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Original Research Article

CONTRASTING LIQUID-BASED PREPARATION (E-PREP) WITH CONVENTIONAL CYTOLOGICAL SMEAR FOR NON GYNAECOLOGICAL SMEARS

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ABSTRACT

Background: To perform liquid-based cytology (LBC) and conventional smears (CS) using fine-needle aspiration for thyroid, breast, and lymph node abnormalities. To evaluate E-PREP's effectiveness in relation to non-gynaecological samples. To contrast E-PREP's benefits and drawbacks for non- gynaecological samples.

Materials and Methods: Samples from patients who visited the cytology department for FNAC of thyroid, breast, and lymph node lesions were used in this prospective investigation. gathering patient clinical data, going to the cytology department for FNAC on lesions in the breast, thyroid, and lymph nodes, and getting ready for CSs and LBP.

Results: The proportion of satisfactory smears is higher in the Liquid-based preparation, at 51.3%. The proportion of inadequate smears is higher in liquid-based preparations, namely at 68.3%. The P value was calculated using the Pearson Chi-Square test, and the resulting P value was 0.254.

Conclusion: The selection between the Conventional technique and LBP relies on the characteristics of the lesion (whether it is solid or cystic) and any additional tests needed for the sample. Every method possesses its own merits and drawbacks. Nevertheless, the integration of two techniques can yield superior smears of higher quality and reduced rates of failure.

Keywords: Fine needle aspiration cytology, Conventional smear, Liquid based preparation, thyroid, breast, lymph node.

INTRODUCTION

Lately, there has been an increasing trend in the adoption of fine needle cytology (FNC) by physicians and pathologists. The FNC technique is characterised by its directness, efficiency, and exceptional accuracy and sensitivity. Two procedures utilised in the field of fine needle cytology are fine needle aspiration cytology (FNAC) and fine needle non-aspiration cytology (FNNAC). The fundamental principle of fine needle aspiration cytology involves extracting cellular material from targeted masses or lesions, typically with strong suction pressure. The process necessitates the use of a needle and a syringe, which should be securely maintained in a syringe holder to facilitate the effortless administration of suction using only one hand. This technique relies on suction and can occasionally result in the formation of a hematoma and the generation of hemorrhagic material.[1,2,3]

The technique called Fine Needle Non Aspiration Cytology (FNNAC), initially pioneered in France, was launched in 1981. It replaces the process of extracting using a syringe with the idea of capillary suction, where a narrow channel (fine needle) is employed to pull in fluid or semifluid material. It is characterised by decreased discomfort, minimised injury, and a focus on the needs of the patient.

The FNAC was primarily used to confirm clinically suspected cases of malignancy and local recurrence of carcinomas, eliminating the need for additional surgical intervention. The clinical use of FNAC extends beyond neoplastic illnesses and includes

non-neoplastic disorders such as inflammatory and degenerative lesions.[3,4]

Liquid-based cytology is an advanced technique used to analyse tiny samples obtained through needle aspiration. Initially developed for cervical cancer screening, this technique is now widely employed for non- gynaecological samples. The fundamental principle of LBC involves acquiring a specimen and immersing it in a preserving solution to maintain its structure, followed by the creation of a single layer of cells through a staining procedure. LBC utilises superior cell preservation procedures that effectively minimise the amount of blood in the background.

The E-PREP system is a cytology processor that utilises liquid-based technology and is equipped with distinctive twin membrane filters. This method allows for the efficient extraction of a significant number of cells and the creation of a single layer sample with outstanding cytological properties. The utilisation of integrated filtration and precipitation techniques has yielded exceptional smear quality. Therefore, the methodology provides improved precision. E-PREP enables the production of a greater quantity of slides, specifically 150 slides each hour.

This study investigated a total of 120 individuals, comprising 40 cases each of thyroid, breast, and lymph node abnormalities. The aim was to evaluate the diagnostic adequacy and precision of both fine-needle aspiration cytology (FNAC) and liquid-based preparation (E-PREP) procedures.[4,5,6]

MATERIAL AND METHODS

This study is a investigation carried out at the Department of Pathology, Government Medical College, Nandyal, Andhra Pradesh, India from July 2023 to November 2023. This investigation employed specimens acquired from 120 patients who underwent fine-needle aspiration cytology (FNAC) for thyroid, breast, and lymph node abnormalities at the cytology department. The cytology division gathers clinical information from patients who have fine-needle aspiration cytology (FNAC) for abnormalities in the thyroid, breast, and lymph nodes. The department does both conventional smears (CS) and liquid-based preparations (LBP).

Inclusion Criteria

1. Noticeable enlargement of the breast, thyroid, and lymph nodes

Exclusion Criteria

1. Swelling in areas other than the breast, thyroid, and lymph nodes
2. Related to non- gynaecological conditions.

RESULTS

Table 1: Thyroid lesions distribution

S.no	Diagnosis	Total
1	Nodular goitre colloidal	21
2	Hashimoto's thyroid	7
3	Lymphocytic thyroid	5
4	Papillary carcinoma	3
5	Colloidal cystic nodule	2
6	Colloid goitre with thyroiditis	2

Table 2: Parameters average score

S. No	Parameters	CS	LBC
1	Blood or clots	1.125	1.751
2	Cellularity	1.654	1.356
3	Cellular Trauma's Degree	1.112	1.455
4	Cellular degeneration degree	0.987	0.897
5	Architecture retention	1.127	0.896

Table 3: Parameters P value (Thyroid)

S. No	Parameters	P Value
1	Blood or clot	0.012
2	Cellularity	0.054
3	Cellular Trauma's Degree	0.685
4	Cellular degeneration degree	0.396
5	Architecture retention	0.086

Table 4: Breast lesions distribution

S. No	Diagnosis	Total
1	Ductal carcinoma	13
2	Fibroadenoma	8
3	Fibroadenoma with fibrocystic disease	4
4	Fibroadenoma with fibroadenosis	4
5	Proliferative breast disease with atypia	2
6	Fibrocystic disease	3

7	Benign phyllodes	2
8	Granulomatous mastitis	2
9	Descriptive (Haemorrhagic)	2

Table 5: Average score of parameters

S. No.	Parameters	Average Score	
		CS	LBP
1	Blood or clot	1.465	1.466
2	Cellularity	1.654	1.946
3	Cellular Trauma's Degree	1.456	0.948
4	Cellular degeneration degree	1.101	0.466
5	Architecture retention	1.123	0.975

Table 6: Parameters P value (Breast)

S. No	Parameters	P Value
1	Blood or clot	0.636
2	Cellularity	0.061
3	Cellular Trauma's Degree	0.033
4	Cellular degeneration degree	0.065
5	Architecture retention	0.054

Table 7: Pearson Chi-Square Test

Technique's comparison (Breast)				
Method	Total cases	Quality of smears		P value
CS	40	Adequate	31	0.845
		Superior	7	
		Unsuitable	2	
LBP	40	Adequate	29	
		Superior	8	
		Unsuitable	3	

Table 8: Smears quality percentage and grading

Grade		Techniques		Total
		CS	LBC	
Adequate	Count	74	71	145
	% within grade	51.1%	48.9%	100%
Superior	Count	35	36	71
	% within grade	24.1%	24.8%	100%
Unsuitable	Count	11	13	24
	% within grade	45.8%	54.1%	100%
Total	Count	120	120	240
	% within grade	50%	50%	100%

Table 9: Pearson Chi –Square Test

Comparison of Both Techniques				
Method	Total cases	Quality of smears		P value
CS	120	Adequate	75	0.135
		Superior	34	
		Unsuitable	11	
LBP	120	Adequate	71	
		Superior	36	
		Unsuitable	13	

DISCUSSION

The predominant method employed for identifying pathological problems in cytology is fine needle sampling, which includes both aspiration and non-aspiration methods. The fundamental principle of fine needle aspiration cytology involves extracting cellular material from targeted masses or lesions, typically by the application of significant suction force. In order to carry out this treatment, it is necessary to employ a needle and a syringe. To ensure smooth suction with one hand, it is crucial to place them in a syringe holder. This treatment employs suction and may sometimes result in the development of a hematoma and the production of hemorrhagic material. The FNAC was initially used to definitively confirm clinically suspected cases of malignancy and local recurrence of carcinomas, eliminating the need for additional surgical intervention. The clinical use of FNAC extends beyond neoplastic situations to include non-neoplastic disorders such as inflammatory and degenerative diseases.[6,7]

The FNAC technique is suitable for detecting easily recognisable superficial lesions, such as those seen in the epidermis, subcutaneous tissue, thyroid, superficial lymph nodes, breast, and salivary gland. The procedure is less strenuous than a biopsy and

entails minimal risk of consequences. The FNAC procedure is performed in the outpatient department, specifically in the radiology theatres. This is categorised as an administrative function. Liquid-based cytology is an advanced technique used to analyse tiny needle aspiration samples. Initially utilised predominantly for the purpose of cervical cancer screening, it is now widely employed for non- gynaecological samples. The fundamental principle of LBC involves gathering a specimen and immersing it in a solution that maintains its integrity. Subsequently, a monolayer of cells is generated with the application of a staining procedure. Liquid-based cytology (LBC) exhibits exceptional cell preservation and substantially decreases the presence of blood in the background.[8,9]

The E-PREP system is a cytology processor that utilises a liquid-based method and includes distinctive twin membrane filters. This method allows for the collection of a significant number of cells and the creation of a single layer sample with outstanding cytological features. The utilisation of integrated filtration and precipitation methods has yielded an exceptional level of clarity in the final product. As a result, the technique yields more precise outcomes. E-PREP enables the production of a higher quantity of slides, specifically 150 slides each hour.[9,10,11]

The acquired smears from both techniques were evaluated using the scoring system developed by Mair et al in 1989 and Bedard Y.C. study. The scoring was based on five objective factors, including background blood or clot, cellularity, degree of cellular trauma, degree of cellular degeneration, and retention of cellular architecture.[10,11]

The number of satisfactory smears, high-quality smears, and unsatisfactory smears obtained from each technique, as well as the overall average score, mean score for each subcategory, and P-value, are compared between the procedures and examined using statistical tests such as the Z-test or Student's t-test.

Thyroid enlargement

In our study, we conducted a thorough review of all observations and data collected from two diagnostic procedures, specifically the Conventional method and LBP, with the goal of finding thyroid enlargement. We performed a thorough computation and analysis of the number of smears that satisfied the criteria for diagnostic adequacy, diagnostic superiority, and those that were considered unsuitable. Furthermore, we calculated the average score for each parameter, the arithmetic mean score, and the P value. Our investigation revealed that the Conventional smear methodology produced a higher quantity of diagnostically suitable smears (25 instances) in comparison to the Liquid-Based Cytology (LBC) method (23 cases). Furthermore, the LBC methodology produced a greater number of diagnostically superior smears (6 cases) compared to the Conventional method (4 cases). Both approaches yielded an equal number of insufficient smears for diagnosis, with only one occurrence each.

Upon comparing the proportions of satisfactory, excellent, and unsatisfactory smears obtained from Conventional smears (CS) and Liquid-Based Preparations (LBP), it was noted that CS yielded a greater percentage of satisfactory smears (52.1%), whereas LBP yielded a higher percentage of excellent smears (60%). The occurrence of diagnostically unsuitable smears produced by both techniques was same, at a rate of 50%. The average score for each parameter in the Conventional smear and LBP of thyroid lesion was calculated. The investigation showed that the average score for background blood was higher in the LBP technique, with a mean value of 1.633.[10,11]

According to the research conducted by Dey P et al, it was found that breast diseases such as fibroadenoma and ductal carcinoma exhibit sufficient cellularity, informative backdrop with stromal elements, and preservation of cellular architecture in LBPs (liquid-based preparations). The present study indicates that the P values for cellularity and retention of cellular architecture were less than 0.05, suggesting statistical significance. In a study conducted by Bedard YC et al, the diagnostic accuracy of CS and LBP was shown to be statistically similar. As to the study conducted, the level of adequacy for CS was 82.4% and for LBP it was 73.7%. The CS approach was diagnostic in 82.4% of cases, whereas the LBP method was diagnostic in 71% of cases. In our investigation, the breast lesions were determined to have a P value of 0.785, indicating that the differences observed were not statistically significant ($P>0.05$). The investigation resulted in an overall P value of 0.785, indicating that the observed differences were not statistically significant ($P>0.05$). The analytical results indicated that the collected smears were inadequate for diagnosis, particularly in comparison to prior studies. Our investigation determined that the fraction of diagnostically inaccurate smears produced by both procedures is equal. While this study did not find any significant differences in average scores, cellular structure preservation, level of cellular damage, and deterioration between Conventional and Liquid based preparation procedures, it has highlighted many practical considerations that need to be considered. Liquid-Based Preparation (LBP) is chosen for cases of Colloid goitre, cystic degeneration in nodular colloid goitre, and colloid nodule due to its efficient preservation of the thyroid follicular cells within the colloid substance.[11,12,13]

Lump in the breast

The comparison of our study with previous studies conducted in the past. The observations and results were compared with the previously mentioned studies. N. Mygdakos et al. did a comparative analysis between CS (caesarean section) and LBP

(low back pain) in samples specifically relevant to non- gynaecology. The study's P value was 0.785, suggesting that the observed alterations did not reach statistical significance. The outcome is less than 0.05. Dey P et al. found that breast illnesses such Fibroadenoma and Ductal carcinoma are associated with low back pain (LBPs) and have a sufficient number of cells, a background that offers valuable information about stromal elements, and a cellular structure that stays undamaged. The present investigation demonstrates that the P values for cellularity and conservation of cellular architecture were both statistically significant, with values below 0.05. A study conducted by Bedard YC et al found that the diagnosis accuracy of CS and LBP was statistically similar. According to the study conducted by Aaron L Shibemba et al, the prevalence of Central Sensitization (CS) was found to be 82.4%, whereas the prevalence of Low Back Pain (LBP) was 73.7%. The CS methodology demonstrated a diagnostic accuracy of 82.4% in the investigated situations, whereas the LBP method exhibited a diagnostic accuracy of 71%. After doing our study, we discovered that the breast lesions had a P value of 0.785, indicating that the observed differences were not statistically significant (P>0.05).[11,12,14]

We conducted a thorough examination of all observations and outcomes derived from two techniques, specifically the Conventional method and LBP, in relation to breast swelling. We assessed the number of smears that satisfied the diagnostic adequacy criteria, those that beyond the diagnostic standards, and those that were considered unsuitable. In addition, we computed the average score for each parameter, the arithmetic mean score, and the related P value. This study evaluated the smears acquired from breast lesions utilising both Conventional smears and Liquid-Based Cytology (LBC). The smears were assessed and classified based on certain criteria, including the presence of blood in the background, the cell count, the extent of cellular injury, the level of cellular degeneration, and the preservation of tissue structure. A higher quantity of diagnostically satisfactory smears was detected in patients suffering from lower back pain (LBP-19 cases, CS- 18 cases). Conventional smears demonstrate a greater level of quality compared to LBP, with 9 cases in CS against only 7 cases in LBP.[13]

In Maheshwari et.al study, a total of 543 women were enlisted during the duration of the study. The women were categorised into two groups based on their cervical cytology results: a positive group (n = 161) and a negative group (n = 382). Neither group exhibited any instances of anal intraepithelial neoplasia. Out of the people with a positive cervical cytology, 99 (61.5%) had negative anal cytology. Similarly, among the participants with a negative cervical cytology, 254 (66.7%) had negative anal cytology. Out of the positive group, 62 anal samples (38.5%) and out of the negative group, 127 anal samples (33.3%) were deemed unacceptable for further evaluation.[13]

The frequency of inaccurate smears is decreased in LBC preparation (LBP-4 cases) compared to Conventional smears (CS-3 cases). An analysis comparing the quality of smears acquired through Conventional smears and Liquid-based preparation found that a larger percentage of high-quality smears were obtained with Conventional smears (56.3%). On the other hand, the Liquid-based preparation method resulted in a higher proportion of satisfactory smears (54%), but also had a higher number of unsatisfactory smears (57.10%). The mean score was computed for each criterion (Background blood or clot, Cellularity, Degree of cellular trauma, Degree of cellular degeneration, and Retention of architecture) in both Conventional smears and Liquid based preparation. The average value of the background blood or clot parameter was greater in patients with lower back pain (LBP), with a mean score of 1.7667. The average cellularity score of Conventional smears was determined to be 1.666, surpassing that of other smear types. The Conventional smear exhibited a greater typical degree of cellular stress, with a mean score of 1.133. The study revealed that there was no significant difference between the two techniques, as indicated by a P value greater than 0.05.[14,15]

Lymph node swelling

The scope of our study encompassed a thorough examination of all observations and data collected from both the traditional approach and the LBP methodology in regards to lymph node enlargement. We assessed the number of smears that met the diagnostic adequacy requirements, those that beyond the diagnostic standards, and those that were considered unsuitable. In addition, we computed the mean score for each parameter. Furthermore, we calculated the mean score and analysed the P value. In our study, we analysed the smears obtained from enlarged lymph nodes using both Conventional and Liquid-based preparation methods. The assessment of the smears was performed by taking into account variables such as the presence of blood and clot in the background, the quantity of cells present, the degree of cellular damage, the level of cellular decay, and the preservation of cellular structure. Our data suggest that the Liquid-based preparation group (LBP-24) had a higher number of smears that met the diagnostic criteria compared to the Conventional group (CS-24). The diagnostic precision of smears obtained from Conventional smears surpasses that of LBP. The items are classified as CS-13 and LBP-3. The frequency of diagnostically inadequate smears is greater in Liquid Based Preparation (LBP) compared to Conventional smears (CS-0, LBP-3). The mean score was computed for each criterion (Background blood or clot, Cellularity, Degree of cellular trauma, Degree of cellular degeneration, and Retention of architecture) in both Conventional smears and Liquid-based preparations. Subjects with low back pain (LBP) showed a greater mean value

for the background blood parameter. The mean score for LBP was determined to be 1.733, whereas the mean score for the typical smear was 1.033. The mean discrepancy between the CS methodology (6.600) and the LBP approach (5.366) is computed to be 1.233. The P value for the mean scores of each technique was determined by a t-test, resulting in a P value of 0.259. The investigation revealed that the observed disparity lacked statistical significance. The outcome is less than 0.05.[11]

We performed a comparison analysis of the P values for each parameter obtained from both procedures using the Pearson Chi-Square test. We conducted a research to compare Conventional smears and Liquid based preparation, focusing on five specific aspects. The statistical significance of the difference was determined by calculating the P value using the Pearson Chi-Square test. The resulting P value was 0.005, indicating a significant difference between the two procedures. The p-value is statistically significant with a significance threshold of 0.05. N. Mygdakos et al. performed a comparative examination of chronic stress (CS) and low back pain (LBP) in samples obtained from non-gynaecological patients. The calculated P value for lymph node lesions in our study was 0.005, indicating that the observed differences were not statistically significant. The outcome is less than 0.05. In our analysis, we documented a total of 120 incidents in total. Among these scenarios, the LBP method produced satisfactory smears in 66 cases, whereas the CS approach produced superior smears in 26 cases. Furthermore, there were 8 occurrences in which the LBP technique resulted in unsatisfactory smudges.[11]

Diaz-Rosario and Kabawat found that there were more premalignant lesions detected on LBP smear preparation compared to conventional smears. In their study, the percentage of low grade squamous intraepithelial lesions increased from 1.6% to 2.7%, and the percentage of high grade squamous intraepithelial lesions increased from 0.3% to 0.5%. Weintraub and Morabia found that the percentage of acceptable cases in LBP was higher (72.2% to 92%) compared to Conventional smears. Bolick et al. found that liquid-based cytology had a higher sensitivity (95.2%) and specificity (58%) compared to conventional pap smear. In contrast, conventional smear had a sensitivity of 85% and specificity of 36%. As reported by Baandrup U et al, conventional smears had a low sensitivity (70%-80%) due to issues with sample collection, insufficiency, and interpretation in cervical smears. Based on the studies conducted by McCrory DC et al, Weintraub J et al, it was found that liquid-based preparation showed a better sensitivity (ranging from 85% to 95%) in detecting abnormalities in cervical smears.[16-20]

The LBP group has a higher proportion of smears that are considered satisfactory (52.4%), whereas the CS technique group has a higher proportion of smears that are considered excellent (61.9%). In addition, the LBP group exhibits a larger proportion of poor smears, amounting to 66.7%. This study aimed to assess the effectiveness of Conventional smears and Liquid based preparation in diagnosing thyroid, breast, and lymph node lesions. We evaluated these methods based on five specific parameters: presence of background blood or clot, cellularity, extent of cellular damage, extent of cellular degeneration, and preservation of cellular architecture. The statistical analysis was conducted utilising the Pearson Chi-Square test, yielding a P value of 0.135. The findings indicated that there was no statistically significant distinction between the two methods, as evidenced by a P value over 0.05.

CONCLUSION

The usual method used for processing samples in Fine needle sampling is preparing smears through air drying and fixing them with alcohol. Using liquid-based preparation is a viable alternate method for managing fine needle aspiration (FNA) samples in non- gynaecological patients. During the LBP method, samples obtained through aspiration are stored in an alcohol fixative and subsequently placed onto LBC slides. The E-PREP system is a cytology processor that uses liquid and has distinctive twin membrane filters. This procedure possesses the capacity to effectively gather a substantial quantity of cells and generate a singular layer of cells with exceptional cytological characteristics. E-PREP enables the production of a larger quantity of slides, specifically 150 slides each hour.

Several studies have shown that non-gynaecological samples produce more favourable results compared to liquid-based cytology when used on non- gynaecological samples. The present work entailed analysing and comparing the mean scores obtained from two methods, specifically Conventional smears and LBP, in samples of thyroid, breast, and lymph node swellings. The Pearson Chi-Square test was used to get the P value, which indicated that there was no statistically significant difference between the two approaches ($P > 0.05$). The choice between the Conventional approach and LBP is based on the properties of the lesion (solid or cystic) and any other diagnostic tests required for the sample. In summary, each method has its own merits and drawbacks, and combining both procedures can result in improved smear quality and decreased failure rates.

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