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The blue magic in aspiration cytology: unfolding the role of toluidine blue. A hospital-based cross-sectional prospective observational study.

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Abstract

Background:
Fine Needle Aspiration Cytology (FNAC) is a widely used diagnostic technique. However, inadequate sampling may compromise diagnostic accuracy. Rapid on-site evaluation (ROSE) using supravital stains such as Toluidine Blue may improve smear adequacy and efficiency.

Aim:
To assess the role of Toluidine Blue Stain (TBS) in on-site adequacy evaluation of FNAC smears and to compare its diagnostic accuracy with Leishman Stain (LS) and Rapid Papanicolaou Stain (RPS).

Materialsand Methods:
This hospital-based cross-sectional prospective observational study included 179 consecutive FNAC cases from various anatomical sites. One unfixed smear per case was stained with 1% TBS for immediate adequacy assessment, while additional smears were stained with LS and RPS for cytomorphological comparison.

Results:
Adequacy with TBS was 97.8% on first aspiration and 100% after re-aspiration. Diagnostic accuracy was 100% for TBS, 97.8% for LS and 82.1% for RPS. TBS provided satisfactory nuclear and cytoplasmic detail across diverse lesions.

Conclusion:
TBS is a rapid, economical and reliable staining technique that enhances on-site adequacy and diagnostic efficiency in routine FNAC practice.

Recommendation:
It is recommended that TBS be integrated into routine FNAC workflow, particularly in high-volume and resource-limited settings, to reduce inadequate sampling rates and improve diagnostic turnaround time. Further large-scale multicentric studies with histopathological correlation are recommended to validate these findings.

Keywords: *Fine Needle Aspiration Cytology, Toluidine Blue, Rapid On-Site Evaluation, Supravital Stain, Diagnostic Accuracy.*
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Introduction

Fine Needle Aspiration Cytology (FNAC) is a well-established, minimally invasive diagnostic technique widely used for the evaluation of palpable and deep-seated lesions. Owing to its simplicity, rapid turnaround time, cost-effectiveness and high diagnostic accuracy, FNAC plays a pivotal role in the initial assessment of lesions involving the breast, thyroid, lymph nodes, salivary glands, soft tissues and various image-guided visceral organs. It significantly aids in distinguishing benign from malignant conditions, thereby guiding clinical management and reducing unnecessary surgical interventions. [1,2]

Despite its advantages, the diagnostic reliability of FNAC largely depends on the adequacy and quality of the aspirated material. Inadequate or poorly cellular smears remain a notable limitation, often leading to inconclusive reports, repeat procedures, diagnostic delays and increased patient anxiety. [3] This challenge has led to the incorporation of Rapid On-Site Evaluation (ROSE), which allows immediate assessment of smear adequacy during the procedure. By facilitating additional passes when required, ROSE has been shown to enhance diagnostic yield and minimize repeat visits. [4]

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An ideal stain for on-site evaluation should be rapid, simple, economical and capable of providing sufficient cytomorphological detail. Supravital stains fulfill these criteria by allowing direct staining of unfixed smears, thereby significantly reducing processing time. [5] Toluidine Blue Stain (TBS), a basic thiazine metachromatic dye with a strong affinity for nucleic acids, provides excellent nuclear detail and satisfactory cytoplasmic contrast within a short staining duration. Although widely used in frozen section evaluation and intraoperative consultations, its application in routine FNAC practice has not been extensively studied. [6]

Conventional stains such as Leishman stain and Rapid Papanicolaou stain are routinely employed in cytology. However, they require fixation and comparatively longer staining times. In high-volume laboratories and resource-limited settings, a rapid and cost-effective alternative without compromise in diagnostic accuracy would be highly beneficial. [7,8]

Accordingly, the present study was designed to assess the effectiveness of Toluidine Blue Stain in evaluating on-site smear adequacy and cytomorphological details in FNAC and to compare its diagnostic performance with Leishman stain and Rapid Papanicolaou stain.

Materials and methods

Study Design and Setting

This hospital-based cross-sectional prospective observational study was conducted in the Department of Pathology at the Employees' State Insurance Corporation (ESIC) Medical College and Postgraduate Institute of Medical Sciences and Research, Bangalore, a tertiary care center providing comprehensive diagnostic services including outpatient consultations, inpatient care and image-guided intervention services. The study was conducted over a period of three months from October to December 2015.

Study Population

A total of 179 consecutive patients undergoing Fine Needle Aspiration Cytology (FNAC) for various palpable and image-guided lesions were included in the study. Aspirations were performed from multiple anatomical sites including thyroid, breast, lymph nodes, salivary glands, soft tissue masses, superficial lesions and image-guided visceral lesions such as liver and lung.

Inclusion Criteria

- All consecutive FNAC cases received during the study period
- Both superficial and image-guided aspirations
- Smears adequate for comparative staining

Exclusion Criteria

- Smears with extensive hemorrhage obscuring cellular details
- Air-dried smears unsuitable for comparative evaluation

Sample Size Determination

A total of 179 participants were included based on consecutive sampling of all FNAC cases presenting during the three-month study period. No formal sample size calculation was performed, as this was a prospective observational study aiming to assess the feasibility and diagnostic accuracy of Toluidine Blue Stain in routine clinical practice. The sample size was determined by the number of eligible cases received within the study timeframe.

Procedure

FNAC was performed using standard aseptic precautions with a 22–23 gauge needle attached to a 10 mL disposable syringe. Multiple smears were prepared from each aspiration.

One unfixed smear from each case was immediately stained with 1% Toluidine Blue Stain (TBS) for rapid on-site evaluation (ROSE). The stain was applied directly to the air-dried smear, allowed to act for approximately one minute and then gently rinsed with distilled water. The smear was examined immediately under the light microscope to assess adequacy.

Additional smears from the same aspiration were processed using routine staining methods:

- Leishman Stain (LS)
- Rapid Papanicolaou Stain (RPS)

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These stained smears were evaluated for detailed cytomorphological assessment.

Parameters Assessed

The following parameters were evaluated and compared among the three staining techniques:

- On-site adequacy
- Staining intensity
- Nuclear detail
- Cytoplasmic detail
- Background clarity
- Diagnostic accuracy

Adequacy was defined as the presence of sufficient representative cellular material to render a cytological diagnosis.

Bias

Potential sources of bias included operator-dependent variation in aspiration technique, subjective interpretation of smear adequacy and variability in staining quality. To minimize bias, all aspirations were performed by a single experienced operator. Adequacy assessment and diagnostic interpretation were performed by two independent pathologists blinded to the staining technique where feasible. Standardized staining protocols were followed for all three stains to reduce technical variability.

Ethical Considerations

Institutional ethical clearance was obtained from the Institutional Ethics Committee of ESIC Medical College and Postgraduate Institute of Medical Sciences and Research, Bangalore (Approval Number: IEC/2015/142; Approval Date: 15th September 2015) prior to commencement of the study.

Informed Consent

Written informed consent was obtained from all participants prior to their inclusion in the study. Participants were informed about the procedure, its purpose and the use of their samples for research. Consent forms were maintained in accordance with institutional guidelines.

Statistical Analysis

The collected data were entered and analyzed using SPSS software version 18.0. Descriptive statistical analysis was performed and results were expressed in percentages.

Results

Participant Flow

A total of 210 consecutive patients undergoing FNAC were screened for eligibility during the study period. Of these, 31 were excluded due to extensive hemorrhage obscuring cellular details (n=18) or air-dried smears unsuitable for comparative evaluation (n=13). The remaining 179 participants were included in the final analysis. All 179 included participants completed the study protocol with no further exclusions or dropouts.

Descriptive Data

Among the 179 participants, the mean age was 45.3 years (range: 18–82 years). There were 102 females (57.0%) and 77 males (43.0%). The site-wise distribution of lesions is shown in Table 3.

On-Site Adequacy

Rapid on-site evaluation using 1% Toluidine Blue Stain (TBS) demonstrated that 175 of 179 cases (97.8%) were adequate on first aspiration. Four cases (2.2%) were initially inadequate; however, immediate re-aspiration performed during the same sitting yielded adequate material in all four cases. Thus, the final adequacy rate achieved with TBS was 100%.

On-site adequacy assessment was not feasible with Leishman Stain (LS) and Rapid Papanicolaou Stain (RPS) due to longer staining duration.

Table 1. On-Site Adequacy Using Toluidine Blue Stain (n = 179)

Stage of Evaluation	Adequate (n)	Inadequate (n)	Adequacy Rate (%)
First aspiration	175	4	97.8

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After re-aspiration	179	0	100

Diagnostic Accuracy

The overall diagnostic accuracy at the time of final cytological interpretation was highest with TBS (100%), followed by LS (97.8%) and RPS (82.1%). Diagnostic accuracy was determined based on final cytological diagnosis and adequacy assessment in the study population.

Table 2. Diagnostic Accuracy of Staining Techniques

Stain	Diagnostic Accuracy (%)
Toluidine Blue Stain	100
Leishman Stain	97.8
Rapid Papanicolaou Stain	82.1

Site-Wise Distribution of Lesions

Table 3. Site Distribution of FNAC Cases (n = 179)

Site	Cases (n)	Percentage (%)
Lymph nodes	49	27.4
Thyroid	38	21
Breast	33	18.4
Soft tissue masses	29	16.2
Superficial lesions	12	6.7
Salivary glands	8	4.5
Image-guided lesions	10	5.6

Organ-Specific Cytological Findings

Thyroid Lesions

Of the 38 thyroid cases, 32 (84.2%) were categorized as benign (Bethesda Category II). Four cases (10.5%) were categorized as follicular neoplasm/suspicious for follicular neoplasm (Category IV) and one case each (2.6%) was reported as suspicious for malignancy (Category V) and malignant (Category VI). No cases were categorized as non-diagnostic (Category I) or atypia of undetermined significance (Category III). Representative cytological features of papillary thyroid carcinoma are shown in Figure 1. Additional thyroid lesions including nodular goitre and follicular neoplasm are illustrated in Figures 2 and 3.

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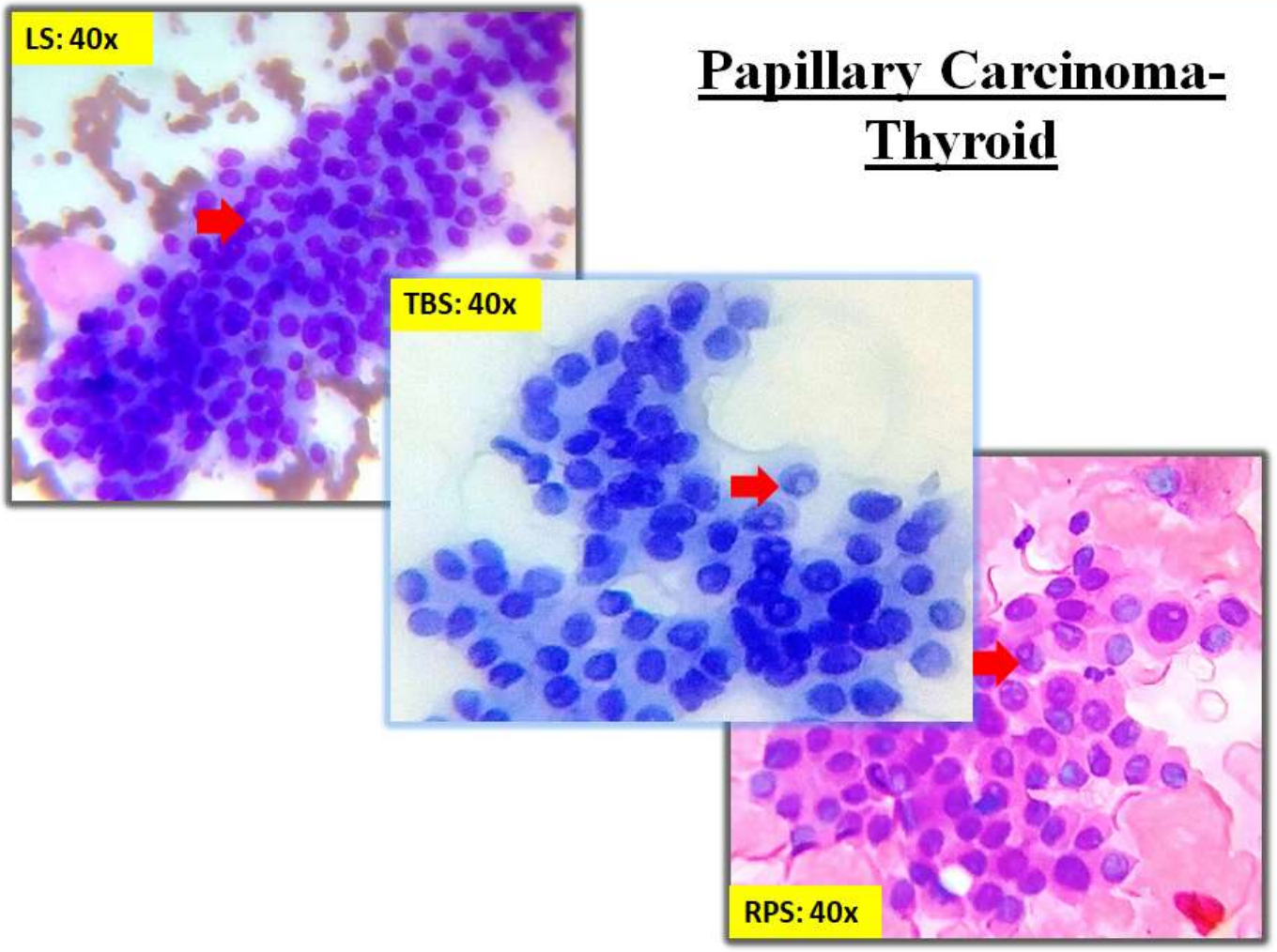

Figure 1: FNAC smears of **Papillary Thyroid Carcinoma** showing papillary fragments with nuclear grooves and intranuclear inclusions: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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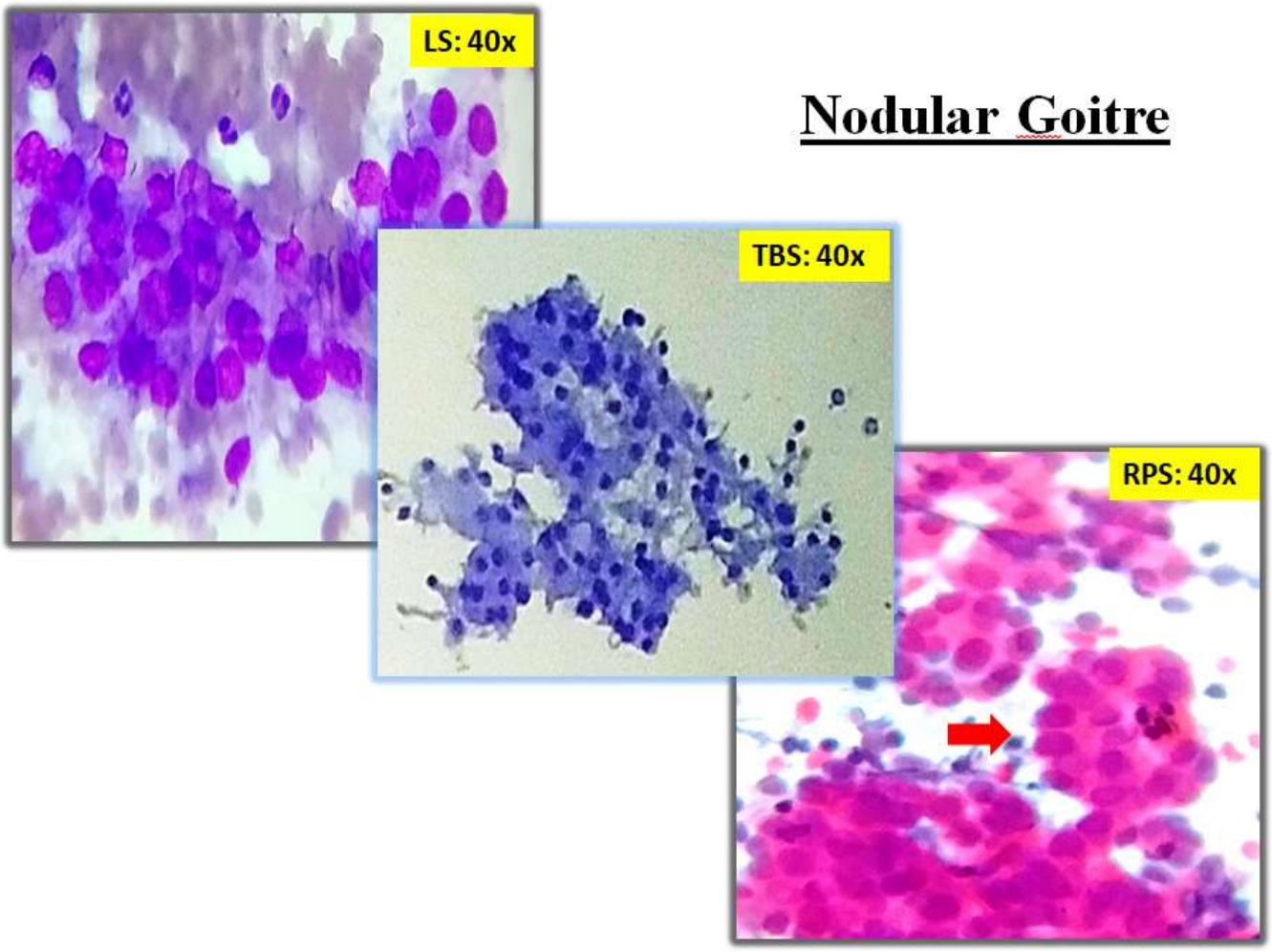

Figure 2: FNAC smears of **Nodular Goitre** showing monolayered sheets of follicular cells having fragile cytoplasm: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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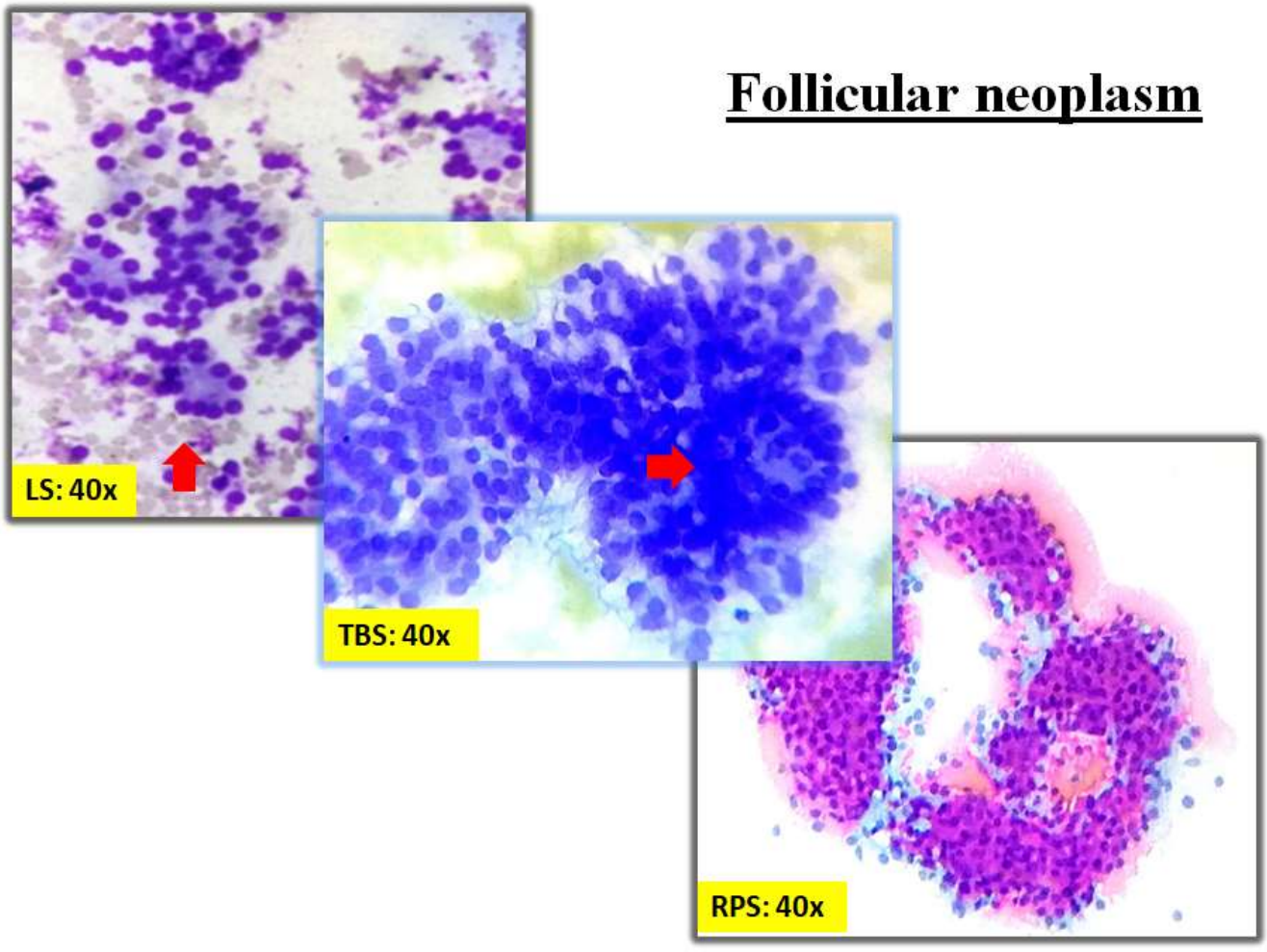

Figure 3: FNAC smears of **Follicular Neoplasm** showing follicular cells in prominent microfollicular & macrofollicular pattern with occasional nuclear overlapping: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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Breast Lesions

Among the 33 breast lesions, 23 cases (69.7%) were benign (C2), 2 cases (6.1%) were atypical (C3), 1 case (3%) was suspicious for malignancy (C4) and 7 cases (21.2%) were malignant (C5). Representative cytological features of fibroadenoma, phylloides tumor and invasive ductal carcinoma are shown in Figures 4, 5 and 6.

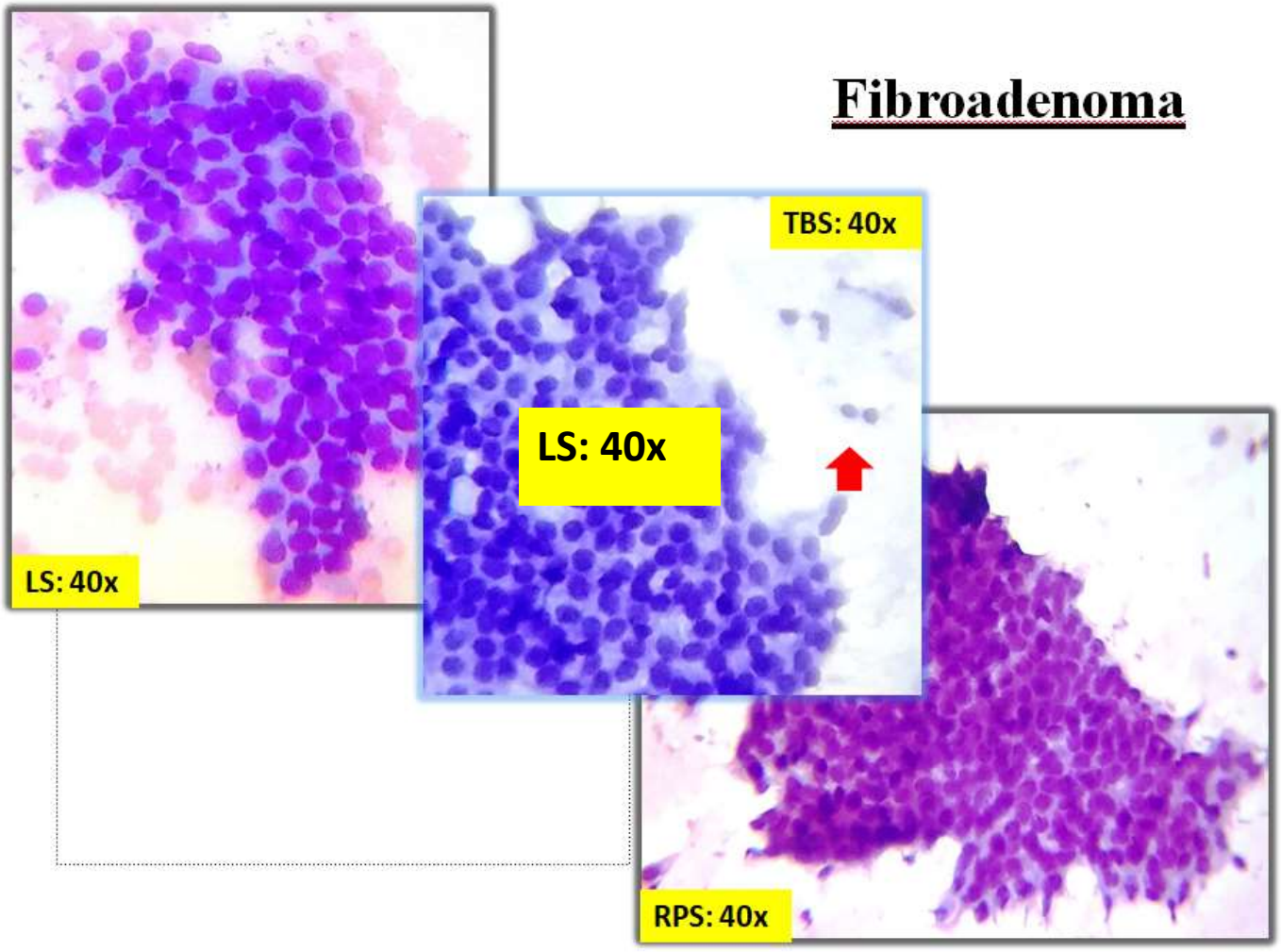

Figure 4: Cytological features of **Fibroadenoma** on FNAC showing cohesive clusters of ductal epithelial cells with bipolar nuclei in the background: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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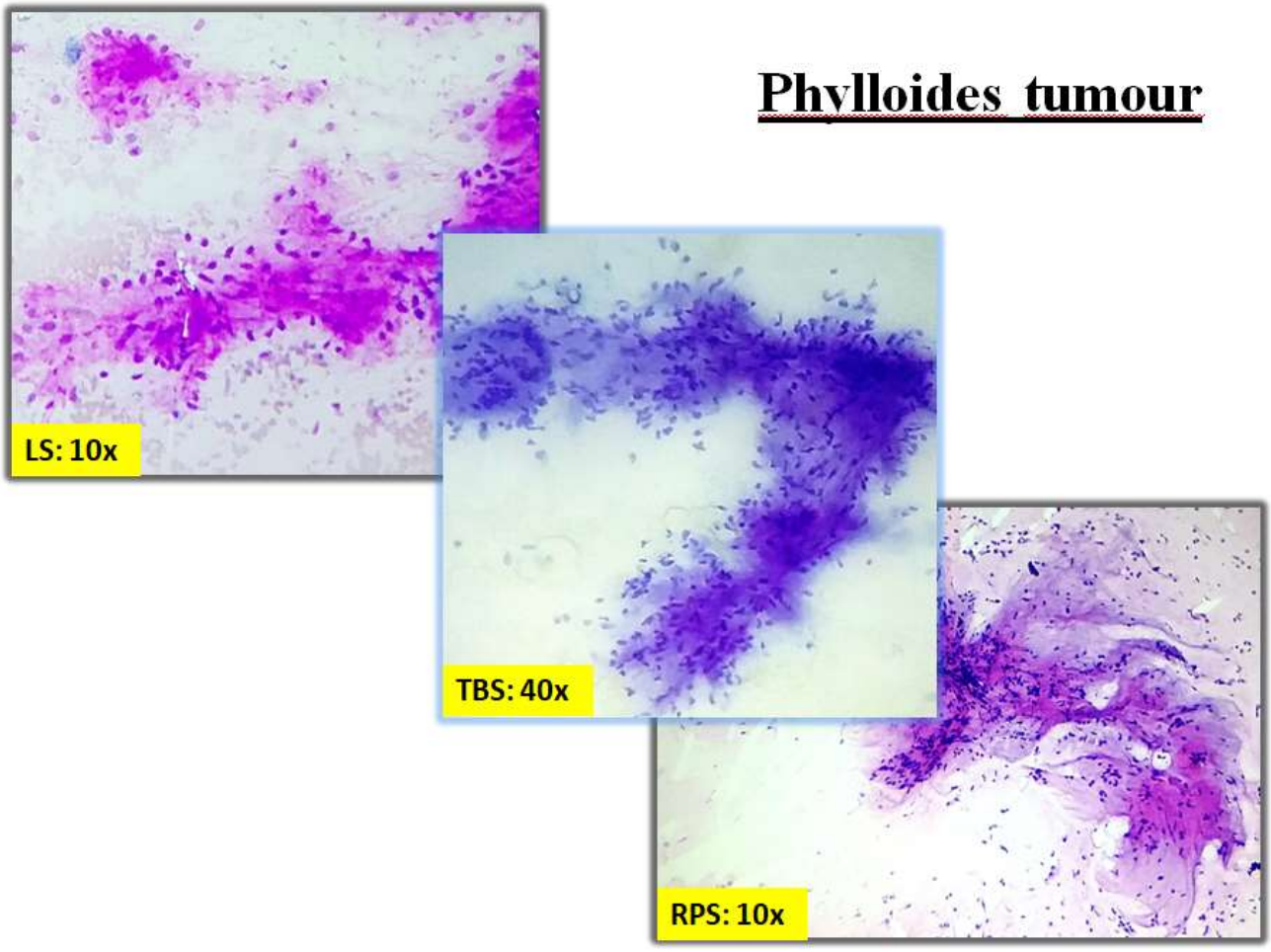

Figure 5: FNAC smears of **Phylloides tumor** showing fragments of spindle cells embedded within fibrous stroma: (A) Leishman stain 10x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 10x.

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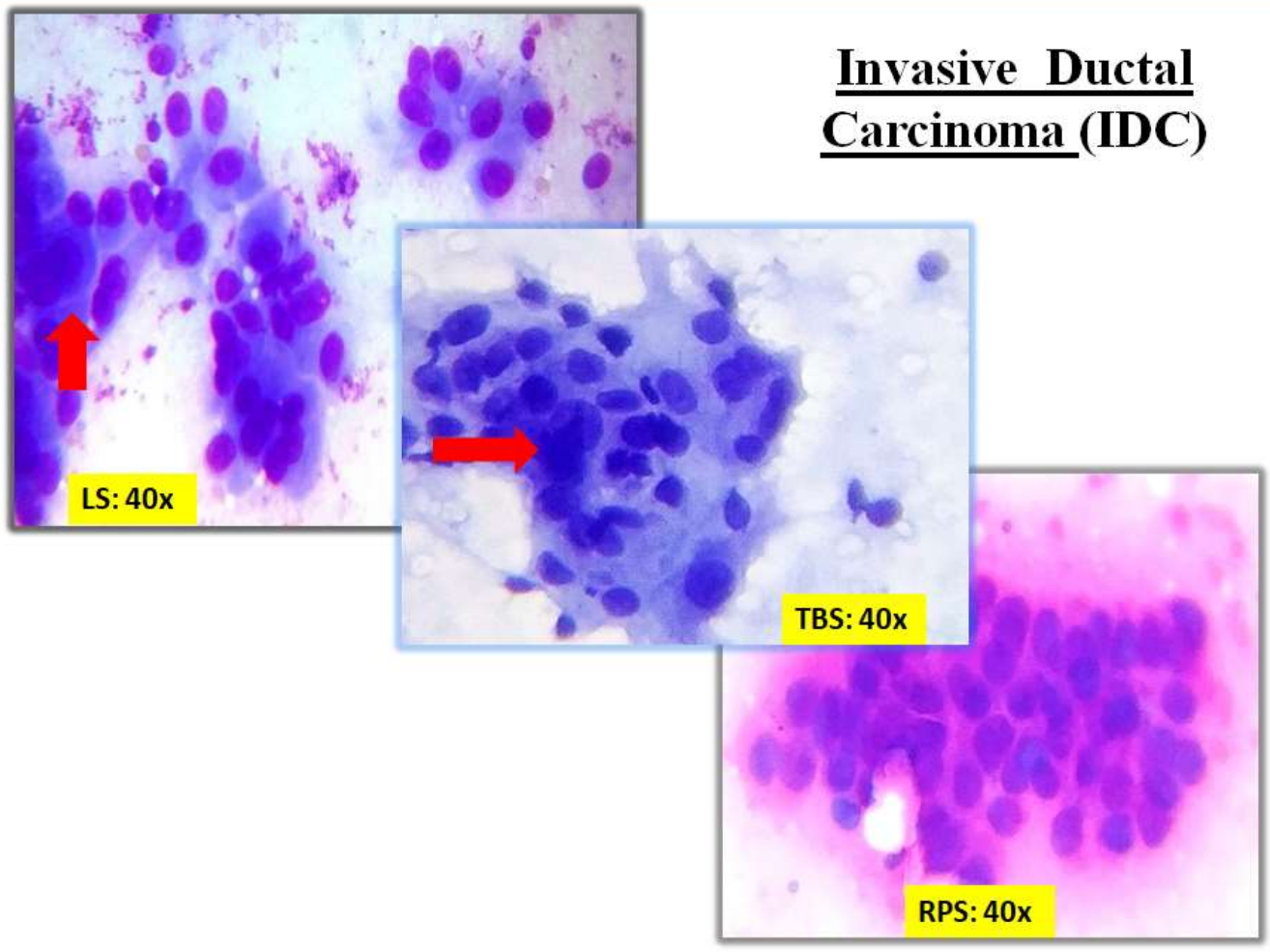

Figure 6: FNAC smears of **Invasive Ductal Carcinoma** showing dyscohesive pleomorphic epithelial cells with prominent nucleoli: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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Lymph Node Lesions

Of the 49 lymph node aspirates, 29 cases (59%) were benign and 20 cases (41%) were malignant. Reactive lymphadenitis (32.7%) and granulomatous lymphadenitis (26.5%) (Figures 7 & 8) constituted the majority of benign lesions. Metastatic squamous cell carcinoma (16%) was the most common malignant lesion (Figures 9–11). Non-Hodgkin's lymphoma is shown in Figure 12.

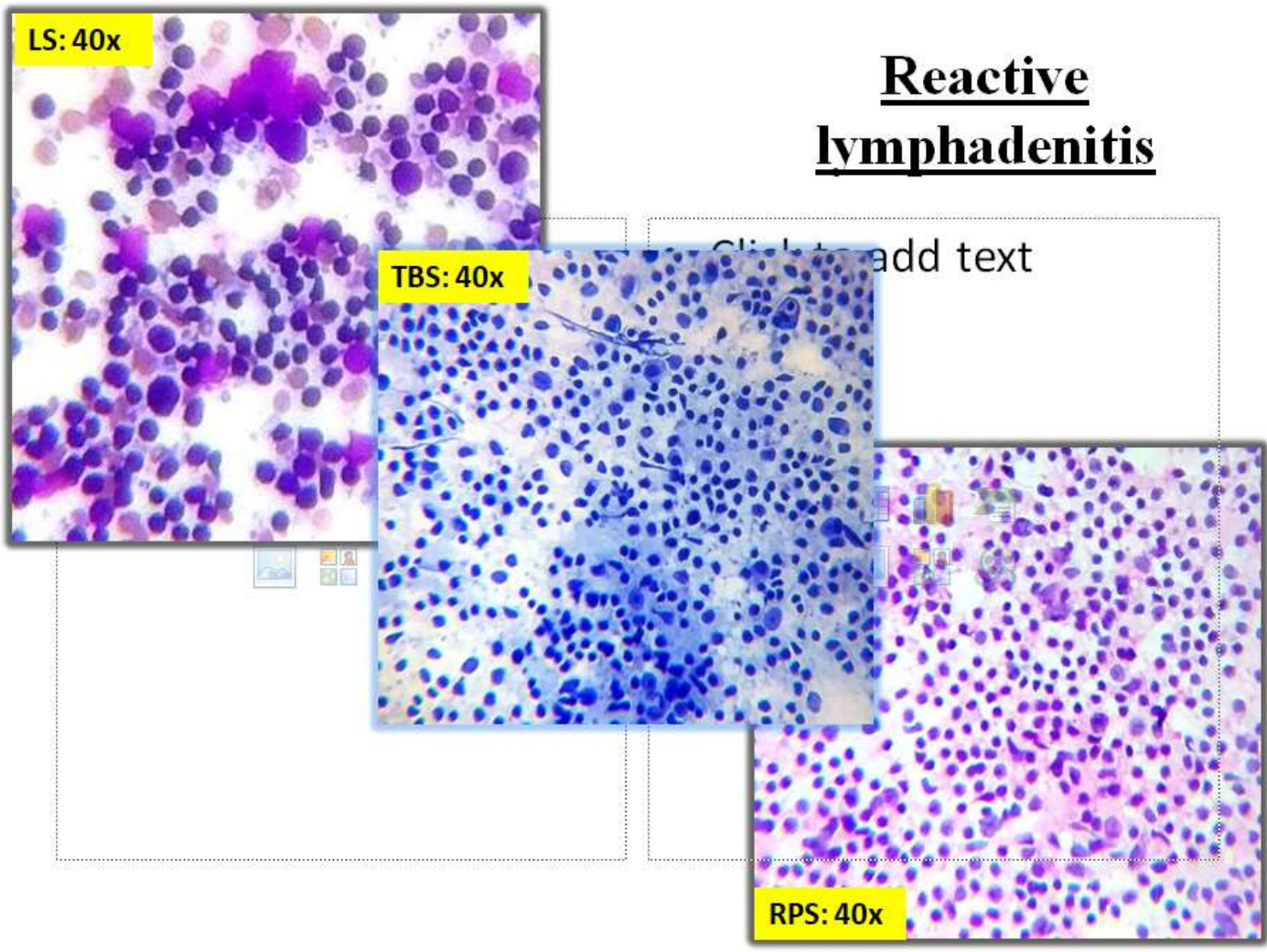

Figure 7: FNAC smears of **Reactive Lymphadenitis** showing polymorphous population of lymphoid series of cells: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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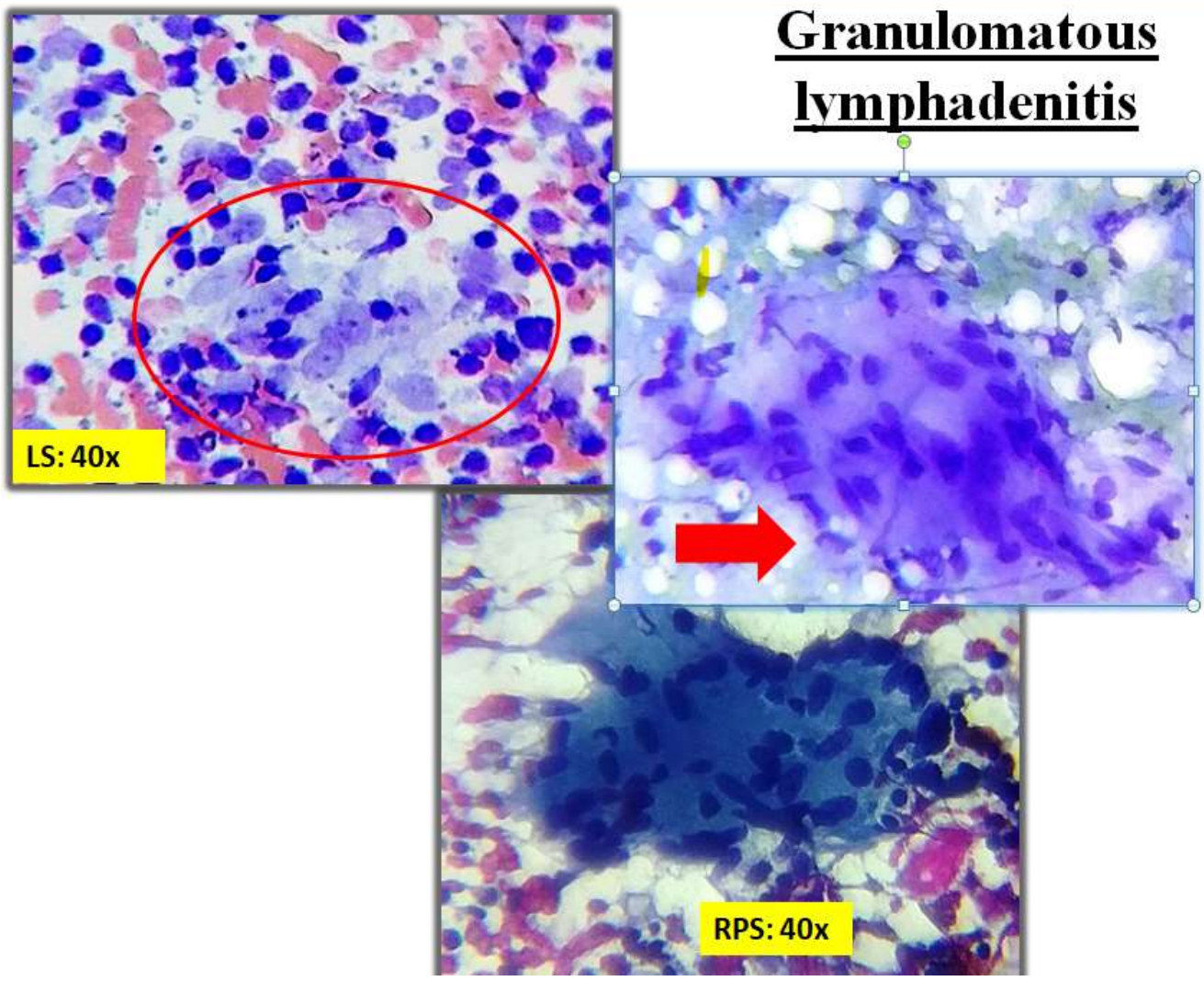

Figure 8: FNAC smears showing **Granulomatous Lymphadenitis** with epithelioid cell clusters: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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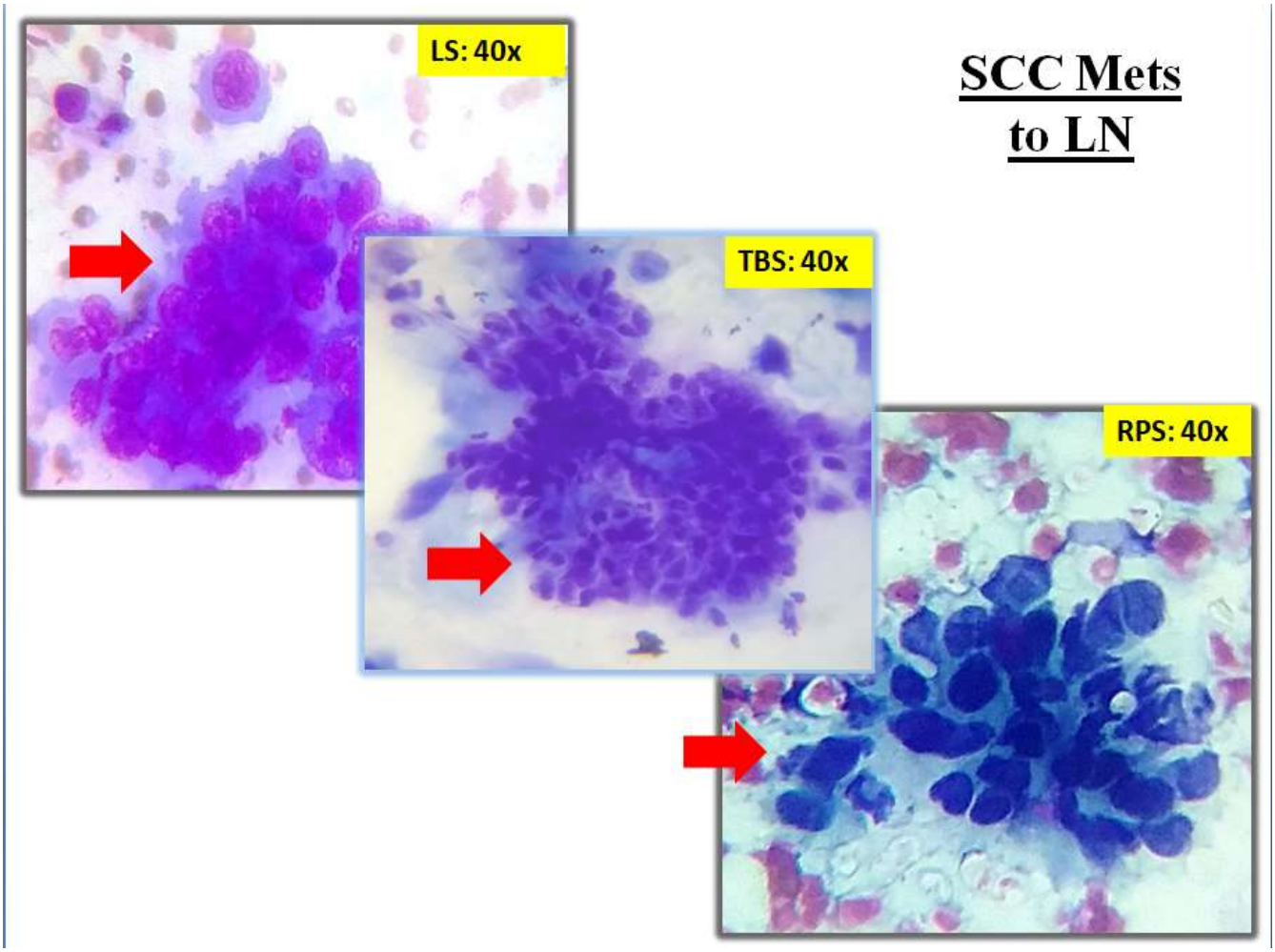

Figure 9: FNAC smears of **SCC Mets. to LN** showing irregular solid sheets of pleomorphic cells having intercellular bridges: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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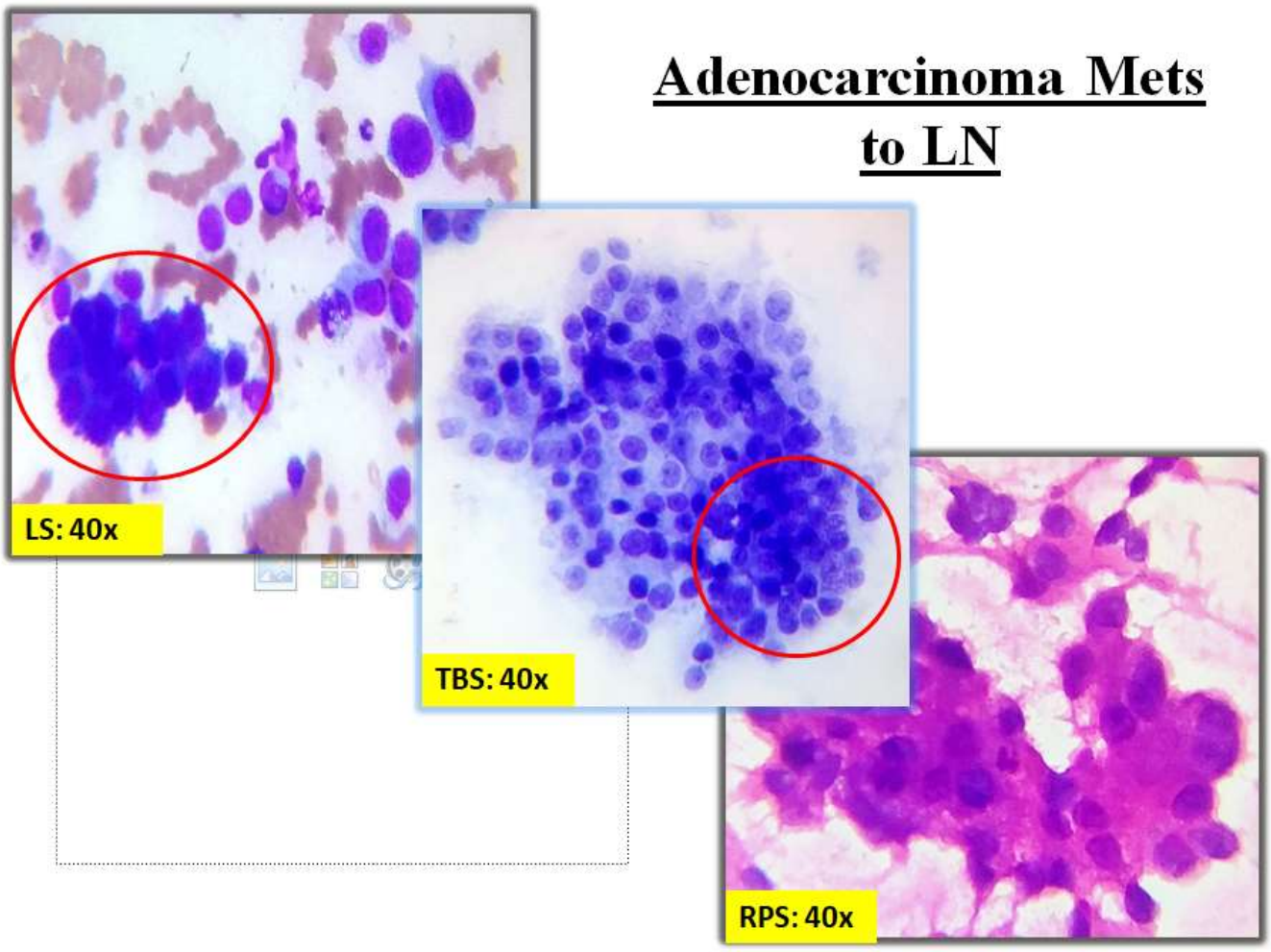

Figure 10: FNAC smears of **Adenocarcinoma Mets. to LN** showing pleomorphic cells tending to form glands with prominent nucleoli in a background of lymphocytes: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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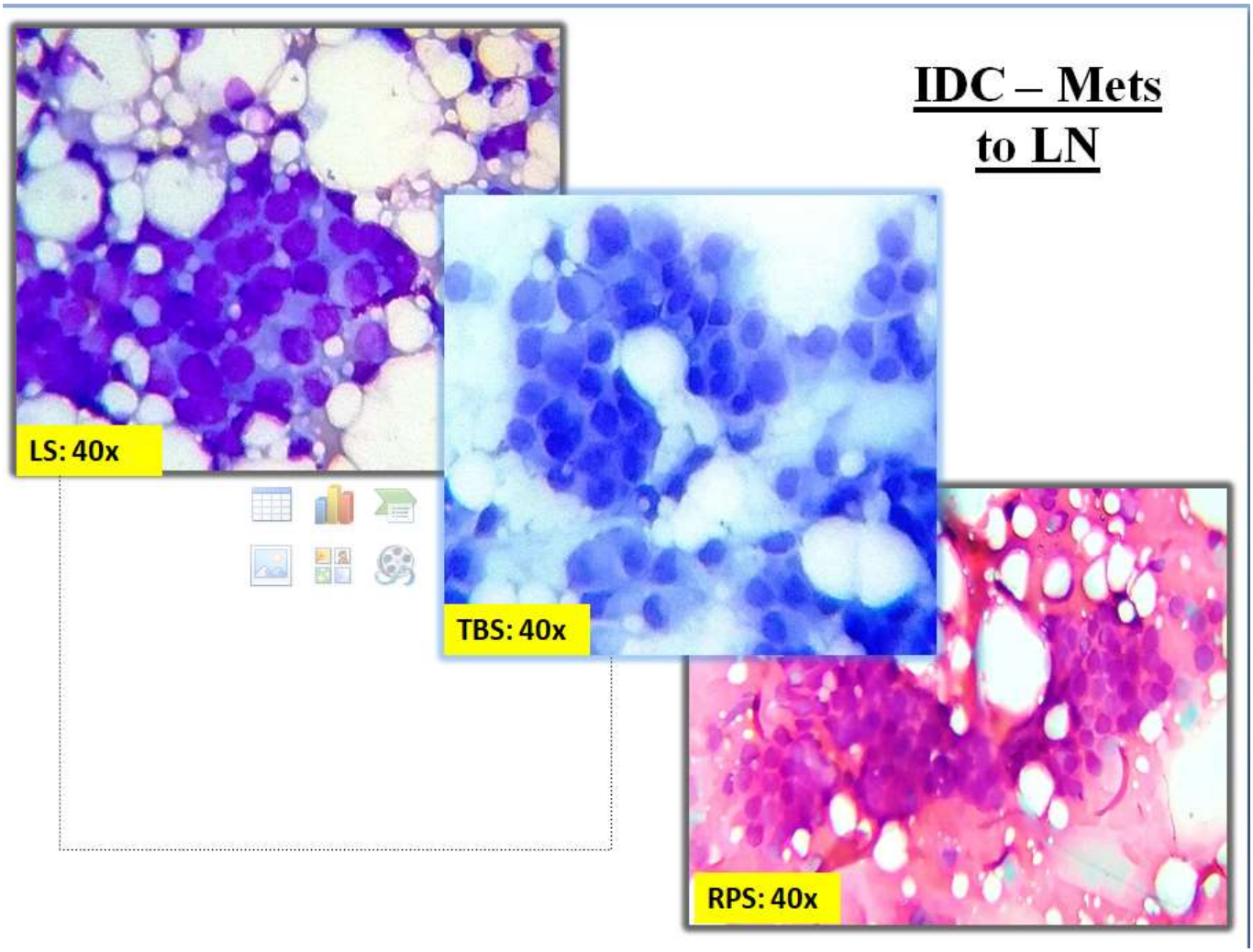

Figure 11: FNAC smears of **Invasive Ductal Carcinoma (IDC) Mets. to LN** showing poorly cohesive pleomorphic ductal epithelial cells with prominent nucleoli in a background of lymphocytes: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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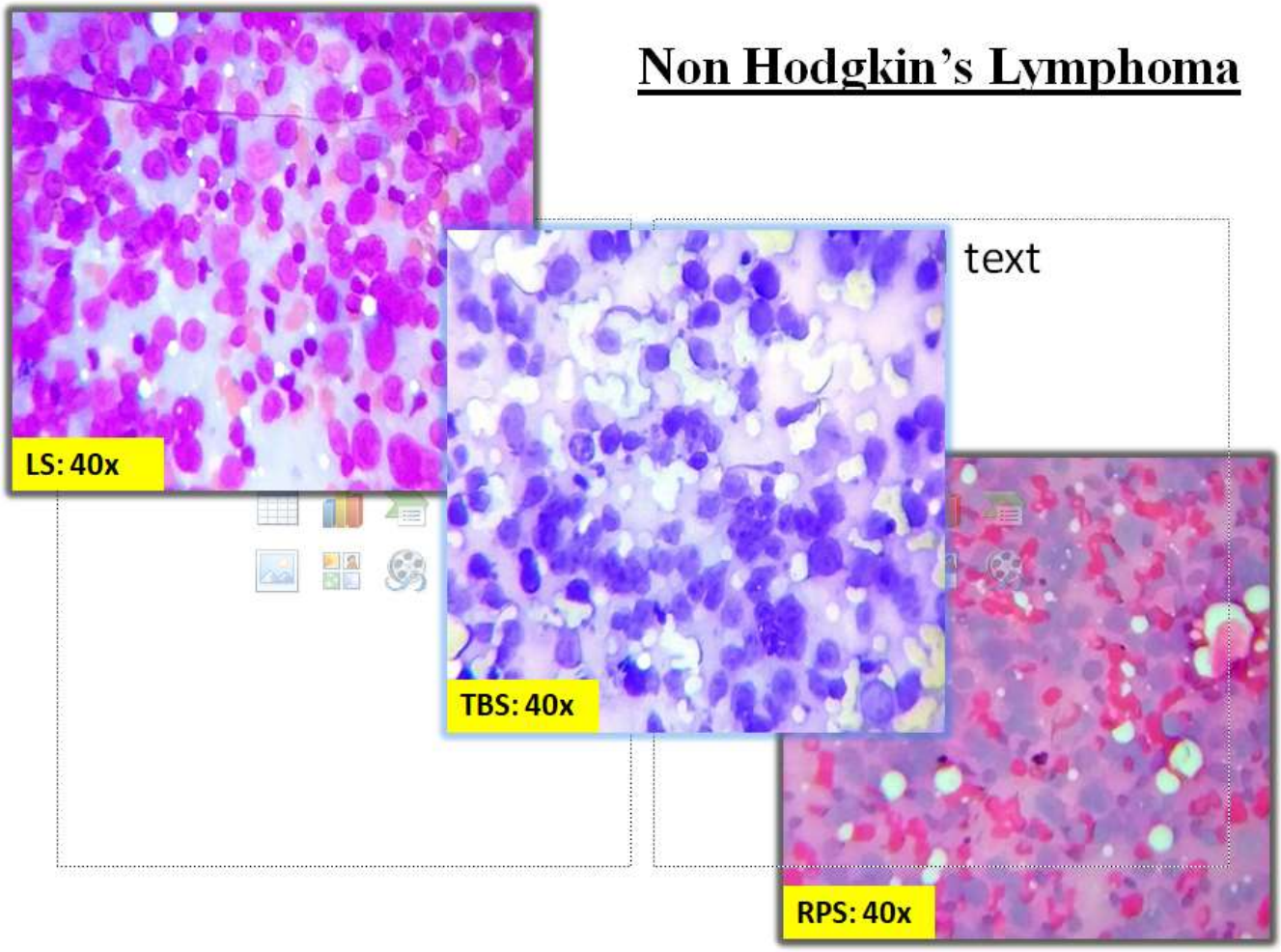

Figure 12: FNAC smears of **Non-Hodgkin's lymphoma** showing monomorphic population of lymphoid series of cells without any RS-like cells: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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Soft Tissue Lesions

Among 29 soft tissue lesions, 27 cases (93%) were benign and 2 cases (7%) were malignant. Lipoma was the most common benign lesion (65.5%) (Figure 13). Malignant soft tissue tumors and small round cell tumors such as Ewing's/PNET are illustrated in Figures 15 and 16.

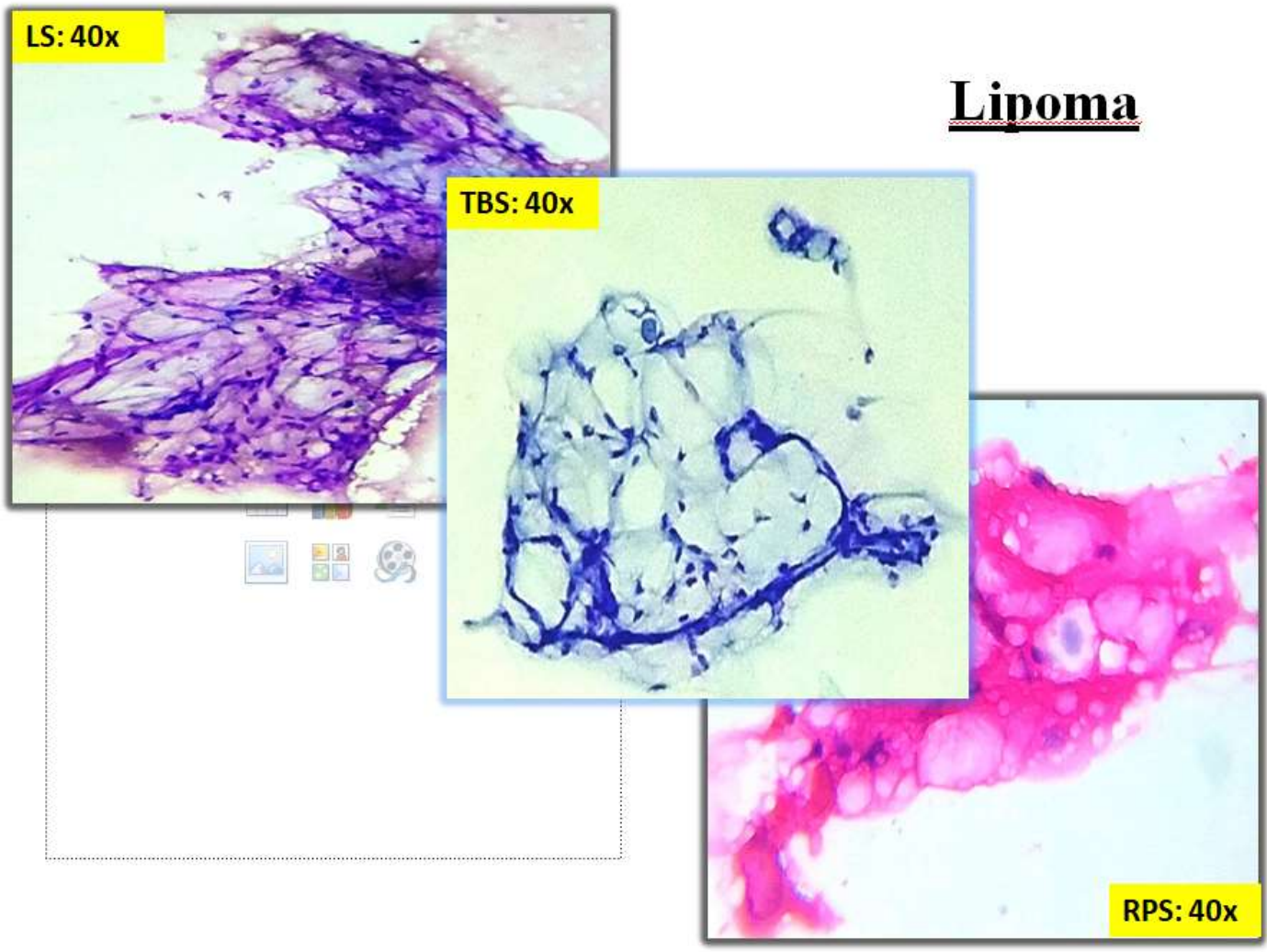

Figure 13: FNAC smears of **Lipoma** showing mature adipocytes within fibrous fragments: (A) Leishman stain40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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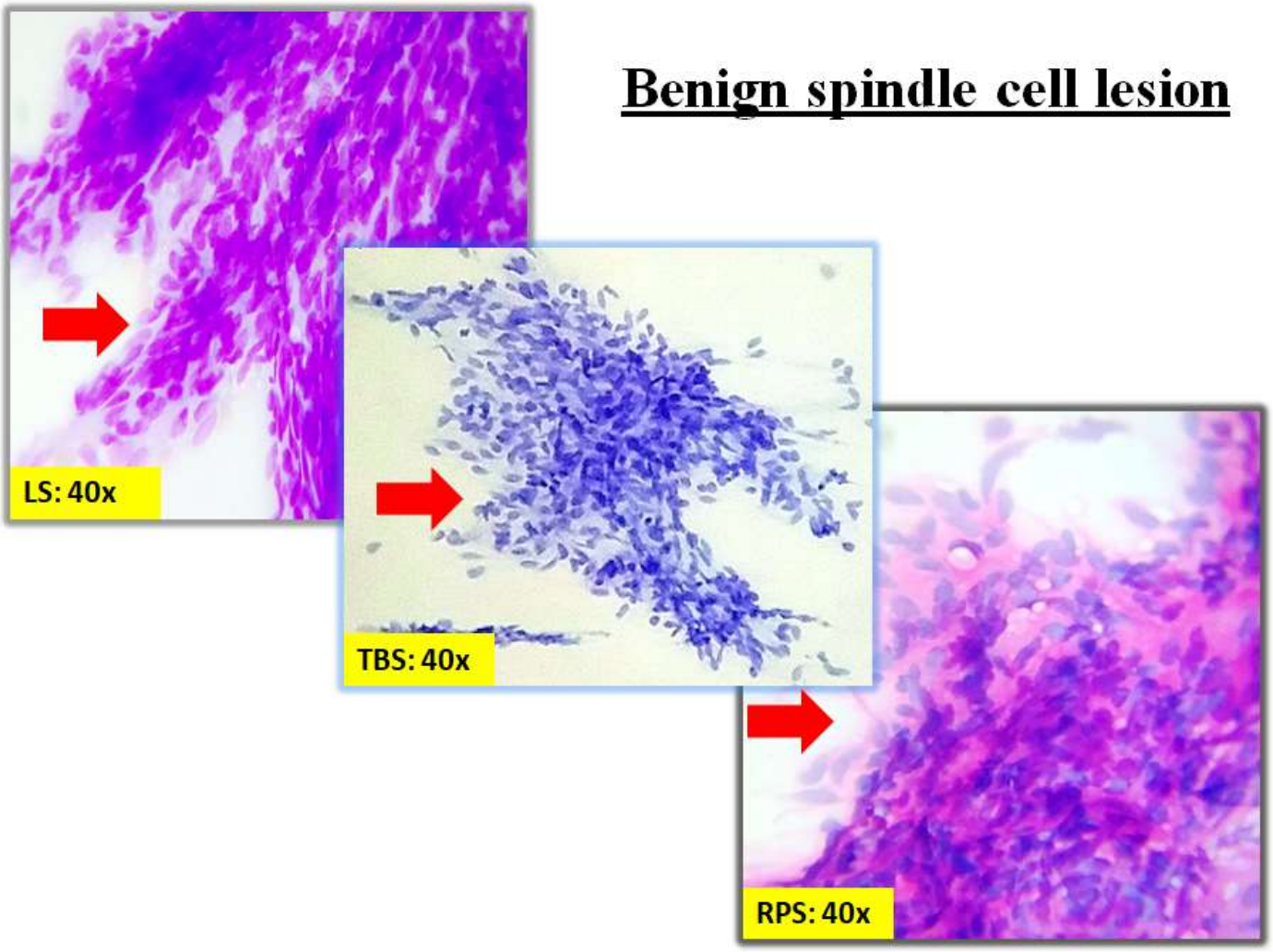

Figure 14: FNAC smears of **Benign spindle cell lesion** showing long and slender cohesive spindle cells with pointed ends: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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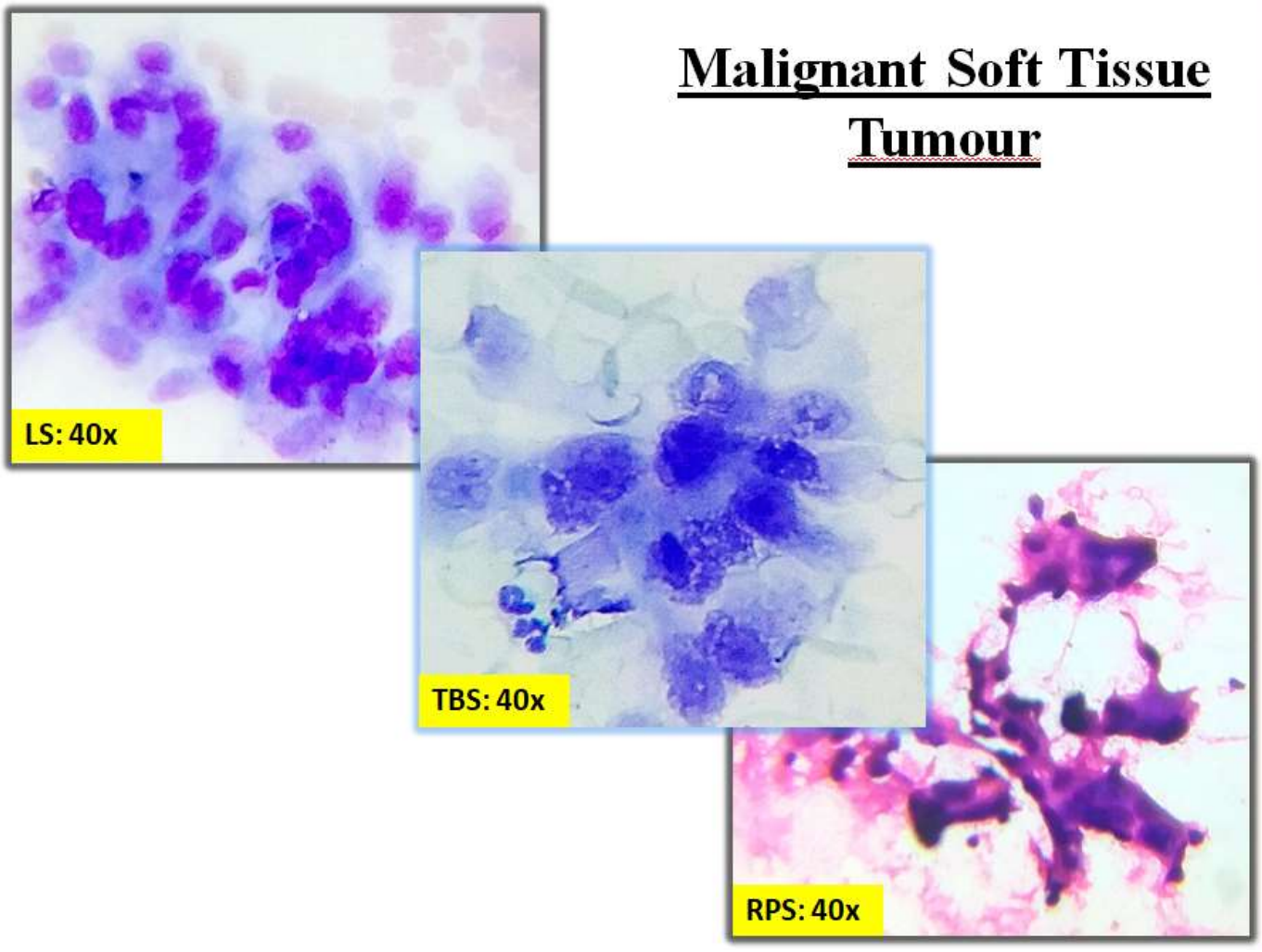

Figure 15: FNAC smears of **Malignant soft tissue tumor** showing poorly cohesive pleomorphic cells with few spindle forms: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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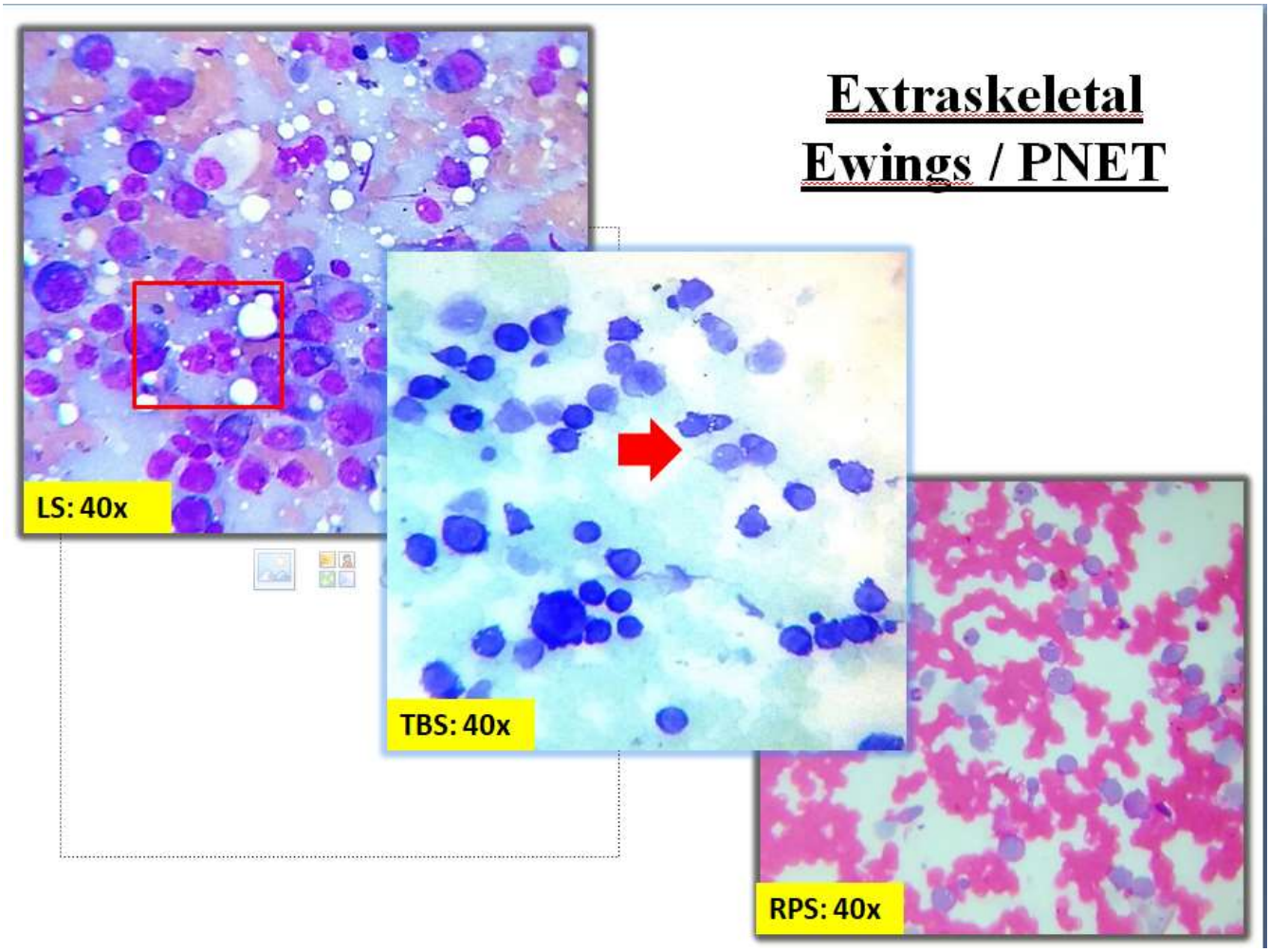

Figure 16: FNAC smears of **Extra-skeletal Ewing's/ PNET** showing dissociated loosely cohesive small round cells with cytoplasmic vacuoles: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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Salivary Gland Lesions

All 8 salivary gland lesions were benign, with pleomorphic adenoma constituting 62.5% (5/8) of cases. Pleomorphic adenoma and sialadenosis are illustrated in Figures 17 and 18.

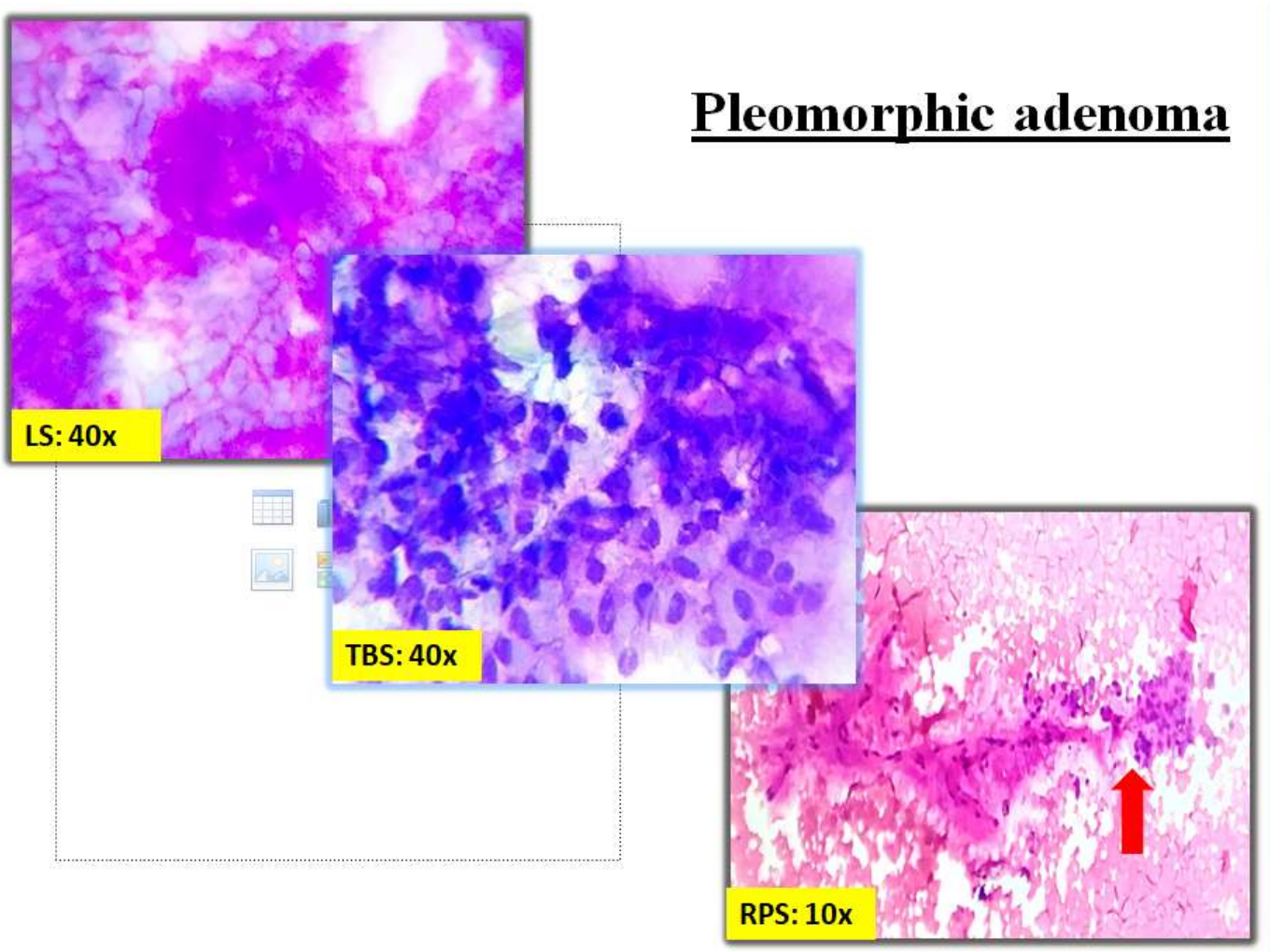

Figure 17: FNAC smears of **Pleomorphic Adenoma** showing epithelial-like cells with fibromyxoid matrix: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 10x.

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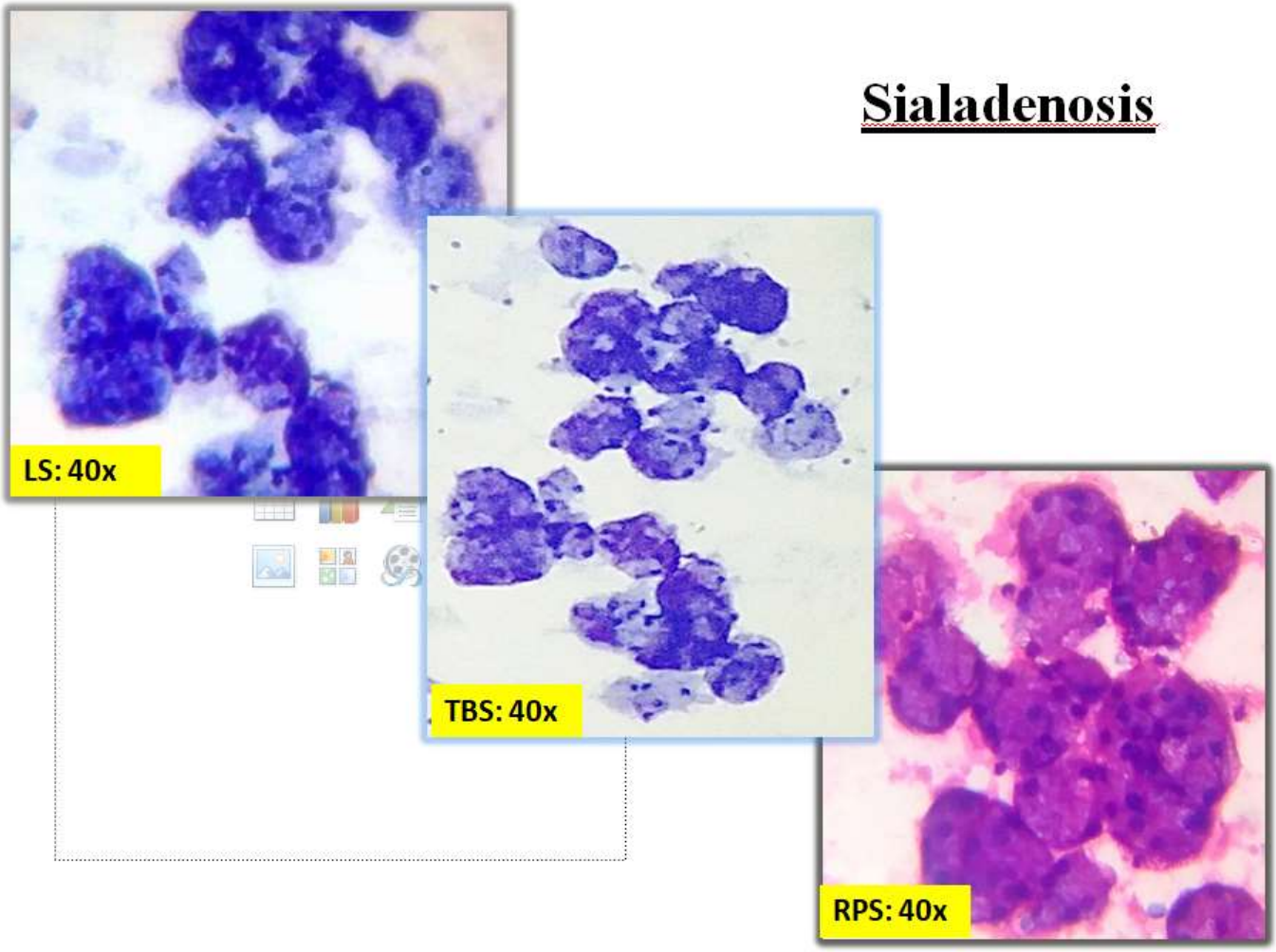

Figure 18: FNAC smears of **Sialadenosis** showing uniform, well –formed hyperplastic acini within fibrovascular stroma: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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Superficial Lesions

Among 12 superficial lesions, 10 cases (83%) were benign (epidermoid cyst) and 2 cases (17%) were malignant (squamous cell carcinoma). Epidermoid cyst is illustrated in Figure 19.

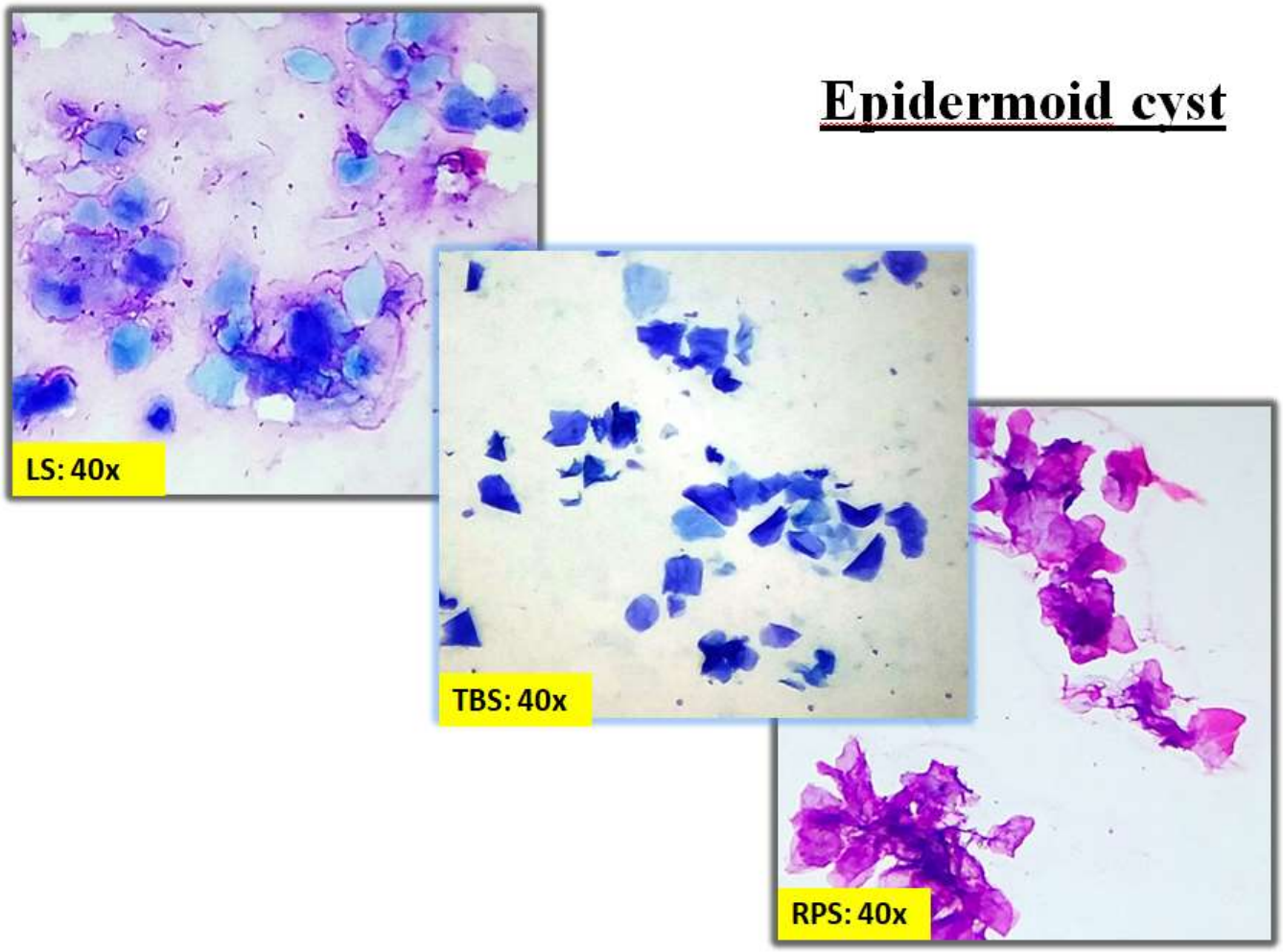

Figure 19: FNAC smears of **Epidermoid Cyst** showing plenty of nucleate & anucleate squames: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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Image-Guided Aspirations

All 10 image-guided aspirates were malignant. Liver lesions constituted 60% (6/10) and lung lesions 40%(4/10). Among liver lesions, hepatocellular carcinoma (Figure 20) accounted for 67% (4/6) and poorly differentiated carcinoma for 33% (2/6). All lung lesions were adenocarcinoma. Additional malignant lesions including poorly differentiated carcinoma - Liver and Lung adenocarcinoma are illustrated in Figures 21 and 22.

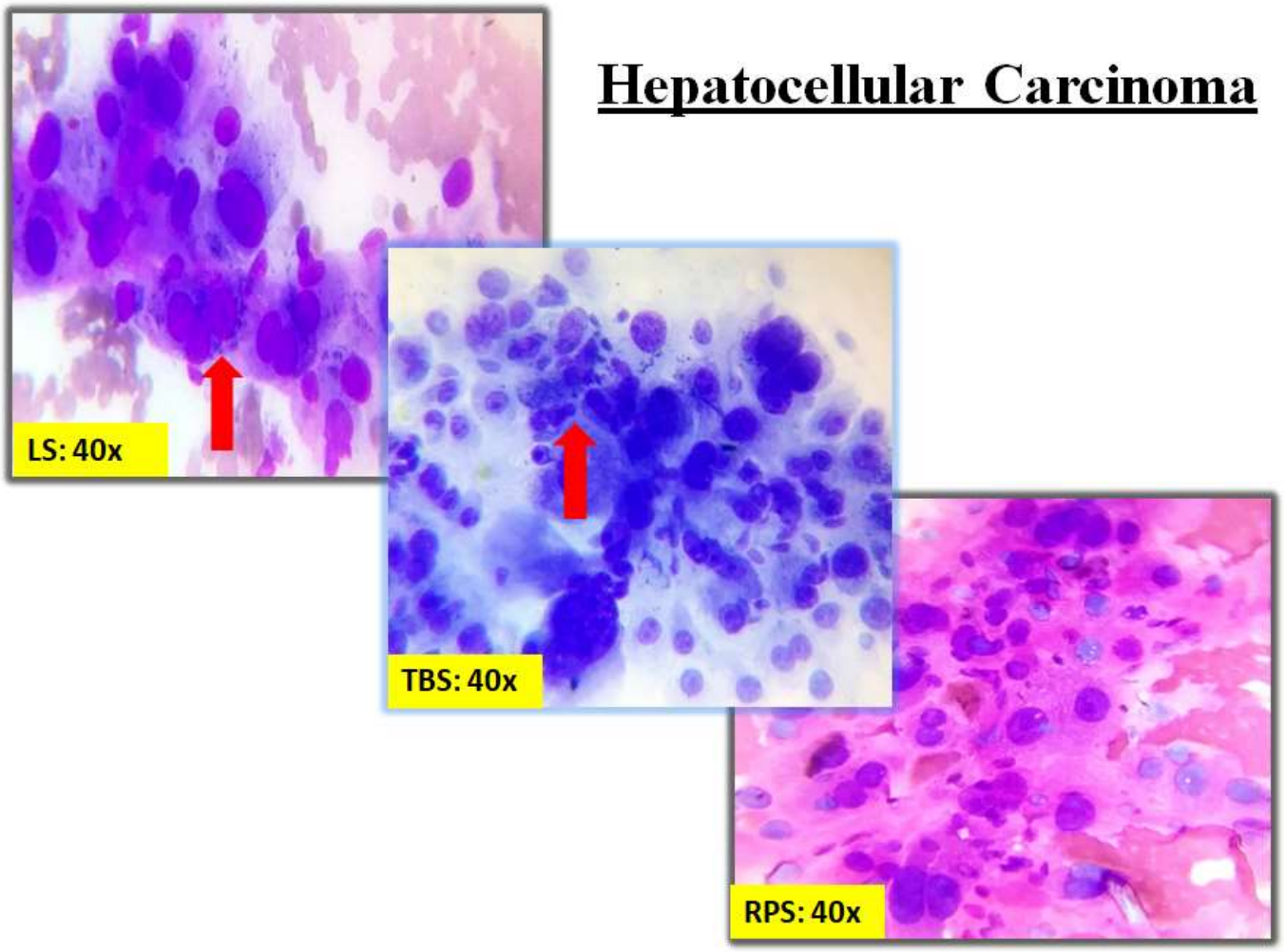

Figure 20: FNAC smears of **Hepatocellular Carcinoma** showing pleomorphic cells with intracytoplasmic bile pigment: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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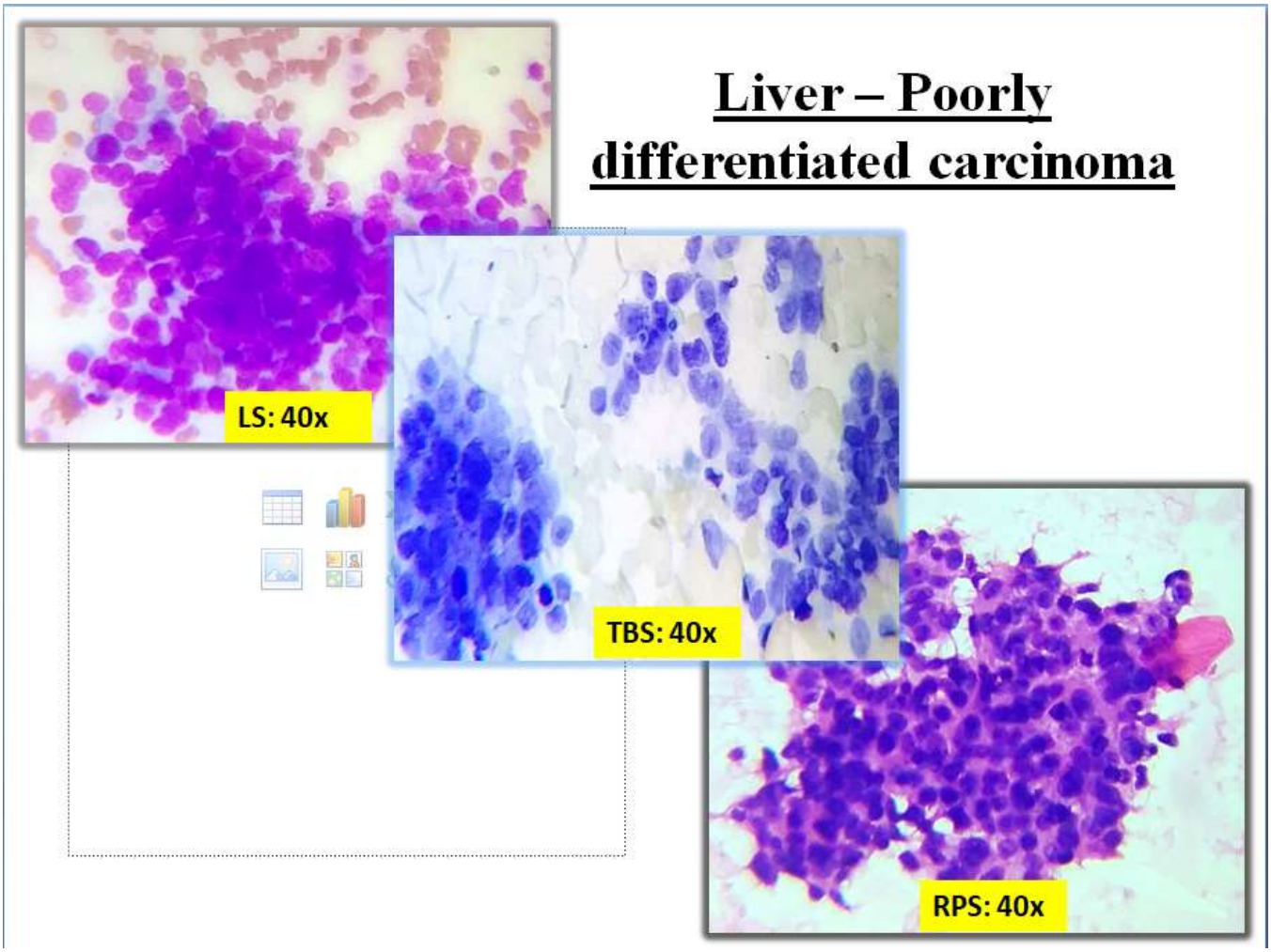

Figure 21: FNAC smears of **Liver (Poorly differentiated carcinoma)** showing poorly cohesive pleomorphic cells: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

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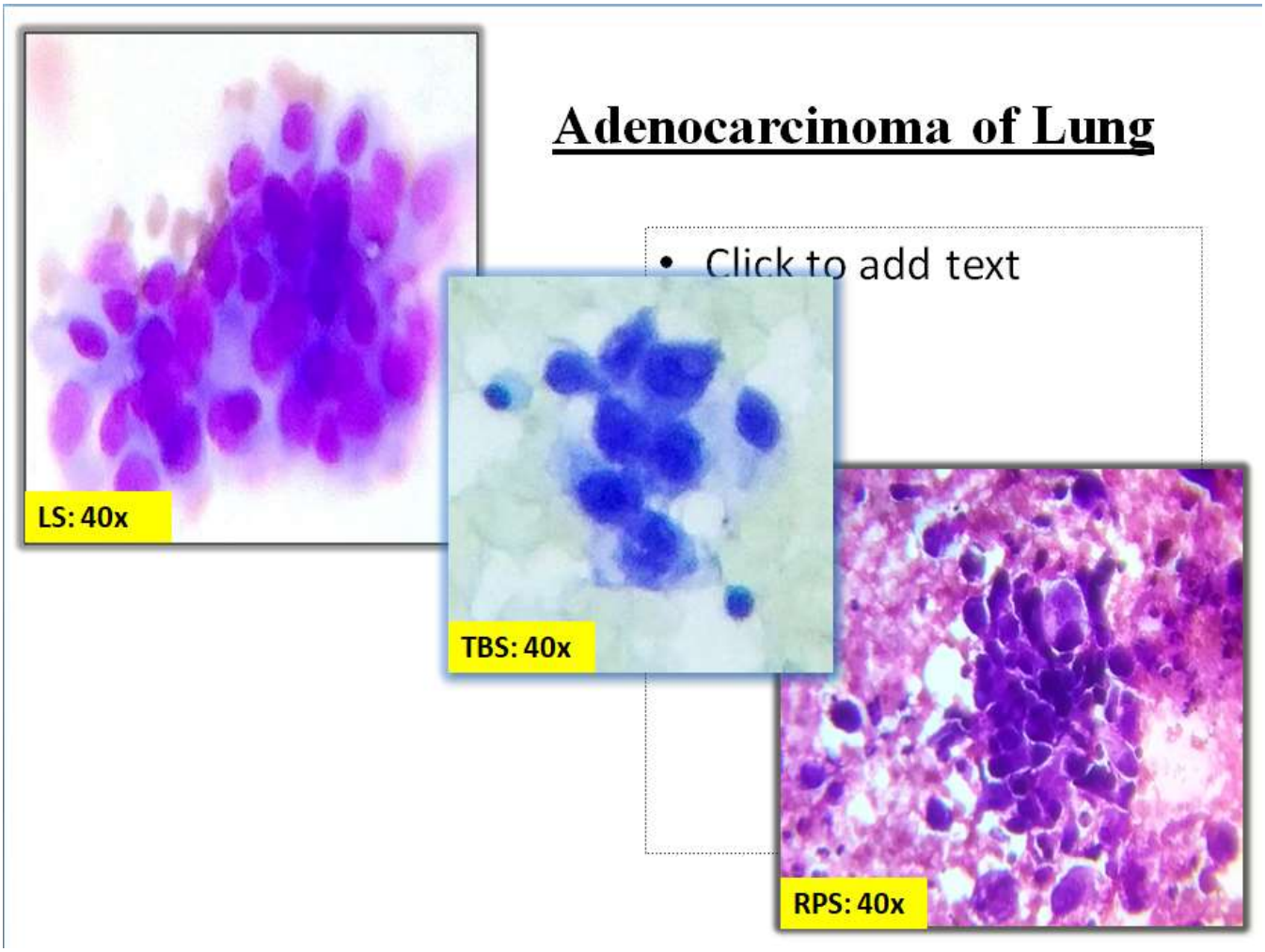

Figure 22: FNAC smears of **Adenocarcinoma of Lung** showing pleomorphic cluster of glandular cells with prominent nucleoli: (A) Leishman stain 40x, (B) Toluidine Blue stain 40x and (C) Rapid Papanicolaou stain 40x.

Comparison of Staining Techniques

Toluidine Blue Stain demonstrated superior practicality in terms of rapid staining time and feasibility of on-site adequacy assessment compared to Leishman and Rapid Papanicolaou stains.

Table 4. Comparison of Cytomorphological and Practical Parameters Among Staining Techniques

Parameter	TBS	LS	RPS
Type of stain	Supravital	Romanowsky-type	Modified Papanicolaou
Fixation required	No	No	Yes
On-site adequacy assessment	Yes	Not feasible	Not feasible
Staining duration	~1 min	~8 min	~5 + 3 min

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Nuclear detail	Good	Good	Good
Cytoplasmic detail	Good	Good	Good
Background clarity	Good	Good	Good
Approximate cost	Rs. 260 / 250 smears	Rs. 180 / 200 smears	Rs. 1745 / 250 smears

Discussion

Fine Needle Aspiration Cytology (FNAC) continues to serve as a primary diagnostic modality for the evaluation of superficial and deep-seated lesions due to its simplicity, rapidity, minimal invasiveness and cost-effectiveness. However, one of the most significant limitations of FNAC remains inadequate sampling, which can lead to inconclusive reports, repeat procedures, delayed diagnosis and increased healthcare burden. [9] Rapid on-site evaluation (ROSE) has been increasingly advocated to overcome this limitation by enabling immediate assessment of smear adequacy and facilitating additional passes during the same sitting. [10]

In the present study, Toluidine Blue Stain (TBS) demonstrated an adequacy rate of 97.8% on first aspiration and 100% after immediate re-aspiration. These findings are comparable with those reported by Ammanagi et al. (2012) [11], who observed that on-site Toluidine Blue staining significantly improved the efficiency of FNAC reporting by reducing inadequate samples and allowing immediate corrective sampling. Similarly, Saba et al. (2015) [12] reported improved adequacy rates and diagnostic efficiency when supravital Toluidine Blue staining was used in comparison with conventional Papanicolaou staining. Sofi et al. (2013) [13] also demonstrated that rapid supravital staining techniques enhanced smear adequacy and reduced the need for repeat aspirations. The high adequacy achieved in the present study reinforces the role of TBS as a valuable adjunct in ROSE.
The overall diagnostic accuracy of TBS in this study was 100%, which was comparable to Leishman Stain (97.8%) and higher than Rapid Papanicolaou Stain(82.1%). Saba et al. (2015) [12] similarly reported high diagnostic concordance between TBS and conventional staining techniques. The slightly lower diagnostic accuracy observed with RPS in the present study may be attributed to staining variability, background artefacts and procedural complexity. In contrast, TBS offers rapid nuclear staining with minimal technical steps, thereby reducing the likelihood of processing-related errors.

Across various anatomical sites, TBS provided satisfactory cytomorphological detail. In thyroid aspirates, characteristic nuclear features of papillary carcinoma, including nuclear grooves and intranuclear inclusions, were clearly visualized. Comparable findings have been reported in studies evaluating rapid supravital staining in thyroid FNAC. In breast lesions, cohesive ductal epithelial clusters in fibroadenoma and dyscohesive pleomorphic cells in invasive ductal carcinoma were distinctly appreciated, consistent with the cytological patterns described by Bukhari et al. (2011) [14] and He et al. (2007) [15] in large FNAC series.

Among lymph node aspirates, reactive lymphadenitis and granulomatous lymphadenitis constituted the majority of benign lesions, while metastatic squamous cell carcinoma represented the predominant malignant category. The ability of TBS to clearly demonstrate epithelioid granulomas, pleomorphic metastatic cells and lymphoid populations further supports its adequacy for routine cytomorphological evaluation. These observations are in agreement with previous studies that have demonstrated reliable nuclear and cytoplasmic detail using supravital staining techniques.

Recent studies have further expanded the applicability of TBS beyond conventional FNAC. Verma and Gupta (2018) [16] reported high concordance rates between supravital staining and conventional cytology across multiple tissue types. Chowdhury et al. (2023) [17] demonstrated high sensitivity and specificity of Toluidine Blue in imprint cytology of bronchoscopic biopsies, supporting its role in rapid preliminary diagnosis. Additionally, large-scale evaluations conducted in recent years have confirmed the reliability of Toluidine Blue in rapid adequacy assessment and preliminary interpretation across diverse anatomical sites.

One of the most significant advantages observed in this study was the rapid staining time of approximately one minute. Compared to Leishman stain, which requires longer staining duration and Rapid Papanicolaou stain, which involves multiple procedural steps, TBS offers a simple and time-efficient alternative. The cost-effectiveness of TBS further enhances its practicality, particularly in high-volume centers and resource-limited settings. By reducing repeat aspirations and minimizing

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patient revisit rates, TBS contributes to improved workflow efficiency and patient satisfaction.

Limitations

The present study has several limitations that should be acknowledged. First, the study was conducted at a single center over a limited duration of three months, which may limit the generalizability of the findings. Second, histopathological correlation was not available for all cases, as surgical excision or biopsy was not performed for every benign lesion. Third, the sample size was determined by consecutive sampling rather than formal sample size calculation, which may affect the precision of diagnostic accuracy estimates. Fourth, while blinding was attempted, complete blinding was not always feasible due to the distinct staining characteristics of TBS. Fifth, the study did not assess inter-observer variability in adequacy assessment. Future large-scale multicentric studies with systematic histopathological confirmation and formal sample size calculations are recommended to provide more robust validation of these findings.

Recommendation

Based on the findings of this study, it is recommended that Toluidine Blue Stain be integrated into routine FNAC practice as a rapid on-site evaluation tool, particularly in high-volume centers and resource-limited settings. Its low cost, minimal staining time and high diagnostic accuracy can help reduce inadequate sampling rates, minimize repeat aspirations and improve patient care. Further research with larger sample sizes and histopathological correlation is recommended to confirm these findings across diverse populations and clinical settings.

Conclusion

Toluidine Blue Stain (TBS) is a rapid, economical and reliable supravital staining technique that significantly enhances on-site adequacy assessment in Fine Needle Aspiration Cytology. In the present study, TBS demonstrated high adequacy rates and diagnostic accuracy comparable to conventional staining methods such as Leishman and Rapid Papanicolaou stains, while offering the distinct advantage of minimal staining time and procedural simplicity. Its ability to provide satisfactory nuclear and cytoplasmic detail across a wide spectrum of lesions supports its practical utility in routine cytopathology. By reducing inadequate smears, minimizing repeat aspirations and improving workflow efficiency, TBS serves as a valuable adjunct in FNAC practice, particularly in high-volume and resource-limited settings.

List of Abbreviations

- FNAC – Fine Needle Aspiration Cytology
- ROSE – Rapid On-Site Evaluation
- TBS – Toluidine Blue Stain
- LS – Leishman Stain
- RPS – Rapid Papanicolaou Stain
- ESIC – Employees' State Insurance Corporation
- LN – Lymph Node
- Mets – Metastasis
- SCC – Squamous Cell Carcinoma
- SPSS – Statistical Package for the Social Sciences

Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

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The data supporting the findings of this study are available from the corresponding author upon reasonable request. Patient confidentiality and institutional guidelines will be respected in any data sharing arrangement.

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