Comparative study of Cyto and Histopathology in Diagnosing Cervical Lymphnodal lesions

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Fine needle aspiration cytology findings are important primary diagnostic tool in cervical lymph nodal enlargement comparable to histopathology but when there is difficulty in diagnosis on cytology, excision biopsy should be done. In this study, we compared the correlation between diagnostic method of cytopathology and histopathology for the evaluation of cervical lymphadenopathy but in lymphoproliferative lesions, excisional biopsy is more important in establishing in evaluation & diagnosis.

Key words: Lymph node, Cervical, FNAC, Histopathology.

INTRODUCTION

Lymph nodes are important part of the immune system. Cervical lymphadenopathy is one of the commonest clinical presentations. Lymph nodes in head and neck form groups and these groups are responsible for draining lymphatic fluid from different regions in the head and neck (The Doctors Lounge, 2007). There are approximately 300 lymph nodes in the neck, and they can be classified in many different ways (Lorenz et al., 2010). One system divides the nodes as follows:

Level I: Submental and submandibular nodes
Level Ia: Submental triangle
Level Ib: Submandibular triangle
Level II: Upper jugular nodes
Level III: Middle jugular nodes
Level IV: Lower jugular nodes
Level V: Posterior triangle group
Level VI: Anterior triangle group
Level VII: Upper mediastinal nodes.

Localized cervical lymphadenopathy is lymph node enlargement that is restricted to cervical area. Most lymphadenopathy is due to a benign self-limited disease such as viral infections, and adenopathy is secondary to an increase in normal lymphocytes and macrophages in response to an antigen.

Other less common mechanisms responsible for adenopathy include lymphadenitis, neoplasia or storage diseases. Tubercular lymphadenitis is one of the commonest causes in developing countries (Shakya et al., 2009). Tubercular lymphadenitis is a common disease in cervical region.

It is more common in children and adolescent age group. Jugulodigastric nodes are more affected. It is not secondary to pulmonary tuberculosis. Superficial cervical lymphadenopathy may be sign of inflammation, metastatic malignancy or malignant lymphoma. Lymph nodes are the most common site of metastatic malignancy and sometimes constitute the first clinical manifestation of the disease. Fine needle aspiration cytology of lymph nodes is excellent first line method for investigating the nature of lesion, as it is economical and convenient alternative to open biopsy (Ahmad Shamshad et al., 2005; Shakya et al., 2009). Usually carcinomas metastasise through lymphatic channels (Henry, 2009). Cervical lymph node metastases from unknown primary sites account for approximately 3% to 9% of all head and neck malignant lesions (Mistry et al., 2008). The frequency of metastatic lymph nodes at various neck levels were as follows:

- Level 1 - 10%
- Level 2 - 75%
- Level 3 - 51.7%
- Level 4 - 13.5%
- Level 5 - 1%
FNAC and histological assessment of such lymph node biopsies help to arrive at an appropriate diagnosis. Further introduction of cytokeratin in immune histochemistry increases the accuracy of the result. It may be difficult to identify nodal involvement by conventional pathological examination of haematoxylin-eosin stained sections particularly when metastases are of microscopic size. Cytokeratin staining of dissected lymph nodes is a useful new diagnostic tool for detecting micro metastatic foci in regional lymph nodes of patients with primary malignancy elsewhere. Because the cytokeratin immunohistochemical method enhances the detection rate of occult micro metastasis in cervical lymph nodes of head and neck carcinoma patients, it may be recommended for routine diagnostic use in patient with negative for a lymph node metastasis on routine haematoxylin-eosin stain. Most cases of cervical lymphadenopathy in all generations are due to infection or benign diseases. Reactive cervical lymphadenitis is common secondary to acute bacterial and viral infections in the Head & Neck region.

Tuberculosis is one of the most common cause of cervical lymphadenopathy in developing countries. Tuberculous lymphadenitis was found to be the commonest form of extrapulmonary tuberculosis, comprising about 75% of the extrapulmonary cases. Present study is an attempt to evaluate lymph node biopsies with special references to metastatic lesions with clinico-pathological correlation.

Causes of Cervical Lymph node Enlargement (Koss, ?)

I. Inflammatory

1. Acute Lymphadenitis
   - Nonspecific
   - Specific e.g. Streptococous, staphylococous, infectious mononucleosis, measles, CMV infection, toxoplasma, cat scratch disease etc.

2. Chronic Lymphadenitis
   a) Nonspecific
   b) Specific e.g. Tuberculosis, syphilis, brucellosis, tularemia, HIV infection, filariasis, actinomycosis etc.

II. Neoplastic

1. Benign
2. Malignant
   - Primary lymphomas and leukemias
   - Secondary
   i. Squamous cell carcinoma from larynx, pharynx, oral mucosa, tongue, tongue, scalp, upper 1/3rd of esophagus
   ii. Adenocarcinoma from GI tract, breast, thyroid, kidney, testis
   iii. Malignant melanoma
   iv. From unknown origin

III. Miscellaneous

- Allergic lymphadenopathy as in serum sickness
- Drug induced
- Post vaccination
- Sinus histiocytosis also called as Rosai Dorfman disease
- Dermatopathic lymphadenopathy
- Chronic pseudolymphomatous lymphadenopathy
- Juvenile rheumatoid arthritis
- Sarcoidosis
- Amyloidosis
- Glycogen and lipid storage diseases
- Connective tissue disorders
- Histiocytic necrotizing lymphadenitis
- Kawasaki disease

Aims and objectives

The current study was undertaken to

- Study the lymph node lesions in various diseases by fine needle aspiration cytology and histopathology.
- To categorize various lymph node lesions into neoplastic and non-neoplastic lesions.
- To correlate the findings of FNAC and histopathology wherever possible.
- To study the morphological features of metastasis in the lymph nodes.
- To suggest the primary site based on lymph node metastasis wherever possible.
- To detect micro metastasis using cytokeratin immunostaining.

MATERIALS AND METHODS

This study was conducted in Department of Pathology, IPGME & R. The present study includes 100 cases from January 2013 to June 2014. All cases of lymph node lesions presenting with superficial cervical lymphadenopathy where FNAC needs to be done and those cases undergoing subsequent biopsy and immunohistochemistry were studied. Total number of cases (who underwent FNAC procedure): 106

Unsatisfactory smear and hence, left out of study: 06

Number of cases those underwent subsequent biopsy: 100

Number of cases where Cytokeratin immunostaining was done: 15

In the present study which studied 100 cases of cervical lymphadenopathy, 61 cases were non-neoplastic lesions and 39 cases were neoplastic lesions and the ratio was 1.56:1. The ratio of non-neoplastic to neoplastic lesions was 6.21:1 in the study of Dash et al., 1996, 1.24:1 in the study of Nada et al., 1996, 6.35:1 in the study of Shamshad et al., 2005 and 4.42:1 in Hirachand et al., 2009 studies. In the study made by Shafullah and Syed Humayun Shah et al., 1999 the incidence of non-neoplastic and neoplastic lesions was 90.6% and 9.6% respectively (Shafullah and Syed Humayun Shah, 1999).

In the present study majority of the patients referred for FNAC were 44(44%) in the age group of 21-40 years similar to the observation of Pandit et al., 1987 - 146(51.05%), whereas in the study of Gupta et al., 2003
most of the patients 532(52.26%) were in the age group of 0-20 years. In the present study majority of the patients 64 were male and 36 were females with male to female ratio of 1.77:1 which is higher that was observed in the study of Hirachand et al., 2009 (male 68 and female 62 with male to female ratio of 1:0.9). Tariq et al., 2008 in their study had 36 males and 34 females with male to female ratio of 1: 2.1. detected with H&E staining but detected on cytokeratin immunoperoxidase staining.

**Table 18. Sensitivity and Specificity of FNAC as compared to Histopathology in the current Study**

<table>
<thead>
<tr>
<th>Histological Diagnosis</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubercular</td>
<td>95.23%</td>
<td>98.27%</td>
</tr>
<tr>
<td>Lymphadenitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive Lymphadenitis</td>
<td>100%</td>
<td>91.35%</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>79.16%</td>
<td>100%</td>
</tr>
<tr>
<td>Secondaries</td>
<td>93.33%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Most of the studies show female predilection. Few studies like Purohit et al and Tripathy SN et al are comparable with the present study. In the present study, tuberculosis accounted for 42% of cases, 19% of cases turned out to be reactive lymphadenitis. Among the neoplastic lesions, 15% cases were found to be metastatic whereas non-Hodgkin’s lymphoma and Hodgkin’s lymphoma accounted for 19% and 5% of cases respectively. In the study made by Jha et al., 2001 of 94 cases, 63.8% of cases were found to be tuberculous lymphadenitis.

In the present study, 68% of patients presented with fever, 27% cases with cough, 12% with loss of appetite, 7% with loss of weight, 2% with dysphagia and 4% with change in voice. Level V group of lymph nodes were most commonly involved in tuberculosis followed by level II. In cases of secondaries, level IV was most commonly involved whereas in cases of lymphomas level V was the most common site. In Jha et al., 2001 series, level II was most commonly involved in cases of tuberculosis. The present study is comparable with that of Baskota et al series where level V was the most common site in cases of tuberculosis. In the present study, non-Hodgkin’s lymphoma to Hodgkin’s lymphoma ratio is 3.8:1 while findings by Peh SC and Shamie et al was 9:1. Raymond Alexandrian study also had a ratio of 5.02:1 which was higher than the present study.

The commonest site of primary in cases of metastatic secondaries was lungs and pancreas by Linderman et al. In the present study, it was thyroid followed by oral cavity and larynx. In the study of Osama Gaber et al, it was possible to establish the primary in 86.7% of cases whereas in the current study it was possible in 14 out of 15 cases (93.33%). 1 case was missed out both in FNAC and histopathological examination which was later confirmed by cytokeratin immunohistochemistry. The primary could not be established due to limited resources in our set up. Zhand et al., 2008 reported that 34 (6.5%) lymph nodes with micrometastasis in 19 patients with cytokeratin. Enepekides et al., 1999 reported a 5% rate of micrometastasis of oral cavity tumors that was not

| Table 16. Comparison of Male to Female Ratio of the Present Study with Other studies |
|-----------------------------------|----------------|----------------|
| Bedi et al, 1999                  | 1:1.7          |                |
| Ammari et al., 1999               | 1:2            | 1.38           |
| Dworski et al., 1989              | 1:1.2          | 1.41           |
| Dandapata et al 1987              | 1:1.4          | 1.41           |
| Purohit et al, 1999               | 1:2            | 1.41           |
| Present Study                     | 1:1.7          | 1.77:1         |

In the study of Chamyal et al., 1997, One hundred and ten cases of cervical lymph node masses were subjected to clinical evaluation and aspiration cytology. Eighty six cases which subsequently underwent open biopsy were subjected to histopathological evaluation. Accuracy, specificity and sensitivity of clinical diagnosis were 86.1%, 93.6% and 91.1% respectively as compared to 88.3%, 97.6% and 93.6% respectively for aspiration cytology. In Shamshad Ahmad et al., 2005 study, correlation of FNAC findings with histopathology; sensitivity and specificity was found to be 91.6% and 99%, respectively. The sensitivity, specificity and diagnostic accuracy was 100% each in malignant lesions. In the current study, the overall accuracy of FNAC as compared to histopathological examination was found to be 93% which is close to most of the other studies in literature.

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The diagnostic accuracy of reactive lymphoid hyperplasia in the current study was 93%. This finding was in agreement with experience of Keith et al., 2007 who reported 88% diagnostic accuracy. However our finding was much lower than that reported by Al-Mulhim et al., 2004 who reported 100% diagnostic accuracy for such cases. However, it is much higher than the study of Hafeez et al., 2011 study who reported an accuracy of
70%. Out of the 7 false positive cases of reactive lymphadenitis, 4 cases turned out to be non-Hodgkin’s Lymphoma, 2 cases were tubercular lymphadenitis and were confirmed on histopathological examination. Remaining 1 case was found to be a metastatic secondary which was missed out after careful microscopic examination and later confirmed by cytokeratin immunostaining with microfoci of malignant cell deposit in the subcapsular space.

Xu et al., 2012 detected micrometastatic disease that was overlooked with H&E staining but was detected with cytokeratin immunoperoxidase staining in the oral cavity, oropharynx, hypopharynx, and larynx tumors of 33 patients. Zhand et al., 2008 reported that 34 (6.5%) lymph nodes with micrometastasis in 19 patients with cytokeratin. Enepekides et al., 1999 reported a 5% rate of micrometastasis of oral cavity tumors that was not detected with H&E staining but detected on cytokeratin immunoperoxidase staining. In the present study, all cases of metastatic carcinoma to the lymph nodes showed exact corroboration with the histopathology.
The diagnostic accuracy of these cases was 100%. Our finding came in close comparison to most investigators who reported more than 90% accuracy rate. While Khajuria et al. reported 87% diagnostic accuracy. Most of the studied metastatic nodes were metastatic squamous cell carcinoma followed by metastatic adenocarcinoma. In the present study, most common histological type was metastatic squamous cell carcinoma followed by metastatic papillary carcinoma of thyroid origin. Similar findings had been documented by other researchers.

FNAC has been widely used for the rapid diagnosis of metastatic malignancies, infectious diseases and reactive lymphoid hyperplasia. However there is a wide divergence of opinions regarding the role of FNAC on primary diagnosis and subclassification of NHL on FNAC smears. This difficulty is particularly problematic when a less than pure monomorphic lymphocyte population exists in the smears. The current study showed a sensitivity, specificity and accuracy of 79.16%, 100% and 95% respectively for Lymphoproliferative disorders. Accuracy of FNAC in diagnosing lymphomas has been reported in the range of 85% by Das DK et al study (Dilip, 2003). Regarding to the 4 false negative cases of non-Hodgkin’s lymphoma, it was found that 3 cases were underdiagnosed as reactive lymphoid hyperplasia on FNAC and re-diagnosed as malignancy on histopathology. 1 case was misdiagnosed as tubercular lymphadenitis.

After the introduction of REAL and WHO classification, the major emphasis has been given on cell morphology and immunophenotype rather than the growth pattern. FNAC combined with Flow cytometry immunophenotype (FCI) may be helpful in accurately subclassifying NHL according to WHO classification. Many of the subtypes of NHL such as Follicular lymphoma (FL) and Mantle cell lymphoma (MCL) which were previously recognized as a pure morphologic entity can be diagnosed by combined use of FNAC and FCI. Although in our study the sensitivity and specificity in diagnosing lymphomas is high but the exact diagnosis and type was made on excisional biopsy. One of the reasons for not completely diagnosing lymphomas is that immunohistochemistry was not applied on cytology in our laboratory.
In the present study, FNAC was reported to have a higher sensitivity in diagnosing Hodgkin’s lymphoma (80%) as compared to non-Hodgkin’s lymphoma (78.94%). In the study of Nasreen Hafeez et al., 2011, the sensitivity for Hodgkin lymphoma was 77.8%. Al Alwanet et al., 1996 concluded 76.9% accuracy rate. However, our result was much lower than that recorded by Al-Alwanet et al. and Das who reported 92% and 90% sensitivity for Hodgkin lymphoma on cytology, respectively. RS cells need to be differentiated from immunoblasts, which typically are not only smaller than RS cells but also lack prominent nucleoli and may have a plasmacytoid appearance. Along with a meticulous search for atypical cells that stand out in a reactive background, clinical suspicion may resolve the issue in these cases to some extent. The presence of atypical mononuclear cells and granulomas together should raise a high index of suspicion for further evaluation.

Hypocellular aspirates from clinically significant large lymph nodes should alert the pathologist to the possibility of fibrosis obscuring the primary pathology. Biopsy is mandatory in such cases. In the study by Singal et al., there were 139 aspirations from cervical lymph nodes with 48 cases of reactive lymphadenitis. On histopathology, 44 cases proved to be correct while 2 cases came out to be tubercular lymphadenitis and 2 cases as Hodgkin’s disease. Patra et al., 1983 included 113 aspirates in this study group in which 44 aspirates of reactive lymphadenitis were confirmed on histopathology in 34 cases, 5 cases of tubercular lymphadenitis were missed. They attributed this failure to missing the exact site of lesion and explained that, had they taken aspirates from multiple sites, they might not have missed these lesions.

**Summary and conclusion**

A total of 106 cases underwent FNAC procedure among which in 6 cases, the cytology smears were found to be unsatisfactory. Hence, these 6 cases were left out of study. Subsequent biopsy was done in those 100 cases and in 15 cases, cytokeratin immunostaining was done. Lymph node aspiration was done at all ages from 0-80 years and the mean age being 37.2 years.

In the present study which studied 100 cases of cervical lymphadenopathy, 61 cases were non-neoplastic lesions and 39 cases were neoplastic lesions and ratio is 1.56:1. Majority of the patients 64 were male and 36 were females with male to female ratio of 1.77:1. 68% of patients presented with fever, 27% cases with cough, 12% with loss of appetite, 7% with loss of weight, 2% with dysphagia and 4% with change in voice. In the present study, tuberculosis accounted for 42% of cases, 19% of cases turned out to be reactive lymphadenitis.

Among the neoplastic lesions, 15% cases were found to be metastatic secondaries whereas non-Hodgkin’s lymphoma and Hodgkin’s lymphoma accounted for 19% and 5% of cases respectively.

Level V group of lymph nodes were most commonly involved in tuberculosis followed by level II. In cases of secondaries, level IV was most commonly involved whereas in cases of lymphomas level V was the most common site. In the present study, non-Hodgkin’s lymphoma to Hodgkin’s lymphoma ratio is 3.8:1. Out of 24 cases of lymphomas, 19 cases (79.1%) were non-Hodgkin’s lymphoma and 5 cases (20.9%) were found to be Hodgkin’s lymphoma. The commonest site of primary in cases of metastatic secondaries was thyroid (4 cases) followed by oral cavity and larynx (3 cases each). In 2 cases primary was in tongue, 1 case each in lung and esophagus and in remaining 1 case, the primary remained unidentified. In case of tubercular lymphadenitis, FNAC had sensitivity, specificity, PPV, NPV and diagnostic accuracy of 95.23%, 98.27%, 97.56%, 96.61% and 97% respectively.

In case of reactive lymphadenitis, FNAC had sensitivity, specificity, PPV, NPV and diagnostic accuracy of 100%, 91.35%, 73.07%, 100% and 93% respectively. In case of lymphomas, FNAC had sensitivity, specificity, PPV, NPV and diagnostic accuracy of 79.16%, 100%, 93.82% and 95% respectively. In case of metastatic secondaries, FNAC had sensitivity, specificity, PPV, NPV and diagnostic accuracy of 93.5%, 100%, 98.83% and 99% respectively. 1 case of metastatic deposit was missed out both in FNAC and histopathological examination which was later confirmed by cytokeratin immunohistochemistry.

The primary could not be established due to limited resources in our set up. In case of Hodgkin’s lymphoma, cytological diagnosis was given in 4 cases in the present study. Typical RS cells were seen in 3 cases and atypical mononuclear cells with prominent nucleoli in the other. 1 case was misdiagnosed as granulomatous lymphadenitis. As Hodgkin’s Lymphoma may have features of prominent granulomatous reaction, we attribute it to be a sampling error and can be minimized by further aspiration and careful examination.

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The overall accuracy of FNAC as compared to histopathological examination was found to be 93%.

**Conclusion**

Fine needle aspiration cytology is a primary diagnostic tool in the evaluation of lymph node lesions, with accuracy comparable to histopathology. At times when there is difficulty in diagnosis on cytology, excision biopsy should be done.

The most frequent causes of cervical lymphadenopathy are tubercular lymphadenitis, reactive lymphadenitis, lymphomas and metastatic malignancies. FNAC alone can help in establishing the diagnosis in large number of cases. FNAC used in conjunction with clinical findings, radiological and laboratory investigations can be a cost effective method. However, in certain situations especially in lymphoproliferative lesions, excisional biopsy is important in establishing correct diagnosis of the nature of the lesion. Cytokeratin staining of dissected lymph nodes is a useful new diagnostic tool for detecting micro metastatic foci in regional lymph nodes of patients with primary malignancy elsewhere. Because the cytokeratin immunohistochemical method enhances the detection rate of occult micrometastasis in cervical lymph nodes of head and neck squamous cell carcinoma patients, it may be recommended for routine diagnostic use in patient with negative for a lymph node metastasis on routine haematoxylin-eosin stain.

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