A Comparative Study of Fine Needle Aspiration Cytology (FNAC) and Immunocyto-chemistry in Preoperative diagnosis of Solitary Thyroid nodules

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ABSTRACT

Introduction: Solitary thyroid nodule is a common entity encountered in day to day clinical practice. The importance of evaluation of a thyroid nodule is the need to exclude malignancy, but the optimum diagnostic strategy is still a matter of debate. **Aims and objectives:** The present study was undertaken to evaluate the utility of fine needle aspiration cytology (FNAC) and immunocytochemistry in the preoperative diagnosis of solitary thyroid nodules. **Methods and Material:** This was a cross sectional study carried out in the Department of Endocrinology, Gauhati Medical College and Hospital, Assam, India from January 2012 to November 2013. The study enrolled 48 patients above 18 years of age with solitary thyroid nodules. Galectin-3 was used as the immunocytochemical marker of interest and was examined in all FNA biopsied specimens. **Results:** FNAC has both high sensitivity as well as specificity for diagnosis of solitary thyroid nodules (100% and 90% respectively); on the other hand immunocytochemistry has a high specificity but low sensitivity (100% and 66.67% respectively). Particularly in nodules with indeterminate cytology, expression of galectin-3 has been found to have a positive predictive value of 100%. **Conclusion:** FNAC remains the gold standard for preoperative evaluation of solitary thyroid nodule. However, in those nodules where FNAC cannot arrive at a definite diagnosis, use of a molecular marker in the form of galectin-3 is promising. Galectin-3 expression can be used as a reliable pre-surgical molecular marker to aid in diagnosis of indeterminate nodules.

Key words: FNAC, Galectin-3, Immunocytochemistry, Solitary thyroid nodule

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INTRODUCTION

Thyroid nodules are common clinical findings and their prevalence in the general adult population ranges from 4 to 7 %.[1] They are more frequently found in women and in the elderly population. The prevalence of thyroid nodules also differs widely depending on the iodine intake by the population living in a defined geographical area[2] and depending on the method used for their identification. For example with the more widespread use of high-resolution ultrasonography (USG) for thyroid imaging, the prevalence of nodules has been estimated to be 19% to 67% in randomly selected individuals.[3-6]

There are limited studies from India on the epidemiology of thyroid nodules. In a study involving 14,762 schoolchildren (56.0% girls and 44.0% boys), aged 6-18 years, with a countrywide representation, the overall prevalence of goiter was 23.0% with a higher frequency in girls.[7] In another Indian study by Marwaha et al [8] which was done in Delhi to find the impact of two decades of universal salt utilization, the prevalence of nodules on palpation was found to be in 1.6% which was lower in men, while the nodule prevalence on ultrasonography was 4.6% in men and 5.6 % in women.

The clinical importance of evaluation of a thyroid nodule is the need to exclude thyroid cancer, which occurs in about 5-10% of the nodules. [9] Thyroid cancer is the most common endocrine malignancy. In most countries the incidence of thyroid cancer has increased during the past few decades and in North America it is one of the most rapidly increasing cancers, representing a major cause of morbidity in premenopausal women.[10, 11]

Professional societies like the American Thyroid Association (ATA) and a consortium of the American Association of Clinical Endocrinologists, the Associatizion Medici Endocrinologi and European Thyroid Association (AACE/AME/ETA) provide similar recommendations for the evaluation and management of patients with thyroid nodules,[12,13] beginning with history and physical examination and then progressing to diagnostic testing which include biochemical parameters like serum TSH, serum thyroglobulin and serum calcitonin; ultrasonography, fine needle aspiration biopsy and finally use of various molecular

markers in inconclusive cases to aid in preoperative decision making.

Thyroid fine needle aspiration cytology (FNAC) is the most reliable, safe, and cost-effective diagnostic tool used in the evaluation of thyroid nodules.[2, 14] However FNAC has well-known limitations in separating benign follicular lesions from well-differentiated carcinomas.[15] The diagnosis of follicular-patterned lesions remains an area which causes lots of confusions for the reporting pathologist and diagnostic criteria in this aspect are highly variable. It is not possible to make a diagnosis of a follicular adenoma or carcinoma based on FNA biopsy alone because this depends on the absence or presence of capsular or lymphovascular invasion, which can only be determined on histology.

The use of immunological markers seems to be an alternative to aid some of the diagnostic challenges in case of these indeterminate nodules. Galectin-3 is a ß-galactosil-binding protein involved in regulating cell-cell and cell-matrix interactions. Galectin-3 has been widely used as a marker of thyroid cancer in immunohistochemistry or immunocytochemistry. Normal thyroid tissue and benign nodules have not been found to express galectin-3, while its expression has been demonstrated in malignant thyroid cells.[16] Galectin-3 is one of the most widely studied markers for malignancy in follicular lesions with indeterminate cytology.[17-19]

Galectin-3 detection by immunohistochemistry or immunocytochemistry has been used to improve diagnostic accuracy in preoperative evaluation of thyroid nodules with variable sensitivities and specificities.[20-23] Keeping in mind the limitations of the available diagnostic methods in establishing thyroid malignancy, the present study was done to find out the relative efficacy of FNAC and galectin-3 expression by immune cyto chemistry in preoperative evaluation of thyroid nodules.

MATERIALS AND METHOD:

The present study was a cross sectional study carried out in the Department of Endocrinology of Gauhati Medical College and Hospital, Assam, India from January 2012 to November 2013. The study enrolled 48 consecutive patients above 18 years of age with solitary thyroid nodules. Patients with diffuse goiter or with

multinodular goiter were excluded from the study. On physical examination a solitary thyroid nodule was defined as a discrete area within the thyroid parenchyma that has a different contour or consistency than the rest of the gland. This was later verified by doing a thyroid ultrasound.

All patients were submitted to thyroid profile (serum T_3, T_4, TSH and TPO antibody), FNAC thyroid and immunocytochemistry for detection of galectin-3. Estimation of T_3, T_4, TSH and TPO antibody were done by chemiluminescent assay using Siemens IMMULITE 1000 analyzer. The results of FNAC were reported according to the current Bethesda system for reporting thyroid cytopathology.[24] We determined the expression of galectin-3(Antigen) in the cells obtained by fine needle aspiration cytology using the Novo Link Polymer Detection System.

Decision for surgery was taken based on clinical judgments and preoperative results of FNAC or immunocytochemistry as well as patients' preference. Twenty (20) out of 48 patients underwent surgery and respective biopsy specimens were taken for final histopathology results (HPE). Thyroid FNAC and immunocytochemistry results were compared to the results of final histological study of the excised specimen in order to calculate the values of the tests. This study was approved by our institutional review board. Descriptive statistics are presented and were compared using the two-sample *t* test (for continuous data) or the chi-square test with Yates' correction (for categorical data). A p value < 0.05 was considered statistically significant.

OBSERVATION AND RESULTS

Our study included 48 subjects presenting with solitary thyroid nodules. The mean age of presentation was 35.64±11.29 years. There were 13(27.03%) male and 35(72.92%) female patients in our study population with a male: female ratio of 1:2.6. (Table 1)

Table: 1 Baseline clinical and biochemical characteristics of subjects: (n=48)

Age of presentation (in years) mean±SD	**35.64±11.29**
Male: Female ratio	1 :2.6
High risk characteristics	
a) Previous history of head and neck irradiation	None
b) Previous history of neck surgery	None
c) Family history of thyroid cancer or other syndromes	None
d) Compressive symptoms	None
e) Lymphadenopathy	3
f) Fixed nodules	None
TSH(mIU/L) (mean±SD)	2.876±4.354
T_3 (nmol/L) (mean±SD)	2.132±1.024
T_4 (nmol/L) (mean±SD)	116.062±31.615
Positive anti TPO antibodies(n)	3

Out of all 48 cases in our study population, FNAC results were interpreted as benign in 35(72.92%), indeterminate in 8(16.67%) and malignant in 5(10.42%) of the subjects. All the malignant nodules on FNAC were reported as papillary carcinoma. (Figure 1 & 2)

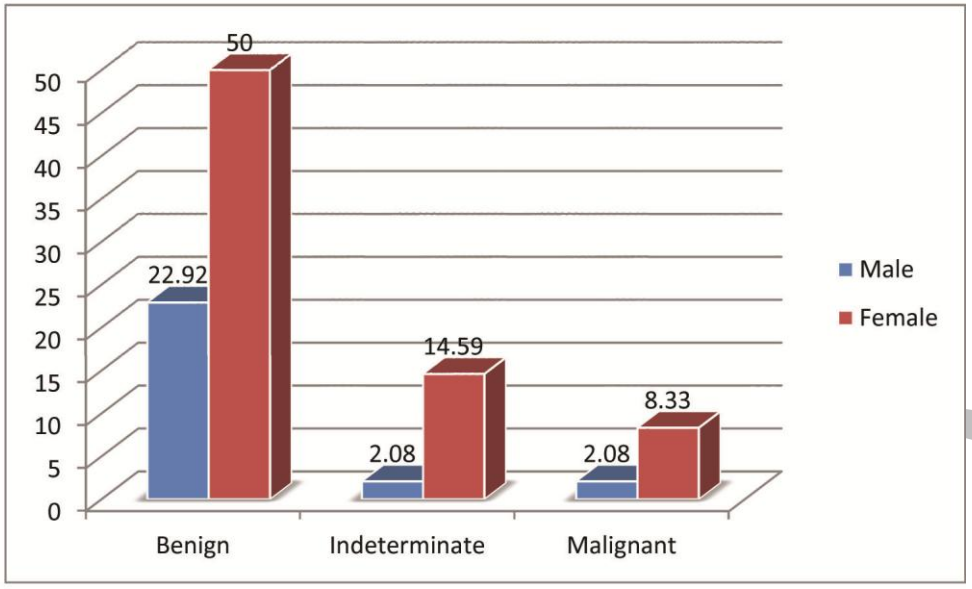

Figure: 1 Distribution of the 48 thyroid nodules according to FNAC results (in per cent)

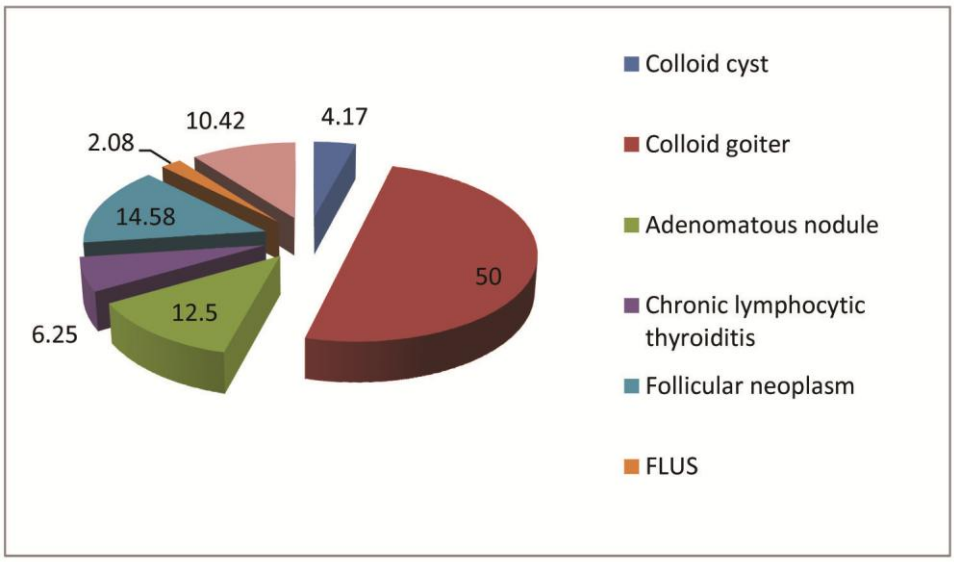

Figure: 2 Various FNAC results in our study population (in per cent): (n=48)

Out of total 48 subjects in our study, immunocytochemistry using galectin-3 was found to be positive in 7 subjects (14.58%) and negative in 41 subjects (85.42%). (Figure 3)

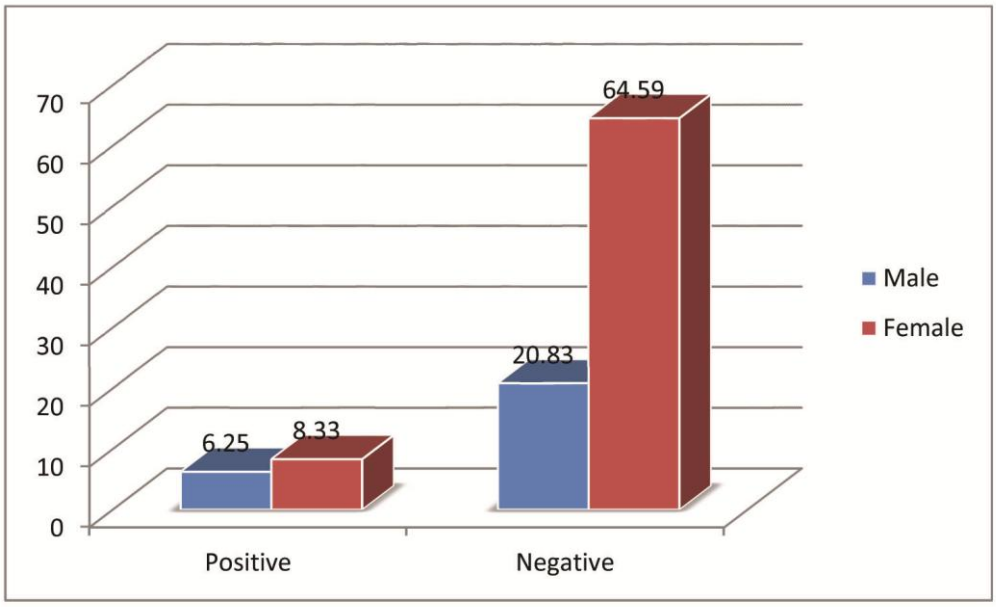

Figure: 3 Distribution of the 48 thyroid nodules according to results of immunocytochemistry (Galectin-3) (in per cent)

The relation between preoperative FNAC and immune-cytochemistry of 48 subjects is shown in Table 2. Of 35 cases which were reported to be benign on cytological analysis, galectin-3 was found to be positive in 2 of them, percentage of positivity in the benign group as a whole being 5.71%. Galectin-3 was positive in 2 of 5 cases with follicular neoplasm while it was positive for the single case with a cytological diagnosis of follicular lesion of undetermined significance (FLUS). The percentage of immune positivity for the indeterminate group as a whole was 37.5%. Of 5 cases with a cytological report of papillary carcinoma, only 2 of them showed positivity for galectin-3(percentage of positivity in the malignant group was 40%).

Table: 2: Relation between FNAC and immunocytochemistry of 48 subjects in our study:

Classification	Galectin-3		% of positivity
	Positive	Negative	
Colloid cyst (n=2)	0	2	0
Colloid goiter (n=24)	1	23	4.17
Adenomatous nodule (n=6)	1	5	16.67
Chronic lymphocytic thyroiditis (n=3)	0	3	0
Follicular neoplasm/suspicious for follicular neoplasm (n=7)	2	5	28.57
Follicular lesion of undetermined significance(FLUS) (n=1)	1	0	100
Suspicious for malignancy (n=0)	-	-	-
Papillary carcinoma (n=5)	2	3	40
Medullary thyroid carcinoma (n=0)	-	-	-
Anaplastic carcinoma (n=0)	-	-	-
Total (n=48)	7	41	14.58

Twenty patients (41.67%) underwent surgery out of which 14 were found to be benign and 6 malignant (percentage of malignancy was 30%). Of the 6 malignant nodules, 5 were papillary carcinoma, one being follicular variant and the remaining one was follicular carcinoma. (Figure 4&5)

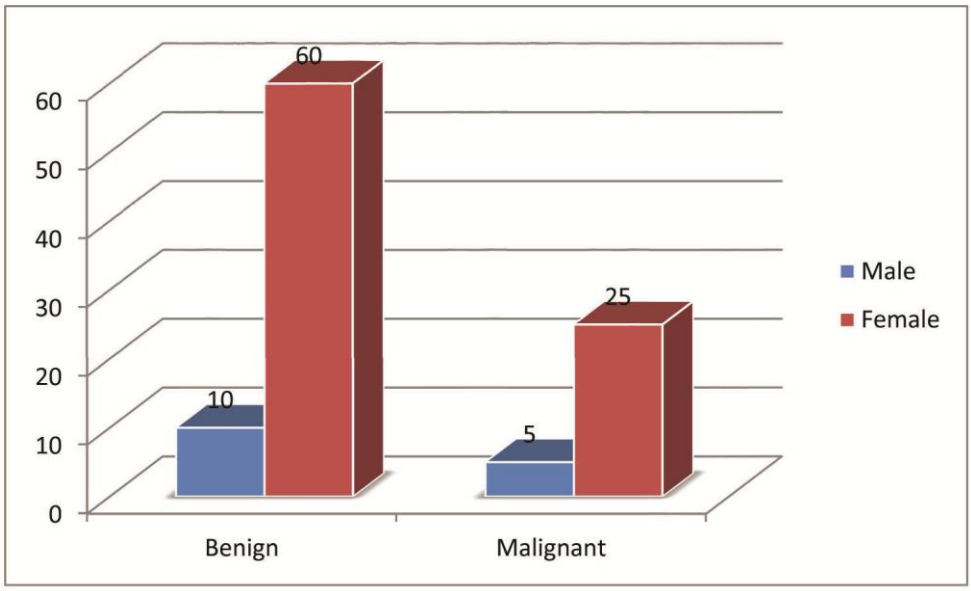

Figure: 4 Distribution of the thyroid nodules according to final histopathology results in subjects undergoing surgery (in per cent): (n=20)

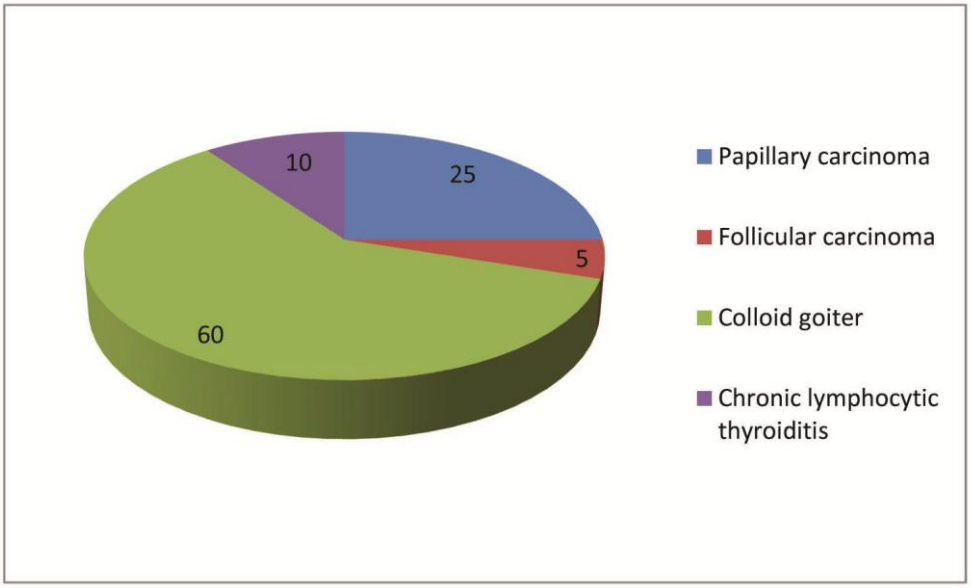

Figure: 5 Histopathology findings in subjects undergoing surgery (in per cent): (n=20)

On comparison of the different baseline clinical and biochemical parameters in between the benign and the malignant groups who underwent surgery, no significant difference was found in respect to age (p=0.523), gender (p = 0.891), mean serum TSH level (p = 0.497), mean T_3 level (p=0.600), mean T_4 level (p=0.345) and thyroid function status (euthyroid, p=0.548; hypothyroid, p=0.666 and hyperthyroid, p=0.548). (Table 3)

Table: 3 Comparison of clinical and biochemical characteristics between benign and malignant groups who underwent surgery: (n=20)

Characters	Benign (n=14)	Malignant (n=6)	P value
Age(in years) (mean±SD)	32.00±12.09	35.50±7.55	0.523
Gender(Female)%	85.71	83.33	0.891
TSH(mmol/L) (mean±SD)	3.82±4.17	5.9±9.7	0.497
T3(nmol/L)	2.05±0.87	1.8±0.73	0.600
Avg. T4(nmol/L) (mean±SD)	114.7±31.95	100.4±24.86	0.345
Euyhyroid (n)	7	4	0.548
Hypothyroid (n)	4	2	0.666
Hyperthyroid (n)	3	0	1.00

Table: 4: Relation between FNAC and histopathology results of subjects undergoing surgery: (n=20)

FNAC(Bethesda classification)	Final histology		% of malignancy
	Benign	Malignant	
Benign (n=9)	9	0	0
FLUS (n=1)	None	1	100
Follicular neoplasm (n=5)	4	1	20
Suspicious of malignancy (n=0)	None	None	None
Malignant (5)	1	4	80

Sensitivity=100%

Specificity=90%

PPV=80%

NPV=100%

Percentage of false positive=10%

Percentage of false negative=0%

Accuracy=92.85%

Table: 5: Relation between Immuno-cytochemistry and histopathology results of subjects undergoing surgery: (n=20)

Galectin-3	Final histology		% of malignancy	p value
	Benign	Malignant		
Positive (n=4)	0	4	100	0.006
Negative (n=16)	14	2	12.5	

Sensitivity=66.67%

Specificity=100%

PPV=100%

NPV=87.5%

Percentage of false positive=0%

Percentage of false negative=33.33%

Accuracy=90%

Table: 6: Relation between FNAC, immunocytochemistry and histopathology of subjects undergoing surgery: (n=20)

FNAC(Bethesda classification)	**Number of patients**	**Galectin-3**		**Final histology**	
				Benign	Malignant
Benign	9	Positive	0	9	0
		Negative	9		
FLUS	1	Positive	1	0	1
		Negative	0		
Follicular neoplasm	5	Positive	1	4	1
		Negative	4		
Suspicious of malignancy	None	Positive	None	None	None
		Negative	None		
Malignant	5	Positive	2	1	4
		negative	3		

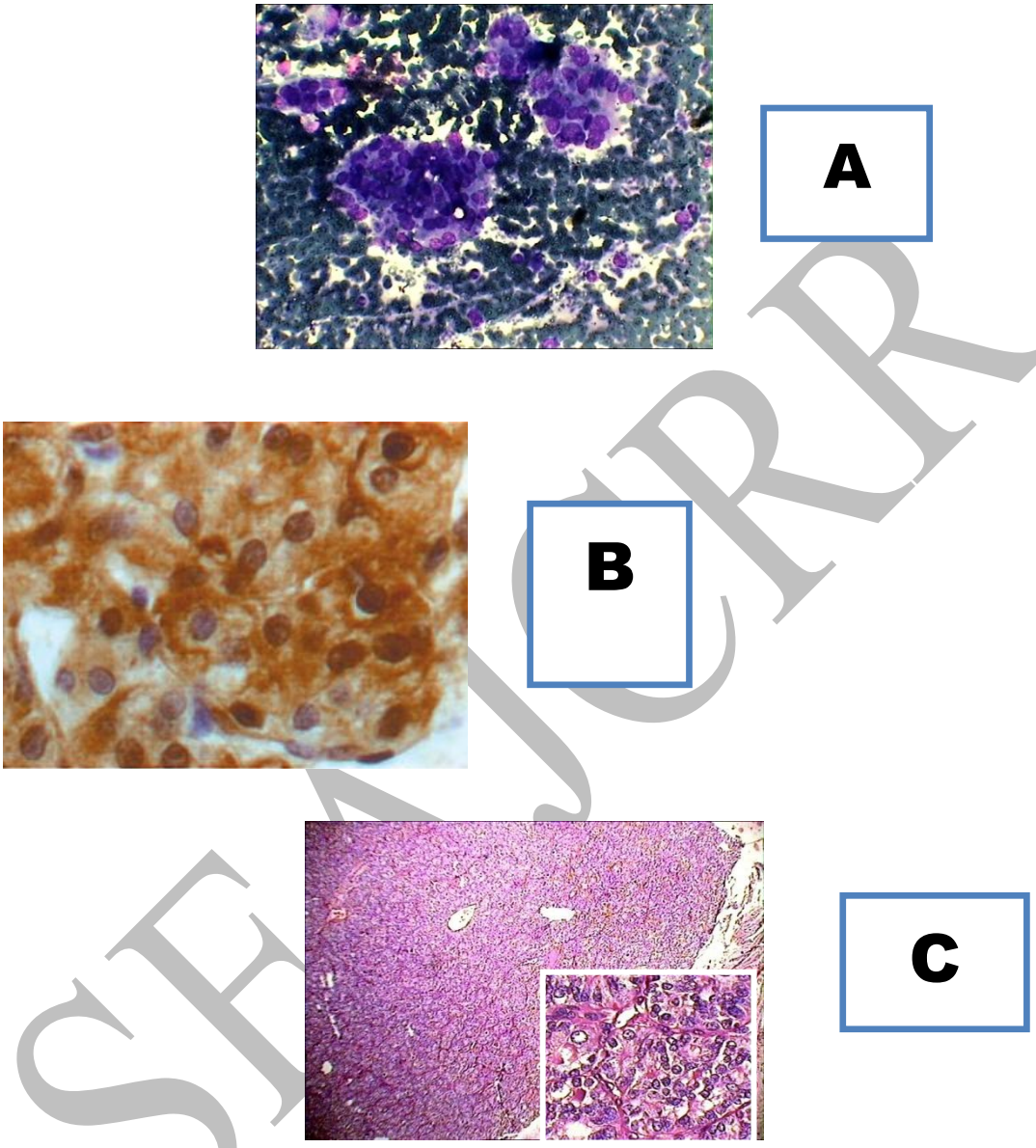

Figure: 6: Galectin-3 immunolocalization in papillary carcinoma (follicular variant). A: Cytopathlogy reported as FLUS; B: Immunocytochemistry showing expression of galectin-3; C: Corresponding histological tissue.

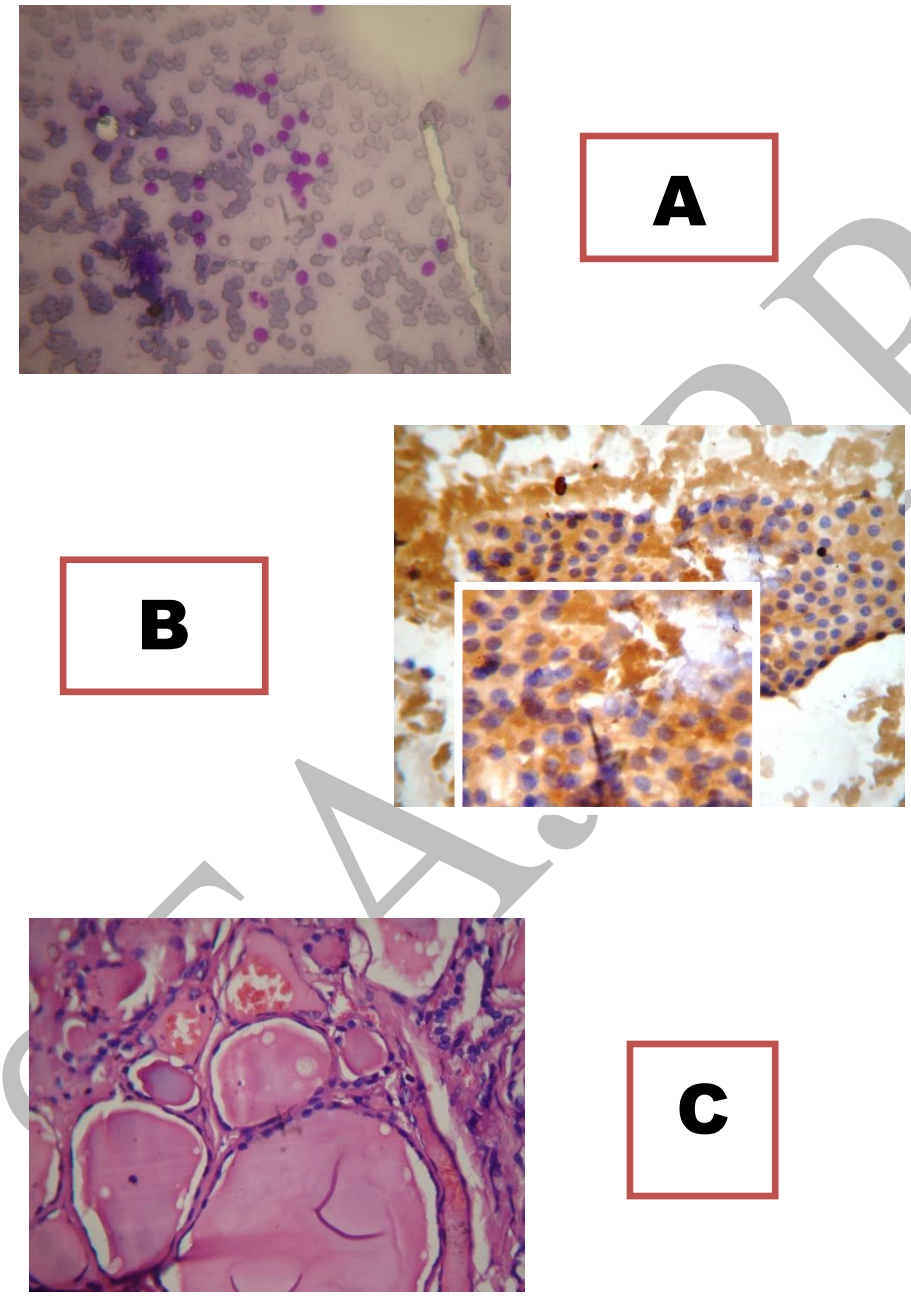

Figure 7: Negative expression of galectin-3 in colloid goiter. A: Cytopathlogy; B: Immunocytochemistry showing negative expression of galectin-3; C: Corresponding histological tissue.

DISCUSSION

The optimum diagnostic strategy for patient with a solitary thyroid nodule is still a matter of debate. The goal of diagnostic workup is to carefully select those patients for surgery who have a high probability of malignancy as well as to exclude safely and effectively those with benign nodules thus avoiding unnecessary surgery. The present study was undertaken to evaluate the utility of FNAC and immunocytochemistry in differentiating benign from malignant thyroid nodules.

The percentage of cancer in thyroid nodules in our study population was found to be 30 %(6 out of 20). Among 1985 study patients by Mary C. Frates et al, [25] 295 (14.9%) subjects were found to have thyroid malignancy. In a study by Rosalinda YA Camargo et al,[26] 274 subjects underwent thyroid surgery out of which only 8 patients (2.91%) had a thyroid cancer. The relatively higher percentage of malignancy in our study is mainly because of the selection bias where subjects either with only high risk characteristics for malignancy on preoperative evaluation or based on personal choice were subjected to surgery.

FNAC is the gold standard for laboratory evaluation of solitary thyroid nodules. Its use in recent years has resulted in a significant decrease in the number of unnecessary surgeries in seemingly benign nodules and helps the clinician to select those patients for surgery who have a high likelihood of harbouring malignancy.

On comparing the results of preoperative FNAC with final histopathology of 20 patients who underwent surgery, all 9 lesions with initial cytological diagnosis of benign nodules were confirmed on subsequent HPE (percentage of false negative=0%). Of 5 cases with cytological diagnosis of follicular neoplasm, 4 had benign and 1 had malignant diagnosis on final histology while the single case with a cytological diagnosis of FLUS was reported to be malignant on final histopathological analysis. Thus, an overall malignancy rate of about 33.33 % for the indeterminate group was found in our study. Among 5 patients with cytological diagnosis of malignant lesion, final histopathology

revealed 1 case as benign (percentage of false positive=10%).

When FNAC reports of 6 patients with thyroid carcinoma were checked, it was found that 4 out of 6 cases had a correct preoperative cytological diagnosis, 1 was reported as follicular neoplasm and 1 case was reported as FLUS.

The overall sensitivity of FNAC in our series was 100%, the overall specificity was 90%, the predictive positive value was 80 % and predictive negative value was 100 %. The sensitivity and specificity of FNAC were 71.43% and 100% respectively according to Altavilla et al, [27] 98% and 99% according to Goellner et al, [28] 93.5% and 75% according to Bouvet et al. [29] Ikram et al has reported sensitivity and specificity for malignancy as 100% which is in accordance with our findings. [30]

False negative FNAC results may occur because of inadequate sampling or misinterpretation of cytology. They are associated with the risk of missing potentially malignant lesions. [31] False negative FNA cytology results occurred in none of our patients. This is consistent with other reports in the literature that suggest a false negative rate of 2% to 7%. [32-37] A false positive cytology report may result in surgical over treatment. False positive FNA cytology results are uncommon and were found in only 1(10%) patient in our series. This finding is also consistent with other reports that cited an incidence of false positive FNAC results ranging from 0% to 9% 9. [32-37]

Out of total 48 subjects in our study, immunocytochemistry using galectin-3 was found to be positive in 7 subjects (14.58%) and negative in 41 subjects (85.42%). Of the 35 cases which were reported to be benign in cytological analysis, galectin-3 was found to be positive in 2 of them, one with colloid goiter and the other with adenomatous nodule. Unfortunately the final histology was available for neither of these subjects. The remaining benign nodules which were negative for galectin-3 and for whom final histology was available (9 cases); all of them were confirmed as benign.

Galectin-3 was positive in 2 of 5 cases with follicular neoplasm, while it was positive for the single case with a cytological diagnosis of FLUS. One of the 2 immunoreactive follicular adenoma cases was later confirmed to be malignant on histopathology while histopathology

was not available for the remaining immunoreactive case. Four out of the 5 follicular adenoma cases with negative galectin-3 were reported to be benign subsequently on HPE and histology was not available for the remainder. Similarly, the single case of FLUS with immunopositivity was also reported to be malignant. Of 5 cases with a cytological report of papillary carcinoma, only 2 of them showed positivity for galectin-3.

Overall in those cases where final histology was available (20 cases), galectin-3 was found to be positive in 4 of the 6 malignant nodules, (3 papillary carcinomas, 1 being follicular variant and 1 follicular carcinoma; percentage of positivity in malignant nodules being 66.67%) with a false negativity of 33.33% and none of the 14 benign nodules (false positivity is 0%).

The overall sensitivity of immunocytochemistry in our series was 66.67 %, the overall specificity was 100%, the predictive positive value was 100 %, the predictive negative value was 87.5 % and the overall accuracy was 90%. Our results were comparable to the findings of Bartolazzi ea al [20] who found sensitivity, specificity, positive predictive value, and diagnostic accuracy of galectin-3 immunodetection as 100%, 98%, 92%, and 99%, respectively using 220 specimens of thyroid nodules obtained preoperatively by ultrasound guided FNAC.

In another study of 125 thyroid aspirates, the sensitivity, specificity, positive predictive value, and diagnostic accuracy of galectin-3 as a single marker in discriminating benign from malignant lesions were 92%, 94%, 95.8%, and 92.8%, respectively. [38]

Use of molecular markers in preoperative diagnosis of thyroid nodules is an area of active research. Particularly, it has been found useful in supplementing the diagnosis of follicular adenoma or carcinoma. No single molecular marker can identify accurately all malignant follicular-patterned lesions; rather the use of combined immunological markers as a panel seems to be an alternative. [15, 20]

CONCLUSION

The present study was undertaken to evaluate the usefulness of FNAC and immunocytochemistry in the evaluation and management of solitary thyroid nodules. While evaluating the sensitivity and specificity of these diagnostic modalities, it was found that FNAC has

both high sensitivity as well as specificity for diagnosis of a solitary thyroid nodule; on the other hand immunocytochemistry has a high specificity but low sensitivity. In our study, the sensitivity and specificity of FNAC and immunocytochemistry were 100% and 90% and 66.67% and 100% respectively.

From our study it was seen that thyroid nodules which were reported as benign in FNA biopsy can safely and reliably be followed up without putting the patients for unnecessary surgical interventions. However, in those nodules where FNAC could not arrive at a definite diagnosis and were reported as indeterminate, use of a molecular marker in the form of galectin-3 was shown to be promising. It can be said that galectin-3 expression can be used as a reliable presurgical molecular marker to aid in diagnosis of indeterminate nodules keeping in mind the fact that a negative galectin-3 expression in an indeterminate nodule does not necessarily exclude malignancy.

Hence a combination of various diagnostic modalities, rather than any single one, will give optimal results and avoid unnecessary surgery as well as help in selecting patients potentially harbouring a malignancy in a thyroid nodule.

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