showed marked enlargement and prominence of the germinal centers. (Fig 2)

The results of this work indicate that benign lymphadenopathy constitutes a significant proportion of findings in aspirates of enlarged lymph nodes (55.3%). It was also proved that cytological examination may not only help to distinguish between benign and malignant types, but may also suggest the nature of the benign process. Out of non-neoplastic lymph node lesions which formed 55.3%, the second most common diagnosis was reactive hyperplasia, which was second to the incidence of tuberculosis in the present study. This figure is correlating with the study of Bezabih M et al (2003)[9]

One case, which was diagnosed as metastatic deposits, and other case that was diagnosed as lymphoma by cytology were turned out to be a reactive lymphadenitis (False Negative -2). These errors were made because of cell with large nucleoli. Some were histiocytes, later recognized because of its size and chromatin pattern. Others, immature lymphocytes from the germinal centers, still have potential for causing misdiagnosis. Errors can be diminished by noting surrounding benign polymorphic cells and occasional phagocytes.

The sensitivity of diagnosing reactive lymphadenitis is 90%

Tuberculous lymphadenitis:

The cytological diagnosis of tuberculous lymphadenitis was made definitely, when granulomas composed of epithelioid cells and Langhans' cells are seen. (Fig 4) but even in the absence of granulomas, necrosis along with the presence of lymphocytes alone gives an indirect evidence of tuberculous lymphadenitis.

Grossly the affected lymph nodes are matted and showed caseous necrosis. (Fig3)

Histopathological examination of tuberculous lymphadenitis showed caseous necrosis, epithelioid cell granulomas and Langhans' giant cells. (Fig5)

In the present study, the sensitivity for tuberculous lymphadenitis is 92.8%. Two cases of tuberculous lymphadenitis diagnosed as reactive lymphadenitis on cytological examination were subsequently found to be tuberculous lymphadenitis in histopathology. Probably, the representative samples were not obtained in these cases.

The diagnostic accuracy in the present study is 96.5%. This is higher than accuracy rate of other research workers. The success result of FNAC in tuberculous lymphadenitis by other research workers were 80% (Bloch, 1967)[10]; 87.18% (Patra et al, 1983)[93]; and 83.33% (Dandapat et al, 1986)[17].

Lymphoma:

Non Hodgkin's Lymphoma

Cytological criteria for diagnosing Non Hodgkin's Lymphoma were monomorphism and macronuleoli. (Fig7, Fig9,Fig10& Fig12)

Although sub classification was attempted, the aim was to distinguish lymphoma from hyperplasia.

Grossly cut surface of enlarged lymph node showed gray white and fish flesh appearance. (Fig6)

Histopathological examination of Small Lymphocytic Lymphoma shows loss of architecture and replaced by diffuse proliferation of well differentiated, mature, small and uniform lymphocytes without cytologic atypia or significant mitoses. (Fig8)

Histopathologically Diffuse Large cell Lymphoma have diffuse pattern of involvement by large cells showing round to oval nucleus and two to three nucleoli, with increased mitotic activity. (Fig11)

In a case of (2946/05) Non Hodgkin's Lymphoma perinodal fatty infiltration was seen. (Fig13)

Reticulin stain in a case of Non Hodgkin's Lymphoma showed a fine, branching reticulin network with pericellular fibrils characterize the lymphoma. (Fig 15)

Peripheral smear showed atypical Lymphoid cells with clefting of the nucleus. (Fig14)

Immuno histochemically three of Non Hodgkin's Lymphoma cases showed CD 20 positivity. (Fig16 &Fig17)

The diagnostic accuracy of Non Hodgkin's lymphoma is 97.7% Aspirate of one

patient was false-positively interpreted as lymphoma, who had a corresponding biopsy showing reactive hyperplasia. This error was made because of the presence of a few cells with large nucleoli. Some of these cells were histiocytes and others were immature lymphocytes from the germinal centers. Lee et al also found difficult in differentiating between reactive hyperplasia and lymphoma by cytology alone.

Hodgkin's Lymphoma:

In Hodgkin's lymphoma, the main cytological feature was polymorphism constituted by immature lymphocytes, eosinophils and Reed-Sternberg cells. (Fig18)

Grossly the cut surface of Hodgkin's lymphoma showed nodular scarring in nodular sclerosis type whereas mixed cellularity type showed abundance of necrosis.

On microscopic examination of nodular sclerosis type of Hodgkin's lymphoma showed variable amount of fibrous tissue and characteristic lacunar type of RS cells. (Fig19)

Immuno histochemical markers CD15 and CD 30 were positive in a case of Hodgkin's Lymphoma mixed cellularity type. (Fig20&Fig21)

The diagnostic accuracy of Hodgkin's lymphma is 98.8%. One case was diagnosed as reactive lymphadenitis in cytology, but showed Hodgkin's lymphoma in histopathology. This may be due to absence of Reed-Sternberg cells in the cytology smears.

During the study period, one case was diagnosed histopathologically as reactive lymphadenitis. After six months, the same case was rediagnosed as suggestive of Hodgkin's Lymphoma cytologically and that was confirmed by histopathology also.

In False negative cases, it may be necessary to repeat FNAC studies, if there is strong clinical suspicious of neoplasm. Sometimes False negative results can be encountered due to small or inaccessible nodes, fibrosis, necrosis or sampling the wrong node.

Gupta et al (1977)[41] who exclusively studied aspiration smears of lymphoma cases also mentioned the possibility of overlap among reactive hyperplasia, lymphocytic lymphoma and Hodgkin's disease.

Gupta et al (1977)[41], Tripathi et al (1985)[121], Mondal et al (1989)[81] and Al-Muhim et al(2004)[1] quoted the diagnostic accuracy in lymphoma as 84%, 80%,96.2% and 88% respectively. In the present study, the diagnostic accuracy was 97.7%.

Leukaemia involving Lymph node:

Histopathologically Acute Lymphoblastic Leukaemia involving the lymph node was appearing to begin at the center of the node residual lymph node structure was seen at the periphery. (Fig 22)

One case was reported as suggestive of lymphoma in F.NAC, tissue section of the same lymph node when correlated with blood smear findings, revealed evidence of leukemia involving the lymph node. In such cases, it is very difficult to differentiate between Non-Hodgkin's lymphoma and leukemic infiltration on the examination of cytological smears alone. Hence, in all cases of suspected lymphoma, details regarding haematological and smear findings and marrow features are essential for correlative study.

Metastatic Deposits:

The FNAC from metastatic carcinoma was cell rich chiefly with cells foreign to lymph nodes, but occasionally, intermingled with lymphoid cells. Specific primary site identification was accurate in many cases.

Cells from squamous cell carcinoma, papillary adeno carcinoma and malignant melanoma were the easiest to identify. Cells from squamous cell carcinoma were single, pleomorphic and had abundant homogeneous keratinized cytoplasm with central nuclei. (Fig25)

Papillary clusters, intranuclear clear areas and rare psammoma bodies were characteristic features of papillary thyroid carcinoma.(Fig27) Metastatic melanoma showed features of isolated oval cells, two or three distinct cell populations based on cell size, multinucleation, macro nucleoli, and intra nuclear inclusions.(Fig26)

Grossly metastatic deposits of malignant melanoma showed brownish melanin pigment deposits. (Fig24) Metastatic deposits of squamous cell carcinoma showed grayish white tumour areas. (Fig23)

Histopathologically, in malignant melanoma lymph node was partially replaced by melanoma cells showing marked pleomorphism and prominent melanin pigments. (Fig 29)

Reticulin stain of squamous cell carcinoma showed thick reticulin fibres surrounding the cords and islands of carcinoma cells. (Fig30)

During the study period, metastatic squamous cell carcinoma was found in maximum number (41.3%) followed by adenocarcinoma (27.6%). This is correlating with the study of Hema arora et al.[46]

In the present study the accuracy rate of diagnosing metastatic carcinoma is 98.8% It is higher than the accuracy reported by Gupta et al (90%)[41] and Tripathi et al (80.2%)[121].

In the present study overall sensitivity, specificity and accuracy of F.N.A.C in superficial lymphadenopathy were 91.6 %, 98.3%, 97 % respectively.

Table 6 shows the comparative study of sensitivity, specificity and accuracy of F.N.A.C in superficial lymphadenopathy with various studies.

Table 7 shows the comparative study of accuracy of individual lesions with various authors.

SUMMARY

The study, FNAC of Lymph node lesions with subsequent correlation of histopathology revealed the following findings.

1. Of the 10,506 general biopsy materials received from Government Rajaji Hospital Madurai during the study period (January 2004 – December 2005), 634 cases of lymph node lesions were encountered with the incidence of 6.03%.
2. During the study period, 388 smears were received for cytological examination.
3. Out of 388 smears, 87 had histopathological correlation.
4. The peak age incidence of non- neoplastic lymph node lesions was in the second decade.
5. Hodgkin's lymphoma occurs in young (4 -30), and old age (45 -79).
6. Non Hodgkin's lymphoma and metastatic deposits commonly occur in the old age.
7. Common site of Lymph node enlargement was cervical group.
8. The most common Non neoplastic lesion in the present study was tuberculous lymphadenitis accounting for 29.9% cytologically and 33.3% histopathologically.
9. Among the malignant lesion, the incidence of metastatic deposits was high and accounting for 34.5% cytologically and 33.33% histopathologically.
10. Among the metastatic deposits, squamous cell carcinoma was the commonest one.
11. Among the non neoplastic lesion tuberculous lymphadenitis had excellent correlation.
12. Three out of 4 cases of Non Hodgkin's lymphoma had bone marrow involvement.
13. Immunohistochemically three cases of Non Hodgkin's Lymphoma showed CD20 positivity.

14. The sensitivity, specificity and accuracy rates of reactive lymphadenitis were 90%, 95.6% and 93.1% respectively.

15. For tuberculous lymphadenitis sensitivity was 92.8%, specificity was 100% and accuracy was 96.5%.

16. The sensitivity of Non Hodgkin's Lymphoma was 100%, the specificity was 97.5%, and accuracy was 97.7%.

17. The sensitivity of Hodgkins Lymphoma was 75%, specificity was 100%, and accuracy was 98.6%.

18. The sensitivity, specificity, and accuracy rates of metastatic deposits were 100%, 98.2%, and 98.8%.

19. The overall sensitivity for F.N.A.C. of superficial lymphadenopathy was 91.6%, specificity was 98.3%, and accuracy was 97%.

CONCLUSION

In summary, “a correlative cytological and histopathological study on superficial lymphadenopathy” revealed the overall sensitivity was 91.6%, specificity was 98.3% and accuracy was 97%. False positive and false negative reports can be minimized by sampling appropriate nodes and by correlative haematologic study.

The results are quite encouraging and FNAC is recommended as the initial diagnostic test in the evaluation of superficial lymphadenopathy. Although FNAC has proven to be a simple, safe, reliable and cost effective diagnostic tool for lymphadenopathies, the limitation of the procedure should be kept in mind and excision biopsy should be used whenever required.

Immunohistochemical staining is useful for confirmation of diagnosis in nodal lesions especially lymphoma and for further classification of lymphomas.

ACKNOWLEDGEMENT

I hereby sincerely thank and acknowledge **The Dean**, Madurai Medical College, Madurai for having permitted me to use the material from Government Rajaji Hospital and Madurai Medical College to carry out this dissertation work.

ANNEXURE -I

PROFORMA

NAME: AGE: SEX:

OCCUPATION: OP/IP NO: UNIT:

CY NO: HPE NO:

CLINICAL SYMPTOMS:

SWELLING: SITE: Cervical / Axillary / Inguinal / Supraclavicular / Others

Duration:

FEVER: Intermittent / Evening rise of Temperature

ANY OTHER SWELLING:

PAST H/O TB: Present /Absent

ON EXAMINATION:

SWELLING: Site:

Size:

Consistency: Firm / Hard

Matted / Discrete

Mobile/ Fixed

ANY OTHER SWELLING:

LIVER: Palpable / not Palpable If Palpable…….cm below Right costal margin

SPLEEN: Palpable / not Palpable If Palpable…….cm below Left costal margin

F.N.A.C:

Cellular / Acellular

Monotonous / Varying population

Epithelioid cells: Present / Absent

Giant cells: Langhan type / other types

R.S. Cells: Present/Absent

Background cells: Lymphocytes / Histiocytes / Eosinophils / Plasma cells / Neutrophils / Tingible body macrophages

Mitotic Activity: Present / Absent

Necrosis: Present /Absent

Metastatic Deposits: Present / Absent

Type of Tumour cells: Squamous cell carcinoma /Adeno carcinoma / Papillary carcinoma/ Malignant melanoma

Cytological Diagnosis:

Histological Data:

Gross appearance :

Microscopic appearance :

Diagnosis offered :

Comments and Remarks:

Investigations:

TC, DC, ESR:

Peripheral Smear & Bone marrow:

Immunohistochemistry:

ANNEXURE –II

PROCEDURES FOR SPECIAL STAINING

I. RETICULIN STAIN[48]

1. Bring sections to water.
2. Treat with potassium permanganate solution for 1-2 minutes.
3. Wash in water, bleach with the potassium metabisulphite solution. Wash well in water.
4. Treat with the iron alum solution for 1 minute.Wash well in tap and then distilled water.
5. Treat with ammonical silver solution for 1 minute.
6. Wash briefly in distilled water and reduce in 10%formalin for 3 minutes.
7. Wash, then tone in 0.2% gold chloride for up to 10 minutes.
8. Rinse in distilled water, then treat with the potassium metabisulphite solution for 1minute.
9. Rinse with distilled water and fix with 5% hypo for 1-2 minutes.
10. Wash, dehydrate, clear, and mount in D.P.X..

Results:

Reticulin fibres	……	Black
Nuclei	……	grey
Collagen	……	grey-purple

IMMUNO HISTOCHEMICAL STAINING [48]

1. Sections to alcohol.
2. Block endogenous peroxidase activity by incubating in hydrogen peroxide solution for 30 minutes.
3. Hydrate sections by passing through graded ethanol series and wash in running water for 15 minutes.
4. Incubate sections in the normal swine serum diluted in Tris/saline for 15 minutes.
5. Drain off excess Tris-buffered normal swine serum.
6. Incubate sections with primary antiserum diluted 1:2000, 1:1000, 1:250 and 1:100 in 1%normal swine serum for 30 minutes.
7. Jet wash off excess antiserum and then wash slides in Tris / saline for three 2 minutes changes.
8. Incubate sections in swine anti-rabbit IgG diluted 1:20 for 30 minutes.
9. Jet wash off excess antiserum and then wash slides for three 2 minutes changes.
10. Incubate sections in PAP complex diluted 1:60 in 1% normal swine serum in Tris / saline for 30 minutes.
11. Jet wash off excess complex and wash in Tris / saline for three 2 minutes changes.
12. Incubate sections in DAB medium for 5 minutes.
13. Wash sections in running water for 10 minutes.
14. Counterstain in alum haematoxylin, dehydrate, clear and mount.

Results:

Reaction product	……	brown
Nuclei	……	blue

ANNEXURE III

Conversion table for the terminology in the updated REAL / WHO classification[13]

ANNEXURE -IV

BIBLIOGRAPHY

1. Al-Mulhim AS, Al-Ghamdi AM, Al-Marzooq YM,Hashish HM, Mohammad HA, Ali AM, Gharib IA: The role of fine needle aspiration cytology and imprint cytology in cervical lymphadenopathy. Saudi Med J: 862-865, 2004.

2. Bailey TM, Akhtar M, Ali MA: Fine Needle Aspiration Biopsy in the Diagnosis of Tuberculosis. Acta Cytol 29: 732-736, 1985.

3. Banks PM: The Pathology of Hodgkin's Disease. Semin Oncol 17: 683-695, 1990a.

4. Banks PM, Metter J, Allred DC: Anaplastic Large Cell (Ki-1) Lymphoma With Histiocytic Phenotype Simulating Carcinoma. Am J Clin Pathol 94: 445-452, 1990b.

5. Behm FG, O'Dowd GJ, Frable WJ: Fine-Needle Aspiration Effects on Benign Lymph Node Histology. Am J Clin Pathol 82: 195-198, 1984.

6. Ben-Yehuda-Salz D, Ben-Yehuda A, Polliack A: Syncytial Variant of Nodular Sclerosing Hodgkin's Disease: A New Clinicopathologic Entity. Cancer 65: 1167-1172, 1990.
7. Berard CW, Greene MH, Jaffe ES : A Multidisciplinary Approach to Non-Hodgkin's Lymphomas. Ann Intern Med 94: 218-235, 1981.

8. Berg JW: The aspiration biopsy smear. In Koss LG (ed). Diagnostic cytology and its Histopathlogic Bases, 1st ed. Philadelphia, JB Lippincott, 311-321, 1961.

9. Bezabih M, Mariam DW: Determination of aetiology of superficial enlarged lymphnodes using fine needle aspiration cytology.East afr Med J. Nov, 80 (11):559-63, 2003.

10. Bloch M: Comparative Study of Lymph Node Cytology by Puncture and Histopathology. Acta Cytol 11: 139-144, 1967.

11. Bottles K, Cohen MB, Brodie H: Fine-Needle Aspiration Cytology of Lymphadenopathy in Homosexual Males. Diagn Cytopathol 2: 31-35, 1986a.

12. Cangiarella J, Symmans WF, Shapiro RL: Aspiration biopsy and the clinical management of patients with malignant melanoma and palpable regional lymph nodes. Cancer 90:162-166, 2000.

13. Christofer DM Fletcher: Diagnostic Histopathology of Tumours. 2nd edition, Churchil

Livingston Publishers; 2002.

14. Colby TV, Hoppe RT, Warnke RA: Hodgkin's Disease: A Clinicopathologic Study of 659 Cases. Cancer 49: 1848-1858, 1981a.

15. Corkill M, Stephens J, Bitter M: Fine Needle Aspiration Cytology of Mycobacterial Spindle Cell Pseudotumor: A Case Report. Acta Cytol 39: 125-128, 1995.

16. Cossman J, Chused TM, Fisher RI, et al: Diversity of Immunological Phenotypes of Lymphoblastic Lymphoma. Cancer Res 43: 4486-4490, 1983.

17. Dandapat, M.C. Panda, B.K., Patra, A.K. and Acharya, N: Diagnosis of tubercular lymphadenitis by Fine Needle Aspiration Cytology. Ind. J. Tub, 34,139-142, 1987.

18. Das DK, Bhambhani S, Pant JN: Superficial and Deep-Seated Tuberculosis Lesions: Fine Needle Aspiration Cytology Diagnosis of 574 Cases. Diagn Cytopathol 8: 211-215, 1992.

19. Das DK, Gupta SK, Datta BN: Fine Needle Aspiration Cytodiagnosis of Hodgkin's Disease and Its Subtypes: I. Scope and Limitations. Acta Cytol 34: 329-336, 1990a.

20. Das DK, Gupta SK, Datta BN: FNA Cytodiagnosis of Non-Hodgkin's Lymphoma and Its Subtyping Under Working Formulation of 175 Cases. Diagn Cytopathol 7: 487-498, 1991a.

21. Das DK, Gupta SK, Datta BN: Working Formulation of the Non-Hodgkin's Lymphomas: A study of Cell Size and Mitotic Indices in Cytologic Subtypes. Diagn Cytopathol 7: 499-503, 1991b.

22. Daskalopoulou D, Harhalakis N, Maouni N: Fine Needle Aspiration Cytology of Non-Hodgkin's Lymphomas: A Morphologic and Immunophenotypic Study. Acta Cytol 39: 180-186, 1995.

23. Dick F, Bloomfield CD, Brunning RD: Incidence, cytology, and histopathology of non-Hodgkin’s lymphoms in the bone marrow, Cancer 33: 1382-1398,1974.

24. Dmitrovsky E, Martin SE, Krudy AG: Lymph Node Aspiration in the Management of Hodgkin's Disease. J Clin Oncol 4: 306-310, 1986.

25. Domagala W, Weber K, Osborn M: Differential Diagnosis of Lymph Node Aspirates by Intermediate Filament Typing of Tumor Cells. Acta Cytol 30: 225-234, 1986.

26. Dorfman RF, Warnke R: Lymphadenopathy simulating the Malignant Lymphomas. Hum Pathol 5: 519-550, 1974.

27. Dunphy CH, Katz RL, Fanning CV, et al: Leukemic Lymphadenopathy: Diagnosis by Fine Needle Aspiration. Hematol Pathol 3: 35-44, 1989.

28. Elavathil LJ, Kahn HJ, Hanna W: Primary Multilobulated B-Cell Lymphoma of the Breast. Arch Pathol Lab Med 113: 1081-1084, 1989.

29. Engzell U, Jakobsson A, Sigurdson A: Aspiration Biopsy of Metastatic Carcinoma in Lymph Nodes of the Neck: A Review of 1101 Consecutive Cases. Acta Otolaryngol 72: 138-147, 1971.

30. Facchetti F, Agostini C, Chilosi M: Suppurative Granulomatous Lymphadenitis: Immunohistochemical Evidence for a B-Cell-Associated Granuloma. Am J Surg Pathol 16: 955-961, 1992.

31. Flanagan NG, Donald F, Harry DS, et al: Non-Hodgkin's Lymphoma and Hodgkin's Lymphoma in the Same Patient. J Clin Pathol 42: 440-441, 1989

32. Forkner CE. Material from lymph nodes of man. II: Studies on living and fixed cells withdrawn from lymph nodes of man. Arch Intern Med, 40: 647-661, 1927.

33. Frable WJ, Kardos TF: Fine Needle Aspiration Biopsy: Applications in the Diagnosis of Lymphoproliferative Diseases. Am J Surg Pathol 12(Suppl 1): 62-72, 1988.

34. Friedman M, Kim U, Shimaoka K: Appraisal of Aspiration Cytology in Management of Hodgkin's Disease. Cancer 45: 1653-1663, 1980.

35. Fulciniti F, Zeppa P, Vetrani A: Hodgkin's Disease Mimicking Suppurative Lymphadenitis: A Possible Pitfall in Fine-Needle Aspiration Biopsy Cytology. Diagn Cytopathol 5: 282-285, 1989.

36. Grieg, E.D. and Gray A.C. Lymphatic gland in sleeping sickness. Br. Med. J. 1904.

37. Guarner J, del Rio C, Carr D: Non-Hodgkin's Lymphomas in Patients With Human Immunodeficiency Virus Infection: Presence of Epstein-Barr Virus by In Situ Hybridization, Clinical Presentation, and Follow-up. Cancer 68: 2460-2465, 1991.

38. Gupta AK, Nayar M, Chandra M: Critical Appraisal of Fine Needle Aspiration Cytology in Tuberculous Lymphadenitis. Acta Cytol 36: 391-394, 1992.

39. Gupta AK, Nayar M, Chandra M: Reliability and Limitations of Fine Needle Aspiration Cytology of Lymphadenopathies: An Analysis of 1,261 Cases. Acta Cytol 35: 777-783, 1991.

40. Gupta RK, Naran S, Lallu, Fauch R: The diagnostic value of Fine needle aspiration cytology in the assessment of a palpable supraclavicular lymph nodes: A study of 218 cases, Cytopathology Aug; 14(14); 201-7, 2003.

41. Gupta S.K. Dutta T.K. and Aikat B.K: Lymph node aspiration biopsy in diagnosis of lymphoma. Ind. J. Path, and Microbiol, 20, 231-237, 1977.

42. Guthrie C.G. Gland puncture as a diagnostic measure. Bull. Johns Hopkins Hosp., 32, 266, 1921.

43. Haque MA, Talukoler SI: Evaluation of fine needle aspiration cytology of lymph nodes in Mymensingh; Mymensingh Med J Jan:12(1):33-35,2003.

44. Harris NL: Lymphoma 1987: An Interim Approach to Diagnosis and Classification. Pathol Annu 27(1): 1-67, 1987.

45. Harry L. Ioachim M.D and Howard Ratech, M.D: Ioachim's Lymph node pathology, Third edition, Lippincott Williams & Wilkins.

46. Hema arora, P.V.Patil M.D: Role of fine needle aspiration cytology in superficial lymphadenopathy,. District Hospital & KLES Hospital and Medical Research Centre, Belgaum , 2000.

47. Jackson H Jr, Parker F Jr: Hodgkin's disease. Gerneral considerations, N Engl J Med,230: 1-8,1944.

48. John D. Bancroft, Harry C. Cook: Manual of Histological Techniques and their Diagnostic Application, first edition, Churchill Livingstone,1999.

49. Jimenez-Heffernan JA, Vicandi B, Lopez-Ferrer P, Hardisson D, Viguer JM. Value of fine needle aspiration cytology in the initial diagnosis of Hodgkin's disease.

50. Juan Rosai MD:Rosai and Akerman Surgical Pathology, ninth edition, Mosby, cha21:1878-2017, 2004.

51. Kaplan HS: Hodgkin's Disease: Unfolding Concepts Concerning Its Nature, Management Prognosis. Cancer 45: 2439-2474, 1980.

52. Kardos TF, Sprague RI, Wakely PE Jr: Fine-Needle Aspiration Biopsy of Lymphoblastic Lymphoma and Leukemia: A Clinical, Cytologic, and Immunologic Study. Cancer 60: 2448-2453, 1987.

53. Katz RL, Gritsman A, Cabanillas F: Fine-Needle Aspiration Cytology of Peripheral T-Cell Lymphoma: A Cytologic, Immunologic, and Cytometric Study. Am J Clin

Pathol 91: 120-131, 1989.

54. Kennedy BJ: Hodgkin's Disease. CA Cancer J Clin 41: 16, 1991.

55. Kim H, Hendrickson MR, Dorfman RF: Composite Lymphoma. Cancer 40: 959-976, 1977.

56. Kjeldsberg CR, Wilson JF, Berard CW: Non-Hodgkin's Lymphoma in Children. Hum Pathol 14: 612-627, 1983.

57. Kline TS, Kannan V, Kline IK: Lymphadenopathy and Aspiration Biopsy Cytology: Review of 376 Superficial Nodes. Cancer 54: 1076-1081, 1984.

58. Kline TS, Neal HS, Holroyde CP: Needle Aspiration Biopsy: Diagnosis of Subcutaneous Nodules and Lymph Nodes. JAMA 235: 2848-2850, 1976.

59. Knowles DM: Immunophenotypic and Antigen Receptor Gene Rearrangement Analysis in T Cell Neoplasia. Am J Pathol 134: 761-785, 1989.

60. Koo CH, Rappaport H, Sheibani K: Imprint Cytology of Non-Hodgkin's Lymphomas: Based on a Study of 212 Immunologically Characterized Cases. Hum Pathol 20: 1-137, 1989.

61. Kornstein MJ, Wakely PE Jr, Kardos TF: Dendritic Reticulum Cells and Immunophenotype in Aspiration Biopsies of Lymph Nodes: Value in the Subclassification of Non-Hodgkin's Lymphomas. Am J Clin Pathol 94: 165-169, 1990.

62. Koss LG, Woyke S, Olszewski W: Aspiration Biopsy. Cytologic Interpretaion and Histologic Bases. 2nd ed, New York, Igaku-Shoin Medical Publishers, Inc,1992.

63. Kumar PV: Soft Tissue Chloroma Diagnosed by Fine Needle Aspiration Cytology: A Case Report. Acta Cytol 38: 83-86, 1994a.

64. Lau S-K, Wei WI, Kwan S: Combined Use of Fine-Needle Aspiration Cytologic Examination and Tuberculin Skin Test in the Diagnosis of Cervical Tuberculous Lymphadenitis: A Prospective Study. Arch Otolaryngol Head Neck Surg 117: 87-90, 1991.

65. Layfield LJ, Glasgow BJ, DuPuis MH: Fine-Needle Aspiration of Lymphadenopathy of Suspected Infectious etiology. Arch Pathol Lab Med 109: 810-812, 1985.

66. Layfield LJ, Heaps JM, Berek JS: Fine-Needle Aspiration Cytology Accuracy With Palpable Gynecologic Neoplasms. Gynecol Oncol 40: 70-73, 1991.

67. Lee Y-TN, Terry R, Lukes RJ: Lymph Node Biopsy for Diagnosis. A Statistical Study. J Surg Oncol 14: 53-60, 1980.

68. Lennert K, Stein H, Kaiserling E: Cytological and Functional Criteria for the Classification of Malignant Lymphoma. Br J Cancer 31: 29-43, 1975a.

69. Lieberman PH, Filippa DA, Straus DJ: Evaluation of Malignant Lymphomas Using Three Classifications and the Working Formulation: 482 Cases With median follow-up of 11.9 Years. Am J Med 81: 365-380, 1986.

70. Lioe TF, Elliott H, Allen DC, Spence RA: The role of Fine needle aspiration cytology in the investigation of superficial lymphadenopathy, Cytopathlogy, Oct;10(5) : 291-7 1999.

71. Lopes Cardozo P: The Significance of Fine Needle Aspiration Cytology for the Diagnosis and Treatment of Malignant Lymphomas. Folia Haematol (Leipzig) 107: 601-620, 1980.

72. Loseke L, Craver LF: The Diagnosis of Hodgkin's Disease by Aspiration Biopsy. Blood 1: 76-82, 1946.

73. Lucas PF: Lymph Node Smears in the Diagnosis of Lymphadenopathy: A Review. Blood 10: 1030-1054, 1955.

74. Lukes RJ, Butler JJ, Hicks EB: Natural History of Hodgkin's Disease as related to its Pathologic Picture. Cancer 19: 317-344, 1966b.

75. Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. Cancer 34: 1488-1503, 1974.

76. Lukes RJ, Collins RD: New Approaches to the Classification of the Lymphoma. Br J Cancer 31: 1-28, 1975b.

77. MacMohan B: Epidemiology of Hodgkin’s disease. Cancer Res,26:1189-1200,1966.

78. Marluce Bibbo: Comprhensive cytopathology, 2nd edition, W.B. saunders company, 1997.

79. Martelli G: Fine needle aspiration cytology in superficial lymph nodes. European journal of surgical oncology, 15:13-6, 1989.

80. Martin HE, Ellis EB. Biopsy by needle pucture and aspiration. Ann Surg;

92:169-181,1930.

81. Mondal A, Mukerjee D, Chatterjee DM, Saha AM, Mukerjee AL: Fine needle aspiration biopsy of cervical lymphadenopathies, J Indian Med Assoc,Dec; 87(12): 281-283,1989.

82. Munker R, Hasenclever D, Brosteanu O, Hiller E, Diehl V: Bone marrow involvement in Hodgkin's disease, an analysis of 135 consecutive cases, J clin Oncol, 13:403-409, 1995.

83. National Cancer Institute (NCI): National Cancer Institute Sponsored Study of Classifications of Non-Hodgkin's Lymphomas: Summary and Description of a Working Formulation for Clinical Usage: The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer 49: 2112-2135, 1982.

84. Nausti JF, Yu G, Boudousquie A, Gupta P: Diagnostic value of lymph node fine needle aspiration cytology : An institutional experience of 387 cases observed over a 5- year period. Cytopathol 11:18-31, 2000.

85. Neiman RS, Barcos M, Bernard C: Granulocytic Sarcoma ; a clinicopathologic study of 61 biopsied cases. Cancer 48:1426-1437, 1981.

86. Neiman RS: Current Problems in the Histopathologic Diagnosis and Classification of Hodgkin's Disease. Pathol Annu 13(2): 289-328, 1978.

87. O'Dowd GJ, Frable WJ, Behm FG: Fine Needle Aspiration Cytology of Benign Lymph Node Hyperplasias: Diagnostic Significance of Lymphohistiocytic Aggregates. Acta Cytol 29: 554-558, 1985.

88. Oertel J, Oertel B, Lobeck H: Cytologic and Immunocytologic Studies of Peripheral T-Cell Lymphomas. Acta Cytol 35: 285-293, 1991.

89. Orell SR, Skinner JM: The Typing of Non-Hodgkin's Lymphomas using Fine Needle Aspiration Cytology. Pathol 14: 389-394, 1982.

90. Pak HY, Friedman NB: Pseudosarcoid Granulomas in Hodgkin's Disease. Hum Pathol 12: 832-837, 1981.

91. Pandit AA, Hkilnani PH, Prayag AS: Tuberculous Lymphadenitis: Extended Cytomorphologic Features. Diagn Cytopathol 12: 23-27, 1995.

92. Pangalis GA, Nathwani BN, Rappaport H: Malignant Lymphoma, Well differentiate Lymphocytic: Its Relationship With Chronic Lymphocytic Leukemia

and Macroglobulinemia of Waldenström. Cancer 39: 999-1010, 1977.

93. Patra, A. K., Nanda, B. K., Mohapatra, B. K. and Panda, A. K.: Diagnosis of lymphadenopathy by fine needle aspiration cytology. Ind. J. Pathol. & Microbiol, 26:273-278, 1983.

94. Patsouris E, Noël H, Lennert K: Histological and Immunohistological Findings in Lymphoepithelioid Cell Lymphoma (Lennert's Lymphoma). Am J Surg Pathol 12: 341-350, 1988.

95. Penny RJ, Blaustein JC, Longtine JA, et al: Ki-1-Positive Large Cell Lymphomas, a Heterogenous Group of Neoplasms: Morphologic, Immunophenotypic, Genotypic, and Clinical Features of 24 Cases. Cancer 68: 362-373, 1991.

96. Pilotti S, Di Palma S, Alasio L, et al: Diagnostic Assessment of Enlarged Superficial Lymph Nodes by Fine Needle Aspiration. Acta Cytol 37: 853-866, 1993.

97. Pontifex AH, Haley L: Fine-Needle Aspiration Cytology in Lymphomas and Related Disorders. Diagn Cytopathol 5: 432-435, 1989.

98. Pontifex AH, Klimo P: Application of Aspiration Biopsy Cytology to Lymphomas. Cancer 53: 553-556, 1984.

99. Radhika S, Gupta SK, Chakrabarti A: Role of Culture for Mycobacteria in Fine-Needle Aspiration Diagnosis of Tuberculous Lymphadenitis. Diagn Cytopathol 5: 260-262, 1989.

100. Ramzy I, Rone R, Schultenover SJ: Lymph Node Aspiration Biopsy: Diagnostic Reliability and Limitations--An Analysis of 350 Cases. Diagn Cytopathol 1: 39-45, 1985.

101. Rappaport H: Tumors of the Hematopoietic System. Atlas of Tumor Pathology. Washington, DC: Armed Forces Institute of Pathology; Sec 3 pt 8, 1966

102. Richard M Demay : The Art & Science of cytopathology American society of clinical pathologists, ASCP Press, Chiacago, 1999.

103. Rilke F, Pilotti S, Carbone A: Morphology of Lymphatic Cells and of their Derived Tumours. J Clin Pathol 31: 1009-1056, 1978.

104. Roy S, Joshi K, Choudhury AR: Nuclear Morphometric Analysis of Non-Hodgkin's Lymphoma. Pathology 21: 100-104, 1989.

105.Russell J, Orell S, Skinner J: Fine Needle Aspiration Cytology in the Management of Lymphoma. Aust NZ J Med 13: 365-368, 1983.

106.Sacks EL, Donaldson SS, Gordon J: Epithelioid Granulomas Associated With Hodgkin's Disease: Clinical Correlations in 55 Previously Untreated Patients. Cancer 41: 562-567, 1978.

107.Saltzstein SL, Ackerman LV: Lymphadenopathy Induced by Anticonvulsant Drugs and Mimicking Clinically and Pathologically Malignant Lymphomas. Cancer 12:164-182, 1959.

108.Schnitzer B: Reed-Sternberg-Like Cells in Lymphocytic Lymphoma and Chronic Lymphocytic Leukemia. Lancet (ii): 1399-1400, 1970.

109.Schultz HB, Ersboll J, Hougaard P: Prognostic Significance of Differential Cell Counts in Non-Hodgkin's Lymphomas. APMIS 96: 361-367, 1988.

110.Singh MK, Verma K, Kapila K. Value of aspiration cytology in diagnosis and subtyping of malignant lymphomas. Indian journal of medical research, 87:32-6. 12, 1988.

111.Sneige N: Diagnosis of Lymphoma and Reactive Lymphoid Hyperplasia by Immunocytochemical Analysis of Fine-Needle Aspiration Biopsy. Diagn Cytopathol 6: 39-43, 1990b.

112.Spieler P, Schmid U: How Exact are the Diagnosis and Classification of Malignant Lymphomas From Aspiration Biopsy Smears? A Comparison between Histologic and Cytologic Diagnoses of 20 Hodgkin's Lymphomas According to Rappaport (1966) and Kiel (1974) Nomenclatures. Pathol Res Pract 163: 232-250, 1978.

113.Spriggs AI, Vanhegan RI: Cytological Diagnosis of Lymphoma in Serous Effusions. J Clin Pathol 34: 1311-1325, 1981.

114.Stani J: Cytologic Diagnosis of Reactive Lymphadenopathy in Fine Needle Aspiration Biopsy Specimens: Acta Cytol 31: 8-13, 1987.

115.Steel BL, Schwartz MR, Ramzy I: Fine Needle Aspiration Biopsy in the Diagnosis of Lymphadenopathy in 1,103 Patients: Role, Limitations and Analysis of Diagnostic Pitfalls. Acta Cytol 39: 76-81, 1995.

116.Stiegler R, Gosse H, Leonhardt P, Gillissen A : Fine needle aspiration cytology as diagnostic bed side method for differential diagnostics of enlarged peripheral lymph nodes, Pneumologie, Jun;57 (6) :322-7,2003.

117.Strickler JG, Medeiros LJ, Copenhaver CM: Intermediate Lymphocytic Lymphoma:

An Immunophenotypic Study With Comparison to Small Lymphocytic Lymphoma and Diffuse Small Cleaved Cell Lymphoma. Hum Pathol 19: 550-554, 1988.

118.Strum SB, Park JK, Rappaport H: Observation of Cells Resembling Sternberg-Reed Cells in Conditions other than Hodgkin's Disease. Cancer 26: 176-190, 1970.

119.Tani EM, Christensson B, Porwit A: Immunocytochemical Analysis and Cytomorphologic Diagnosis on Fine Needle Aspirates of Lymphoproliferative Disease. Acta Cytol 32: 209-215, 1988.

120.Tani EM, Löwhagen T, Nasiell K,: Fine Needle Aspiration Cytology and Immunocytochemistry of Large Cell Lymphomas Expressing the Ki-1 Antigen. Acta Cytol 33: 359-362, 1989a.

121.Tripathy SN, Mishra N, Patel NM, Samantray DK, Das BK, Mania RN: Place of aspiration biopsy in the diagnosis of lymphadenopathy. Indian J Tub, 32:130-4, 1985.

122.Tseng FY, Hsiao YL, Chang TC: Cytologic features of metastatic papillary thyroid carcinoma in cervical lymph nodes, Acta Cytol, Nov-Dec;46(6) : 1043-8, 2002.

123.Tubbs RR, Fishleder A, Weiss RA: Immunohistologic Cellular Phenotypes of Lymphoproliferative Disorders: Comprehensive Evaluation of 564 cases Including 257 Non-Hodgkin's Lymphomas Classified by the International Working Formulation. Am J Pathol 113: 207-221, 1983.

124.Ustun M, Risberg B, Davidson B, Berner A: Cystic change in metastatic lymph nodes; a common pitfall in fine needle aspiration cytology, Diagn Cytopathol. Dec; 27 (6): 387-92, 2002.

125.Van de Schoot L, Aronson DC, Behrendt H, Bras J: The role of Fine needle aspiration cytology in children with persistent or suspicious lymphadenopathy;Paediatric Sur J, Jan;36(1):7-11, 2001.

126.Van der Valk P, Meijer CJLM: The Non Hodgkin's Lymphomas: Old and New Thinking. Histopathology 13: 367-384, 1988.

127.Van Heerde P, Go DMDS, Koolman-Schellekens MA: Cytodiagnosis of Non-Hodgkin's Lymphoma: A Morphological Analysis of 215 Biopsy Proven Cases. Virchows Arch 403: 213-233, 1984.

128.Vargas J, Arguelles M, Nevado M: Fine Needle Aspiration Biopsy of a Cutaneous Relapse of Lymphomatoid Granulomatosis: A Case Report. Acta Cytol 37: 205-208, 1993.

129.Verma K, Rao AV, Kapila K: Cytologic Diagnosis of an Acute Myeloid Leukemia Infiltrate in a Lymph Node. Acta Cytol 35: 373-374, 1991.

130.Vonderheid EC, Tam DW, Johnson WC: Prognostic Significance of Cytomorphology in the Cutaneous T-Cell Lymphomas. Cancer 47: 119-125, 1981.

131.Warnke RA, Kim H, Fuks Z: The Coexistence of Nodular and Diffuse Patterns in Nodular Non-Hodgkin's Lymphomas:Significance and Clinicopathologic Correlation. Cancer 40: 1229-1233, 1977.

132.Winter JN, Marder RJ, Hauck WW: Phenotypic Analysis in Diffuse, Large Cell Lymphoma: Clinical and Histologic Associations. Am J Clin Pathol 85: 425-432, 1986.

133.Young NA, Al-Saleem T. Diagnosis of lymphoma by fine needle aspiration cytology using the revised European-American classification of lymphoid neoplasms. Cancer, 87: 325–345, 1999.