ORIGINAL ARTICLE

Diagnostic accuracy of cytological smears versus cell block preparation in malignant fluid aspirates with histopathological correlation

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Introduction: Serous effusion cytology is a minimally invasive, readily accessible and affordable diagnostic technique.It has several advantages like aiding the clinician to formulate a differential diagnosis, assess impact of therapeutic intervention, monitor the course and predict the prognosis of the disease. It also permits one to follow the impact of therapeutic interventions and monitor prognosis of the disease. **Aim and Objective**: To study the diagnostic accuracy of both cytological smear and cell block preparation in the malignant fluid aspirates and correlating their results with histopathological findings. **Materials and Methods**: About 50 fluid samples were divided into two halves; one part was routinely processed for centrifuged cytological smears and other half was subjected to cell block procedure with plasma thromboplastin technique. **Results**: In our study, cell blocks showed sensitivity of 91%, specificity of 100%, positive predictive value (PPV) of 1% and negative predictive values (NPV) of 0.6% and accuracy of 0.92 whereas conventional smears showed sensitivity of 86.36%, specificity of 100%, PPV of 1%, NPV of 5% and accuracy of 0.88. **Conclusion**: Cell block is an outstanding complementary technique for improving the cytodiagnosis in effusion studies when applied in conjunction with smears offering more accuracy, diagnostic yield and higher sensitivity.

KEY WORDS: Cytological smear; cell block; fluid cytology; malignant aspirate

INTRODUCTION

The advent of serous effusions cytology started back in the 19th century with Lucke and Kleb being the prime investigator who identified malignant cells in ascitic fluid.[1] Since then, effusion cytology has been regularly reported in medical literature and has now become a routine diagnostic modality worldwide.

Serous effusion refers to the accumulation of excess fluid within the body cavities like the pleural, pericardial and peritoneal cavity. Effusion consistently offers information about an underlying pathology and in clinical practice is considered as important diagnostic sample. Serous effusion cytology is a minimally invasive, readily accessible and affordable diagnostic procedure. It is widely used in the initial evaluation of causation of fluid collection in the body cavities. This routine cytopreparatory technique consists of sample centrifugation, smearing of the cell deposits and papanicolaou staining.[2] It has the following advantages first, assist the clinician to formulate and point out the causation of effusion and formulate a list of differential diagnosis. Second, it also permits one to follow the impact of therapeutic interventions and monitor prognosis of the disease.[3]

Cytodiagnosis by conventional smears have lower sensitivity due to overcrowding of cells, cell loss and different laboratory processing methods.[4] Hence, conventional cytological smear poses a diagnostic difficulty in accurate identification of malignant or reactive mesothelial cells.[5] A cell block

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is a type of preparation by which cytological material is compacted into a pellet and embedded in paraffin blocks for further processing.[6,7] Cell blocks have several advantages over cytological smears which may facilitate the diagnosis of malignancy[8] such as a better visualisation of the tissue architecture, an easier cytomorphological differentiation between reactive mesothelial cells, mesothelioma or metastatic adenocarcinoma. The possibility of processing multiple sections for immunocytochemistry (ICC) and other specialized tests.[8]

Studies such as Bhanvadia *et al.*, Farahani *et al.* have also shown discrepancy in the results even though cell block and cytological smear have the same sensitivity.[3,9] To assess this difference in results there is a need to study the diagnostic performance of cytological smears and cell block preparation in malignant fluid aspirates with histopathological correlations. This evaluation can further establish the role of cell blocks as an additional technique for improving diagnostic accuracy of serous effusion cytology.[10]

METHODOLOGY

This was a hospital-based prospective observational study. It was carried out in the Department of Pathology, Rohilkhand Medical College and Hospital, a tertiary care hospital in Rohilkhand region, Bareilly, U.P. The study duration was 1 year from 1st November 2019 to 31st October 2020. Fifty fluid samples received in the department for malignant cell examination were included in the study period. Any sample which was inadequate for evaluation was excluded from the study.

Fluid Collection

Standard precautions were observed while collecting the fluid samples. Along with patient demographics other details such as clinical and radiological findings including purpose, date and time of collection and receiving the sample in the laboratory were obtained from the patient's records. Separate consent was not needed from the patients other than that obtained before fluid tap by the treating physician. All fluid samples were initially examined for characteristics such as amount, color, appearance, consistency, temperature, and evidence of clotting. The samples were subsequently processed before microscopic examination.

Fluid Processing

The fluid samples were divided into two equal parts. First part was used for conventional cytological smear preparation while the second part was used for cell block preparation. A representative volume of the sample fluid (10–15 ml) was centrifuged at 2000 rpm for 10 min. Subsequently, majority of the supernatant was gently decanted without disrupting the intact sediment. Smears were made from this sediment containing the newly formed cell button. Thereafter, the sediment was spread gently on the slide to make a thin evenly spread smear. Minimum of two smears were prepared one was air dried stained with Geimsa and another was wet fixed stained with Papanicolaou stain. The ideal fixative was used, i.e., 95% ethanol.

The other part of the fluid sample is centrifuged at 2500 rpm for 10 min. Supernatant is removed and the fresh unfixed sediment deposit is mixed with two drops of pooled plasma. Subsequently, two drops of thromboplastin are added and mixed. This mixture is allowed to stand for 2 min. The resultant clot is wrapped in a premoistened filter paper and placed in a cassette. The tissue cassette is fixed into a jar containing buffered formalin fixative for at least 4 h. After this, cell blocks are embedded in paraffin and sectioned at 4–6-µ thickness. They will be stained with the Hematoxylin and Eosin stain. Special stains such as the Periodic acid Schiff with diastase staining, immunohistochemical (IHC) and mucicarmine stain can be performed wherever necessary.

Fluid Examination and Reporting Outcomes

The fluids were examined and reporting was done as per institutional guidelines. In order to facilitate comparison between the two methods we simplified the diagnosis as benign and malignant. For cell block we considered no significant findings, few atypical cells and negative as benign category while for cytological smear, few atypical cells and negative were categorised as benign. The gold standard against which the diagnosis was compared was the final diagnosis that was recorded using all available patient data such as clinical features, medical history, biochemistry, histopathology, clinical imaging and other relevant investigations.

Statistical Analysis

The patient data were recorded as per the proforma in a secure Microsoft Excel database. The statistical analysis and the graphs were prepared using IBM Statistical Package for Social Sciences Version 26.

OBSERVATIONS AND RESULTS

The study comprised a total of 50 samples out of which 21 (42%) were pleural and 29 (58%) were peritoneal fluid. The age of the patients in the study group ranged from 17 to 90 years with maximum peak between 41 and 50 years. The mean age was 52.52 ± 15.91 years. There were 17 males (34%) and 33 females (66%) among the total cases in the study.

Fluid Distribution

Majority of the samples were pleural fluids (52.4%) in males and (47.6%) in females, followed by peritoneal fluid (20.7%) in males and (79.3%) in females. Other type of fluids such as pericardial, cerebrospinal fluid, BAL, synovial fluid, fluid from cystic lesions were not received during the study period and hence were not taken among the study group.

Clinical Features

The patients who were provisionally diagnosed with pleural effusion presented with chest pain in about 27 cases, whereas others had various types of lesions in the abdominal cavity and presented with ascites, abdominal discomfort and distension in

27 cases. Other symptoms like shortness of breath were present in 33 cases, fever in eight cases and cough in 16 cases were also frequently shown [Table 1].

Fluid Characteristics

The various characteristics of fluid such as volume, color, appearance, and clot formation were also taken into consideration to assess the physical nature of the fluid. Depending upon the color, the fluids were red in 19 cases (38%), yellow in 21 cases (42%) followed by pale yellow in 7 cases (14%) and reddish brown in three cases (6%). Based on appearance, they were categorized as clear fluid in 8 cases (16%), slightly hazy in 6 cases (12%) and hazy in 36 cases (72%). The presence of clot formation was seen in 32 cases (64%) whereas 18 cases (36%) showed no clot formation.

Gross Specimens and their Histopathology Diagnosis

Out of 50 cases, histopathology specimens were not received for 36 cases (72%) for microscopic examination. Among the rest, there were 6 specimens (12%) of uterine mass with bilateral adnexa, 3 (6%) pleural biopsies, 3 (6%) peritoneal biopsies and 2 (4%) colonoscopic biopsies were processed and reported for confirming the diagnosis. The distribution of specimens and their microbiological diagnosis are shown in Figures 1 and 2.

Outcome Reporting

In cell blocks, 40 (80%) out of 50 cases were positive for malignant cells, only 1 case (2%) was showing few atypical cells, 3 cases (6%) were negative for malignant cells, 6 cases (12%) had no significant findings. In smear cytology, 37 (74%) out of 50 cases were positive for malignant cells, only 1 case (2%) was suspicious for malignant cells, 11 cases (22%) showed few atypical cells and 1 case (2%) was negative for the malignant cells. For the malignant final diagnosis, most of the fluids were peritoneal (26 vs. 18 pleural) and were from female subjects (21 out of total 26). This trend was seen consistently in both malignant cell blocks and malignant cytological smears. We calculated the performance metrics for the two techniques using the final diagnosis as the gold standard. For cell block the sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) and accuracy were 90.91%, 100%, 1, 0.6 and 0.92, respectively. For cytological smear the sensitivity, specificity, PPV, NPV and accuracy were 86.36%, 100%, 1, 0.5 and 0.88, respectively. Cohen's κ was then used to determine if there was agreement between cytological smear and cell block. There was moderate agreement between the two tests, $\kappa = 0.419$ (95% confidence interval [CI]: 0.121 and 0.717 $P = 0.003$).

DISCUSSION

We received a total of 50 fluid samples that were subjected to conventional smear preparation and cell block method. Out of 50, majority of them were peritoneal effusions (29), followed by pleural fluids (21). The studies by Khurram *et al.*[11] and Shivakumarswamy *et al.*[12] showed similar fluid distribution to our study. In studies by Thapar *et al.*,[4] Padmavathi *et al.*,[13] Matreja *et al.*,[5] Bansode *et al.*,[14] Agrawal *et al.*[15] and Kumar *et al.*[16] the most common effusion was these pleural followed by the peritoneal effusions. The random nature of case selection based on strict inclusion and exclusion criteria's may have led to the difference in fluid samples studied across various studies.

In the present study, majority of the samples were pleural fluids followed by peritoneal fluids. Both modalities, the

Table 1: Shows the gender wise distribution of patient complaint in the study

Patient complaint	Gender				Total (*n*=50)
	Male		Female		
	Frequency (*n*)	Percentage (%)	Frequency (*n*)	Percentage (%)	
Chest Pain					
Yes	12	44.4	15	55.6	27
No	5	21.7	18	78.3	23
Abdominal discomfort					
Yes	4	14.8	23	85.2	27
No	13	56.5	10	43.5	23
Shortness of breath					
Yes	15	45.5	18	54.5	33
No	2	11.8	15	88.2	17
Cough					
Yes	7	43.8	9	56.3	16
No	12	28.6	30	71.4	42
Fever					
Yes	5	62.5	3	37.5	8
No	12	28.6	30	71.4	42

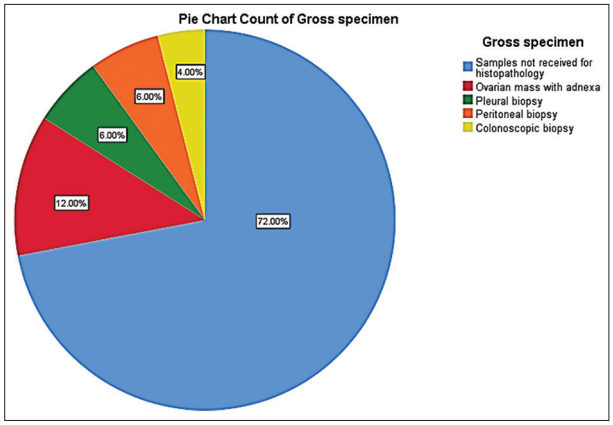

Figure 1: Shows the pie chart distribution for gross specimen in the study

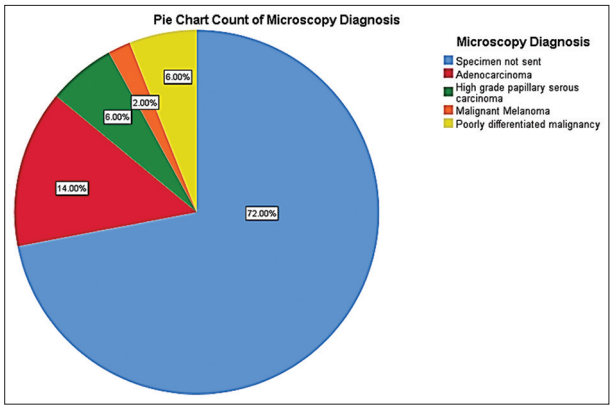

Figure 2: Shows the pie chart distribution for microscopic histopathological diagnosis in the study

cytological smears and cell blocks were separately categorized and subsequently compared. The fluid samples were similarly divided into the two equal halves, one part was routinely processed for cytological smear method and the other half was subjected to cell block procedure according to plasma thromboplastin method, routinely processed and stained with hematoxylin and eosin dye. In the study, it was found that on cytological smear, 74% cases were positive for malignancy, 22% showed few atypical cells, 2% were suspicious of malignancy and 2% were negative for malignancy. In Bansode *et al.*,[14] on conventional smear, out of 19 cases in suspicious category malignancy was confirmed in one case and showed acinar architecture were diagnosed as adenocarcinoma on cell block, 11 cases were suspicious on cell block also and as IHC which was also an added advantage to cell block, six cases were then diagnosed as negative and five cases were as positive for malignancy. Similar findings were found in the studies of Shivakumarswamy *et al.*[12] and Thapar *et al.*[4]

There were 14 cases in this study which were accompanied by the gross specimens in the histopathology section. The gross histopathology specimens were not received for 36 cases (72%) for microscopic examination. Among the rest, there were 6 specimens (12%) of uterine mass with bilateral adnexa, 3 (6%) pleural biopsies, 3 (6%) peritoneal biopsies and 2 (4%) colonoscopic biopsies were routinely processed, stained by the hematoxylin and eosin stain and reported for confirming the diagnosis.

The microscopic evaluation in our study showed mainly adenocarcinomas (14%) which included metastatic adenocarcinoma (2%), well differentiated adenocarcinoma (2%), non-small cell variant of adenocarcinoma (2%) and papillary mucinous adenocarcinoma of intestinal type (2%). Other microscopic findings which were appreciated during the study were high grade papillary carcinoma (6%), malignant melanoma (2%) and poorly differentiated malignancy (6%). Two representative cases are shown in Figures 3 and 4.

In our study, on cell blocks 80% cases were positive for malignancy, 12% had no significant findings, 2% were suspicious of malignant cells and 6% were negative for malignancy. Cell blocks confirmed the cytological smear diagnosis in majority of cases and correlation with histopathologic findings in some cases established a specific diagnosis. Cell blocks showed sensitivity of 90.91 %, specificity of 100 %, PPV of 1%, NPV of 0.6% and accuracy of 0.92. Conventional cytological smears showed sensitivity of 86.36%, specificity of 100%, PPV of 1%, NPV of 0.5% and accuracy of 0.88. There was moderate agreement between the two tests, $\kappa = 0.419$ (95% CI: 0.121 and 0.717 $P = 0.003$). Increased accuracy of cell blocks over the cytological smears in diagnosing the malignancy making cell block more superior than smears was also noted by Matreja *et al.*[5] and Thapar *et al.*[4]. Also, Farahani *et al.*[9] concluded that serous effusion cytology showed high specificity and moderate sensitivity while evaluation of these effusions. There were 21 cases on cytological smear and also the cell block were found to be positive for malignancy in Bansode *et al.*[14] and the diagnostic yield of 6.33% for malignancy was significantly increased by cell block method. Similar findings were also reported by Padmavathi *et al.*[13] on cell block method increasing diagnostic utility by 10%. The cell block technique has not only increased the positive results but it has also helped to demonstrate better architectural patterns which could be of great help in approaching the correct diagnosis of the primary site. Similar findings are also reported by Agrawal *et al.*[15] The diagnostic accuracy of these conventional cytological smear was 76% while that of cell block was 93% as discussed by Khurram *et al.*[11] Over the last decade, it is evident that ICC is superior to conventional techniques in the diagnostic workflow of effusion cytology. Other ancillary techniques such as special stains, molecular studies such as Polymerase Chain Reaction, Fluorescence *in-situ* hybridization (FISH), gene fusion analysis, protein overexpression and next generation sequencing may be helpful in subtyping of numerous malignancies for therapeutic purposes. These developments are exciting and will definitely be game changers in the field of effusion cytology.

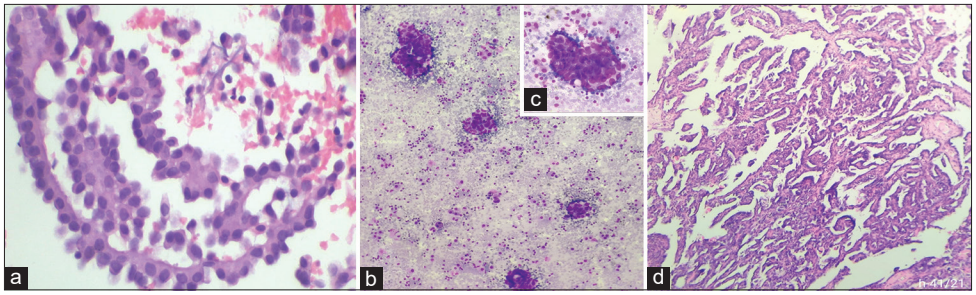

Figure 3: (a) Shows round to oval shaped neoplastic cells with numerous papillae suggestive of papillary serous carcinoma on cell block (H&E; 40×). (b and c) Shows irregular clusters of pleomorphic neoplastic cells with coarse chromatin and inset shows magnified view of these malignant cells (L&G; 40×). d from the papillary growth shows tumor cells invading the stroma arranged as papillae favoring high grade papillary serous carcinoma (H&E;40×)

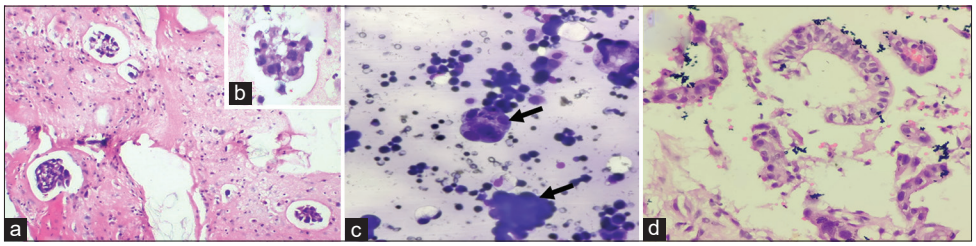

Figure 4: (a) Shows cluster of malignant cells in a cluster forming a acini like appearance and inset (b) shows magnified view of the malignant cells favoring adenocarcinoma on cell block (H&E; 10×). (c) Shows tight clusters of atypical epithelial cells (L&G; 40×). (d) Shows numerous glands favoring adenocarcinoma (H&E, 40×)

CONCLUSION

Cell block is an outstanding complementary technique for improving the cytodiagnosis in effusion studies when applied in conjunction with cytological smears. The technique offers more accuracy, diagnostic yield and higher sensitivity than conventional smears. Cell blocks are helpful in establishing the primary site for malignancy in effusion and metastatic lesions by providing higher cellularity, various better architectural patterns and morphological features with additional yield for the malignant cells as compared to the conventional cytological smears. Hence, they appear to form a bridge between cytopathology and histopathology. Cell block technique is central for the future of cytology as it is less invasive to the patient, offers numerous advantages and more can be done with less material.

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