



## ORIGINAL RESEARCH ARTICLE

## EXFOLIATIVE CYTOLOGY ANALYSIS FROM DIFFERENT SITES OF THE BODY

Sushna Maharjan<sup>1\*</sup>, Sabin Ranabhat<sup>1</sup>, Mamata Tiwari<sup>1</sup>, Anita Bhandari<sup>1</sup>, Bidur Prasad Osti<sup>1</sup>, Puja Neopane<sup>2</sup><sup>1</sup> Department of Pathology, Chitwan Medical College Teaching Hospita, Bharatpur, Chitwan, Nepal<sup>2</sup>Department of Oral Medicine and Pathology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan**\*Correspondence to:** Dr. Sushna Maharjan: Associate professor, Department of pathology, Chitwan Medical College, Bharatpur, Chitwan, Nepal.

E-mail: sushnamaharjan74@gmail.com

## ABSTRACT

Background: Cytological study of body fluids a non-invasive, simple procedure, relatively inexpensive, and helps in faster reporting that has high population acceptance. The present study aims to determine the proportion of malignant and non malignant lesions. Methods: A total of 1129 specimens of exfoliative cytology were examined during the period over five years from year January 2011 to December 2016 at Department of Pathology at Chitwan Medical College Teaching Hospital, Nepal. The fluid received was centrifuged at 3000 revolutions per minute for five minutes. Smears were made from the sediment. Two to three slides were air dried and stained with Giemsa stain. One slide was immediately fixed in 95% alcohol and stained with Papanicolaou (Pap) stain for cytological evaluation. Results: Pleural fluid was most common specimen (49.3%) with peritoneal fluid (32%), pericardial fluid (1%), BAL (7.8%), CSF (4%), sputum (3.4%), synovial fluid (2%) and urine (0.5%) specimens. Male to female ratio was 1.33:1. Maximum cases belonged to age group of 50-69 years. Only (5.8%) 67 cases were neoplastic. The maximum cases of malignant neoplasm were detected in pericardial fluid and pleural fluid, each 21 (1.8%) cases, and 20 (1.7%) cases BAL. Malignant effusions were detected in 46 (4%) cases. Maximum malignant cases were adenocarcinoma (71.6%), followed by squamous cell carcinoma (20.8%). Conclusion: Exfoliative cytology should be suggested in all cases of effusion and suspected malignancies which helps in reaching at a particular diagnosis and aids in further management.

**Key words:** Adenocarcinoma, Body fluids, Effusion, Exfoliative cytology, Neoplastic.

## INTRODUCTION

Cytological study of body fluids is widely accepted method for diagnosis because it is non-invasive, simple procedure, relatively inexpensive, and also helps in faster reporting.

In 1860, Beale described the morphology of malignant cells in sputum of oropharyngeal carcinoma.<sup>1</sup> However, the application of exfoliative cytology was restricted to gynecological diagnosis previously but the introduction of new methods of staining and collection of specimens by Papanicolaou and Traut changed the trend.<sup>2,3</sup> Exfoliative cytology analysis was further extended into oral cavity when comparative studies were conducted to study the cervical and oral cytology in menstrual cycles.<sup>4</sup>

In cytology, there are two main types of specimens:

gynecological and non-gynecological. Gynecological specimens are usually Pap (cervical) smears. Non-gynecological specimens fall into two categories-exfoliative and fine needle aspiration samples. Exfoliative specimens come from areas of the body where cells are shed (or scraped/brushed) from the lining of organs or tissues various fluids. The sites of exfoliative specimens include: lungs-sputum, bronchial washings or brushings; lower urinary tract-urine; chest and abdominal cavities-pleural, pericardial and peritoneal fluid; central nervous system-cerebrospinal fluid (CSF); and gastrointestinal tract (GIT)-esophageal, stomach or intestinal brushings.<sup>5</sup>

The aim of the present study is to evaluate exfoliative

cytology at Chitwan Medical College Teaching Hospital (CMCTH) and determine the proportion of malignant and non malignant lesions.

**MATERIAL AND METHODS**

**Study design**

This is a descriptive and cross sectional study conducted over five years from year January 2011 to December 2016 at Department of Pathology, CMC, Nepal. All exfoliative specimens received for cytological evaluation were included for the study. Specimens containing degenerated cells with inconclusive diagnosis were excluded from the study. The fluid received was centrifuged at 3000 revolutions per minute for five minutes. The supernatant was discarded. The remaining sediment was transferred with the help of pipette onto glass slides and spread evenly. Two to three slides were air dried and stained with Giemsa stain. One slide was immediately fixed in 95% alcohol and stained with Papanicolaou (Pap) stain for cytological evaluation.

**Data collection and analysis**

Data were entered and analyzed using Statistical

Package for Social Sciences 20.0 (SPSS 20.0). These data were presented in tables and bar charts as frequency and percentage.

**RESULTS**

A total 1129 specimens of exfoliative cytology were examined during the period of study. Out of them 557 (49.3%) cases were pleural fluid, 362 (32%) cases peritoneal fluid, 11 (1%) cases pericardial fluid, 89 (7.8%) cases BAL, 44 (4%) cases CSF, 38 (3.4%) cases were sputum, 22 (2%) cases synovial fluid and 6 (0.5%) cases urine specimens (Figure 1). There were 645 (57%) male patients and 484 (43%) female patients (Table 1) with M:F=1.33:1. The youngest and the oldest patients were of ages 1 year and 100 years, respectively. Maximum number of patients, 428 (38%) were in age group of 50-69 years (Table 2).

**Table 1: Distribution of patients by sex**

Sex	No. of patients (%)
Male	645 (57)
Female	484 (43)
<b>Total</b>	<b>1129 (100)</b>

**Table 2: Distribution of different types of specimen according to age group**

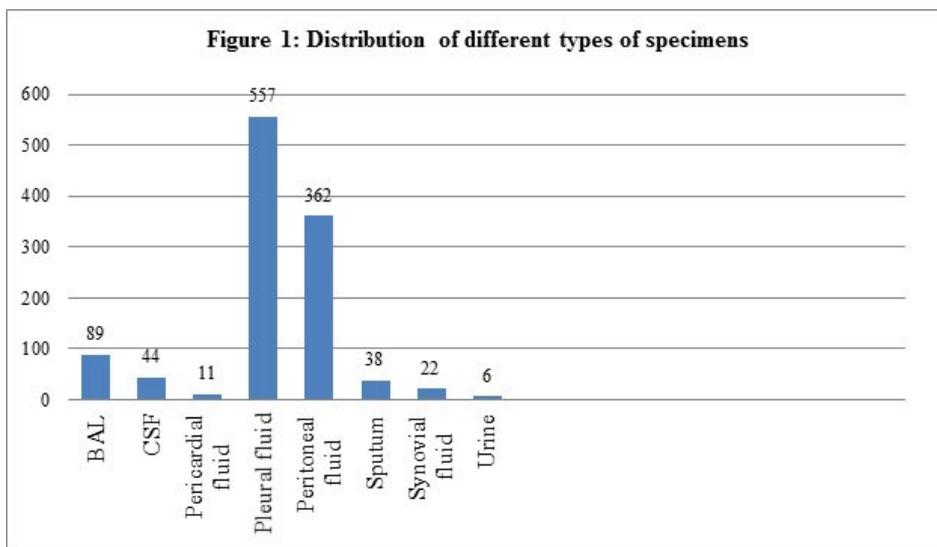
Age group (yrs)	BAL(n)	CSF(n)	Pericardial fluid (n)	Pleural fluid (n)	Peritoneal fluid (n)	Sputum (n)	Synovial fluid (n)	Urine (n)	Total n (%)
0-9	0	2	0	4	3	0	0	0	9 (0.8)
10-19	2	6	2	38	10	0	2	0	60 (5.3)
20-29	5	8	0	74	28	0	3	0	118 (10.5)
30-39	1	6	0	63	52	1	2	0	125 (11)
40-49	10	5	2	69	61	5	4	1	157 (14)
50-59	28	9	1	86	81	11	4	0	220 (19.5)
60-69	23	3	5	84	70	13	5	5	208 (18.4)
70-79	16	3	1	88	41	6	1	0	156 (13.8)
80-89	4	2	0	43	15	2	0	0	66 (5.8)
90-99	0	0	0	7	1	0	1	0	9 (0.8)
≥100	0	0	0	1	0	0	0	0	1 (0.1)
Total n (%)	89 (7.8)	44 (4)	11 (1)	557 (49.3)	362 (32)	38 (3.4)	22 (2)	6 (0.5)	1129 (100)

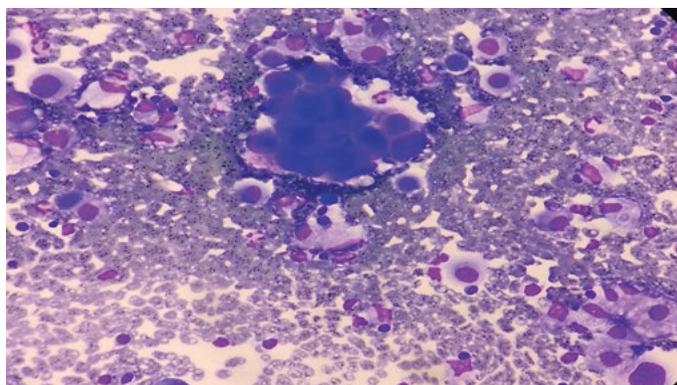
**Table 3: Incidence of neoplastic and non neoplastic lesions in different types of exfoliative cytology specimens**

Specimens	Neoplastic lesions n(%)	Non-neoplastic lesions n(%)	Total n(%)
BAL	20 (1.7)	69 (6.1)	89 (7.8)
CSF	0 (0)	44 (4)	44 (4)
Pericardial fluid	4 (0.4)	7 (0.6)	11 (1)
Peritoneal fluid	21 (1.8)	341 (30.2)	362 (32)
Pleural fluid	21 (1.8)	536 (47.5)	557 (49.3)
Sputum	0 (0)	38 (3.4)	38 (3.4)
Synovial fluid	0 (0)	22 (2)	22 (2)
Urine	1 (0.1)	5 (0.4)	6 (0.5)
Total n (%)	67 (5.8)	1062 (94.2)	1129 (100)

**Table 4: Incidence of malignancy in different types of exfoliative cytology specimens and their distribution according age and sex**

Age group (yrs)	BAL (n)		Pericardial fluid (n)		Pleural fluid (n)		Peritoneal fluid (n)		Urine (n)	
	M	F	M	F	M	F	M	F	M	F
0-9	0	0	0	0	0	0	0	0	0	0
10-19	0	0	0	0	0	0	0	0	0	0
20-29	0	0	0	0	1	0	0	0	0	0
30-39	0	0	0	0	0	3	0	1	0	0
40-49	0	1	0	0	0	5	1	2	0	0
50-59	2	4	0	0	2	1	1	3	0	0
60-69	2	4	2	1	4	0	1	5	0	1
70-79	5	1	1	0	2	0	2	2	0	0
80-89	0	1	0	0	1	1	1	2	0	0
90-99	0	0	0	0	0	0	0	0	0	0
≥100	0	0	0	0	0	1	0	0	0	0
<b>Total (n)</b>	<b>9</b>	<b>11</b>	<b>3</b>	<b>1</b>	<b>10</b>	<b>11</b>	<b>6</b>	<b>15</b>	<b>0</b>	<b>1</b>





**Figure 2: Microphotograph showing metastatic cluster of adenocarcinoma of lung in pleural fluid. (Giemsa stain, 40X)**



**Figure 3: Microphotograph showing small cell carcinoma of lung in BAL. (Giemsa stain, 40X)**

Table 3 showed a total of 67 (5.8%) cases of neoplastic lesions, all of those cases were malignant neoplasm. The maximum cases of malignant neoplasm were detected in pericardial fluid and pleural fluid, each comprising 21 (1.8%) cases, and BAL comprising 20 (1.7%) among all types of exfoliative cytology. Malignant effusions were detected in 46 (4%) cases.

The maximum specimens, i.e. 557 (49.3%) were pleural fluid in this study (Figure 1). Most of the patients were in the age of 50-79 years (Table 2). Male patients had pleural effusion commonly, comprising 348 (62.5%) and remaining 209 (37.5%) patients were female. M:F ratio was 1.7:1. Malignant pleural effusion was interpreted in 21 (3.8%) cases out of 557 pleural effusions and 1.8% among total 1129 cases (Table 3); [10 (1.8%) male and 11 (2%) female (Table 4)]. Remaining were non neoplastic lesions.

A total of 362 (32%) cases of peritoneal fluid were

evaluated during the study period (Figure 1). Most of the patients were in the age of 50-69 years (Table 2). There were 174 (48%) male patients and 188 (52%) female patients (M:F=1:1.08). Malignancy was detected in 21 (5.8%) cases out of 362 peritoneal effusions and 1.8% among total 1129 cases (Table 3); [6 (1.7%) male and 15 (4.1%) female (Table 4)].

There were only 11 (1%) cases of pericardial fluid (Figure 1). Maximum patients were in age of 60-69 years (Table 2). Malignant pericardial effusion was found in 4 patients i.e. 36.4% out of 11 pericardial effusions and 0.4% of total 1129 specimens (Table 3); [3(27.3%) male and 1 (9.1%) female (Table 4)].

Out of 1129 specimens, 89 (7.8%) were BAL (Figure 1). Most of the patients were in the age of 50-69 years (Table 2). Fifty (56.2%) were male and 39 (43.8%) were female with M:F ratio of 1.3:1. There were 20 malignant cases (Table 3); [9 (10.1%) male and 11 (12.4%) female (Table 4)].

All 44 (4%) cases of CSF, 38 (3.4%) cases of sputum and 22 (2%) cases of synovial fluid cytology had no malignancy (Figure 1 and Table 3). There were 28 (63.6%) male and 16 (36.4%) female in study of CSF cytology (M:F=1.8:1). Twenty four (63.2%) male and 14 (36.8%) female were observed in the study of sputum cytology (M:F=1.7:1). There were 11 (50%) each male and female patients in the study of synovial fluid (M:F=1:1).

Six samples of urine were received for cytology (Figure 1) among which 1 male patient of aged 64 years was diagnosed as suspicious for malignancy with atypical cells (Table 3). There were 4 (66.7%) male and 2 (33.3%) female (M:F=2:1).

Among 67 malignant cases, 44 (65.7%) were observed in age ranging from 50-79 years in our study. Malignancy was found in 29 (43.3%) male and 38 (56.7%) female with M:F ratio of 1:1.3 (Table 4). Most of the malignant neoplasms, 48 (71.6%) were adenocarcinoma. All 21 cases of malignant peritoneal effusion and all 4 cases of pericardial effusion were metastatic adenocarcinoma. Out of 21 cases of malignant pleural effusion, 20 cases were metastatic adenocarcinoma (Figure 2). In BAL cytology, adenocarcinoma was diagnosed in 3 cases out of 20 malignant cases. The second common malignancy [14 (20.8%)] observed was squamous

cell carcinoma, all were diagnosed in BAL cytology. Three (4.5%) cases were small carcinoma of lung detected (Figure 3) in BAL cytology. There was 1 (1.5%) case of Non Hodgkin lymphoma in pleural effusion. The other 1 (1.5%) case of malignancy was found in urine cytology, which was reported as positive for malignancy. Biopsy from the urinary bladder was sent for histopathological examination and diagnosed as Transitional carcinoma of urinary bladder.

## DISCUSSION

In the present study, maximum specimens were pleural fluid followed by peritoneal fluid among total aspirated fluids. Males were commonly affected compared to females. Most patients belonged to age group of 50-59 years followed by 60-69 years. Several other studies observed maximum cases of peritoneal fluid followed by pleural fluid with male > female. They found maximum affected patients in age group of 30-40 years followed by 40-50 years.<sup>6-9</sup>

We interpreted a total of 5.8% malignant lesions in exfoliative cytology (Table 3) out of which 4.1% were malignant effusions. Pradhan et al.<sup>6</sup> and Joshi et al.<sup>9</sup> observed malignant effusions more frequently in 18.66% and 22.66% whereas Gupta et al.<sup>7</sup> had only 5.4% malignant effusions. (9) The present study showed maximum number of malignant effusions in the age group of 60-69 years with male preponderance, M:F ratio being 1.16:1 (Table 4). Joshi et al.<sup>9</sup> reported maximum malignant effusions in the age group of 21-40 years with M:F ratio being 1.13:1.

Malignant pleural effusion was found in wide range of 1.5% to 38.23% in several studies.<sup>6-13</sup> We detected 3.8% of malignant pleural effusions [1.8% male and 2% female (Table 3 and 4)]. In other studies,<sup>6,10</sup> male patients encountered malignant pleural effusion more commonly than in female patients (M:F=1.4:1).

Peritoneal effusion was found commonly in 51-60 years (24.3%) in the report by Dowerah et al.<sup>14</sup> similar to our study. Many authors<sup>6,8,10,14,15</sup> reported malignant peritoneal effusion in 2.3% to 24.2% but other authors<sup>7,9</sup> observed malignant peritoneal effusion as the commonest malignancy in their reports constituting 50% and 55.88% respectively. We interpreted only 5.8% of malignant peritoneal

effusions. Female patients had malignant peritoneal effusion commonly than male patients in our study likewise in study by Ekpe et al.<sup>16</sup>

Pericardial effusions were observed less frequently in many studies<sup>6,7,9,10</sup> ranging from 0.54% to 3.25%. Gupta et al.<sup>7</sup> interpreted a case of malignant pericardial effusion showing metastatic deposits of breast carcinoma in a male. However, Pradhan et al.<sup>6</sup> reported malignancy in 21% of pericardial effusion, all of those were metastatic adenocarcinoma and all were male patients. Joshi et al.<sup>9</sup> found 5.88% malignancy pericardial effusion whereas in the study by Grandi et al.<sup>10</sup> all were inflammatory pericardial effusion only. In our study, pericardial effusion was observed in 1% only (Figure 1) similar to the other above mentioned studies. Malignant pericardial effusion was seen in 36.4% of out of total pericardial effusion in this study which is higher than in other studies.

Cytological examination of synovial fluids is important to differentiate the inflammatory and non-inflammatory joint diseases.<sup>17</sup> The careful observation of cellular changes in synovial fluid allows a good understanding of the pathogenetic mechanisms.<sup>18</sup> Ostovic et al.<sup>19</sup> diagnosed 70.4% of inflammatory diseases with male predominance.<sup>17</sup> In our study we also reported predominantly (96.6%) inflammatory diseases in synovial fluid. We found majority of the synovial fluids transudative in nature and most of them were showing neutrophilic predominance which is supported by other authors.<sup>7,8</sup> Cytologic examination for tumor cells has not been extensively studied because of the fact that invasion of the joint space by tumor is rare.<sup>19</sup>

The spread of cancer into the central nervous system is a serious problem leading to neurological symptoms and rapid mortality. CSF cytology is currently considered the gold standard for diagnosis of leptomeningeal metastasis.<sup>20</sup> According to Glass et al.<sup>21</sup> a positive CSF cytology is a reliable indicator of CNS malignancy and reflects leptomeningeal tumor. Gondos<sup>22</sup> found malignant cells in 9.5% of CSF examination and it has been found to be of particular value in the diagnosis of metastatic carcinoma, lymphomatous and leukemic involvement of the meninges and certain primary CNS tumors. We did not find any malignancy in CSF cytology and majority showed lymphocytic predominance, which is also

supported by Gupta et al.<sup>7</sup>

BAL has gained wide acceptance as a tool for diagnosing lung cancer, which was originally popular as a therapeutic tool for pulmonary conditions such as pulmonary alveolar proteinosis, cystic fibrosis and intractable asthma.<sup>23,24</sup> Bhagat et al.<sup>25</sup> detected malignant lesions in 3/13 BAL samples which were found to have malignancy on histopathological evaluation as well. This indicates that BAL Cytology can be useful to diagnose malignant lesions of lung with certainty.

Humphrey et al.<sup>26</sup> performed lung cancer screening with chest x-ray with or without sputum cytology that showed benefit in case-control studies, however didn't show benefit in randomized control trials. Sputum cytology examination is useful for early detection in populations at high risk for lung cancer.<sup>27</sup>

Ammanagi et al.<sup>28</sup> included 36 patients with clinically suspected carcinoma of lung in their study. Twenty cases were confirmed as lung malignancy by histopathologic and/or radiographic and clinical evidences. Among these 20 confirmed cases, sputum cytodiagnosis was positive for malignancy in 12 (60%) cases that means the pickup rate of lung cancer by sputum cytology was 60%.

Voided urinary cytology is a useful noninvasive technique because of its high specificity. It has a relatively high sensitivity at detecting high-grade lesions; however, its sensitivity is less for low-grade papillary tumors. Nabi et al.<sup>29</sup> had mentioned that repeat urine cytology examination should be performed after six to eight weeks if patients with suspicious urine cytology have a negative initial evaluation. Those patients who have persistent positive cytology or recurrent hematuria need further careful evaluation, including assessment of the prostate because most patients in this group are found to have an underlying malignancy. Siddappa et al.<sup>30</sup> found 32.5% with positive finding of atypical cells, 9.5% among them had malignancy on both histopathological examination and cystoscopic findings and remaining were of reactive origin. We had only one case of suspicious of malignancy with atypical cells in urine cytology which was diagnosed as Transitional carcinoma of urinary bladder on histopathology.

## CONCLUSION

Exfoliative cytology should be suggested in all cases of effusion and suspected malignancies which helps in reaching at a particular diagnosis and aids in further management.

## ACKNOWLEDGEMENT

We would like to acknowledge Mr. Anil Shah for his technical assistance.

## REFERENCES

1. Beale LS. Examination of sputum from case of cancer of the pharynx and adjacent parts. Arch Med 1860;2:44–6.
2. Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smear. The Commonwealth Fund, New York, 1943;S1– 47.
3. Papanicolaou GN. A survey of the actualities and potentialities of exfoliative cytology in cancer diagnosis. Ann Intern Med 1949;31:661–74.
4. Ziskin DE, Moulton R. A comparison of oral and vaginal epithelial smears. J Clin Endocrinol 1948;8:146–65.
5. Shirley SE, Escoffery CT. An introduction to cytology: principles and practice [Internet]. 2005 [cited 2.18.2017].
6. Pradhan SB, Pradhan B, S Dali. Cytology of body fluids from different sites: an approach for early diagnosis of malignancy. J Nep Med Assoc 2006;45:353–6.
7. Gupta R, Dewan D, Raina R, Gupta M. Exfoliative cytology of body fluids: a study from provincial hospital of Jammu region, India. Int J Res Med Sci 2016;4(4):1016-9.
8. Shulbha VS, Dayananda BS. Cytology of body fluids-an aid to primary diagnosis. Indian Journal of Pathology and Oncology 2015;2(2):81–3.
9. Joshi A, Mahajan N, Karmakar PJ. Diagnostic utility of various techniques used in body fluid cytology. J Dent Med Sci 2014;13(1):13-8.
10. Grandhi B, Shanti V, Rao MN, Reddy CV, Venkata MMK. The diagnostic utility of cell block as an

- adjunct to cytological smears. *Int J Med Res Health Sci* 2014;3(2):278–84.
11. Dagli AF, Kucuk S, Sezer M, Ucer O. Cytopathologic diagnosis in pleural effusion and cyto-histopathologic correlation. *Turkish Journal of Pathology*. 2011;27(1):12-6.
  12. Verma SK, Dubev AL, Singh PA, Tewerson SL, Sharma D. Adenosine deaminase (ADA) level in tubercular pleural effusion. *Lung India* 2008;25(3):109-10.
  13. Cakir E, Demirag F, Aydin M, Erdogan Y. A review of uncommon cytopathologic diagnosis of pleural effusions from a chest diseases center in Turkey. *Cytojournal* 2011;8(1):1-7.
  14. Dowerah E, Das S. Cytological evaluation of peritoneal fluid with special reference to malignancy. *Int J Bio Res* 2014;5(6):396–8.
  15. Junaid TA, Odor EIA. Cytologic diagnosis of ascitic fluid in Ibadan, Nigeria. *J Natl Med Assoc*. 1980;72(7):669-72.
  16. Ekpe EEL, Omotoso AJ. The sensitivity of cytology in the differential diagnosis of ascites among adult Nigerians in a Tertiary Health Institution. *Sch J App Med Sci* 2015; 3(2F):969-72.
  17. Ostović KT, Kaić G, Ostović I, Skoro M, Novak NP, Morović-Vergles J. The importance of urgent cytological examination of synovial fluids in differentiation inflammatory and non-inflammatory joint diseases. *Coll Antropol* 2010;34(1):145–52.
  18. Freemont AJ. Role of cytological analysis of synovial fluid in diagnosis and research. *Ann Rheum Dis* 1991; 50(2): 120–3.
  19. Teloh HA. Clinical Pathology of Synovial Fluid. *Ann Clin Lab Sci* 1975;5(4):282-7.
  20. Chamberlain MC, Glantz M, Groves MD, Wilson WH: Diagnostic tools for neoplastic meningitis: Detecting disease, identifying patient risk, and determining benefit of treatment. *YSONC* 2010, 36:S35-45.
  21. Glass JP, Melamed M, Chernik NL, Posner JB. Malignant cells in cerebrospinal fluid (CSF): The meaning of a positive CSF cytology. *Neurology* 1979;29(10):1369-75.
  22. Gondos B. Cytology of cerebrospinal fluid: Technical and diagnosis considerations. *Ann Clin Lab Sci* 1976; 6(2): 152-7.
  23. Johnston WW, Elson CE. Respiratory tract. In: Bibbo M, editor. *Comprehensive cytopathology*, 2nd ed. Philadelphia: W. B. Saunders Company; 1997. p. 325-401.
  24. Rogers RM, Braunstein MS, Shurman JF. Role of bronchopulmonary lavage in the treatment of respiratory failure: a review. *Chest* 1972;62(5):Suppl:95S-106.
  25. Bhagat VM, Tailor HJ, Patel PR, Adajania M, Mantri P. Utility of bronchoalveolar lavage in cytology; a study at tertiary care hospital. *Asian Pac J Health Sci* 2014;1(4):362-4.
  26. Humphrey LL, Teutsch S, Johnson MS. Lung cancer screening with sputum cytologic examination, chest radiography, and Computed Tomography: An update for the U.S. preventive services task force. *Ann Intern Med* 2004;140:740–53.
  27. Thunnissen FBJM. Sputum examination for early detection of lung cancer. *J Clin Pathol* 2003;56:805-10.
  28. Ammanagi AS, Dombale VD, Miskin AT, Dandagi GL, Sangolli SS. Sputum cytology in suspected cases of carcinoma of lung (Sputum cytology a poor man's bronchoscopy!). *Lung India* 2012; 29(1):19–23.
  29. Nabi G, Greene D, Donnel MO. Suspicious urinary cytology with negative evaluation for malignancy in the diagnostic investigation of haematuria: how to follow up? *J Clin Pathol* 2004;57:365–8.
  30. Siddappa S, Mythri K, Kowsalya R. Cytological findings in routine voided urine samples with hematuria from a tertiary care center in south India. *Journal of Cytology / Indian Academy of Cytologists* 2012;29(1):16-9.