



Original Research Article

Exfoliative cytology of different body fluids- an important aid to primary diagnosis

Avani Tiwari¹, Ajay Singh Thakur^{1,*}, Pradeep Chandrakar², Amit Choraria³, Vivek Choudhary²

¹Dept. of Pathology, Regional Cancer Centre, Pt. Jawaharlal Nehru Memorial Medical College, Raipur, Chhattisgarh, India

²Dept. of Radiation Oncology, Regional Cancer Centre, Pt. Jawaharlal Nehru Memorial Medical College, Raipur, Chhattisgarh, India

³Dept. of Surgical Oncology, Regional Cancer Centre, Pt. Jawaharlal Nehru Memorial Medical College, Raipur, Chhattisgarh, India



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ABSTRACT

Background: Cytological examination of exfoliated cells in various effusion fluids is very challenging and of paramount importance for early diagnosis and management of various pathological processes. It is of utmost significance in identifying malignant cells and hence throws light on the cause, staging and prognosis of cancer.

Aims and Objectives: This study was carried out to know the different trends of various types of effusions for various pathological processes with an emphasis on malignant effusion in a tertiary care centre.

Materials and Methods: Retrospective, analytical, observational study done over a period of one year from January 2019 to December 2019. A total of 418 cases including peritoneal and pleural and cerebrospinal fluid and urine were analyzed. Samples were centrifuged for five minutes at 2000 rpm and smears prepared from deposit were stained by Papanicolaou (PAP) and May-Grunwald- Giemsa (MGG) stains.

Results: Out of 418, 221 were peritoneal effusions, 172 were pleural, 20 cerebrospinal fluids and 5 urine. Out of 221 cases of peritoneal effusions, 144 were non neoplastic and 61 were malignant effusion. Out of total 172 pleural effusions 139 were non neoplastic and 27 neoplastic. Commonest malignancy in peritoneal and pleural fluid was adenocarcinoma from ovary and lung respectively.

Conclusions: Cytological evaluation of various fluids is simple, rapid, inexpensive and less invasive tool with high accuracy and thereby reducing the need for invasive investigations. It is especially helpful in evaluating and staging malignancies thereby guiding the clinician in further management.

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1. Introduction

There are three major cavities in the body: pleural, pericardial, and peritoneal. These cavities have parietal and visceral layers, both lined by mesothelial cells overlying the submesothelial stromal matrix tissue.¹ The parietal and visceral layer is separated by thin layer of lubricating fluid which facilitates the movement of both membranes against each other in the absence of disease.² However,

in pathologic states, these cavities develop spontaneous effusions attributable to various pathophysiological processes. This fluid provides a clinically useful specimen for cytological evaluation to diagnose the underlying pathologic process, such as infections, inflammation, neoplasia, etc.³ Tapping and analyzing these fluids in terms of biochemical parameters and cytology not only serves in diagnosis and therapeutic intervention but also aids in staging, treatment outcome, disease monitoring and prognosis.^{4,5} The diagnostic yield of effusion fluid is higher than needle biopsy since the cell population present

* Corresponding author.

E-mail address: ajay.jnm@gmail.com (A. S. Thakur).

in the sediment is representative of a much larger surface area.^{6,7} Almost 20% of the effusions examined are directly or indirectly related to the presence of malignant disease with carcinoma of the lung as the most common underlying cause.^{5,8} Many studies were performed previously on different fluids, few focusing on single fluid. This study was carried out to know the different trends of various types of effusions with an emphasis on malignant effusion.

2. Materials and Methods

Our study is a descriptive, analytical and retrospective observational study, undertaken in the department of pathology in a tertiary care cancer centre. The duration of the study was one year; from January 2019 to December 2019. Inclusion criteria were samples from pleural effusion, peritoneal effusion, cerebrospinal fluid (CSF) and urine. Cases from either sex of any age group were included in the study. Exclusion criteria were body fluids other than those in the inclusion criteria were excluded. Complete clinical history including clinical examination along with all relevant blood, serum and radiological investigations of the patients were noted from the medical records of the patients. All the samples received were immediately processed. In case of delayed submission beyond working hours, samples were received and stored overnight in refrigerator at temperature of 2-6 °C. Sample volume ranged from 2 ml to 2000 ml. The gross appearance of the fluid was assessed. For hemorrhagic fluids, glacial acetic acid was used as a hemolysing agent and then processed routinely. Classification into transudate or exudate was done on the basis of gross appearance (clear or turbid), protein content and cell count (low or high) present in the fluid. The fluid with protein more than 3 gm/100 ml was taken as exudate and less than 3 gm/100 ml as transudate.⁷ The fluids were centrifuged at 2000 revolution per minute (rpm) for five minutes to produce uniform suspension of cells. Both wet fixed (methyl alcohol) and air dried smears were made and stained with Papanicolaou (PAP) and May-Grunwald-Giemsa (MGG) stains respectively. PAP stain helped in better interpretation of nuclear features and MGG stain for cytoplasmic features. The stained smears were studied on light microscopy and evaluated for cellularity, predominant cell type, size, architecture (acini / sheets/ 3-dimensional balls/ papillae/ rosette, singly scattered), nuclear and cytoplasmic features, chromatin, degree of inflammation, reactive changes and other background features. All the data was analyzed and summarized.

3. Results

A total of 418 fluids were examined cytologically which included pleural, peritoneal, cerebrospinal fluid and urine specimens. The age ranged from 5 years to 82 years. Female preponderance was observed with M: F ratio of 1:1.55. The

most common fluid was peritoneal fluid, 221 (52.9%) cases, followed by pleural fluid, 172 (41.1%) cases, CSF 20 (4.8%) and least common was urine, 5 (1.2%) cases (Table 1). The most common age group affected was 5th decade followed by 6th decade. (Table 2)

All individual cases were categorized into 3 major categories; benign/negative for malignancy, suspicious for malignancy and positive for malignancy (Table-3). The smears which were diagnosed as positive for malignancy were further typed on the basis of cytomorphology. Those which could not be typed were subsequently followed and the diagnosis was rendered on the basis of histopathology and immunohistochemistry (IHC), wherever necessary.

Out of 418 cases of cytological specimens, 305 (73%) were negative for malignancy. In peritoneal fluid 65.2% (144/221), pleural fluid 80.8% (139/172), CSF 85% (17/20) and in urine all the specimens (5/5) were present in this category. These cases included smears which were predominantly inflammatory (acute, chronic as well as mixed) or reactive, having mesothelial cells and macrophages in abundance.

22 out of 418 cases (5.3%) were kept in the category of suspicious of malignancy. These cases did not show definitive features of malignancy. However showed presence of atypical looking cells, either obscured by too much of hemorrhage, inflammation, necrosis, etc or had low cellularity with changes depicting only a doubt of malignancy. Presence of reactive mesothelial cells, close mimickers of malignancy also raised suspicion of malignancy in few cases. Few of these cases lost follow-up and few underwent repeat cytological or histopathological test rendering a definite diagnosis.

Malignant cells were detected definitely in 91 (21.8%) cases. Maximum number of malignant effusions were peritoneal (67%) followed by pleural (29.7%) and cerebrospinal fluid (3.2%). Urine specimen did not show any malignancy.

Out of total 221 cases of peritoneal effusion, maximum number of cases (64 cases) were observed in the age group of 41-50 years (Table 2) and with female preponderance; M: F ratio of 1:2.94. Total 124 cases were transudate and 97 cases were exudate in nature. Cytological examination of smears revealed that out of 97 cases of exudate, 61 were malignant effusion and 36 were non- malignant effusion (Table 4). Transudates had protein level less than 3 gm% and exudates had more than 3 gm%. Out of 61 cases of malignant effusion 39 cases (64%) were metastasis from ovarian malignancy (Figure 1) followed by 6 cases (9.8%) from gall bladder malignancy. Least common cases included metastasis from lung, esophagus, buccal mucosa, bile duct malignancy along with chronic myelogenous leukemia (CML); one case each (1.6%). (Table 5) (Figures 2 and 3)

A total of 172 (41.1%) cases were of pleural effusion. Similar to peritoneal fluid the most common age group

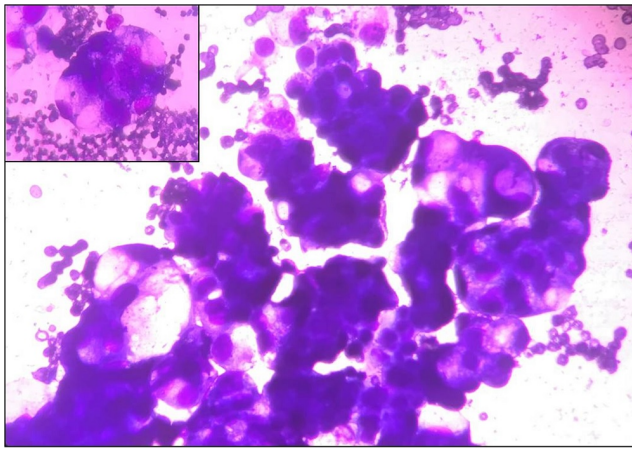


Fig. 1: Signet ring adenocarcinoma in peritoneal fluid: Photomicrograph showing three dimensional clusters of atypical signet ring cells. (MGG, 400X). Inset: Multivacuolated typical signet ring cell with hyperchromatic nuclei. (MGG, 400X)

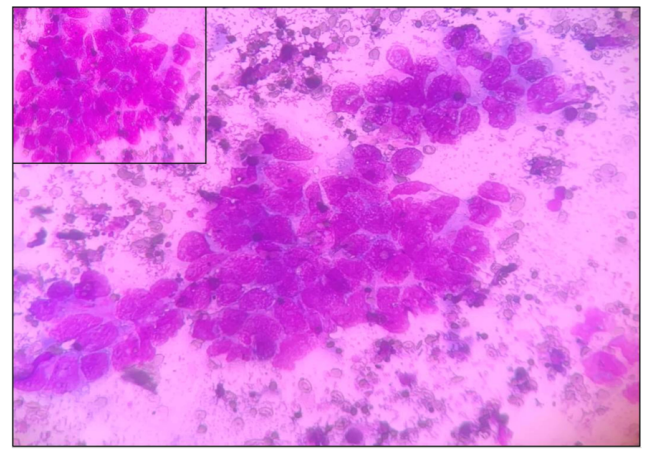


Fig. 3: Metastatic germ cell tumor in peritoneal fluid. Photomicrograph showing sheets of malignant cells with vacuolated cytoplasm, hyperchromatic nuclei and prominent nucleoli. (MGG, 400X)

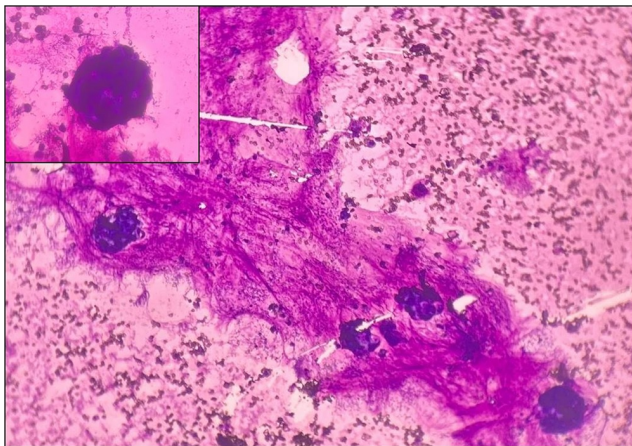


Fig. 2: Mucinous adenocarcinoma in peritoneal fluid. Photomicrograph showing extracellular mucin with three dimensional cell clusters of malignant cells. (MGG, 200X). Inset: Higher magnification (MGG, 400X)

affected is 41-50 years (45 cases) (Table 2) and with slight male preponderance; M: F ratio of 1.1:1. Total 94 cases were transudate and 78 cases were exudate in nature. Out of 78 cases of exudate, 27 cases were malignant effusion and 51 cases were non-malignant effusion (Table 4). Out of 27 cases of malignant effusion 11 cases (40.7%) were metastasis from lung malignancy (Figures 4 and 5) followed by 5 cases each (18.5%) from breast and ovarian malignancy. (Table 6)

Out of 20 cases of CSF maximum numbers of cases (nine cases) were observed in 0-10 years with a male preponderance of 2.3: 1. Seventeen cases were transudate and three cases were exudate in nature. All the three exudative CSFs were malignant in nature; two cases were from acute lymphoblastic leukemia (ALL) (Figure 6) and

one case was from medulloblastoma.

Five urine specimens were received. The age group of the patients was 51-70 years and all were males. All the cases showed inflammatory picture predominantly comprising of neutrophilic infiltrate and bacteria. None of the cases showed presence of malignant cells.

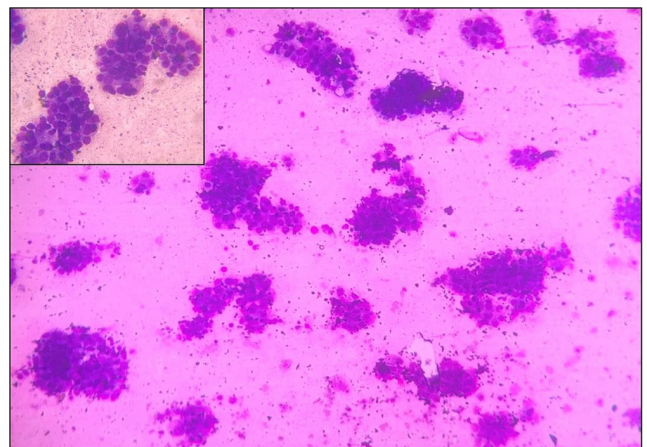


Fig. 4: Papillary adenocarcinoma in pleural fluid: Photomicrograph showing papillary fragments and clusters with focal acinar arrangement. (MGG, 200X). Inset: Higher magnification (MGG, 400X)

4. Discussion

The history of serous effusion cytology can be traced back to the 19th century. Lucke and Klebs were apparently the first investigators who recognized the presence of malignant cells in an ascitic fluid in 1867. In 1882 Quincke was credited for detailed descriptions of ovarian and lung cancer

Table 1: Distribution of cases according to the type of specimen and their gender wise incidence.

Type of Specimen	Number of Cases	Male	Female
Peritoneal	221 (52.9%)	56	165
Pleural	172 (41.1%)	89	83
CSF	20(4.8%)	14	06
Urine	05(1.2%)	05	0
Total	418 (100%)	164(39.2%)	254(60.8%)

CSF- Cerebrospinal fluid

Table 2: Age wise distribution of cases in peritoneal and pleural effusion, CSF and urine

Age in years	Peritoneal fluid	Pleural fluid	CSF	Urine	Total
0-10	2	2	9	0	13
11-20	3	3	6	0	12
21-30	28	12	1	0	41
31-40	33	34	3	0	70
41-50	64	45	0	0	109
51-60	56	32	1	2	91
61-70	28	32	0	2	62
71-80	6	9	0	1	16
>80	1	3	0	0	4
Total	221	172	20	5	418

CSF- Cerebrospinal fluid

Table 3: Distribution of cases on the basis of diagnosis

Site	Benign/negative for malignancy	Suspicious of Malignancy	Malignant	Total
Peritoneal	144 (65.2%) (47.2%)	16 (7.2%) (72.7%)	61 (27.6%) (67%)	221 (100%)
Pleural	139 (80.8%) (45.6%)	6 (3.5%) (27.2%)	27(15.7%) (29.7%)	172 (100%)
CSF	17 (85%) (5.6%)	0 (0%) (0%)	3(15%) (3.2%)	20 (100%)
Urine	5 (100%) (1.6%)	0 (0%) (0%)	0 (0%) (0%)	5 (100%)
Total	305 (100%)	22 (100%)	91 (100%)	418 (100%)

CSF- Cerebrospinal fluid

Table 4: Distribution of transudate and exudate on cytological examination

Type of fluid	Transudate	Exudate	
		Non malignant	Malignant
Peritoneal fluid	124	36	61
Pleural fluid	94	51	27
CSF	17	0	3
Urine	0	5	0

CSF- Cerebrospinal fluid

cells in serous effusions. Since that time reports on effusion cytology have started to appear in the medical literature, and serous effusion cytology now is a routine diagnostic procedure worldwide. Further with the advent of lumbar puncture in the year 1891, in Germany CSF cytological examination was introduced in the field of cytopathology.⁷ In the current scenario the cytological examination of effusion has become a complete diagnostic modality which aims at pointing out the etiology of effusions.⁹

Mechanism of formation of abnormal fluid in the body cavity works on the principle of 'Starlings Law'. This states that fluid is accumulated when there is decrease

in the plasma colloidal pressure and increased capillary hydrostatic pressure.⁹⁻¹¹ However; it is not always possible to characterize a fluid into an exudate or transudate. This provides only a general guideline for possible underlying etiology. Hence, fluid protein is used as a basis to distinguish between exudates and transudates.⁹

In the present study of 418 cases of fluids, the age ranged from 5- 82 years. The age range in most of the other studies were from first to ninth decade which was in concordance to our study.^{4-7,9,10,12-15} Male preponderance was found in most of the studies^{4,5,7-10,12-14} except studies by Ayyagari S et al⁶ and Gupta R et al¹⁵ who found female preponderance

Table 5: Distribution of peritoneal fluid according to the diagnosis and the associated malignancies

Primary site of malignancy	Benign/ Negative for malignancy	Suspicious of malignancy	Malignant	Total
Ovary	49	9	39	97
Stomach	19	0	6	25
Gall bladder	9	1	5	15
Liver	9	0	0	9
Unknown primary	10	2	2	14
Cervix	10	1	3	14
Pancreas	8	0	0	8
Breast	6	0	0	6
Kidney	6	0	0	6
Colon	4	2	0	6
Lung	4	0	1	5
Esophagus	3	0	1	4
Testes	1	1	2	4
Anal canal	3	0	0	3
GE Junction	2	0	0	2
Soft tissue sarcoma	1	0	0	1
HL	1	0	0	1
Endometrium	1	0	0	1
Buccal mucosa	0	0	1	1
CML	0	0	1	1
Total	144	16	61	221

GE- Gastroesophageal, HL- Hodgkin lymphoma, CML- Chronic myelogenous leukemia

Table 6: Distribution of pleural fluid according to the diagnosis and the associated malignancies.

Primary diagnosis/ site of malignancy	Benign/ Negative for malignancy	Suspicious of malignancy	Malignant	Total
Lung	45	1	11	57
Unknown primary	22	3	3	28
Breast	17	1	5	23
Pneumonia	20	0	0	20
Ovary	6	0	5	11
Cervix	8	1	0	9
PTB	8	0	0	8
Buccal mucosa	4	0	1	5
Liver	3	0	0	3
Gall bladder	2	0	0	2
Stomach	0	0	2	2
HL	2	0	0	2
Soft tissue sarcoma	1	0	0	1
MRCT	1	0	0	1
Total	139	6	27	172

PTB- Pulmonary tuberculosis, HL- Hodgkin lymphoma, MRCT- Malignant round cell tumor

similar to the present study.

Most common fluid received was peritoneal fluid (52.9%), followed by pleural fluid (41.1%), cerebrospinal fluid (4.8%) and urine (1.2%). The present study correlated with the findings of Chakrabarti et al,⁸ Shulbha et al,¹⁰ Bhagat et al,¹³ Bhade et al¹⁴ and Gupta et al.¹⁵ Other authors found pleural fluid as the commonest fluid.^{4-7,9,12} This could be attributed to the various epidemiological factors. In our study the difference may be due to more number of cases of abdominal malignancies.

In 221 cases of peritoneal fluid, most common age group involved was 41-50 years with a female preponderance; M: F ratio of 1: 2.9. These findings were in concordance with Ayyagari et al⁶ and Chakrabarti et al⁸ who also observed female preponderance with M: F ratio of 1: 2.6 and 1: 1.4 respectively. However age group affected was different.^{6,8} Cytological examination revealed that 124 cases (56%) were transudate and 97 cases (44%) were exudate in nature. Out of 97 cases of exudative effusion, 61 were malignant in nature. Most studies showed similar findings as in our

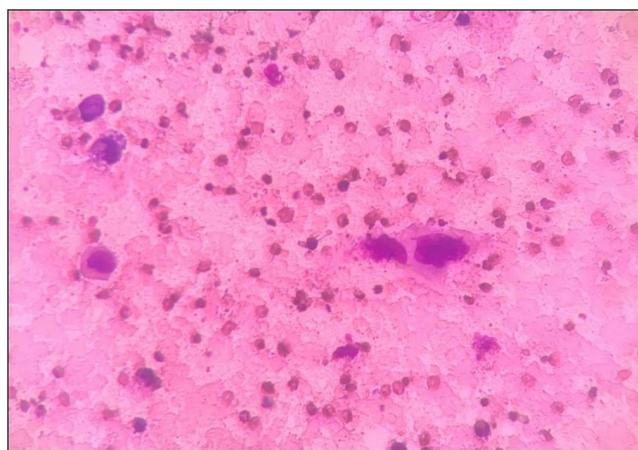


Fig. 5: Metastatic squamous cell carcinoma in pleural fluid. Photomicrograph showing singly scattered atypical cells with squamous differentiation in a known case of SCC lung. (MGG, 400X)

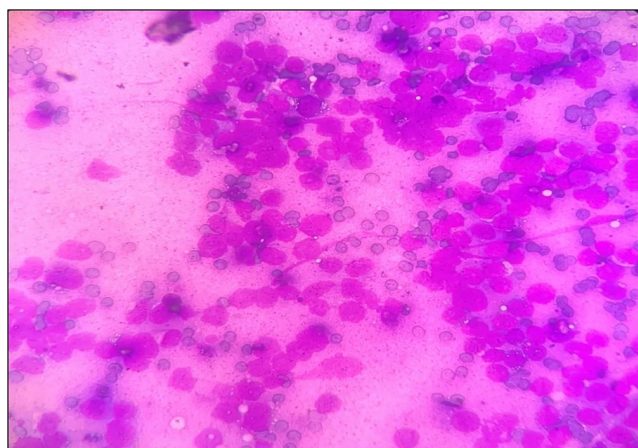


Fig. 6: Photomicrograph showing acute lymphoblastic leukemia cells in CSF. (MGG, 400X)

study, transudate being more common.^{6,8–10} Studies showed that in case of peritoneal effusion, ovarian malignancy was the most common primary site shedding malignant cells in peritoneal fluid.^{6,8–10} Our study also revealed similar trend. This may be due to female preponderance in our study and adenocarcinoma of the ovary being the most common abdominal malignancy in women.¹⁶ Rest of the malignant peritoneal effusions were from malignancies of stomach, gall bladder, lung, esophagus and buccal mucosa. Jha et al.¹⁷ in their study of effusion cytology in patients with simultaneous malignancy and ascitis, found gastric malignancy as the most common primary carcinoma affecting peritoneum. However we had only six cases of carcinoma stomach (2nd most common) affecting the peritoneal fluid. Infiltration of peritoneal fluid by CML is a rare extramedullary manifestation.¹⁸ We also found a rare case of CML infiltrating to the peritoneal fluid.

Pleural fluid was found as the second most common effusion fluid having 172 cases (41.1%) similar to the observation by various authors.^{8,10,13–15} Most common age group involved was 41-50 years. There was a slight male preponderance having M: F ratio of 1.1: 1, this was in concordance with Hathila et al⁵ and Chakraborti et al.⁸ Microscopic examination and biochemical analysis revealed that 94 cases (54.6%) were transudate and 78 cases (45.4%) were exudate in nature similar to the observation by other authors.^{8,10} However, Sudha et al⁶ and Kumawat et al⁹ observed that lymphocyte rich exudates were more common. Primary adenocarcinoma of lung was the most common malignancy in malignant pleural effusion. Sudha et al,⁶ Chakraborti et al⁸ and Gupta et al¹⁹ also established similar findings. It can be because of the male predominance and lung carcinoma being the most common malignancy in males, these trends were observed. Next to lung carcinoma, breast and ovary contributed to the pleural effusion as they are the most common malignancies in females.¹⁶

CSF remained the third most common fluid in many of the studies.^{4,7,12–15} This corroborates with our finding; CSF being the third most common fluid (4.8%). Shulbha et al¹⁰ in their study on cytology of body fluids found a relatively higher percentage of CSF samples (25.9%) in their study which was even more than pleural fluid (24.1%). This finding may be due to inclusion of increased number of pediatric population in their study. Most of the patients in our study were in the age group of 0-10 years which is similar to the findings of Saba et al¹² and Bhagat et al.¹³ Seventeen (85%) out of 20 cases were transudate in nature and three cases were exudate. All three cases were malignant (15%); two cases were from ALL and one case was from medulloblastoma. Identifying blast cells and their percentage in the CSF is an important prognostic factor in pediatric ALL and determines the incidence of relapse and need for the change in treatment protocol.²⁰ Tertirov et al²¹ determined the role of early CSF examination in detecting true cases of early tumor dissemination and is important for determining both treatment and prognosis. It is important to identify infectious causes of exudative CSF effusion for early diagnosis, improvement in prognosis and reduce spread of disease and complication.^{10,14} Study done by Shulbha et al¹⁰ found *Cryptococcus* in CSF and Bhade et al¹⁴ found tuberculosis as the cause of exudative effusion. However, we did not find any infective cause in our study. This may be because our centre is a tertiary care referral cancer centre.

Urine cytology for screening of transitional cell carcinoma (TCC) has been used for long time. Despite the advent of several newer techniques for screening and diagnosis of urothelial malignancies, cytomorphology still remains an important tool. "Atypical cells" in urine have been recognized and studied time and again. The accurate interpretation of the character of "Atypical cell"

in urine is a major challenge for cytopathologists.²² Five urine specimens with suspected cases of carcinoma urinary bladder presenting as intermittent hematuria in elderly patients were included in our study. The reason for less number of urine specimens may be due epidemiological factors. All the cases however, showed cytological features of urinary tract infection. None of the cases showed presence of malignant cells.

Almost all the studies came across difficulty in interpretation of malignancy due to the presence of reactive mesothelial cells which are a very close mimicker of malignancy as they also have the tendency of rosette formation, pseudoacini or acini, with or without prominent nucleoli.^{4–6} In our study, we also found 22 out of 418 cases (5%) of various fluids being reported as suspicious of malignancy. Unfortunately, 14 cases lost follow-up and the remaining 5 out of 8 cases presented with massive effusion and positive for malignancy on follow-up. 3 cases were of bronchiectasis with pleural effusion.

There is an increased role of cytocentrifuge and cell block study which not only increases the cellularity, but cellular morphology, nuclear and cytoplasmic details, are better appreciated. We can reduce false negative results and increase diagnostic sensitivity and specificity. Also, cell block carries advantage of performing immunohistochemistry which helps in the diagnosis and can be used for typing of tumor without invasive tissue biopsy.^{4,23,24}

5. Conclusion

Preliminary cytological analysis of various fluids remains the simple, relatively painless, convenient, less time consuming, cost effective, first line method in arriving at the diagnosis and to understand the disease progression. This thereby reduces the need for invasive investigations and their related complications. Cytological analysis of serous effusions have a better diagnostic performance vis-a-vis needle biopsy as the population of cells acquired in a sediment is representative of a larger surface area than the latter. It is especially helpful in evaluating and staging malignancies thereby guiding the clinician in further management. This result in the upstaging or down staging of tumor and thereby affects treatment plan and prognosis for the patient. Some cases may present major interpretative challenges to the pathologist like presence of reactive mesothelial cells which at times poses difficulty in diagnosis, being close mimicker of malignancy. These limitations can be overcome by cell block, histopathology and immunohistochemistry that are usually diagnostic.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

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Author biography

Avani Tiwari, Assistant Professor

Ajay Singh Thakur, Assistant Professor

Pradeep Chandrakar, Associate Professor

Amit Choraria, Assistant Professor

Vivek Choudhary, Director, Professor and Dean

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