Original article:

Diagnosis of lymphadenopathies by FNAC: a prospective study

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Abstract:

Objective: To determine the incidence of various pathological lesions involving lymph nodes by using simple and easy diagnostic tool (FNAC) and prove the diagnostic importance of FNAC.

Materials and methods: Our study included 1926 cases of lymphadenopathy, between January 2012 and December 2013. Patients with palpable lymph nodes anywhere in the body with a valid requisition were included in the study. All the demographic details, history and clinical findings of the swelling are taken by the attending pathologist.

Results: Our study included 1926 cases of lymphadenopathy, we reported 1136 cases (59%) as granulomatous lymphadenitis, 500 cases (26%) of Non-specific lymphadenitis, 232 cases (12%) of Lympho-proliferative disorders (Hodgkin’s and Non-Hodgkin’s disease) 58 cases (03%) metastases from primary site. Our study also showed the incidence of lympho-proliferative diseases in association with Human Immuno-deficiency Virus (HIV) infection (144 cases). Majority of the granulomatous lymphadenitis cases were associated with HIV cases constituting about 66.5% (756 cases).

Conclusion: Fine Needle Aspiration Cytology is a simple, safe, rapid, cost effective and reasonably accurate method of establishing the diagnosis of lymphadenopathy. Overall accuracy of FNAC in comparison with the histopathological study is very high and may obviate the need of excision biopsy when the findings are compatible with the clinical diagnosis. The high accuracy rate of aspiration cytology of lymph nodes calls for its wide application in daily practice.

Keywords: Lymphadenopathy, Lympho-proliferative disorder, FNAC.

Introduction: Lymphadenopathy is one of the commonest clinical presentations of patients, attending the outdoor clinics in most hospitals. The aetiology varies from an inflammatory process to a malignant condition. The use of fine needle aspiration cytology (FNAC) in the investigation of lymphadenopathy has become an acceptable and widely practiced, safe, simple, rapid, painless and minimally invasive technique. FNAC is highly cost effective out-patient procedure and has high accuracy. FNAC was initially conceived as a means to confirm a clinical suspicion of local recurrence or metastasis of known malignancy without subjecting the patient to further surgical intervention. Many clinicians feel that this remains the most important contribution of the technique from practical point of view, but the clinical value of FNAC is not limited to neoplastic conditions. FNAC is also valuable in the diagnosis of inflammatory, infectious, reactive, degenerative conditions. Samples which were aspirated from the swelling can be used for microbiological and biochemical analysis in addition to cytological preparations. Now –a-days FNAC is used as a first line investigative technique in any palpable and visible swellings of the body. Few of the differential diagnoses of the lymphadenopathy include reactive hyperplasia/ inflammatory conditions, granulomatous disorders and malignancy.
Thus FNAC gives a clear picture of the case/cases requiring further investigations, medical management, surgical intervention or just clinical follow-up. FNAC is a valuable diagnostic technique with least number or nil contraindications. Shakya et al. also mentioned that the cytology is more readily accepted for the evaluation of deeply seated lymph nodes (i.e. surgically inaccessible) with primary lymphoma or for medically unfit patients for surgery. We planned study to determine the incidence of various pathological lesions involving lymph nodes by using simple and easy diagnostic tool (FNAC) and prove the diagnostic importance and accuracy of FNAC.

MATERIALS AND METHODS
Our study included 1926 cases of lymphadenopathy, between January 2012 and December 2013. Patients with palpable nodes were referred to department of pathology after clinical examination by the clinician and surgeon. Routine FNAC was performed by the attending pathologist on receiving the requisition duly filled by the consultant surgeon. All the demographic details of the patient, history and clinical findings of the swelling are taken by the attending pathologist and entered in a cytology register. Under aseptic precautions, aspiration of enlarged lymph nodes was performed free hand using a 23 G needle with 5 cc or 10 cc syringe. The cellular aspirate is spread on a dust-free glass slide and immediately transferred to coplin jars filled with alcohol for fixation. After fixation, slides are stained with Hematoxylin and Eosin (H & E). Diagnosis was given on the same of FNAC procedure. Granulomatous inflammation is recognized cytologically by observing aggregates of histiocytes with or without associated multinucleated giant cells. A dirty necrotic background would suggest caseation and possibly tuberculosis. In cases where an infective aetiology was suspected, needle washings were sent for bacteriological culture and sensitivity. When TB was suspected, an additional sample was sent for culture and slides were also stained with Ziehl-Neelsen methods to detect acid fast bacilli (AFB) directly. The eventual diagnosis of granulomatous inflammation by FNAC was confirmed either by other supportive laboratory investigations or by surgery in difficult or in doubtful cases where the definitive opinion is not possible. In non-specific or reactive lymphadenopathy cases, a repeat FNAC was done after a period of 7 to 10 days after a course of antibiotic therapy. In Lympho-proliferative disorders, malignancies or metastases, FNAC diagnosis was reconfirmed by histopathological examination.

RESULTS
In our study, we reported 1926 cases presented with lymphadenopathy at various locations of the body, most common being cervical lymphadenopathy.

1) In a total study of 1926 cases of lymphadenopathy, we reported 1136 cases (59 %) as granulomatous lymphadenitis, 500 cases (26 %) of Non-specific lymphadenitis, 232 cases (12%) of Lympho-proliferative disorders (Hodgkin’s and Non- Hodgkin’s disease, 58 cases (03%) metastases from primary site.

2) Our study also showed the relative incidence of lympho-proliferative diseases in adults(218 cases) and pediatric population (14 cases) and also incidence of lympho-proliferative diseases in association with Human Immuno-deficiency Virus (HIV) infection (144 cases). (FIGURE 1 & 2)

3) Among the total incidence of granulomatous lymphadenitis reported, 71 % (806 cases) of
the cases were reported in adults and 29% (330) of cases were reported in the pediatric population. Majority of the granulomatous lymphadenitis cases were associated with HIV cases constituting about 66.5% (756 cases). (FIGURE 3 & 4)

FIGURE 1 – Incidence of Lymph proliferative diseases in adults (218 cases) and paediatric age group (14 cases)

FIGURE 2- Incidence of Lympho-proliferative diseases associated with HIV (88 cases)
FIGURE 3- Incidence of granulomatous lymphadenitis in adult (806 cases) and pediatric population (330 cases)

FIGURE 4- Incidence of Granulomatous Lymphadenitis in HIV patients (756 cases).

DISCUSSION
The well-defined role of FNAC in the investigation of lymphadenopathy has previously been studied.\(^2,3\) In the context of granulomatous disorders, the possible aetiology is wide and the use of FNAC with other ancillary tests (microbiological, immunohistochemical, radiological, biochemical and special staining techniques) is useful for obtaining a definitive diagnosis. The algorithm shows a useful classification of the aetiology of granulomatous lymphadenopathy. (FIGURE 5)
Aetiologies of Lymphadenopathies:

**FNAC OF LYMPHADENOPATHY**

<table>
<thead>
<tr>
<th>Infective</th>
<th>Neoplastic</th>
<th>Lymphoma</th>
<th>Carcinoma</th>
</tr>
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<tbody>
<tr>
<td>Tuberculosis</td>
<td>Metastatic</td>
<td>Hodgkin’s Lymphoma</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>Atypical Mycobacterium</td>
<td></td>
<td></td>
<td>Non-Hodgkin’s Lymphoma</td>
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<td>Sarcoidosis</td>
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<tr>
<td>Toxoplasmosis</td>
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Tuberculosis cervical lymphadenopathy usually present with multiple lymph node enlargements, less constitutional symptoms, tuberculosis is an important public health problem \(^{(4)}\) and it is commonest cause of infectious diseases affecting the lymphatic tissues of the body \(^{(5)}\). The diagnosis is based on high index of doubt with hematological and pathological investigations this diseases can be diagnose with FNAC (FIGURE 7). In our study family history was not present in most of the patients while commonest age group affected was 18 to 35 years, and male were predominant, the upper deep jugular nodes in posterior triangle were most commonly involved. Tuberculosis lymphadenopathy constituted the commonest lesion followed reactive lymphadenitis, lymphoproliferative disorders and metastatic malignancies, which is correlating with most of the studies of Indian authors \(^{(6-10)}\). Maximum AFB positivity is seen in cases showing caseous necrosis with occasional epithelioid cells. Sometimes in absence of AFB positivity the diagnosis of highly suspicious of tuberculosis was given in these lesions with strong clinical suspicion, high ESR and chest X-
ray findings. The tuberculosis in HIV positive cases showed caseous necrosis, while granulomas were less and ill formed.

A suspicious clinical history of TB (pyrexia, night sweats, recent travel to endemic areas, no previous BCG vaccination) coupled with positive aspirate, blood, sputum or urine tests for AFB and good response to anti-tuberculous therapy supports the diagnosis of TB. One disadvantage is the inherent delay in culture result, but it is anticipated that as polymerase chain reaction and other amplification techniques become more common, detection time for the organism will shorten, improving the value of FNA in clinical practice. The presence of epithelioid cells either with caseation or positive Ziehl-Neelsen stain for acid-fast bacilli appears to be the best criterion to diagnose tuberculous lymphadenitis by fine needle aspiration. Bezabih et al found FNAC reliable in helping to avert more invasive surgical procedures undertaken in the diagnosis of tuberculous lymphadenitis\(^{(11)}\).

The typical FNAC features of toxoplasmosis include the presence of follicular hyperplasia with secondary germinal centre rich in macrophages, presence of groups of epithelioid cells and presence of monocytoid histiocytes have been previously described\(^{(12,13)}\). A combination of FNA features with positive serological testing and history of animal contact gives the diagnosis of toxoplasmosis and thus avoids unnecessary surgical excision. Sarcoidosis is a disease of unknown aetiology that can be characterized by the histological hallmark of epithelioid non-caseating granulomas, usually accompanied by multinucleated giant cells. Although there is no single gold standard test, the important role of FNAC in histological diagnosis and its under utilization was highlighted by Tambouret \textit{et al}\(^{(14)}\). We agree with the authors that FNAC used in conjunction with clinical findings, radiological and laboratory investigations can be a cost effective method in diagnosis toxoplasmosis.

The reactive pattern is variable depending on the degree of stimulation, the number and size of the germinal centres and on whether the sample derives mainly from a germinal centre or from interfollicular or paracortical tissue. Germinal centre material is represented by poorly defined tissue fragments of poorly cohesive cells. These fragments include centroblasts, centrocytes, tingible body macrophages and a number of lymphocytes which adhere to the syncytial cytoplasm of dendritic reticulum cells. Dendritic reticulum cells have oval or round nuclei with a smooth nuclear membrane, a coarsely granular, uniformly distributed chromatin and small distinct nucleoli. The cytoplasm is dispersed in the background. A smear, which derives mainly from interfollicular tissue, consists predominantly of lymphocytes with a variable but much smaller number of scattered immunoblasts, plasma cells, non-specific histiocytes and endothelial cells. (FIGURE 8)

The main features which distinguish a reactive process from lymphoma are:

\begin{itemize}
  \item \textit{a)} A mixed population of lymphoid cells representing the whole range of lymphocyte transformation from small lymphocytes to immunoblasts and plasma cells.
  \item \textit{b)} A predominance if small , sometimes slightly larger stimulated lymphocytes , which have small round nuclei and a characteristic chromatin pattern of large ill-defined condensations
\end{itemize}
c) Centroblasts and centrocytes associated with dendritic reticulum cells and tingible body macrophages derived from germinal centres. FNAC as a first line screening method has been recommended in suspected malignancy (3&15). Granulomas may be encountered in both Hodgkin’s disease and non-Hodgkin’s lymphoma, particularly T-cell lymphoma(16) Hodgkin’s lymphoma is characterized by the classic Reed Sternberg cells in a background of sarcoid-like granuloma, reactive lymphoid cells and occasional eosinophils. Occasionally, lymph nodes containing metastatic carcinoma may also show features of granuloma. Previous reports have been described in metastatic nasopharyngeal carcinoma, seminoma and malignant melanoma where granulomas were seen mimicking granulomatous lymphadenitis. Histologically, non-caseating granuloma composed of epithelioid histiocytes with multinucleated giant cells are seen, but these can be indistinguishable from granulomatous inflammation from other causes. A series by Khurana et al (17) highlighted the difficulties encountered in making a definitive diagnosis of malignant neoplasm that mimics, or occurs, in association with granuloma. Granulomatous inflammation found in lymph nodes draining carcinomas is a recognized phenomenon. Such phenomenon is reported in pulmonary small cell carcinoma, malignant melanoma, papillary thyroid carcinoma, gastric carcinoma, and rhabdomyosarcoma. This has been suggested to be either a response to necrotic material or an immunological T-cell mediated hypersensitivity reaction to cell surface antigens. However, the precise mechanism is largely speculative as the exact tumor or host factors that enable such a response remain unknown. We agree with Lui et al (18) in their pragmatic approach of diligent examination of FNAC slides combined with ancillary clinical, serological and imaging investigations in the drainage areas to identify any occult malignancy.

In the present study 232 cases of lymphomas have been reported 163 cases of Hodgkin’s lymphoma which was diagnosed on FNAC due to preserved Reed Sternberg (RS) cells (FIGURE 9 & 10). In lymphoma group, with presence of RS cell, the cytological diagnosis of Hodgkin's lymphoma is easy. FNAC is helpful in diagnosis of Hodgkin's lymphoma though biopsy is recommended for confirmation and classification. Cytological sub-typing of lymphoma in FNAC smears is difficult and requires extensive experience. It can be possible where the team of experience pathologists and oncologists are present. It is believed by many authors that in non-Hodgkin's lymphoma biopsy are mandatory, and we also believe in it. Below mentioned algorithm is useful in sub-typing the Lymphomas on FNAC. (FIGURE 6)
ALGORITHM

CLASSIFICATION OF LYMPHOMA

FNAC LYMPHOID POPULATION OF CELLS

- Monotonous population
  - Predominant cell type
    - Small
      - Lymphocytic Lymphoma
    - Medium
      - Burkitt’s Lymphoma
    - Large
      - Large B cell Lymphoma

- Heterogenous population
  - Mainly small cells
    - a) Reactive hyperplasia
    - b) Follicular Lymphoma (Gr 3)
    - c) Marginal Zone lymphoma
  - Large cells
    - a) Reactive Immunoblastic
    - b) Follicular Lymphoma
    - c)Peripheral T cell lymphoma
    - d) Lymphoplasmacytic Lymphoma

- Pleomorphic population
  - Predominant cell type
    - Small
      - Reactive hyperplasia
    - Medium
      - Follicular Lymphoma
    - Large
      - Lymphocyte Predominant Hodgkin’s Lymphoma
      - Lymphocyte Rich Hodgkin’s Lymphoma
      - T-cell rich B cell Lymphoma
      - Anaplastic large cell lymphoma
      - Large cell Pleomorphic lymphomas (T-Cell & B-Cell type)
      - True histiocytic lymphoma (Histiocytic sarcoma)
      - Anaplastic plasmacytoma
CONCLUSION

Fine Needle Aspiration Cytology is a simple, safe, rapid, cost effective and reasonably accurate method of establishing the diagnosis of lymphadenopathy. Non representative sampling usually in small metastatic deposits may be an important cause of false negative diagnosis and granulomatous reaction to metastatic tumors may be misinterpreted as granulomatous lymphadenitis on FNAC. In such cases biopsy should be advised to support or confirm the FNAC diagnosis. Whenever necrosis is abundant in cases of metastatic malignancy, cytomorphological interpretation of smears should be done with caution. Overall accuracy of FNAC in comparison with the histopathological study is very high and may obviate the need of excision biopsy when the findings are compatible with the clinical diagnosis. The high accuracy rate of aspiration cytology of lymph nodes

FIGURE 7- Smear shows clusters of epithelioid cells, lymphocytes, plasma cells, caseous necrosis. (TB lymphadenitis)

FIGURE 8- Smear shows small and large lymphocytes, tingible body macrophages, centroblasts, centrocytes in a hemorrhagic background. (Reactive Lymphadenitis)

FIGURE 9- Smear shows polymorphous population of cells comprising of lymphocytes, eosinophils, occasional plasma cells and large mono-nucleate and bi-nucleate pleomorphic Reed-Sternberg (RS) cells in a hemorrhagic background (Hodgkin’s Lymphoma).

FIGURE 10- Smears shows bi-nucleate pleomorphic Reed-Sternberg (RS) cell.
calls for its wide application in daily practice. Thus in conclusion this simple procedure should be advocated by the clinicians so that early diagnosis of cervical lymphadenopathy is possible in shortest period of time and also makes the clinician decide about the treatment so as to prevent the progress of the disease.

REFERENCES


