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RESEARCH ARTICLE

COMPARISON OF CYTOMORPHOLOGICAL DETAILS IN LIQUID BASED CYTOLOGY AND CONVENTIONAL CYTOLOGY IN FINE NEEDLE ASPIRATES OF PLEOMORPHIC ADENOMA

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ABSTRACT

Introduction: Liquid based cytology which is at present the standard protocol in cervical cytology samples is also being increasingly used for evaluation of non-gynecological specimens. There are few studies comparing LBC with conventional smears (CS) in salivary gland (SG) fine-needle aspiration biopsies (FNAB) and most of them have been done on Thin Prep. This study compares the cytomorphological details of Sure Path and conventional cytology in cases of pleomorphic adenoma.
Aim: The aim of our study is to compare conventional smears and liquid based cytology (SurePath) in FNA of Pleomorphic adenoma in terms of cytomorphological details, adequacy and ease of interpretation.
Materials and Methods: We conducted a prospective observational comparative study which included 47 cases of Pleomorphic adenoma. Both CS and LBC (SurePath) smears were prepared as per standard protocols and examined.
Results and conclusion: In terms of adequacy and cellularity, conventional smears were better than LBC. Diagnostic accuracy of both the techniques in cellular cases however was comparable. Ease of interpretation was better with conventional smears due to abundant chondromyxoid stroma, an important clue in the diagnosis of pleomorphic adenoma. Nuclear details and background were better in LBC as compared to CS.

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INTRODUCTION

The World Health Organization has described 45 morphologic types of primary salivary gland tumors (Barnes *et al.,* 2005). Pleomorphic adenoma (PA) is the most common salivary gland tumor in both children and adults (Heaton *et al.,* 2013). Fine-needle aspiration (FNA) is a well-established sensitive and specific diagnostic procedure for salivary gland lesions that is easy to perform, rapid, minimally invasive and is well tolerated and accepted by the patients. Fine needle aspiration cytology is highly accurate in diagnosing Pleomorphic Adenoma. Diagnostic difficulties may arise in scenarios such as cellular specimens with sparse or absent matrix, lesions with adenoid cystic like areas, lesions with focal cytologic atypia and lesions with squamous and/or mucinous metaplasia (Pusztaszeri *et al.,* 2009). Liquid-based cytology (LBC) which was originally introduced for Pap smears has been increasingly adopted by many laboratories for non-gynecological specimens because of its many advantages.

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However, the utilization of LBC for fine-needle aspiration (FNA) has been controversial, particularly in sites such as the salivary gland where the architecture, extracellular matrix material and inflammatory component play a major role in the diagnosis.

Aim and Objectives

In this study, we compared conventional cytology with liquid based cytology (Sure Path) in fine needle aspirates of pleomorphic adenoma in terms of cytomorphological details, ease of interpretation and adequacy.

MATERIALS AND METHODS

We conducted a prospective observational comparative study which included 47 cases of pleomorphic adenoma. This study was conducted in a tertiary care based hospital over a time span of 1 year (September 2015 – September 2016). Detailed history and radiological details of the patients were recorded. Physical examination of the salivary gland swelling was done. The salivary gland lesion was aspirated using 22 gauge needle and 20 ml syringe and the material was expelled on a slide to make conventional smears.

Another pass was done and the material was collected in a vial with BD Cytorich™ (Tripath imaging, Burlington, NC 27215 USA) Red Preservative for Liquid based cytology. The smears were prepared and stained as per standard protocol and interpreted by two cytopathologists independently.

RESULTS

A total of 47 conventional and LBC smears of pleomorphic adenoma were prepared and analysed. Most of the patients presented between 3rd and 4th decade of life. Males accounted for 38.3% (18/47) while females accounted for 61.7% (29/47) of all the cases. In majority of cases, parotid gland was involved (38/47) followed by submandibular gland (6/47) and minor salivary glands (3/47). In conventional cytology, all the smears (47/47) were found to be adequate while in SurePath, 38/47 (80.85%) of the smears were adequate (Table 1). The cytomorphological features that were compared between conventional and LBC smears included cellularity, architecture, nuclear details, cytoplasmic details, chondromyxoid stroma and background.

The cytomorphological details of parameters observed in LBC and Conventional smears of the cases which were found to be adequate in both conventional cytology and Liquid based cytology (38/47) (Table 2). It was observed that cellularity, architecture and chondromyxoid stroma were observed better in conventional smears as compared to Surepath smears. Nuclear details were better appreciated in Surepath smears while cytoplasmic details were same between the two techniques.

The background as expected was clear in Surepath smears as there was no blood and blood derived debris. The interpretation was easier with Conventional cytology as compared to Liquid based cytology. Histopathological correlation was available in 34 cases. The diagnostic accuracy of both the cytopreparatory techniques was 100%, however 26/34 (76.47%) cases were more easily diagnosed by conventional smears, 3/34 (8.82%) by LBC smears while in 5/34 (14.7%) cases, the ease of interpretation was same by both the techniques.

Table 1. Comparison of Adequacy in Conventional and SurePath samples (N=47)

Technique	Total no of cases = 47			
	Adequate		Inadequate	
	No	%	No	%
CS	47	100.00	0	0
LBC	38	80.85	9	18.75

Table 2. Cytomorphological Details of Parameters observed in LBC and Conventional Cytology (Inadequate cases of LBC excluded) (n=38)

Features	Conventional better		Convention equal to LBC		LBC Better	
	No.	%	No.	%	No.	%
Cellularity	25	65.8	10	26.3	3	7.8
Architecture	26	68.4	11	28.9	1	2.6
Nuclear details	0	0	5	13.1	33	86.8
Cytoplasmic details	3	7.8	28	73.6	7	18.4
Chondro-myxoid stroma	34	89.4	4	10.5	0	0.00
Background	0	0.00	0	0.00	38	100.00
Ease of Interpretation	29	76.31	5	13.1	4	10.5

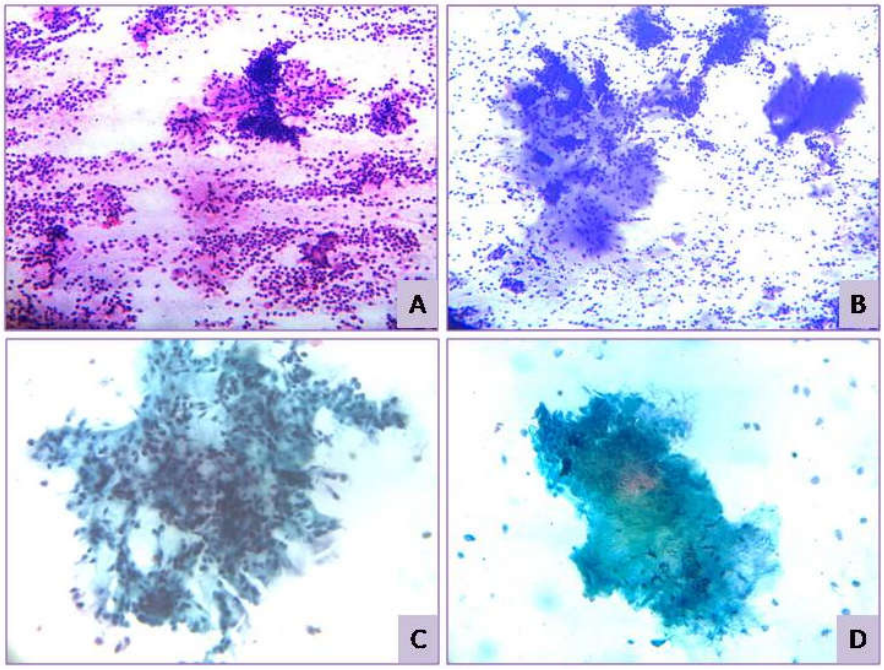

Fig.1. Pleomorphic adenoma showing benign ductal cells and myoepithelial cells admixed with chondromyxoid stroma[Conventional smear A-H&E stainx10, B-Giemsa stain,x10], [C&D-Liquid based cytology smears Pap stainx40&x20]

DISCUSSION

Fine needle aspiration is a sensitive, specific and invaluable tool for diagnosis of salivary gland neoplasms. As early as in 1933, Stewart published his experience of aspiration of 66 mixed tumors of the salivary glands (Stewart, 1933). Liquid based cytology is a system of smear preparatory technique that was introduced in 1996 (Hees and Lebeau, 1933; Lee *et al.,* 1996). At present, LBC is the standard protocol for smear preparation in cervical cytology and it has been gaining enthusiasm for use in non-gynecological fine needle aspirates as well but it is not a preferred method in non-gynecology specimens. We found that conventional cytology was easier to interpret as compared to Sure Path cytology in fine needle aspiration of Pleomorphic adenomas.In terms of adequacy, conventional smears were found to be adequate in all the cases while liquid based cytology was adequate in 38/47 cases. This was also seen in other studies like Parfitt *et al* (2007) (Parfitt *et al.,* 2007) where the reason of inadequacy was use of split sample technique. In our study, we did two separate passes for CS and LBC. Sampling error may have led to inadequacy in LBC. Also in Sure Path, centrifugation is done two times and the material is decanted during which loss of cells may have occurred. There are two schools of thoughts for sampling in LBC along with CS. Some people advocate split sample technique while others advocate a separate pass for collection of sample for Liquid based cytology. In split sample technique, a single pass is made and conventional smear is prepared first, the remaining material in hub of the needle is rinsed in the preservative fluid for LBP. Due to this bias, loss of cellularity occurs in LBP. In our study, two separate passes were done. The material aspirated in first pass was smeared on slides for conventional cytology. The material aspirated in second pass was rinsed in preservative fluid for LBP. Loss of cellularity may have occurred during the second pass if the needle did not strike the site of lesion or due to hemorrhage after the first pass. Cellularity and architectural arrangement was better preserved in Conventional smears. In conventional smears, the cells were seen arranged in sheets, papillary fragments and large clusters while in LBC smears, there was fragmentation of cells into smaller clusters and an increase in number of single lying cells in the background.

The fragmentation in LBC occurs during the centrifugation step. In a study done by Hoda RS (Hoda, 2007) in FNA cases where LBP were prepared from needle/syringe rinse after initial CS preparation, significant loss of cells was seen. Chondromyxoid stroma was better visualized and was seen in abundance in majority of cases in conventional smears. However, in none of the cases it was better in Liquid Based Preparation. In literature it is reported that background matrix is altered in both quantity and quality in Liquid Based Preparation. However, most of the studies have been done on ThinPrep technique. In SurePath, it is seen that the loss of extracellular elements is less pronounced (Hoda, 2007). It was noted that in Liquid Based Preparations extracellular material such as colloid, mucin, amyloid, chondroid and myxoid appeared as small dense droplets or acquired a filamentous and/or moth-eaten appearance that made it indistinguishable from fibrin (Michael and Hunter, 2000). Similar diagnostic accuracy with both techniques was reported by Biscotti *et al* (1995) (Biscotti *et al.,* 1995) in thyroid aspirates, although they pointed out that colloid was decreased and appeared as droplets in Liquid Based Preparations. Nuclear details and cytoplasmic details were well preserved in Liquid Based Cytology.

In the present study, nuclear details were observed better in LBC as compared to CS. Similar findings were reported by Parfitt JR *et al* (2007) (Parfitt *et al.,* 2007) and Chung Shan Leung *et al* (1997) (Chung Shan Leung *et al.,* 1997). LBC is superior to conventional preparations with regard to clearer background and reduced obscuring artifacts such as crush, air drying and obscuring blood as seen in all other studies. In our study, LBC was better than CS with regard to the background in all the cases. In Sure Path technique, background is removed when it is layered on density gradient reagent. Also, Sure Path retains background inflammatory infiltrate in 69.5% cases as compared to 56% cases in Thin Prep (Hoda, 2007). Therefore, Pleomorphic adenoma was more easily diagnosed by conventional smears due to better cellularity, architectural preservation and abundant chondromyxoid stroma. However, LBC may be used as a complementary technique along with conventional cytology. In our setup where cost affordability is a major constraint, we prefer conventional cytology over Liquid Based Cytology.

Conclusion

Though the diagnostic accuracy of both conventional cytology and Sure Path LBC is similar in cases of Pleomorphic adenoma, Conventional cytology should be preferred since it is easier to interpret and more cost effective than LBC.

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