

ACCURACY AND DIAGNOSTIC UTILITY OF FROZEN SECTION IN THYROID LESIONS: A COMPARATIVE STUDY

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ABSTRACT

Introduction: Many surgeons perform intra operative frozen section to overcome the limitations of FNAC, to differentiate benign lesions from malignant lesions and to determine the extent of surgery. However, the use and cost effectiveness of FS is controversial in follicular lesions. Nevertheless FS is most useful in cases where FNA results are indeterminate or suspicious and it is a cost effective way to avoid second surgical procedure if a total thyroidectomy is indicated. **Aim:** To study the diagnostic utility of frozen section and fine needle aspiration cytology thyroid lesions utilizing final histopathology diagnosis as gold standard. **Methods:** The present study was conducted on 237 cases of thyroid tissue submitted for frozen section for a study period of 5 years. Among those, 95 cases had pre-operative FNAC done. **Results:** The sensitivity, specificity and diagnostic accuracy of FNAC was 42.86%, 96.72% and 82.93%, whereas that of FS was comparatively superior (82%, 100% and 95.65% respectively). **Conclusion:** Thus, the overall sensitivity, specificity and diagnostic accuracy of FS was superior to that of FNAC in the present series. The main limitations of FS and FNAC in our study were- Capsular / vascular invasion in follicular neoplasm was not detected in many cases and papillary microcarcinomas were missed.

Key words: FNAC, Frozen section, Thyroid lesions.

INTRODUCTION

The thyroid gland is affected by many pathological lesions which is manifested either by diffuse pattern of involvement or which produce nodules. It has been estimated that 4-10% of the population will have a clinically evident thyroid nodule for which a large number of patients seek medical attention; the incidence of malignancy in such solitary thyroid nodules varies from 5% to 30% [1,2].

The traditional approach for the evaluation of patients with thyroid pathology includes detailed clinical history, physical examination, thyroid function tests, ultrasonography and computed tomography [3,4]. But none of these tests conclusively distinguish benign from malignant lesions, and in malignant lesions when to operate or to what extent.

In the past five or six decades, Fine needle aspiration cytology (FNAC) has been increasingly utilized in the investigation of the thyroid nodules. The main purpose

of the test is to distinguish nodules with malignant cytology which require surgery from those with benign cytology. Preoperative FNAC is considered safe, accurate and well tolerated [5, 6]). The use of FNA has reduced the number of surgeries done for benign lesions [7]. There is also increase in the yield of malignancies found in surgically excised specimens.

The main limitations of thyroid FNA include inability to distinguish follicular adenoma from carcinoma [8]. To overcome the limitation of FNA, to differentiate benign lesions from malignant lesions and to determine the extent of surgery many surgeons perform intra operative frozen section [9].

As far as frozen section (FS) in thyroid is concerned it can reliably diagnose papillary, medullary and anaplastic thyroid carcinoma [10]. Its utility in follicular neoplasm and cost effectiveness is most debatable [11].

With the increasing use and acceptance of FNAC, the use of FS has become controversial. Nevertheless FS is most useful in cases where FNA results are indeterminate or suspicious [12,13] and it is a cost effective way to avoid second surgical procedure if a total thyroidectomy is indicated [14].

MATERIALS AND METHODS

Study design: Observational study



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Ethics approval: the study was approved by hospital review board.

Study location: Apollo hospital, Hyderabad

Study duration: Period of five years 2012-2016

Inclusion criteria: All the thyroid cases received for frozen section

Sample size: The present study was conducted on 237 cases of thyroid tissue submitted during thyroid surgery for frozen section evaluation. Among these, 95 cases had preoperative FNAC done.

FNAC: Conventional smears, both air dried and fixed are prepared from each FNAC aspirate and stained with Giemsa and Pap stain respectively.

Methodology:

FS: Minimum two representative samples are taken in capsulated nodules, with the section representing the tumor, capsule and the adjacent non-neoplastic tissue. Two or three frozen sections (5 micron thick) are placed on a glass slide and stained with rapid H&E.

PHE (Permanent histopathological examination): Once the frozen section diagnosis is done, the specimen is fixed in 10% neutral buffered formalin overnight. Multiple sections are then taken from the representative area.

Study definitions and study analysis: The results of frozen section were analyzed and compared with final histopathological diagnosis and also with preoperative FNAC results wherever applicable. The results are categorised as follows.

- a. **Categorization of cytological findings:** Inadequate for evaluation, Benign, Suspicious for malignancy and Malignant
- b. **Categorization of frozen section results:** Benign, Deferred and Malignant

- c. **Categorization of final HPE results:** Benign and Malignant

Statistical analysis: Sensitivity, specificity and diagnostic accuracy was assessed for FNAC and frozen section. 'p' value was calculated using Chi-Square test. Statistical significance was defined as p <0.05 and finally the sensitivity, specificity, diagnostic accuracy of FS was compared with that of FNAC. The added benefit of FS over FNAC, its diagnostic utility and pitfalls were analyzed.

RESULTS

The most common lesion on FNAC was Suspicious of follicular neoplasm (Bethesda category IV) [previously reported as hyperplastic nodule / Follicular Neoplasm]. The most common lesion on permanent HPE was nodular goiter.

Table 1. Correlation of frozen section with final HPE diagnosis (n=237)

Frozen (No. of cases, Percentage)	Final HPE		
	Category	No. of cases	Percentage
Benign (166, 70.04%)	Benign	157	66.24%
	Malignant	9	3.80%
Deferred (30, 12.66%)	Benign	9	3.80%
	Malignant	21	8.86%
Malignant (41, 17.30%)	Benign	0	0
	Malignant	41	17.30%

Table 2. Correlation of FNAC and frozen section diagnosis with final HPE diagnosis (n=95)

FNAC	FROZEN		HPE	
	Category	No. of cases	No. of Benign Cases	No. of Malignant cases
Benign (71, 74.74%)	Benign	57(60%)	54(56.84%)	3(3.16%)
	Deferred	10(10.53%)	5(5.26%)	5(5.26%)
	Malignant	4(4.21%)	0(0%)	4(4.21%)
Inadequate for evaluation (13, 13.68%)	Benign	9(9.47%)	9(9.47%)	0(0%)
	Deferred	1(1.05%)	0(0%)	1(1.05%)
	Malignant	3(3.16%)	0(0%)	3(3.16%)
Suspicious for malignancy (9, 9.47%)	Benign	2(2.1%)	1(1.05%)	1(1.05%)
	Deferred	3(3.16%)	1(1.05%)	2(2.1%)
	Malignant	4(4.21%)	0(0%)	4(4.21%)
Malignant (2, 2.11%)	Malignant	2(2.11%)	0(0%)	2(2.11%)

Table 3. Comparison of FS vs FNAC (with inclusion of suspicious cases)

	FNAC with inclusion of suspicious cases	Frozen
Sensitivity	42.86%	82%
Specificity	96.72%	100%
Diagnostic Accuracy	82.93%	95.65%

Table 4. Pitfalls in frozen section are:

Detection of capsular /Vascular invasion in Follicular neoplasms.
Detection of papillary microcarcinoma.
Diagnosis of Follicular Variant of papillary thyroid carcinoma.
Papillary hyperplasia mistaken for papillary carcinoma

Table 5. Pitfalls in FNAC are:

Inadequate sampling
Follicular neoplasms - to differentiate between hyperplastic nodule & follicular adenoma
To differentiate between follicular adenoma & carcinoma
Cystic lesions harbouring malignancy
Papillary hyperplasia mistaken for papillary carcinoma

DISCUSSION

On FS, majority of cases were in benign category (166, 70.04%). 30 cases were deferred (12.66%) to permanent sections and 4 were in malignant category (17.30%). Of the 9 malignant cases, which were diagnosed as benign and turned out to be malignant on frozen section, three of them were papillary microcarcinoma measuring less than 1 cm occurring in a predominant lesion of multi nodular goitre in 2 cases and follicular adenoma in 1 case. These cases were missed on FS as only 2 sections were studied during FS. The other 6 were diagnosed as follicular carcinoma on final HPE but reported as benign (follicular neoplasm/dominant nodule) on FS as there was no capsular or vascular invasion evident on the frozen sections studied.

The dilemma regarding the presence or absence of capsular/vascular invasion is well documented in literature [1,2]. Unless the entire capsule is evaluated on permanent section, the possibility of malignancy cannot be completely ruled out. The assessment of capsular and vascular invasion is limited by sampling error, thickness and irregularity of capsule, vessel distortion and collapse [1,2]. In few cases it is difficult to differentiate

entrapment of cells in thyroid capsule from capsular invasion on FS.

Out of 30 cases (12.66%) deferred, 21 were malignant on final HPE. Among the benign lesions, follicular adenoma and multi nodular goitres constituted the majority. Two cases suspicious of papillary carcinoma on FS turned out to be colloid goiters with papillary hyperplasias. The papillary foci seen on FS were suspicious of papillary carcinoma however the nuclear features were not evident. The nuclear features pathognomic of papillary carcinoma are caused due to formalin fixation and are difficult to appreciate on frozen section. This is one of the pitfalls in FS [15].

Carcinomas less than 1 cm, called as micro carcinomas, have been widely implicated as a cause of missed carcinoma on FS and FNAC [16,17]. In the present study, 9 incidental papillary micro carcinomas were detected on final HPE section, 5 were missed on FS. This may be attributed to sampling error, a limitation which is shared by both FNAC and FS. Both of these techniques sample only a small part of a lesion and thus miss small foci of carcinoma, leading to false negative diagnosis. These small carcinomas would be picked up only on permanent paraffin sections after extensive sampling of suspicious areas grossly [18].

Out of the 41 cases (17.30%) diagnosed as malignant on FS, all of 41(17.30%) turned out be malignant on permanent section with no false positive results. Among these, 28 cases were papillary carcinomas, 3 being papillary micro carcinomas correctly detected on FS.

Two are follicular variants of papillary carcinoma, diagnosed as follicular carcinoma on FS – another pitfall in diagnosis of follicular variant of papillary carcinoma in FS, as it is based on identification of typical nuclear features of papillary carcinoma including ground glass nuclei, nuclear grooves, and nuclear pseudo inclusions which is not clearly evident on FS. FS evaluation has poor detection rate of diagnosing follicular variant of papillary carcinoma due to loss of nuclear details caused by freezing of the tissue [1]. The diagnostic limitation of detection of follicular variant of papillary carcinoma has been well documented in the literature.

In the present study, FS was able to accurately diagnose malignancies with no false positives. The histological subtypes in our study also correlated well with the final HPE subtype except for follicular variant of papillary carcinoma. Shaha A et al have observed that one of the major discrepancies between FS and permanent HPE in patients with malignancy is determination of cell type, also supported by Charu Batra et al [1].

71 cases (74.74%) were diagnosed as benign lesions on FNAC, 12 turned out to be malignant on final HPE. Among 57 benign cases on FS, 54 were confirmed as

benign on permanent HPE. Other 3 turned out be follicular carcinoma, where there was no capsular or vascular invasion evident on FS. This malignancy was missed on FNAC and FS. This limitation is shared by both FS and FNAC.

The ability of FNAC to differentiate between benign and malignant lesion in follicular neoplasm of thyroid reduces the clinical utility of this technique [7]. This is because capsular or vascular invasion which is the criteria for follicular carcinoma cannot be made on cytology smears. Approximately 20% of all follicular tumours ultimately prove to be malignant [16]. In the present study, about 12% of cases diagnosed as follicular neoplasm turned out to be malignant which is lower comparatively. Because of this risk, many surgeons recommend total thyroidectomy in this group of patients [17].

One case was follicular adenoma with incidental papillary micro carcinoma. One of the reason for low sensitivity rate of FNAC may be due to incidental papillary micro carcinomas as pointed out by many authors [17,19] either due to inadequate sampling , or error in interpretation [2,17]. One case was diagnosed as colloid goitre with papillary hyperplasia on FNAC as the nuclear features typical of papillary carcinoma were lacking in this particular case. This case later turned out to be papillary carcinoma on FS and permanent sections.

Thus contrary to many studies which suggested that FS can be omitted when FNAC suggests a benign diagnosis [20] the present study detected 4 malignancies on FS, for which a benign diagnosis was made on FNAC and thus FS was proved to be beneficial. Fatma Althoubaity suggested that routine FS should be done even in benign aspirates for a better informed diagnosis which is useful in extent of thyroid resection⁽²⁾.

False negative rates of FNAC in various studies range from 3.3 to 16.1% [21] In the present study it is 12.63%. Therefore negative FNA and FS results must be viewed with caution in view of these false negative being noted. 13 cases (13.68%) were labelled as inadequate for evaluation, either due to not satisfying the minimum criteria of six clusters containing 10 follicular cells or cyst fluid only or blood only. Among these, 4 cases were confirmed as malignant on final HPE.

One case which was sparsely cellular on FNAC and was deferred for permanent section for suspicious of capsular invasion turned out to be invasive follicular carcinoma on final HPE sections. Three cases, two of which were sparsely cellular and other which contained only cyst fluid and colloid turned out to be papillary carcinoma on FS and was confirmed as the same on permanent sections.

The reduced sensitivity of FNAC in majority of papillary carcinomas presenting as cystic masses, and thus missed on FNAC is well documented in literature [1]. This also forms the basis for explanation of non-diagnostic or unsatisfactory category of Bethesda system for reporting thyroid cytopathology [22] that when only cyst macrophages are seen on the smears, the parenchyma of the nodules has been not sampled and one cannot exclude a cystic papillary carcinoma. The incidence of such nodules varies from 15% to 30% and the malignancy risk in such nodules is 4%⁽²²⁾

Thus to conclude, 13 cases which were inadequate to evaluate on FNAC, FS was able to identify 3 malignancies. Many authors suggest the use of FS in inadequate aspirates and as a guide to thyroidectomy [1,2]. 9 cases (9.47%) were suspicious for malignancy on FNAC (all of which were suspicious for papillary carcinoma). Among these 9 cases of FNAC, 7 cases were confirmed as malignant on final HPE.

Role of FS in cases of FNA suspicious of papillary carcinoma is subjected to various controversies. Franco Luchini et al [11] and Li Volsi VA et al [23] recommended FS as most useful for confirmation when FNAC is suspicious of papillary carcinoma. However study by Jerry C. Roach et al concludes where FNAC is highly suggestive of papillary carcinoma, FS adds nothing to the management and should not be performed [14].

9 cases which were suspicious for malignancy, FS was able to diagnose four cases of malignancy. Malignancy rate in suspicious category is 16-54 % [24] and according to guidelines of the Papanicolaou society of cytology for practice of thyroid FNAC, this should be around 15% [4]. In the present series, the malignancy rate in this category is 7.3% which is lower than reported. Out of the 2 cases (2.11%) diagnosed as malignant on FNAC, both were malignant on FS and on subsequent permanent HPE as well. One case was papillary carcinoma thyroid, confirmed as the same; while other was medullary carcinoma on FNAC, confirmed as papillary carcinoma on FS and final HPE. The accuracy of FNAC in detecting cancer is well documented in literature. Boyd LA et al [25] suggested that FS adds very little to intraoperative decision making in patients diagnosed as cancer by FNAC. However, Chow et al concluded FS is indicated if preoperative FNAC suggested malignancy in order to determine the extent of operation [26] and to avoid surgical over treatment.

For statistical evaluation of FNAC, 'inadequate for evaluation' category was excluded.

The sensitivity was 42.86%, specificity was 96.72% and diagnostic accuracy was 82.93%. In literature the sensitivity of FNAC varies from 13-97 [1,2]. The specificity of FNAC in detecting malignancy varies from 60-100⁽¹¹⁾ and the diagnostic accuracy of FNAC in literature varies

from 65-96 [18]. The sensitivity, specificity and accuracy of FNAC are in the reported range in the literature and are comparable to other studies previously done.

For statistical evaluation of frozen section, deferred category was excluded. The sensitivity, specificity and diagnostic accuracy was 82%, 100% and 95.65% respectively. P-value was significant (0.000001) which was calculated using Chi-square test. The sensitivity of FS in the literature varies from 50-100% [1-3]. The specificity of FS in the literature varied from 92-100% [2] and the diagnostic accuracy of FS varies from 87-97 [3,18]. The sensitivity, specificity and accuracy of FS of the present study were in the reported range in literature.

The overall sensitivity, specificity and diagnostic accuracy of FS is superior to that of FNAC in the present series. Similar conclusion was made in studies done by Charu Batra et al [1], Fatma Althoubaity [2]. However, study by Boyd L.A et al showed no significant difference in sensitivity, specificity or accuracy between FS and FNA [25].

The variation in sensitivities and specificities in the literature reflect how the authors choose to define, classify and enter the suspicious results into the data base [15,21]. Few authors included deferred category of FS in benign group, and suspicious category in benign group in FNAC [25]. In the present series, for statistical evaluation, deferred category was excluded from FS. Statistical evaluation for FNAC was done both by inclusion and exclusion of suspicious cases into malignant category separately as done by Charu Batra et al [1].

FNAC is primarily a screening test; hence a high sensitivity for malignancy is required at the cost of lowered specificity. In contrast, FS aims at high specificity at the loss of some sensitivity. Therefore the two procedures are regarded as complementary with a better yield of accuracy [27].

CONCLUSIONS

The overall sensitivity, specificity and diagnostic accuracy of FS was superior to that of FNAC in the present series. Our study detected 4 malignancies on FS, for which a benign diagnosis was made on FNAC, thus emphasizing the role of FS even in benign aspirates. FS plays an important role in operative management on lesions which are inadequate on FNAC as our study was able to identify 3 malignancies in this category. And FS was able to confirm 4 cases of malignancy out of 9, which were suspicious for malignancy on cytology emphasizing the importance of FS in these cases as well.

Limitations: The main limitations of FS and FNAC in our study were- Capsular / vascular invasion in follicular neoplasm was not detected in many cases and papillary microcarcinomas were missed.

Conflict of interest: Nil

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