Fine needle aspiration cytology (FNAC), a handy tool for metastatic lymphadenopathy
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INTRODUCTION
Lymph nodes are common site of metastases for different cancers. Thus clinical recognition and urgent diagnosis of palpable lymphadenopathy is of paramount importance specially to differentiate between inflammatory lesions or metastatic or primary neoplastic tumor.

Although open biopsy with histological examination of excised tissue still remains the golden standard for diagnosis of lymph node tumors, yet FNAC (Fine needle aspiration cytology) has now become an integral part of the initial diagnosis and management of patients presenting with lymphadenopathy. This simple technique has gained wide acceptance since it offers a high degree of accuracy, lending itself to out patients diagnosis and thus reducing the cost of hospitalization. The results of FNAC compare favourably with those of tissue biopsies and in some situations the aspirate has qualities of a microbiopsy. Suspicious or doubtful situations should be resolved by surgical biopsy and further by immunocytochemistry and molecular techniques whenever required. The aim of the present study is to highlight the role of FNAC in diagnosis of metastatic lesions of lymph nodes in a resource challenged environment like ours.

MATERIAL AND METHODS
The present study was conducted on 275 patients having enlarged lymph nodes with a clinical suspicion of primary or secondary malignancy in lymph node, over a period of 2½ years from January 2005 to July 2007. A detailed history, clinical examination and relevant investigations were done. FNAC of the enlarged lymph nodes was performed and smears were stained with Papanicolaou and Haematoxylin-Eosin stain. Lymph node biopsy was performed in selected cases and sections were stained with Haematoxylin-Eosin stain. Special stains and immunohistochemical staining was done, where a diagnosis was deferred. In patients with first clinical presentation as lymphadenopathy, FNAC diagnosis of metastasis was rendered, the primary site was searched for and a biopsy was taken from the primary site. Relevant clinical examination and radiological investigations were subsequently advised to search for the primary site.

OBSERVATIONS
275 cases were studied in which 221 (80.4%) were metastatic tumors of lymph node. Rest 42 (15.3%) cases were lymphoma and 12 (4.4%) cases clinically suspected for malignancy turned out to be reactive lymph nodes and are excluded from our study. Out of these 221 cases, 150 (67.9%) were metastatic squamous cell carcinoma (SCC)
followed by carcinoma breast 25 cases (11.3%) and adenocarcinoma 20 cases (9.0%). The rest included 4 (1.8%) each carcinoma thyroid and small cell carcinoma lung, 3 (1.3%) each of mucoepidermoid carcinoma and round cell tumors, 2 (0.9%) cases of melanoma and 1 case (0.4%) of transitional cell carcinoma (Table 1). There were 9 cases (3.2%) of undifferentiated tumors. The highest number of lymph nodes involved were the cervical group, being 134 (89.3%), and 7 (4.7%) supraclavicular in metastatic SCC alone. Total cervical lymph nodes were 164 (74.2%), followed by axillary group in 21 (9.5%) cases (Table 2). The size of the lymph nodes was >2 cms in majority of the cases (120 cases-80%) of metastatic SCC, whereas in only 30 cases (20%) the size was < 2 cms. In metastatic SCC, the primary site was detected in 65 cases (43.3%), whereas 55 cases (36.6%) presented first as lymph node enlargement and retrospectively the primary tumor was searched for, whereas in metastatic adenocarcinoma, primary tumor was first detected in 10 cases (50%).

Table 1: Distribution of primary site metastatic to lymph nodes

<table>
<thead>
<tr>
<th>Type of malignancy</th>
<th>No. of cases</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>150</td>
<td>67.87</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>20</td>
<td>9.04</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>25</td>
<td>11.31</td>
</tr>
<tr>
<td>Medullary carcinoma (Thyroid)</td>
<td>04</td>
<td>1.80</td>
</tr>
<tr>
<td>Small cell carcinoma (Lung)</td>
<td>04</td>
<td>1.80</td>
</tr>
<tr>
<td>Muco-epidermoid carcinoma</td>
<td>03</td>
<td>1.35</td>
</tr>
<tr>
<td>Round cell tumor</td>
<td>03</td>
<td>1.35</td>
</tr>
<tr>
<td>Melanoma</td>
<td>02</td>
<td>0.90</td>
</tr>
<tr>
<td>Transitional cell carcinoma (Urinary bladder)</td>
<td>01</td>
<td>0.45</td>
</tr>
<tr>
<td>Poorly or undifferentiated carcinoma</td>
<td>09</td>
<td>4.07</td>
</tr>
<tr>
<td>Total</td>
<td>221</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Localized lymphadenopathy was the predominant finding in 150 cases (100%) of SCC and also 20 cases (100%) of adenocarcinoma. In metastatic SCC, 110 cases (73.3%) had single lymph node, discrete group in 100 cases (66.7%) and fixed in 80 cases (53.3%), non-tender being 90 cases (60%). Cystic degeneration or necrosis was found in 40 cases (26.7%) and fungating ulcerating mass in 10 cases (6.7%) of SCC. In contrast 3 cases (15%) showed cystic change and 2 cases (10%) showed fungating mass in adenocarcinoma while 5 cases (20%) showed cystic change and 4 cases (16%) showed fungating ulcerating mass in metastatic carcinoma breast.

FNAC of lymph nodes with metastatic SCC (150 cases) showed well differentiation in 68 cases (45.3%), moderate differentiation in 52 cases (34.7%) and poor differentiation in 27 cases (18%). Smears showed isolated cells or clusters of keratinizing malignant squamous cells with or without evidence of keratin formation; cells had distinct cell borders, hyperchromatic nucleus with coarse chromatin (Fig.1). Eosinophilic keratinized cells were better appreciated by Pap staining. Biopsy showed replacement of lymph node partially or completely by metastatic squamous cells. In 3 cases (2%), necrosis and cystic change was seen in FNAC with no definite malignant cells but some atypical degenerated cells were seen in the background and histopathology showed evidence of metastatic SCC.

FNAC of lymph nodes with adenocarcinoma showed cells usually arranged in cohesive groups of various sizes with lymphocytes in background in most cases. The cell groups were either arranged in ball like clusters, papillary fragment, loose cluster or acini with central lumina. Cells showed eccentric nucleus, mostly with prominent nucleoli and evidence of mucin production in the form of cytoplasmic vacuolation (Fig.2). On histopathology, lymph nodes were seen to be infiltrated by malignant cells forming glandular/acinar structures.

Immuno-histochemistry was helpful in making the diagnosis.
in 2 (0.9%) cases each of melanoma and neuroblastoma respectively, and 4 (1.8%) cases of small cell carcinoma lung. Smears from melanoma showed necrosis, intra and extracellular melanin along with large pleomorphic cells (Fig.3). The presence of fine, granular melanin pigment in the cytoplasm is a helpful identifying feature for melanoma.

Nine (4.1%) cases were poorly differentiated or undifferentiated tumors on FNAC. Three patients had mass in lung, one had a large occipital mass while five had multiple lymphadenopathy. Smears showed large pleomorphic cells with high nucleocyttoplasmic ratio and prominent nucleoli. Binucleate and multinucleate forms were also seen. Biopsy and immunochemistry could not be done in these cases as they were lost to follow up. FNAC in these cases helped in confirming the diagnosis of malignancy.

Cyto-histological correlation was done and the diagnostic accuracy for metastatic lymph nodes was 97.9%, with sensitivity of 97.9% & specificity of 100%.

DISCUSSION

Lymph node aspiration has a very important role in the diagnosis of malignant lymphadenopathies especially in a developing country like ours where the cost of hospital stay and surgical procedures cannot be borne by the patient.5

In the present study overall frequency of malignancy was found to be higher in males (M:F = 2.4: 1). This may be because of decreased incidence of various addictions in females. Similar observations were made by Haque and Talukder6, and Steel et al7.

In our study, 221 cases (80.4%) were metastatic tumors of lymph node while 42 cases (15.3%) were primary tumours i.e. lymphomas, an observation similar to Chhotray and Acharya8, and Frable Wh9. However, Nada Al Alwan et al10 found metastatic involvement of nodes to be less as compared to lymphomas, a first attributed to geographical variation.

The maximum number of aspirations was done from cervical lymph nodes (74.2%). This may be due to the easy accessibility of cervical lymph nodes for examination and evaluation, besides a large number of cases (40%) in our study had metastatic head and neck malignancy, a finding similar to Chhotray and Acharya8, and Frable Wh9, who also found cervical lymph nodes to be most commonly involved. However, Ojo et al10 found axillary nodes to be most common site of involvement followed by cervical lymph nodes.10

Among the metastatic tumors, squamous cell carcinoma was the most common tumor followed by carcinoma breast and adenocarcinoma. Our findings are similar to studies conducted by Chhotray and Acharya8, Frable Wh9 and Pilotti et al4 where squamous cell carcinoma predominated over adenocarcinoma.

In metastatic squamous carcinoma (SCC), head and neck was the most common site of primary. Cancer of mouth/oropharynx is the most frequent cancer in males (16.4%) and third most frequent in females (8.8%) in India.13 Oral cancers account for a large number of malignancies, especially in a country like ours and they often present as cervical lymphadenopathy. Hence, FNAC can play a very important role in early diagnosis and timely intervention in
metastatic cancers, especially in resource challenged environment like ours.

In 28 (18.7%) cases of metastatic SCC and 2 (10%) cases of adenocarcinoma, primary was not found even after thorough evaluation of the patients and most of these patients were lost for follow up. Pilloti et al,\textsuperscript{4} reported that 14 (10.9%) cases out of 128 total cases were suspicious for metastasis but could not be confirmed, with no primary site detectable.

An important clue to the diagnosis of metastatic SCC is the presence of necrosis and keratinization, which is better appreciated on Pap stain than on H & E stain. SCC can be easily confused with a cystic lesion or pilomatrixoma, especially when head and neck region is involved.\textsuperscript{14} In our study there were 3 (1.3%) cases which showed only necrosis and/or cystic change on FNAC but revealed SCC on histology. The cytologic appearance of squamous cell carcinoma depends upon the degree of differentiation by the tumor. Keratinizing cancers are readily identified when cells with abundant sharply demarcated dense eosinophilic cytoplasm and pyknotic nuclei are present in smears. Non keratinizing squamous cell carcinoma are represented by round, oval or polygonal cells with sharply demarcated pale cytoplasm and coarsely granular nuclear chromatin.\textsuperscript{12}

Cytologic features may give clues to site of origin in adenocarcinomas. Gastric carcinomas showed large signet cells with intracytoplasmic mucin while columnar cells with elongated palisading nuclei in a necrotic background suggested a colonic tumor. Many times, immunostaining for keratin 7 and 20 play an important role in determining the origin of adenocarcinomas.\textsuperscript{14}

Small cell carcinomas may be easily confused with small cell lymphoma on FNAC and therefore immunostaining with keratin and leukocyte common antigen (LCA) plays an important role as SCC are positive for keratin and negative for LCA.\textsuperscript{14} 4 cases of small cell carcinoma lung metastatic to cervical and supraclavicular lymph nodes showed neoplastic cells in aggregates and flat sheets with high nucleo-cytoplasmic ratio and nuclear moulding in a background of lymphocytes. This was confirmed by immunohistochemistry. Pilloti et al,\textsuperscript{4} have found similar results.

In the present study mucoepidermoid carcinoma of salivary gland metastatic to cervical lymph nodes was seen in 3 patients, and retrospectively the preimary tumor was discovered in parotid (1 case-0.4%) and minor salivary gland (2 cases-0.9%). Sheahan et al,\textsuperscript{15} also noted that FNAC helped in diagnosis of metastatic mucoepidermoid carcinoma.

Melanomas metastazing to lymph nodes can usually be diagnosed on FNAC by the presence of melanin in malignant cells. However, it can be confused with metastatic SCC when pigmentation is not found.\textsuperscript{16} Pilloti et al detected 16 cases of melanoma metastatic to axillary nodes.\textsuperscript{4}

There were 3 cases (1.3%) of round cell tumour of which 2 (0.9%) were neuroblastomas and one patient was lost for follow up. Neuroblastomas are one of the commonest abdominal malignancies in young children.\textsuperscript{17} FNAC followed by biopsy and immunostaining (synaptophysin positive) confirmed the diagnosis of neuroblastoma. These tumors are usually positive for neuron specific enolase (NSE), synaptophysin, S-100 protein and glial fibrillary acidic protein.\textsuperscript{18}

Out of 221 cases, FNAC was correlated with biopsy in 191(86.4%) cases. Out of these 121 cases (63.3%) were correlated with biopsy/FNAC of the primary site while in 70 cases (36.6%) correlation could be done with biopsy of lymph node alone. 30 cases (13.6%) could not be followed up. Correct correlation was possible in all the cases except 4 patients with enlarged lymph nodes which showed necrosis and cystic change on FNAC, but were found to be metastatic carcinoma when biopsy was performed. Hence there were 4 false negative cases & no false positive case of metastasis in lymph node. Sheahan et al,\textsuperscript{15} in their study of 42 cases found 5 false negative cases which were all cystic nodal metastasis in neck. Steel et al,\textsuperscript{7} commented that degenerated squamous cells in cystic lesions might mimic malignancy. Chottray & Acharya found 3.43% false negative and no false positive case in their study of metastatic lymphadenopathies.\textsuperscript{5}

Khurana et al,\textsuperscript{19} found that exuberant granulomatous response along with necrosis and inflammation as a cause for false negative diagnosis in metastatic SCC. An important diagnostic pitfall for FNAC, especially in a country like ours where tuberculosis is rampant, is seen in differentiating malignancy from tuberculosis. Many a times FNAC may reveal only necrosis as was seen in 3 of our cases which on histology revealed SCC.

Thus the diagnostic accuracy for metastatic lymph nodes was 97.9%, with sensitivity of 97.9% & specificity of 100%. Similar findings were observed by Kim et al who found them to be 97.9%, 97.9%, 99.1% respectively.\textsuperscript{20} Various studies conducted by Lee et al\textsuperscript{2} and Nada al Alwan et al\textsuperscript{9} also had somewhat similar result.
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Poorly differentiated tumors were difficult to interpret on FNAC. Even the biopsy of these tumors was inconclusive. In such cases immunostaining was required to reach a diagnosis. Similar opinion was expressed by Kline et al., in their study of malignant lymphadenopathy.

Partial lymph node involvement is the main cause of false negative report in metastatic carcinoma. Micro-metastases is also unlikely to be sampled even by repeated aspirations. SCC are prone to undergo necrosis. An aspirate from such a cervical node may be mistaken for branchial cyst. Cystic nodes in neck may also represent metastasis of papillary carcinoma of thyroid. Poorly differentiated malignancies, undifferentiated carcinomas make it difficult to differentiate between epithelial tumors, lymphomas, melanomas and sarcomas. Primary site identification for metastatic squamous cell carcinoma, adenocarcinoma and melanoma has high specificity. But the primary site identification for other tumors maybe difficult. Recognition of cells from malignant carcinoid, bronchogenic carcinoma, thyroid carcinoma and endometrial adenocarcinoma is troublesome.

The accuracy of cytology, immunocytochemistry and transmission electron microscopy (TEM) using biopsy results as gold standard in diagnosing tumor category are 78% by cytology and 91% by the latter. But in a resource challenged environment like ours, FNAC still remains the most acceptable, cheap and easily accessible modality for the diagnosis of metastatic lymphadenopathy.

CONCLUSION

FNAC is a logical extension of the more formalized biopsy procedure, lending itself to saving of time and cost, and is convenient for both patient and physician in the management and follow up of malignant lymphadenopathies. FNAC helps in defining the tumor type while clinical history and investigations help in identifying the tumor site.

References

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