Accuracy of Cytological Analysis in Metastatic Lymphadenopathy; a Retrospective Study.

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ABSTRACT

Background: Presently, fine needle aspiration (FNA) biopsy is the preliminary, non-invasive test for diagnosis of suspected lymphadenopathy. Apart from its diagnostic yield, it helps identifying the origin, grading and typing of the metastatic lesions in many cases. **Methods:** In the present study, data of 369 patients with suspected neoplastic lymphadenopathy presenting to the surgical and medical outpatient department of our institute over a period of 3 years was collected. FNA biopsy was performed on the most prominently visible and palpable lymph node. The use of special stains was performed in selected cases. **Results:** Of 369 cases studied, cytological diagnosis was offered in 358 cases, while unsatisfactory smears were reported in 11 cases. Of these 358 cases where a cytological diagnosis of either primary or metastatic lymphadenopathy was given, 244 were males and 114 were females. Metastatic tumors in the lymph nodes were reported in 307 cases and lymphoma in 51 cases. The distribution of lymphadenopathy revealed involvement of cervical group in 222 cases, axillary group in 57 cases, supraclavicular in 28 cases & inguinal in 24 cases. The commonest primary tumor, metastasizing to lymph nodes was squamous cell carcinoma (52.44%). **Conclusion:** In our study, FNA biopsy proved to be a safe and non-expensive technique that provided a high diagnostic accuracy with zero false positive results, confirmed the presence of secondaries where primary tumor was evident and guided the response to medical treatment.

Keywords: Fine needle Aspiration (FNA) Biopsy, metastatic, lypmhadenopathy, lymphoma, cytology.

INTRODUCTION

Metastasis of various aggressive neoplastic lesions to lymph nodes is a known phenomenon. Though, clinical recognition of a neoplastic lesion by detailed history taking and examination is frequently possible by experienced clinicians, true identification of the metastatic or primary nature, morphology, grade and typing requires diagnostic work up. Occasionally, lymphatic metastasis is the first sign of malignancy with early detection, playing a pivot role in management and prognosis.

In developing countries, where tubercular lymphadenitis is commonly reported, a large percentage of cases with persistent mass in neck turn out to be malignant on cytological examination. In contrast, persistent neck masses in paediatric population rarely turn out to be malignant.[1] The primary malignancies of lymph nodes, i.e. the lymphomas usually range from 2% to 15.3% among all aspirates. Data from previous studies report that the incidence of metastatic lymphadenopathy varies from 65.7% to 80.4% in Indian subcontinent region[2].

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Fine Needle Aspiration Cytology (FNAC), now more appropriately called as Fine Needle Aspiration (FNA) Biopsy, has an established role in the initial diagnosis and guiding initial treatment for cases presenting with lymphadenopathy. Although, for more confirmatory details, open biopsy with histopathological and immunochemical examination of suspected lesions still remains the gold standard. Since, this procedure is minimally-invasive, performed on out-patient basis and offers a fairly high degree of accuracy, it has gained wide-spread acceptance. Increasing literature support and newer studies with the elaboration of common pitfalls during analysis have improved the sensitivity and accuracy of FNA in all anatomic sites.[2-4] This studies was undertaken with the aim of reviewing the role of cytological diagnosis in lymph nodes with suspected or unsuspected malignancies, observing common diagnostic pit falls and discussing their remedies.

MATERIALS AND METHODS

After taking approval from institutional review board, the data of a 3 year period from 2010 to 2012 were collected to evaluate the diagnostic efficacy of fine needle aspiration biopsy in metaplastic lymphadenopathies of primary or secondary origin, anywhere in the body. All the patients that underwent FNAB gave written informed consent for the same as per institutional guidelines. The details of history, clinical examination and relevant investigations were noted in all the cases as per available records. In all cases, FNA of suspected lymph nodes was performed using 22-24 gauge disposable needles attached to a 10 ml syringe. The smears were stained with Papanicolaou, Leishman, Hematoxylin and Eosin stains. Where-ever tissue samples were available

sections were stained and processed with Hematoxylin and Eosin stain. Cyto-morphological features like cell population, cellular pattern, individual cell morphology, etc. were assessed under low and high power microscopy. Special stains like Periodic acid Schiff (PAS) and immunological stains were done where ever necessary. In cases where a cytological diagnosis of metastatic lesion was offered, an excision biopsy and radiological investigations were advised in search of primary site. In cases with inadequate cellularity or where necrotic material or pus fluid was aspirated an ultrasound/ CT guided FNA was advised. In cases where a tissue section was made available, histo-cytological correlation was done for re-evaluation and comparison of techniques.

RESULTS

During the 3 year period, FNA was performed in a total of 369 cases with clinical suspicion of malignancies in the lymph node, of which the diagnosis was rendered in 358 cases. The size of lymph nodes ranged from 0.5-5 cm in diameter in most of the cases, while the average size was between 1-2.5 cm. Unsatisfactory smears were reported in 11 cases as repetition of the procedure also failed to deliver satisfactory material in these cases. The cause for unsatisfactory smears in such cases was either very scant cellularity or poor quality of smears due to drying artefact or inadvertent mixing with blood. Of the 358 cases reported with neoplastic lymphadenopathy, 307 cases were identified as metastatic tumors in lymph node and 51 cases were of lymphoma.

In the given 358 cases, the gender distribution revealed male preponderance with 244 cases males and 114 cases females. The maximal number of lymph nodes involved, were of cervical group with 222 cases, followed by axillary group in 57 cases [Table 1].

Table 1: Involvement of various group of lymph nodes.

Age Group	**No. of Cases**
0-15	3
16-30	34
31-45	50
46-60	113
>60	158
Total	358
Group of lymph node	**No. of cases (%)**
Cervical	222 (72.3)
Axillary	57 (18.5)
Supraclavicular	28 (9.1)
Inguinal	24 (7.8)
Mediastinal	18 (5.8)
Mesenteric	9 (2.9)
Total	**358**

Out of the 307 cases of metastatic lesions, 161 cases were squamous cell type, 47 cases were invasive ductal and 30 cases were adenocarcinoma [Table 2].

Table 2: Distribution of cases according to the type of malignant lesion.

Type of carcinoma	**No. of cases (%)**
Squamous cell carcinoma	161 (52.44)
Breast carcinoma (Invasive Ductal Ca)	47 (15.3)
Adenocarcinoma	30 (9.7)
Poorly differentiated carcinoma	24 (7.8)
Mucoepidermoid carcinoma	12 (3.9)
Small cell carcinoma	9 (2.9)
Large cell carcinoma	8 (2.6)
Endocrinal type	7 (2.2)
Thyroid carcinoma	4 (1.3)
Small round cell tumor	2 (0.6)
Adnexal	1 (0.3)
Nasopharyngeal carcinoma	1 (0.3)
Melanoma	1(0.3)
Total	**307**

Tumors metastasizing to the lymph node most commonly originated in the head and neck region followed by breast & respiratory system.

The commonest variety of metastatic carcinoma was a squamous cell type, with primary site detectable in 121 cases. The common primary sites involved were tongue, alveolus, buccal mucosa and larynx in 107 cases. Other sites included lung, oesophagus, cervix, skin and penis. The smears constituted either isolated or clusters of malignant squamous cells with or without keratin formation. These malignant cells had well defined cell borders and hyperchromatic nuclei with coarse chromatin [Figure 1]. Eosinophilic keratinized cells were better appreciated with Papaniculaou stain. In 19 cases, necrosis and cystic change were observed with few degenerated and viable malignant cells in the background. In 6 cases only necrotic material was observed, so false negative diagnosis was given. These, however, proved to be metastatic lymph nodes when were subjected to histopathological examination.

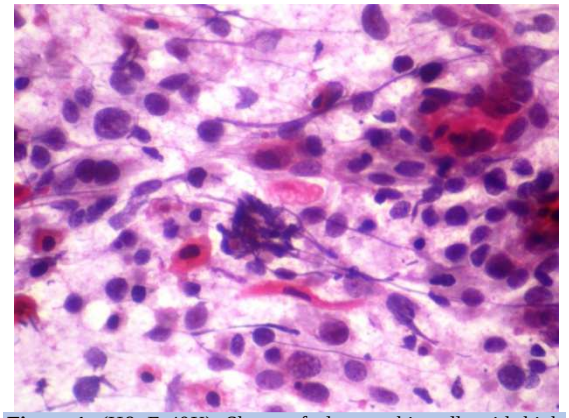

Figure 1: (H& E 40X): Sheets of pleomorphic cells with high N:C ratio with some cells revealing keratinization.

Metastasis of adenocarcinoma was observed in 30 cases, with lung (10 cases) being the commonest primary site followed by GIT, gall bladder and ovary. Primary site could not be found out in 11 cases. Cytological smears showed cells usually arranged in cohesive groups, ball like clusters, papillae and acini. The cells had eccentric nuclei and prominent nucleoli with mucin production as evidenced by cytoplasmic vacuolation [Figure 2].

In the metastatic lesions from lung, apart from squamous and adenocarcinoma, 9 & 8 cases were labelled to be of small cell and large cell types, respectively. Sub-typing of metastatic carcinoma cells was not achieved in 24 cases due to lack of differentiating features, so these cases were labelled into poorly differentiated categories. Cytokeratin immunostaining was done in some cases where morphology was not clear cut evidence for epithelial origin of the neoplasm.

Smears showed large pleomorphic cells with high nucleo-cytoplasmic ratio and prominent nucleoli. Bi- and multinucleate forms were also seen. Fourteen of these cases had a mass in lung and FNA from these masses showed similar cytological features. In the remaining 10 patients, primary site could not be ascertained as these patients were lost to follow up.

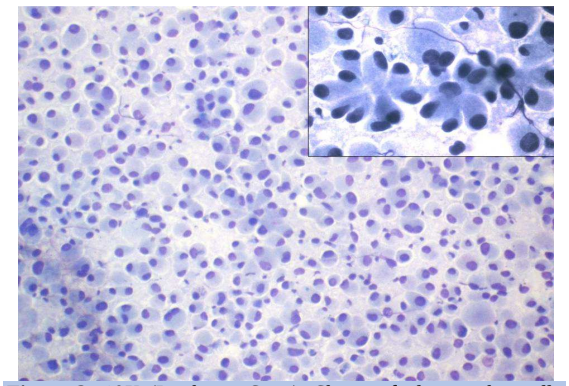

Figure 2: 10X (Leishman Stain): Sheets of pleomorphic cells having eccentric nuclei and abundant finely vacuolated cytoplasm. (Insat 40X) showing pleomorphic cells forming vague glandular pattern.

Metastasis from thyroid was reported in 4 cases. This comprised of metastasis from papillary carcinoma in 3 cases and medullary carcinoma in one case. Smears in papillary carcinoma comprised of follicular cell arranged in the papillae. Cells exhibit optical clearing of nuclear chromatin, nuclear grooving and intranuclear cytoplasmic pseudoinclusion [Figure 3]. Spread of primary salivary gland tumor was suspected in 12 cases with features suggestive of mucoepidermoid carcinoma. These cases were later on confirmed on histopathological examination.

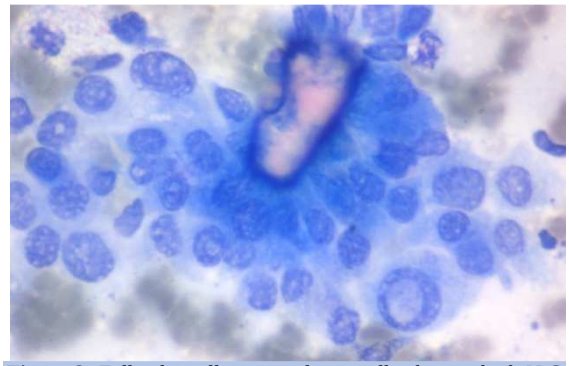

Figure 3: Follicular cells arranged in papillae having high N:C ratio and clearing of nuclear chromatin. One cell exhibit intranuclear cytoplasmic pseudoinclusion.

In addition to the common tumors metastasizing to lymph nodes, there were certain unusual tumors that posed difficulty in diagnosis. These included two cases of small round cell tumor [Figure 4], of which one was confirmed to be neuroblastoma and other nephroblastoma on histopathology. One case of metastatic malignant melanoma was also encountered in axillary lymph node [Figure 5].

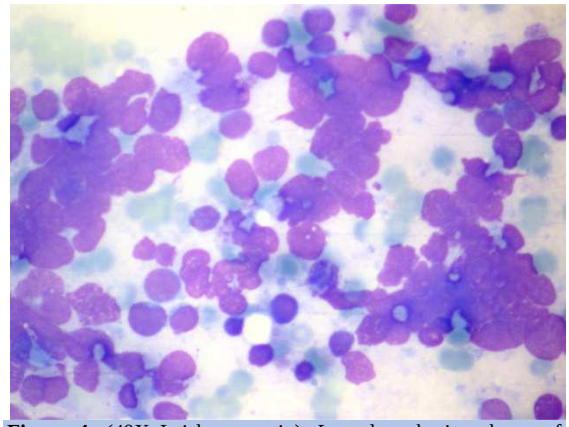

Figure 4: (40X Leishman stain): Loosely cohesive sheets of uniform round cells with high N:C ratio, opened up chromatin and scanty cytoplasm.

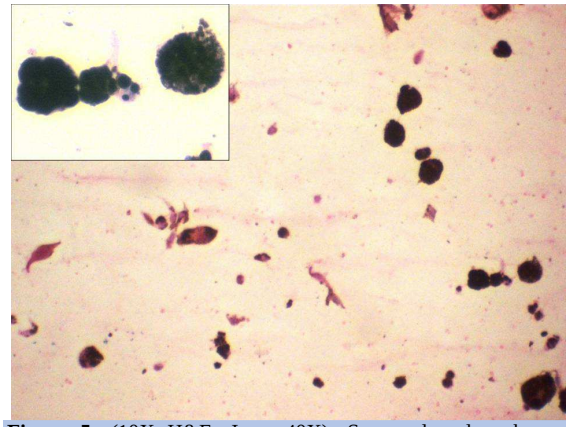

Figure 5: (10X H&E; Insat 40X): Scattered melanophages exhibiting black melanin intracytoplasmic pigment.

Of the 51 cases of primary tumors of lymph node, 39 cases were of Non-Hodgkin's lymphoma and 12 cases of Hodgkin's lymphoma, which were confirmed on lymph node biopsy.

In cases where a history of primary malignancy with cytological evidence of lymph node metastasis was available, excision biopsy was deferred.

Cyto-histopathological correlation was done in 88 cases and the diagnostic accuracy for metastatic lymph node was 93.18% with sensitivity of 93.18% and specificity of 100%. In histologically confirmed cases, 51 were of lymphoma and 37 cases turned out to be metastatic.

DISCUSSION

In present study, the neoplastic involvement of lymph nodes was predominantly due to metastatic lesions (85.75% cases) while rest cases (14.25%) were attributed to a primary involvement i.e. lymphomas. Similar observations were made by Kiran *et al*[1], Chhotray *et al*[5]and Frable *et al*[6]. However, Alwan *et al*[7]reported the metastatic involvement of nodes to be less as compared to lymphomas in his studies, a finding which can be attributed to geographical variation.

Another observation in our study was a higher incidence of malignancy in males (M: F= 2.14:1). It is in concordance with findings reported by other researchers of the South-East Asian zone like Mehrotra *et al*[8] (3.8:1) & Bhattarcharjee *et al*[9] (2.9:1), but different from that reported from western world (Engzell *et al*[10]1.07:1 and Ustun *et al*[11]1:2).

We performed maximum number of aspiration from cervical nodes (62.01%), which can be attributed to a large number of cases having head and neck malignancy, along with ease of accessibility for examination and procedural performance.

We could identify the primary site of malignancy in approximately 93% cases of metastatic lesions with the help of clinical data and FNA in our study. With the support of clinical history and investigations, FN Aproved to be helpful in defining the tumor type in most of the cases. Facundo *et al*,[12] were able to find primary in 59% cases, while the efficiency increased to 95% in combination with immunochemistry. These findings again establish the role of FNA in the identification of occult primary sites and typing of tumor cells.

In the present study, metastasis of squamous cell carcinoma to the lymph nodes was the most frequently observed lesion, i.e. in 161 of 307 aspirates (52.44%), while head and neck were the commonest site of primary tumor. The data is similar to the results obtained by the Kiran Alam *et al*[1], Bagwan *et al*[2], Ustun *et al*[11], but contrary to that reported by Chih Hsu *et al*[13] as his study was carried out in China, where nasopharyngeal carcinoma is endemic. In differentiated squamous carcinomas, cells may be arranged in tight clusters or remain as loosely scattered single cells. These cells have hyperchromatic irregular nuclei with varying degrees of keratinization.[14] Rarely, the bronchial or epidermal cysts with mature squamous cells may resemble a differentiated metastatic carcinoma.

Cytological evaluation can also give clue to the site of origin of adenocarcinoma. Gastric carcinomas can be identified by the presence of large signet cells with intra-cytoplasmic meanwhile colonic tumours show columnar cells with typically elongated palisading nuclei with necrosis in the background.[15,16]

The presence of metastatic undifferentiated carcinomatous cells in lymph nodes raises the likely possibility of primary tumor to be from nasopharynx or lung.[17] In nasopharyngeal carcinoma cells appear large with a primitive looking, round vesicular nuclei, prominent nucleoli and an ill-defined cytoplasm. Cytological features in small cell carcinoma of lung reveal naked cells with scanty cytoplasm, ovoid nuclei, indistinct nucleoli and salt and pepper type chromatin.

Owing to availability of tissue specimen, histo-cytological correlation could be performed in 88 cases. Cytological finding correlated well with histological ones in 82 cases except in 6 patients where necrosis and cystic changes were reported on FNA. Here, the lymph nodes had undergone enlargement following necrosis around the metastatic invasion, as was evident on histological examination. This led to aspiration of superficially encasing cheesy and necrotic material, thus leading to erroneous cytological diagnosis. Henceforth, our analysis revealed 6 false negative cases with false positive cases. In a study of 42 cases by Sheahan *et al*[18], 5 false negative cases were reported which all were cystic nodal metastasis involving cervical lymph nodes. Similarly, Chottray & Acharya[5] reported 3.43% false negative and zero false

positive cases which strongly points towards the efficacy of cytological analysis in ruling out false positive errors. Sometimes, degenerated squamous cells in cystic lesions mimic malignant lesions due to irregular atypical cellular appearance[16]. An exaggerated granulomatous response to metastatic cells incites inflammation and necrosis leading to false negative results in evaluating squamous cell carcinoma[17]. Another important diagnostic pitfall of FNA is seen in differentiating malignancy from tuberculosis[17].

In our study, cytological analysis of metastatic lymphadenopathy revealed a diagnostic accuracy of 93.18%, sensitivity of 93.18% & specificity of 100%. Similar findings were observed by Kim *et* al[4] who found these variables to be 97.9%, 97.9% and 99.1% respectively. Studies conducted by Lee *et al*[19] and Alwan *et al*[7] also had somewhat similar result. In our previous study in paediatric cases, the positive predictive value of FNA in diagnosing malignancy was 100%, though, here, the sample size of malignant lesions was limited[20].

Regional metastasis is one of the most important factors in the prognosis and treatment of patients with malignancy. Hypocellularity or inadequate sampling on FNA biopsy is commonly dealt with the aid of imaging techniques like CT, MRI and sonography. They help in localizing the pathological areas, identify solid areas in a cystic swelling, but differentiating between benign, malignant or metastatic nature of a lesion on imaging basis is a limitation[15]. Recently, newer techniques such as radio-immunoscintigraphy or positron emission tomography have been explored for identifying and staging malignant lesions, but these expensive techniques still have to prove their value in clinical practice.

CONCLUSION

FNAB is of considerable value in the documentation of metastasis disease and staging in known primary and occult tumors. It has high diagnostic accuracy with bare minimal false positive results while diagnosing a suspected malignant lymphadenopathy.

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