ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 03 | March - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARISON OF BODY FLUID CELL COUNTING BETWEEN AUTOMATED HEMATOLOGY ANALYZER AND MANUAL MICROSCOPIC METHOD

Pathology

Dr Purbita Chatterjee	Resident , Department Of Pathology , Subharti Medical College
Dr Sangeeta Sharma	Professor , Department Of Pathology, Subharti Medical College
Dr Navya Jaiswal	Assistant Professor , Department Of Pathology, Subharti Medical College
Dr Rani Bansal	Professor , Department Of Pathology, Subharti Medical College
Dr Anjali Khare	Professor And HOD , Department Of Pathology , Subharti Medical College
Dr Archita Kansal Tiwari*	Assistant Professor , Department Of Pathology , Subharti Medical College *Corresponding Author
Dr Vertika Sharma	Resident , Department Of Pathology, Subharti Medical College

ABSTRACT

Cytological study of fluids is an inexpensive, simple procedure and has significant utility in diagnosing neoplastic and non neoplastic lesions. The cytological examination of fluids in combination with physical examination helps identify aetiologic agents, follow the natural process of the disease and monitor the response to the treatment.Aim: To determine the diagnostic utility of serosal fluid cytology and analyse the incidence of neoplastic and non neoplastic lesions using serous fluid cytology.Materials and Methods: This was a descriptive cross-sectional study comprising 311 cases conducted in a tertiary care hospital.Purposive sampling was used to recruit the participants. All the patients with pleural effusions, ascites or in whom Cerebral Spinal Fluid (CSF), pericardial and synovial fluids examination was indicated were included. The provisional diagnosis was obtained from case sheets, including relevant clinical information. Smears were prepared from freshly tapped specimens without adding anticoagulants and were processed by routine, conventional smear technique. The data were analysed using the SPSS version 22.0 for Windows. Numerical variables were reported as frequency and percentage. The chi-square test was used wherever necessary, and the p-value less than 0.05 were considered significant.Results: The peritoneal fluid was the most common fluid collected in the present study, followed by pleural fluid and CSF. The malignancy rate in the present study was 19 (10.4%) of peritoneal fluid, 6 (5.9%) for pleural fluid, and 2 (4.1%) for CSF. Conclusion: Adenocarcinoma was the most common malignancy found in this study, which was in concordance with the research conducted earlier, where gold standard investigations confirmed the findings. In the peritoneal fluid, most of the patients had cirrhosis and tuberculosis. In pleural fluid and cerebrospinal fluid, most of them had tuberculosis and chronic inflammatory conditions, respectively. Previous researchers confirmed similar findings in their studies. It is seen that malignant and benign conditions like tuberculosis can be diagnosed well with effusion cytology.

KEYWORDS

serosal cytology, CSF, pleural , automated analyser

INTRODUCTION

The pleura, pericardium, and pelvis are all covered in serous body cavities, which are mesothelium-lined potential spaces(peritoneum). A tiny amount of fluid, called an ultrafiltrate of plasma, normally aids in the movement of the organs. Fluid may build up and cause an effusion if the generation and resorption of this plasma ultrafiltrate are not correctly balanced [1].

Effusion is the accumulation of fluid in the body cavity. This fluid during disease process undergoes abnormal and disproportionate qualitative and quantitative changes[2]. Pathological conditions like congestive heart failure, hepatic cirrhosis, hypoproteinemia, infections like bacterial pneumonia and tuberculosis, neoplastic conditions like mesothelioma, metastatic carcinoma, and other conditions like trauma, pancreatitis, and peritonitis are the common cause of pleural and peritoneal effusion. Exudative and transudative kinds of effusion exist. Transudative cells have lower levels of protein than exudative cells and a higher overall cell count, low number of total cells [2]. This study was done to compare the results of cytological analysis of body fluids by manual and automated method.

MATERIALS AND METHODS

The present study was a hospital based prospective cross-sectional study conducted in the Department of Pathology at Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital from August 2020-Nov 2022.

Sample Size: 311 cases
Study Design- Hospital Based Cross-sectional Study

Inclusion Criteria

- All body fluid sample

Exclusion Criteria

- Insufficient quantity <1ml.
- Fluids with coagulum, purulent and flocculent samples, turbid samples
- Pediatric age group.
- Sputum and seminal fluid

Procedure

- Fluids were collected in a sterile wide-mouth universal container with proper labellingand identification number.
- Processing was done immediately after receiving.
- If there was any delay in transport it was refrigerated at 4 degree Celsius.
- Essential clinical details were recorded.
- Physical examination (volume, color, appearance and coagulum) and microscopic examination of fluid in the form of TLC and DLC was done.
- For TLC fluid was examined manually by modified Neubauer chamber and automated analyzer HORIBA H2500.

For DLC, after centrifugation for (1500 rpm for 5 min/ cytospin) is stained with May Grunwald Ginemsa (MGG) for dry smear and Haematoxylin and Eosin (H&E) and Papanicolaou (Pap) for wet smear. Smears are examined and cytological findings were noted. Biochemical Analysis of the fluid was recorded.

Manual Method

- An improved Neubauer chamber (Ref. Photomicrograph 2) was used to count the total WBC's in the BF's.
- The WBC's were dyed using WBC diluting fluid (Turks-water: glacial acetic acid: gentian violet =97:2:1) and then the haemocytometer's counting chamber was filled using a glass

capillary at a 400x magnification (Fluid depth at 0.1 mm).
- The total number of cells in the four large corner primary squares containing 16 smaller secondary squares each with area of 0.004 mm2 was counted.
- In cases where there was very low WBC count, all 9 primary squares were counted. 1:10 or 1:20 was used depending on the number of cells in specimen.
- WBCs/µL = Total number of cells x dilution factor Volume.

Automated Method
- Using automated analyzer Horiba H2500 (Ref. Photomicrograph 1) with a specific BF mode, the total WBC and limited differential counts (PMNs and MNs) were determined.
- The equipment uses flow cytometry and hydrodynamic focusing technologies to identify and cluster each cell type. Sample pre-preparation is not required prior to its analysis.
- Analyzer aspirates 85 µL of sample in open mode for one time analysis and uses nucleic acid dye, fluorescent light scatter (Size of cell) with side scatter (Inner complexity) light and fluorescence intensity (DNA/RNA concentration) to determine the total and limited differential counts in the _DIFF' channel.
- Cells with increased fluorescence such as macrophages and mesothelial cells are also counted in DIFF scattergram.
- —WBC abnormal scattergram flag will be generated when cells present in sample exceeds instrument cut-off value

RESULTS

The present cross-sectional study was conducted in the Department of Pathology at Subharti Medical College and associated Chhatrapati Shivaji Hospital. A total of 311 body fluids samples were evaluated from Dec. 2020 to May 2022. Cytological analysis was done in all cases and clinicopathological correlation was done, and comparative analysis of body fluid was done by automated and manual method in 232 cases. Age of the patients in the present study ranged from 21-89 years. Out of 311 samples, maximum were in the age group of 18-30 years (24.76%) followed by 41-50 years (23.47%).

Males (57.23%) were comparatively more as compared to females (42.77%) in present study.

Based upon the biochemical analysis and cell count, body fluids were divided into transudate and exudate. Exudative fluid was found in 58.52% of the cases while transudative fluid in 41.48% of the cases.

Transudative nature was found maximum in CSF fluid(30.55%) while exudative nature was revealed mostly in ascitic fluid(47.59%). When transudate/exudate distribution was compared according to fluid type using chi square test, statistically significant difference was found as $p<0.05$.

Correlation Of Clinical Diagnosis With Different Type Of Body Fluids.

Clinical Diagnosis	Frequency(n)	Percentage(%)
Peritoneal Fluid (n=148)		
Chronic Liver Disease	103	69.59
SBP (Subacute Bacterial Peritonitis)	15	10.13
Abdominal Tuberculosis	20	13.51
Pancreatitis	10	6.75
Pleural Fluid (n=66)		
TB	38	57.57
Pneumonia	26	39.39
CHF	2	3.03
CSF (n=93)		
Meningitis	92	98.92
VP Shunt	1	1.07
Other Inflammatory Condition	1	1.07
Pericardial Fluid (n=2)		
Myocardial Infraction	2	100%

Ascitic (47.59%) was the most common type of fluid obtained in this study followed by CSF (30.55%) and pleural fluid (21.22%). Pericardial fluid (21.22%) was the least common type of fluid received. Total cell counts of body fluid (cells/cubic mm) was found more in automated method as compared to manual method. When total cell count was compared between automated and manual method using t test, statistically significant difference was found as $p<0.05$.

Mean polymorphs (%) was found more in automated method as compared to manual method with statistically significant difference was found as $p<0.05$.

Mean lymphocytes (%) reported by automated method and manual method was 51.11±8.17 and 32.48±6.28 respectively. When lymphocytes (%) was compared between automated and manual method using t test, statistically significant difference was found as $p<0.05$.

Mean RBC (cells/cubic mm) was found more in automated method as compared to manual method. When RBC was compared between automated and manual method using t test, statistically significant difference was found as $p<0.05$

DISCUSSION

Effusion cytology dates back to the 19th century. Since then, effusion cytology has gained tremendous importance in the medical literature. The cytological interpretation of individual cells that are exfoliated into these fluids is of paramount importance since they provide an insight into the diagnostic, prognostic and therapeutic aspect of various pathological process in the body. A better knowledge of spectrum of clinical history and clinical signs of pleural, peritoneal, pericardial analysis, along with radiological, biochemical and cytological evaluation of the fluids helps in narrowing the diagnostic dilemma faced by physicians and helps in better management of patients. Body fluids are segregated into transudates and exudates. Cytological analysis comprises of microscopic examination, wherein the total and differential cell count is performed using a hemocytometer. In our study automated hematology analyzers (Horiba H2500) with a Body Fluid Mode (BFM) are provided for the extension of its function in the analysis of cells in BFs.[3]

Due to dearth of literature in automated vs manual microscopy studies, the present cross-sectional study was conducted in the Department of Pathology at Subharti Medical College and associated Chhatrapati Shivaji Hospital among 311 body fluids samples received in In the present study majority of cases were peritoneal, (47.59%) was the most common type of fluid obtained in this study followed by CSF (30.55%) and pleural fluid (21.22%). Pericardial fluid (21.22%) was the least common type. It is comparable to the studies [4] . In contrast study [5] showed pleural fluid is predominant. This difference could be because of nature of cases received in health care facilities and selection of cases based on inclusion and exclusion criteria. The aim of the study was to compare cytological analysis of body fluids by manual vs automated method with reference to a) Total cell count b) Differential cell count and clinicopathological correlation of the fluid.[6]

The present study showed slightly male predominance over female, male (57.23%) and female (42.77%).

Based on biochemical analysis fluids were divided into exudative and transudative type of effusion. Transudative has less protein content, low total cell count while exudative have high protein content and raised total cell count.[7] Out of 311 cases, 129(41.48%) were transudative fluid and 182(58.52%) were exudative fluid. Transudative nature was found maximum in CSF fluid while exudative nature was revealed mostly in ascitic fluid. When transudate/exudate distribution was compared among the study subjects according to fluid type using chi square test, statistically significant difference was found as $p<0.05$.[8]

Sheetal at al[35] assessed 375 samples comprising of peritoneal fluid 183(48.8%), followed by pleural fluid 102(27.2%), cerebrospinal fluid 49(13.1%), pericardial fluid 24(6.4%) and synovial fluid 17(4.5%)[9].He distributed cases on the basis of effusion with majority of patient had cirrhosis 95(51.9%) followed by tuberculosis 24(13.1%) and cancer 19(10.4%). Similarly Anita b et al conducted a study on 70 patients comprising of pleural fluid (31), pericardial fluid(5) and peritoneal fluid(34).He did clinic cytological correlation with cirrhosis of liver(70.6%) followed by abdominal tuberculosis(23.6%).Both the studies are similar to our study where clincocytological correlation was done of 311 body fluid samples with chronic liver disease 65.5% followed by tuberculosis(57.7%).[10]

CONCLUSION

Evaluation of body fluid is simple, quick safe and cost-effective process which helps the treating clinicians to reach a diagnosis and to understand the disease progression. The automated approach performed the bodily fluid analysis with great, fundamental accuracy. For the assessment of cerebral fluid, pleural fluids, and the ascitic fluids cell counts, an automated approach is a helpful extra tool, particularly in an emergency situation. It provides the reproducibility, precision, accuracy, rapid and reliable data on RBC and WBC count, and is easier for quality control and standardization than manual method. Fully automated analysis meets time and quality requirements and are objective in material handling. Moreover, automated method would bring homogeneity between different laboratories. Further studies with large number of samples are required to draw a definite conclusion.

Acknowledgement : None
Informed Consent: written informed consent was taken from patients
Ethical Approval: ethical committee approval was taken from the institutional committee of ethics.
Source Of Funding- funding source was self
Conflict Of Interest – there was no conflict of interest

REFERENCES

1. Hussian AN. The lung. In; Kumar V, Abbas AK, Aster JC (eds), Robbins and Cotran Pathologic Basis of Disease. 1st Edition. Elsevier India. 2014; 15:669- 726.
2. Shivankumarswamy U, Arakeri SU, Karigowdar MH, Yelikar BR. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. Journal of Cytology.2012; 29(1):11-5.
3. Bhanvadia VM, Santwani PM, Vachhani JH. Analysis of diagnostic value of cytological smear method vs cell block method in body fluid cytology: a study of 150 cases. Ethiop J Health Sci.2014; 24(2):125-31.
4. Bales CE, Laboratory techniques. In; Koss LG, Melaned MR (eds) Koss' Diagnostic Cytology and It's Histopathologic Basis. 5th Edition. Wolters Kluwer; New York. 2006:1467-512.
5. Thapar M, Mishra RK, Sharma A and Goyal V. Critical analysis of cell block versus smear examination in effusions. J Cytol.2009; 26(2): 60-4
6. Shubhada B, Nayak S, Kumbhalkar D. Evaluation of Cell Block Technique in the Cytodiagnosis of Body Fluids. 2013; 4(7):87-94.
7. Madakshira MG, Kolavadi SS, Varma V, Bhardwaj R. Cell block- A useful adjunct in cytopathology of serous effusions. National Journal of Lab Med.2017; 6(2):26-31.
8. Chu AY, Litzky LA, Pasha TL, Zhang PJ. Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. Journal of Modern Pathology. 2005; 18: 105–10.
9. Reagan JW. Exfoliative Cytology of Pleural, Peritoneal and Pericardial Fluids. CA Cancer J Clin. 1960; 10: 153-9.
10. DeMay RM. Fluids. In; The Art and Science of Cytopathology.2nd Edition. Erik and Lisa Tanck Hongkong. 2012: 268-89.