



STUDY OF UTILITY OF MANUAL LIQUID-BASED CYTOLOGY AND CONVENTIONAL SMEARS IN THE EVALUATION OF VARIOUS FINE-NEEDLE ASPIRATION SAMPLES

Dr Dhananjay Prasad

Assistant Professor, Department of Pathology, Rims, Raipur, CG, India- 492006

Dr Vanita Bhaskar

Assistant Professor, Department of Pathology, Rims, Raipur, CG, India- 492006

Dr Brajendra kumar

Professor, Department of Medicine, RIMS, Raipur, CG, India - 492006

ABSTRACT

Liquid-based cytology (LBC) preparation is a way to improve and refine the fine-needle aspiration (FNA) samples. Our study was undertaken to evaluate the utility of manual LBC (MLBC) and CS preparations in various FNA samples. In this cross-sectional study, a total of 100 FNA samples from various anatomical sites were evaluated using MLBC and CS preparations. Cellularity, blood, informative background, monolayers, cell architecture, cytoplasmic, and nuclear preservation were compared with MLBC and CS preparations by Wilcoxon signed rank test. $P < 0.05$ is considered statistically significant. MLBC preparations were superior to CS preparations in view of absence of blood and debris ($P = 0.001$), presence of monolayers ($P < 0.001$), and preservation of cytoplasmic ($P = 0.001$) and nuclear details ($P = 0.001$). However, no statistically significant differences were found between MLBC and CS preparations with regard to cellularity ($P = 0.157$), informative background ($P = 0.083$), and architecture ($P = 0.739$). MLBC preparations in FNAC are a safe, easy, and less time-consuming procedure, and it may have promising diagnostic value in the evaluation of FNA samples from various anatomical sites. However, the use of both MLBC and CS preparations is recommended to achieve optimal diagnostic yield.

KEYWORDS

Conventional smears (CS); fine-needle aspiration (FNA) cytology; liquid-based cytology (LBC); manual liquid-based cytology (MLBC)

INTRODUCTION:-

The advantages of liquid-based cytology (LBC) include rapid fixation, even distribution of cells over a smaller slide area, and decreased obscuring background elements, such as blood, inflammation, and mucus. Also, standardized LBC fixation provides advantages for centralized laboratories, especially when FNA procedures are carried out without rapid assessment. LBC preparations provide material for ancillary techniques, and their routine application in pathology laboratories with specific reference to procedure standardization and the opportunity to store cells are significant benefits of nongynecological LBC, which can be used in different organs for different applications. Only a few studies have addressed usage regarding FNA of different organs such as breast, salivary gland, thyroid gland, lymph nodes, bone and soft tissue prepared by LBC technique. The present study was undertaken to evaluate the utility of manual LBC (MLBC) and conventional smears (CS) in various FNA samples.

MATERIALS AND METHOD:-

This cross-sectional study was conducted in the Department of Pathology RIMS, RAIPUR, from JANUARY 2016 to December 2016. Informed consent was obtained from all patients before the initiation of the study. A total of 100 FNAs from various sites, such as lymph node, thyroid, breast, salivary gland, and soft tissue, were included.

In each site, FNA was performed using a 23-gauge needle with 5 mL syringe. In each case, two passes were made. The first pass was made for CS and the second pass was made for MLBC preparations. For CS, the sample was placed directly on the slide and the smears were made. For MLBC preparation, the material was preserved in alcohol-based liquid preservative for minimum of half an hour. The material was centrifuged at 1,500 rpm for 5 min. The supernatant was discarded and the pellet was agitated to get a homogenous sample. One drop of normal saline was added to the pellet and it was mixed well. A volume of 50 µL of diluted pellet was placed on clean slides with a drop of fixative solution. Stains such as May-Grunwald Giemsa (MGG), Hematoxylin and Eosin (H & E) and Papanicolaou stain (Pap) were used for staining the CP and MLBC preparations. Special staining with stains, such as Ziehl-Neelsen (ZN), for acid-fast bacilli (AFB) was performed as and when required. The representative CS and MLBC preparations

were compared by a semiquantitative scoring system using several criteria, namely cellularity, blood, informative background, monolayers, cell architecture, and cytoplasmic and nuclear preservation using the Wilcoxon signed rank test on the IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows (version 20.0. Armonk, New York: IBM Corporation). $P < 0.05$ is considered statistically significant.

RESULTS:-

Among the 100 FNA samples, anatomical sites were lymph node ($N = 22$) (10 reactive hyperplasia, 6 granulomatous lymphadenitis, and 2 acute suppurative lymphadenitis, lymphoma and metastatic carcinoma), thyroid ($N = 41$) (23 nodular colloid goiter, 14 thyroiditis, and 4 carcinoma), breast ($N = 23$) (12 fibroadenoma, 5 breast abscess, 4 fibrocystic disease, and 2 ductal carcinoma), salivary gland ($N = 8$) [2 chronic sialadenitis, 2 cystic lesions, and 4 pleomorphic adenoma (PA)], and soft tissue ($N = 6$) [4 benign spindle cell lesions and 2 sarcoma].

Among the 100 FNA samples, 42 cases underwent surgical intervention and corresponding final histopathological diagnoses were available. The comparison of fine-needle aspiration cytology (FNAC) diagnoses of CS and MLBC preparations with corresponding histopathological diagnoses shown in Table 1.

Final Histopathological diagnoses	Number of cases	FNAC diagnoses			
		CS		MLBC	
		Benign	Malignant	Benign	Malignant
Lymph node	3				
Reactive lymphadenitis	3	3	0	3	0
Granulomatous lymphadenitis	3	3	0	3	0
Non-Hodgkin's lymphoma	1	0	1	0	1
Metastatic carcinoma	2	0	2	0	2
Thyroid	17				
Nodular colloid goiter	8	8	0	8	0
Hashimoto's thyroiditis	5	5	0	5	0
Follicular carcinoma	2	0	2	0	2
Papillary carcinoma	2	0	2	0	2
Breast	9				
Fibroadenoma	5	5	0	5	0
Fibrocystic disease	2	2	0	2	0
Invasive ductal carcinoma	2	0	2	0	2
Salivary gland	4				
Pleomorphic adenoma	2	2	0	2	0
Lymphoepithelial cyst	2	2	0	2	0
Soft tissue	3				
Benign fibrous histiocytoma	2	2	0	2	0
Necrotizing fasciitis	1	0	1	0	1
Undifferentiated pleomorphic sarcoma	1	0	1	0	1

Table1:- Comparison of FNAC diagnoses of CS and MLBC preparations with corresponding histopathological diagnoses (N = 42)

According to the Wilcoxon signed rank test, the present study showed that MLBC preparations were superior to CS preparations in view of absence of blood and debris ($P = 0.001$), presence of monolayers ($P < 0.001$), and preservation of cytoplasmic ($P = 0.001$) and nuclear details ($P = 0.001$). However, no statistically significant differences were found between LBC and CS preparations with regard to cellularity ($P = 0.157$), informative background ($P = 0.083$), and architecture ($P = 0.739$) Table 2.

FNAC features	Present study		Mygdakos et al. ¹⁰		Koybasioğlu et al. ¹⁰	
	Wilcoxon signed rank test*		Wilcoxon signed rank test*		ANOVA*	
	Z	Asymptomatic signed test	Z	Asymptomatic signed test	F	P
Cellularity	-1.414 (a)	0.157	-1.352*	0.131	5.250	0.023
Blood	-3.343 (b)	0.001	-6.553*	<0.001	272.499	0.000
Background	-1.720 (b)	0.083	-1.969*	0.057	77.712	0.000
Monolayer	-3.891 (b)	0.001	-6.111*	<0.001	2.231	0.134
Cell architecture	-0.333 (a)	0.739	-0.299*	0.865	2.589	0.109
Cytoplasmic details	-3.234 (a)	0.001	-3.197*	<0.001	3.869	0.051
Nuclear details	-3.694 (a)	0.001	-3.197*	<0.001	0.720	0.398

*Based on negative ranks. †Based on positive ranks. P < 0.05 is statistically significant. ‡Statistical analysis

Table2:- Comparison of MLBC and CS preparations of the present study and the published studies

In lymph node lesions, all the cases were diagnosed on MLBC preparations. Immature lymphoid cells, Reed-Sternberg cells were better recognized in monolayers. Squamous cells were visualized with well-preserved keratin in metastatic squamous cell carcinoma. There was difficulty in the identification of granulomatous lesions and lymphoglandular bodies. In cases of thyroid lesions, amount of colloid was diminished significantly and it was dense, fragmented, or in droplets. There was difficulty in identifying nuclear grooves and pseudoinclusions in cases of papillary carcinoma. Hence, MLBC preparations should be interpreted with great caution and CS should always be employed for the arriving of diagnosis. All the cases of breast lump were interpreted correctly by MLBC preparation even though a stromal fragment/chondromyxoid matrix was altered or diminished. For the salivary gland swelling, in the diagnosis of PA, support of CS needed due alteration in the chondromyxoid matrix. In soft-tissue lesions, MLBC preparation showed good results due to clean background.

DISCUSSION:-

From the clinician's standpoint, LBC technique is far easier, quicker, and safer and requires less skill. From the pathologist's standpoint, the advantages of using the LBC technique are no to minimal confounding factors (blood, debris and necrotic materials), excellent cell preservation, lesser fixation artifacts (air-drying artifacts), even distribution and less overlapping of the cells and fewer numbers of slides requiring examination.

However, because of the chemical influences of the fixation medium and the physical forces of processing techniques, it tends to produce certain cytomorphological alterations and artefacts: smaller cell clusters and sheets and breakage of papillae; altered cell distribution with more discohesion and slightly more three-dimensional clusters; attenuated chromatin details with prominent nucleoli and smaller cell size; intranuclear inclusion is difficult to visualize; altered background matrix in both quantity and quality; aggregation of lymphocytes and markedly decreased number of extracellular particles; and small mononuclear cells, red blood cells, and myoepithelial cells.

Garbar et al., done a study on FNAC of lymph node with CS and LBC at two university hospitals and authors concluded that despite the cost, the efficiency of lymph node FNAC is identical between CS and LBC. However, LBC preparation of the present study was superior to CS in certain aspects viz., easy visualization of immature lymphoid cells and Reed-Sternberg cells and presence of monolayering. Only cytological features which confirm that samples were aspirated from lymph node is lymphoglandular bodies. In the present study, it was not seen in the background of MLBC prepared slides and also we found difficulties in the recognition of granulomatous conditions; hence, the current study support the need of CS in the evaluation of lymphadenopathy. Amount of colloid in the background plays an important role in the

diagnosis of follicular lesions of thyroid. In this study, the amount of colloid on MLBC preparations was diminished and appear dense, fragmented, and in droplets. Nuclear grooves and pseudoinclusions were less apparent in papillary carcinoma. Similarly, few workers demonstrated these problems in their study. However, Lee et al., observed that background material were slightly superior in LBC preparation than CS preparation. In thyroid lesions, the present study found that MLBC preparations should be interpreted with great caution and CS should always be employed to confirm the diagnosis.

All the cases of breast lump were diagnosed on MLBC preparation in the current study. The diagnosis of fibroadenoma was rendered on the basis of visualization of ductal cell aggregates and bipolar cells even though stromal fragments were altered or diminished. However, the diagnosis of fibroadenoma seems to be most problematic on LBC preparations, with some studies showing a low diagnostic rate compared to CS and false-positive diagnoses while overclassifying fibroadenomas as atypical or suspicious. Based on the presence of rich cellularity, detailed nuclear features, and clean background, the diagnosis of carcinoma made in the present study. Both LBC and CS preparations have comparable performance for the detection of breast carcinoma. In the evaluation of salivary gland lesions, there are important morphological differences with respect to the quantity and appearance of stroma. In this study, chondromyxoid matrix was condensed and fragmented on MLBC preparations; hence, support of CS was needed for the diagnosis of PA. The diagnostic yield appears to be greater in CS than that in LBC preparation in the diagnosis of PA, which is the most commonly rendered diagnosis in salivary gland lesions.

In the current study, there was statistically significant differences between MLBC and CS preparations in view of absence of blood and debris, presence of monolayers, and preservation of cytoplasmic and nuclear details ($P = 0.001$). However, no statistically significant difference was found between these two groups with regard to cellularity, informative background, and architecture ($P > 0.05$). These findings were in accordance with the studies done by Tripathy et al., Mygdakos et al., and Dey et al. Koybasioğlu et al. compared ThinPrep and CS in head and neck FNAC and found that LBC preparations were superior to CS preparations with regard to cellularity, informative background, and cytoplasmic details ($P < 0.005$); however, the presence of monolayers, cell architecture, and cytoplasmic and nuclear details were not statistically significant between two groups ($P > 0.05$).

CONCLUSION:-

Our study proved that MLBC preparation in FNAC is a safe, easy, and less time-consuming procedure, and it may have promising diagnostic value in the evaluation of FNA samples from various anatomical sites. However, the use of both MLBC and CS preparations is recommended to achieve optimal diagnostic yield.

REFERENCES:-

- Zeppa P. Liquid-based cytology: A 25-year bridge between the Pap smear and molecular cytopathology. *Acta Cytol* 2014;58:519-21.
- Ramos MA, Cury Fde P, Scapulatempo Neto C, Marques MM, Silveira HC. Micronucleus evaluation of exfoliated buccal epithelial cells using liquid-based cytology preparation. *Acta Cytol* 2014;58:582-8.
- Mygdakos N, Nikolaïdou S, Tzilivaki A, Tamiolakis D. Liquid Based Preparation (LBP) cytology versus Conventional Cytology (CS) in FNA samples from breast, thyroid, salivary glands and soft tissues. Our experience in Crete (Greece). *Rom J Morphol Embryol* 2009;50:245-50.
- Tripathy K, Misra A, Ghosh JK. Efficacy of liquid-based cytology versus conventional smears in FNA samples. *J Cytol* 2015;32:17-20.
- Koybasioğlu F, Onal B, Şimşek GG, Yilmazer D, Han Ü. Comparison of ThinPrep and conventional smears in head and neck fine needle aspiration cytology. *Turk Patoloji Derg* 2008;24:159-65.
- Ren S, Solomides C, Draganova-Tacheva R, Bibbo M. Overview of nongynecological samples prepared with liquid-based cytology medium. *Acta Cytol* 2014;58:522-32.
- Garbar C, Rimmelink M, Masciaux C. Fine needle aspiration cytology of lymph node: Experience of 2 university hospitals with conventional smears and liquid-based cytology. *Acta Cytol* 2008;52:418-23.
- Afiy AM, Liu J, Al-Khafaji BM. Cytologic artifacts and Pitfalls of thyroid fine-needle aspiration using ThinPrep: A comparative retrospective review. *Cancer* 2001;93:179-86.
- Dey P, Luthra UK, George J, Zuhairy F, George SS, Haji BI. Comparison of ThinPrep and conventional preparations on fine needle aspiration cytology material. *Acta Cytol* 2000;44:46-50.
- Lee KR, Papillo JL, St John T, Eyerer GJ. Evaluation of the ThinPrep processor for fine needle aspiration specimens. *Acta Cytol* 1996;40:895-9.