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Applicability of On-Site Evaluation of Cervical Cytology Smears Stained with Toluidine Blue to Reduce Unsatisfactory Results

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Keywords
Cervical cytology · Papanicolaou · Rapid on-site evaluation · Toluidine blue · Unsatisfactory rate

Abstract
Introduction: Carcinoma of the uterine cervix is a major health problem faced by Indian women. Screening techniques like visual inspection with acetic acid, Lugol's iodine, Papanicolaou smear, and human papillomavirus DNA testing have been suggested. Pap smear is a simple, safe, cost-effective, and reliable technique used for screening cervical lesions. Rapid on-site evaluation (ROSE) using the 1% aq. toluidine blue staining method has been less studied in cervical cytology. ***Materials and Methods:*** Our study was a prospective study done over a period of 2 years. All the cervical cytology smears were reported as per the Bethesda system 2014. Rapid stain using aqueous toluidine blue (1%) and conventional Pap stain was done on the smears received. ***Results:*** We evaluated a total of 1,300 cases, with 97.6% satisfactory samples. The spectrum of cases included 96.3% of negative for malignancy cases (including bacterial vaginosis, trichomonas, candida, and atrophic smears), atypical squamous cell of undetermined significance and atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion in 0.5% cases, low-grade squamous intraepithelial lesion and high-grade intraepithelial lesion in 0.3% cases, squamous cell carcinoma in 0.3% cases, and atypical glandular cells/adenocarcinoma in 0.2% cases. Turnaround time was within 48 h in 77% cases. With rapid stain, our unsatisfactory rate was reduced from 12% to approx. 2.4%. ***Conclusion:*** ROSE has been attempted on routine FNA cytology samples with success. However, the use of ROSE in cervical cytology has not been attempted to date. Lower unsatisfactory rate is an important indicator for the successful implementation of cervical cancer screening technique.

Introduction

Carcinoma of the uterine cervix is a major health problem faced by Indian women, and every year, approximately 120,000 women develop this disease [1]. India accounts for 15.2% of the total cervical cancer deaths in the world [1, 2]. Although the incidence of carcinoma cervix has declined in the urban population, in the rural areas it still continues to be highly prevalent [3]. The usual 10–20 years of the natural history of progression from mild dysplasia to carcinoma cervix makes this cancer a relatively early preventable disease and provides the rationale for screening [3, 4].

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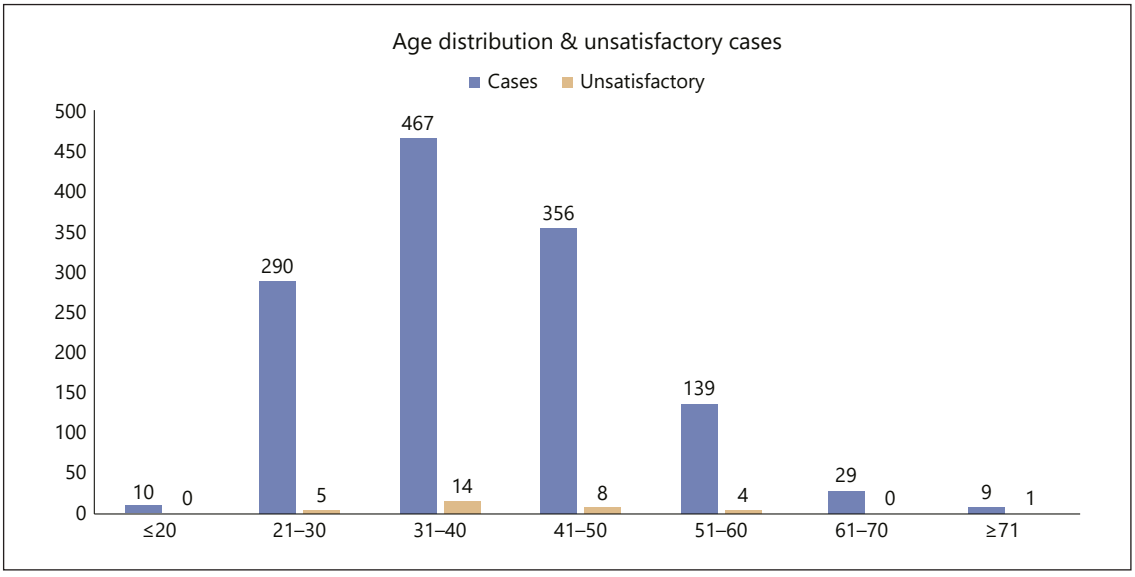

Fig. 1. Age distribution of cases along with unsatisfactory cases in each age group.

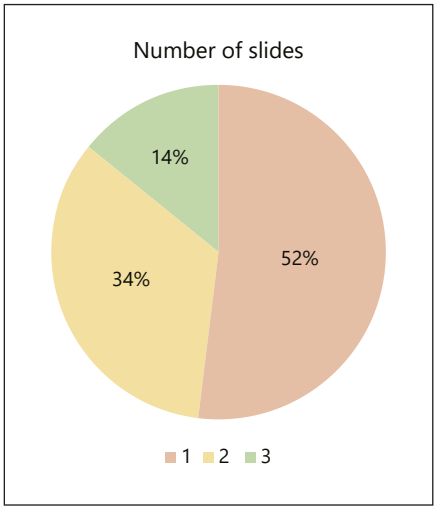

Fig. 2. Number of slides prepared and cases.

The international medical community has also recognized that cervical cancer is preventable and cervical cytology screening has been hailed as the most successful cancer screening method in medical history [4, 5]. The quality of cervical cytology screening can be assessed by quality indices such as relative percentages of the various diagnoses, including the unsatisfactory (US) rate and the atypical squamous cells(ASC) to squamous intraepithelial lesion (SIL) ratio [3, 5]. The correlation of cytological diagnosis with available histological follow-up allows the evaluation of false-positive and false-negative cases [6–8].

Rapid stain using toluidine blue, a supravital stain can accentuate good cytological and nuclear details, enabling the three-dimensional view of cells in wet mount film. It is simple, easily available, cost-effective, and used for quick reporting [9, 10]. There are very few studies on the utility of toluidine blue in cervical cytology [11, 12]. Our aim was to evaluate the role and advantages of rapid stain in cervical smear cytology using toluidine blue. To correlate the US rate before and after implementation of rapid stain. To correlate the findings in toluidine blue and final cytological stain.

Materials and Methods

Our study was a prospective study done on all cervico-vaginal smears received in the Department of Pathology over a period of 2 years (January 2020–December 2021). Females ≥18 years of age and sexually active were included in the study. A total of 1,300 cases were included in the study. The Pap smears were collected in the Department of Obstetrics and Gynaecology by trained expert gynecologists. To obtain a Pap smear, a speculum is inserted into the vagina and a spatula is inserted into the cervical external os and twirled around to collect a sample of cells which is then smeared on two glass slides. The slides with the duly filled cervical cytology requisition form were received by the pathology department for processing. Demographic details/relevant clinical information like age, sex, site of Pap smear, etc., were collected from the cervical cytology requisition form [7, 9, 10]. Rapid stain – 1% aqueous toluidine blue stain (TBS) – was used for assessing the adequacy of

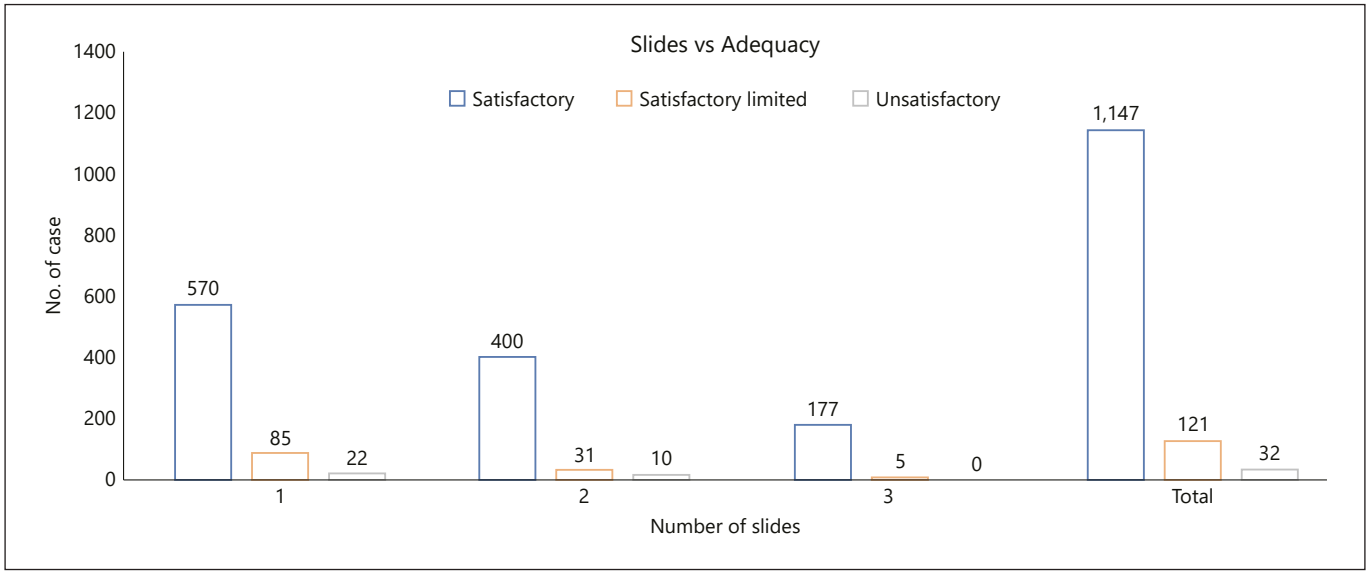

Fig. 3. Adequacy rate among the number of slides prepared.

the sample on the slides sent to laboratory and making a provisional diagnosis, following which the toluidine blue slides were returned for conventional Pap stain [11]. The results from the techniques were compared to evaluate the efficacy of the rapid stain technique using conventional Pap smear as the gold standard. Information regarding the adequacy of the sample was immediately informed to the consultant in case of inadequate smears for the repeat procedure. The rapid stain was compared with conventionally stained Pap smears for cytomorphology staining, timing, and efficiency of stains [12–15]. Wherever possible correlation of histopathology findings with the cytology was done.

Results

The study was done over a period of 2 years, and a total of 1,300 cases were included. The various age groups of patients were included, most common in the 3rd decade with 467 cases (36%), and a mean age was of 39 years (Fig. 1). The overall cases were categorized based on adequacy into (a) satisfactory in 1,268 cases (97.6%) and (b) US due to limited squamous cellularity or obscuring inflammation in 32 cases (2.4%).

We analyzed the cases based on the number of slides received. There were 677 cases (52%), wherein only one slide was received for reporting. The satisfactory rate was only 96.8% (655 out of 677 cases), and the US rate was 3.2% (22 out of 677 cases). Two slides were received in 441 cases (34%), and the satisfactory rate was 97.7% (431 out of 441 cases). The highest satisfactory rate was in cases, wherein 3 slides were received (180 cases/99%) out of the 182 cases (14%) in this group. Also, no US cases were noted (Fig. 2, 3).

We had noted the last menstrual period (LMP) for the cases received in the department. 180 cases (14%) were in postmenopausal phase, vault smear in 34 cases (3%), within 5 days of a menstrual period in 57 cases (4%), no mention of LMP in 129 cases (10%) (Fig. 4).

The spectrum of cases reported during the study period was analyzed. Negative for inflammation/malignancy was reported in 704 cases (54%) (Fig. 5), reactive cellular changes associated with inflammation in 250 cases (19%), a shift in flora suggestive of bacterial vaginosis (BV) in 181 cases (14%) (Fig. 6), trichomonas vaginitis in 03 cases (0.3%), fungal organism consistent with candida sps. in 65 cases (05%) (Fig. 7), atrophic smear in 48 cases (3.8%) (Fig. 8), ASC-US in 06 cases (0.5%), and atypical squamous cell cannot exclude high-grade squamous intraepithelial lesion (ASC-H) in 02 cases (0.2%). Similarly, the low-grade SIL was reported in 01 cases (0.1%) and high grade in 02 cases (0.2%). Squamous cell carcinoma (SCC) was reported in 03 cases (0.3%) (Fig. 9). We also had one case each of atypical glandular cells of undetermined significance and adenocarcinoma. There were 32 unsatisfactory cases (2.4%) (Table 1).

We were also able to note the findings of candida, trichomonas, BV, and atypical cellular features on toluidine blue. A comparison of spectrum of infectious etiology was made between the rapid on-site evaluation

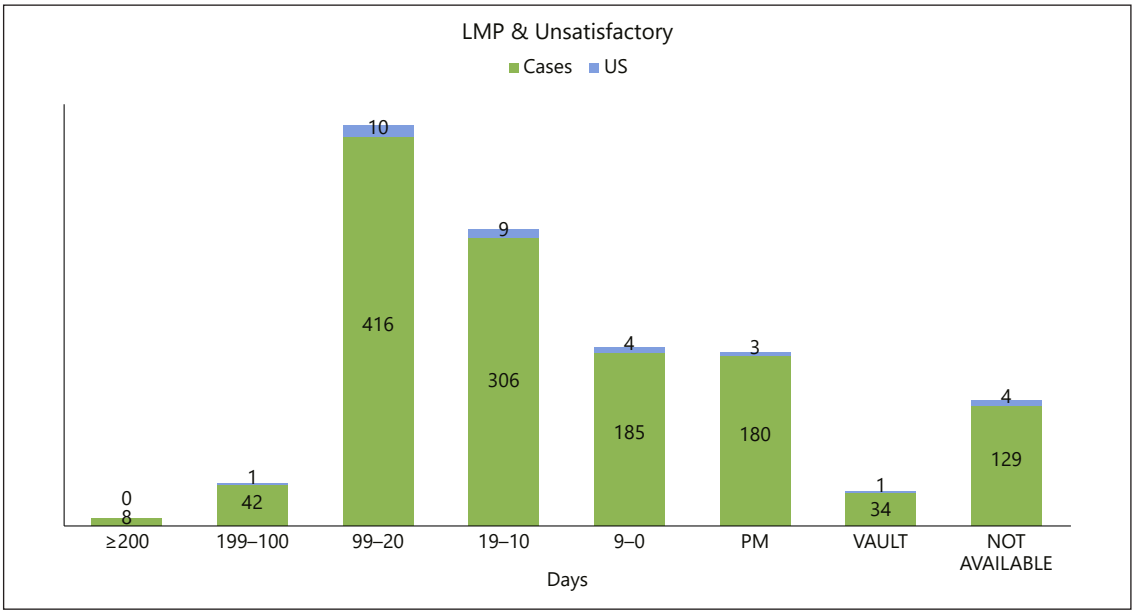

Fig. 4. Comparison of LMP and unsatisfactory cases.

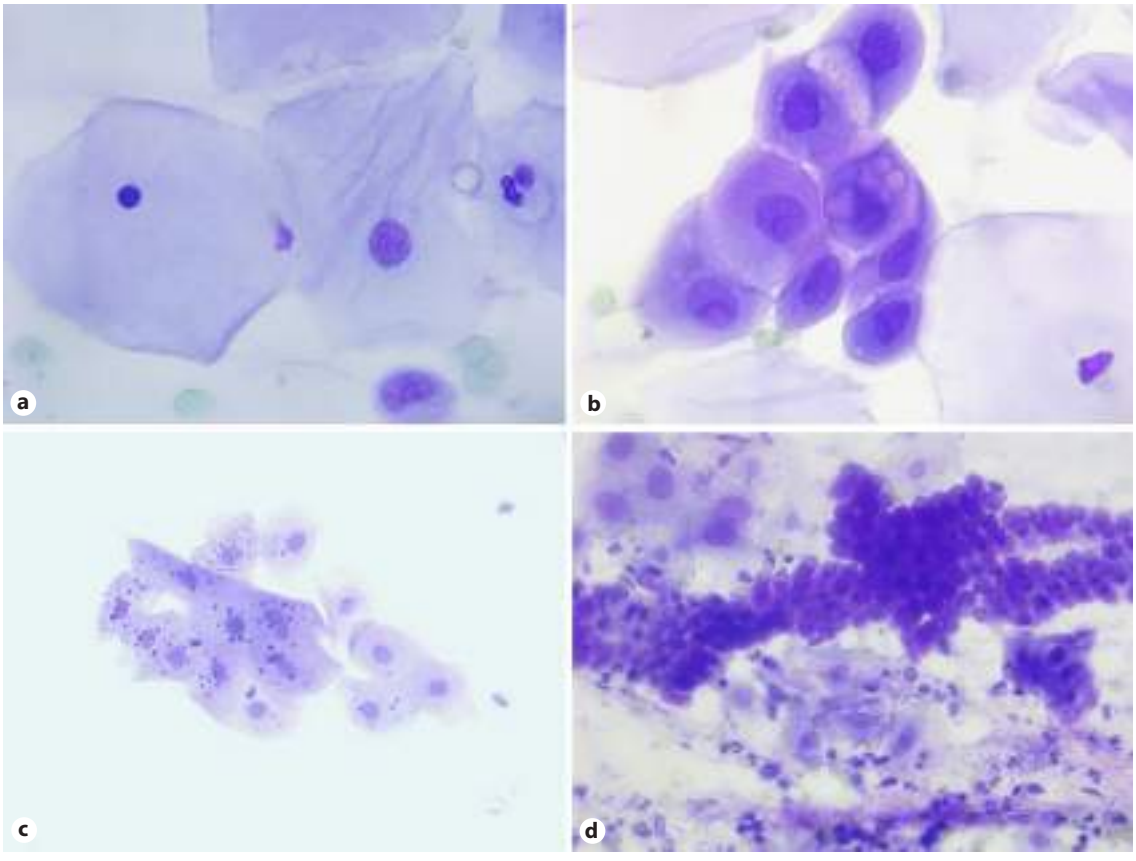

Fig. 5. a–d Normal Pap smear with superficial, intermediate, and parabasal cells along with keratohyaline granules in few superficial squamous epithelial cells. Clusters of endocervical cells are also seen (Tol blue, ×20, ×40, ×100).

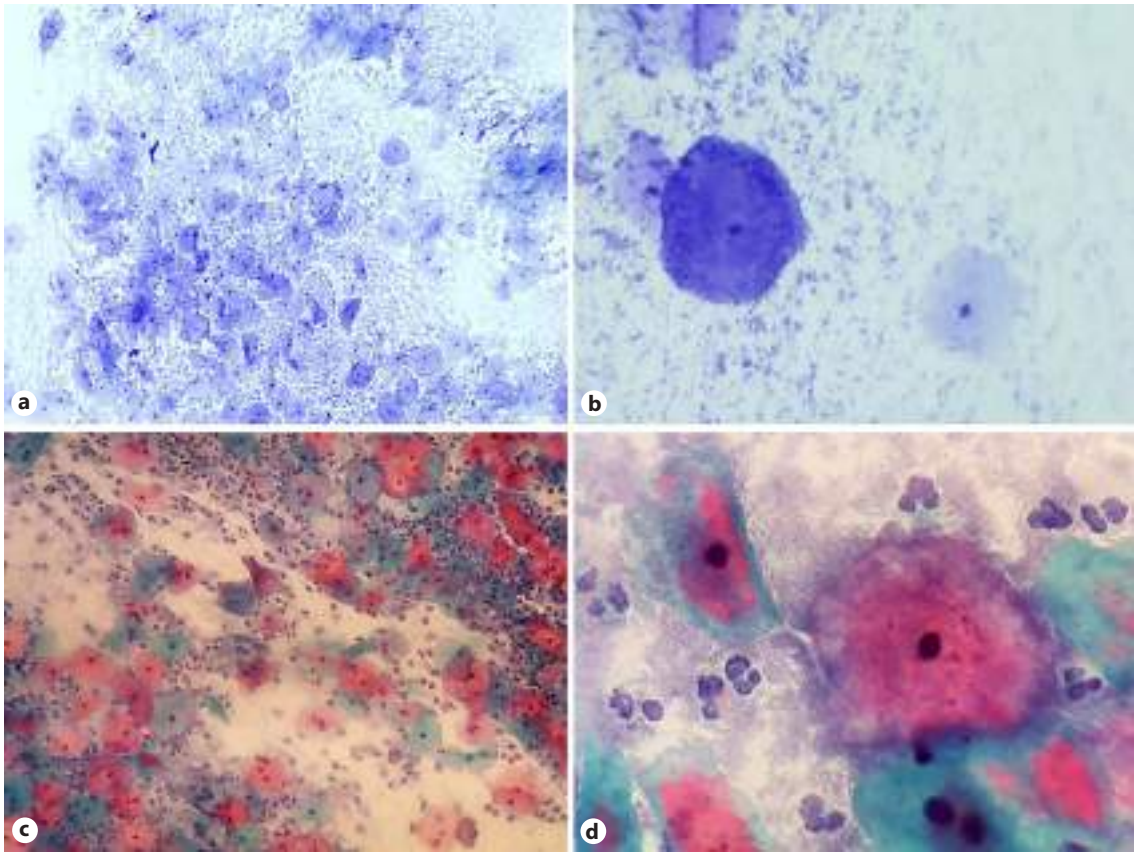

Fig. 6. a–d Pap smear of bacterial vaginosis showing plenty of coccobacilli and clue cells (Tol blue ×10, ×40; PAP ×10, ×40).

(ROSE) cases and pilot study cases. We had slightly higher detection rate of BV-14% (181 cases) and candidiasis 5% (65 cases) as compared to the pilot study cases of 12.3% and 2.2%, respectively.

Turnaround time was analyzed in our study group. We had same-day (<24 h) release of the reports in 400 cases (31%), 24–48 h in 594 cases (46%), 48–72 h in 194 cases (15%), and 73–96 h in 112 cases (09%) (Fig. 10).

A retrospective analysis of 357 cases for 3-month duration prior to the study was done to look for an US rate, and it was found to be 12.1%. The majority of the US cases had only 1 slide received for reporting, i.e., 35 out of 245 cases with a single slide (14.2%). The positivity index in the pilot study was only 0.92%. These data had helped us to understand the scenario of the screening method.

The quality indices were performed on the study group of 1,300 cases. The positivity index was 1.26%, high-grade squamous intraepithelial lesion (HSIL) % was 0.15%, ASC% was of 50%, and ASC:SIL ratio was of 2.6. However, our positivity index was lower than the normal range of 3–10% [16]. The possible cause can be the lower number of cases and our institute not being an exclusive cancer center, wherein a greater number of positive cases can be expected. The US rate was 12.1% before the implementation of the rapid stain method. However, with the implementation of rapid stain, the US rate was 2.4%.

Toluidine blue was used in our study to have an impression on adequacy. The added benefit was the stain quality on rapid technique which was also helpful in making a provisional diagnosis. There was concordance between the provisional and final cytological diagnoses in 97% cases.

Histopathology correlation and follow-up were available in 112 cases. Majority of the cases where cytology was negative for intraepithelial malignancy (NILM) were reported as nonspecific chronic cervicitis (59%) and endocervical polyp (26%). One case each of ASC-H and HSIL along with three cases of SCC in cytology was diagnosed

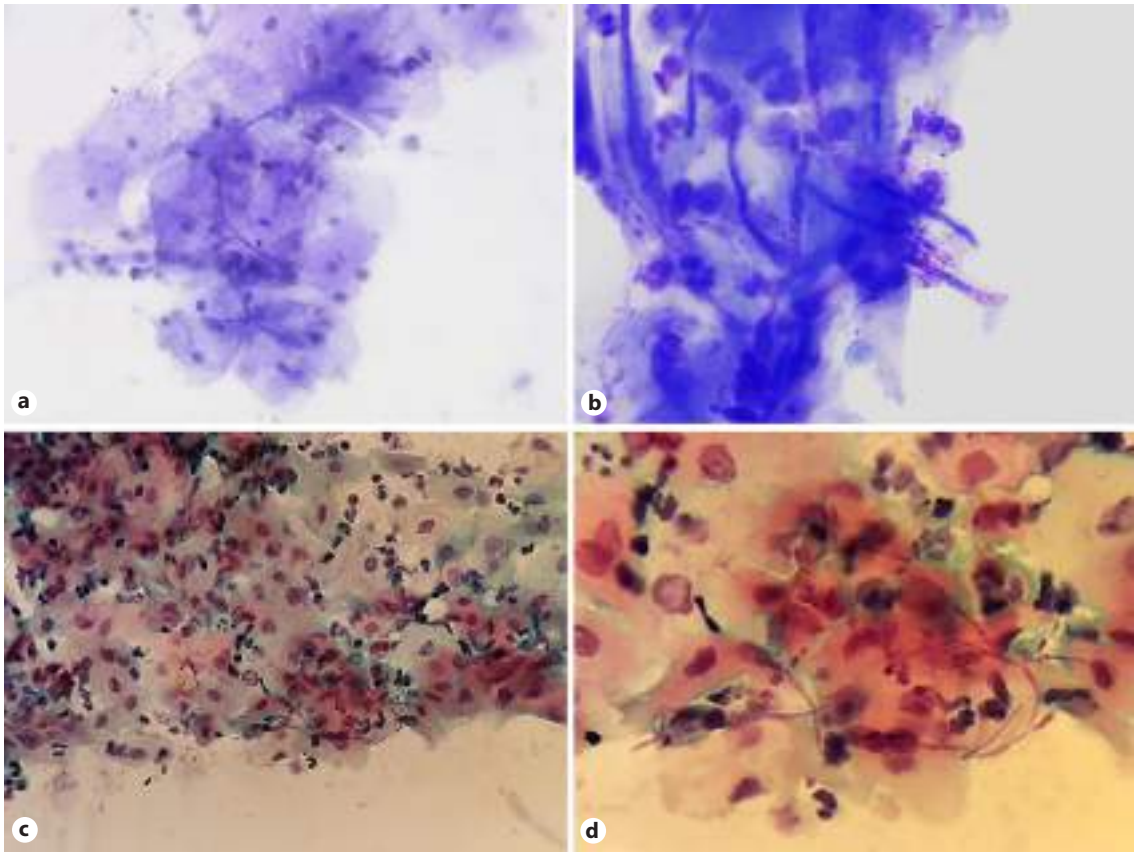

Fig. 7. a–d Pap smear showing the fungal elements with branching septate hyphae along with superficial squamous epithelial cells (Tol blue ×10, ×40; PAP ×10, ×100).

as nonkeratinizing SCC on histopathology. Single case of adenocarcinoma was also confirmed on histopathology (Table 2).

Discussion

Cervical carcinoma is the major health problem faced by Indian women. Approximately 120,000 women are diagnosed with the disease yearly [1]. Around 15.2% of cases of cervical carcinoma deaths in the world are from India. Even though the incidence of carcinoma cervix has declined in the urban population, it is still a major concern in the rural population. In view of the 10–20 year progression of the disease from mild dysplasia to cancer, it makes cervical cancer a relatively early preventable disease [2, 3]. "Preventable but not prevented" is the reality of cervical cancer in developing countries like India [13].

The Papanicolaou stain is used as a method for screening of cervical cancer even though it is a time-consuming process and with the requirement of a significant amount of alcohol in processing the slides [14–16]. Dighe et al. [17] had used 1% acetic acid in replacement of alcohol in the steps of Papanicolaou stain. Although it is a cheaper method compared to alcohol, it still has limitations in the form of suboptimal staining [17]. Ultrafast and rapid Pap is less time-consuming but requires the use of alcohol [18–20].

In cervico-vaginal cytology, the implementation of toluidine blue has been limitedly used [6, 21]. Our study was to evaluate the helpful staining properties of toluidine blue in mass screening for inflammatory lesions and SIL lesions. Rapid stain with toluidine blue can be used at campsite screening, since it requires very less time for staining, rapid interpretation on the adequacy, and lesser turnaround time. TBS method requires approximately 3 min compared to 10 min of rapid economic acetic acid Papanicolaou and 30 min of conventional Pap.

TBS is a rapid method for assessing adequacy and making a provisional diagnosis. Before the implementa-

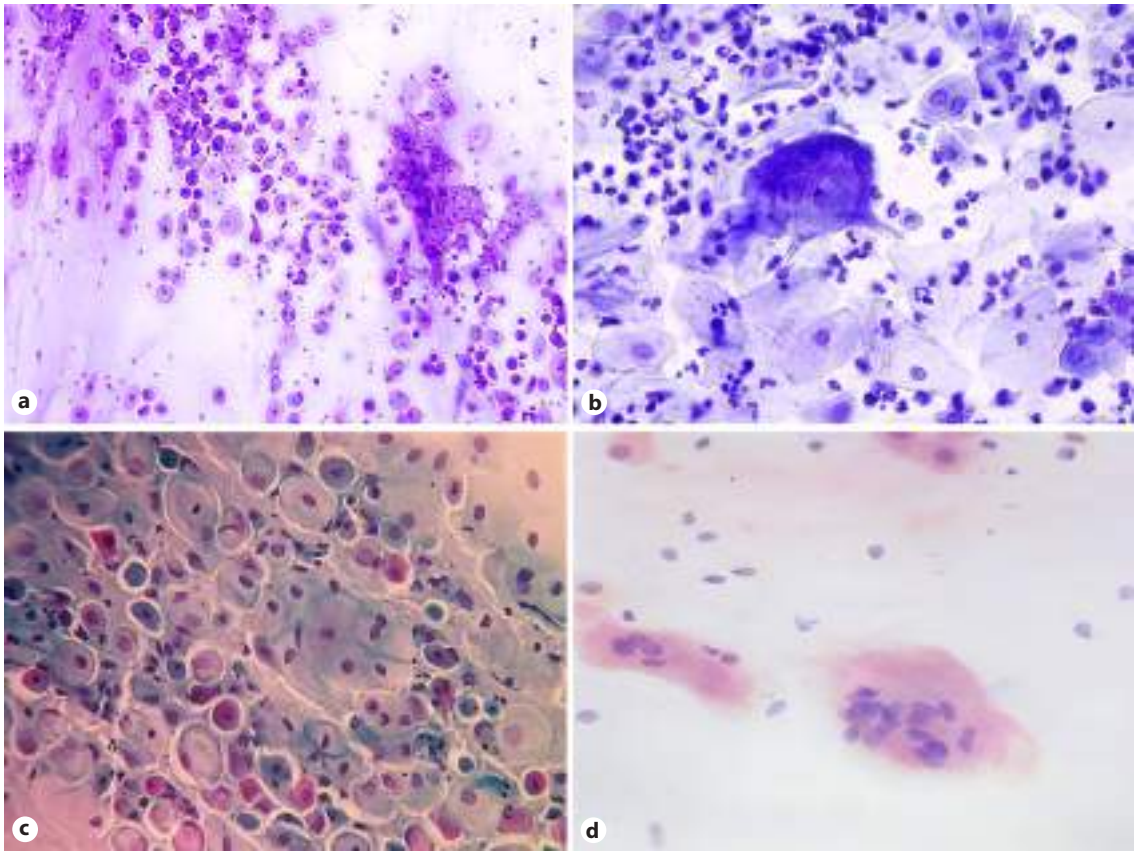

Fig. 8. a–d Pap smear showing predominantly parabasal and basal cells along with occasional giant cells in a case of atrophic smear (Tol blue, ×10, ×40; PAP ×40).

tion of TBS, the inadequacy rate was 12% as compared to 2.4% after implementation of TBS. Thus, ROSE with TBS reduced the inadequacy rate significantly. The smear inadequacy was informed to the treating physician who then could proceed to repeat the smear in the same visit, thus reducing the patients' visits to the outpatient department. In a country like ours, due to socioeconomic struggles and resource constraints, many times patients are lost to follow-up after one or two OPD visits. The success of our screening program thus will definitely be improved if we could reduce the rate of inadequacy.

Studies done by Kothari et al. [9], Saba et al. [22], Ammanagi et al. [10] have successfully tested the TBS method in conventional fine needle aspiration cytology (FNAC) smears [9, 23]. TBS has been proved as diagnostic adjunct in detection of asymptomatic oral SCC and its utility in early detection of oral malignancies [23, 24]. TBS can be used in campsite, wherein the absence of trained personnel, failure to obtain adequate smear, and incorrect interpretation being the various factors which can make the cervical cancer screening programs difficult to achieve the desired target of detection of cancer in early stages [16, 25].

We evaluated the 32 US cases for the possible causes. It was found that the number of slides received in these cases was only one slide (22 cases). The possible cause of inadequacy was due to nonrepresentative sampling in the single slide sent to the laboratory for reporting. The remaining 10 cases had an increased inflammatory cell obscuring the epithelial cells, possibly due to sampling being during the menstrual phase and patients not wanting to repeat the procedure on the same day.

Although rapid stain in the form of toluidine blue has been very rarely used in Pap smear, we tried to compare our study with few other studies. Studies done by Atilgan et al. [21] (2.1%) and Nagose et al. [6] (2.71%) had near about similar US rates as our study 2.4%. However, the number of cases in their study was 32,026 and 6,647 re-

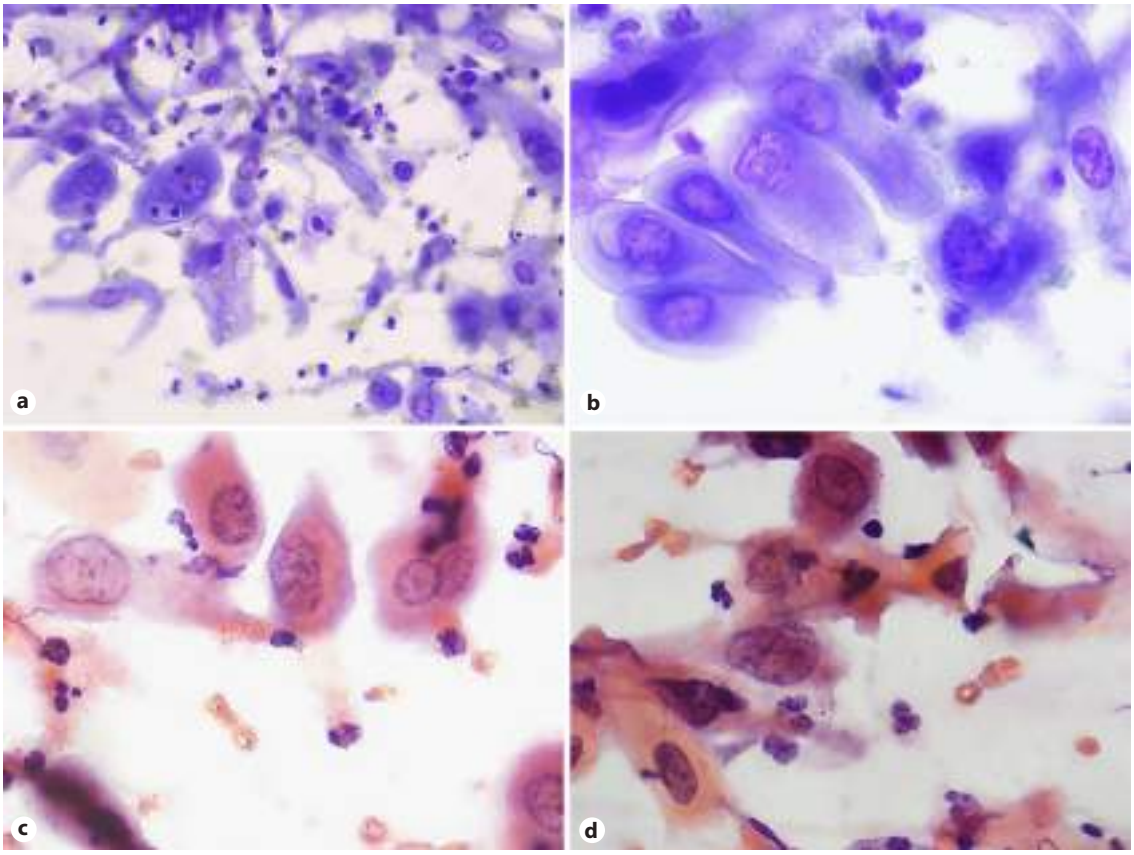

Fig. 9. a–d Pap smear showing the atypical tadpole like cells along with intense keratinization of the cells along with increased N:C ratio, irregular nuclear chromatin in a case of SCC (Tol blue, ×10, ×40; PAP ×10, ×40).

Table 1. Spectrum of cases as per Bethesda system for our study

Bethesda system 2014	Cases	%
US	32	2.4
NILM	705	54
REACTIVE	250	19
BV	181	14
TV	3	0.3
CANDIDA	65	5
ATROPHY	48	3.8
ASC		
ASC-US	6	0.5
ASC-H	2	0.2
SIL		
LSIL	1	0.1
HSIL	2	0.2
Squamous cell carcinoma	3	0.3
AGUS	1	0.1
Adenocarcinoma	1	0.1
Total	1,300	100

US, unsatisfactory; NILM, negative for intraepithelial malignancy; REACTIVE, reactive cellular changes associated with inflammation; BV, bacterial vaginosis; TV, trichomonas vaginalis; AGC-US, atypical glandular cell-undetermined significance; ASC-US, atypical squamous cell-undetermined significance; ASC-H, atypical squamous cell cannot exclude HSIL; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

spectively, which is very high compared to our study without the implementation of ROSE [6, 21]. The regular implementation of ROSE will help in further reduction of US rates. Patil et al. [11] had not included the US cases in the study although the use of toluidine blue was done with 94.7% cases benign NILM (Table 3).

In 2015, Saba et al. [22] used the TBS staining in FNAC and concluded that TBS study of FNAC improves the adequacy rate by minimizing the smearing and drying artifact, and loss of cell sample during fixation and staining also increases diagnostic accuracy. The use of ROSE was also a helpful factor for early identification of infectious conditions such as bacterial vaginosis, candida and trichomonas with characteristic clue cells, budding yeast forms and pear shape with blue bob respectively in TBS method. This can help us in better identification of the organisms, in view of the crisper staining [11]. Another advantage of provisional diagnosis with TBS is that it allows for better triage of samples, and in select cases with candida, trichomonas vaginalis, etc., treatment can be given to the patient in the same visit, once a provisional

Table 2. Correlation of PAP smear and histopathology

Cytology	Histopathology				
	No treatment	Chronic cervicitis	Endocervical polyp	SCC	Adenocarcinoma
US	0	9	0	0	0
NILM	8	56	28	0	0
ASC-US	2	1	1	0	0
ASC-H	0	0	0	1	0
LSIL	1	0	0	0	0
HSIL	0	0	0	1	0
SCC	0	0	0	3	0
Adenocarcinoma	0	0	0	0	1
Total	11	66	29	5	1

US, unsatisfactory; NILM, negative for intraepithelial malignancy; ASC-US, atypical squamous cells-undetermined significance; ASC-H, atypical squamous cell cannot exclude HSIL; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

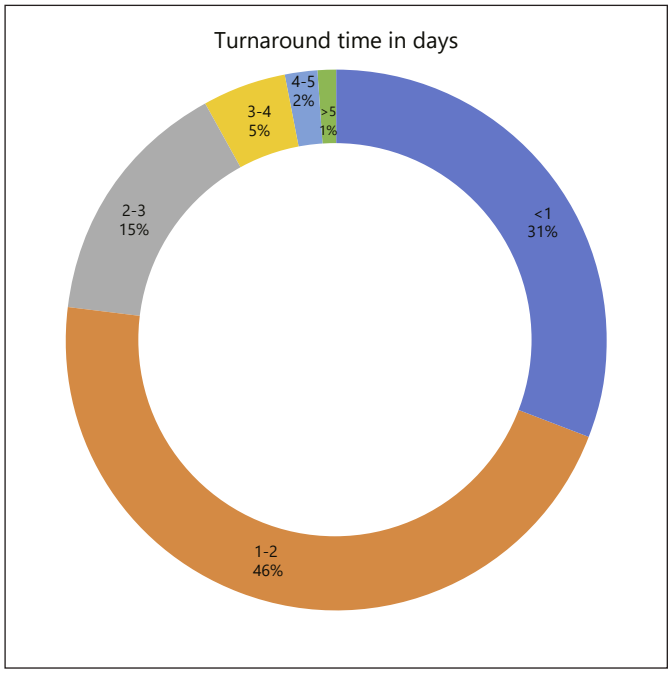

Fig. 10. Turnaround time and distribution of cases.

diagnosis is made on toluidine blue. Our study had a slightly higher diagnostic rate for infectious condition, i.e., BV and candidiasis.

There is varying opinion regarding the use of single slide or double slide for sample collection. However, there is no significant difference between the two methods. Even use of Ayre's spatula or cytobrush can alter the adequacy rate on slides. Single slide can encourage the cytopathologist to give more attention on the slide and look for atypical cells. Single slides can reduce the daily screening workload of laboratory, cost for staining, and more sample collection [26, 27]. Our study had a higher US rate with single possibly due to limited area on the slide to spread the sample and excess mucous. Lack of experience could be a cause for US rate, but in our study the physician collecting samples had minimum of 4 years and maximum of 12 years post-residency experience.

Cervical cytology is largely dependent on factors, i.e., expertise of the person performing the procedure, date of collection in menstruation cycle, method of sample collection, smear making, and type of sampling devices. Added to these factors would be the proper immediate fixation of smears and better staining for interpretation of slides by the cytopathologist [28–30]. ROSE can act as a connecting link between the various phases of screening process, by immediate rapid screening, we can guide the clinician for a repeat sample on-site and also be used as an added tool for making a provisional diagnosis [9]. Even a single slide from the multiple representative area can be of help in collection of the sample and better screening.

Conclusion

TBS method is a rapid technique for mass screening of cervical cytology in resource-limited settings of India. Not only does it reduce the turnaround time, but it also increases the possibility of detection of infectious conditions. ROSE in cervical cytology has been an untouched

Table 3. Correlation with other studies

Author	Narasimha et al. [25]	Atilgan et al. [21]	Bamanikar et al. [30]	Nagose et al. [6]	Patil et al. [11]	Pilot study without ROSE	Final study with ROSE
Year	2011	2012	2013	2017	2018	2019	2021
Method	Routine	Routine	Routine	Routine	Toluidine blue	Routine	Toluidine blue + routine
Sample size	1,531	32,026	560	6,647	240	357	1,300
NILM	65.18	95	89	96.1	94.7	87	96.1
ASU-C/H	3.1	2.0	2.3	0.5	3	0.5	0.7
LSIL/HSIL	4.0	0.6	2.2	0.6	2	0	0.3
SCC	1.24	0.01	0.5	0.1	0.4	0.2	0.3
AGUS/ADE	2.0	0.2	0.1	0.1	0	0	0.2
NO							
US	24.3	2.1	5.71	2.71	Not included	12.1	2.4

domain to explore. It can be used as alternative to rapid Pap for screening of cervical carcinoma in resource-limited settings.

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Statement of Ethics

Written informed consent was obtained from all participants to participate in the study. The study protocol was reviewed and approved by IEC, AIIMS, Mangalagiri, approval number AIIMS/MG/IEC/2020-21/30.

Conflict of Interest Statement

The authors declare no conflicts of interests.

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Author Contributions

Tummidi Santosh carried out concepts and design, and literature search, participated in clinical study and manuscript preparation, and will stand as guarantor also. Arundhathi Shankaralingappa and Vijayan Sharmila carried out data acquisition, data analysis, and clinical study. All the authors have read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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Rapid on-site evaluation and cell blocks: getting the most from the least invasive method in cytopathology

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Rapid on-site;
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Toluidine blue;
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Introduction Fine needle aspiration cytology (FNAC) sampling is a minimally invasive procedure done to identify the pathology behind super cial and deep-seated lesions. Rapid on-site evaluation (ROSE) can be an adjunct to the FNACs. Our study aimed to identify the role of ROSE in diagnostic adequacy and to check the bene t of cell block (CB)/cell buttons prepared from the ROSE samples.

Material and methods A prospective study was conducted where all patients referred for FNAC were included. ROSE using 1% aqueous toluidine blue stain and CB/cell button preparations were done for the identi cation of various cytological lesions.

Results Among 600 cases included in the study most common age group was third and fourth decades with a mean age of 41.6 years and M: F ratio of 1:1.7. Ultrasound-guided procedures were done in 20% of cases. CB preparation was available in 14% of cases. Most CBs were from the cases wherein ROSE was performed 81% (77 out of 86), with CB helping in making an accurate diagnosis in 17% of cases. Lymph nodes 26%, and thyroid 23% were the most common sites for sampling with the highest number of repeat procedures from non-ROSE cases (14%). The non-diagnostic rate for non-ROSE cases was 7.7% (23/300) even after the repeat procedures as compared to 1.3% (4/300) for ROSE. Three slides on average were consumed in ROSE-performed procedures, as compared to an average of 5 slides in non-

Statement of Ethics: Written informed consent was obtained from all participants. The study protocol was reviewed and approved by IEC, AIIMS, Mangalagiri approval number IEC/AIIMS/MG/2019-20/29.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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ROSE. The average turnaround time was 1.7 days for non-ROSE cases and 1.05 for ROSE cases respectively. Cyto-histopathological correlation was available in 40% of cases with a sensitivity of 98.1%, specificity of 96.7%, positive predictive value of 90%, negative predictive value of 99.4%, and diagnostic accuracy of 97%. The correlation of CB, number of slides consumed, and turnaround time among the 2 groups were statistically significant (*P* value 0.001).

Conclusions ROSE is a method used to assess material aspirated at the time of FNAC procedures to determine the adequacy and to an extent to identify whether the lesion is neoplastic or non-neoplastic. CBs have helped in increasing diagnostic accuracy apart from the fact that the paraffin-embedded tissue material can be used for further studies.

Introduction

Fine needle aspiration cytology (FNAC) dates to around the 1950s. However, the idea of obtaining cells and tissue fragments through a needle introduced into the abnormal tissue was by no means new.[1] In the mid-19th century, Kun (1847), Lebert (1851), and Menetrier (1886) employed needles to obtain cells and tissue fragments to diagnose cancer.[2]

Rapid on-site evaluation (ROSE) for FNACs can be highly effective in many clinical settings. The decrease in non-diagnostic specimens has been reported to drop from 15%-47% to 4%-23%, depending on the fine needle aspiration (FNA) sites.[3-6] Toluidine blue is a supra-vital stain that accentuates good nuclear details and enables a 3D view of cells in wet mount films.[6] It is easily available, cost-effective, and can be used for quick reporting. Very aptly said ROSE can be called the *frozen section of cytology".*[2,7,8]

The use of cellblocks (CBs) in routine non-gynecologic cytopathology varies in each institution. A good CB preparation depends on the presence of adequate cellular aggregates or tissue fragments or a solid visible cell pellet after specimen centrifugation or fixation.[2,9] Our study was performed to evaluate the role and advantages of ROSE in cytology sampling, and its correlation with final cytological diagnosis. The efficacy of CB/cell button preparations in various samples aspirated by FNAC was also studied.

Materials and methods

A prospective study was conducted where all patients referred for FNAC were included in an alternative random sequence mode and was carried out in a tertiary care hospital in southern India. The study was done after obtaining ethical clearance from the institute (IEC/AIIMS/MG/2019-20/29). All patients advised for FNAC were included in the study. The demographic data and relevant clinical information like age, sex, site of FNAC, etc. were collected from the cytology requisition form developed in-house [Fig. 1]. The FNAC procedure was done using 22-25 g needles with or without suction using a 10-ml syringe. No local anesthesia was used. Aspirated material was smeared onto a slide and screened using 1% aqueous toluidine blue for adequacy. The smears were wet fixed by 5 dips in the absolute alcohol, followed by 10 dips in 1% aqueous toluidine blue solution, slowly tap water washed to remove the excess stain and seen under the microscope. After adequacy evaluation and provisional diagnosis, the slides were returned to Papanicolaou (PAP) stain. Further material as per the requirement was collected for the CB/button technique. A maximum of 3 passes were attempted depending on the sample aspirated and material for the CB. The detailed case history of the patient was recorded while performing FNAC and consent of the patient regarding the procedure was taken after proper explanation of the procedure to the patient.

Results

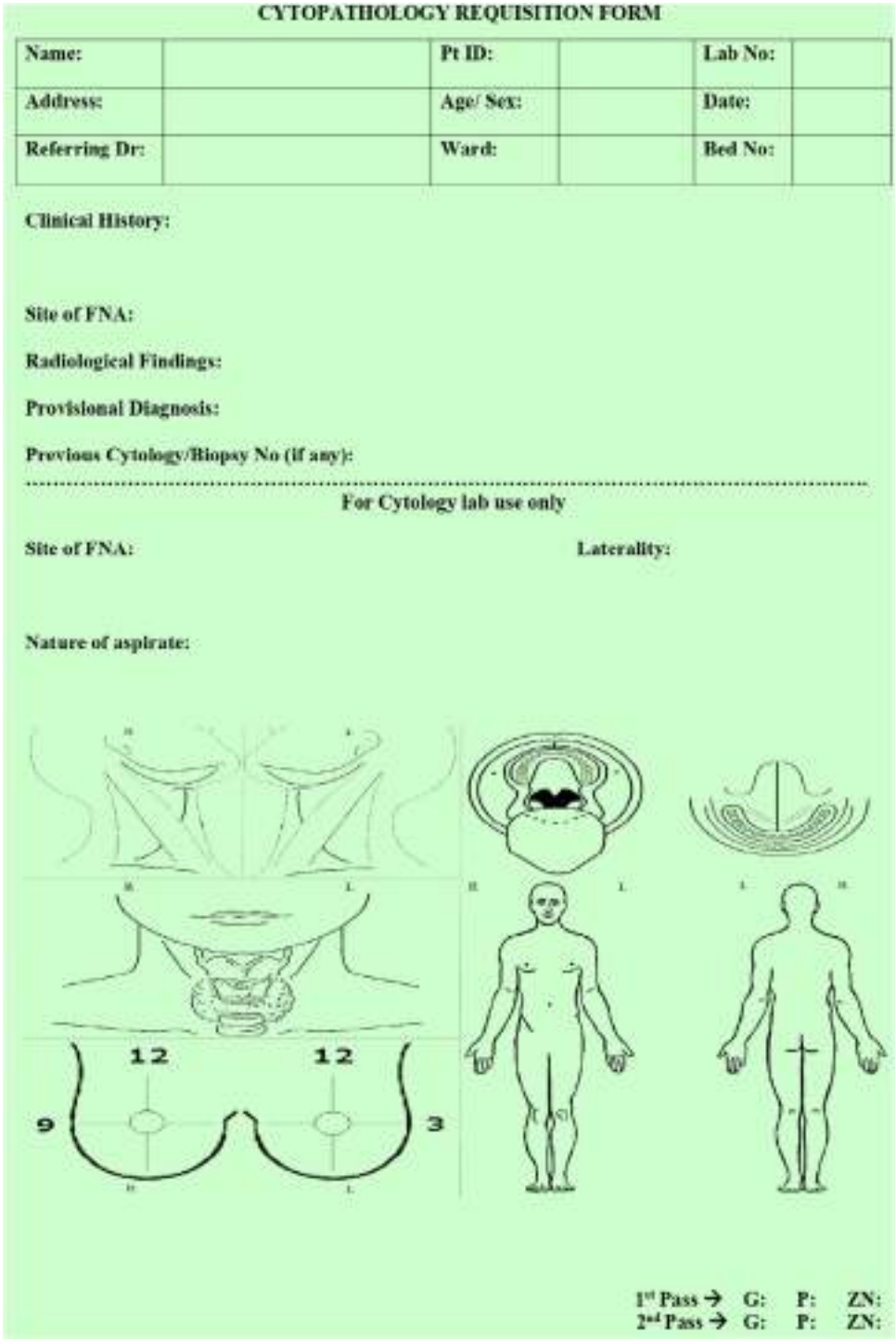
Our study included a total of 600 FNAC samples from various sites. The details of the patients were collected in a standardized worksheet [Fig. 1]. The most common age group was third decade (26%) followed by fourth decade (19%) with a mean age of 41 years. Majority of the cases were females (64%) with M: F ratio of 1:1.7. The spectrum of organs involved was 26% lymph node, 23% thyroid, 21% soft tissue, 18% breast, 5% salivary gland, and 07% of others (tzanck smears, axilla, liver, and pancreas) [Fig. 2].

FNACs were divided into ultrasound-guided and non-ultrasound-guided groups. Ultrasonography (USG) guided cases being 20% (122 cases) of the total 600 cases of which thyroid (52%; 62/122) was the most common site under guidance followed by breast (28%; 34/122) and lymph nodes (11.5%;15/122). Among the non-ultrasound-guided FNACs performed in 478 cases (80%), lymph nodes were the most common at 29.5% (141 cases), soft tissue at 26% (122 cases), breast and axilla at 17% (82 cases), and thyroid being 16% (76 cases) [Fig. 3].

During the guided FNACs, radiologists were responsible for localization of the lesion followed by pathologists performing the sampling and smears. The non-ultrasound-

guided FNACs were solely sampled and smeared by the pathologist.

ROSE was done in 300 alternative samples and equivalent non-ROSE samples of 300. Among the 300 cases wherein ROSE was performed, lymph nodes (27%; 82/300) cases were the common site followed by thyroid (24.3%; 72/300), breast/axilla (20%, 61/300). For the non-ROSE sites most common were soft tissue swellings (26.3%; 79/300) followed by lymph node (25%; 74/300) and thyroid (22%, 66/300) [Fig. 3].


CYTOPATHOLOGY REQUISITION FORM

Name:		Pt ID:		Lab No:	
Address:		Age/ Sex:		Date:	
Referring Dr:		Ward:		Bed No:	

Clinical History:

Site of FNA:

Radiological Findings:

Provisional Diagnosis:

Previous Cytology/Biopsy No (if any):

For Cytology lab use only

Site of FNA: Laterality:

Nature of aspirate:

1st Pass → G: P: ZN:
2nd Pass → G: P: ZN:

Figure 1 Case record form for clinical history and details collection.

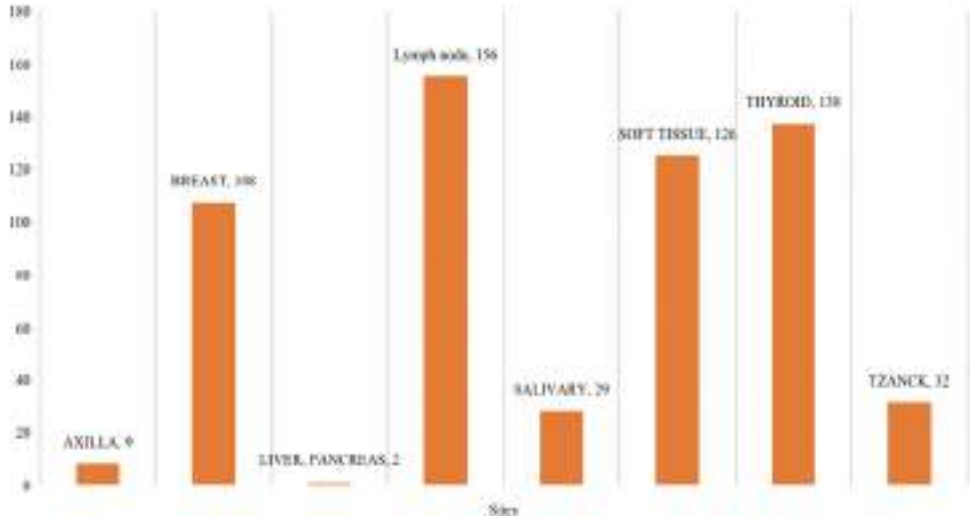


Figure 2 Chart showing the spectrum of organs involved. LN, lymph node.

Cell block and cytology

A total of 86 cases had CB preparations (14.3%), of which non-ROSE cases had only 3% (9/300) CB preparations. The cases included 4 ductal carcinoma breasts, 2 cases of metastatic lymph nodes, 1 each of fungal infection [Fig. 4A, B], pleomorphic adenoma [Fig. 5A, B], and anaplastic thyroid carcinoma. In 26% (77/300) of ROSE-performed cases CB preparation was done [Fig. 3]. The most common organ was the breast in 32 cases, the lymph node in 19 cases, the thyroid in 14 cases, soft tissue in 7, the salivary gland in 4 cases, and in 1 case from liver. There were 3 cases (4%, 3/77) in CB which were inconclusive due to low cellularity and not helpful in diagnosis. The major dif culty was in thyroid cases since the morphology was not much appreciated for atypia of undetermined signi cance or follicular neoplasms [Fig. 6A, B], whereas in papillary carcinoma thyroid, the nuclear and morphological features were better in CB preparations [Fig. 7A-D]. Our study has 17% cases (13/77) where the CBs helped make an accurate diagnosis as compared to the nal cytological diagnosis. Cases such as metastatic squamous cell carcinoma [Fig. 8A, B], phyllodes tumor [Fig. 9A-D], non-Hodgkin lymphoma of the thyroid [Fig. 10A-C], Rosai-Dorfman syndrome [Fig. 11A, B],

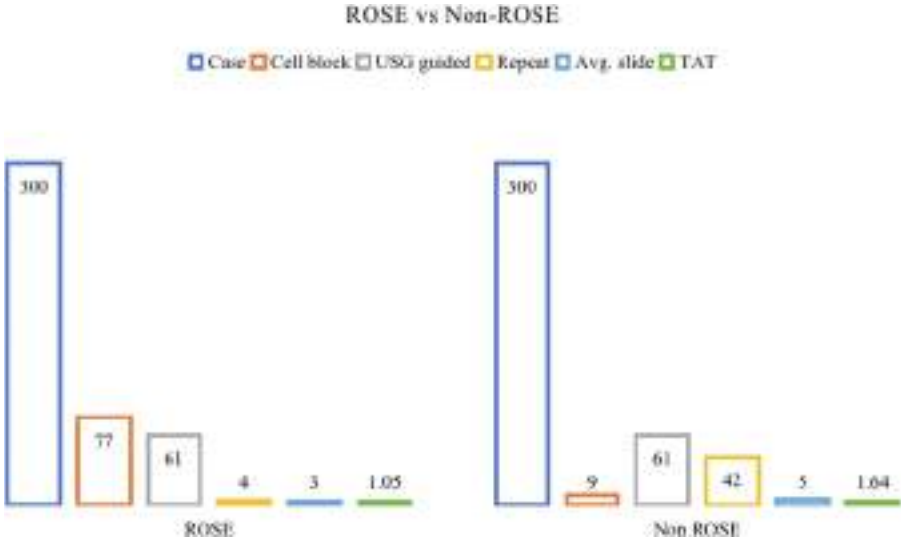


Figure 3 Chart showing the number of cases included, cellblock preparation, USG-guided procedures, number of repeat FNACs, non-diagnostic rates, average slides consumed, and turnaround time. FNAC, Fine needle aspiration cytology; ROSE, rapid on-site evaluation; TAT, turnaround time.

Table 1 Comparison of ROSE versus non-ROSE cases yield for cellblock, repeat procedures, slides consumed and TAT.

Characteristic	ROSE N, n 300[a]	ROSE Y, n 300[a]	value
Cellblock			0.001
No	291 (97)	223 (74)	
Yes	9 (3.0)	77 (26)	
Repeat			0.001
N	258 (86)	296 (99)	
Y	42 (14)	4 (1.3)	
Slides	4 (4, 6)	4 (3, 4)	0.001
TAT	1 (1, 2)	1 (0, 1)	

Abbreviations: ROSE, rapid on-site evaluation; TAT, turnaround time.
[a]n ( ); Median (IQR).

insulin-induced amyloidoma [Fig. 12A, B], and ductal carcinoma breast [Fig. 13A, B] were con rmed using CBs/button methods, the material of CBs was used for ancillary tests like special stains and immunohistochemistry (IHC). Cell block preparation in non-ROSE cases was lower, since the pathologists performing the sampling followed by the smear making were not sure of the sample adequacy and provisional diagnosis. In ROSE cases, a provisional diagnosis was avalibale on-site and the pathologist had a better idea about the sample to be aspirated for further cell block or special stains. The cell block in ROSE vs non-ROSE cases was statistically signi cant (*P*-value 0.001) (Table 1).

Repeat, non-diagnostic, and turnaround time

We had 7.7% (23/300) non-diagnostic reports released among the non-ROSE procedures which included 3.7% (11/300) of soft tissue swellings, 2.0% (6/300) of thyroid lesions, 1.3% (4/300) of lymph node lesions, 1 case each in breast and salivary gland. In comparison to the above, among ROSE-performed cases, the non-diagnostic rate was 1.3%, which included 1 case each from breast, lymph node, thyroid, and salivary gland.

The repeat in procedures on a different day was done in 14% (42/300) of cases, and repeat procedures had cellularity with a valid cytology report in 11% (33/300) of cases. Only 1.3% (4/300) of cases were repeated for ROSE-performed cases and they were non-diagnostic on repeat procedures [Fig. 3 and Table 1].

The repeat procedures have also led to increased slide consumption. On average 4 slides were consumed in the 600 cases. ROSE-performed procedures had a maximum of 6 slides (average 3) whereas in non-ROSE cases the maximum slides consumed were 14 (average 5) [Fig. 3] and were statistically signi cant (*P* value 0.001) with T-test [Fig. 14 and Table 1].

Correlation of cytology with nal histopathology was available in 40% (241/600) cases. The validity parameters were calculated including all atypical, suspicious, and malignant cases as positive with a sensitivity of 98.1%, speci city of 96.7%, positive predictive value of 90%, negative predictive value of 99.4%, and diagnostic accuracy of 97% [Table 2].

The turnaround time (TAT) ranged from 24 hours to as long as 10 days, with the average being only 1.5 days. TAT was 1.7 days on average for non-ROSE cases and 1.05 for ROSE cases [Fig. 3], which was statistically signi cant (*P* value 0.001) and T-test done [Fig. 15 and Table 1]. Risk of malignancy (ROM) was calculated for broad categories of non-diagnostic 0%, benign 0%, atypical 36%, suspicious 88%, and malignant 100% [Table 2].

Discussion

FNAC is an essential basic diagnostic technique used for the diagnostic evaluation of super cial and deep lesions. The technique was reported as early as 1847 when Kun et al

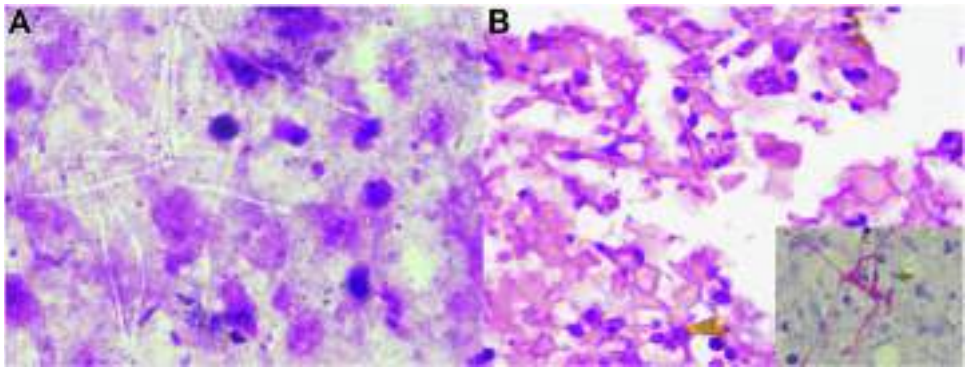


Figure 4 A, Inflammatory smear with fungal elements: Cytosmears show a necrotic background with negative stained fungal hyphae with few degenerated cells. B, Cell block preparation shows the inflammatory tissue with areas of necrosis and fungal elements. Periodic acid-Schiff (PAS) stain is showing positive for the fungal elements *(insert)* (Tol. Blue x40; H&E x40, PAS x40).

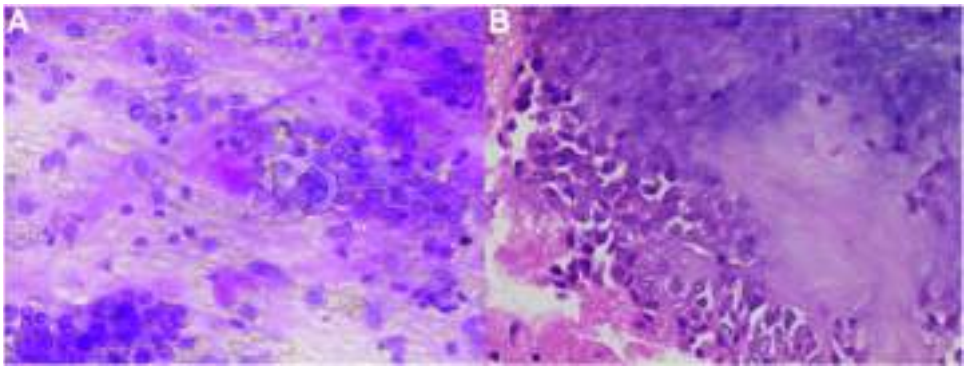


Figure 5 A, Pleomorphic adenoma of salivary gland (MILAN IVA): Cytosmears show ductal epithelial cells in clusters and sheets along with a few plasmacytoid forms. The epithelial cells are seen melting with the brillary chondromyxoid background. B, Cellblock preparation shows the abundant brillary chondromyxoid stroma along with ductal epithelial cells (Tol. Blue x40; H&E x40).

described a "new instrument for the diagnosis of tumors".[2] The initial skepticism among pathologists and clinicians regarding the utility of FNAC has gradually diminished and the simple technique is now practised worldwide for early diagnosis and further studies.[3,10]

ROSE has been used to assess material aspirated at the time of FNAC procedures for determining the adequacy and to an extent to identify whether the lesion is neoplastic or non-neoplastic. It also helps in triaging material for further studies, ie, special stains, CB, recommending a core biopsy, flow cytometry, and molecular analysis.[11]

FNAC has been gaining more importance day by day in patient evaluation and personalized medicine using newer prognostic markers which makes the method more critically important.[12] However, the procedure s success depends on the sample collected for the CB. CB provides the platform for applying necessary ancillary tests for precise diagnosis and personalized molecular evaluation.[13] Hence the importance of FNAC samples becomes crucial in molecular testing and to ensure that proper and suf cient sample is aspirated, the role played by cytopathologists in ROSE becomes valuable. Studies have shown that a higher quality of CB provides better utilization of IHC.[13]

FNAC from super cial and deep-seated sites has been performed for diagnostic purposes. The maximum utility of ROSE has been evaluated when the FNAC is being done from the deep-seated lesion, computed tomography guided FNAs, Endobronchial ultrasound-guided transbronchial ne needle aspiration, deep-seated lymphadenopathy, or internal organs since an unsatisfactory sample would lead to a delay in diagnosis and treatment.[14] However, it has also been performed in routine super cial lesions such as breast, soft tissue, lymph nodes, thyroid, and recently in gynecological smears.[3,10,12,15] A cytopathologist being available all the time during the ROSE procedure requires an investment of time and cost.[16] Hence centers have looked for alternative evaluators in the form of cytotechnologists, radiologists, and pulmonologists.[17] Our study has implemented ROSE in daily settings for routine FNACs with the help of trainees to reduce the cost burden borne by the patient for procedures.

ROSE has been put in the forefront of patient care and it is viewed as the intraoperative consultation or frozen section

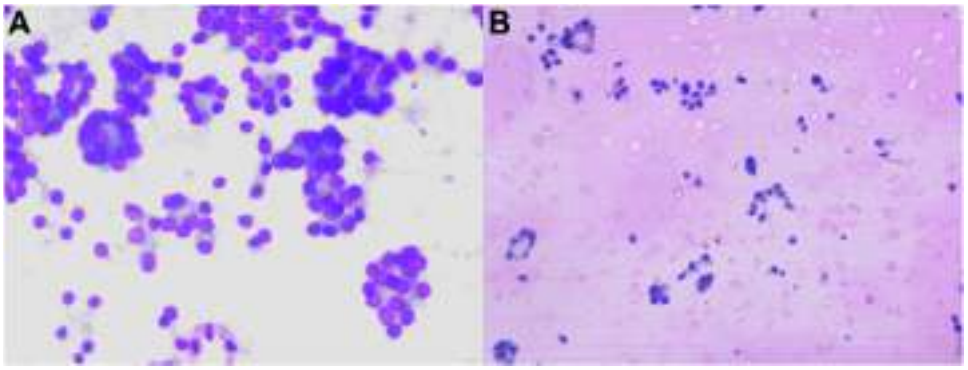


Figure 6 A, Follicular neoplasm (Bethesda IV): Cytosmears show thyroid follicular cells arranged in repetitive micro-follicular patterns with uniform and bland nuclear features. B, Cellblock preparation shows only a few benign-looking thyroid follicular cells along with a thick colloid in the background (Giemsa x40; H&E x20).

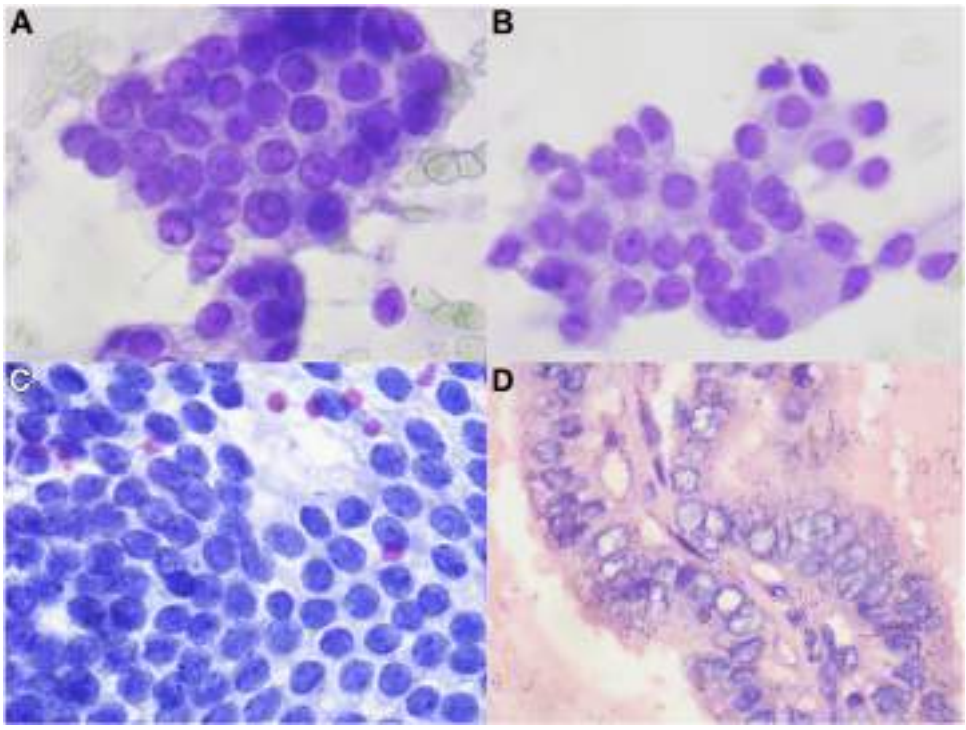


Figure 7 A-C, Malignant, Papillary carcinoma thyroid (Bethesda VI): Cytosmears are cellular and show uniform-looking thyroid follicular cells with round to oval nuclei, nuclear indentation, prominent nucleoli, nuclear grooving, and moderate to scant cytoplasm. D, Cell block preparation shows papillae lined by thyroid follicular cells with overcrowding, palisading, nuclear clearing, and occasional groves (Tol. Blue x100; PAP x40; H&E x100).

of cytology.[3,8,18] In some cases this procedure may preclude the need for those procedures and save the time of surgical pathologists and surgeons, potentially decreasing the time of surgical procedures. Regular implementation of the procedure helps in reduced stay of patients in hospital and fewer diagnostic tests.[8,19] ROSE-assisted FNAs with clinic-radiological correlation have been the main pillar for setting up various diagnostic clinics in hospitals.[18] Meena et al had shown that the ROSE with gross on-site examination has a very high sensitivity of 98% for sample adequacy, with gross examination-positive slides being prioritized for microscopic evaluation.[20]

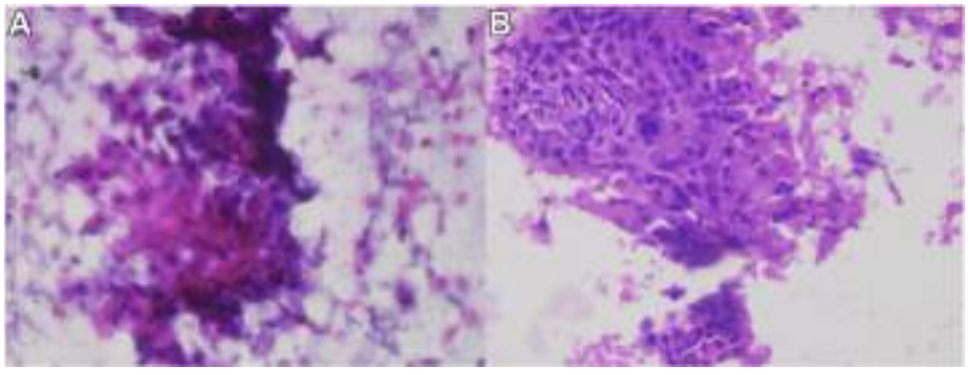


Figure 8 A, Metastatic squamous cell carcinoma (Sydney VI): Cytosmears show atypical keratinized squamous cells with hyperchromatic nuclei and irregular nuclear membrane along areas of necrosis in the background. B, Cell block preparation shows dysplastic squamous cells with hyperchromatism, prominent nucleoli, and loss of intercellular bridging (PAP x40; H&E x20).

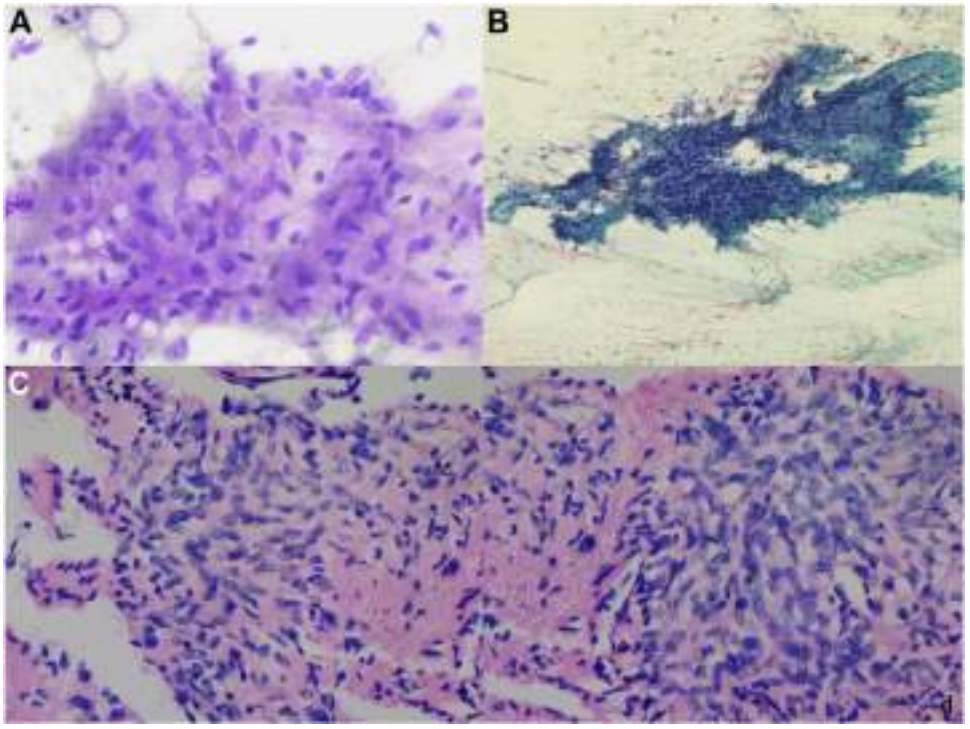


Figure 9 A, B, Benign phyllodes tumor (Yokohama III): Cytosmears are showing brillary cellular stromal tissue with plump oval to spindle cells. C, D, Cell block preparation shows cellular stromal fragments with few mitotic gures (arrow) (Tol. Blue x100; PAP x20; H&E x100).

Our study had very high repeat procedures among non-ROSE cases (14%; 42/300) as compared to 1.3% for ROSE-assisted FNAs. The repeat procedures lead to an increased nancial burden to the patient, delay in report, pressure on staff and cytopathologists for reprocedure, and wastage of slides and reagents, all of which have a direct effect on the cost per test.

CBs have been routinely prepared from the residual cytological fluid samples or extra material of aspirates.[21] Although CB is routinely used for pleural, peritoneal

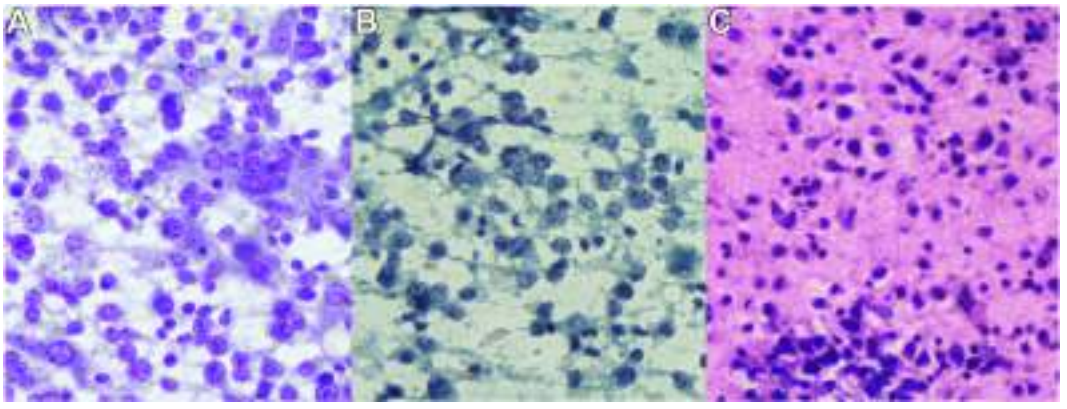


Figure 10 A, B, Non-Hodgkin lymphoma- Diffuse large B cell lymphoma (DLBCL) (Sydney VI): Cytosmears are cellular and show thyroid follicular cells scattered singly with numerous lymphocytes. C, Cell block preparation shows mature small lymphocytes and occasional thyroid follicular cells on a lympho-glandular background (Tol. Blue x100; PAP x40; H&E x40).

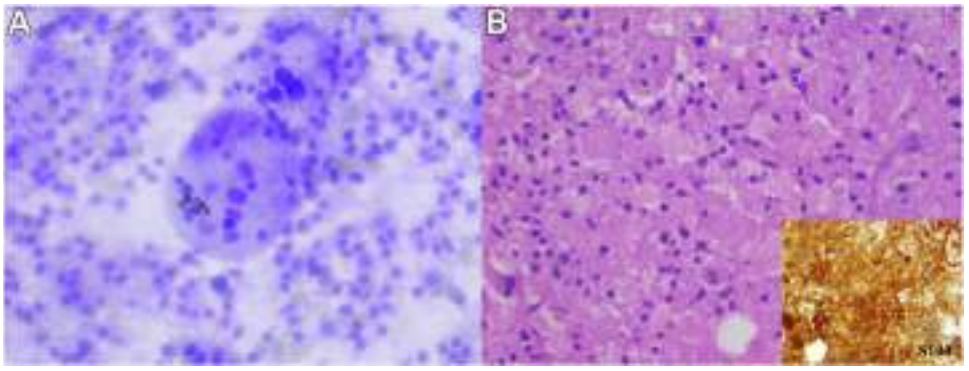


Figure 11 A, Rosai-Dorfman disease (Sydney II): Cytosmears show numerous lymphocytes along with emperipolesis having engulfed mature lymphocytes and plasma cells. B, Cell block preparation showing the foamy macrophages with engulfed small lymphocytes and plasma cells, with Immunohistochemistry positive for S100 (insert) (Tol. Blue x100; H&E x40).

fluids, and bronchial washings, FNA samples can also be used for further workup.[2] A good CB can be very useful in architectural pattern identi cation, archival purposes, immunohistochemical staining as well as cytochemical stains, and molecular diagnostics, ie, fluorescence in situ hybridization, polymerase chain reaction, and next-generation sequencing.[22-28] CBs have shown better sensitivity (90%) and speci city (100%) in comparison to conventional cytology (70% and 100%) and LBC (73.3% and 100%) preparations.[29] Nathan et al had near equal sensitivity of smears (90.6%) and CB (89.4%).[9] Basnet et al had a sensitivity of 91.8% for smears compared to 95.9% for CB[30] [Fig. 10].

CB preparation has evolved from the simple sedimentation techniques to the addition of centrifugation to increase cellularity, or the use of cellular adjuvants to increase cellular cohesiveness. The various types of methods include normal saline needle rinse, tissue coagulum clot method/cell button, thrombin clot, agar embedding, collodion bag, Shandon cyto-block, microwave technique, and automated methods.[25,31-34] Our study used 2 methods, for cases where the gross on-site evaluation was satisfactory-tissue coagulum thread method, and for diluted samples/fluid-like aspirates thrombin method was used.[35,36]

Our study had a slightly lower sensitivity (90%) and diagnostic accuracy (95.5%) in comparison to ROSE nd-ings with cytology. The review of CBs showed that one case of thyroid reported as follicular neoplasm on cytology and in CB showed few scattered dissociated thyroid follicular cells without any pattern [Fig. 6A, B]. Similarly a case of suspicion for ductal carcinoma on cytology, showed sparse atypical ductal cells on CB, and hence a descriptive report was issued on CB [Fig. 13A, B].

However, we also encountered some rare and unique cases where in CB had played an important role in providing material for further workup. A case of

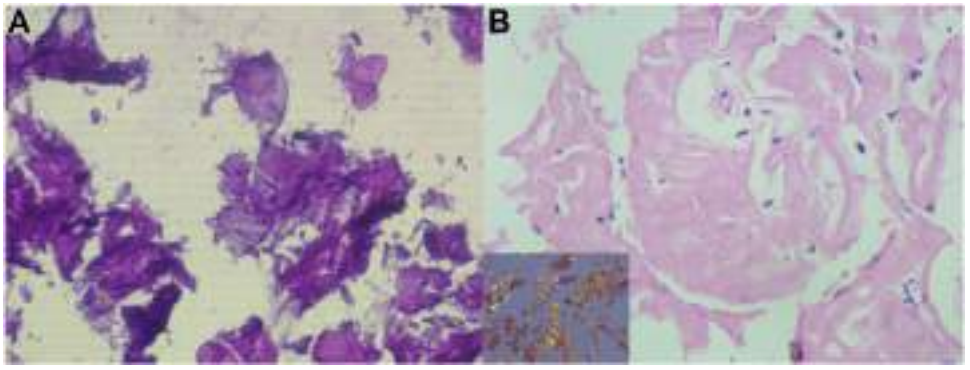


Figure 12 A, Insulin-induced amyloidoma: Cytosmears show homogenous cellular material with few adipocytes. B, Cell block preparation shows homogenous acellular material with congo red positive and birefringence in polarized microscopy (Tol. Blue x100; H&E x40).

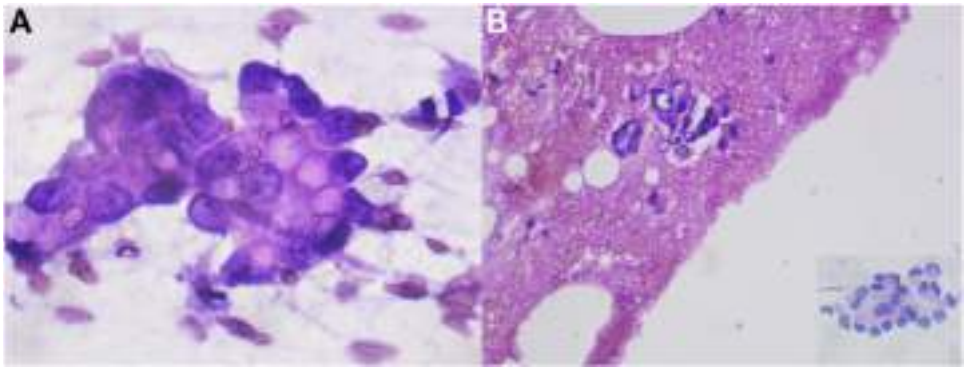


Figure 13 A, Cytosmears show few ductal cells with moderate pleomorphism and cytoplasmic vacuolation. B, Cell block preparation shows few ductal cells with hyperchromatic nuclei and PAS-positive hyaline globules in the cytoplasm (insert) (PAP x100; H&E x40).

subcutaneous swelling con rmed as insulin-induced amyloidoma from CB [Fig. 12A, B],[37] a thyroid swelling which on FNAC showed monomorphic lymphoid cells was positive for CD 20, B- cell lymphoma-2 (BCL-2) on IHC and was nally signed as diffuse large B cell lymphoma.[38] Cases of breast lumps which on FNA were dif cult to diagnose as benign phyllodes or broadenoma were successfully diagnosed on CB as stromal fragments with atypia and mitotic gures were evident [Fig. 9A-D]. CB was very useful in detecting lesions due to fungal infections as special stains could be easily applied on tissue sections. The fungal lesion was also a condition wherein a CB sample was used for special stains in our study.[39] The use of telecytology using Whole Slide Imaging along with ROSE has also been tried in literature making it an upcoming source of second opinion in nongynecologic cytopathology.[40-42]

Rapid stains such as rapid hematoxylin and eosin, ultrafast PAP, toluidine blue, brilliant cresyl blue, Rapid PAP, and Diff-Quik have been used in the past.[43] Air-dried methods carry the risk of aerosol generation and alter the nuclear features.[44] As compared to various methods earlier, toluidine blue is one of the cost-effective methods for staining and has the bene t of returning the slides to routine PAP or hematoxylin and eosin stain retaining the wet xation with morphologic details.[10,15,43,45]

FNAs along with ROSE have shown a signi cant reduction in the cost to the patient and laboratory by as much as 35%. Our study too has shown similar signi cant bene ts wherein 14% repeat procedures in non-ROSE with an average being 5 slides per procedure as compared to 1.3% in ROSE cases with an average of 3 slides per procedure.[3,46]

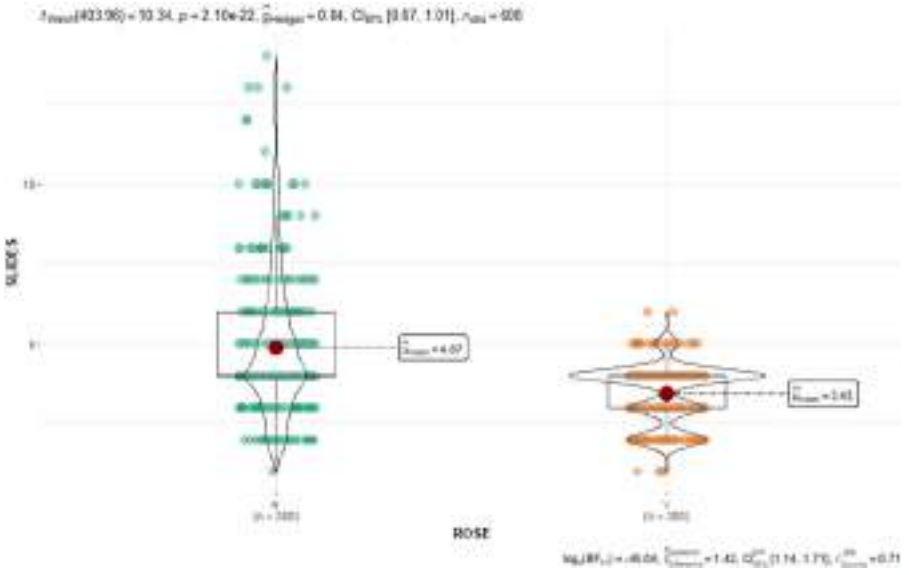


Figure 14 Means and standard deviations of number of slides in ROSE-assisted FNA group versus non-ROSE group. CI, con dence interval; ROSE, rapid on-site evaluation.

Table 2 Various validity parameters showing the correlation between ROSE, nal cytology, cellblock, and histopathology.

Validity parameters	ROSE versus nal cytology (follow-up cases)	ROSE versus nal cytology (n 300)	Cytology versus CB (n 600)	Cytology versus Histopathology (total follow-up)
Sensitivity	96.7	95	90	98.1
Speci city	100	100	100	96.7
Positive predictive value	100	100	100	90
Negative predictive value	99.1	99.24	92.4	99.4
Diagnostic accuracy	99.3	99.3	95.5	97.1
Histopathology	145 out of 300	300	86	241

Abbreviations: CB, cellblock; ROSE, rapid on-site evaluation.

CBs and cytology reports have been rendered simultaneously in various centers.[47,48] However in a country like India where there are resource, manpower, and cost constraints; cytology is the sample reported and CBs are only prepared wherein the ROSE has given a clue of atypia, suspicious, or malignancy. Even the chance of losing samples during processing, and limited tissue would delay the routine cytology reports. The average TAT in ROSE-performed samples (1 day) was lower in comparison to non-ROSE cases (1.6 days). Reports performed on later dates were the reason behind the increased TAT in non-ROSE cases. CB samples had separate TAT (3 days) and were independent of the cytology reports.

The limitation of our study was the sample size and IHC not being done in all CBs in-house due to the limited availability, even then the ef cacy of both ROSE and CBs can be appreciated.

Conclusions

Our study was an attempt to look for the bene ts of using ROSE and CB in diagnostic cytopathology services. Not only were these methods of added advantage to the better patient care services, but they also helped in reducing the TAT, better patient compliance, and reduction in consumption of laboratory reagents and consumables effectively leading to lesser burden on the lab. We recommend the use of ROSE in routine super cial and deep-seated FNACs along with CB in cases where the ROSE has nd-ings of suspicious or malignant.

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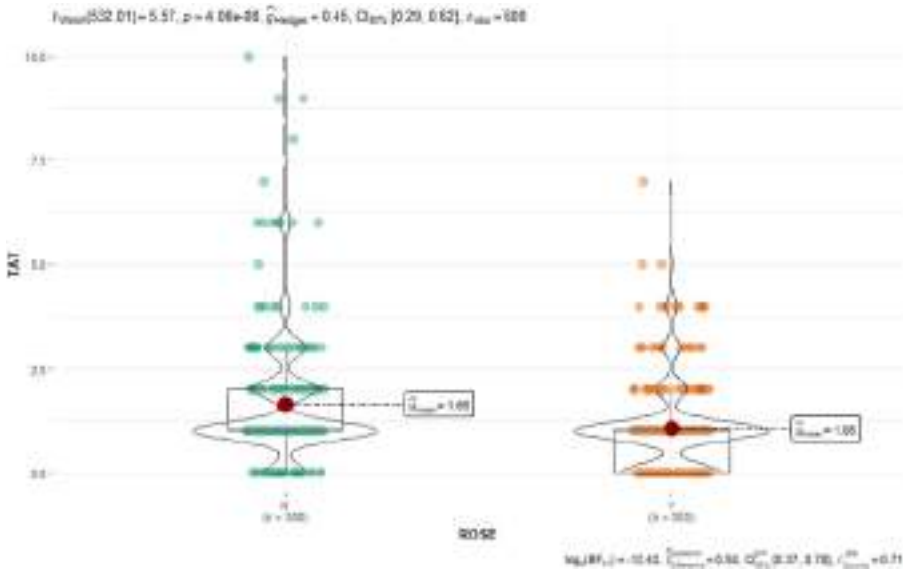

Figure 15 Means and standard deviations of TAT in ROSE-assisted FNA group versus non-ROSE group. CI, con dence interval; ROSE, rapid on-site evaluation; TAT, turnaround time.

Con ict of interest disclosures

The authors made no disclosures.

CRediT authorship contribution statement

Tummidi Santosh: Writing review & editing, Writing original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Arundhathi Shankaralingappa:** Writing review & editing, Supervision, Resources, Methodology, Formal analysis. **Rajeev Aravindakshan:** Software, Methodology, Formal analysis.

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