

ROLE OF TOLUIDINE BLUE STAINING, TO REDUCE THE NON-DIAGNOSTIC REPORTING OF FINE NEEDLE ASPIRATION CYTOLOGY

SABA K.,¹ SAJJAD Y.,² MAJEED T.³ AND BALOCH B.K.⁴

¹Departments of Pathology, ²Plastic Surgery, Lahore General Hospital, ³Department of Gynae. and Obst., King Edward Medical University, ⁴Department of Electrical Engineering UET, Lahore

ABSTRACT

Background and Objective: Fine needle aspiration cytology is becoming the essential domain of diagnostic modalities but its reliability depends upon its adequacy and quick reporting onsite. A cross sectional study was conducted to compare the FNAC smears adequacy by toluidine blue, a supravital stain (TBS) and Papanicolaou stain (PAPS).

Materials and Methods: The accuracy of 2 stains were compared to detect the adequacy of FNAC, smears of 200 aspirates, from different organs (TBS and PAPS). The values were calculated in percentages and 2x2 table calculated the accuracy of two methods.

Results: The diagnostic adequacy was 100% of TABS as compared to 84% of PAPS. The accuracy of lymph nodes, salivary glands, breast and thyroid by TABS was same. Commonest pathology (66.3%) in lymph node swellings was caseating granulomas (57/86 cases) consistent with tuberculosis followed by reactive lymphadenitis (20/86 cases; 23.3%). Neoplastic lesions were less common comprising of lymphoreticular disorder 2/86 (2.3%), Hodgkin lymphoma (one case; 1.16%) and one case (1.16%) of non-Hodgkin Lymphoma. The 25/37 (67.56%) breast lesions were benign as compared to 9/37 (24.32%) malignant cases while only 2/37(5.4%) cases, were suspicious. Benign thyroid lesions were more frequent comprising of 51 (72.27%) cases. One case (1.5%) of papillary carcinoma was found while 13/66 (19.7%) cases were follicular lesions. Regarding salivary gland lesions, there were 4 (36.4%) cases of pleomorphic adenoma and 3 (27.3%) cases of non-specific sialadenitis. There was one case (9%) of each of mucoepidermoid carcinoma, adenoicycstic carcinoma and benign cyst.

Conclusion: TBS may be used onsite reporting of FNAC smearsto improves the diagnostic accuracy.

Key Words: Fine needle aspiration cytology, Supravital stain, Breast, Thyroid, Lymph nodes, salivary glands.

INTRODUCTION

The FNAC is being commonly used now a days for rapid diagnosis of different lesions of lymph nodes, thyroid, breast, salivary glands and different other lesions but poor preparations and cumbersome staining techniques make this procedure non-diagnostic FNAC and decrease the accuracy. The reliability of this technique depends upon its onsite and accurate reporting to the physicians. This also needs the adequate aspiration and good staining background for the pathologist to make their diagnosis accurate and definite. It has been seen that some rapid staining procedures are being adopted by the pathologists to give their report onsite or in time.¹⁻⁴

The issue of repeat aspiration in FNAC is largely unaddressed. Repeat diagnostic procedures may be distressful both to the patient and the aspirator, and impose unnecessary workload on the laboratory. It was suggested that repeat of FNAC should be discouraged

for the cost effectiveness for any diagnostic procedure for the management of patients disease.⁵

Re-aspiration of FNAC at the first visit, following an adequacy assessment by rapid staining, limits visits and shortens the time for definitive management, treatment, thus critically affecting morbidity. In the long run it saves time, money and manpower.⁶⁻⁷

Many times FNAC is repeated to arrive at a conclusive diagnosis. Usually inadequate smears or non-diagnostic cytological reports and FNAC from different topographical sites e.g., Breast, Thyroid, Lymph nodes and other palpable swellings may contribute to repeats of this diagnostic modality to decrease the extra work for the diagnosis of these cases. Repeat FNA's just prolongs the time and increases the cost of management.⁸⁻¹²

There are many conventional stains being used to stain the FNAC smears. Giemsa, PAPS or Haematoxylin and eosin (H&E) stains are commonly used but the-

se take a little longer time to prepare and full – length laboratory setup is required to use these stains. There is an ever – increasing demand for rapid diagnosis in cytology. Quick staining techniques are routinely being used worldwide.¹³⁻¹⁶

The study was conducted to compare the accuracy of adequacy of smears stained with TBS and PAPS.

MATERIAL AND METHODS

The accuracy of 2 stains were compared to detect the adequacy of FNAC, smears of 200 aspirates, from different organs(TBS and PAPS). The values were calculated in percentages and 2x2 table calculated the accuracy of two methods. The study was conducted at the department of Pathology, King Edward Medical University, Lahore sine 1.08.2014 to 31.12.2014.

Fine needle aspiration cytology, was performed by using the needles of 21 – 23 G. The needles were attached to 5 – 10 ml syringes. The aspirate was obtained by agitating the needle tip within the lesion. Then, the aspirates were expressed over the slide to make the smears. In many wet film studies, aspirated material was expressed over slides. The slides were immediately dipped 10 times in the 0.5% toluidine stain solution. The slides were washed with tap water three times. Water mounting with a coverslip was done. The under surface of the slides were wiped with tissue paper. Evaluation under the microscope for the technical adequacy and preliminary diagnostic impression, particularly concerning the presence of atypia in the cells and possible neoplasia was done. FNAC was repeated in cases of inadequate smears. Suppose, the materials were scanty and adhered to the hub of needle, the needle was rinsed with 0.5% toluidine blue stain. Then, it was expressed over slide, covered with cover slip, and sealed with DPX. Now the adequacy, morphology of wet film preparations were interpreted, documented in the cytology requisition form and compared later on with conventional papanicolaou stained smears.

RESULTS

There were 200 patients on whome FNAC was performed. There were 152 females and 48 males. The gender ratio was 1:3.16 with percentage of 24% males and 76% females. The overall mean age was 31.08 ± 15.74 years (Females: 31.09 ± 15 and males: 31.06 ± 18 years) (Table 1 and Figure 1).

Lymph nodes lesions predominated with frequency of 86/200 (43%) followed by thyroid 66/200 (33%), Breast, 37/200 (18.5%) and Salivary glands 11/200 (5.5%) respectively (Figure 1).

Commonest lesions (66.3%) diagnosed in lymph node swellings was Caseating granulomas (57/86 cas-

Table 1: Patient’s age and gender who presented for FNAC.

	N (1:3.16)	Minimum Years	Maximum Years	Mean Years	%age
Patient age	200	5	80	31.08 ± 15.74	
Female	152	5	80	31.09 ± 15.00	76%
Males	48	10	80	31.06 ± 18	24%

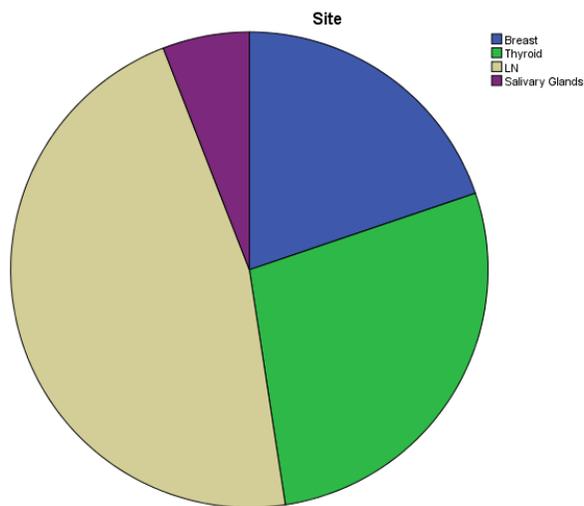


Fig. 1: Topographic distribution of lesions which presented for FNAC.

es) consistent with tuberculosis followed by reactive lymphadenitis (20 cases; 23.3%). Neoplastic lesions were less common comprising of lymphoreticular disorder 2/86 (2.3%), Hodgkin lymphoma (one case; 1.16%) and one case (1.16%) of non-Hodgkin lymphoma (Table 2). In our study 67.56% cases of breast FNAC were of class II, while only 24.32% (9) cases were of Class V. Remaining cases of FNAC of breast lesions were of class III and IV (Table 2).

FNAC of thyroid was also reported adequately by TABS, 51 (72.27%) cases were of category II, 01 case (1.5%) of category VI (papillary carcinoma) and 13 cases (19.7%) were of category IV Bethesda reporting for thyroid cytology. One case (1.5%) was of undetermined significance of category III was reported by this method (Table 2).

We reported the FNAC stained with TABS with same confidence, 4 (36.4%) cases of pleomorphic adenoma and 3 (27.3%) cases of nonspecific sialadenitis (Non-specific sialadenitis). There was one case (9%), each of mucoepidermoid carcinoma, adenoid cystic carcinoma and benign cyst (Table 2).

No inadequate smear was reported by TBS because

all the inadequate smears were re-pri-cked onsite, if found inadequate but 32 FNAC smears were found inadequate stained by PAPS (Table 3 – 4).

DISCUSSION

The FNAC is a reliable and worthwhile onsite technique, used for diagnosis of various diseases. It is an easy technique being used all over the world for rapid diagnosis for an early management of patients in pre-operative and pretreat-ment investigation. Nowadays its impor-tance has risen as an adjunct to clinical, radiological and other laboratory data for better management to improve pati-ent’s prognosis. In spite of its impor-tance and popularity, FNAC has its own advantages, pitfalls and limitations. The main reasons of its limitations are un-satisfactory or non-diagnostic inadequ-ate smears which needs revisiting of pat-ients. Inspissation of cells in aspirated materials in hub and needle lumen is another reason of low efficiency of FN-AC. Inexperienced hands it may cause distortion of cells during aspiration or exporting materials on the slides during the making of smears. Clotting may cau-se poor FNAC quality and relationship of cellular morphology with stromal components.¹⁶⁻²⁰

The TBS technique is a way of mar-king tissue for rapid microscopical ex-amination. It gives blue staining for nu-cleic acids and polysaccharides and also increases the sharpness of nuclear de-tails on cytological smears. Toluidine bl-ue staining is not a reliable method for rapid staining and diagnosis.¹⁷⁻¹⁸

The sample size of our study was 200 cases comprising of breast, thyroid, lymphnodes and salivary glands swell-ings. This was consistent with other stu-dies mentioned in literature conducted to compare this supravital stain with conventional stains.¹⁵⁻¹⁶

The overall mean age was 31.08 ± 15.74 with a range of 5 – 80 years (fema-les, 31.09 ± 15 and males was 31.06 ± 18 years). This is almost consistent with other studies conducted locally for eva-luation of FNAC results.¹⁹⁻²¹

The age was not consistent with some other stud-ies conducted to evaluate FNAC results which could be due to topographic selection of different lesions and sample size.²²⁻²³

Table 2: Showing distribution of different Lymph Nodes lesions which presented for FNAC.

Pathology (Lymph nodes = 86, Thyroid = 66, Breast = 37, Salivary Gland Lesion = 11)	Frequency	%age
Caseating Granuloma (n = 86)	57	66.3
Reactive Nonspecific	20	23.30
Pyogenic Lymphadenitis	04	4.60
Lymphoreticular Disorder	02	2.30
Hodgkin Lymphoma	01	1.16
Non Hodgkin Lymphoma	01	1.16
Metastatic Lesions of Breast	01	1.16
Category – II (Thyroid)	51	72.27
Category – III (Thyroid)	01	1.50
Category – VI (Thyroid)	13	19.7
Category – VI (Thyroid)	01	1.5
Class – II (Breast)	25	67.56
Class – 3 (Breast)	01	2.7
Class – 4 (Breast)	02	5.4
C – 5 (Breast)	09	24.32
Pleomorphic adenoma of Salivary Gland	04	36.4
Non-specific Inflammation of Salivary Gland	03	27.2
Mucoepidermoid Carcinoma of Salivary Gland	01	9.1
Adenoid Cystic Carcinoma of Salivary Gland	01	9.1
Benign Cyst of Salivary Gland	01	9.1
Squamous Cell Carcinoma of Salivary Gland	01	9.1

The females were predominated as compared to males, comprising of 152 female and 48 males. The gender ratio was 1:3.16 with percentage of 24% males and 76% females. The overall gender ratio was not consistent with literature either locally or internat-

ionally. Again the difference may be due to sampling techniques.²³

In a total of 200 cases, there were 86 cases of lymph node pathology, which underwent FNAC. The most common pathology was seen in cervical lymph nodes. There were 78.9% neck swellings either anterior or posterior cervical lymphadenopathy. Only 7% submandibular, 6% axillary lymph nodes, 6% post auricular lymph nodes, 2.3% inguinal lymph nodes, 1.16%

and 1.16% pre-auricular lymph nodes were involved. Submental and pre-auricular lymph nodes swellings were the least common lesions which presented for FNAC in our study. The findings are consistent with other studies because neck has been reported the commonest site of peripheral lymph node swellings and is commonly encountered in FNAC procedures.²⁴

FNAC is commonly used in the diagnosis of lump breast because it is an excellent, safe, and economical

Table 3: Calculated cost effectiveness for TBS and Pap staining Procedure for 200 Patients.

Test Name (n=200)	Kit Price (Rs.)	Alcohol Price (Rs.)	Infra-structure Table, Chair, Staining Jars, Electricity, Water	Technician	Attendant	Total Price (Rs.)	Per test Cost (Rs.)	Non-Diagnostic Results
TBS	9000	0	0	0	10000	19000	95	0
Pap	18000	22,000	22,000	15000	10000	87,000	435	32

Table 4: Benefits for TBS and Pap staining Procedure for 200 Patients.

		TBS		PAP	
		Yes	No	Yes	No
<i>Economic Benefits to patients</i>	Time saving	200	0	168	32
	Patient	200	0	168	32
	Pathologist	200	0	168	32
	Patients Attendants	200	0	168	32
<i>Patients Indirect costs</i>	Travelling saved	200	0	168	32
	Lodging saved	200	0	168	32
	Opportunity cost saved	200	0	168	32
	Improved lab turnover	200	0	168	32
	Diagnostic yield	200	0	168	32
<i>Humanitarian Benefits</i>					
	Patients suffering Reduced	200	0	168	32
<i>Quality of work</i>					
	Better outcome	200	0	168	32
<i>Quantity of work</i>					
	Revisiting for re-aspiration	0	200	32	168
	Lab Turnover	200	0	168	32

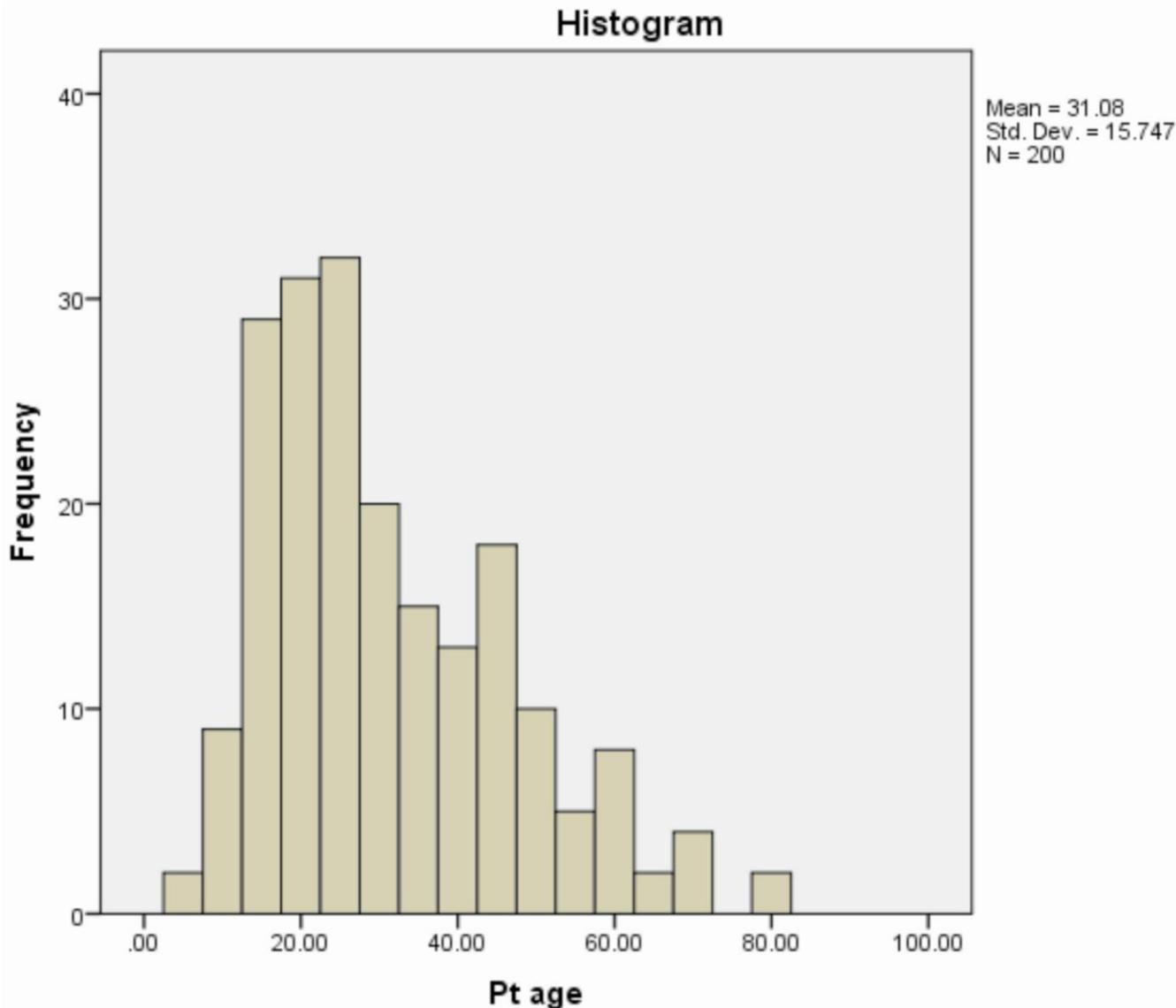


Figure 2: Age distribution of the patients in the study who presented for FNAC.

diagnostic modality even on-site for quick reporting with minimal inexpensive equipment as a simple procedure.²⁵⁻²⁷

In our study, 37 patients presented with lumps in breast for FNAC. UOQ swellings were the commonest site of lesions comprising of 83.8% followed by UIQ with 8% swellings. There was one case of each site from LOQ, areola and axillary tail. The diagnostic yield as accurate for breast FNAC by TABS as reported by others.¹¹

Aspiration of the thyroid in patients with colloid goiter showed the characteristic thick colloid as patchy blue coloured material and thin colloid as purplish pink coloured granular material. Follicular cells were seen as round mild pleomorphic cells with scanty cytoplasm and mild nuclear anisokaryosis. Aspirate from

papillary carcinomas showed papillary sheets of monomorphic cells with moderate pale pink cytoplasm, large round uniform nucleus with prominent small basophilic nucleolus and some nuclei with inclusions.²⁵

There were 66 cases of thyroid swellings who gave consent for this FNAC study. Right lobe was involved predominantly, in 47% patients followed by 33.33% cases with left lobe swellings. Only 13.63% patients presented with swelling of whole thyroid glands while 9% cases came with isthmus nodules. Solitary thyroid nodules (STN) were the more frequent lesions in thyroid patients and were seen in 69.7% cases. Some cases of multinodular goiters (21.2%) and diffusely enlarged thyroid gland (9.1%) also came for FNACs. First time we used the Bethesda Reporting System for reporting FNAC of thyroid nodules according to the literature

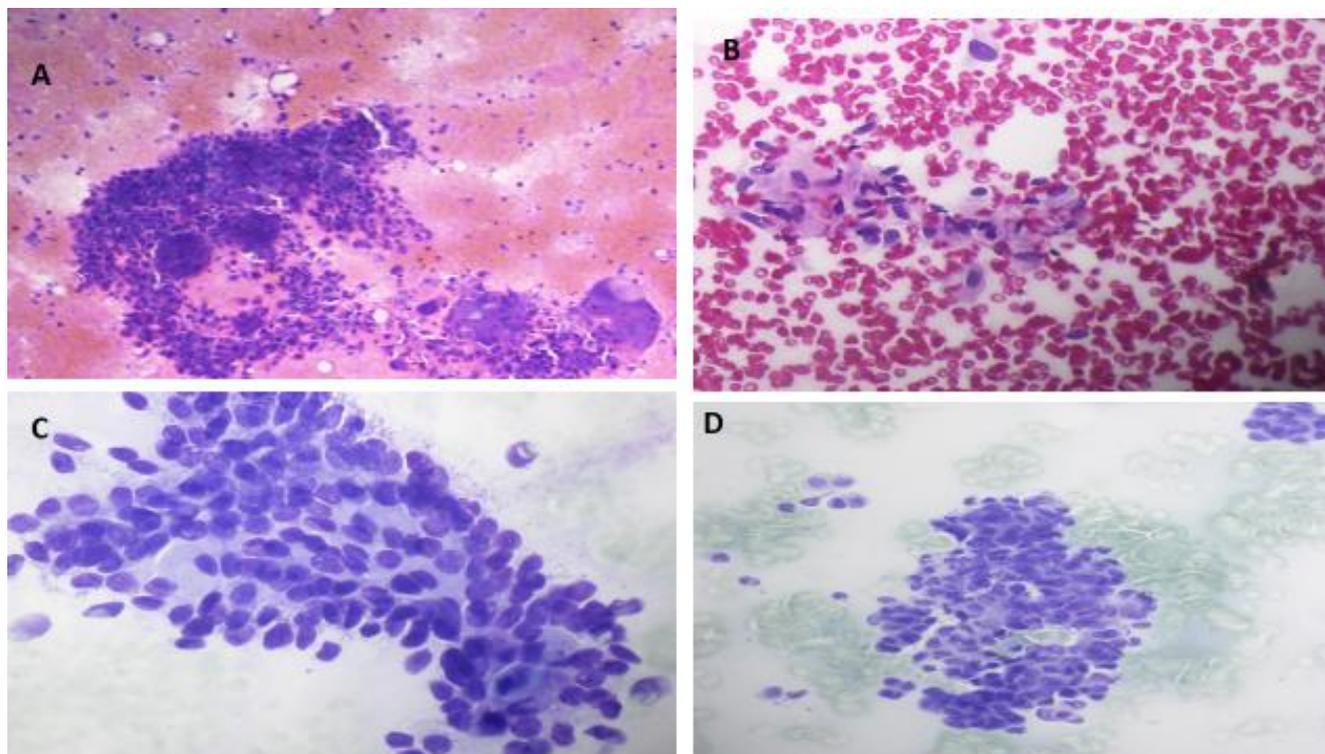


Fig. 3: Photomicrographs (TABS 20x), A: adenoid cystic carcinoma, B: Granuloma, C: Ductal Carcinoma, D: Papillary carcinoma.

and used six diagnostic categories to standardize communication of thyroid fine – needle aspiration (FNA) interpretations between clinicians and cytopathologists.²⁸

The “TBSRTC system for the onsite reporting of thyroid nodules by TABS was as good as reported by Basharat et al, but contradicted by the findings of Park et al.²⁹⁻³⁰

The reporting of pleomorphic adenoma of various salivary gland lesions was similar as reported by previous studies, in our study it was (36.4%). The frequency of various malignant neoplasm in previous studies vs. our study is variable, i.e., mucoepidermoid carcinoma 24.6% vs. 9%, acinic cell tumor 12% vs. 9%, adenoid cystic carcinoma 18% vs. 9%. The difference in frequency may be again due to our small sample size as compared to other studies.²⁵

A repeat FNAC in the same sitting on these 32 patients showed adequate material in all of our patients. Therefore, for 100%, a decision could be made at the very first visit to plan further management on TBS smears. These findings are also consistent with Ammanagi et al who found 98% adequate smears on repeat on site procedure.¹³

Our study employed TBS, which is a cheap and easily available supravital stain and results from our study are promising in terms of adequacy assessment as compared to conventional Pap stained smears.

The morphological features of cells from all smears were excellent by this supra-vital stain; even nuclear and cytoplasmic preservation was fine but less superior to smears stained by PAPS. Stain the “TBS” technique was good to reduce the need for revisiting the patients due to inadequacy of smears. The diagnostic yield was 100% by “TBS” as compared to “PAPS”. By TABS, we issued the reports within few minutes (3 minutes), onsite, while one day was given for completing the stain and reporting of smears by PAPS. The findings are consistent with Ammanagi et al,¹³ who found 28/200 (14%) unsatisfactory on the first attempt as assessed by rapid staining. These included 38% of thyroid cases, 10.4% of breast lump cases and 03.2% of lymph node cases as compared to our study showing 31% of thyroid, 31% of breast, 33% of lymph nodes and 03% of Salivary glands. In our study the inadequate smears were more frequently seen in lymph node as compared to Ammanagi et al.¹³

No inadequate smear was reported by TBS because all the inadequate smears were re-pricked onsite, if found inadequate but 32 FNAC smears were found inadequate stained by PAPS as was not possible to re-prick immediately due to complex staining procedure of PAPS. On the basis of this reporting the yield of accuracy of TABS was 200/200 (100%) as compared to PAPS 168/200 (84%).

It is **concluded** from this study that the accuracy

of fine needle aspiration diagnosis by supravital rapid stain TBS was 100 as compared to 84% of the Papanicolaou stained smears due to high percentage of inadequacy by this conventional technique.

Author's Contributions

Saba K was the main author who conceived, designed and did statistical analysis and editing of manuscript. Yawar Sajjad helped manuscript writing, designing the research. Tayyaba Majeed and Bakhat Yar Khan Baloch helped in preparing the draft of manuscript.

ACKNOWLEDGEMENTS

We are grateful for the cooperation of my colleagues, especially Ali Afzal for his moral support made me to complete the article without which it was impossible to do this research. We are also thankful to Pathology technical staff, Tariq and Irfan. We have some great word for Prof Rakhshinda Bajwa, who helped us in each and every steps of research.

REFERENCES

- Ducatman BS, Hogan CL, Wang HH. A triage system for processing fine needle aspiration cytology specimens. *Acta Cytol.* 1989; 33 (6): 797-9.
- Verma K, Tiwari MC, Agarwal J, Kapila K. Diagnostic accuracy of immediate interpretation of fine needle aspirates. *Indian J Med Res.* 1991; 94: 197-9.
- Stewart CJ, Stewart IS. Immediate assessment of fine needle aspiration cytology of lung. *J Clin Pathol.* 1996; 49 (10): 839-43.
- Silverman JF, Finley JL, O'Brien KF, Dabbs DJ, Park HK, Larkin EW, et al. Diagnostic accuracy and role of immediate interpretation of fine needle aspiration biopsy specimens from various sites. *Acta cytologica.* 1989; 33 (6): 791-6.
- Goyal R, Garg PK, Bhatia A, Arora VK, Singh N. Clinical audit of repeat fine needle aspiration in a general cytopathology service. *J Cytol.* 2014; 31 (1): 1-6.
- Alsohaibani F, Girgis S, Sandha GS. Does onsite cytotechnology evaluation improve the accuracy of endoscopic ultrasound-guided fine-needle aspiration biopsy? *Can J Gastroenterol.* 2009; 23 (1): 26-30.
- Sofi LA, Sherwani RK, Hasan M, Nobin H. On-spot brilliant cresyl blue staining in fine needle aspiration, a novel technique for cytological diagnosis. *Acta Cytol.* 2013; 57 (6): 641-5.
- Graciano AJ, Chone CT, Fischer CA, Bublitz GS, Peixoto AJ. Repeated fine-needle aspiration cytology for the diagnosis and follow-up of thyroid nodules. *Braz J Otorhinolaryngol.* 2014; 80 (5): 422-7.
- Christopher MM, Hotz CS. Cytologic diagnosis: expression of probability by the clinical pathologists. *Vet Clin Pathol.* 2004; 33 (2): 84-95.
- Gomez-Macias GS, Garza-Guajardo R, Segura-Luna J, Barboza-Quintana O. Inadequate fine needle aspiration biopsy samples: pathologists versus other specialists. *Cytojournal.* 2009; 6: 9.
- Kocjan G. [Needle aspiration cytology of the breast: current perspective on the role in diagnosis and management]. *Acta Med Croatica.* 2008; 62 (4): 391-401.
- Borget I, Vielh P, Leboulleux S, Allyn M, Iacobelli S, Schlumberger M, et al. Assessment of the cost of fine-needle aspiration cytology as a diagnostic tool in patients with thyroid nodules. *Am J Clin Pathol.* 2008; 129 (5): 763-71.
- Ammanagi AS, Dombale VD, Patil SS. On-site toluidine blue staining and screening improves efficiency of fine-needle aspiration cytology reporting. *Acta Cytol.* 2012; 56 (4): 347-51.
- Chang MC, Chen RD, Ho WL. [Intraoperative cytology: the use of Liu's stain for immediate diagnosis]. *Zhonghua Yi Xue Za Zhi (Taipei),* 1993; 51 (5): 368-75.
- Sumathi S, VR. M. Supravital-stained wet film study of fine needle aspirates: A reliable supplementary diagnostic procedure. *Clin Cancer Investig.* 2012; 1: 135-99.
- Sumathy C, Suresh Durai J, Swaminathan K, Vallimananan S, SA. M. Supravital Stained Rapid Wet Mount Preparation of Fine Needle Aspirates – A Cytomorphological Study. *Tirunelveli e J Medical Sciences* 2012; 3: 62-6.
- Joy MP, Iyer VK, Aron M, Kapila K, Verma K. Rapid staining using toluidine blue: a reliable method for quick diagnosis in ultrasound-guided aspiration cytology. *Indian J Pathol Microbiol.* 2003; 46 (4): 589-92.
- Hsieh MH, Hsiao YL, Chang TC. Fine needle aspiration cytology stained with Rius method in quicker diagnosis of medullary thyroid carcinoma. *J Formos Med Assoc.* 2007; 106 (9): 728-35.
- Zhang S, Ivanovic M, Nemcek AA, Jr., Defrias DV, Lucas E, Nayar R. Thin core needle biopsy crush preparations in conjunction with fine-needle aspiration for the evaluation of thyroid nodules: a complementary approach. *Cancer,* 2008; 114 (6): 512-8.
- Jorda M, Rey L, Hanly A, Ganjei-Azar P. Fine-needle aspiration cytology of bone: accuracy and pitfalls of cytodiagnosis. *Cancer,* 2000; 90 (1): 47-54.
- Bukhari MH, Niazi S, Hanif G, Qureshi SS, Munir M, Hasan M, et al. An updated audit of fine needle aspiration cytology procedure of solitary thyroid nodule. *Diagn Cytopathol.* 2008; 36 (2): 104-12.
- Sherwani RK, Akhtar K, Ray PS, Basha M. The significance of microbiopsies in cytological smears. *Indian J Pathol Microbiol.* 2013; 56 (3): 200-3.
- Raghuvver CV, Leekha I, Pai MR, Adhikari P. Fine Needle Aspiration cytology versus Fine Needle Sampling without aspiration. A prospective study of 200 cases. *Indian J Med Sci.* 2002; 56 (9): 431-9.
- Biswas G, Das A, Halder D, Mukherjee A, Dutta S, Sinha R. Clinico-pathological correlates of cervical lymphadenopathy: a hospital based study. *Indian J Otolaryngol Head Neck Surg.* 2013; 65 (Suppl. 1):42-7.
- Rosai J. Major and minor salivary glands. In: Ackerman's surgical pathology. 10th ed. New York: Mosby Co; 2010: 817-40 p.
- He Q, Fan X, Yuan T, Kong L, Du X, Zhuang D, et al. Eleven years of experience reveals that fine-needle aspiration cytology is still a useful method for preoperative diagnosis of breast carcinoma. *Breast,* 2007; 16 (3): 303-6.
- Bukhari MH, Arshad M, Jamal S, Niazi S, Bashir S, Bakhshi IM, et al. Use of Fine-Needle Aspiration in the Evaluation of Breast Lumps. *Patholog Res Int.* 2011; 2011: 23-5.

28. Nemenqani D, Yaqoob N. Fine needle aspiration cytology of inflammatory breast lesions. *J Pak Med Assoc.* 2009; 59 (3):1 67-70.
29. Ludvikova M, Kholova I. [Up-to-date experience with the international classification system Bethesda 2010 for thyroid fine-needle aspirate: a review]. *Cesk Patol.* 2014; 50 (3): 155-60.
30. Park JH, Yoon SO, Son EJ, Kim HM, Nahm JH, Hong S. Incidence and malignancy rates of diagnoses in the Bethesda system for reporting thyroid aspiration cytology: an institutional experience. *Korean J Pathol.* 2014; 48 (2): 133-9.