A COMPARATIVE STUDY OF CYTOLOGIC EVALUATION BETWEEN CONVENTIONAL METHOD & LIQUID BASED CYTOLOGY IN PLEURAL, PERICARDIAL & PERITONEAL FLUIDS

Pathology

Dr. R. P. Siddiqui	Associate Professor, Department of Pathology, Pt. JNM Medical College and Dr. BRAM Hospital, Raipur (Chhattisgarh).
Dr. Mohd. Jafar Memon	Assistant Professor, Department of Pathology, Pt. JNM Medical College and Dr. BRAM Hospital, Raipur (Chhattisgarh).
Dr. Shraddha Sahu*	Postgraduate Student, Department of Pathology, Pt. JNM Medical College and Dr. BRAM Hospital, Raipur (Chhattisgarh). *Corresponding Author

ABSTRACT

Background- The diagnostic detection of malignant cells in effusions serves as a mainstream diagnostic tool and a predictor of the spread of diseases. **Aim-** To compare diagnostic efficacy of liquid based cytology (LBC) with conventional cytopreparatory techniques of pleural, peritoneal and pericardial fluids. **Methodology** - 110 peritoneal, pleural and pericardial effusions were received from indoor of various departments, microscopic examination was carried out by conventional and SurePath LBC method. For each case scoring was done for cellularity, cell morphology, distribution and background. They were classified as Non-neoplastic, suspicious for malignancy and malignant. **Results** - LBC showed better cell yield, morphology, distribution and background. Non-neoplastic lesions were maximum in number and constituted 78 (70.9%) cases, 8 (7.3%) cases were suspicious for malignancy, 22 (20%) malignant cases and 2 cases were unsatisfactory for evaluation. **Conclusion-** compared to conventional method, LBC was superior in making diagnosis.

KEYWORDS

Peritoneal, pleural, pericardial fluid, LBC, conventional method

INTRODUCTION

The pleural, pericardial, and peritoneal cavities are lined by a single layer of flat mesothelial cells called the serosa. The parietal and visceral layers are separated by a cavity containing only a small amount of pleural, pericardial and peritoneal fluid, enough to lubricate the adjacent surfaces. In disease states, a greater amount of fluid, an effusion accumulates.^{1,2}

The diagnostic detection of malignant cells in effusions serves as a mainstream diagnostic tool and a predictor of the spread of diseases with impact on prognosis and upstages prognostic morbidity. Hence the routine cytological preparations represents a simple, safe and cost effective method limiting the amount of issues associated with biopsy procedures.^{3,4,7}

Liquid based preparation (LBC), which has been approved by the US Food and Drug Administration since 1996 (and was originally developed for cervical samples), has gained popularity as an alternative technique for the collection and preparation of cytologic specimens from many different sites in the body, including effusions, with good results.^{8,9,10}

In recent years liquid based cytology has emerged as an alternative to conventional cytopreparatory (CP) methods. Liquid based cytology (LBC), enables cells to be suspended in a monolayer. It makes better cytological assessment possible with improved sensitivity and specificity, since fixation is better and nuclear details are well preserved in the technique.^{1,11}

MATERIAL AND METHOD

The present study was an observational, prospective type of cross-sectional study. This study was conducted in Cytology Laboratory, Department of Pathology, Pt. J.N.M. Medical College & Dr. B.R.A.M. Hospital, Raipur (Chhattisgarh), India. A total of 110 cases of peritoneal, pleural & pericardial effusions were collected & examined from indoor of various departments of hospital. The fluids not freshly collected (>24 hrs of collection of fluids) were excluded.

After receiving the sample, gross examination of fluid was done for – Volume, colour, appearance, then further processing was done. Two types of techniques were involved in this study- Conventional cytology method and Liquid based cytology method. For this fluid was divided into two parts for conventional and LBC.

For CP slides were made from the centrifuge deposit. For LBC sediment obtained from centrifuge deposit was mixed with cytorich fluid and left for 30 min, vortexed and processed in the automated

processor according to manufacturer's recommendation. Both the slides were stained with H & E, PAP and MGG stain; examined under microscope and scoring was done.^{12,13}

Table 1 - Showing Scoring System

Parameter	Quantitative Description	Point Score
Background blood or proteinaceous material	1. Large amount, great compromise in diagnosis	0
	2. Moderate amount, diagnosis possible.	1
	3. Minimal, diagnosis easy	2
Amount of cellular material	1. Minimal to absent, diagnosis not possible.	0
	2. Sufficient for cytodagnosis.	1
	3. Abundant, diagnosis simple	2
Cell morphology, cellular degeneration and trauma	1. Marked cellular degeneration, diagnosis not possible.	0
	2. Moderate cellular degeneration, diagnosis possible.	1
	3. Minimal cellular degeneration, diagnosis easy.	2
Distribution of cells	1. Totally in the periphery or sparsely distributed.	0
	2. Combination.	1
	3. Evenly distributed.	2

RESULTS

A total of 110 cases of effusion were taken during period of study, among which 73 cases were of peritoneal & 33 were of pleural effusion and 04 cases were of pericardial fluid.

Table 2 - Analysis Of Conventional And Liquid Based Cytology Technique Under Various Diagnostic Categories

S.N.	Diagnostic categories	Method			
		Conventional		LBC	
		No. of all cases	%	No. of all cases	%
1.	Non-neoplastic	77	70%	78	70.90%
2.	Suspicious of malignancy	11	10%	08	7.27%
3.	Malignant	19	17.27%	22	20%
4.	Unsatisfactory	03	2.73%	02	1.83%
	Total	110	100%	110	100%

Table 3 - Statistical Analysis Of All Morphological Parameters Of Peritoneal, Pleural And Pericardial Fluids

Parameters	Type of fluids	LBC Superior	Conventional Superior	Equivalent	P-value
Cellularity	Peritoneal	47 (64.3%)	03(4.2%)	23(31.5%)	<0.0001
	Pleural	17 (51.5%)	01(3%)	15(45.5%)	<0.0001
	Pericardial	01 (25%)	00	03(75%)	0.79
Cell Morphology	Peritoneal	49 (67.2%)	01(1.3%)	23(31.5%)	<0.0001
	Pleural	18 (54.5%)	01(3%)	14(42.5%)	<0.0001
	Pericardial	01 (25%)	0	03(75%)	0.25
Cell distribution	Peritoneal	56 (76.7%)	0	17(23.3%)	<0.0001
	Pleural	26 (78.7%)	0	07(21.3%)	<0.0001
	Pericardial	02 (50%)	0	02(50%)	0.16
Background	Peritoneal	70 (95.8%)	0	3(4.2%)	<0.0001
	Pleural	30 (90.9%)	0	03(9.1)	<0.0001
	Pericardial	03 (75%)	0	01(25%)	0.05

Table4-Overall Comparative Analysis Of Conventional Technique With LBC

Conventional smear	NM in LBC	Suspicious in LBC	Malignant in LBC	Unsatisfactory in LBC	Total	p Value
NM	72	02	01	02	77	X ² =137.57 P<0.0001
Suspicious	05	06	00	00	11	
Malignant	00	00	19	00	19	
unsatisfactory	01	00	02	00	03	
Total	78	08	22	02	110	

Out of 110 cases, 2 unsatisfactory and 1 negative for malignancy cases in CP were reported as malignant in LBC. Out of 11 cases diagnosed as suspicious in conventional, 5 cases were given negative for malignancy and 6 cases were given suspicious by LBC smears. This study showed liquid based cytology was better than conventional method and difference was found to be highly significant statistically.

Fig 1- Bar diagram showing overall comparative analysis of conventional technique with LBC.

Fig-2Highly cellular smear showing metastatic deposits of IDC breast in pleural fluid, showing clusters and discrete population of moderately pleomorphic cells having hyperchromatic nuclei and prominent nucleoli

with moderate to abundant cytoplasm in clear background (400x, MGG stain, LBC)

Fig–3. Sparsely cellular smear showing metastatic deposits of Ca cervix in peritoneal fluid, showing few discrete and small sheet of squamous cells in haemorrhagic, necrotic and inflammatory background (100x, H&E stain, CS)

Fig – 4. Smear showing metastatic deposits of Ca cervix in peritoneal fluid, showing discrete and sheet of squamous cells in mildly haemorrhagic and inflammatory background (100x, H&E stain, LBC)

Fig–5. Smear showing a microfilaria in peritoneal fluid in inflammatory background (400x, PAP stain, LBC)

Fig–6. Smear showing sheets of benign mesothelial cells in haemorrhagic background (400x, H&E stain, CS)

Fig–7. Smear showing sheets of benign mesothelial cells in clear background (400x, H&E stain, LBC)

DISCUSSION

Out of 73 peritoneal fluids, maximum number of cases obtained score 2 when cellularity (68.49%), cell morphology (76.72%) and distribution (83.56%) were considered, which was in concordance with study of P.

U Swathy et al¹². 95.88% cases obtained a score of 2 in our study while maximum number of cases obtained score 1 in study done by P.U Swathy et al.

Out of 33 peritoneal fluids, maximum number of cases obtained score 2 when cellularity (78.78%), cell morphology (78.78%) and distribution (93.93%) were considered, was in concordance with study of **P. U Swathy et al**¹². 96.96% cases obtained a score of 2 in our study while maximum number of cases obtained score 1 in study done by **P. U Swathy et al**¹².

We found a better cellular yield by LBC (p-value <0.0001) as compared to CP in pleural (51.5%) and peritoneal fluid (64.3%) which was in concordance with the study of **P U Swathy et al**¹² (p-value=0.004), **Babloyan et al**¹⁴, **Amiri et al**¹⁵, **Rishu et al**¹ and **Esther et al**¹⁰.

In contrast no statistical difference / better cellularity was observed in CP by **Dadich et al**¹⁶ (p-value=0.22), **Amiri et al**¹⁵ (p-value=0.13) and **Alwahaibi et al**¹⁷.

Superior morphological details, better cellular distribution and cleaner background were observed with LBC (p-value <0.0001) in pleural and peritoneal fluid in our study and this was in concordance with study of **P U Swathy et al**¹² (p-value=0.000 and 0.001). Better architectural preservation, nuclear details, better staining, preservation of nuclear-cytoplasmic ratio and more 3-dimensional clusters were noted in the study of **Amiri et al**¹⁵, **Rishu et al**¹, **Nasuti et al**¹⁸, **Esther et al**¹⁰, **Ruchita et al**¹⁹, **Gabriel C et al**²⁰. In contrast **Dadich et al**¹⁶ stated that CP provided significantly sharper cytomorphologic features (p-value=0.05).

In present study the non-neoplastic lesions were maximum in number which constituted 70.9%, this is in concordance with study of **Rishu et al**¹, **Amiri et al**¹⁵ & **Ji Hae et al**²¹. Suspicious of malignant cases were 7.27% of cases, **Ji Hae et al**²¹ reported 16.45% of suspicious cases. Among all neoplastic cases, 20% cases were reported as malignant in present study, this is showing concordance with study of **Rishu et al**¹ that reported 16% cases as malignant.

The non-neoplastic cases in peritoneal fluid in our study were 78.8% which was in concordance with study of **Rishu et al**¹ (70%). 57.59% cases of pleural fluid were non-neoplastic in our study while in studies of **Esther et al**¹⁰, **Nasuti et al**¹⁸ and **Porntip et al**²² the non-neoplastic cases constituted 78.9%, 19% and 13.6% respectively. 50% cases of pericardial fluid were non-neoplastic in our study while in studies of **Esther et al**¹⁰ and **Porntip et al**²² the non-neoplastic cases constituted 6.8% and 5.4% respectively.

In present study suspicious cases in peritoneal fluid constituted 4.10%, while in studies of **Amiri et al**¹⁵, **Rishu et al**¹, **Rossi et al**¹ and **Nasuti et al**¹⁸ suspicious cases were 6.3%, 2.7%, 2.05% and 4.7% cases respectively. 12.12% cases were reported as suspicious in pleural fluid of our study while in the study of **Esther et al**¹⁰ suspicious cases were 2.93%. In our study suspicious cases were 25% in pericardial fluid while in study of **Esther et al**¹⁰ it constituted 0.34% cases.

In present study malignant cases of peritoneal fluid were 16.5% cases which were comparable with studies of **Rishu et al**¹ and **Porntip et al**²² constituting 16% and 19% cases respectively. 27.25% cases of pleural fluid were malignant in our study, while **Esther et al**¹⁰ and **Porntip et al**²² reported 10.12% and 35.45% cases respectively. In pericardial fluid 25% cases were reported malignant, **Esther et al**¹⁰ and **Porntip et al**²² reported 0.78% and 0% cases malignant.

To conclude in our study out of 7 suspicious cases reported in CP in peritoneal fluid 4 cases were reported as negative for malignancy in LBC which may be due to better cellular morphology and background in LBC smears. One case reported as unsatisfactory in CP of peritoneal fluid was given malignant in LBC favouring better cellular yield and morphology.

Similarly in pleural fluids out of 22 negative for malignant cases in CP, 2 were reported suspicious, 1 malignant and 1 unsatisfactory in LBC which may be due to better cellular yield and morphology in LBC smears.

In pericardial fluid no difference in reporting of negative for malignancy, suspicious, malignant and unsatisfactory in CP and LBC

was noted which may be due to less number of cases (4/110) in our study.

Overall out of 110 cases in our study, 2 unsatisfactory and 1 negative for malignancy cases in CP were reported as malignant in LBC. Out of 11 cases diagnosed as suspicious in conventional, 5 cases were reported as negative for malignancy in LBC smears which may be due to better cell morphology and background. Moreover LBC requires lesser screening time as compared to CP.

Limitations - Despite of a number of advantages of LBC there are few limitations which include increase in man-hour work and increased cost.

CONCLUSION

LBC in cytology laboratory has increasingly played an important role in diagnosis of body cavity fluids. Our study establishes the superiority of LBC over conventional preparations with regard to cell yield, preservation of cell morphology, uniformity of cell distribution and background. In addition the screening time was reduced in LBC prepared slides due to concentration of cells in a small circular area and clean background devoid of RBC's and debris. In our study few of the unsatisfactory and negative for malignant cases in conventional preparation were reported as malignant in LBC thus indicating improved diagnostic accuracy. There are few limitations of LBC like increased man-hour work, increased cost and altered morphology of cells including small cell clusters, more discohesion with attenuated chromatin details, prominent nucleoli and small cell size. So interpretation of LBC requires an experienced cytopathologist who is familiar with the cytomorphological alteration of the cells in this method.

REFERENCES-

- 1) Rishu Mittal et al. (2016) Study of Ascitic Fluid by Liquid Based Cytology and Its Comparison with Conventional Cytosmears and Cell Block Preparations. *Annals of International Medical and Dental Research*: Vol (2), Issue 6.
- 2) Edmund S. Cibas cytology diagnostic principles and clinical correlates 3rd edition 2009.
- 3) Esther Ross et al. (2017) The Role of Liquid Based Cytology and Ancillary Techniques in the Peritoneal Washing Analysis: Our Institutional Experience. 10.1371/journal.pone.0168625 January 18, 2017.
- 4) Lee YM, Hwang JY, Son SM, Choi SY, Lee HC, Kim EJ et al. (2014) Comparison of diagnostic accuracy between cellprep Plus and ThinPrep liquid-based preparations in effusion cytology. *Diagn Cytopathol* 2014; 42: 384-90.
- 5) Gong Y, Sun X, Michael CW, Attal S, Williamson BA, Bedrossian CWM. (2003) Immunocytochemistry of serous effusion specimens: A comparison of ThinPrep vs cell block. *Diagn Cytopathol* 2003; 28: 1-5.
- 6) Bibbo M, Wilbur DC (2008) Comprehensive cytopathology; in: Marluce B and Nasuti JF (eds): *Evaluations of the Sample in Smears and Liquid-Based Preparations*. Philadelphia, Saunders, 2008; 65-74.
- 7) Bedrossian CWM. (1998) Diagnostic problems in serous effusions. *Diagn Cytopathol* 1998; 19: 131-7.
- 8) Renshaw AA, Dean BR, Antman KH, Sugarbaker DJ, Cibas ES. (1997) The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest*. 1997; 111: 106-109.
- 9) Jing X, Li QK, Bedrossian U, Michael C. (2013) Morphologic and immunocytochemical performances of effusions cell blocks prepared using 3 different methods. *Am J Clin Pathol*. 2013; 139: 177-182.
- 10) Esther Diana Rossi et al. (2015) The Role of Liquid-Based Cytology and Ancillary Techniques in Pleural and Pericardial Effusions: An Institutional Experience. January 14, 2015 in Wiley Online Library.
- 11) Sakamoto H, Takenaka M, Ushimaru K, Tanaka T. (2012) Use of Liquid-Based Cytology (LBC) and Cell Blocks from Cell Remnants for Cytologic, Immunohistochemical, and Immunocytochemical Diagnosis of Malignancy. *Open Journal of Pathology*, 2012; 2(3): 58-65.
- 12) Swathy, P U et al. (2015) Comparison between Liquid Based Cytology and Conventional Cytopreparatory Methods in Body Cavity Fluids: A Study of 100 Cases: <http://repository-tnmgrmu.ac.in/id/eprint/7987>
- 13) Archana Joshi, Nidhi Mahajan, Karmarkar. (2014) Diagnostic utility of various techniques used in body fluid IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 13, Issue 1 Ver. V. (Jan. 2014), 13-18.
- 14) Babloyan S, Voulgaris Z, Papaefthimiou M, Beglaryan G, Kyroudi A, Karakitsos P. (2009) Comparison of quality between conventional and thinprep cytology in investigation of patients with epithelial ovarian cancer. *The New Armenian Medical Journal* 2009; 3(3): 22-28.
- 15) Amiri et al. (2019) Comparison of Conventional Cytology, Liquid-Based Cytology, and Cell Block in the Evaluation of Peritoneal Fluid in Gynecologic Malignancies [krager](https://doi.org/10.1155/2019/1234567). 2019 Jan; 63(1): 63-72.
- 16) Hrishikesh Dadhich et al. (2016) A Comparative Analysis of Conventional Cytopreparatory and Liquid Based Cytological Techniques (Sure Path) in Evaluation of Serous Effusion Fluids Wiley Online Library *Diagnostic Cytopathology*, Vol. 00, No 00.
- 17) Nasar Yousuf Alwahaibi, Nasra Said Alnoumani, Usha Rani Bai. (2014) Comparison of ThinPrep® and Conventional Preparations for Peritoneal and Pleural Cytology Smears. *Annual Research & Review in Biology* 2014; 4(20): 3139-49.
- 18) Joseph F. Nasuti et al. (2001) Diagnostic Value of Liquid-Based (ThinPrepT) Preparations in Nongynecologic Cases Wiley Online Library *Diagnostic Cytopathology*, Vol 24, No 2.
- 19) Ruchit et al. (2017) Impact of SurePath® liquid-based preparation in cytological analysis of peritoneal washing in practice of gynecologic oncology *J Cytol*. 2017 Apr-Jun; 34(2): 95-100.
- 20) Udaysmith S., Arakeri SU, Karigowdar MH, Yelikar B. (2012) Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology *J Cytol* 29(1), 2012; 11-15.

- 21) Ji Hae et al. (2009) Comparison of Cytologic Evaluation between Conventional Method and CellprepPlus(R) Liquid-Based Cytology in Body Fluid. *The Korean Journal of Pathology* 2009; 43: 448-52.
- 22) Pornpip Jangsiriwitayakorn et al. (2018) Comparative Analysis of Modified Liquid-Based Cytology and CytoRich Red Preparation in Assessment of Serous Effusion for Cancer Diagnosis 2018; 19(6): 1571–1575.