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COMPARISON OF QUALITY OF SMEAR IN CONVENTIONAL SMEAR TECHNIQUE AND LIQUID BASED CYTOLOGY OF BODY CAVITY FLUIDS: A CROSS-SECTIONAL ANALYTIC STUDY.

Pathology

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ABSTRACT

Introduction: Cytological evaluation of body cavity fluids provides information about various inflammatory lesions and infections, it also helps in staging, prognosis and management of malignancies. For the cytopreparation of body fluids, many techniques are used. Liquid based cytology (LBC) is an emerging technique which provides a homogenous and clear sample that covers a small portion of slide thereby reducing screening time. The main aim of this study is to find out the better method in view of quality of smear between conventional smear technique and liquid based cytology. **Materials and Methods:** In this study 75 body cavity fluid samples were processed using conventional centrifugation method and LBC technique. Slides were prepared and observed under microscope to compare the performance of both technique in terms of 'quality of smear'. **Results:** LBC technique showed better results in case of cellularity, uniform cell distribution, cleaner background and cell morphology. While in case of preservation of architecture both techniques (CS & LBC) provided equivalent result. **Conclusion:** Liquid based cytology (A monolayered slide preparation technique) has outperformed conventional smears and offers a preferable diagnostic tool for cytopathologists in screening various cytology smears and saving the valuable time.

KEYWORDS

LBC, conventional centrifugation, quality of smear, body cavity fluids.

INTRODUCTION

Body cavities like pleural, peritoneal, pericardial, are lined by a single layer mesothelial cells known as serosa. In normal condition this layer contains small amount of fluid for lubrication of adjacent surfaces. In order to diagnose the causative factor behind any effusion, we require cytological examination of the serous fluid[1]. Cytological evaluation provides information about various inflammatory lesions and infections of serous membranes.[2] it also helps in staging, prognosis and management of malignancies. For the cytopreparation of body fluids, many techniques are used like conventional centrifugation, cytocentrifugation, membrane filtration sediment, cell block preparation and liquid-based preparation. Each method has its own pros and cons.[3] In conventional methods poor fixation, air drying artifacts, thick smears, overlapping of cells, presence of other materials like blood cells, inflammatory cells etc. hinders in proper diagnosis, as well as sometimes false negative results are also obtained[4]. Liquid based cytology technique has been approved by the US food and drug administration and was originally developed for gynaecological samples, but has also gained popularity for cytological evaluation of non-gynaecological specimens like body cavity fluids[5]. The basic principle of LBC is providing a homogenous and clear sample that covers a small portion of slide. It is a technique in which samples are collected into a liquid fixative solution and then a monolayer of cells is formed on the slide for microscopic evaluation[6].

The purpose of this study was to compare LBC technique with conventional smear technique in assessing the quality of smear on the basis of five parameters- Cellularity, Cell distribution, Background, Cell morphology and Architecture.

MATERIALS AND METHODS

The present study was conducted in the department of Pathology of Maharaja Agrasen Medical College, Agroha, Hisar, Haryana over the span of one year. It was a hospital based cross sectional analytic study. Ethical clearance was obtained from Institute's ethical committee(MAMC/IEC/2023/35).

Inclusion Criteria: Fluid samples from peritoneal, pleural, synovial, ascitic.

Exclusion Criteria: Fluid samples other than peritoneal, pleural, synovial, ascitic. FNAC samples and soft tissue samples.

Methodology

75 fluid samples (5-10ml) from body cavities (pleural, peritoneal, ascitic and synovial) were taken and volume, colour, turbidity of the fluids was recorded. This fluid was divided into two parts for preparing smears by conventional and LBC techniques. Smear prepared from both techniques were assessed by the single observer on the basis of five criteria- Cellularity, Cell distribution, Background, Cell morphology and Architecture. Mair et al. scoring was applied to each parameter and given a score 0,1 or 2 based on appearance of slide under microscope as shown in table 1. On the basis of cumulative score, smears were categorized into three categories as given in table 2.

Statistical Analysis

Data was entered in Microsoft excel spreadsheet to calculate percentage. Chi square test was applied, and result was analysed using SPSS software version 20.0. A p-value <0.05 was considered as statistically significant.

Table 1: Table Showing Mair Et Al Scoring.

S. NO	Criteria	Qualitative And Quantitative Discription	Point Score
1.	Cellularity	A) Minimal to absent, diagnosis not possible	0
		B) Sufficient for cytology diagnosis	1
		C) Abundant, diagnosis simple	2
2.	Cell distribution	A) Totally in the periphery or sparsely distributed.	0
		B) Combination	1
		C) Evenly distributed	2
3.	Background blood or proteinaceous material	A) Large amount, great compromise in diagnosis.	0
		B) Moderate amount, diagnosis possible	1
		C) Minimal, diagnosis easy	2
4.	Cell morphology, cell degeneration and trauma	A) Marked cellular degeneration diagnosis not possible	0
		B) Moderate cellular degeneration diagnosis possible	1
		C) Minimal cellular degeneration diagnosis easy	2

5.	Architecture	A) Not preserved	0
		B) Partially preserved	1
		C) Well, preserved	2

Table 2: Table Showing Smear Quality On The Basis Of Cumulative Score.

S.NO	CATEGORY	TOTAL SCORE	SMEAR QUALITY
1.	I	0-3	Unsuitable for diagnosis.
2.	II	>3-6	Adequate for diagnosis.
3.	III	>6-10	Diagnostically superior smear.

RESULTS

Out of 75 fluid samples 66.7% were male and 33.3% were females. In case of fluid type 61(81.3%) were pleural fluid, Ascitic 7(9.3%), peritoneal 6(8%) and Synovial 1(1.3%). On the basis of diagnosis 64 samples (85.3%) were non-malignant and 11(14.7%) were malignant.

In case of cellularity, the score of 2 is seen maximum in case of LBC (47 Samples), while in conventional 32 samples secured score 2. So, in case of cellularity, LBC is better than CS. [Table 3]

In case of uniform cell distribution, 41 samples of CS secured score 1, while 20 samples were at score 2. In LBC 25 samples secured score 1, while 47 samples obtained score 2. So, in case of uniform cell distribution, LBC is preferrable than CS. [Table 4]

In case of background, 4 samples of CS secured score 2, while in LBC 33 samples secured score 2. LBC proved to be more promising than CS in cleaner background. [Table 5]

In LBC 17(22.7%) samples secured score 2, while only 8(10.7%) samples of CS secured score 2. This clearly states that LBC presented with excellent preservation of cell morphology as compared to CS. [Table 6]

In the present study it was found that when considering the parameters of architecture, the number of cases having score 2 in LBC was 15(20%) and CS was 7(9.3%). While 63(84%) of CS and 58(77.3%) of LBC samples were at score 1. The proportion of cases with score 1 and 2 in LBC and CS did not differ significantly, which states that in this criterion both techniques performed equally. [Table 7]

The total score was divided into 3 categories i.e. category I, II, III. As shown in Table 2. It was seen that LBC obtained more scoring in category III (62%) i.e. smear prepared of 47 samples out of 75 were diagnostically superior type. So, in case of diagnostically superior smear LBC excelled over conventional technique. [Table 8]

DISCUSSION

In the present study LBC technique promises better cell yield as compared to CS because in LBC cells are concentrated over a small area on the slide proving cell enrichment. The data obtained from this study is in concordance with study done by P U Swathy et al[7] and Rishu Mittal et al[8]. Other supporting studies are done by R.P Siddiqui et al[9], Babloyan et al[10] and Charlotte Gabriel et al[11].

LBC presents better cell uniformity as compared to CS, this is proved in study done by Shyam H Nemade et al[3] where 155 cases of malignant fluid samples were studied. Other studies done by Nasar Yousuf Alwahaibi et al[4], R.P Siddiqui et al[9]and Charlotte Gabriel et al[11] support the same findings. In our study we also found similar data. This states that LBC technique proved to be better than CS. This finding may be justified by the reason that in case of LBC technique a homogeneous monolayer of cells is formed over a pre-defined small area marked on the slide, without any overlapping of cells, this provides a clearer view of cells under microscope.

In our study, LBC slides presented with cleaner background, contributing in easy and quick diagnosis as compared to CS because the fixative used in LBC technique, dissolves all the unwanted material present. Hence, presenting with much cleaner background. This finding is in concordance with studies done by Shyam H Nemade et al[3], Hrishikesh Dadhich et al[12], Nasar Yousuf Alwahaibi et al[4], Babloyan et al[10], Nalwa et.al[13], Sonti Sulochana et al[14], R.P Siddiqui et al[9] and P U Swathy et al[7].

In case of cell morphology preservation, 17(22.7%) samples of LBC slides secured score 2, while only 8(10.7%) samples of CS secured score 2. This clearly states that LBC technique samples presented with better preservation of cell morphology as compared to CS. Hoda RS et al[15] and Priya Singh et al[16] in their studies observed the same result. Other supporting studies are done by Shyam H Nemade et al[3], Charlotte Gabriel et al[11], Nasar Yousuf Alwahaibi et al[4], Dr. R.P Siddiqui et al[9], P U Swathy[7], Reshma Wankhede et al[4].

In our study it was found that when considering the parameters of architecture, the proportion of cases with score 1 and 2 in LBC and CS did not differ significantly, which states that in this criteria both techniques performed equally. The probable reason behind this may be due to cell shrinkage and fragmentation of clusters observed in LBC technique, cells appear to be smaller than their normal appearance due to the use of preservative. Shyam H Nemade et al[3] stated the same findings.

The fixative solution used in LBC technique clears all hemorrhagic and proteinaceous material present in background, presenting clear view of cells. Cells were concentrated in small area on the slide in a homogenous monolayer, no overlapping of cells, which reduces slide screening time. No air-drying artifacts like as in CS method were seen in LBC smear slides, which otherwise hinders diagnosis. Better cell yields and excellent preservation of cytomorphological features were seen in LBC technique. Due to all above facts LBC technique is more promising in providing diagnostically superior smears as compared to CS technique.

In a study done by Shyam H Nemade et al[3], more no. of samples of LBC were in category III. R P Siddiqui et al[9] and Charlotte Gabriel et al[11] in their studies concluded that LBC was superior over conventional technique and can safely replace other types of wet- fixed preparations, resulting in enhanced specimen quality. In the present study it was found that 47(62.7%) of LBC and 21(28%) samples of CS smears were in category III. So, the main objective of this study to find out the better method in view of quality of smear among conventional smear technique and liquid based cytology is well achieved.

CONCLUSION

Our study establishes the superiority of Liquid based cytology over Conventional Smear with regard to reduce screening time as it offers good cellularity, uniform cell distribution, clearer background and well-preserved cell morphology. However, with regard to architecture both Liquid based cytology and Conventional Smear showed similar results. There were few limitations of LBC like; increased men hour work, increased cost and less number of malignant sample size. In spite of these few limitations Liquid based cytology (a monolayered slide preparation technique) has outperformed conventional smears and offers a preferable diagnostic tool for cytopathologists in screening various cytology smears and saving the valuable time.

Table no.3: Table Showing Cellularity Score.

Method	Score 0	Score 1	Score 2
Conventional	7(9.33%)	36(48%)	32(42.67%)
LBC	1(1.33%)	27(36%)	47(62.67%)

Table no.4: Table Showing Uniform Cell Distribution Score.

Method	Score 0	Score 1	Score 2
Conventional	14(18.6%)	41(54.6%)	20(26.6%)
LBC	3(4%)	25(33.3%)	47(62.66%)

Table no.5: Table Showing Cleaner Background Score.

Method	Score 0	Score 1	Score 2
Conventional	13(17.33%)	58(77.33%)	4(5.33%)
LBC	2(2.66%)	40(53.33%)	33(44%)

Table No.6: Table showing Cell Morphology, Cell Degeneration and Trauma Score.

Method	Score 0	Score 1	Score 2
Conventional	7(9.3%)	60(80%)	8(10.7%)
LBC	2(2.7%)	56(74.7%)	17(22.7%)

Table No.7: Table Showing Architecture Score

Method	Score 0	Score 1	Score 2
Conventional	5(6.7%)	63(84%)	7(9.3%)
LBC	2(2.7%)	58(77.3%)	15(20%)

Table No.8: Table Showing Statistical Analysis of Smear Quality on the Basis of Total Score

S.no	Category	Smear quality	Total score		Percentage	
			CS	LBC	CS	LBC
1.	I (Score 0-3)	Unsuitable for diagnosis	11	2	14.7%	2.6%

2.	II (Score > 3-6)	Adequate for diagnosis	43	26	57.3%	34.7 %
3.	III (Score > 6-10)	Diagnostically superior smear	21	47	28 %	62.7%

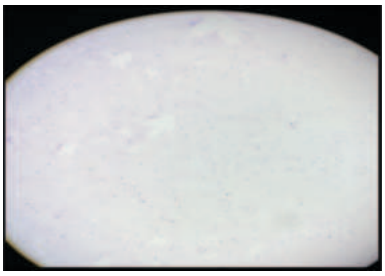

Fig-1: Slide Showing Low Cellularity in CS Technique. (100X; H&E stain)

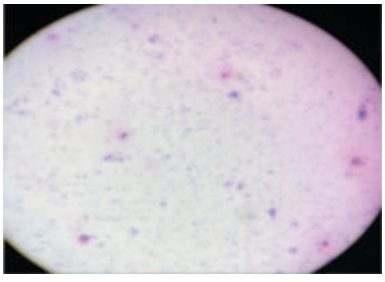

Fig-2: Slide Showing High Cellularity in LBC Technique. (100X; H&E stain)

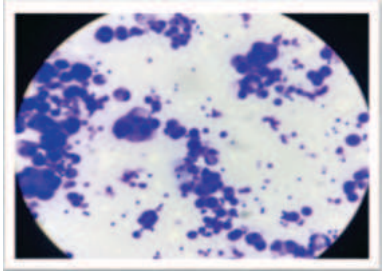

Fig 3: Slide showing overlapping of Atypical cells in CS technique. (400X; H&E stain)

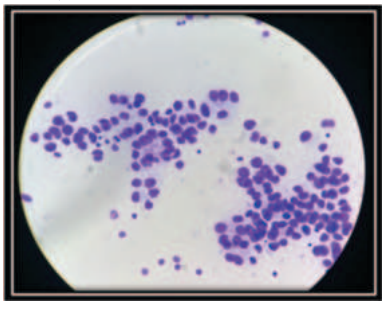

Fig-4: Slide Showing Uniform Distribution of Atypical Cells in LBC Technique. (400X; H&E stain)

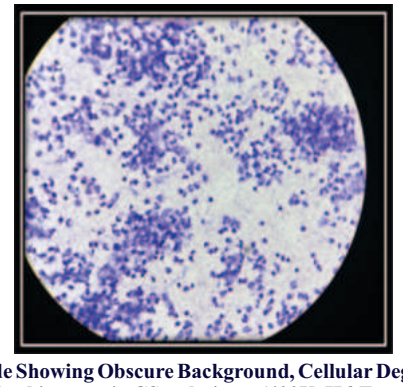

Fig-5: Slide Showing Obscure Background, Cellular Degeneration and Poor Architecture in CS technique. (400X; H&E stain)

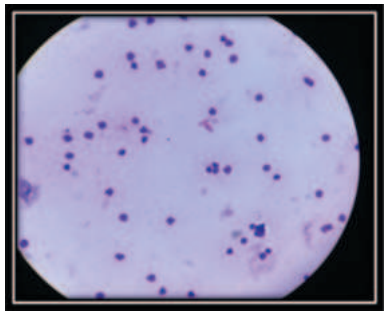

Fig-6: Slide Showing Clean Background, Minimal Cell Degeneration in LBC technique. (400X; H&E stain)

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