Original article:

Cytologic evaluation of lymphadenopathy in a tertiary care hospital of central India

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Abstract:

Introduction - With the increasing costs of medical facilities, any technique which speeds up the process of diagnosis, limits the physical and the psychological trauma to the patient and saves the expenditure of hospitalization, is of tremendous value. Fine needle aspiration cytology (FNAC) reduces the necessity to perform excision biopsy in many cases, thus saving patients from surgical complications. FNAC of lymph nodes also helps the surgeon to select, guide and modify the treatment plan in patients who require surgeries. To study the different cytomorphological patterns associated with various lymphadenopathies in a tertiary referral hospital of central India.

Method- A cohort study was conducted over a five year period from January 2011 to December 2015. FNAC of 480 patients with lymphadenopathy was performed after informed consent. Smears were prepared, stained and studied.

Results- The age of the patients ranged from 2 yrs to 90 yrs with a male to female ratio of 1:1.1. Maximum incidence of lymphadenopathy was seen in third decade of life. Cervical region was reported as most frequent site of lymphadenopathy. Reactive hyperplasia of lymph node and tuberculous lymphadenitis were the common benign lesions observed and squamous cell carcinoma was the most common metastatic lesion in our study.

Conclusion- FNAC is safe, simple and inexpensive definite diagnostic procedure to render a prompt diagnosis in cases of reactive, infective & neoplastic lymphadenopathies and lymph node biopsy is required only in inconclusive cases or sometimes in the cases of malignancy. Patients with reactive cytological changes and who clinically also appear benign, can avoid unnecessary surgery. FNAC can also pick up unsuspected malignancies.

Key words: FNAC, Lymphadenopathy, Lymph node

Introduction:

The incidence of lymphadenopathy appears to be increasing all over the world. Lymphadenopathy is defined as an abnormal increase in size of lymph node of any site with measurement more than 1 cm. in diameter and/or altered consistency. Lymph nodes are frequently enlarged in various regional and systemic diseases and sometimes may be the only clinical finding or one of the several nonspecific findings. Also due to their easy accessibility make them among the most commonly aspirated organs for diagnostic purposes. Inflammatory and immune reactions are the most frequent causes of lymphadenopathy and are self limiting in majority of

cases. Lymphoid tissue undergoes reactive changes to a wide variety of antigenic stimuli. Lymph nodes are also affected commonly by tuberculosis, lymphomas and by metastasis of malignant neoplasm from regional and distant organs.

Although fine needle aspiration (FNA) of lymph nodes for diagnostic purpose was first done by Griey and Gray in 1904 in cases of sleeping sickness,[1] the technique developed gradually, until 1921, when Guthrie tried to correlate FNA results with various disease processes.[2] Since then the journey of FNAC has come a long way in conjunction with sophisticated imaging guided procedures to become an important diagnostic tool. Nevertheless, the few cells that are obtained from the lymph node aspiration are often sufficient to make the diagnosis, and biopsies are usually required only in inconclusive cases or in lymphomas.

Material and Methods:

A hospital based descriptive observational study was conducted among patients with various lymphadenopathies over a period of five years from January 2011 to December 2015. All the admitted as well as out-patients who have lymphadenopathy and are referred by the clinical departments of our hospital to Department of Surgical pathology for FNAC were included in the study. Exclusion criteria were- 1) Known and already diagnosed cases of lymphadenopathy. 2) All patients referred for FNAC and having swelling other than related to lymph nodes. Prior to Fine Needle Aspiration (FNA), epidemiological data, history, clinical findings and provisional clinical diagnosis were noted in a pretested proforma. Written informed consent was obtained from all the patients. FNA was performed with the help of a 23 gauge disposable needle attached to a 10cc syringe. Guidance of USG/CT was taken in cases of deep seated lymph nodes. Specimen so obtained was expelled on 3-4 glass slides according to the amount of material and smears were prepared, wet fixed in 95% alcohol or air dried followed by Papanicolaou (PAP) and Giemsa stain respectively. Gram's and/or Ziehl-Neelsen's (Z-N) stain were used wherever indicated. The slides were mounted and studied under microscope. Lesions were categorized based on cytology. Various epidemiological factors were studied and data was analyzed.

Results:

During a period of 5 years FNA from enlarged lymph nodes was done in 480 patients**.** The age of the patients ranged from 2 yrs to 90 yrs with a male to female ratio of 1:1.1. Maximum number of cases, i.e., 24.58% (n=118) were reported in third decade of life whereas second highest number of cases (16.46%, n=79) were seen in second decade. The least number of cases (0.42%, n=2) were reported in ninth decade of life [Table 1].

Cervical region (79.37%, n=380) was reported as most frequent site of lymphadenopathy in the present study followed by inguinal (7.08%, n=34) and than axillary (5%, n=24). Multiple sites were involved in 4.17% (n=20) of cases in our study [Table 2]. The size of the lymph nodes was found to be less than or equal to 2 cm. in 84.59% of the benign cases and more than 2 cm. in 70.21% of malignant cases. Smears in 3.3% (n=16) cases were unsatisfactory due to insufficient material aspirated whereas cytologic diagnosis could be offered in 96.7% (n=264) cases. No complications were observed in any patient following fine needle aspiration and all patients tolerated the procedure very well.

The benign lesions seen in our study were tuberculous lymphadenitis (41.81%, n=194), reactive hyperplasia of lymph node (32.11%, n=149), acute suppurative lymphadenitis (2..59%, n=12), chronic non-specific lymphadenitis (1.94%, n=9) and histiocytic necrotizing lymphadenitis/ Kikuchi-Fujimoto disease (0.66%, n=3) [Figure 1A,

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1B, 1C and 2A, 2B]. One case of histoplasmosis was reported in which PAS positive spores of Histoplasma were seen in the cytoplasm of macrophages as well as extracellularly [Figure 1D]. We have also reported one case each of lepromatous lymphadenitis and cystic lymph node. On the other hand malignant lesions reported in the present study comprised of Hodgkin's Lymphoma (1.08%, n=5), Non-Hodgkin's lymphoma (2.8%, n=13), granulocytic sarcoma (0.21%, n=1) and Metastatic lymphadenopathy (16.16%, n=75) [Table 3, Figure 2C, 2D and 3A to 3D]. Males show predominance in most of the benign and malignant lesions except among cases of reactive hyperplasia of lymph nodes where almost equal incidence was noted in both genders.

Out of 16.16% (n=75) cases of metastatic deposits, 45.33% (n=34) were of metastatic squamous cell carcinoma, 30.66% (n=24) metastatic adenocarcinoma, 14.66% (n=11) metastatic poorly differentiated carcinoma, 2.66% (n=2) metastatic neuroblastoma and 1.33% (n=1) each of metastatic papillary carcinoma, metastatic melanoma and metastatic small cell carcinoma. In 2.66% (n=2) cases, a diagnosis of metastatic undifferentiated malignant tumor was made as the cytological features were not indicative of a specific tumor type [Table 4].

Discussion:

In the present study, a slight female preponderance was noted with a Male : Female ratio of 1:1.1 which correlated with the studies of Narang RK *et al*,[3] Nidhi *et al*[4] and Ageep *et al*[5] who reported M:F ratio of 1:1.2. In few other studies like those of Bharadwaj K *et al*[6], females outnumbered males. In contrast Hirachand S *et al*[7] [1.1:1], Gupta R *et al*[8] [1.2:1], Chawla N *et al*[9] [1.3:1], Chawla N *et al*[10] [1.44:1], Quadri SK *et al*[11] [1.5:1] and Bargotra R[12] [1.59:1] have reported male preponderance in their studies.

Lymphadenopathy can be found in patients ranging from an early to advanced age. This was correlated with our findings as we found youngest patient of 2 years and oldest patient of 90 years in our study. The peak incidence of benign lesions was in the 3rd decade while the peak incidence of malignant lesions was in the 5th decade. These findings correlated with that of Ahmad SS *et al*,[13] Sarda AK[14] *et al* .

The most frequent site of lymphadenopathy in our study was cervical region [79.37%], followed by inguinal and than axillary. Quadri SK *et al*[11] [76%], Mohanty R *et al*[16] [66.48%]; Ahmad *et al*[13] [73.5%], Hirachand S *et al*[7] [50.76%] and Nidhi P *et al*[4] [90%] have also reported cervical region as the most common site of involvement in their studies. Multiple sites were involved in 4.17% of cases in our study. The size of the lymph nodes was found to be less than or equal to 2 cm. in 84.59% of the benign cases and more than 2 cm. in 70.21% of malignant cases. Hafez NH[15] *et al*. have reported less than 2 cm. sized lymph nodes in 87.5% of the benign cases and more than 2 cm sized lymph nodes in 78.9% of malignant cases.

In the present study benign causes (Inflammatory and reactive hyperplasia) contributed to 79.74% of total 464 cases while malignant ones (primaries and secondaries) constituted only 20.26%. Many studies from India and other developing countries show similar trend. Ahmed SS et al[13] have reported 86.4% benign lesions, Gupta R *et al*[8] have reported 88.11% benign lesions and Bargotra R[12] has reported 78.6% of benign lesions amongst various lymphadenopathies.. In a study from Nepal, Hirachand S *et al*[7] have reported 81.5% of benign and 18.5% of malignant lesions. Studies from Pakistan (Khan *et al*[17] and Fatima *et al*[18]) also showed 92% and 73.2% of benign cases respectively. Whereas the studies carried out in western countries show predominance of malignant

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lesions over benign lesions. Steel *et al*[19] have reported 59% of malignant lesions and 34% of benign lesions in a similar study. Hafez NH *et al*[15] have reported 69.4% malignant lesions and 30.6% benign lesions in a study from Eastern country. Few studies from India also showed higher incidence of malignant lesions the reason may be that most of such studies were conducted in referral cancer hospitals.

Percentage of unsatisfactory smears due to insufficient aspirated material as reported by various previous authors ranged from 3.33%[9] to 15.2%.[20] We reported 3.33% (n=16) of smears as unsatisfactory in our study, which is in the acceptable range of less than 10-15%.[21] The unsatisfactory smears were mainly from the lymph node swellings smaller than 1 cm. in diameter.

Tuberculosis was the most common (41.81%) benign cause of lymphadenopathy in our study. The high incidence of tuberculous lymphadenitis may be because our institute caters to patients belonging mostly to the lower socio-economic group. Biswas G *et al*[22] (45.4%), Nidhi P *et al* (55%%) and Bhatt *et al*[23] (51.9%) have also observed tuberculous lymphadenitis as the most common benign lesion in their studies. Surase Sanjay *et al*[24] have also reported high incidence (62.89%) of tuberculous lymphadenitis in a large study. We have also used similar diagnostic cytologic criteria for tuberculous lymphadenitis as used by Surase Sanjay *et al*[24] viz. presence of characteristic epithelioid cell clusters with or without Langhan's giant cells with or without necrotic material. Z-N staining of all such purulent and cheesy aspirates which were suspicious of tuberculosis but did not showed epithelioid granuloma or Langhan's giant cells, was done to confirm the diagnosis. Although granulomas in lymph node are seen in response to a wide variety of infectious causes and non-infectious causes (benign as well as malignant), tuberculosis is so common in low-income countries like India that every clinically relevant case of granulomatous lymphadenitis should be considered as tuberculous lymphadenitis, unless proved otherwise.

Reactive hyperplasia of lymph node may be due to any infectious (including tuberculous) or malignant lesion at any other site. In our study, although reactive hyperplasia was the most common cause of lymphadenopathy in females, overall it was second most common cause after tuberculosis. However many previous authors like Bargotra R[12], Mohanty R *et al*[16] (34.36%), Chawla N *et al*[9] (41.7%) and Gupta R *et al*[8] (41.6%) have reported reactive hyperplasia as most common cause of lympadenopathy in their studies. The present study also comprised 1.94% of cases diagnosed as chronic non-specific lymphadenitis on cytology. Mohanty R et al[16] and Haque and Talukdar[25] have reported incidence of chronic non-specific lymphadenitis to be 2.8% and 2.63% respectively in their studies. We have reported three cases of Kikuchi-Fujimoto disease on cytology. Such cases should be differentiated from lymphomas.

Malignancies in lymph nodes in our country are predominantly metastatic in nature with an incidence varying from 65.7%[26] to 90%[27] of malignant lymph node lesions, while lymphomas range from 2%[28] to 18%[16]. In our study, metastatic lymphadenopathy was reported in 79.78% while lymphomas in 19.14% of total 94 malignant lesions. Out of 18 cases of lymphomas, Non Hodgkin's lymphoma was reported in 72.22% (n=13) and Hodgkin's lymphoma in 27.78% cases (n=5). This is in accordance with the study of Gupta R et al[8] who have reported Non Hodgkin's lymphoma in 61.5% and Hodgkin's lymphoma in 38.5% cases. Chawla N et al[9] have also reported Hodgkin's lymphoma in 33.3% cases of total lymphoma cases. Although false negative rate of FNAC is higher in cases of lymphomas.[29,30] One rare case of monocytoid type of

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granulocytic sarcoma or myeloid sarcoma was also reported in our study. Smears in this case showed dispersed population of monocytoid cells with erythroblasts without presence of lymphoglandular bodies. The cytochemical stains showed strong NSE positivity and MPO negativity, giving way to the final diagnosis.[31]

Amongst the metastatic tumors, squamous cell carcinoma was the most common followed by adenocarcinoma in our study. However in females, Metastatic adenocarcinoma was commoner than metastatic squamous cell carcinoma. Hirachand S *et al*[7] and Mohanty R *et al*[16] have also reported squamous cell carcinoma as the most common metastatic lesion in various lymphadenopathies. Similarly Babu GS *et al*,[32] Gupta R *et al*[8] and Hafez NH *et al*[15] have also reported squamous cell carcinoma as most common metastasis followed by adenocarcinoma amongst various lymphadenopathies. Two cases of metastatic neuroblastoma in our study have multiple metastatic foci including involvement of bone marrow and orbital bone at the time of presentation. We had one unsuspected case of metastatic melanoma in inguinal lymph node from an undiagnosed primary in the leg. We could also diagnose a case of metastatic papillary carcinoma and could suggest primary in the thyroid.

Conclusion:

Despite its limitations and pitfalls, FNAC is a good first line screening method for evaluating the cases of lymphadenopathies even in a tertiary care hospital, avoiding the need of biopsy in many cases. The optimum success of the procedure requires interdisciplinary approach and communication among the clinician, radiologist and pathologist. FNAC helps in categorizing the cause of lymph node enlargement as reactive change, infective/inflammatory, lymphoma, metastatic etc. FNAC not only pick up unsuspected metastatic deposits but in most conditions give a clue regarding site of primary. In patients with known histologically proven malignancy at primary site, a cytological diagnosis of metastasis helps in avoiding unwanted surgery for confirming metastasis.

Table 1. Age and Gender-wise distribution of various lymphadenopathy cases

S.No	Age group	No. of Cases			
		Male	Female	Total	Percentage
1	0-10	33	23	56	11.67
2	11-20	36	43	79	16.46
3	21-30	40	78	118	24.58
4	31-40	35	39	74	15.42
5	41-50	24	33	57	11.87
6	51-60	31	14	45	9.37

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7	61-70	19	15	34	7.08
8	71-80	09	06	15	3.12
9	81-90	02	00	02	0.42
Total		229 (47.7%)	251 (52.3%)	480 (100%)	

Table 2. Distribution of various lymphadenopathy cases according to site of involvement.

S.No	Site of Lymphadenopathy	Male n(%)	Female n(%)	Total Cases	Percentage
1	Cervical LN	171 (75%)	209 (82.94%)	380	79.2
2	Axillary LN	12 (5.26%)	12 (4.76%)	24	5.0
3	Inguinal LN	21 (9.2%)	13 (5.16%)	34	7.0
4	Abdominal/Retroperitoneal LN	5 (2.2%)	1 (0.4%)	6	1.3
5	Occipital LN	1 (0.44%)	2 (0.8%)	3	0.6
6	Preauricular LN	0	2 (0.8%)	2	0.4
7	Mediastinal LN	0	1 (0.4%)	1	0.2
8	Parasternal LN	1 (0.44%)	1 (0.4%)	2	0.4
9	Epitrochlear LN	1 (0.44%)	1 (0.4%)	2	0.4
10	Post auricular LN	0	1 (0.4%)	1	0.2
11	Infraclavicular LN	0	1 (0.4%)	1	0.2
12	Paragastric LN	0	1 (0.4%)	1	0.2
13	Femoral LN	1 (0.44%)	0	1	0.2
14	Cheek / Angle of Mouth	2 (0.88%)	0	2	0.4
15	Multiple sites	13 (5.7%)	7 (2.78%)	20	4.2
	Total-	228	252	480	100

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Table 3. Various benign and malignant lesions diagnosed on fine needle aspiration of lymph nodes.

S. No.	Cytological Diagnosis	Male n(%)	Female n(%)	Total Cases	Percentage
1	Reactive Hyperplasia- LN	74 (26.24%)	75 (41.2%)	149	32.11
2	Tuberculous lymphadenitis	128 (45.39%)	66 (36.26%)	194	41.81
3	Acute suppurative lymphadenitis	7 (2.48%)	5 (2.74%)	12	2.59
4	Chronic non specific lymphadenitis	6 (2.12%)	3 (1.64%)	9	1.94
5	Histiocytic necrotizing lymphadenitis/ Kikuchi-Fujimoto disease	0	3 (1.64%)	3	0.66
6	Lepromatous lymphadenitis	0	1 (0.54%)	1	0.21
7	LN involvement by Histoplasma	1 (0.35%)	0	1	0.21
8	Cystic lymph node	0	1 (0.54%)	1	0.21
9	Granulocytic Sarcoma	1 (0.35%)	0	1	0.21
10	Non-Hodgkin's lymphoma	11 (3.90%)	2 (1.1%)	13	2.8
11	Hodgkin's lymphoma	3 (1.06%)	2 (1.1%)	5	1.08
12	Metastasis	51 (18.08%)	24 (13.18%)	75	16.16
	Total-	282	182	464*	100

*Note- 16 cases are excluded due to unsatisfactory smears.

Table 4. Various metastatic lesions diagnosed on fine needle aspiration of lymph nodes.

S. No.	Cytological Diagnosis	Male n(%)	Female n(%)	Total cases	Percentage
1.	Squamous cell carcinoma	26 (50.98%)	8 (33.33%)	34	45.33%
2.	Adenocarcinoma	14 (27.45%)	9 (37.5%)	23	30.66%
3.	Poorly differentiated carcinoma	6 (11.64%)	5 (20.83%)	11	14.66%
4.	Small cell carcinoma	1 (1.96%)	0	1	1.33%
5.	Papillary thyroid carcinoma	0	1 (4.17%)	1	1.33%
6.	Malignant Melanoma	1 (1.96%)	0	1	1.33%
7.	Neuroblastoma	2 (3.92%)	0	2	2.66%
8.	Undifferentiated tumor	1 (1.96%)	1 (4.17%)	2	2.66%
	Total-	51	24	75	100

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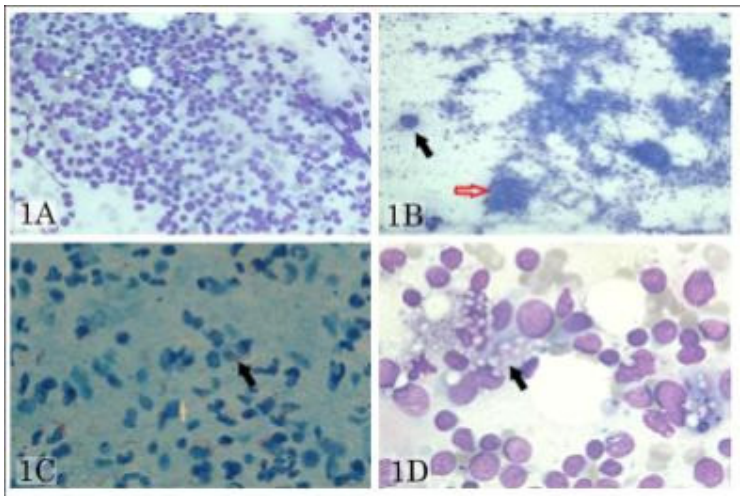

Figure 1A: Polymorphic population of lymphoid cells in reactive hyperplasia of lymph node (Giemsa, 400X).

Figure 1B : Epithelioid cell granulomas (red arrow) and giant cell (black arrow) in tuberculous lymphadenitis (Giemsa, 100X).

Figure 1C: Acid fast beaded bacilli (arrow) in tuberculous lymphadenitis (Ziehl-Neelsen's stain, 1000X).

Figure 1D: Spores of Histoplasma (arrow) in the cytoplasm of macrophages and extracellularly (Giemsa, 1000X).

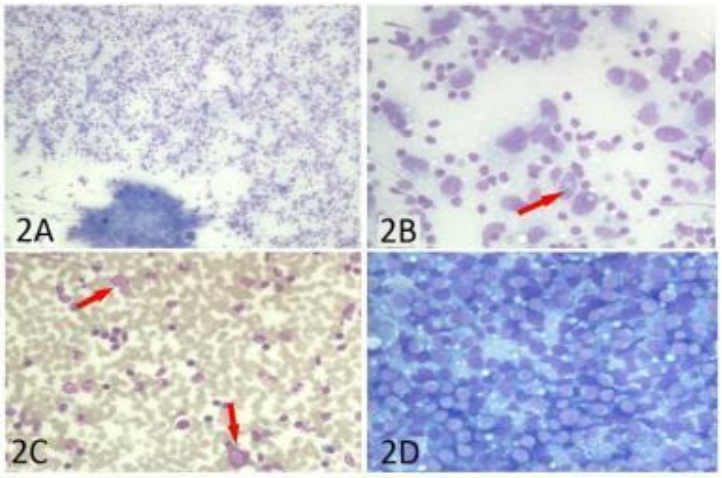

Figure 2A: Hypercellular smear showing crescentic and plasmacytoid histiocytes with mixed population of lymphoid cells in Kikuchi- Fujimoto disease (Giemsa, 100X).

Figure 2B: Smear of Kikuchi- Fujimoto disease showing crescentic histiocytes with punched out nucleoli (arrow) along with mixed population of lymphoid cells and karyorrhectic debris in the background (Giemsa, 400X).

Figure 2C: Reid-sternberg cells (arrow) in Hodgkin's lymphoma (Giemsa, 400X).

Figure 2D: Dispersed monomorphic population of large lymphoid cells in Non-Hodgkin's lymphoma (Giemsa, 400X).

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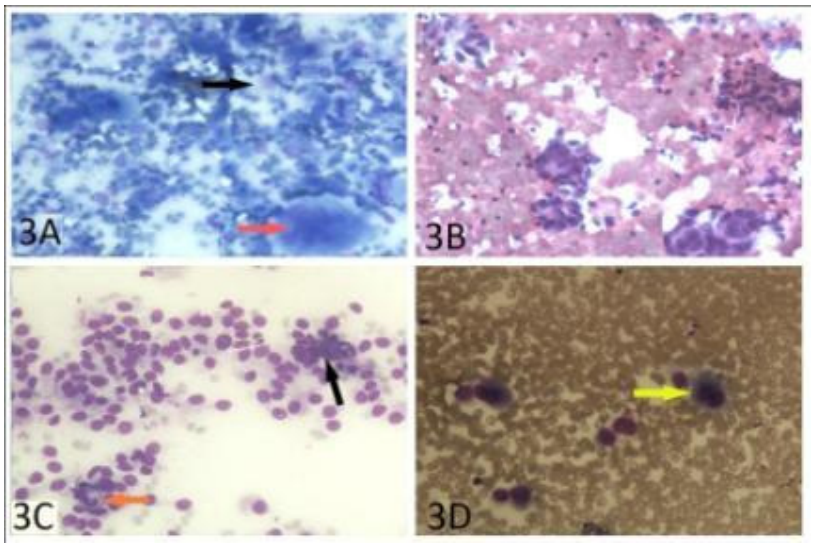

Figure 3A: Giant cell (red arrow) and atypical squamous cells (black arrow) in a case of metastatic squamous cell carcinoma (Giemsa, 400X).

Figure 3B: Atypical epithelial cells forming acini over a background of lymphocytes and blood in Metastatic Adenocarcinoma (Giemsa, 400X).

Figure 3C: Smear of Metastatic papillary carcinoma showing follicular epithelial cells forming a micropapillae (black arrow) along with calcification (orange arrow), (Giemsa, 400X).

Figure 3D: Malignant melanocytes (yellow arrow) in a case of metastatic melanoma (Giemsa, 400X).

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