Original Article

Diagnostic Accuracy of Frozen Section in Comparison With Fine Needle Aspiration Cytology in Thyroid Lesions – A Prospective Study

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ABSTRACT

Background and Objectives: Thyroid gland because of its superficial location is amenable to direct physical examination. Clinical assessment of thyroid lesions by means of physical examination, thyroid scans and ultrasonography is not completely reliable. Fine needle aspiration cytology (FNAC) is the most cost-effective, safe and an initial diagnostic test in the pre-operative evaluation of thyroid lesions. Many surgeons use intraoperative frozen section biopsies to confirm the diagnosis of FNAC and guide the extent of thyroidectomy. Therefore, the objective of the present study was to assess the diagnostic accuracy of frozen section in comparison with fine needle aspiration cytology and histopathology in thyroid lesions.

Materials & Methods: Fine needle aspiration cytology and frozen section (FS) were evaluated in 59 patients. FNAC was classified as benign, malignant and suspicious, and FS was classified as benign, deferred and malignant. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for both FS and FNAC.

Results: Out of 59 patients, 3 were positive for malignancy, 51 were benign, and five were suspicious on FNAC. Over all accuracy of fine needle aspiration cytology was 93%. Five were positive for malignancy, 50 were benign and 4 were "deferred" in frozen section. Over all accuracy of frozen section was 96%.

Discussion: Fine needle aspiration cytology can be used primarily to select patient for surgery and frozen section can be employed to plan the extent of surgery. Frozen section had a good accuracy, sensitivity and negative predictive value compared to fine needle aspiration cytology.

Keywords: Fine Needle Aspiration, Cytology, Frozen Section, Thyroid Nodule

Introduction

Thyroid gland is unique among endocrine glands in many ways. It is the largest of all the endocrine glands in the body, and because of its superficial location, is amenable to direct physical examination, fine needle aspiration and surgical biopsy (1).

Clinical assessment of thyroid lesions by means of physical examination, thyroid scans and ultrasonography is not completely reliable. Fine needle aspiration cytology (FNAC) is the most cost-effective, safe and an initial diagnostic test in the pre-operative evaluation of thyroid lesions. An adequate thyroid aspirate is necessary for the interpretation of FNAC (2). The diagnostic dilemma of the cytologist occurs in lesions of thyroid which are diagnosed as atypical or suspicious of malignancy in 15-30% cases (3). Many surgeons use intraoperative frozen section biopsies to confirm the diagnosis of FNA cytology and guide the extent of thyroidectomy (4). However, there are few comprehensive studies in India regarding comparison of frozen section diagnosis with FNAC and histopathological diagnosis.

Therefore, the aim of the present study was to assess the diagnostic accuracy of frozen section in comparison with fine needle aspiration cytology and histopathology in thyroid lesions and also to emphasize the significance of frozen section requirement in thyroid lesions.

Materials and Methods

All cases clinically presenting with thyroid lesions were referred to the Department of Pathology, BLDE University, Bijou, India for FNAC. Frozen section and histopathological evaluation were taken as subjects for this prospective study from Aug 2009 to July 2011.

Overall prevalence rate of thyroid lesions in India is 36% (5). The calculated sample size works out to be 58 with allowable error of 35%, using the formulae $n=4pq/L^2$. All the cases of thyroid lesions admitted in to our hospital from Aug 2009 to July 2011 were included in the study, to arrive at the expected sample size.

Statistical Package for the Social Sciences (SPSS) 14 software was used to analyze the data. Frequencies and percentages were used to describe the data.

An inclusion criterion was FNAC, frozen section and histopathological examination performed as diagnostic procedures in the thyroid lesions. Swellings in front of the neck other than thyroid lesions were excluded for the study.

Detailed history and clinical examination was done as per the proforma, Informed consent and institutional ethical clearance were undertaken. Preoperative FNAC, intraoperative frozen section and histopathological examination was performed. Criteria for adequacy of fine needle aspiration material was six clusters of thyroid follicular cells in at least two slides prepared from two separate aspirates or 5-6 groups of follicular cells with more than 10cells/group and abundant colloid.

Categorization of patients based on FNAC diagnosis and frozen section diagnosis was done and histopathological sections were used to assess the accuracy of FNAC and frozen section. Patients were classified in to benign, suspicious and malignant on the basis of their FNAC report. Suspicious cases included follicular neoplasm, suspicious for papillary formation. Frozen section reports were also classified into benign, deferred and malignant.

Results

All 59 patients with clinically palpable thyroid nodule first undergoing FNAC and then frozen section were included in the study and were later compared with final histopathological

examination. Of 59 patients, 50 patients were female and 9 were male. The youngest patient was 18 years (female) and the oldest patient was 68 years (female). The highest incidence of a solitary nodule in our series was observed in the 4th decade of life.

Among the 59 patients histological examination revealed benign lesions in 52 cases (88%), the commonest being the multinodular goitre with 20 cases (33.8%), followed by 18 cases (30%) of nodular (colloid) goitre, 8 cases (13.5%) of Hashimotos Thyroiditis and one case of hyperplastic goitre. Follicular adenoma was diagnosed in 5 cases (8.47%). Malignancy was found in 07 cases (11%). Papillary carcinoma was the commonest malignancy accounting for 6 cases (10.16%) and there was a single case of follicular carcinoma.

Fine Needle Aspiration Cytology Analysis:

A definitive cytological diagnosis was obtained in 54 cases (91%). Fifty-one of these were benign, three were malignant. The remaining five cases were suspicious on FNAC. Following histological assessment of suspicious cases, one of the 5 cases proved to be malignant (minimally invasive follicular carcinoma) and the other 4 were benign (Table 1).

Table 1- Comparison of fine needle aspiration cytology with final histological diagnosis with statistical evaluation

FNAC	Total No. of Cases	Histological Diagnosis	
		Malignant	Benign
Malignant	03	03	00
Suspicious	05	01	04
Benign	51	03	48
Total	59	07	52

Foot Note:

Sensitivity of fine needle aspiration cytology in

diagnosing malignancy - 42%
Specificity of fine needle aspiration cytology in diagnosing malignancy- 100%
Positive predictive value – 100%
Negative predictive value – 92%
Accuracy – 93%
P- value- Not significant (*P* >0.05)

Frozen Section Analysis

Frozen section evaluation suggested that 50 lesions were benign and this was confirmed histologically in 49 cases (98%). While one lesion (2%) was subsequently recognized as follicular carcinoma and this patient underwent second operation. In four cases (6.7%) the intra-operative diagnosis was deferred to definitive evaluation (Table 2).

Table 2- Comparison of frozen section with final histological diagnosis with statistical evaluation

Frozen section	Total No. of Cases	Histological Diagnosis	
		Malignant	Benign
Malignant	05	05	00
Deferred	04	01	03
Benign	50	01	49
Total	59	07	52

Foot Note:

Sensitivity of frozen section in diagnosing malignancy - 71%
Specificity of frozen section in diagnosing malignancy - 100%
Positive predictive value-100%
Negative predictive value- 96%
Accuracy- 96%
P- value- Not significant (*P* >0.05).

Table 3 shows the correlation of FNAC, frozen section and final histopathological diagnosis. Three patients diagnosed as malignant on FNAC, all turned out be malignant of frozen section and as well as on final histopathology.

Table 3- Results of fine needle aspiration cytology and frozen section
in 59 cases and their correlation with the final histopathological diagnosis

FNAC* No. of patients with diagnosis	Frozen section No. of patients with diagnosis	Final histopathological diagnosis No. of patients
3 Malignant	3 Malignant	3 Malignant (Papillary Carcinomas)
51 Benign (Figure 1)	a) 46 Benign	a) 46 Benign
	b) 3 Deferred (Figure 2)	b) 2 Benign and 1 Malignant (Papillary carcinoma – Figure 3),
	c) 2 Malignant	c) 2 Malignant (1 – Follicular variant of Papillary carcinoma and 1- Papillary carcinoma)
5 Suspicious	a) 4 Benign	a) 3 Benign and 1 Malignant
	b) 1 Deferred	(Minimally invasive follicular carcinoma) b) 1 Benign

* Fine Needle Aspiration Cytology

Fifty one cases diagnosed as benign on FNAC were subjected to frozen section and reported as 46 benign, 2 malignant and 3 were deferred on frozen section to histopathology for further evaluation. On histopathology, all 46 cases diagnosed as benign on frozen section turned out to be benign, 2 malignant cases turned to be malignant (PCT), out of 3 deferred cases 1 turned to be malignant (PCT) on histopathology and others benign.
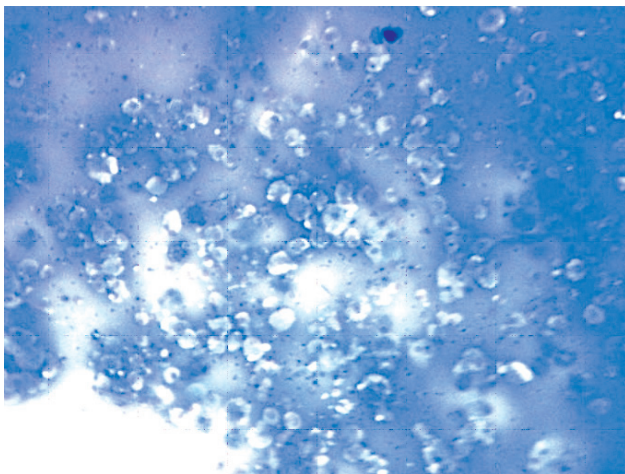
Out of Fifty cases which were diagnosed as benign on FNAC, three turned out to be malignant on histopathology. Of which one case which was benign on FNAC was deferred on frozen section (because of papillary fronds) was turned out to be papillary carcinoma on histopathology (Fig. 1-3). Other two cases diagnosed as benign on FNAC were diagnosed as malignant on frozen section, and were later confirmed by histopathology.
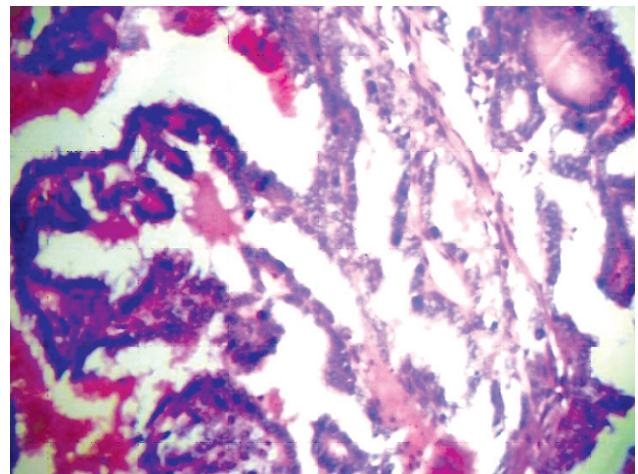

Fig. 1: Photomicrograph showing cystic macrophages (Benign on Cytology – Giemsa stain ×200)

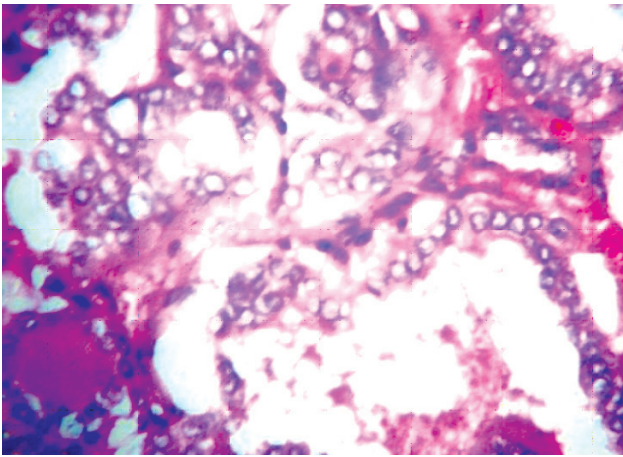**Fig. 2:** Photomicrograph showing papillary fronds (Deferred on Frozen section – Rapid H & E stain ×200)

Fig. 3: Photomicrograph showing nuclear features of papillary carcinoma of thyroid (On Histopathology – H & E ×400)

Five cases which were suspicious on FNAC were subjected to frozen section, where it was diagnosed as benign in 4 cases and one was deferred to histopathology. On histopathology, a case diagnosed as benign on frozen section was confirmed as minimally invasive follicular carcinoma. One case deferred on frozen section to histopathology was reported as benign lesion on histopathology.

Statistical Evaluation

1. For statistical evaluation of FNAC, the classification of "suspicious" was included in the benign group, because of the effect on the surgical decision.

2. In FNAC there were no false positive cases, 3 true positive cases, 4 false negative cases and 52 true negative cases.

3. For statistical evaluation of frozen section, the deferred samples were classified in the benign group.

4. In FS, there were no false positive cases, 5 true positive cases, 2 false negative cases and 52 true negative ones.

5. Sensitivity was 42% for FNAC and 71% for FS, specificity 100% for FNAC and 100% for FS, positive predictive value 100% for FNAC and 100% for FS and negative predictive value 92% for FNAC and 96% for FS.

6. Accuracy was 93% for FNAC and 96% for FS.

7. Accuracy was higher for FS than for FNAC in surgical management of the thyroid lesions.

Discussion

Thyroid nodules are common clinical lesions and most are ultimately determined to be benign, but approximately 5% are malignant (1). Recent literature suggests that each patient with a thyroid nodule should undergo a complete evaluation that includes a detailed history and physical examination (6).

A variety of tests have been employed to separate benign from malignant thyroid nodules. These tests include isotope scanning and FNA. Combined use of isotope scanning, fine needle aspiration cytology, and histopathology of thyroid offers the best diagnostic strategy (7).

The estimated prevalence of palpable thyroid nodules (TN) is 5%, but using neck ultrasonography one half of population may have a thyroid nodule (8).

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 the sample is the same material. In the present study, a total of 50 patients (84%) were female. There is a significant female preponderance in our series, which compares favorable with study done by Srinivas *et al.* (9) which shows 71% female preponderance.

The youngest patient was 18 years (female) and the oldest patient was 68 years (female). The highest incidence of a solitary nodule in our series was observed in the 4th decade of life i.e. in 17 patients, which compares favorable with study done by Srinivas *et al.* (9) in 25 patients.

Benign lesions were found in 52 cases (88%), which can be compared (77%) with study done by Srinivas *et al*. (9), the commonest in our series being the multinodular goitre with 20 cases (33.8%). Malignancy was found in 7 (11%) of the cases. Papillary carcinoma was the commonest malignancy accounting for 6 cases. The incidence of malignancy in our series is comparable to the incidence of malignancy in literature that is 11-23% (9).

According to Batra *et al*. (10) sensitivity, specificity and accuracy of frozen section was reported to be 81.8%, 100% and 95.4% respectively as compared to 60%, 100%, and 89.4% of that of FNAC.

Anton *et al*. (11) suggests, gross examination of the thyroid gland at frozen section contributes more pertinent information than the microscopic evaluation. For instance, although papillary carcinomas can demonstrate protean features, ranging from cystic to solid to large infiltrating masses, the presence of a sclerotic, sometimes calcified nodule or a cystic nodule with recognizable papillary structures may be seen and is very helpful in arriving at the correct diagnosis. In the present study during frozen section gross features were helpful in arriving diagnosis. Almeida *et al*. (12) states that the frozen section is only indicated when the FNA reports follicular neoplasm.

Rosai *et al*. (13) believe that the presence of capsular invasion is best assessed by gross examination. In addition, carcinomas often will demonstrate a thicker, more irregular capsule than adenomas. In the present study, there was a case in which we could not demonstrate a thicker and an irregular capsule on gross but on histopathology it turned out to be a minimally invasive follicular carcinoma.

The ability to differentiate malignant nodules from benign nodules of the thyroid was the main problem in the field of thyroid surgery. The most important limitation of FNAB was its ability to diagnose the folicullar lesions. Besides that, its dependency on the experience of the physician who performed this method, and the clinical interpretation of the results could influence the diagnosis (14).

Determinations of sensitivity and diagnostic accuracy are affected by how the author of a given study chooses to define and classify suspicious FNA results (15). The inclusion of suspicious FNA diagnoses with clearly malignant FNA cases tends to increase the sensitivity of FNA for detecting thyroid cancer, while decreasing the specificity and overall accuracy of the test.

In the present study, for statistical evaluation of FNAC, the classification of "suspicious" was included in the benign group, because of the effect on the surgical decision. If suspicious group was included in malignant group then sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy would have been 57%, 92%, 50%, 94% and 88% respectively.

And in frozen section, the deferred samples were classified in the benign group. If deferred group was included in malignant group then sensitivity, specificity, PPV, NPV and accuracy would have been 85%, 94%, 66%, 98% and 93% respectively.

In the present study, sensitivity in diagnosing malignant lesions was less compared to other studies done because we included the suspicious/ indeterminate group in the benign category for calculation purpose. Other parameters were comparable with other studies. Sensitivity, Specificity and diagnostic accuracy in diagnosing malignant lesions by frozen section was 71%, 100% and 96% respectively which can be compared with the studies already done (9, 10) (Table 4).

Table 4- Comparison of sensitivity, specificity and accuracy
of fine needle aspiration cytology and frozen section with medical literature

Biopsy technique (References)	Sensitivity (%)	Specificity (%)	Accuracy (%)
FNAC (present study)	42	100	93
(9)	66	95	89
(10)	60	100	89.4
(2)	88	89	91
Frozen section (present study)	71	100	96
(9)	86	100	96
(10)	81.8	100	95.4
(2)	93	100	97

In our study frozen section is considered to be more sensitive, specific and accurate in diagnosis of the thyroid lesions. FNAC is good for selecting patients who need surgery but relatively low accuracy do not permit adequate planning of the extent of surgery. Frozen section had high specificity (100%) and led to a reduction in the number of late re-operation. Had the surgical decision been based on FNAC only, 4 patients would have required second operation.

The specificity of FS was 100%, as there were no false positives. Only in 4 cases FS was deferred, out of which one was Papillary carcinoma and the other were benign lesions on final histological diagnosis.

The evaluation of nuclear features by frozen section is limited (11). So, it becomes necessary for those cases in which the possibility of papillary carcinoma exists there intraoperative touch imprints or scrape preparations be performed to discern and document the characteristic nuclear features. In the present study, we did intraoperative imprint smears and could demonstrate nuclear features of papillary carcinoma and all turned out to be papillary carcinomas on histopathological evaluation. In addition, a case of follicular variant of papillary carcinoma was diagnosed intraoperatively with the aid of imprint smear.

There was a case reported benign in frozen section with the final histological diagnosis of minimal invasive follicular carcinoma. The assessment of capsular and vascular invasion by frozen section examination is seldom helpful (16). It is limited by sampling error, variable thickness and irregularities of capsule, as well as blood vessel distortion and collapse. The rate of follicular cancer among follicular neoplasms is low, that is about 15% (17). One quarter of these lesions will begin with extrathyroidal invasion or local node involvement, which will be apparent on exploration and thus preclude the need for frozen section (17).

The frequency of malignancy in indeterminate FNA cytology in our series was 20%, comparable to the 13-36% in literature (5). The frequency of malignancy in deferred group in frozen section in our series was 25%, comparable to the 15-35% in literature (4).

Many papers on the diagnostic sensitivities of FNAC for thyroid nodules exist in the literature, showing a wide range from 43 – 100% and specificity from 47 – 100%. Factors contributing to this broad range of sensitivity and specificity are the handling of suspicious cases, adequacy of sample, sampling techniques, experience of pathologist in interpretation, length of follow up and inclusion of suspicious/indeterminate cases in the category of false negative diagnosis (18).

Ongoing cyto-frozen-histological correlation is an important quality control assurance measure and allows labs to calculate their false positive and false negative rates. Hence, fine needle aspiration and frozen section are invaluable tools in the management of thyroid lesions with good accuracies.

The limitation of the study was in the study design, done by collecting data from a tertiary hospital not from the community and the study has not included inter or intra observer bias.

Conclusion

To conclude, fine needle aspiration cytology (FNAC) can be used primarily to select patient for surgery and frozen section can be employed to plan the extent of surgery. Frozen section can be avoided when the FNAC result is consistent with malignancy, but could be of value when the FNAC result is reported as suspicious, inadequate, or even benign. In our study, frozen section had a good accuracy, sensitivity and negative predictive value compared to fine needle aspiration cytology.

Acknowledgments

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