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Diagnostic Efficacy of Manual Liquid Based Cytology in Fine Needle Aspiration Samples

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Abstract

Introduction: Liquid based cytology (LBC) has played a pivotal role in exfoliative cytology. However it can be implemented in Fine needle aspiration (FNA) samples. Automated techniques have been widely used, but limited due to cost and availability. *Aim*: The objective of the study was to assess the diagnostic efficacy of Manual LBC in FNAC samples. *Methods*: In this prospective study, a total of 60 FNA samples from various anatomical sites were evaluated. Smears were made from conventional preparation (CP) and manual LBC (MLBC) preparation. Both CP and MLBC preparations were compared for cellularity, background, monolayers, cell architecture, cytoplasmic and nuclear details by a semiquantitative scoring system. P<0.005 was considered statistically significant. *Results*: Diagnostic accuracy was better in MLBC to CP in view of absence of blood and debris, presence of monolayers and preservation of nuclear and cytoplasmic details. However, with regard to cellularity, informative background and cell architecture there was no statistical significance. *Conclusion*: MLBC performed on FNA samples can be safe, cost effective promising diagnostic technique in combination with CP to achieve high diagnostic yield.

Keywords: MLBC; CP; FNA Samples**.**

Introduction

Exfoliative liquid based cytology (LBC) was initially used on gynecological pap smears to almost three decades which includes the preparation and evaluation of cells collected in a liquid fixative [1]. However, LBC can be performed on non-gynecological fine needle aspiration (FNA) samples like breast, thyroid, lymph nodes, salivary gland, bone and soft tissue [2].

LBC has advantages of improved sensitivity and specificity since cell fixation is better with well preservation of nuclear details and thus lowering the rate of unsatisfactory cytology samples [3].

The residual cell suspension can be used for ancillary techinques like Immunocytochemistry. The present study was undertaken to evaluate the diagnostic efficacy of Manual LBC (MLBC) in FNA samples [4].

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Materials and Methods

This study was conducted on patients attending Cytopathology department at our institution from June 2016 to November 2016. Informed consent was taken from all patients. 60 FNA samples from various sites like lymph nodes, breast, salivary glands, thyroid, bone and soft tissue were included in the study.

Two passes were made in each case using a 23G needle attached to a 10ml syringe. First pass was made for conventional smear (CS) and the second pass was made for MLBC preparation. CS was made by directly placing the sample on the smear.

The sample obtained from the second pass was preserved in an alcoholic fixative for 25 minutes. Later

the material was centrifuged for 2000 rpm for 10 minutes. After discarding the supernatant, the pellet was agitated to get a uniformhomogenous sample. 50µl of sample was taken on clean slides and smears were made. Hematoxylin & Eosin (H&E) stain, Papanicoloau (Pap) stain and May GrunwaldGeimsa (MGG) stain were done for both CS and MLBC smears. Special stainswere done wherever required. Two observers examined the CS and MLBC smears on different occasions without knowing the diagnosis.

A semi quantitative scoring system was used to compare representative CS and MLBC smears using several criteria consisting of cellularity, blood, informative background, monolayers, cell architecture, nuclear and cytoplasmic preservation using the Wilcoxon signed rank test of the IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows (version 20.0.or Windows (version 20.0. Armonk, New York: IBM Corporation). Every cytology diagnosis was recorded and tabulated to get P value. The lesser the P value, the more accurate is the result. P<0.05 is considered statistically significant as shown in Table 1.

Results

Among the 60 FNA samples, anatomical sites were lymph node (n=14) (7 reactive lymphadenitis, 2 granulomatous lymphadenitis, and 1 acute suppurative lymphadenitis, 1 Lymphoma and 3 cases of metastatic deposits), thyroid (n=26) (14 Nodular goiter, 8 thyroiditis and 4 papillary carcinoma of thyroid), Breast (n=15) (8 Fibroadenoma, 4 Ductal carcinoma, 2 Fibrocystic disease and 1 Breast abscess), salivary gland (n=3) (2 pleomorphic adenoma, 1 chronic sialadenitis) and soft tissue (n=2) (1 benign spindle cell tumor and 1 high grade sarcoma).

Final histopathologicaldiagnosis was available in 24 cases among 60 FNA samples. The comparison of FNA cytology diagnosis of CS and MLBC preparation with corresponding histopathological diagnosis is shown Figure 1.

According to the Wilcoxon signed rank test, MLBC preparation contained adequate diagnostic cells in all cases and were greatly superior to CS with respect to presence of monolayers (P<0.001), absence of blood

Table 1: Semiquantitative scoring system used in various FNA smears

Cytological features	Score 0	Score 1	Score 2	Score 3
Cellularity	Zero	Scanty	Adequate	Abundant
Background blood and debris	Zero	Occasional	Good amount	Abundant
Informative background	Absent	Present	-	-
Monolayer	Absent	Occasional	Good Amount	-
Cell Architecture	Non recognized	Moderately recognized	Well recognized	-
Cytoplasmic details	Poor	Fair	Good	Excellent
Nuclear details	Poor	Fair	Good	Excellent

Table 2: Comparison of FNAC diagnosis of CS and MLBC preparations with corresponding HP diagnosis

Final Histopathological Diagnosis	No. of Cases (n=24)	FNAC			
		CS		MLBC	
		Benign	Malignant	Benign	Malignant
Thyroid (n=10)					
Nodular Colloid Goitre	5	5	0	5	0
Hashimoto's Thyroidities	3	3	0	3	0
PCT	2	0	2	0	2
Breast (n=6)					
Fibroadenoma	2	2	0	2	0
Ductual carcinoma	3	0	3	0	3
Fibrocystic disease	1	1	0	1	0
Lymphnode (n=5)					
Reactive Lymphadenitis	2	2	0	2	0
Metastatic carcinoma	2	0	2	0	2
Lymphoma	1	0	1	0	1
Salivary Gland (n=2)					
Pleomorphic adenoma	2	2	0	2	0
Soft Tissues (n=1)					
BFH	1	1	0	1	0

Table 3: Statistical Results – Comparative Analysis

FNAC features	Present Study Wilcoxon Signed Rank		Arul P. Wilcoxon Signed Rank		Mygdakos et al Wilcoxon Signed Rank	
	Z	Asymptomatic Signed test	Z	Asymptomatic Signed test	Z	Asymptomatic Signed test
Cellularity	-1.312	0.143	-1.414	0.157	-1.352	0.131
Blood	-3.143	0.001	-3.343	0.001	-6.553	0.001
Background	-1.432	0.068	-1.732	0.083	-1.997	0.057
Monolayer	-3.862	0.001	-3.987	0.001	-6.111	0.001
Cell Architecture	-0.343	0.761	-0.333	0.739	-0.299	0.865
Cytoplasmic details	-3.324	0.001	-3.234	0.001	-3.197	0.001
Nuclear Details	-3.564	0.001	-3.494	0.001	-3.197	0.001

and debris (P=0.001) and preservation of nuclear and cytoplasmic details. However MLBC showed cellularity, preserved cell architecture and informative background as good as CS, expressed by no statistical significant values (P=0.143, P=0.761, P=0.068) respectively.

In interpretation of thyroid gland lesions, MLBC had diminished amount of colloid compared to conventional smears. Epithelial crowding was more evident in MLBC than CS. In cases of breast lump, MLBC smears were as good as CS.

Comparison data for FNA lymph node enlargement suggested that the Reed –Sternberg (RS) cells were easily recognized on MLBC than CS. However visualization of lymphoglandular bodies was difficult to indentify in MLBC.

For the salivary gland mass, in the diagnosis of pleomorphic adenoma, the chondromyxoid matrix was diminished and fragmented in MLBC compared to CS.

In soft tissue lesions, MLBC preparation showed good results due to the clean background.

Discussion

Although LBC has enjoyed favorable reports of evaluation of both gynecological and non gynecological specimens from a good number of studies in view of increased cellularity, lack of obscuring background, improved morphology and decrease in unsatisfactory yield, LBC tends to produce certain cytomorphological alteration and artifacts like small cell clusters, breakage of papillae, altered and diminished background matrix due to chemical influences of fixatives used and the physical forces of processing techniques. Therefore the cytopathologists should be very cautious in reporting LBC preparation to avoid misinterpretations if LBC is the only methodology employed [5].

Arul P in his study concluded that MLBC in lymph node lesions was superior to CS in certain aspects like easy visualization of immature lymphoid cells and RS cells, but found difficulty in identification of lymphoglandular bodies and granulomas [6]. However a study done by Garbar et al, stated that despite the cost, the efficiency of lymph node FNAC is identical between CS and LBC [7]. The present study had similar results as that of Arul P.

In FNAC of thyroid lesions, Lee et al, observed in his study that informative background material were slightly superior in LBC preparation than CS preparation [8]. However in our study and study by Arul P, colloid is diminished and appeared fragmented and dense.

In evaluation of breast lesions, cases of breast lump were diagnosed on MLBC in the present study, however diagnosing fibroadenoma on LBC preparation had low diagnostic rate compared to CS and false positive diagnosis while overclassifyingfibroadenomas as atypical or suspicious for malignancy. Dey et al, stated that the diagnosis of ductal carcinoma was easier on MLBC due to clean background and detailed nuclear features of tumor cells [9].

In our study, the quality of chondromyxoidstroma in salivary gland lesions was altered and was condensed and fragmented in MLBC compared to CS preparation, hence support of CS was needed for the diagnosis of pleomorphic adenoma.

In the current study, there was statistically significant differences between MLBC and CS preparations in view of preservation of nuclear and cytoplasmic details, absence of blood and debris and presence of monolayers (P=0.001). However, no statistically significant difference was found between two groups with regard to cellularity, architecture and informative background (P>0.005). These finding were

in accordance with studies done by Arul P, Mygdakos et al and Tripathy et al as depicted in Figure 2 [10,11].

Conclusion

MLBC preparation may have a promising diagnostic value in FNAC of mass lesions from various anatomical sites as it is safe, easy and less consuming procedure. However, the use of both MLBC and CS preparations is recommended to achieve high diagnostic yield.

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