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Pathology

DIAGNOSTIC APPLICATION OF CK19 AND CD56 IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING FOLLICULAR EPITHELIAL CELL-DERIVED THYROID NEOPLASMS.

Dr. Muni Bhavani Itha*	Assistant Professor, Department of Pathology, KMCH, Guntur, Andhra Pradesh, India. *Corresponding Author
Dr. V R Krishna Prasanthi. M	Post Graduate, Department of Pathology, Dr. PSIMS & RF, Vijayawada, Andhra Pradesh, India.
Dr. Kalyan Chakravarthi. V	Professor, Department of Pathology, Dr. PSIMS&RF, Vijayawada, Andhra Pradesh, India.
Dr. Ranga Rao. Diddi	Professor & Head, Department of Pathology, Dr. PSIMS & RF, Vijayawada, Andhra Pradesh, India.

ABSTRACT **INTRODUCTION** The thyroid is an endocrine organ that controls the basal metabolic rate, drives somatic and psychic growth, and is involved in calcium metabolism. Different tumors types may arise in the thyroid -gland, represent the most common neoplasms of the endocrine system, and pose a significant diagnostic challenge to pathologists, surgeons, and oncologists. Based on standard histomorphologic features, the accurate categorization of follicular-patterned thyroid lesions is not always straightforward in most cases. Differential diagnostic criteria, which are often subtle and subjective, that contribute to diagnostic problems. Over the last few decades, considerable progress has been made in tumor molecular gene profiling and diagnostic immunohistochemistry in differentiating follicular epithelial cell-derived thyroid neoplasms with overlapping histomorphologic features.

MATERIAL AND METHODS: A descriptive study on thyroid neoplasms was conducted in the Department of Pathology, Tertiary care teaching hospital; over two years period. A total of 50 cases of thyroid neoplasms were studied in detail correlating the clinical, radiological, and histopathological findings. The biopsy material was provided by the department of surgery and ENT and also from outside sources. The types of specimens included were total thyroidectomy specimens and hemithyroidectomy specimens. A complete and detailed histopathological examination has been carried out and immunohistochemistry was done using mouse anti-human CK19 and CD56 antibodies during the study period.

AIM: The present study's main objective is to see how CK19 and CD56 immunohistochemical markers are useful in diagnosing thyroid pathology, both alone and in combination.

RESULTS: The sensitivity, specificity, PPV, NPV, and diagnostic accuracy of CK19 and CD56 immunohistochemistry markers expression in thyroid neoplasms were evaluated both separately and in combination and it was found that each studied marker was sensitive and specific for certain thyroid lesions but the sensitivity, specificity, and diagnostic accuracy were significantly improved when two markers were used together.

CONCLUSION: In summary, as no marker by itself has a superior diagnostic value, a combination of markers may be helpful for more accurate diagnosis in diagnostically challenging cases. In the present study, the use of combined markers (CK19 and CD56) improves the sensitivity, specificity, and diagnostic accuracy to 100%, 96.7%, and 98% which is higher than when a single marker alone is used. Therefore, in this study, we evaluated the diagnostic utility of a panel consisting of CK19 and CD56 immunohistochemistry to distinguish between benign and malignant thyroid neoplasms.

KEYWORDS : Biomarker, Cytokeratin-19, Cluster of Differentiation 56, Pathology, Thyroid Carcinomas, Papillary.

INTRODUCTION

The thyroid is an endocrine gland that controls the basal metabolic rate, drives somatic and psychic growth, and is involved in calcium metabolism. Different rates of growth and biological aggressiveness cause a range of different tumors types to arise in the thyroid gland, each with a variable natural history.[1]

According to the Global Cancer Statistics GLOBOCAN 2018 report estimates that out of 18.1 million new cases (17.0 million excluding non-melanoma skin cancer) thyroid cancers accounts for 5,67,233(3.1 % of global cancer diagnosis) and out of 9.6 million cancer deaths (9.5 million excluding non-melanoma skin cancer) thyroid cancers accounts for 41,071 (0.4% of global cancer deaths) worldwide. Women have a global thyroid incidence rate of 10.2 per 100,000, which is three times greater than men and it accounts for 5.1 percent of the total female cancer burden, or 1 in every 20 cancer diagnoses in 2018.[2]

The majority of thyroid carcinomas (95%) are formed from follicular epithelial cells and are highly differentiated, such as papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), with a small percentage of poorly differentiated carcinomas (PDCs) and undifferentiated carcinomas (UDCs). Medullary carcinomas (MCs) are produced from parafollicular C cells, which make up around 3% of all thyroid carcinomas.[3]

Since the early 1980s, the incidence of thyroid cancer has been steadily rising in many countries, owing to an increase in papillary thyroid cancer diagnosis due to diagnostic alterations, advances in the detection and diagnosis, and perhaps changes in the prevalence of risk factors.[4]

The increase in thyroid cancer incidence is almost entirely attributed to PTC, such increase reflects the true increase in PTC and with a minor component of over-diagnosis of PTC. The follicular, medullary, and anaplastic cancer rates were relatively unchanged did not change significantly.[5-7]

Most of the thyroid neoplasms are relatively easy to interpret, but the accurate classification of certain follicular-patterned thyroid lesions can exhibit diagnostic difficulties, such as in differentiating hyperplastic nodular goiter with focal areas of papillary budding and nuclear clearing may be confused with PTC, Follicular adenoma from follicular carcinoma and Encapsulated follicular variant of papillary carcinoma (E-FVPTC) from other follicular neoplasms mainly from the follicular adenoma, when FVPTC demonstrates nuclear morphology of conventional PTC only in focal areas.

It's because the application of the diagnostic criteria for various follicular lesions has been proven to be subjective and contextual. Intra and inter-observer discrepancies among pathologists are well documented in the diagnosis of follicular patterned thyroid lesions with histomorphological overlapping features.

Conventional methods such as clinical history taking, physical examination, thyroid function testing, thyroid imaging, and ultrasound are all traditional ways used for evaluating thyroid lesions. However, in some instances, they are unable to distinguish between benign and malignant neoplasms. The most conclusive/ definitive diagnosis comes from a histopathological examination of surgically removed thyroid tissues.

However, thyroid neoplasms with nodular architecture, a follicular patterned growth, and ambiguous nuclear characteristics often pose diagnostic difficulties during the histopathological examination of resected thyroid specimens. In such cases, the use of ancillary diagnostic techniques such as immunohistochemistry, flow cytometric analysis has become an integral part of pathology practice which can improve diagnostic accuracy when combined with standard morphologic criteria, since over-or-under diagnosis may have a great impact on patient management and prognosis.

Thyroid transcription factor 1 (TTF1), Hector Battifora Mesothelial Cell-1 (HBME-1), Galectin-3, and Cytokeratin19 are the most commonly employed antibodies in thyroid pathology, among a wide range of biomarkers. However, no single marker is sufficiently sensitive to make a clear diagnosis.[8-10] CD 56 is a newly described, "promising" marker in thyroid pathology, however, there is limited and unclear literature data to date.[11]

The majority of studies have evaluated the expression of individual markers in varied thyroid lesions, whereas a few have investigated the expression of multiple markers. In our study, we investigated the diagnostic utility of a panel of two markers (CD56 and CK-19) in diverse follicular-derived thyroid lesions, both alone and in combination. Our goal was to investigate the sensitivity and specificity of these indicators in differentiating the follicular cell-derived thyroid neoplasms with morphologically overlapping features.

Cytokeratin19

The cytoskeleton is made up of intermediate filaments called cytokeratin. Cytokeratin19 is a type 1 cytokeratin with a low molecular weight that is present in a range of simple and glandular epithelia, both normal and their neoplastic counterparts. It is crucial in maintaining the structural integrity of the cells.

CK19 expression is generally undetectable in the thyroid gland's follicular epithelium. However, a focal staining pattern may be detectable in normal thyroid tissue, particularly in inflamed tissue. The positive expression may also be detectable in follicular epithelial cells of lymphocytic thyroiditis and follicular neoplasms. Numerous studies have shown that CK19 has a strong and diffuse staining pattern in PTC and overexpression of the CK19 is a promising indicator of PTC. [8-11]

Cluster of differentiation CD56

CD56 is a neural cell adhesion molecule (NCAM) that facilitates homotypic and heterotypic cell-cell adhesion via homophilic binding processes. It is part of a glycoprotein family that is important for cell binding, migration, and differentiation. It is found in thyroid follicular epithelial cells. In some cancers, loss of CD56 expression is linked to a higher risk of metastasis and a worse prognosis. CD56 expression was negative in PTC but was found to be diffusely positive and strong in benign thyroid lesions.[9-11]

MATERIAL AND METHODS

This was a descriptive study on thyroid neoplasms conducted in the Pathology Department of a Tertiary Care Teaching Hospital over a two-year period. A total of 50 cases of thyroid neoplasms were studied in detail correlating the clinical, radiological, and histopathological findings. The biopsy material was provided by the department of surgery and ENT and also from outside sources The types of specimens included were total thyroidectomy specimens and hemithyroidectomy specimens.

Multiple sections were studied from each tumor by the paraffin embedding technique. The tissue has been fixed in 10% formalin and is processed in an automatic tissue processor and later paraffin embedding has been done. Sections of 4 to 5 µ thickness were cut on a rotary microtome and the routine stain which has been used for all the tumors was Harris hematoxylin and eosin. A detailed microscopic examination has been carried out and immunohistochemistry was done using mouse anti-human CK19 and CD56 antibodies during the two-year study period.

Classification of thyroid neoplasms was done according to the Modified Version of WHO Classification of Nonmedullary Thyroid Tumors (2017).[12]

- Statistical analysis was made by using percentages and the Chi-square test.

INCLUSION CRITERIA

- **All (follicular epithelial cell-derived) benign and malignant tumors of the thyroid.**

EXCLUSION CRITERIA

- **Thyroid tumors of C-cell origin.**
- **Non-epithelial thyroid tumors.**
- **All non-neoplastic lesions.**

Expression of CK19:
The cellular localization of immunoreactivity with CK19 was **cytoplasmic staining with a frequent enhancement of the adjacent cell membrane.**

A section of the **Conventional-PTC** case was taken as a **positive control for CK 19.**

Expression of Cd56:
The cellular localization of immunoreactivity with CD56 was a **membranous expression with or without cytoplasmic staining** of the cell.

The normal thyroid tissue section was taken as a **positive control for CD 56.**

Negative controls:
Negative controls were done by excluding the primary antibody and its replacement with PBS.

Scoring for the immunohistochemical markers
A semi-quantitative assessment of immunohistochemical scoring was performed. For both the antibodies, immunoreactivity was considered positive if >10% of follicular epithelial cells were stained.

Table 1: Scoring of immunohistochemical markers.

score	Immunoreactivity (% of cells with positive expression)
0	Positive staining in < 10% of cells.
+1	Positive staining in 10-25% of the cells
+2	Positive staining in 26-50% of the cells
+3	Positive staining in > 50 of the cells.

Score 0 is considered as negative, and score 1 to 3 is considered as positive.

Table 2: Properties of the primary antibodies used in the present study.

Antigen	Clone	Source	Dilution	Supplier
Cytokeratin19	RCK 108	Mouse mAb	Ready to use	DAKO
Cluster of differentiation CD 56	123C3	Mouse mAb	Ready to use	DAKO

mAb – monoclonal antibody

Figure 1: Monoclonal mouse anti-human antibodies CK19 and Cd56.

RESULTS

Table 3: Distribution of thyroid neoplasms

Histopathology	Frequency	Percent (%)
Benign	30	60.0
Malignant	20	40.0
Total	50	100.0

In our study, out of 50 cases of thyroid neoplasms, benign thyroid neoplasms were the most common neoplasms accounting for 30 cases (60%)followed by malignant neoplasms accounting for 20cases (40%).

Table 4: Distribution of both benign and malignant thyroid neoplasms concerning gender.

Sex	Histopathology				Total	
	Benign		Malignant			
	Count	%	Count	%	Count	%
Female	26	86.7%	15	75.0%	41	82.0%
Male	4	13.3%	5	25.0%	9	18.0%
Total	30	100.0%	20	100.0%	50	100.0%

Out of 50 cases of thyroid neoplasms, female preponderance was seen in 41 cases accounting for 82%.

Table 5: Distribution of both benign and malignant thyroid neoplasms concerning age.

Age	Histopathology				Total	
	Benign		Malignant			
	Count	%	Count	%	Count	%
21-30	13	43.3%	4	20.0%	17	34.0%
31-40	14	46.7%	6	30.0%	20	40.0%
41-50	1	3.3%	4	20.0%	5	10.0%
51-60	2	6.7%	3	15.0%	5	10.0%
61-70	0	0.0%	3	15.0%	3	6.0%
Total	30	100.0%	20	100.0%	50	100.0%

Out of 50 cases of thyroid neoplasms, most of the cases were in the age group of 31-40 accounting for 20 cases (40%).

Table 6: Distribution of thyroid status in both benign and malignant neoplasms

Thyroid Status	Histopathology				Total	
	Benign		Malignant			
	Count	%	Count	%	Count	%
Euthyroid	28	93.3%	10	50.0%	38	76.0%
Hyperthyroid	1	3.3%	0	0.0%	1	2.0%
Hypothyroid	1	3.3%	10	50.0%	11	22.0%
Total	30	100.0%	20	100.0%	50	100.0%

Out of 50 cases of thyroid neoplasms, 38 cases (76%) were in the euthyroid state.

Table 7: Distribution of thyroid neoplasms and their variants

Histopathology	Frequency	Percentage
C-PTC	7	14.0
C-PTC with HT	4	8.0
E-PTC	1	2.0
MPTC	1	2.0
FV-PTC	3	6.0
FTC	4	8.0
FA	25	50.0
FA with HT	3	6.0
HCA	2	4.0
Total	50	100.0

Table 8: Distribution of papillary thyroid carcinoma and its variants

PTC	Frequency	Percent
Conventional – PTC (C-PTC)	11	68.8
Encapsulated – PTC (E-PTC)	1	6.3
Follicular Variant – PTC (FV-PTC)	3	18.8
Micro Papillary Thyroid Carcinoma (MPTC)	1	6.3
Total	16	100.0

Out of 16 cases of PTC, the Conventional/ Classic variant of PTC was the most common variant accounting for 11 cases (68.8%).

Table 9: Expression of CK19 in various thyroid neoplasms.

Histopathology	CK19								Total	
	1+		2+		3+		Ng			
	Count	%	Count	%	Count	%	Count	%	Count	%
C-PTC	0	0.0%	0	0.0%	7	46.7%	0	0.0%	7	14.0%
C-PTC HT	0	0.0%	0	0.0%	4	26.7%	0	0.0%	4	8.0%
E –PTC	0	0.0%	0	0.0%	1	6.7%	0	0.0%	1	2.0%
FTC	1	20.0%	1	14.3%	0	0.0%	2	8.7%	4	8.0%
FV-PTC	0	0.0%	1	14.3%	2	13.3%	0	0.0%	3	6.0%
MPTC	0	0.0%	0	0.0%	1	6.7%	0	0.0%	1	2.0%
FA	1	20.0%	5	71.4%	0	0.0%	19	82.6%	25	50.0%
FA with HT	1	20.0%	0	0.0%	0	0.0%	2	8.7%	3	6.0%
HCA	2	40.0%	0	0.0%	0	0.0%	0	0.0%	2	4.0%
Total	5	100.0%	7	100.0%	15	100.0%	23	100.0%	50	100.0%

Table 10: CK19 immunoreactivity in thyroid neoplasms with histopathology correlation.

Histopathology	Ck19				Total
	1+	2+	3+	Ng	
Benign	4	5	0	21	30
	13.3%	16.7%	0.0%	70.0%	100.0%
Malignant	1	2	15	2	20
	5.0%	10.0%	75.0%	10.0%	100.0%
Total	5	7	15	23	50
	10.0%	14.0%	30.0%	46.0%	100.0%
Chi-square value = 33.10; df = 3; P<0.001					

Table 11: Association between histopathological diagnosis and immunohistochemical diagnosis. concerning CK19 expression.

CK19	Histopathology		Total
	Malignant	Benign	
Malignant	18	9	27
	90.0%	30.0%	54.0%
Benign	2	21	23
	10.0%	70.0%	46.0%
Total	20	30	50
	100.0%	100.0%	100.0%

Table 12: Expression of CD56 in thyroid neoplasms.

Histopathology	Cd56								Total	
	1+		2+		3+		Ng			
	Count	%	Count	%	Count	%	Count	%	Count	%
C-PTC	2	66.7%	0	0.0%	0	0.0%	5	29.4%	7	14.0%
C-PTC HT	0	0.0%	0	0.0%	0	0.0%	4	23.5%	4	8.0%
E-PTC	0	0.0%	0	0.0%	0	0.0%	1	5.9%	1	2.0%
FTC	1	33.3%	0	0.0%	0	0.0%	3	17.6%	4	8.0%
FV-PTC	0	0.0%	1	25.0%	0	0.0%	2	11.8%	3	6.0%
MPTC	0	0.0%	0	0.0%	0	0.0%	1	5.9%	1	2.0%
FA	0	0.0%	2	50.0%	22	84.6%	1	5.9%	25	50.0%
FA with HT	0	0.0%	1	25.0 %	2	7.7%	0	0.0%	3	6.0%
HCA	0	0.0%	0	0.0%	2	7.7%	0	0.0%	2	4.0%
Total	3	100.0 %	4	100.0 %	26	100.0 %	17	100.0 %	50	100.0 %

Table 13: CD56 immunoreactivity in thyroid neoplasms with histopathology correlation.

Histopathology	Cd56				Total
	1+	2+	3+	Ng	
Benign	0	3	26	1	30
	0.0%	10.0%	86.7%	3.3%	100.0%

Malignant	3	1	0	16	20
	15.0%	5.0%	0.0%	80.0%	100.0%
Total	3	4	26	17	50
	6.0%	8.0%	52.0%	34.0%	100.0%
Chi-square value = 42.95; df = 3; P<0.001					

Table 14: Association between histopathological diagnosis and immunohistochemical diagnosis concerning CD56 expression.

Cd56	Histopathology		Total
	Malignant	Benign	
Malignant	16	5	21
	80.0%	16.7%	42.0%
Benign	4	25	29
	20.0%	83.3%	58.0%
Total	20	30	50
	100.0%	100.0%	100.0%

Table 15: Association between histopathological diagnosis and immunohistochemical diagnosis by using combined CK19 and CD56 expression.

CK19+CD56	HP		Total
	Malignant	Benign	
Malignant	20	1	21
	100.0%	3.3%	42.0%
Benign	0	29	29
	0.0%	96.7%	58.0%
Total	20	30	50
	100.0%	100.0%	100.0%

Table 16: Tumour Markers & its Sensitivity, Specificity, PPV, NPV, and Diagnostic accuracy

Type of Marker	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
CK19	90%	70%	66.67%	91.30%	78%
CD56	80%	83.3%	76.2%	86.20%	82%
CK19+CD56	100%	96.7%	95.3%	100%	98%

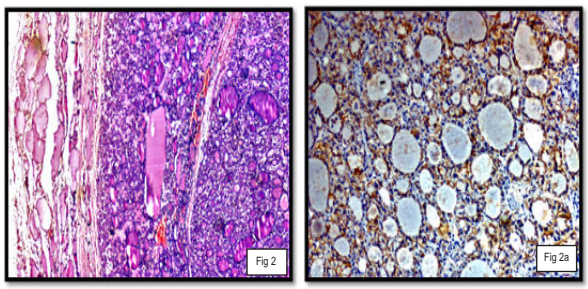

Figure 2: H&E stained photomicrograph (4x magnification) showing Follicular adenoma with adjacent compressed normal thyroid.

Figure 2a: Photomicrograph (10x magnification) showing Follicular adenoma with diffuse and strong / score +3 CD56 membranous positivity in the follicular epithelial cells.

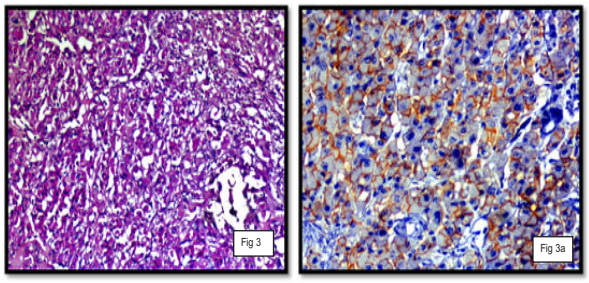

Figure 3: H&E stained photomicrograph (10x magnification) showing Hurthle cell adenoma composed of cells with abundant eosinophilic granular cytoplasm with small round nuclei.

Figure 3a: Photomicrograph (10x magnification) showing Hurthle cell adenoma with diffuse and strong / score +3 membranous positivity for Cd56.

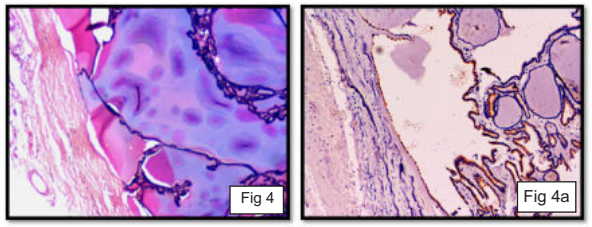

Figure 4:H&E stained photomicrograph showing macrofollicular variant of Follicular adenoma (10x magnification).

Figure 4a: Photomicrograph (10x magnification) showing macrofollicular variant of FA with focal and weak cytoplasmic CK19 positivity

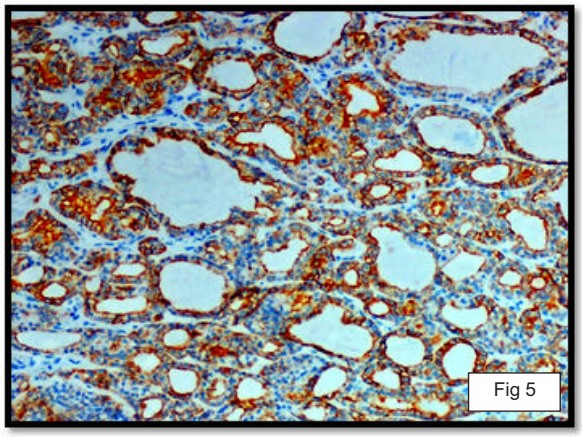

Figure5: Photomicrograph (4x magnification) showing FV-PTC with diffuse and strong/score +3 CK19 expression.

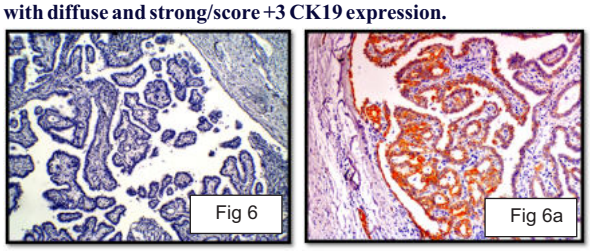

Figure 6: Photomicrograph (4x magnification) of conventional papillary thyroid carcinoma showing negative expression for Cd56.

Figure 6a: Photomicrograph (4x magnification) showing conventional papillary thyroid carcinoma with diffuse and strong cytoplasmic expression for Ck19.

DISCUSSION:

The results obtained from the present study were compared with the similar studies available in the literature.

In the present study, a total of 50 thyroid neoplasms were studied, benign thyroid neoplasms were the most commonly encountered accounting for 30 cases (60% of total thyroid neoplasms) followed by malignant thyroid neoplasms accounting for 20 cases (40% of total thyroid neoplasms) shown in table 3. Similar findings were observed with the study by Ijomone E.A. et al.[13]

Gender distribution (shown in table 4)
Out of 50 cases, 41 cases (82%) were showed female preponderance and the remaining 9 cases (18%) were showed male preponderance. Of these 86.7% (26 /30 cases) of benign neoplasms and 75% (15/20 cases) of malignant neoplasms showed female preponderance. In our study, female preponderance was observed with male to female ratio was 1:4.5. Our study finding is comparable with the studies by Ijomone E.A. et al.[13] from Southern Nigeria, Lanier A.P., et al.[14] from the USA and Ahmed et al. [15] from Saudi where female to male ratios were 5:1, 2.5:1, and 1.01: 1.08 respectively.

Age distribution (shown in table 5)
Our study includes cases as young as 21 years old female and as old as 68 years old female. Of 50 cases of thyroid neoplasms, the majority

occurred between the age group of 31-40 accounting for 40% (20/50 cases) followed by 21-30 years accounting for 34% (17/50 cases).

Benign thyroid neoplasms showed peak age incidence in the age group of 31-40 years accounting for 46.7% (14/30 cases). Similar findings were reported in the studies of Anidi et.al. [16] in Enugu and Ijomone E.A.et al [13] in Southern Nigeria where the peak age incidence was respectively in the fourth and fifth decades of life.

Malignant thyroid neoplasms displayed peak age occurrence between the age group of 31-40 years accounting for 30% (6/20 cases). This is in concordance with the study by O.O. Ariyibi et al.[17] in Nigeria.

Thyroid status in both benign and thyroid malignant neoplasms (shown in table 6)

In the present study out of 30 cases of benign neoplasms 28 cases were found to be in euthyroid status accounting for 93.3% followed by hyperthyroid and hypothyroid status was each one case. Out of 20 cases of malignant neoplasms, 10 cases were in euthyroid status and the remaining 10 cases show hypothyroid status (elevated TSH levels).

In the present study, benign neoplasms were associated with euthyroid status, and the likelihood of thyroid cancer increases with higher serum TSH concentration (hypothyroid status). Similar findings were observed with a study by Haymart MR et al.[18]

Benign neoplasms (shown in table 7)
In the present study, benign neoplasms represented 60% (30/50 cases). A follicular adenoma is the most common tumor encountered accounting for 28 cases and followed by hurthle cell adenoma, 2 cases. in our study, similar study findings were observed by Ijomone E.A.et al [13]where benign neoplasms account for 52.4% and study by S Gole et al.[19] where a total of 246 cases of thyroid neoplasms were studied for 12 years, out of which benign neoplasms account for 71.54% (176/246 cases).

Malignant neoplasms (shown in table 7, 8)
Out of 50 cases, malignant neoplasms account for 20 cases (40% of total thyroid neoplasms). Present study findings were comparable with the study by Ijomone E.A.et [13] and S Gole et al.[19] where malignant neoplasms account for47.6%, 28.45% respectively.

Papillary thyroid carcinoma was the most common thyroid malignancy, accounting for 32% of total thyroid neoplasms (16/50 cases), and was the most common thyroid malignancy accounting for 80% (16/20 cases). Present study findings were comparable with studies by Ariyibi o.o. et al.[17] from Nigeria, Lanier A.P.et al.[14] from USA, Khan et al.[20] from Rawalpindi, Ahmed et al [15] from Saudi Arabia where the percentage of occurrence of papillary thyroid carcinoma was 41.8%, 90%, 60%, 80% respectively.

Criteria used for the diagnosis of papillary carcinoma included nuclear features (ground glass nuclei, grooving, pseudo inclusions, and nuclear overlapping), papillary patterns, psammoma bodies, lymphocytic infiltrate, tumor encapsulation along with more specific features such as clear cells, tall cells, columnar cells, hurthle cell change, etc.

In the present study, out of 16 cases of PTC, 4 cases(4/16 cases, 25%) have been associated with Hashimoto's thyroiditis. Patil PV et al. [21] reported that the frequency of papillary thyroid carcinoma in Hashimoto's thyroiditis varies between 0.5% to 23.7% and Kollur SM et al.[22] the study had shown the incidence rate of Hashimotos thyroiditis coexistent with thyroid neoplasm was 15%. A peritumoral inflammatory response is not designated as Hashimoto's thyroiditis or lymphocytic thyroiditis.

In the present study, out of 50 cases of thyroid neoplasms, 7 cases (14%) were associated with Hashimotos thyroiditis. Out of 7 cases, 4 cases were C-PTC and 3 cases were follicular adenoma. Presents study findings were compared with Kollur SM et al. study.[22]

Papillary thyroid carcinoma and its variants: (shown in table 8)
Of 16 cases of PTC, 11 cases(68.8%) were classic/ conventional PTC, 3 cases (18.8%) of follicular variant of PTC(FV-PTC) followed by Encapsulated PTC and Micro papillary thyroid carcinoma (MPTC) each one case (6.25%) respectively. The majority of papillary thyroid carcinomas fit into classical papillary carcinoma (11/16 cases), very much similar to the study by Muhammad Muzaffar et al. [23]and the second most common variant of PTC was FV- PTC, which is somewhat lower when compared with a figure of 31.6% incidence found in a study carried out by Lin HW et al.[24]

Follicular thyroid carcinoma
In our study out of 20 cases of malignant thyroid neoplasms, 4 cases were follicular carcinoma accounting for 20% and 8% of total thyroid neoplasms (50 cases of total thyroid neoplasms). Present study findings were comparable with studies by Ariyibi o.o. et al [17], Khan et al. [20] and Woolner et al.[25] were percentages of occurrence of follicular thyroid carcinomas were 32%, 24%, and 17.2% respectively.

Ck19 expression in the studied thyroid neoplasms: (shown in table 9-11, 16,18)

Among 30 cases of benign thyroid neoplasms positive expression was observed in only 9/30 cases (30%), among which score +1 expression was observed in two cases of follicular adenoma and two cases of HCA. Five cases of follicular adenoma had shown a score of +2 expression. None of the benign thyroid neoplasms had shown score +3 expression. The remaining 21/30 cases (70%) had shown negative expression, score 0. In all the 9/30 cases of benign thyroid neoplasms the expression is focal and patchy staining pattern, we did not find any strong CK19 positivity in any of the benign thyroid neoplasms.

Among 20 cases of malignant thyroid neoplasms, positive expression was observed in 18/20 cases (90%), which includes all the cases of classic/conventional PTC – 11/20 cases (score +3 expression), all the 3 cases of FV-PTC (one case with score +2 expression and 2 cases with score+3 expression), E-PTC and MPTC, each case had shown score +3 expression and 2/4 cases of FTC also had shown positive expression (one case with +1 expression and other cases with +2 expression). The remaining 2 cases of FTC had shown negative expression. None of the PTC cases had shown negative expression. All the 16 cases of PTC independent of its variant had shown positive expression.

The relationship between the benign and malignant groups was statistically significant (P<0.001). In the present study, the sensitivity and specificity of CK19 in differentiating malignant and benign thyroid neoplasms were 90% and 70% respectively. Present study findings were compared with other studies shown in table 17.

Table 17: Comparison of IHC marker CK19 sensitivity and specificity in thyroid neoplasms, with other studies.

Study	Ck19 Sensitivity	CK19 Specificity
Barroeta et al.- 2006 [26]	100%	70%
Park YJ, et al.-2007 [27]	90%	83%
Liu YY et al.-2008 [28]	56%	98%
Barut F, et al.- 2010 [29]	92%	78%
Song Q et al.-2011 [30]	96%	74%
Nechifor-Boilă A et.al-2014 [11]	45%	100%
Dunderovic et al.-2015 [31]	75%	71%
Present study	90%	70%

Table 18: Sensitivity, Specificity, PPV, NPV, and Diagnostic accuracy of CK19 marker.

Type of marker	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
CK19	90%	70%	66.67%	91.30%	78%

In the present study, the chief utility of CK19 lies in its highest sensitivity for PTC. All most all cases of PTC independent of its variant had shown diffuse and strong /score + 3 expressions except one case which had shown score+2 expression and none of the cases had shown negative expression. Hence negative staining for CK19 is strong evidence against PTC.

Among benign neoplasms, the majority of follicular adenomas 21/30 cases had shown negative/absent expression, while remaining cases of follicular adenomas had shown focal and weak expression. Follicular carcinomas also showed a variable degree of CK 19 immunoreactivity. In follicular adenomas and follicular carcinomas, none of the cases had shown score +3 / diffuse and strong CK 19 expression.

Because of lower specificity, its immunoreactivity in follicular lesions may limit its utility as a diagnostic marker. Hence, in the above-mentioned studies and our analyses CK-19 alone was not useful in

differentiating the follicular thyroid lesions.

CD 56 expression in the studied thyroid neoplasm: (shown in table 12-14, 16,20)

Among 30 cases of benign thyroid neoplasms, positive expression was observed in 29/30 cases (96.7%), among which 24 cases of follicular adenoma and 2 cases of hurthle cell adenoma had shown score +3 expression, whereas 3 cases of follicular adenoma had shown score +2 expression. Only one case of follicular adenoma had shown negative expression.

Among 20 cases of malignant thyroid neoplasms, positive expression was observed in 4/20 cases (20%), which includes 2 cases of C- PTC which had shown focal and patchy staining score +1 immunoreactivity. one case of follicular thyroid carcinoma, and one case of FV- PTC had shown score+1 and +2 expression respectively. None of the malignant neoplasms had shown a score of +3 CD56 expression. Negative expression was observed in 16/20 cases (80%), which includes 13 cases of PTC, 3 cases of FTC, and one case of FA.

CD 56 has a statistically significant p-value ($P<0.001$) in differentiating the malignant and benign neoplasms. In the present study sensitivity and specificity of CD56 in differentiating malignant and benign thyroid neoplasms were 80% and 83.3% respectively.

The present study findings correlated with a study by Alshenawy H.A.[32] where Sensitivity and specificity were 80% and 90% respectively. (other comparative studies were shown in table 19).

Table 19:Comparison of IHC marker CD56 sensitivity and specificity in thyroid neoplasms with other studies.

Study	Cd56 Sensitivity	CD56 specificity
Hanan Alsaeid Alshenawy[32]	80%	90%
HENG MA et al.[33]	79.1%	100%
Nechifor –Boila A et.al[11]	81.8%	63.6%
Mi Kyung Shin et. al [34]	95%	72.73%
Present study	80%	83.3%

In the present study, CD56 showed an absent expression in the majority of the PTC cases accounting for 81.25% (13/16 cases). Almost all cases of benign thyroid neoplasms 29/30 cases (96.7%) had shown diffuse and strong CD 56 immunoreactivity. In our study, diffuse and strong CD56 positivity indicates the benign nature, and the loss of expression of CD56 indicates the malignant nature of the lesion.

Recently, El Demellawy et al.[32,22] has suggested that CD56 is of great value in selecting PTC from other follicular cell-derived thyroid lesions/tumors, with a 100% sensitivity and a 100% specificity.

Table 20: Sensitivity, Specificity, PPV, NPV, and Diagnostic accuracy of CD56 marker

Type of marker	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
CD56	80%	83.3%	76.2%	86.2%	82%

In the present study, CD56 was found to be the most specific marker and valuable negative diagnostic marker for distinguishing PTC from benign thyroid lesions. Strong and diffuse expression of CD56 indicates the benign nature of the lesion.

Combined marker (CK19+CD56) expression in thyroid neoplasms and their diagnostic value*:* **(**shown in table 15, 16)

In the present study, CK19 had shown high sensitivity and low specificity and CD56 had shown low sensitivity and high specificity, thus to improve the overall diagnostic accuracy of these markers**, the** present study aimed to analyze the sensitivity and specificity of these combined markers CK19+CD56 to differentiate benign and malignant thyroid neoplasms (shown in table 15,16 and 21). Comparison of combined marker CK19+CD56 sensitivity and specificity with other studies were given in table 22.

Table 21: Sensitivity, Specificity, PPV, NPV, and Diagnostic accuracy of combined markers CK19+CD56 in differentiating benign and malignant thyroid neoplasms:

Type of marker	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
CK19+CD56	100%	96.7%	95.3%	100%	98%

Table 22: Comparison of combined marker CK19+CD56 sensitivity and specificity with other studies.

Study	(Ck19+CD56) Sensitivity	(Ck19+CD56) Specificity
Present study	100%	96.7%
Nechifor –Boila A et.al[11]	90.9%	63.3%
Hanan Alsaeid Alshenawy[32]	70%	82%

In the present study, the use of combined markers (CK19+CD56) improves the sensitivity, specificity, and diagnostic accuracy to 100%, 96.7%, and 98% respectively, which is higher when compared to sensitivity, specificity, and diagnostic accuracy of each marker alone is used in differentiating benign and malignant thyroid neoplasms of follicular epithelial cell origin.

SUMMARY:

In the present study, benign thyroid neoplasms are the most common than malignant thyroid neoplasms. Most thyroid tumors are observed in the age group of 31 - 40 years. Females were commonly affected than males with the male to female ratio being 1:4.5. A follicular adenoma is the most common benign thyroid neoplasm and papillary thyroid carcinoma is the most common thyroid malignancy. The classical/conventional type of PTC was the most common variant encountered.

CK19 expression:

- In the present study, CK19 has the highest sensitivity 90%. All the cases of papillary thyroid carcinoma had shown diffuse and strong positive expression. A negative expression is a good evidence against PTC.
- Variable CK 19 immunoreactivity was observed in some of the benign and malignant follicular thyroid neoplasms; Because of its lower specificity, the expression of CK19 in follicular lesions may limit their utility as a diagnostic marker, hence CK-19 alone was not useful in differentiating the follicular thyroid neoplasms.

CD56 expression:

- In the present study, most of the benign thyroid neoplasms had shown positive expression, reported that strong and diffuse expression of CD56 indicates the benign nature of the lesion.
- In the present study, the majority of the malignant thyroid neoplasms had shown negative expression, reported that weak/absent expression of CD56 indicates the malignant nature of the lesion. CD56 serves as a negative diagnostic marker for distinguishing PTC from benign thyroid lesions with morphologically overlapping features.

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In conclusion, whereas no single marker has a greater diagnostic value, a combination of markers may be useful for more precise diagnosis in challenging cases. In this study, using combination markers (CK19and CD56) increased sensitivity, specificity, and diagnostic accuracy to 100%, 96.7 percent, and 98 percent, respectively, compared to using a single marker alone.

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