

Frozen Section as an Aftermath to Fine Needle Aspiration Cytology in Thyroid Lesions

VIDYASHREE VITTAL ANCHAN, PARINITHA SADASHIVAPPA SANGAM, DINESH UDIPI SHASTRI, VANDANA UDAYKUMAR GRAMPUROHIT, HEPHZIBAH S RANI

ABSTRACT

Introduction: Study of thyroid diseases are of significant importance as it has implications on medical and surgical management. The choice of the diagnostic method used is also vital as it may influence the quality of the outcome. Fine Needle Aspiration Cytology (FNAC) has for long been a useful, cheap and safe procedure in preoperative evaluation of thyroid lesions. Frozen sections on the other hand can confirm the FNAC diagnosis and it also serves as an intraoperative guide to decide the extent of the thyroid surgeries.

Aim: To assess the diagnostic accuracy of FNAC and frozen sections and to determine the utility of frozen sections in thyroid lesions.

Materials and Methods: This five year retrospective study was conducted between July 2010 to July 2015. All patients undergoing both FNAC and frozen sections for thyroid lesions were included in the study. Results were then compared with the gold standard which is histopathology. SPSS 20.0 software was used to analyse the data. Descriptive statistics was applied to draw conclusions. Sensitivity, specificity,

Positive Predictive Value (PPV), Negative Predictive Value (NPV) and diagnostic accuracy were calculated for both FNAC and frozen sections.

Results: Among 70 cases, FNAC was reported as non-neoplastic in 43 cases among which eight cases differed on histopathology. Total 27 cases reported as neoplastic of which eight cases differed on histopathology. On frozen sections, among 43 reported as non-neoplastic and 27 as neoplastic, only four cases in each differed on histopathology studies. On the final histopathology sections, 43 were non-neoplastic and 27 were neoplastic lesions (9 benign, 18 malignant). FNAC sensitivity was 70.37%, specificity was 81.39%. Frozen section sensitivity was 85.18%, specificity was 90.69%. The difference was statistically significant.

Conclusion: In the present study, frozen sections had better sensitivity and specificity compared to FNAC in thyroid lesions. Thus, concluding that FNAC can definitely help the clinician to stratify the patients and select them for surgery but frozen section can confirm the FNAC diagnosis and guide the extent of thyroidectomy.

Keywords: Dyshormonogenetic goitre, Histopathology, Thyroidectomy

INTRODUCTION

FNAC is now the first line diagnostic test with the main purpose of confirming benign lesions and thus reducing unnecessary thyroid surgeries [1-4]. In some cases, it provides a definitive diagnosis of malignancy, tumour typing and therefore helps in comprehensive surgery with therapy planning in a single step [5].

Though, worldwide literature claims high accuracy of thyroid cytology, there are also limitations of this procedure [4]. However, frozen section helps as an intraoperative guide for thyroid surgeries with many surgeons using it to confirm the FNAC diagnosis and guide the extent of thyroidectomy [6].

Due to the more widespread use of FNAC, the role of frozen sections has reduced. The accuracy of FNAC in diagnosing papillary carcinoma thyroid and also the need for extensive sampling of tumour capsule to diagnose follicular carcinoma in

frozen sections, the number of intraoperative consultation has steadily reduced [5]. In spite of medico-economic advantage of frozen sections as it allows one stage total thyroidectomy and its accuracy for diagnosing thyroid cancer, it remains a subject of controversy [7,8]. The aim of this study was to assess the diagnostic accuracy of FNAC and frozen sections and to determine the utility of frozen section in thyroid lesions.

MATERIALS AND METHODS

It is a retrospective study from July 2010 to July 2015 conducted in the Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad which is a Tertiary Care Hospital. The sample size was calculated using the formula $N=4pq/L2$. The cases where FNAC was performed but frozen section was not opted during surgery were excluded from the study. Similarly, the cases where frozen section was

opted but FNAC was not performed prior were also excluded from the study.

A total of 70 cases were included in the study wherein all three of FNAC, frozen section and final histopathology was performed at our institute. FNAC using 23 gauge disposable needles without local anaesthesia was performed by pathologists who prepared the smears from the aspirates. Air dried smears were stained by May Grunwald-Giemsa (MGG) stain and alcohol fixed smears were stained by Hematoxylin and Eosin stain and Papanicolaou stain. Criteria for adequacy for FNAC material was six clusters of thyroid follicular cells in atleast two slides prepared from two separate aspirates. For frozen section, the tissue slices were cut in a cryostat Leica CM1510-1 at 6 micrometer (-21°C) and stained with rapid Hematoxylin and Eosin stain. Multiple sections were examined to make the final histopathological diagnosis. Automated tissue processor Leica 1020 was used. Institutional ethical clearance was obtained.

STATISTICAL ANALYSIS

The data collected was tabulated and analysed by proportions and percentages. Descriptive statistics were applied to draw conclusions. Statistical Package for the social Sciences (SPSS) version 20.0 software was used to analyse the data. Statistical test like sensitivity, specificity, diagnostic accuracy, positive and negative predictive value were calculated.

RESULTS

During the study period total of 1034 thyroid FNAC procedures were performed. Among these, frozen section was opted during surgery in 70 cases. Of these, 16 cases (22.8%) were males and 54 (77.2%) were females. The youngest patient was 18 year and oldest patient was 85 year. Mean age was 40.75±14.37 years. Most common age group for both benign and malignant lesion was 31-40 years.

On FNAC, 43 cases were reported as non-neoplastic lesions and 27 were neoplastic. Among non-neoplastic lesions eight cases differed on histopathology. Four cases reported as nodular goitre on FNAC were papillary carcinoma on histopathology. One case reported as adenomatoid goitre on FNAC was Follicular Tumour of Uncertain Malignant Potential (FT-UMP). Two cases of nodular goitre reported on FNAC proved to be Hurthle cell adenoma on histopathology. One case reported as hyperplastic nodule on FNAC proved to be follicular adenoma on histopathology [Table/Fig-1].

Among 27 cases of neoplastic lesions on FNAC, eight cases differed. Two cases reported as medullary carcinoma, three cases reported as suspicious for papillary carcinoma and one as follicular lesion of undetermined significance on FNAC were all nodular goitre on histopathology. One case reported as follicular neoplasm on FNAC proved to be Hashimoto's thyroiditis and one case reported as follicular neoplasm on FNAC proved to be dysplastic nodular goitre [Table/Fig-1].

On frozen section, 43 cases were reported as non-neoplastic

and 27 cases as neoplastic. Among cases reported as non-neoplastic on frozen section four cases differed. One case reported as nodular goitre on frozen proved to be follicular variant of papillary carcinoma and two cases reported as nodular goitre proved to be micropapillary carcinoma. One case reported as non-neoplastic lesion on frozen proved to be Squamous Cell Carcinoma (SCC) on histopathology [Table/Fig-2].

Among 27 cases reported as neoplastic on frozen section, four cases differed. One case reported as suspicious for papillary carcinoma and another case as follicular adenoma on frozen were nodular goitre on histopathology. One case reported as follicular carcinoma on frozen section was dysplastic nodular goitre on histopathology and one case reported as benign follicular lesion on frozen section was Hashimoto's thyroiditis [Table/Fig-2].

Among 70 patients, histopathology revealed non-neoplastic lesion in 43 cases (61.4%) and 27 (38.6%) were neoplastic. Among non-neoplastic lesions, the commonest was nodular goitre with 41 cases (58.57%). Benign neoplasm was seen in nine cases (12.85%), most common being follicular adenoma

FNAC	Total no. of cases	Histological Diagnosis		
		Non-neoplastic lesion	Benign neoplastic lesion	Malignant neoplastic lesion
Non-neoplastic lesion	43	35	3 (2- Hurthle cell adenoma, 1- Follicular adenoma)	5 (4- Papillary carcinoma, 1- FT-UMP)
Benign neoplastic lesion	9	4 (1- Dysplastic nodular goitre, 1- Hashimoto's thyroiditis, 2- Nodular goitre)	5	0
Malignant neoplastic lesion	18	4 (4- Nodular goitre)	1 (1- Follicular adenoma)	13
Total	70	43	9	18

[Table/Fig-1]: Comparison of FNAC with histopathologic diagnosis.

Frozen Section	Total no. of cases	Histological Diagnosis		
		Non-neoplastic lesion	Benign neoplastic lesion	Malignant neoplastic lesion
Non-neoplastic lesion	43	39	0	4 (3- Papillary carcinoma, 1- SCC)
Benign neoplastic lesion	14	2 (1- Nodular goiter, 1- Hashimoto's thyroiditis)	8	4 (2- Hurthle cell carcinoma, 1- Micropapillary carcinoma, 1- FT-UMP)
Malignant neoplastic lesion	13	2 (1- Nodular goitre, 1- Dysplastic nodular goitre)	0	11
Total	70	43	8	19

[Table/Fig-2]: Comparison of frozen section with histopathologic diagnosis.

Type of Lesion		No. of cases	Percentage
Non-neoplastic lesion (43 cases)	Nodular goitre	41	58.57
	Dyshormono-genetic goitre	1	1.43
	Hashimotos thyroiditis	1	1.43
Benign (9 cases)	Follicular adenoma	7	10
	Hurthle cell adenoma	2	2.85
Malignant (18 cases)	Papillary carcinoma	11	15.7
	Hurthle cell carcinoma	3	4.3
	Follicular tumour of uncertain malignant potential	2	2.85
	Medullary carcinoma	1	1.43
	Squamous cell carcinoma	1	1.43
Total		70	100

[Table/Fig-3]: Histopathologic diagnosis.

in seven cases (10%) and malignancy was found in 18 cases (25.7%). Papillary carcinoma was the commonest malignancy accounting for 11 cases (15.7%) [Table/Fig-3].

In our study, 14 cases were follicular neoplasms. Among which seven cases were follicular adenoma, two cases were Hurthle cell adenoma, two were FT-UMP and three were Hurthle cell carcinoma. Among seven cases of follicular adenoma, on FNAC only five cases were reported as follicular neoplasm and one case was reported as nodular goitre and one case as suspicious for papillary carcinoma. On frozen section, all these cases were reported as follicular neoplasm with no evidence of capsular invasion in the sections studied. Among two cases of Hurthle cell adenoma, both were reported as nodular goitre on FNAC, whereas on frozen sections one case was reported as Hurthle cell neoplasm and other was reported as nodular goitre. Of two cases of FT-UMP, on FNAC one was reported as follicular neoplasm and one as nodular goitre whereas on frozen, one was reported as follicular neoplasm without capsular invasion in the sections and one was reported as follicular carcinoma. Among three cases of Hurthle cell carcinoma, all the three were identified as Hurthle cell neoplasm on FNAC. But on frozen one could be clearly called as Hurthle cell carcinoma and two were called Hurthle cell adenoma.

Among 11 cases of papillary carcinoma in our study, seven were reported as papillary carcinoma on both FNAC and frozen section. In four cases, it was missed on both FNAC and frozen section. Of which two cases were follicular variant of papillary carcinoma, two were micropapillary carcinoma.

FNAC sensitivity was observed to be 70.37%, specificity was 81.39%, diagnostic accuracy was 77.14%, PPV was 70.37%, and NPV was 81.39%. Frozen section sensitivity was observed to be 85.18%, specificity was 90.69% and diagnostic accuracy was 88.57%. The PPV and NPV for frozen was 85.18% and 90.69% [Table/Fig-4-6].

Parameters	Kumar M et al., [1] (n=59)	Batra C et al., [2] (n=50)	Prades JM et al., [7] (n=202)	Pai SB et al., [10] (n=64)	Present study (n=70)
Age (in years)	18-68	7-70	14-89	20-73	18-85
Sex (Female)	50 (84%)	41 (82%)	166 (82%)	46 (71%)	54 (77%)
Non-neoplastic	47 (79.6%)	35 (70%)	110 (54.4%)	36 (56%)	43 (61%)
Benign	5 (8.4%)	2 (4%)	48 (23.76%)	13 (20.31%)	9 (12.9%)
Malignant	7 (11.86%)	13 (26%)	49 (24.24%)	15 (23.43%)	18 (25.7%)

[Table/Fig-4]: Comparison of different parameters with other studies.

FNAC	Kumar M et al., [1] (n=59)	Batra C et al., [2] (n=50)	Prades JM et al., [7] (n=202)	Pai SB et al., [10] (n=64)	Nart D et al., [11] (n=291)	Present study (n=70)
Sensitivity	42%	69.2%	35.7%	66%	43%	70.37%
Specificity	100%	80%	99.2%	95%	68%	81.39%
Accuracy	93%	77.1%	84.1%	89%	73%	77.14%
PPV	100%	-	93.7%	83%	80%	70.37%
NPV	92%	-	83.3%	90%	72%	81.39%

[Table/Fig-5]: Comparison of statistical values of FNAC with other studies.

Frozen section	Kumar M et al., [1] (n=59)	Batra C et al., [2] (n=50)	Prades JM et al., [7] (n=202)	Pai SB et al., [10] (n=64)	Nart D et al., [11] (n=291)	Present study (n=70)
Sensitivity	71%	81.8%	55.8%	86%	76%	85.18%
Specificity	100%	100%	99.1%	100%	82%	90.69%
Accuracy	96%	95.4%	89%	96%	84.5%	88.57%
PPV	100%	-	95%	100%	82.8%	85.18%
NPV	96%	-	88.6%	96%	85%	90.69%

[Table/Fig-6]: Comparison of statistical values of frozen section with other studies.

DISCUSSION

Evaluation of FNAC and intraoperative diagnosis are traditional components in the management of thyroid lesions. FNAC is indeed a true corner stone for the preoperative evaluation of thyroid lesions [5,9]. FNAC is an accurate preoperative procedure in diagnosing thyroid lesions and helps in distinguishing non-neoplastic and neoplastic conditions for the purpose of selecting patients that need surgical intervention [6]. Kraemer 16 rightly says 'As the surgeon drives, pathologists posts the road signs' [2]. Frozen sections in addition to FNAC helps the surgeon in decision making during surgery. Some surgeons still demand frozen section confirmation to overcome cytological error [9]. Comparative study of FNAC and frozen section with histopathology has an advantage of being comprehensive. Merits and demerits

of these diagnostic methods can be better understood because source of sample is the same material [1]. There are very few studies highlighting the importance of intraoperative consultation in India and there are no studies done in this region of Karnataka.

In the present study of 70 cases, a total of 54 (77%) were females. Kumar M et al., and Prades JM et al., reported female preponderance of 84% in their 59 case series and 82% in their 202 case series respectively [1,7]. Study by Pai SB et al., and Batra C et al., show 71% of 64 cases and 82% of 50 cases to be female respectively [2,10].

In present study, the mean age was 40.75 years. This is comparable with study done by Batra C et al., which showed the mean age of 40.5 years [2]. We found that the majority of patients i.e., 24 cases (34.28%) in our study were in their fourth decade of life. This is in accordance with study done by Pai SB et al., where 39% and Kumar M et al., where 28% were in fourth decade [1, 10]. Batra C et al., shows most of the patients in their fourth and fifth decade of life forming 52% of the study group [2]. Most common age group for malignant lesion in our study was fourth decade (7.45%) [Table/Fig-4].

Majority of the cases in our study had non-neoplastic lesion i.e., 43 cases (61%). Study by Kumar M et al., Prades JM et al., Batra C et al., and Pai SB et al., reported 79.6%, 54.4%, 70% and 56% respectively to be non-neoplastic lesion [1,2,7,10]. Among these the commonest non-neoplastic lesion in our case series was nodular goitre with 41 of total cases (60%). Kumar M et al., Prades JM et al., and Batra C et al., reported 64%, 45.54% and 66% respectively as nodular goitre in their study [1,2,7].

Neoplastic lesion was found in 27 cases (nine were benign and 18 malignant). Most common benign lesion in our study was follicular adenoma with seven cases (10%). Pai SB et al., Kumar M et al., Prades JM et al., and Batra C et al., showed 10.93%, 8.47%, 21.28% and 4% to have follicular adenoma [1,2,7]. Most common malignant lesion in our study was papillary carcinoma with 11 cases (15.7%). Pai SB et al., Kumar M et al., and Prades JM et al., reported 21.8%, 10%, 18.8% cases of papillary carcinoma and showed papillary carcinoma as the commonest malignant neoplasms in their study [1,7,10]. Nart D et al., reported 64 among 291 cases as papillary carcinoma (29.9%) [11].

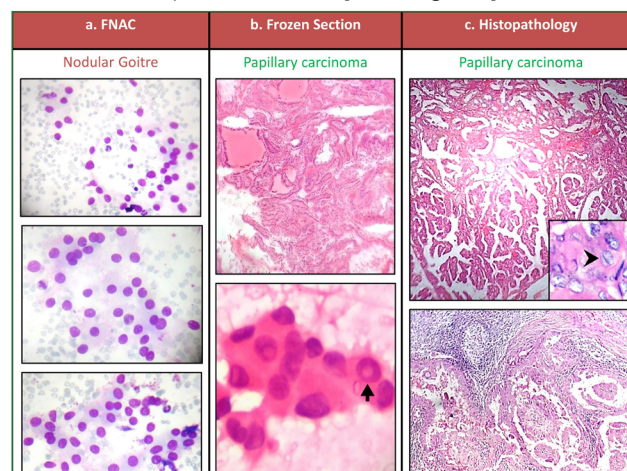
In the present study, one case of papillary carcinoma was reported as nodular goitre on FNAC due to low cellularity and the absence of nuclear features in the smears studied [Table/Fig-7a]. However, the same case on frozen sections showed the papillary pattern with the presence of intra nuclear cytoplasmic inclusions and was reported as papillary carcinoma [Table/Fig-7b]. Histopathological sections showed papillary arrangement with intra nuclear cytoplasmic inclusions and confirmed the diagnosis of papillary carcinoma [Table/Fig-7c].

Another case of nodular goitre was reported as suspicious for papillary carcinoma on FNAC due to the deceptive papillary pattern of arrangement, intra-nuclear cytoplasmic inclusions

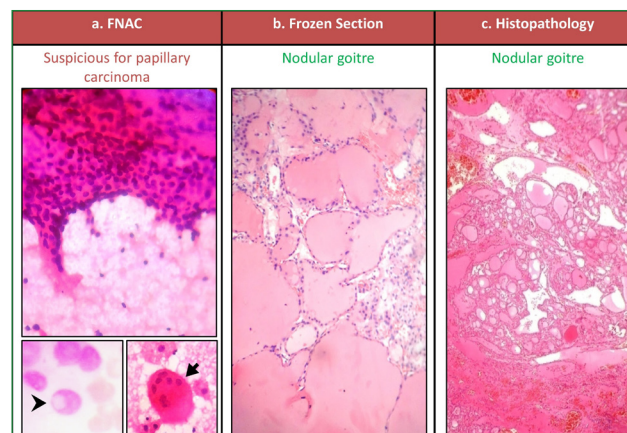
and multinucleated giant cells [Table/Fig-8a]. However, on frozen sections only the colloid filled follicles were noted without any nuclear findings suggesting papillary carcinoma and thus, this case was correctly diagnosed as nodular goitre [Table/Fig-8b]. Histopathology confirmed the diagnosis of Nodular goitre [Table/Fig- 8c].

A case of follicular adenoma was reported rightly as follicular neoplasm on FNAC where the FNAC smears showed cells arranged in repetitive follicular pattern [Table/Fig-9a]. Frozen section could further categorise the case as follicular adenoma due to the absence of capsular invasion [Table/Fig-9b]. Histopathological sections clearly showed absence of capsular invasion and confirmed the diagnosis of Follicular adenoma [Table/Fig-9c].

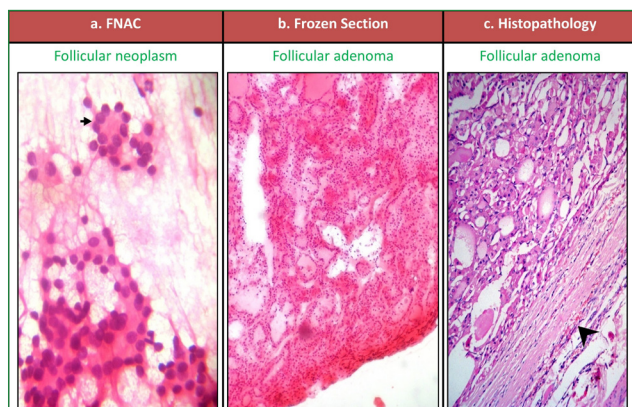
Similarly, a case of Hurthle cell carcinoma was labelled as Hurthle cell neoplasm on FNAC [Table/Fig-10a] but on frozen



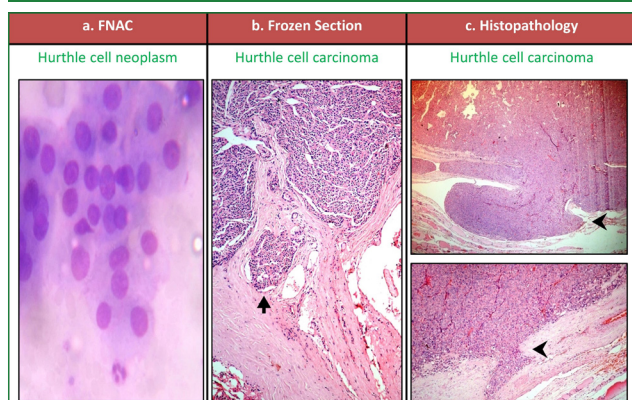
[Table/Fig-7]: Case of Papillary carcinoma reported as Nodular goitre on FNAC (a) but correctly reported as Papillary Carcinoma on frozen section (b) due to presence of papillary pattern and intranuclear cytoplasmic inclusions. Histopathological sections (c) showing papillary pattern and nuclear grooves.



[Table/Fig-8]: Case of nodular goitre reported as suspicious for papillary carcinoma on FNAC (a) due to the presence of false papillae, intranuclear cytoplasmic inclusions and multinucleated giant cells but correctly reported as nodular goitre on frozen section (b). Histopathological sections showing nodular goitre (c).



[Table/Fig-9]: Case of follicular adenoma reported as follicular neoplasm on FNAC (a) due to repetitive follicles but further classified as follicular adenoma on frozen section (b) due to the absence of capsular invasion. Histopathological sections showing follicular Adenoma (c).

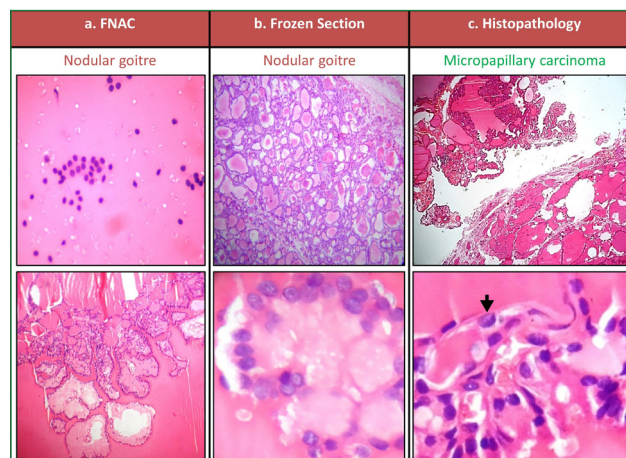


[Table/Fig-10]: Case of Hurthle cell carcinoma reported as Hurthle cell neoplasm on FNAC: a) but further classified as Hurthle cell carcinoma on frozen section: b) due to the presence of capsular invasion. Histopathological section showing Hurthle cell carcinoma (c).

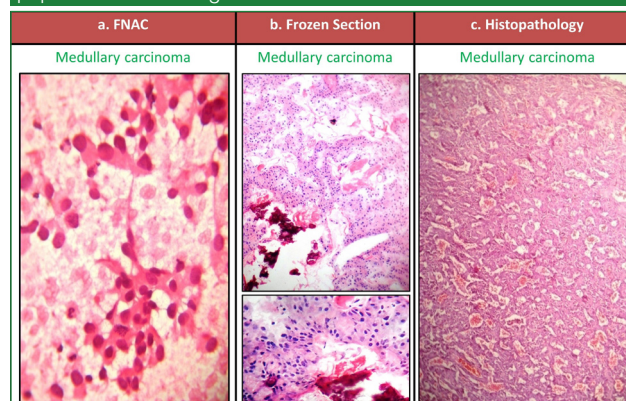
sections the capsular breach could be demonstrated and was categorised further as Hurthle cell carcinoma [Table/Fig-10b]. On histopathology, the capsular invasion was evident and the diagnosis of Hurthle cell carcinoma was confirmed [Table/Fig-10c].

One case of micropapillary carcinoma was missed both on FNAC and frozen sections. The FNAC smears from the aspirate showed plenty of colloid with few follicular cells and this case was reported as nodular goitre [Table/Fig-11a]. Even the frozen section on the tissue sampled showed only colloid filled follicles and thus was also reported as nodular goitre [Table/Fig-11b]. However, the histopathological sections showed foci of micropapillary pattern with presence of nuclear grooves and was thus reported as micropapillary carcinoma [Table/Fig 11c]. It is premised that this area was not aspirated on FNAC and this foci was not sampled on frozen section.

An interesting case of medullary carcinoma was reported correctly both on FNAC and frozen and was also confirmed on histopathology [Table/Fig-12].



[Table/Fig-11]: Case of micropapillary carcinoma reported as Nodular goitre on both FNAC (a) and frozen section (b) due to absence of nuclear features for micropapillary carcinoma. Histopathological sections proved to be micropapillary carcinoma (c) showing foci of papillae with nuclear grooves.



[Table/Fig-12]: Case of medullary carcinoma on both FNAC (a) and frozen section (b). Histopathological sections (c) of medullary carcinoma.

Follicular lesions can be misinterpreted as nodular goitre on FNAC when there is sparse cellularity and when not many repetitive follicles are seen in the smears. Hurthle cell neoplasms can be misdiagnosed as nodular goitre as these hurthle cells could be misinterpreted as hurthle cell change in nodular goitre. Micropapillary carcinoma can be missed on FNAC when the representative area is not aspirated during FNAC. Sometimes nodular goitre can be called suspicious on FNAC when the cellularity is less and when the nuclear features are deceptive. At such a time, intraoperative frozen sections can give additional information on diagnosis and help in limiting the surgery.

Follicular carcinoma may not always be diagnosed on frozen sections due to limited sections taken during intraoperative consultation. But this draw back can be overcome sometimes by gross inspection of the specimen. As believed by Rosai J et al., gross examination can best show the capsular invasion. Thicker and more irregular capsule is seen in carcinoma than adenoma [12]. The possible cause for misdiagnosis of follicular variant of papillary carcinoma could be the follicular pattern

of arrangement of follicular cells and the failure to appreciate nuclear characteristics on frozen sections. As the area involved in micropapillary carcinoma is very small, these cases can be missed on frozen sections if the representative bits are not taken. As told by Anton RC et al., frozen sections can give pertinent information by gross examination. Though papillary carcinoma can be solid to cystic to infiltrating, the presence of sclerosis or calcification or cystic change with recognisable papillary structures can help in correctly diagnosing papillary carcinoma [13].

The sensitivity, specificity, diagnostic accuracy, PPV and NPV of FNAC in our study was 70.37%, 81.39%, 77.14%, 70.37% and 81.39% respectively. The values obtained from other studies are shown in [Table/Fig-5]. The sensitivity, specificity, diagnostic accuracy, PPV and NPV of frozen section in our study was 85.18%, 90.69%, 88.57%, 85.18% and 90.69% respectively. The values obtained from other studies are shown in [Table/Fig-6]. There is significant higher diagnostic accuracy in frozen sections compared to FNAC in thyroid lesions.

Frozen sections compliment FNAC and helps the surgeon in decision making during surgery. Nodular goitre being the most common lesion, limited operations such as lobectomy and isthmectomy are usually performed by using frozen sections as the final guide for the extent of thyroidectomy. If malignancy is identified on frozen sections, then extended surgery is performed. Frozen sections are of value in confirming the cytological diagnosis. Unnecessary extensive surgeries and second operations can be considerably reduced with frozen sections as an intraoperative guide [6].

Both FNAC and frozen sections in thyroid lesions are not without limitations as the opinions are subjective due to varied thyroid pathologies.

FNAC diagnosis may be hindered due to the nature of the procedure, technical error and inadequate slide preparations. FNAC has a major drawback as it cannot differentiate between follicular adenoma and follicular carcinoma. Detecting micropapillary carcinoma due to occult lesion is also a major challenge. Nodular goitre with extensive papillary features also imposes major difficulty in the cytological diagnosis [14-16].

Frozen section too has a handful of limitations. Increased cost, prolonged time, sampling error especially in small lesions, tissue artefact and wasting of specimen which would be necessary for final histopathology are the common disadvantages of intraoperative frozen section. As the FNA and frozen section are not necessarily interpreted by the same Pathologist, it is prone to subjective error. On the contrary, the frozen section interpreter is not always blind to the FNA diagnosis [14-16].

The study has its limitations as well. As this was a retrospective study, clinical details were collated from the hospital archives. No personal evaluations of the patients could be performed. Technical errors if any could not be validated. The frozen section interpreter was not necessarily blind to FNAC diagnosis.

Accurate diagnosis of thyroid lesions using either FNAC or

frozen section is important in planning clinical management and delivery of appropriate one stage surgical management. FNAC can avoid unnecessary surgery on benign nodules and thyroiditis. On the other hand, frozen section can be opted in neoplasms and suspicious cases. It can confirm FNAC diagnosis and identify malignancies in indeterminate or unsatisfactory FNAC smears. Intraoperative frozen section plays a vital role in deciding between total thyroidectomy and lobectomy. A standardised language in frozen section can help the surgeon in decision making. Frozen section compliments FNAC and enables one step surgery. Systematic implementation of frozen section is economically justified [14,17-22].

CONCLUSION

In our study, frozen sections had better sensitivity, specificity and diagnostic accuracy than FNAC. Combining FNAC and frozen sections helps in intraoperative decision making. FNAC can be used to select patient for surgery and intraoperative frozen sections can be used to augment this diagnostic modality for planning surgical management.

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AUTHOR(S):

1. Dr. Vidyashree Vittal Anchan
2. Dr. Parinitha Sadashivappa Sangam
3. Dr. Dinesh Udipi Shastri
4. Dr. Vandana Udaykumar Grampurohit
5. Dr. Hephzibah S Rani

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Pathology, East Point College of Medical Sciences and Research Centre, Bengaluru, Karnataka, India.
2. Associate Professor, Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India.
3. Professor, Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India.

4. Professor, Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India.
5. Associate Professor, Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Parinitha Sadashivappa Sangam,
Associate Professor, Department of Pathology,
SDM College of Medical Sciences and Hospital,
Sattur, Dharwad-580009, Karnataka, India.
E-mail: drparinithabhat2@gmail.com

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