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Original Research Article

Histopathology of Thyroid Lesions with Special Reference to Expressionof Cytokeratin 19 and Galectin-3 in Thyroid Neoplasms at Silchar Medical College and Hospital

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Conflict of interest: Nil

Abstract:

The diseases of thyroid lesions comprise conditions related to thyroid hormone deficiency (hypothyroidism), those with excessive release of thyroid hormones (hyperthyroidism), and mass lesions of the thyroid. Thyroid lesions can be divided into two major types: those with a diffuse pattern of growth and those with nodule formation. The diffuse pattern of growth is seen mainly affecting the entire gland like in hyperplasia and thyroiditis, whereas mass lesions of the thyroid include a vast array of developmental, inflammatory, and neoplastic diseases. Despite using routine investigations like thyroid function tests and radio imaging like ultrasonography and scintigraphy, it becomes challenging to diagnose these thyroid nodules as they cannot discriminate between benign and malignant. Thyroid neoplasm presents a diverse range of tumors with varying biological behavior, although the majority of them can be easily interpreted by characteristic histopathological features. The vast majority of thyroid cancer patients can be cured by initial treatment, and this claims the need for precise and accurate diagnosis of the indolent stage. Immunohistochemical markers are used for the evaluation of thyroid lesions and the markers are studied in normal tissues as well as in benign and malignant tumors with the hope of finding significant differences between these groups. The present study was undertaken to assess the expression of immunohistochemical markers, cytokeratin 19 and galectin 3, for predicting the behaviour of various thyroid neoplasms and support in diagnosing the tumors. Cytokeratin 19 is considered to be useful for the diagnosis of thyroid malignancies, especially in papillary carcinoma and in cytologically suggestive but indeterminant cases. The sensitivity and specificity of CK19 as a single marker are reported to be as high as 92% and 97%, respectively. Galectin-3 (GAL-3) is a 31kDa galactosidase binding lectin found in the cytoplasm and nuclear compartment, playing an important role in cell-cell, cell-matrix interaction, and also in pre mRNA splicing. The implication of this marker has been seen in the regulation of normal cellular proliferation, apoptosis, as well as in malignant transformation and metastasis of cancer cells. The majority of immunohistochemical studies found that galectin-3 was differentially expressed in thyroid carcinoma compared with benign thyroid lesion. In the present study, we have taken CK 19 and Gal-3 to study their potential applications in the differential diagnosis between malignant and benign thyroid neoplasms. A combined panel of IHC using different antibodies has been suggested for the improvement of diagnostic accuracy of the follicular-patterned neoplasms.

Keywords: Thyroid Neoplasms, Cytokeratin-19, Galectin-3.

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Introduction

The thyroid gland was named by Thomas Wharton, an English physician and anatomist, in 1656.The word thyroid was derived from the Greek word “thyros”, meaning shield, as it is located in the anterioraspect of the neck. [1 In the 19th century, the physiology of the gland and its pathological enlargement or goiter was described. The pathological lesions of the thyroid gland are familiar among the general population and constitute a large proportion of endocrine referrals. The knowledge in the field of thyroid pathology has been growing rapidly in recent years, facilitating not only early and accurate diagnosis for patients, but also in the management of the patients. The diseases of thyroid lesions comprise conditions related to thyroid hormone deficiency (hypothyroidism), those with excessive release of thyroid hormones (hyperthyroidism), and mass lesions of the thyroid. [2] Thyroid lesions can be divided into two major types: those with a diffuse pattern of growth and

those with nodule formation. The diffuse pattern of growth is seen mainly affecting the entire gland like in hyperplasia and thyroiditis, whereas mass lesions of the thyroid include a vast array of developmental, inflammatory, and neoplastic diseases. Thyroid nodules can be clinically diagnosed in 4-7% of the general population and are detected incidentally on ultrasonography in 19-67%. Of all palpable thyroid nodules, 5% are only malignant and the majority are asymptomatic. [3] Despite using routine investigations like thyroid function tests and radio imaging like ultrasonography and scintigraphy, it becomes challenging to diagnose these thyroid nodules as they cannot discriminate between benign and malignant.4 Thyroid neoplasm presents a diverse range of tumors with varying biological behaviour, although the majority of them can be easily interpreted by characteristic histopathological features. The vast majority of thyroid cancer patients can be cured by initial treatment and this claims the need for precise and accurate diagnosis of the indolent stage. Immunohistochemical markers are used for the evaluation of thyroid lesions and the markers are studied in normal tissues as well as in benign and malignant tumors with the hope of finding significant differences between these groups. The present study was undertaken to assess the expression of immunohistochemical markers, cytokeratin 19 and galectin-3, for predicting the behaviour of various thyroid neoplasms and support in diagnosing the tumors. Cytokeratin 19 (CK 19) is a low molecular weight, type I intermediate filament which maintains the structural integrity of epithelial cells.5 It is expressed in the simple epithelium of the thyroid gland. [6] Cytokeratin 19 is considered to be useful for the diagnosis of thyroid malignancies, especially in papillary carcinoma and in cytologically suggestive but indeterminant cases. [7,8,9] The sensitivity and specificity of CK19 as a single marker are reported to be as high as 92% and 97%, respectively. [10] Galectin-3 (GAL-3) is a 31kDa galactosidase binding lectin found in the cytoplasm and nuclear compartment, playing an important role in cell-cell, cell-matrix interaction, and also in pre mRNA splicing. [11,12] The implication of this marker has been seen in the regulation of normal cellular proliferation, apoptosis, as well as in malignant transformation and metastasis of cancer cells.12 The majority of immunohistochemical studies found that galectin-3 was differentially expressed in thyroid carcinoma compared with benign thyroid lesions. [13] GAL-3 has been studied in many cancers, including breast carcinoma, [14] hepatocellular carcinoma, [15] colorectal carcinoma, [16] and gastric carcinoma. [17]

Materials and Methods:

The present study was a Hospital based prospective cross-sectional study undertaken to study the histopathology of thyroid lesions and expression of Cytokeratin 19 and Galectin-3 in thyroid neoplasms with the help of immunohistochemical analysis. The present study was undertaken in the Department of Pathology, Silchar Medical College and Hospital, Silchar for 1 year (June, 2020 to May, 2021. There was a total of 42 resected specimens, clinically presenting with thyroid swelling, submitted to the Dept. of Pathology, SMCH for histopathological examination. 12 thyroid neoplasms were diagnosed (5 benign, 7 malignant). Therefore, immunohistochemistry with CK 19 and Gal- 3 was done on these 12 neoplasms as per IHC protocol. Patients of all age groups and sex were included. A histopathological examination performed as a diagnostic procedure in thyroid swelling will be included.

Swellings in the front of the neck other than thyroid swelling and patients with bleeding disorders will be excluded. Parameters studied were detailed clinical history and routine investigations after taking consent from the patients, hospital records of the patients, macroscopic and detailed gross examination of the thyroid specimens received, the location of the resected specimens were recorded, microscopic examination of biopsy tissues, immunohistochemistry paraffin embedded tissue in histopathologically diagnosed thyroid neoplasms .The study tools were predesigned proforma, usual pathological instruments – like scalpels, scalpel blades, forceps, knives, 10% formal saline, various grades of alcohol, xylene, paraffin, incubator, microtome, various stains, slides, cover slips etc. For the Immunohistochemical study – Tris buffer, EDTA buffer, Poly-L-lysine, microwave, Perox free blocking reagent, Super block, Anti-Polyvalent HRP Polymer HRP Label, Primary antibody, DAB chromogen and binocular microscopes were used.

Plan for Analysis of Data: All data were collected, compiled and subjected to suitable statistical analysis. For statistical analysis Chi-square test and Fisher's exact test were used. A p value of less than 0.05 was considered statistically significant. The Statistical software SPSS Version 20 was used for the analysis of the data, and Microsoft Office Word and Excel 2007 have been used to generate graphs, tables, etc.

Methodology

Thyroid resected specimens were fixed in 10% formalin and brought to the laboratory. A detailed gross examination was done and recorded systematically. The specimens were then sectioned from representative areas and the following techniques were observed in preparing the slides for histopathological examination. Tissue sections (3-5mm) made from different representative areas and biopsy samples were fixed for 24 hours in 10% formalin Tissue blocks was prepared by transferring

the tissue from the wax bath to a Leukhart "L" block filled with molten wax. The tissue were inverted to make the surface to be cut free of air bubbles and oriented so that this surface rests on the base of the mould. The blocks were quickly cooled. The L shapes were removed when the paraffin was hard enough along with the number tag. The paraffin section thus obtained was cut in a rotary microtome to obtain a 5µ (micron) thick section. The ribbons of sections were spread in hot water at 37^{O} C for expansion of the curled section and then lifted out on albumenized slide. The slides were then incubated at 56 ^{O}C in a hot air oven to remove the paraffin wax and fix the tissue to the slides. The paraffin was cleaned from the section in two changes of warm xylene. The sections were then stained in Hematoxylin & Eosin (H&E) stain.

Results and Observations:

The present study titled "Histopathology of thyroid lesions with special reference to expression of cytokeratin 19 and galectin-3 in thyroid neoplasms at SMCH." was undertaken in the Department of Pathology, Silchar Medical College & Hospital, Silchar during the study period of June, 2020 to May, 2021. It was a hospital based, cross-sectional observational study. A total of 42 resected specimens of thyroid, clinically presenting with thyroid swelling were submitted to the Department of Pathology, Silchar Medical College & Hospital, Silchar for histopathological examination. Age, sex distribution and clinical profile were documented in all 42 cases. 7 cases turned out to be malignant neoplasms and 5 were found to be benign neoplasms (follicular adenoma) on histopathological examination. So immunohistochemistry with cytokeratin 19 and galectin-3 was done as per IHC protocol in these 12 cases only.

Immunohistochemistry with cytokeratin 19 and galectin-3 in these 12 cases were reported as the extent and intensity of cytoplasmic immunoexpression.

1: Age distribution of patients:

Table 1: Age distribution of patients:

Age group in years	Number of patients	Percentage (%)
0-9	0	0
10-19	3	7.14
20-29	15	35.71
30-39	9	21.43
40-49	8	19.05
50-59	5	11.91
60-69	2	4.76
Total	42	100

The age of the patients ranged from 15 to 66 years with a median age of 35 years. The mean age was 35.21years with a standard deviation of 13.32 years. Majority of patients were in the age group of 20-29 years, 15 cases (35.71%), followed by the age group of 30-39 years with 9 cases (21.43%).

2: Sex distribution of thyroid swelling among various age groups:

Table 2: Sex distribution of thyroid swelling among various age groups:

Age inyears	Male	Female	Total
0-9	0	0	0
10-19	1	2	3
20-29	2	13	15
30-39	1	8	9
40-49	3	5	8
50-59	1	4	5
60-69	0	2	2
Total	8	34	42

Among the 42 patients, 34 (80.95%) were female and only 8 (19.05%) were males. In males the highest incidence was found in the age group of 40-49 years comprising of 3 patients. In females the highest incidence was in the age group of 20-29 years with 13 cases followed by 30-39 years with 8 cases. The mean age for males was 35.75±13.56 years and the mean age for females was 35.09±12.47 years. The difference was found to be statistically not significant (p= 0.650).

3. Consistency of thyroid swelling:

Table 3: Consistency of thyroid swelling

Consistency	Number	Percentage
Solid	19	45.24%
Cystic	13	30.95%
Mixed	10	23.81%

Majority of thyroid lesions 19 (45.24%) were solid in consistency, followed by 13 cystic cases (30.95%) and mixed type accounting for 10 cases (23.81%).

4. Location of the thyroid swelling:

Table 4: Location of the thyroid swellings

Location	Number	Percentage
Right Sided	24	57.1
Left Sided	12	28.6
Bilateral	5	11.9
Midline	1	2.4

Among the various thyroid swellings, 24 were located on the right side, and 12 on the left side. 5 cases presented with bilateral neck swelling and 1 case of colloid goiter presented as midline swelling.

5. Cytological diagnosis of thyroid swelling:

Table 5: Cytological diagnosis of thyroid swelling:

FNAC Diagnosis		Number OfPatients	Percentage(%)
Benign	Colloid goiter (CG)	24	57.2
	Multinodulargoiter (MNG)	5	11.9
	Lymphocytic thyroiditis (LT)	3	7.1
	Total	32	76.2
Suspicious	Follicular Neoplasm(Fn)	5	11.9
Malignant	Papillary thyroid Ca (PTC)	5	11.9
	Medullary Ca of Thyroid (MCT)	0	0
	Total	5	11.9

FNAC was done in all 42 cases of thyroid swelling for pre-operative diagnosis. Majority of the swellings were found to be benign (32). Of these, colloid goitre accounted for 24 cases, followed by MNG with 5 cases, LT with 3 cases. 5 cases were diagnosed as follicular neoplasm. Remaining 5 cases were diagnosed as malignant neoplasm.

6. Histopathology of thyroid lesion

Table 6: Benign vs malignant lesions on histopathology

Diagnosis	Number of patients	Percentage(%)
Benign	35	83.33
Malignant	7	16.67
Total	42	100

On histopathological examination, out of the 42 patients, 35 (83.33%) were found to have benign disease while 7 (16.67%) were diagnosed as having malignant disease.

7. Benign vs malignant lesions in various age groups:

Table 7: Age distribution of benign and malignant thyroid lesions

Age group inyears	No of benign cases	No of malignantcases	Total number ofpatients
0-9	0	0	0
10-19	3	0	3
20-29	14	1	15
30-39	8	1	9
40-49	7	1	8
50-59	2	3	5
60-69	1	1	2
Total	35	7	42

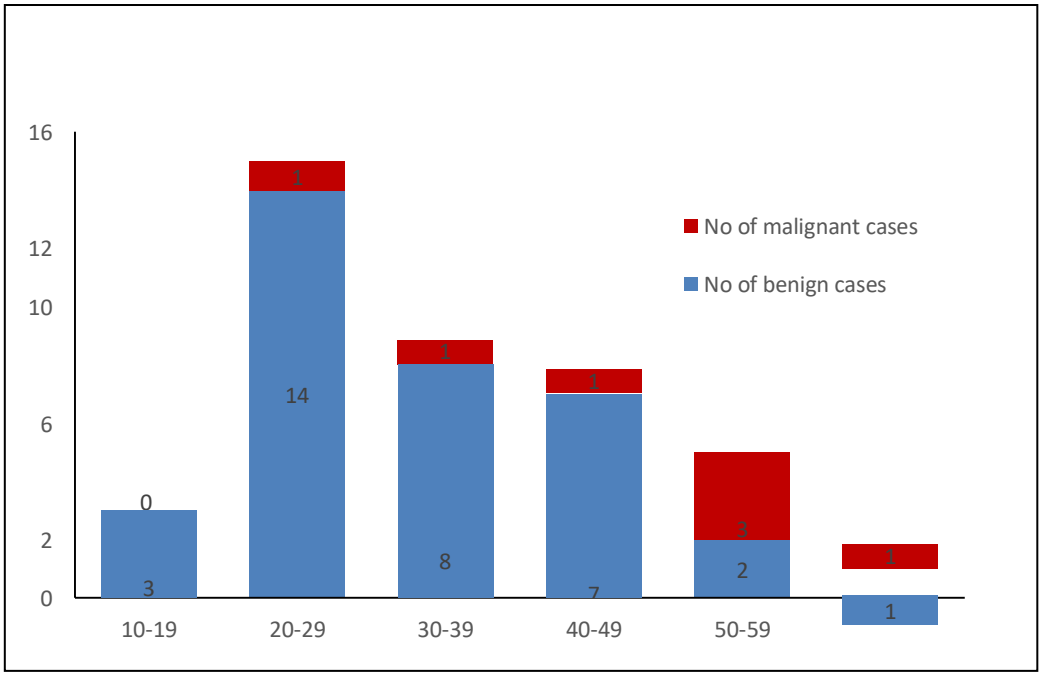

Figure 1: Bar diagram showing age distribution of benign and malignant thyroid lesions

On histopathological examination, benign lesion was most common in the age group of 20-29 years with 14 cases (33.33%), followed by 30- 39 years with 8 cases (19.05%). Malignant lesions were most common in the age group 50-59 years with 3 cases (7.14%) . The mean age for benign thyroid mass was found to be 32.79±12 years. The mean age for malignant thyroid mass was found to be 47.36±13.80 years. The difference was found to be statistically not significant (p=0.066).

8. Histopathological diagnosis of malignant thyroid lesion:

Table 8: Spectrum of malignant thyroid lesion

Histological type	Number of cases
Papillary thyroid carcinoma (PTC):	5
Classical (PC)	3
Follicular variant (FVPTC)	2
Follicular thyroid Ca (FTC)	1
Medullary Ca of thyroid (MCT)	1

Malignant thyroid lesion accounted for 7 (16.67%) cases out of the total 42 cases studied. These included 5 cases of PTC, 1 cases of FTC, 1 cases of MCT. 2 patients were male and 5 were female. 6 of these exhibited solid consistency and 1 had mixed consistency.

The average age of presentation 47.36±13.80 years.PTC: accounted for 5 cases out of which 3 were female and 2 male. Mean age was 42.50±13.04 years. Most of these presented with B/L swelling in the neck. 4 of the swellings had solid consistency while 1 had mixed consistency. FTC: accounted for 1 cases of a 59 year female. The swelling were solid in consistency. MCT: included 1 cases of a 66 year female. The swelling was solid in consistency.

9. Histopathological diagnosis of benign thyroid lesions:

Table 9: Spectrum of benign thyroid swelling

Type of benign thyroid swelling	Number of cases
Colloid goiter	22
Multi nodular goiter	6
Lymphocytic thyroiditis	2
Follicular adenoma	5
TOTAL	35

Benign thyroid lesion accounted for 35 (83.33%) cases out of the total 42 cases studied. These included 22 cases of CG, 6 cases of MNG, 5 cases of FA and 2 cases of LT. 6 patients were male and 29 were female. 13 of these exhibited cystic

consistency, 13 exhibited solid consistency and 9 had mixed consistency.

The average age of presentation was 32.79±12 years.CG: accounted for 22 cases; of which 18 were female and 4 male. Mean age was 31.77±10.77 years. 12 of these had cystic consistency, 4 had solid consistency while 6 had mixed consistency MNG: accounted for 6 cases; 1 were male and 5 female. Mean age was 37.83±17.51 years. 2 of the swelling were solid, 3 were mixed and 1 cystic. FA: included 5 cases; 4 female and 1 male. Mean age was 30.50±11.40 years. All five were solid in consistency.LT: accounted for 2 cases both of which were female. Mean age was 34.50±14.14 years. Both the swellings had solid consistency.

10. FNAC and Histopathology correlation:

Table 10: Correlation between findings of FNAC and HPE in case of benign and malignant thyroid swelling

FNAC Diagnosis	Number	HPE		Diagnostic Accuracy
		Benign	Malignant	
Benign	32	30	2	97.1%
Malignant	5	0	5	

Immunohistochemistry

11. Staining pattern of CK 19 in different histological types of thyroid neoplasm: IHC was done in all 12 cases of thyroid neoplasm. Staining was positive in thyroid malignancies but was negative in case of benign follicular adenomas.

Table 11: Intensity of staining of CK-19 expression in different histological types of thyroid neoplasm

Stainingof CK 19	FA	PC	FVPTC	FTC	MCT	Total
0	3	0	0	0	0	3
1+	2	0	0	1	1	4
2+	0	0	1	0	0	1
3+	0	3	1	0	0	4
Total	5	3	2	1	1	12

Among the thyroid neoplasms, CK 19 expression was negative in all 5 cases of follicular adenoma. Out of these, 2 cases of follicular adenoma showed low or weak staining (less than 5%) and 3 cases showed no staining for CK 19.

In case of malignant thyroid neoplasms, positive CK 19 expression (high staining more than 5%) was seen in 5 cases (71.43%) and remaining 2 cases (28.57%) showed negative CK 19 expression (weak staining less than 5%). The difference between the expression of CK 19 in benign and malignant thyroid lesion was found to be statistically significant (p=0.014).High CK 19 expression was observed in all cases of PTC and its follicular variant. CK 19 showed weak expression in 1 case of FTC and 1 case of MCT.

12. Staining pattern of CK 19 in papillary thyroid carcinomas

Table 12: Intensity of staining pattern of CK 19 expression in papillary thyroid carcinomas

Staining intensity of CK-19	0	1+	2+	3+	Total
No of PC	0	0	0	3	3
Percentage ff Positivity	0%	0%	0%	100%	100%
No of FVPTC	0	0	1	1	2
Percentage of Positivity	0%	0%	50%	50%	100%

In the present study, staining intensity of CK 19 in classical papillary carcinoma shows strong and diffuse positivity in cytoplasm of cells (3+) in all the 3 cases. In follicular variant of papillary carcinoma 1 case showed 2+ positivity and 1 case showed 3+ positivity.

13. Staining pattern of CK 19 in follicular adenoma and follicular carcinoma

Table 13: Intensity of staining pattern of CK 19 in follicular adenoma and follicular carcinoma

Staining intensity ofCK-19	0+	1+	2+	3+	Total
No of FA	3	2	0	0	5
Percentage of Positivity	60%	40%	0%	0%	100%
No of FTC	0	1	0	0	1
Percentage of Positivity	0%	100%	0%	0%	100%

CK 19 expression was negative in all 5 cases of follicular adenoma. Out of these, 2 cases of follicular adenoma showed low or weak staining (less than 5%) and 3 cases showed no staining for CK 19. CK 19 expression in one case of FTC, showed weak staining (less than 5%).

14. Staining pattern of GAL-3 in different histological types of thyroid neoplasm: GAL-3 expression was studied in all 12 cases of thyroid neoplasm.

Table 14: Intensity of staining pattern in different histological types of thyroid neoplasm

Staining ofGAL-3	FA	PC	FVPTC	FTC	MCT	TOTAL
0+	4	0	0	0	0	4
1+	1	0	1	0	0	2
2+	0	1	1	1	1	4
3+	0	2	0	0	0	2
TOTAL	5	3	2	1	1	12

Among the thyroid neoplasms, high Gal-3 expression was seen in all 6 cases of malignancies except one case of papillary carcinoma while low Gal-3 expression was seen in all 5 cases of benign follicular adenoma. The difference was statistically significant (p=0.003).

15. Staining pattern of GAL-3 in papillary thyroid carcinoma:

Table 15: Intensity of staining pattern of Gal-3 expression in papillary thyroid carcinoma

Staining Of GAL-3	0	1+	2+	3+	TOTAL
No Of Pc	0	0	1	2	3
Percentage Of Positivity	0	0	33.33	66.67	100
No Of FVPTC	0	1	1	0	2
Percentage Of Positivity	0	50	50	0	100

Gal-3 showed high expression in 4 cases of papillary carcinoma whereas in 1 case of follicular variant of papillary carcinoma it showed low expression (1+) The difference was statistically not significant(p=0.171).

16. Staining pattern of GAL 3 in follicular adenoma and follicular carcinoma:

Table 16: Intensity of staining pattern of Gal-3 in follicular adenoma and follicular carcinoma

Staining pattern ofGAL 3	0	1+	2+	3+	TOTAL
No Of FA	4	1	0	0	5
Percentage Of Positivity	80	20	0	0	100
No Of FTC	0	0	1	0	1
Percentage Of Positivity	0	0	100	0	100

Gal- 3 was negative in all 5 cases of benign follicular adenoma. It showed low or weak staining in 1 case and in 4 cases showed no staining. In 1 case of follicular carcinoma, Gal-3 showed moderate intensity of staining (2+). The difference between the two was found to be statistically significant (p=0.014).

17. Statistical analysis of CK 19 and GAL-3:

Table 17: Statistical analysis of CK 19 and GAL-3

IHC Marker	Sensitivity	Specificity
CK 19	71.43%	100%
GAL-3	85.71%	100%
CK 19 & GAL-3	100%	100%

For statistical evaluation of sensitivity and specificity, sensitivity is the number of carcinomas with positive results as a percentage of total number of carcinomas. Whereas specificity is the number of benign lesions with negative results as a percentage of total number of benign lesions. In the study we found CK 19 alone has sensitivity of 71.43% and specificity of 100%. Gal-3 alone has sensitivity of 85.71% and specificity of 100%. When both the markers are combined the sensitivity is 100% and specificity is 100%.

Discussion: The present study titled "Histopathology of thyroid lesions with special reference to expression of Cytokeratin 19 and Galectin-3 in thyroid neoplasms at Silchar Medical College and Hospital" was undertaken in the Department of Pathology, Silchar Medical College & Hospital, Silchar during the study period of June, 2020 to May, 2021. A total of 42 cases of thyroid specimens were studied during this period.

Clinical history and routine investigations available with the patients were recorded. Of the 42 cases, 5

cases were benign and 7 cases were malignant lesions of the thyroid. The other 30 cases were of various non-neoplastic thyroid lesions. Histopathological examination of the samples received was done. Immunohistochemistry was done using CK 19 and Gal-3 and their expressions were studied in 12 cases of thyroid neoplasms. The results obtained were compared with those of other previously conducted studies.

1. Age distribution: In this study, most of the patients were in the age group of 20-29 years, 15 cases (35.71%), followed by age group of 30-39 years with 9 cases (21.43%). Thus 57.14% patients were of the age group 20-39 years. Age ranged from 15 to 66 years with a median age of 35 years. Mean age was 35.21 years with a standard deviation of 13.32 years. The youngest patient was 15 years (female) with colloid goitre and the oldest patient was 66 years old with medullary carcinoma of thyroid. The findings are consistent with several other studies.

Table 18: Age distribution of patients with thyroid swelling

Age group in years	Number of patients	Percentage (%)
0-9	0	0
10-19	3	7.14
20-29	15	35.71
30-39	9	21.43
40-49	8	19.05
50-59	5	11.91
60-69	2	4.76
Total	42	100

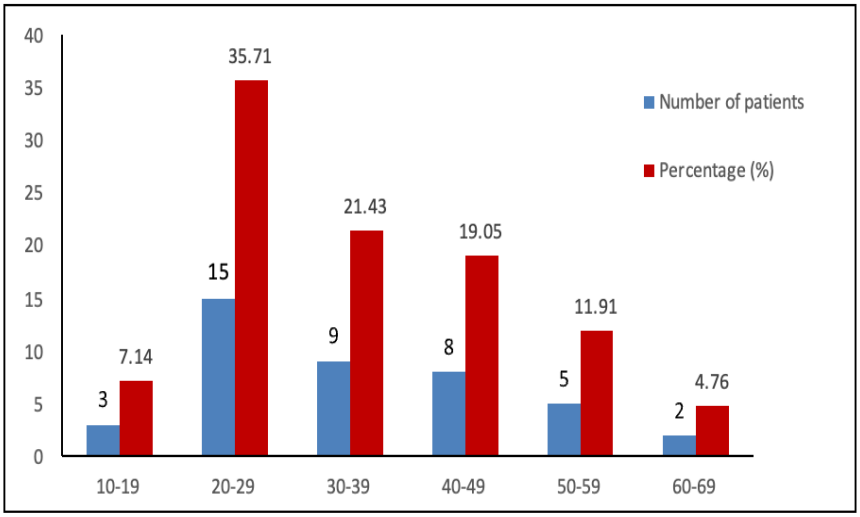

Figure 2: Bar diagram showing age distribution of patients with thyroidswelling

2. Sex distribution:

Table 19: Sex distribution of thyroid swellings in various age groups

Age inyears	Male	Female	Total
0-9	0	0	0
10-19	1	2	3
20-29	2	13	15
30-39	1	8	9
40-49	3	5	8
50-59	1	4	5
60-69	0	2	2
Total	8	34	42

In the present study, 34 patients (80.95%) were females and only 8 (19.05%) were males. In males the highest incidence was found in the age group of 40-49 years comprising of 3 patients. In females the highest incidence was in the age group of 20-29 years with 13 cases followed by 30-39 with 8 cases. The mean age for males was 35.75±13.56 years and the mean age for females was 35.09±12.47 years. The difference was found to be statistically not significant (p=0.650).

3. Consistency of thyroid lesions:

Table 20: Consistency of thyroid lesions

Consistency	Number	Percentage
Solid	19	45.24%
Cystic	13	30.95%
Mixed	10	23.81%

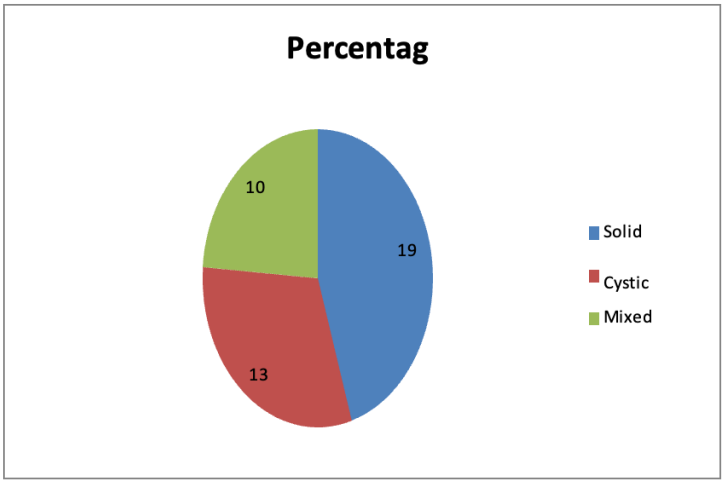

Figure 3: Consistency of Thyroid Swelling

In the present study, majority of thyroid lesions 19 (45.24%) were solid in consistency, followed by cystic 13 cases (30.95%) and finally mixed type accounting for 10 cases (23.81%).

4. Site of the swelling:

In our study, 24 swellings were located on the right side, and 12 on the left side. 5 cases presented with bilateral neck swelling and these included 4 cases of PTC, 1 case of colloid goitre. 1 case of colloid goitre presented as midline swelling.

5. Cytological findings in thyroid lesion: FNAC was done in all 42 cases of thyroid swelling for pre-operative diagnosis. Majority of the swellings were found to be non-neoplastic thyroid lesion (total 32). Out of these, colloid goitre accounted for 24 cases, followed by MNG with 5 cases and LT with 3 cases. 5 cases were diagnosed as follicular neoplasm. Remaining 5 cases were diagnosed as PTC.

6. Histological diagnosis if thyroid lesion:

In the present study, out of the 42 patients, 35 (83.33%) were found to have benign disease while 7 (16.67%) were diagnosed as having malignant disesase on histopathological examination.

7. Benign lesions:

Out of the benign, a vast majority of the lesions were non- neoplastic of which colloid goitre comprised the highest number of cases i.e. 22 followed by multinodular goitre with 5 cases and lymphocytic thyroiditis with 2 cases.

Colloid goitre is the most common benign lesion. In our study, among the benign neoplastic cases, all the cases were diagnosed as follicular adenoma.

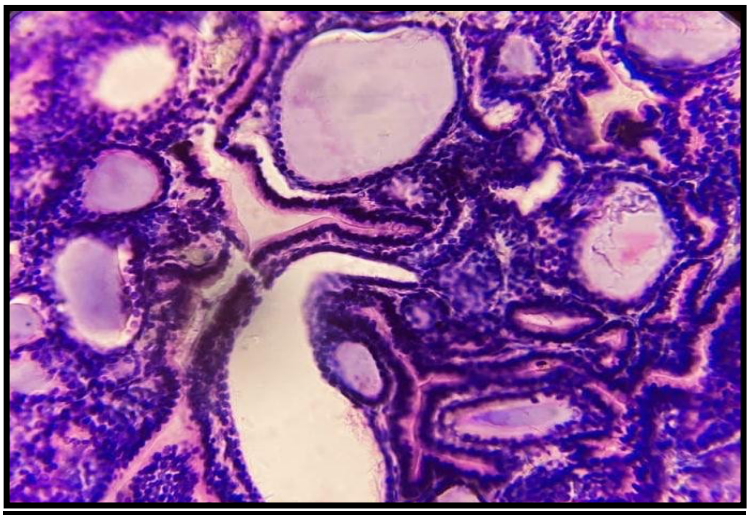

Figure 4: H&E section of colloid goitre(10x)

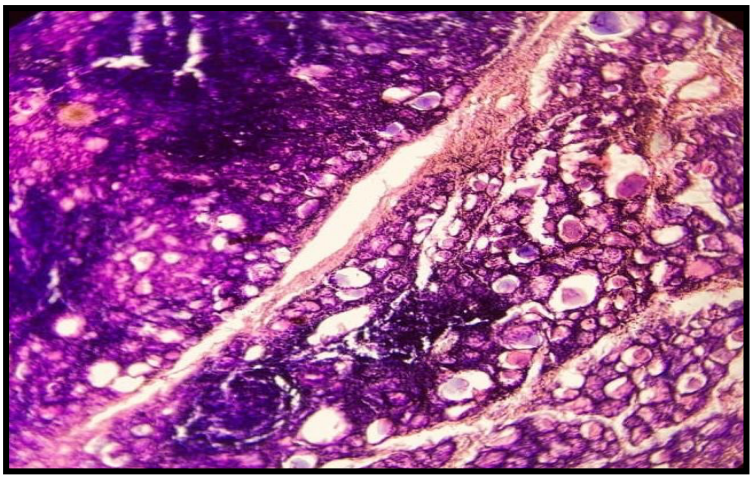

Figure 5: H&E section of lymphocytic thyroiditis(10x)

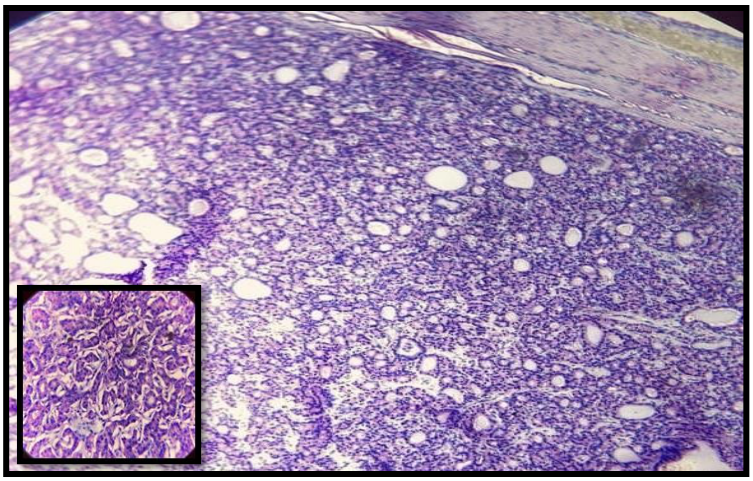

Figure 6: H&E section of follicular adenoma (10x)

8. Malignant lesions: In our study of 42 cases, among the 12 malignant thyroid lesions, papillary thyroid carcinoma was the most common which comprised of 5 cases, followed by 1 case each of follicular thyroid carcinoma and medullary thyroid carcinoma.

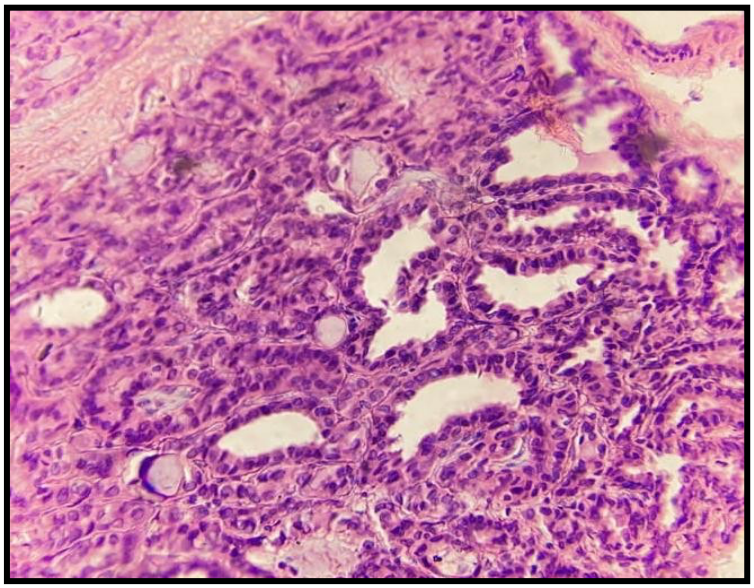

Figure 7: H&E section of papillary thyroid carcinoma(10x)

9. Age distribution in benign and malignant groups:

On histopathological examination, benign lesion was most common in the age group of 20-29 years with 14 cases (33.33%), followed by 30-39 years with 8 cases (19.05%). Malignant lesions were most common in the age group 50-59 years with 3 cases (7.14%). The mean age for benign thyroid mass was found to be 32.79±12 years. The mean age for malignant thyroid mass was found to be 47.36±13.80 years. The difference was found to be statistically not significant (p=0.066). Arif et al, [115] in their study found that the mean age for benign lesion was 49.8±13.5 years and the mean age for malignant lesion was 47.2±13.4 years and the difference was not statistically significant.

10. FNAC and Histopathology correlation:

In our study, the diagnostic accuracy came out to be 97.1%

Immunohistochemistry:

In our study, among the thyroid neoplasms, immunostaining with CK 19 was negative in all cases of benign follicular adenoma and among malignant neoplasm it was positive (high staining more than 5%) in 5 cases (71.43%) and remaining 2 cases (28.57%) showed negative expression (staining less than 5%). We also found a statistically significant correlation in the expression of CK 19 in benign and malignant thyroid lesion. (p=0.014) which implies that CK 19 might have a role in differentiating malignant and benign thyroid nodules.

Among the histological types of malignancies, CK 19 expression was found to be highest in PTC, both classical and its follicular variant. All the 5 cases of papillary carcinoma showed strongly and diffusely positive staining. The expression CK 19 in FA was low or negative in all 5 cases whereas in 1 case of follicular carcinoma it showed low expression (1+). Thus CK 19 reactivity is limited in distinguishing follicular adenoma and follicular carcinoma.

The expression of Gal-3 was low or negative in all 5 cases of benign follicular adenoma whereas high expression of Gal-3 was seen in (4/5) cases of PTC, 1 cases of FTC and 1 case of MTC. The difference was statistically significant (p=0.003). Gal-3 staining frequency of the papillary carcinomas was seen in 4/5 cases. It was higher in all 3 cases of classical papillary thyroid carcinoma and out of 2 cases of follicular variant of papillary carcinoma 1 had low staining (1+). In our study we found Gal-3 expression to be low or negative in all 5 cases of benign follicular adenoma and higher in 1 case of FTC and the difference was statistically significant (p=0.0140.In our study, sensitivity of CK 19 and Gal-3 was found to be 71.43% and 85.71% respectively.

The specificity of both CK 19 and Gal-3 was found to 100%. When both the markers are combined the sensitivity and specificity was found to be 100%. When both CK-19 and Gal-3 combined the sensitivity and specificity was found to be 93.2% and specificity 100%.Our study confirmed that expression of CK 19 and Gal-3 is a useful panel for the diagnosis of papillary and follicular carcinoma of the thyroid and is also helpful in differentiating benign from malignant thyroid neoplasms. However, our study has limitations in that the number of malignant cases was lower and we did not get cases of anaplastic and poorly differentiated carcinomas. The clinical utility of CK 19 and Gal-3 in thyroid carcinoma patients has to be further defined by prospective studies with larger sample sizes.

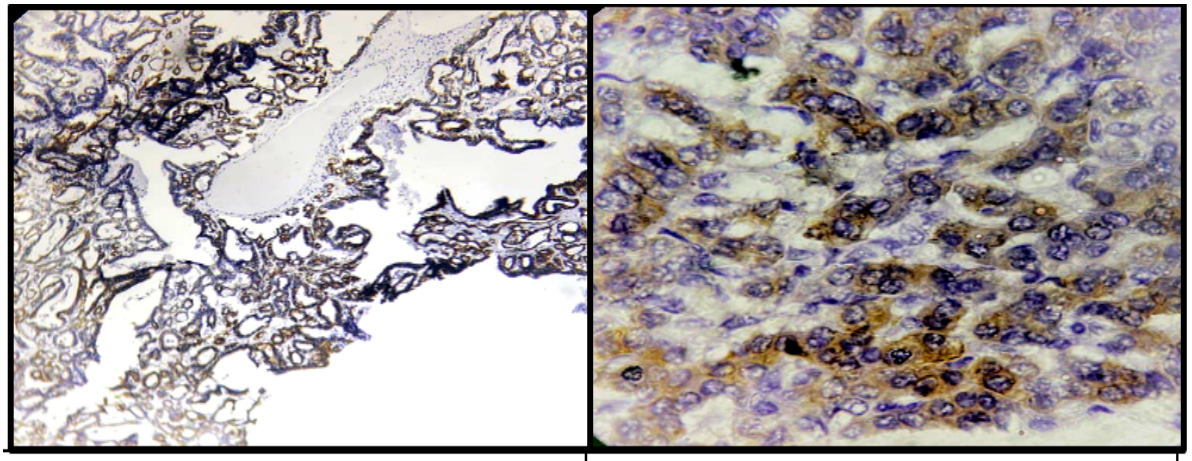

Image 5: Strong cytoplasmic CK19 positivity in follicular variant of papillary thyroid cancer (scanner view)

Image 6: Moderate cytoplasmic positivity of Galectin-3 in papillarythyroid carcinoma (40x)

Figure 8 and 9

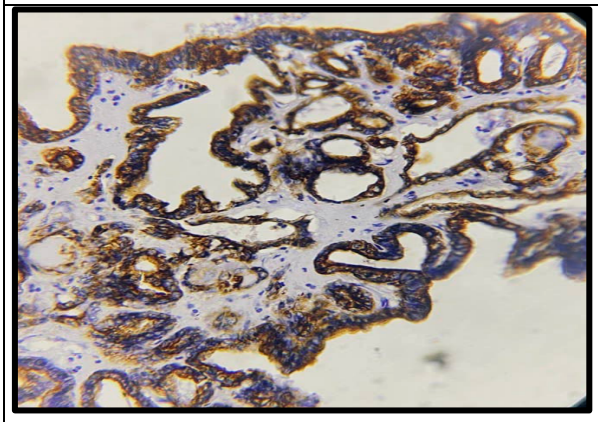

Figure 10: Strong cytoplasmic CK19 positivity in classical papillary thyroid cancer(10x)

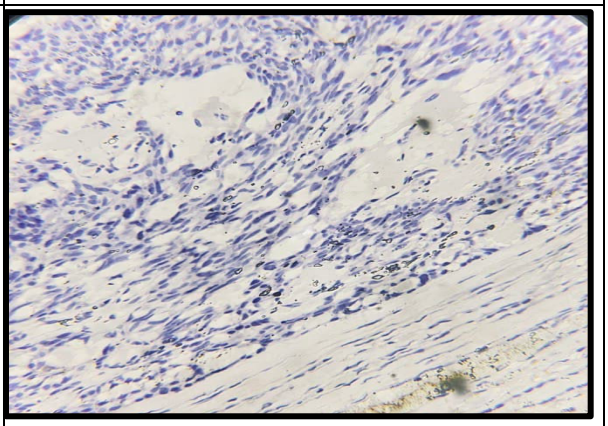

Figure 11: Negative staining of Galectin-3 in follicular adenoma (10x)

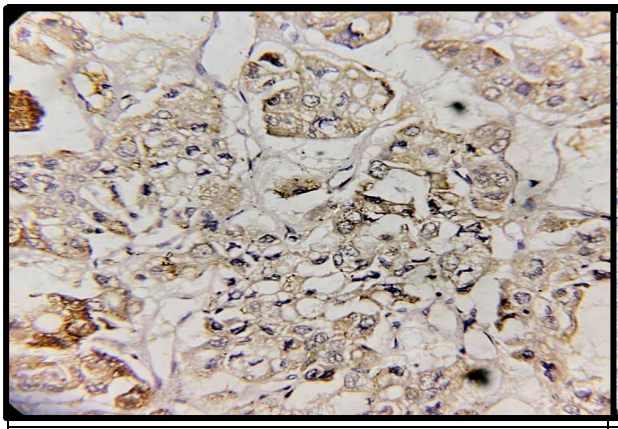

Figure 12: Moderate staining of Galactein3 in follicular carcinoma (40x)

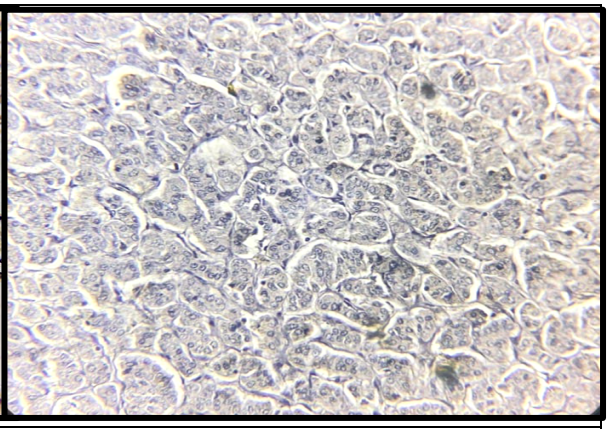

Figure 13: Weak cytoplasmic positivity of CK19 in follicular carcinoma(40x)

Conclusion:

There is a wide spectrum of thyroid lesions with variable morphologies, and the most common manifestation of thyroid disease is goitre followed by MNG. Thyroid lesions commonly present in the third or fourth decade of life, and the majority of them turn out to be non-neoplastic or benign nodules. However, a small percentage of the thyroid nodules are malignant neoplasms. A female preponderance is seen in almost all the varied thyroid lesions, including carcinomas. The most common malignant neoplasm of the thyroid is PTC. The majority of the malignant thyroid neoplasms are well-differentiated lesions, and the majority of subtypes (except MCT) arise from the follicular epithelium.

PTC is diagnosed on the basis of nuclear features and has a wide range of variants like follicular variant, tall columnar, diffuse sclerosing, oncocytic etc. Follicular neoplasms are classified based on the presence or absence of capsular and/or vascular invasion. The majority of thyroid neoplasms can be readily diagnosed by characteristic histopathological features, but in some lesions like follicular-patterned lesions (eg. FA, FTC, FVPTC, oncocytic variant of PTC, etc), difficulty arises in diagnosing as benign or malignant lesions. Hence, immunohistochemistry, molecular profiling etc are needed in diagnosing such challenging cases. A combined panel of IHC using different antibodies has been suggested for the improvement of diagnostic accuracy of the follicular-patterned neoplasms.

In the present study, we have taken CK 19 and Gal-3 to study their potential applications in the differential diagnosis between malignant and benign thyroid neoplasms. CK 19 showed varying intensities of staining in different thyroid lesions and was found to be a sensitive and specific marker in the diagnosis of papillary carcinoma and its variants. Although CK 19 does not differentiate between follicular adenoma and follicular carcinoma, it helps in differentiating follicular variants of papillary carcinoma from follicular carcinoma. But a definite conclusion cannot be made in this regard as the study is limited to a minimum number of cases.

Gal-3 showed higher sensitivity to follicular carcinoma. In the present study, we have found a

significant correlation in the diagnosis of follicular adenoma and carcinoma using Gal-3, which is difficult by histopathology alone. Thus, using both CK19 and Gal-3 helped in differentiating benign and malignant thyroid neoplasms. However, the clinical utility of CK 19 and Gal-3 in thyroid neoplasms has to be further defined by prospective studies with larger sample sizes which might help in providing more accurate, scientific and relevant conclusion.

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