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Research Article

NIFTP Categorization of the Thyroid Lesions, A New Concept Versus Papillary Carcinoma with IHC Markers (HBME-1, CK-19, CD56 AND P63)

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List of Abbreviations

PTC – Papillary Carcinoma Thyroid

NIFTP– Non-invasive Follicular neoplasm with papillary Like Nuclear Features

FT – UMP – Follicular Tumor with Uncertain Malignant Potential

WDT– UMP – well Differentiated Tumor with Uncertain Malignant potential

H and E – Haematoxylin and Eosin

IHC – Immunohistochemistry

CK – 19 - Cytokeratin - 19

HBME –1 - Hector Battifora Mesothelial - 1

ICD – International Classification of Diseases

EPON - Encapsulated papillary oncocytic neoplasms

+VE - Positive

-VE - Negative

Introduction

Thyroid malignancy is the most common endocrine malignancy.[1] Majority of thyroid tumors are primary and epithelial. They are preferably divided into 3 major categories depending on the cell types involved.

1. Tumors exhibiting follicular cell differentiation
2. Tumors exhibiting C-cell differentiation
3. Tumors exhibiting follicular and C-cell differentiation

Papillary carcinoma thyroid is well differentiated tumor, derived from follicular epithelial cells which may or may not be surrounded by a capsule. It is defined by the presence of distinctive nuclear features (PTC-type) and require presence of either papillae or invasion of the surrounding parenchyma.

Whereas NIFTP is entirely surrounded by a capsule or well circumscribed with a smooth contour facing the adjacent parenchyma. It has follicular growth pattern with no papillae but the nuclear alterations are widespread and well developed.[2]

Accurate classification of follicular pattern thyroid lesions is not always an easy task on routine H AND E stain due to overlapping histomorphology. Hence, NIFTP is often under-diagnosed as follicular adenoma or over-diagnosed as encapsulated variant of PTC.[3]

This study focuses on preventing such misdiagnosis using immunohistochemical markers – HBME-1, CK-19, p63 AND CD56.

Aims and Objectives

1. To categorize NIFTP from PTC
2. To evaluate expression of HBME-1, CK-19, p63 AND CD56 in NIFTP and PTC.
3. To prevent misdiagnosis of NIFTP.

Methodology

Total of 80 total thyroidectomy and hemithyroidectomy specimens submitted to the department of Pathology, SVS Medical College, Mahabubnagar for histopathological evaluation.

The period of prospective and retrospective study was from OCTOBER 2018 to September 2021.

Inclusion Criteria:

40 cases each, diagnosed as PTC and NIFTP on histopathological examination.

Exclusion Criteria:

Benign neoplasms and other malignancies f thyroid were excluded, since the target study is 80 cases (all females), all the cases beyond 80 were excluded in view of IHC limitations.

Clinical data was obtained from the patient's outpatient and inpatient records and requisition forms accompanying the specimens to the department.

On arrival to the department, the specimens were adequately fixed in 10% neutral buffer formalin followed by the evaluation of gross features.

The gross details of specimen submitted for evaluation of malignancy were observed and recorded.

Then the representative tissue from thyroidectomy specimen was subjected to routine processing of paraffin embedding.

Four to five micron thick sections were taken from paraffin embedded blocks, stained with haematoxylin and eosin [H and E] stain and studied.

Representative tissue bits were taken from the original blocks and a tissue microarray was assembled for the study of IHC markers

Procedure for Hematoxylin and Eosin Staining

- Deparaffinise in Xylene – 2 changes – 5 minutes each.
- Wash in absolute alcohol – 1 change – 3 minutes.
- Wash in water for 3-5 minutes.
- Stain with haematoxylin for 5 minutes. o Place in running tap water for bluing for 3-5 minutes.
- Dip in acid alcohol – 1dip.
- Wash in water for 3-5 minutes.
- Stain with eosin for 1 – 2 dips.
- Wash in water for 1-2 dips.
- Dip in alcohol – 1dip.
- Blot, dry and mount in DPX.

Preparation of Reagents for IHC

Tris EDTA Buffer (Antigen Retrieval)

- TRIS – 1.21gms (Hydroxymethylemethylamine)
- EDTA - 370mgs
- Distilled water – 1000ml (pH 9)

TBS Buffer (Wash Buffer)

- TRIS buffer solution – 5ml
- Distilled water – 95ml
- Substrate buffer – 1ml
- DAB reagent - 20µl

Procedure for IHC Staining

- Deparafinisation in xylene
- Rehydration in graded alcohols followed by tap water and distilled water wash.
- Transfer the TRIS buffer to microwave oven bowl and boil at 800watts for 5 min.
- Transfer the slides into boiled TRIS buffer and boil at 800watts for 5min, 640 watts for 10min and 480 watts for 5min
- Remove the slides from the microwave and bring them to room temperature.
- Wash them with distilled water. o Wash the slides with wash buffer.
- Peroxidase blocking is done for 15min
- Wash with wash buffer twice for 3min each time.
- Cover the whole section with primary antibody for 30min
- Wash with wash buffer for 3min
- Cover the whole slide with secondary antibody (HRP) for 30min
- Wash with wash buffer twice for 3min each time.
- Cover the entire slide with DAB for 10min
- Wash with wash buffer followed by tap water
- Counter stain with hematoxylin for 30seconds
- Wash with tap water followed by liquor ammonia
- Dehydration with graded alcohols
- Clearing with xylene
- Mount with DPX
- Examine under microscope

Procedure for Manual Tissue Microarray

- Donor block of tissue was taken and the tumor area was marked
- Punch biopsy needle of bore size 8 was taken and the representative tissue from the donor block was separated.
- The first tissue in each block was marked as an index tissue for identification.

- The tissue bits were placed in the recipient block with proper labelling and numbering.
- 20 tissue bits were taken in each block in a serial order.
- Embedding was done carefully while retaining the position of each tissue bit.

Assessment of Expression of IHC Markers

S.NO.	GRADING	PERCENTAE OF CELLS EXPRESSING HBME-1
1.	0	0%-10%
2.	1+ (focal)	10%-50%
3.	2+ (diffuse)	>50%

Table 1: HBME-1 Scoring pattern

S.No	Grading	Percentage of cells expression CK 19
1	0(negative)	No positively stained cells
2	1+ (focal positive)	<25% of positively staining cells
3	2+ (positive)	25 – 50% of positively staining cells
4	3+ (diffuse positive)	>50% positively staining cells

Table 2: CK-19 Scoring pattern

S.No.	Score	Intensity
1.	0	Nil
2.	1+	Weak
3.	2+	Moderate
4.	3+	Strong

Table 3: CD56 and p63 Scoring pattern

Observation and Results

The present study was conducted in the department of pathology, SVS Medical college, Mahabubnagar. 80 Thyroidectomy specimens were evaluated from October 2018 to September 2021 . Of these 80 cases, 40 cases were diagnosed as PTC and the other 40 cases were diagnosed as NIFTP on routine HPE.

Age group of study included, PTC between 20 and 69 years of age where as NIFTP between 18 and 50 years of age.

AGE IN YEARS	FREQUENCY	PERCENTAGE
11-20	1	2.5%
21-30	11	27.5%
31-40	12	30%
41-50	09	22.5%
51-60	03	7.5%
61-69	04	10%
Total	40	100%

Table 4: Age Distribution of PTC

In the present study majority of PTC cases were seen in the 3rd and 4th decades (47.5%) where as least number of cases were seen in 2nd decade.

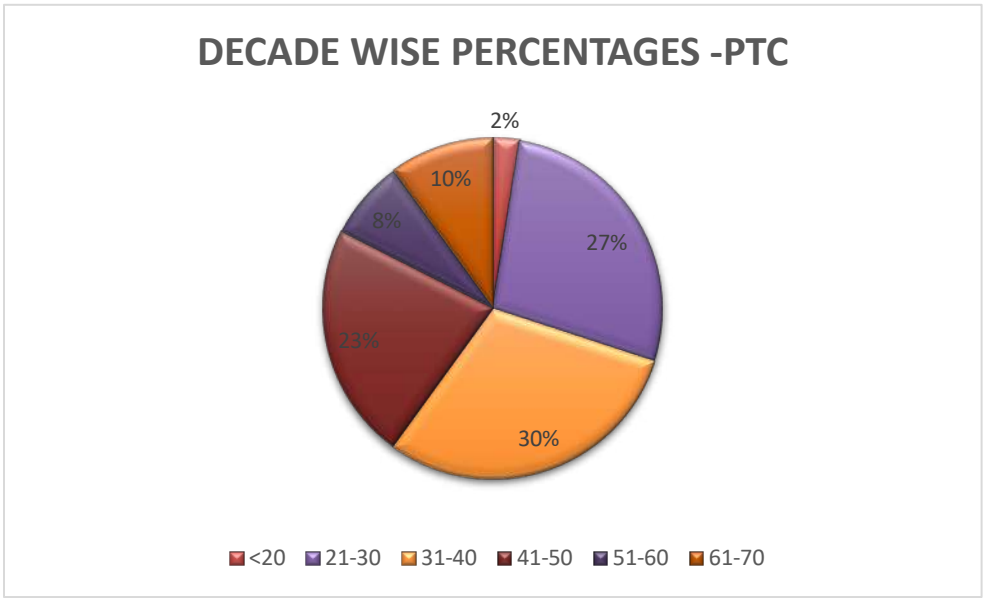

Figure 1: Pie diagram showing decade wise distribution of PTC

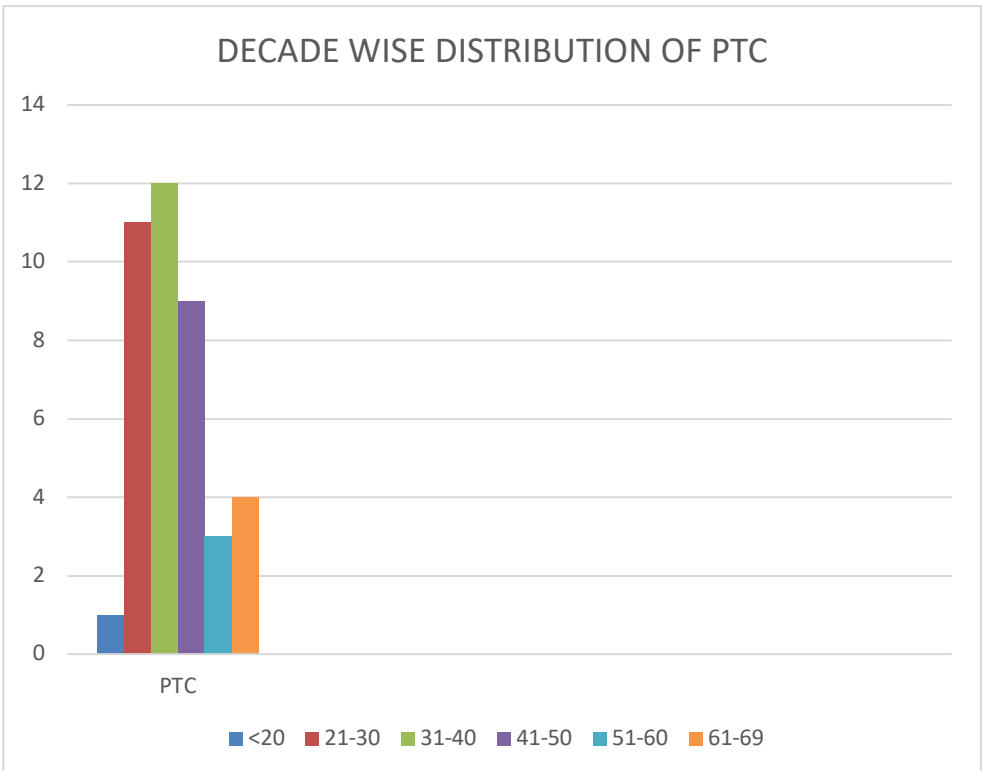

Figure 2: Clustered columns showing age distribution of PTC, most common in 4th decade followed by 3rd decade

AGE IN YEARS	NO. OF CASES	PERCENTAGE
11-20	03	7.5%
21-30	18	42.5%
31-40	14	35%
41-50	05	12.5%
Total	40	100%

Table 5: Age distribution of NIFTP

In the present study majority of NIFTP cases were seen in the 3rd decade (42.5%) where as least number of cases were seen in 2nd decade.

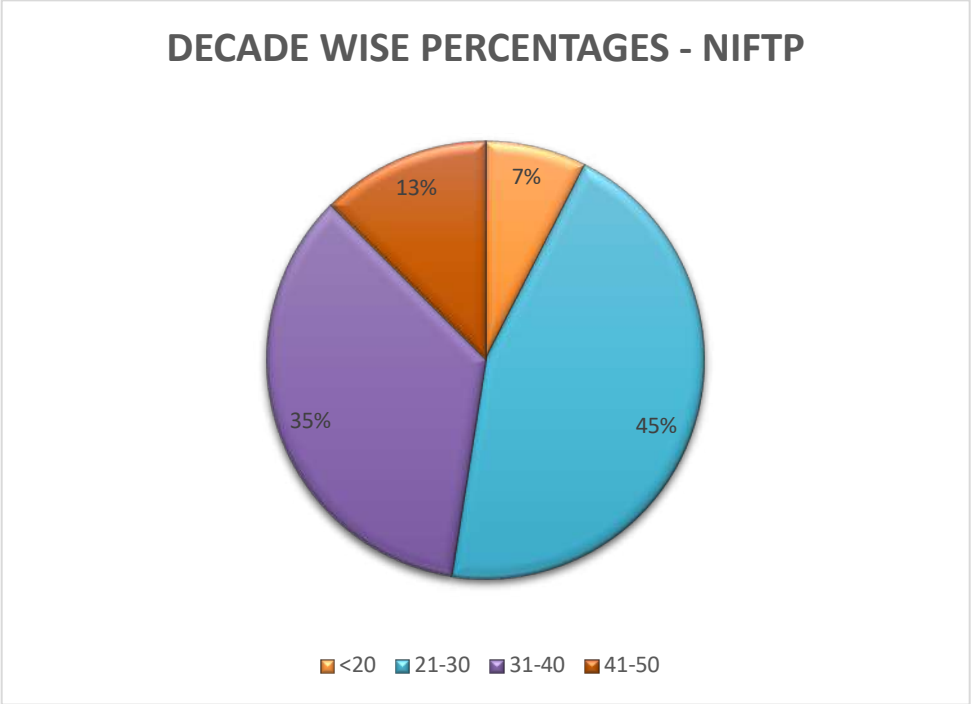

Figure 3: Pie diagram showing decade wise distribution of NIFTP

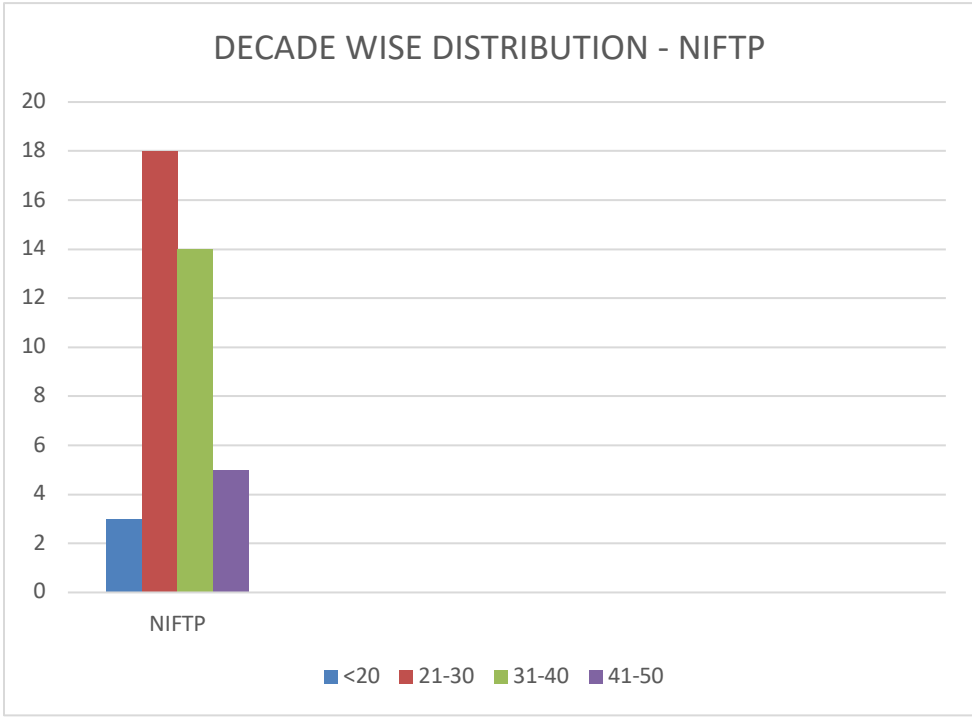

Figure 4: Clustered columns showing age distribution of NIFTP, most common in 3rd decade followed by 4th decade

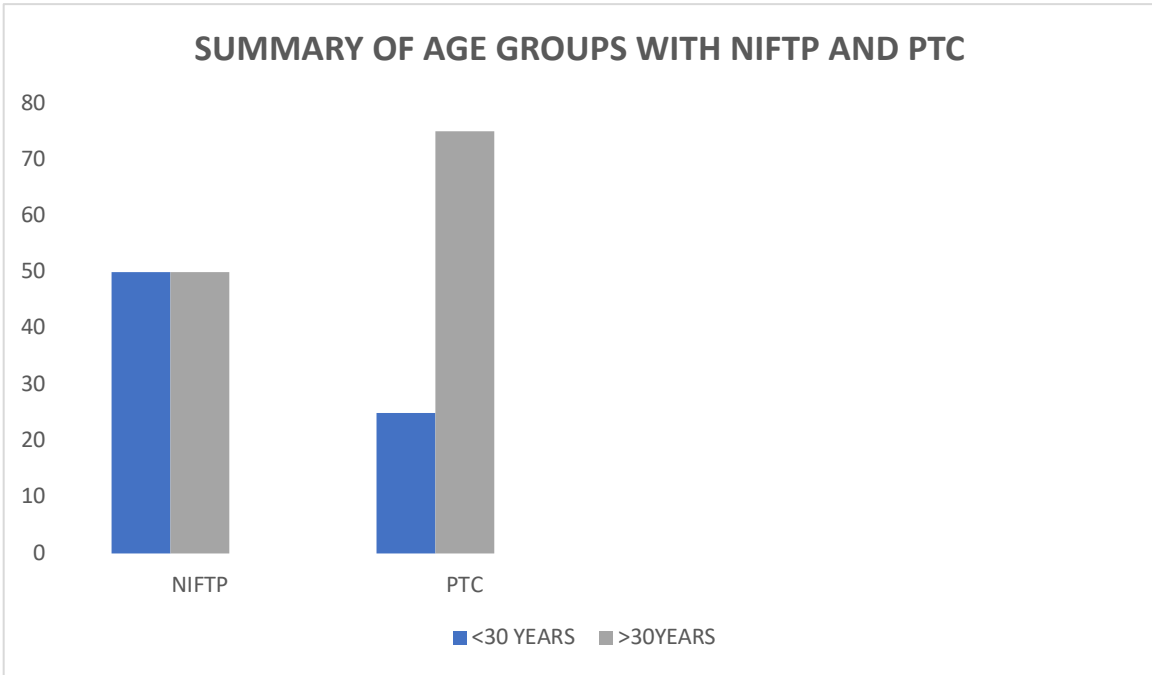

Figure 5 : Showing incidence of NIFTP and PTC before and after 3rd decade

The above chart shows 50% females with NIFTP appeared to be below 30 years of age where as only 25% females of PTC were under that age group.

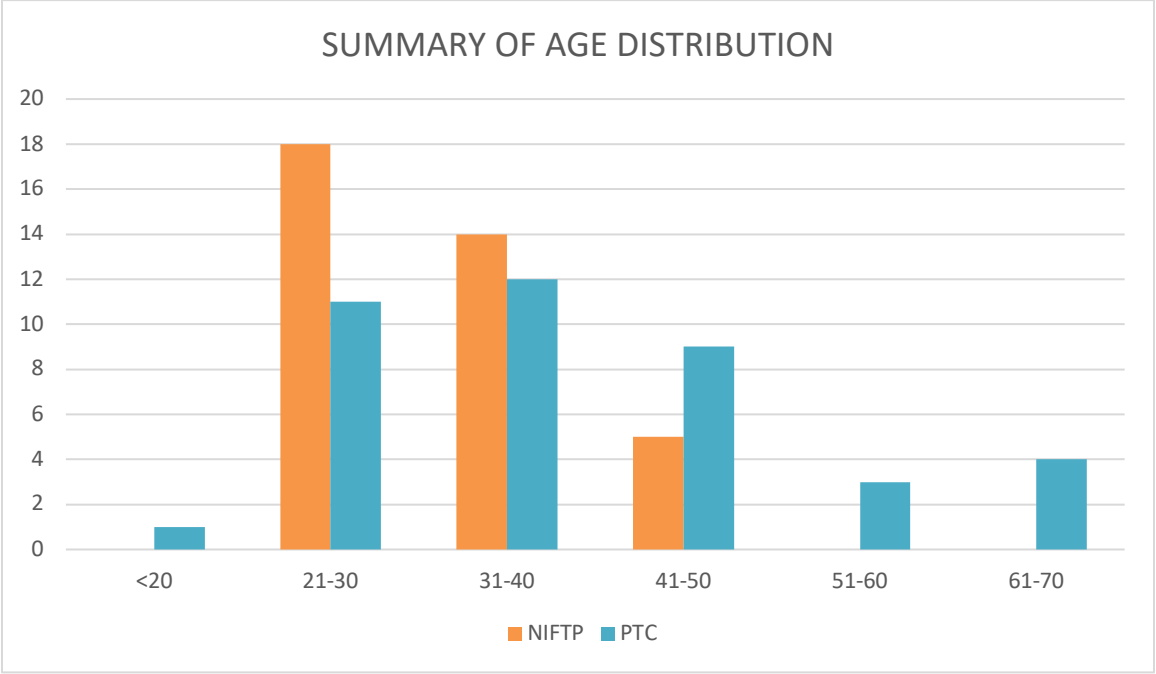

Figure 6: Summary of age groups with NIFTP and PTC.

The above chart shows that NIFTP cases were more in 3rd decade followed by 4th decade whereas PTC cases were more in 4th decade followed by 3rd decade.

TUMOR TYPE	NO. OF POSITIVE CASES	NO. OF NEGATIVE CASES
NIFTP	8 with 1+	32
PTC	32 with 2+	8

Table 6: (HBME-1) Expression in NIFTP AD PTC

Showing number of diffusely positive PTC cases and focally positive NIFTP cases with membrane expression of HBME-1.

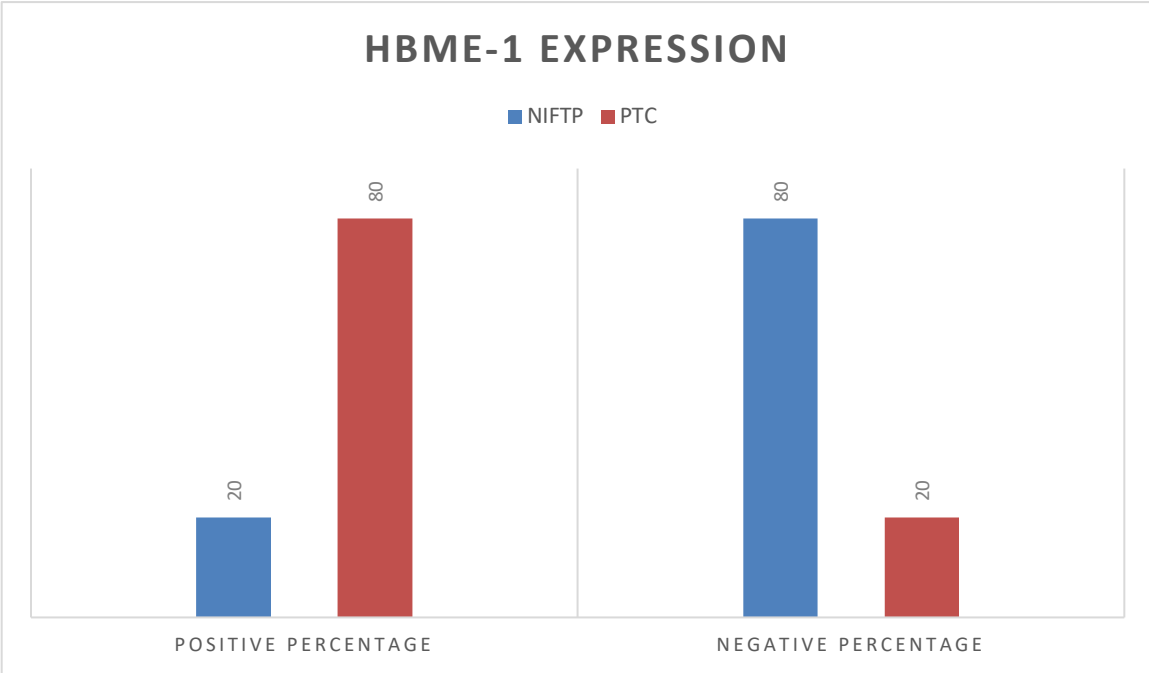

Figure 7: Showing percentage of HBME -1 positive cases in both the study groups

NIFTP	24	16
PTC	30	10

Table 7 : Expression of CK-19 IN NIFTP and PTC

The table above is showing number of diffuse cytoplasmic and membranous positive cases of PTC and focally positive cases of NIFTP on CK-19 stain.

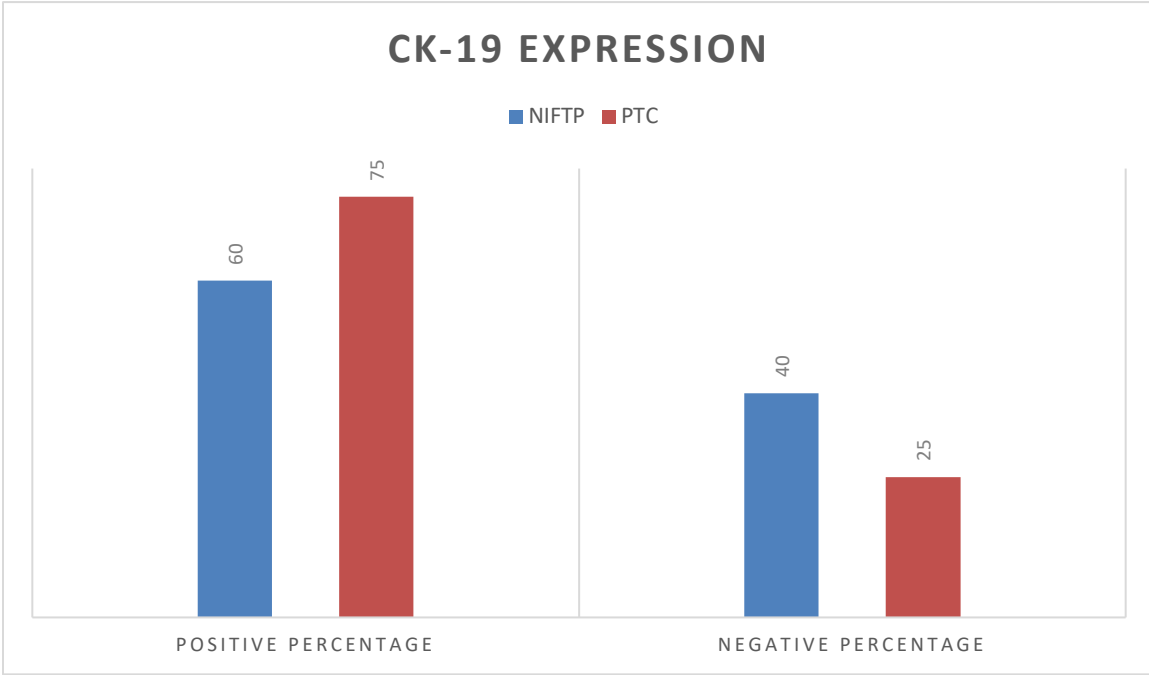

Figure 8: Showing positive and negative percentages of CK-19 in both the study groups

TUMOR TYPE	NO. OF POSITIVE CASES	NO. OF NEGATIVE CASES
NIFTP	0	40
PTC	0	40

Table 8: Expression of CD-56 IN NIFTP and PTC

Showing no case of NIFTP and PTC stained positive with CD-56

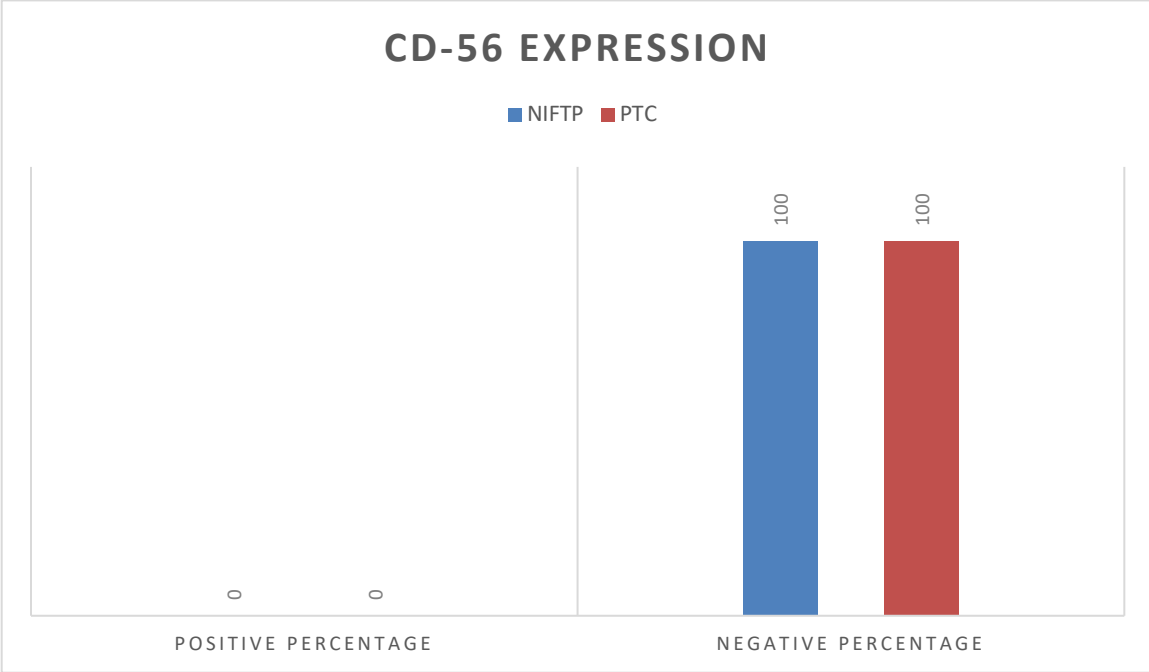

Figure 9: Showing 100% cases with no CD-56 expression

TUMOR TYPE	NO. OF POSITIVE CASES	NO. OF NEGATIVE CASES
NIFTP	0	40
PTC	24	16

Table 9: Expression of p^{63} IN NIFTP and PTC

The above table is showing number of cases with moderate to strong nuclear positivity in PTC and entirely negative expression in NIFTP on p63 stain.

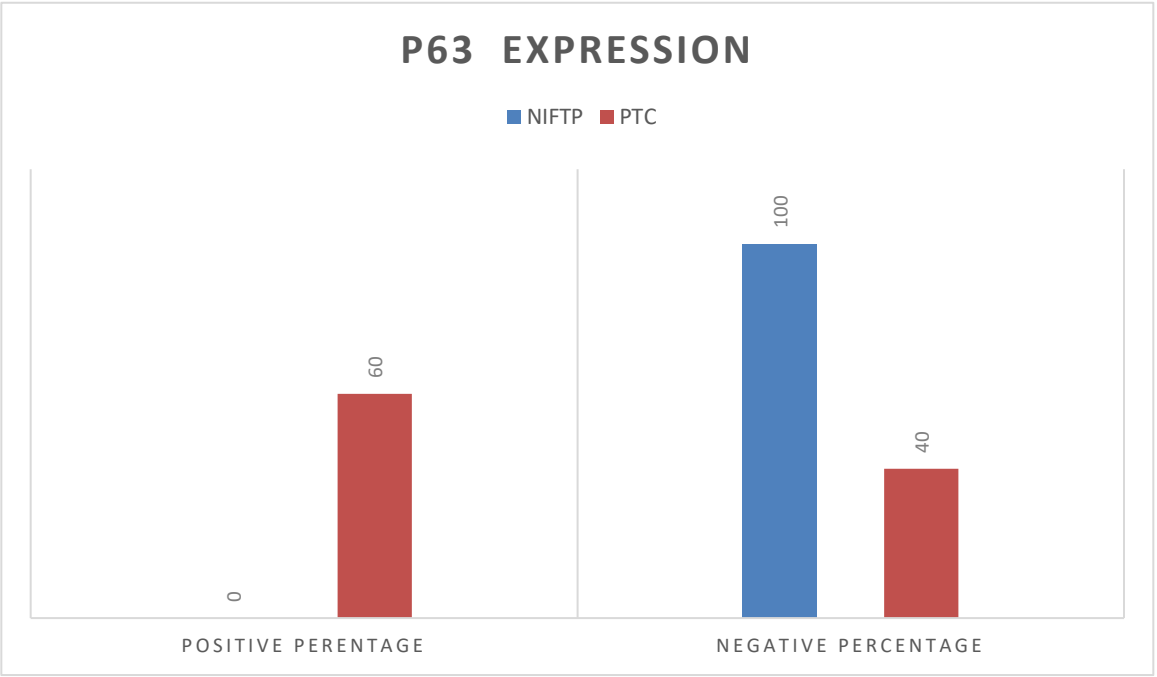

Figure 10: Showing 100% negative expression in NIFTP and 60% positive expression in PTC on p63 stain

Sensitivity and Specificity of HBME-1, CK-19 & P^{63}

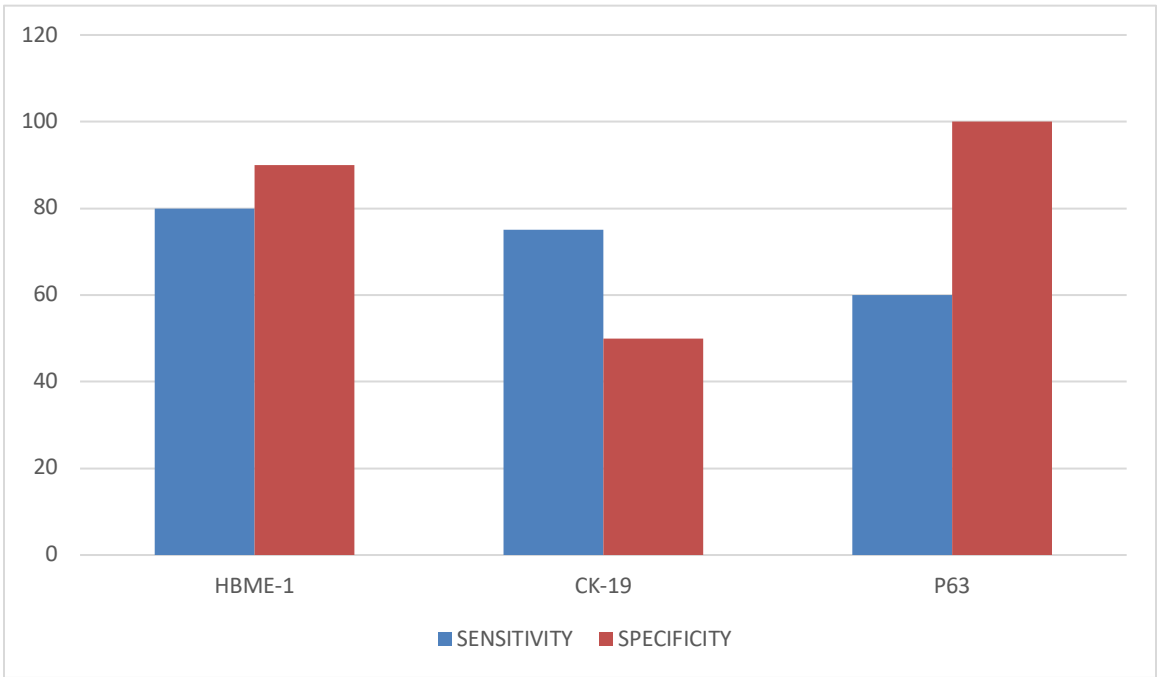

Figure 11: Showing HBME-1 as most sensitive and P63 as most specific of all the markers used in the current study

Figure 12: Cut section of thyroid specimen showing grey white lesion in the right lobe

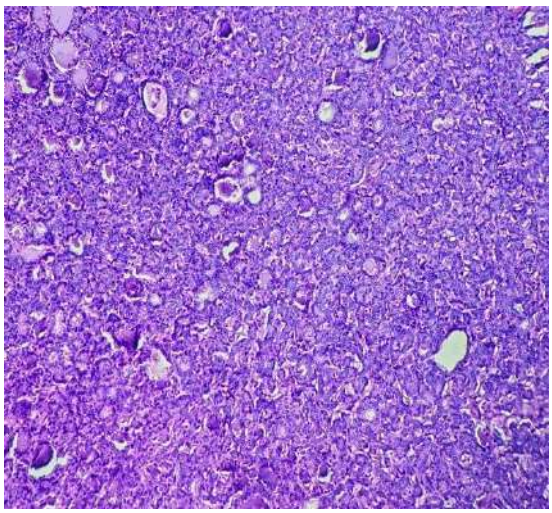

Figure 13: H&E stain – NIFTP showing compactly arranged follicular pattern (10x)

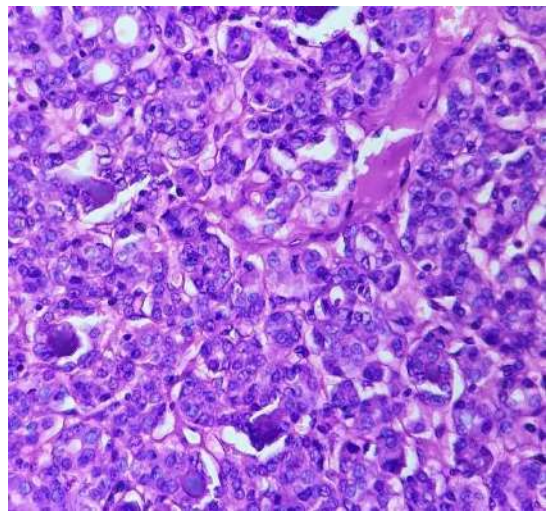

Figure 14: H&E stain – NIFTP showing PCT like nuclear features (40x)

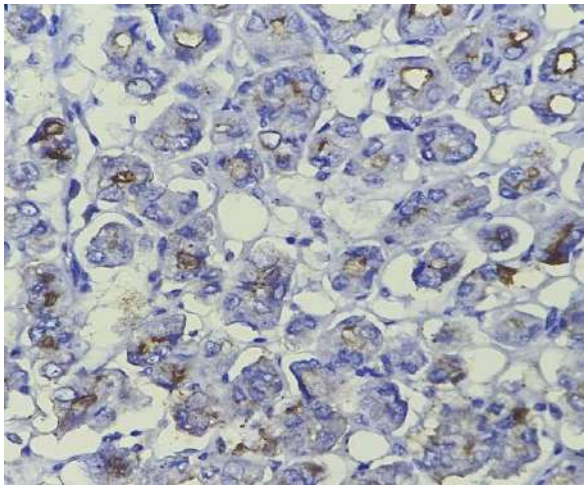

Figure 15: NIFTP showing no membrane activity with HBME-1

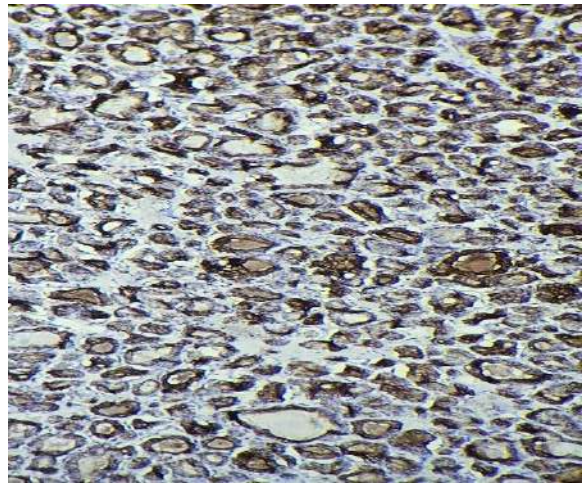

Figure 16: NIFTP showing membrane and cytoplasmic expression of CK-19

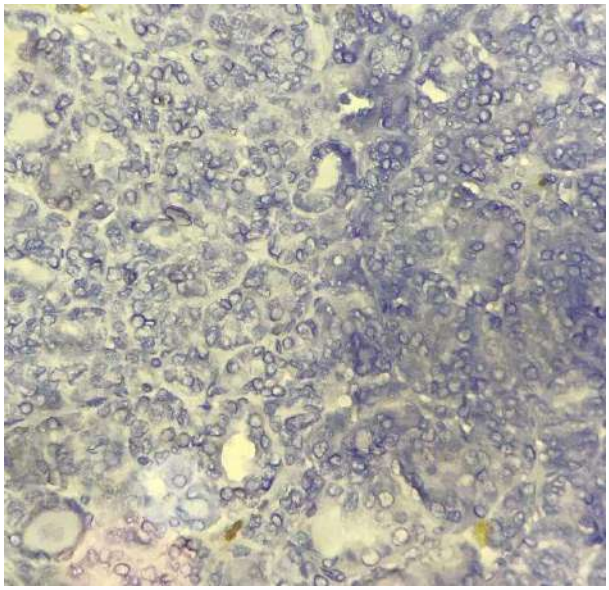

Figure 17: NIFTP showing no reactivity on CD-56 stain

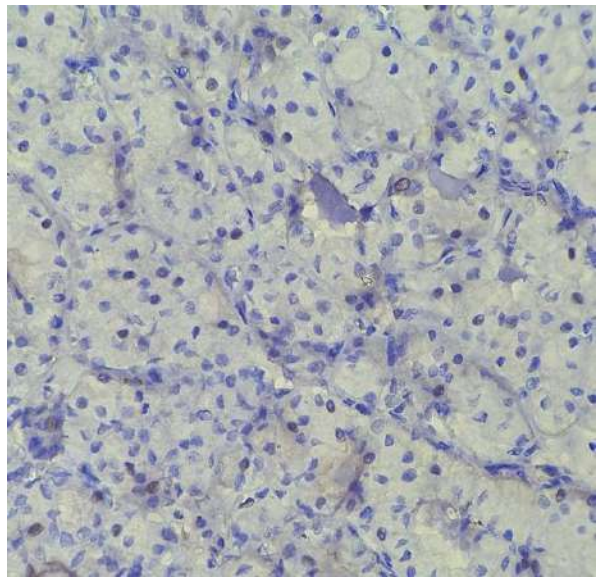

Figure 18: NIFTP showing negative p63 staining

Figure 19: Cut section of thyroid specimen showing grey white solid lesion in right lobe

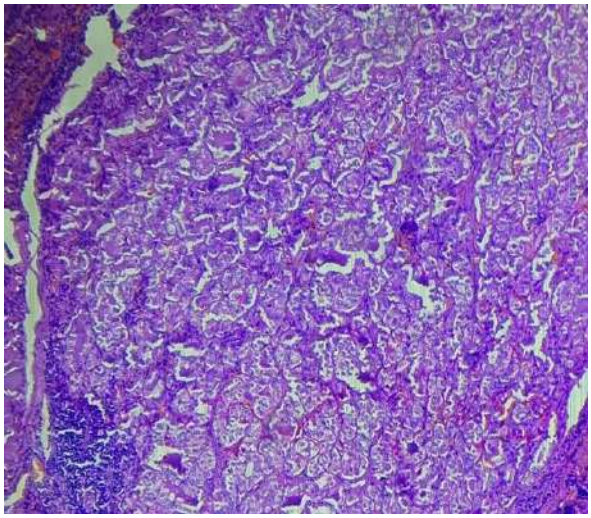

Figure 20: H&E stain – PTC showing micopapillary pattern (10x)

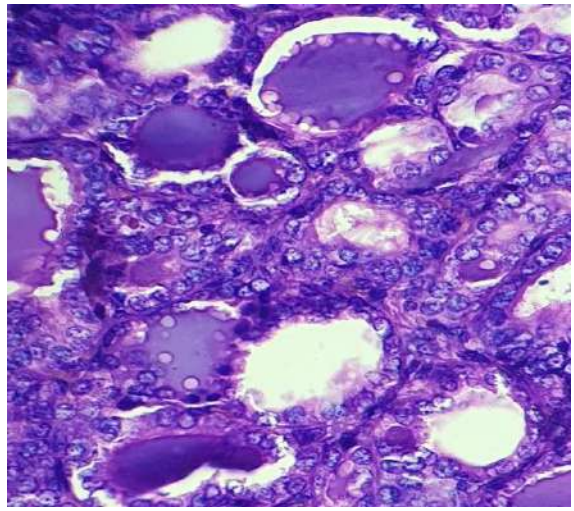

Figure 21: H&E stain – PTC showing orphan annie eye nuclei with thickened nuclear borders (40x)

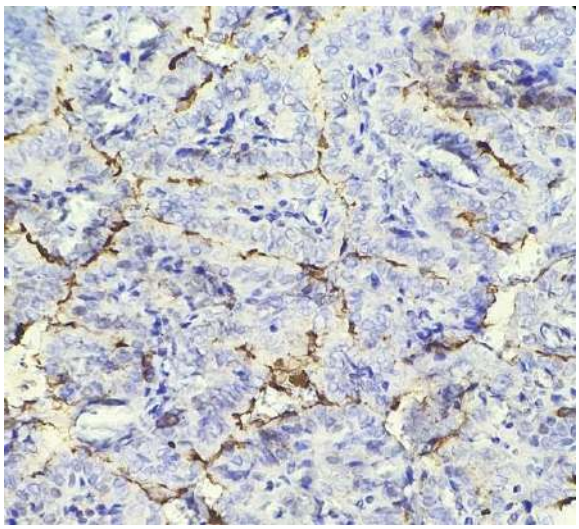

Figure 22: HBME-1 showing weak membrane positivity in PTC

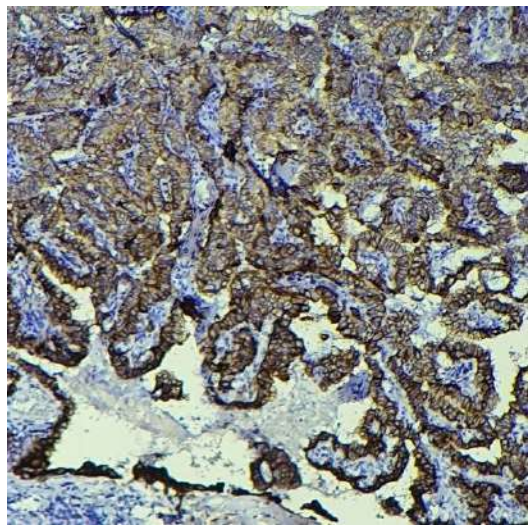

Figure 23: CK-19 showing strong expression in PTC

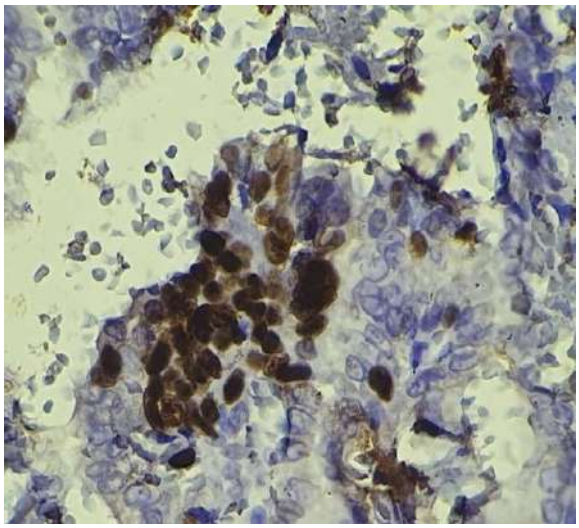

Figure 25: p63 showing strong nuclear expression in PTC

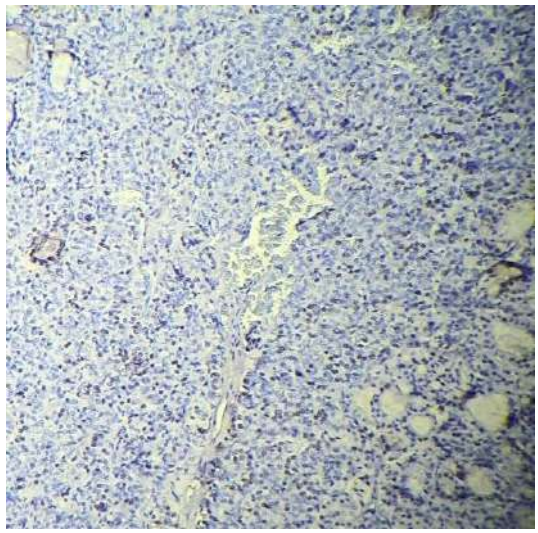

Figure 26: CD56 showing negative expression in PTC

Figure 27: Microarray blocks

Figure 28: IHC staining in process

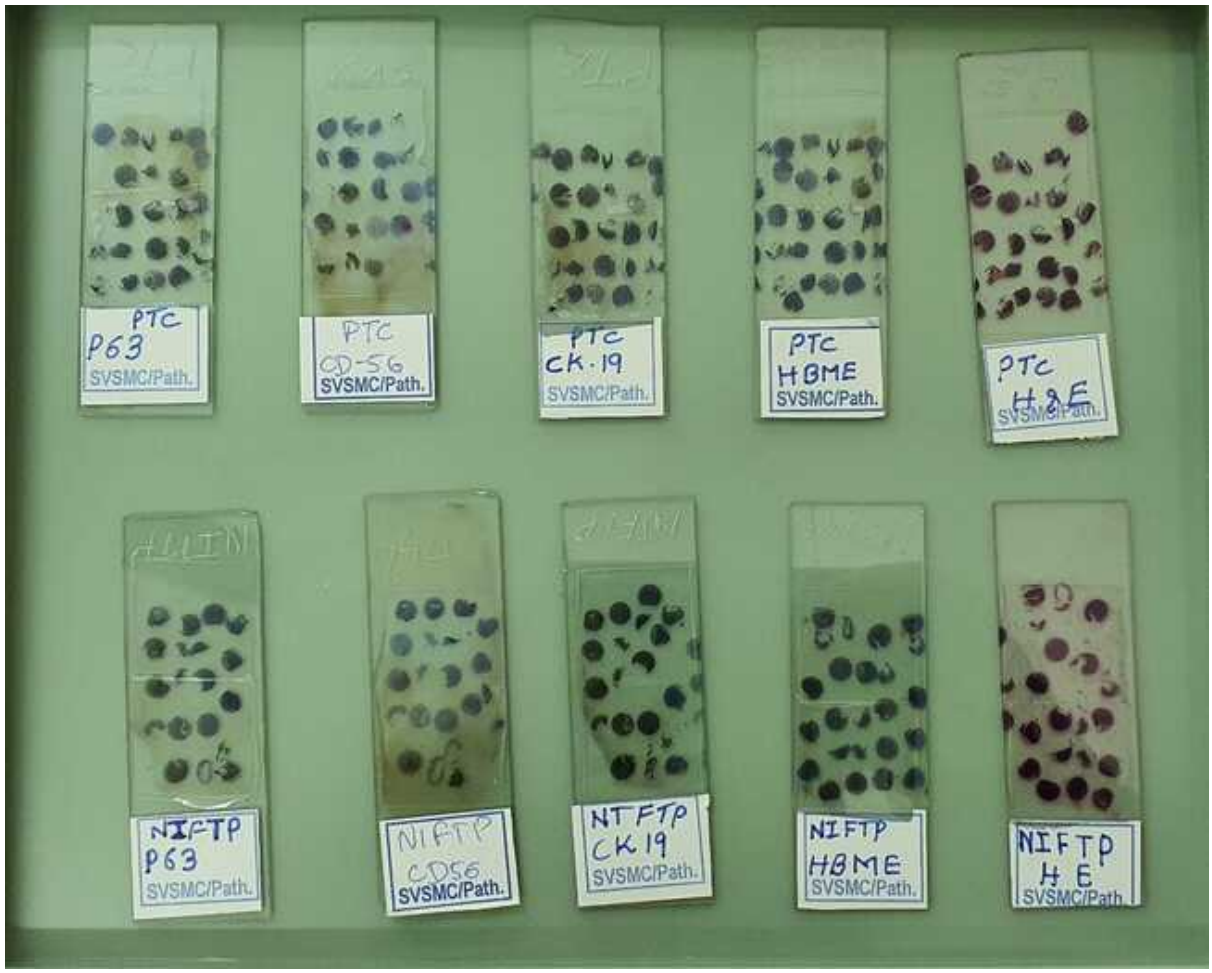

Figure 29: Stained microarray slides

Discussion

Thyroid lesions are common worldwide and they are more common in females in the age group between 35 to 65 years. Globally as of 2015, 3.2 million people had thyroid cancer. Over a decade, the incidence rate of thyroid cancer in India in women increased from 2.4 to 3.9 and in men from 0.9 to 1.3, a relative increase of 62% and 48% respectively(01-Jun-2018). Thyroid tumours are the most common endocrine tumours comprising 6 to 10 %. Every year about 3.8% of new cases of thyroid cancer occur. Hence for better clinical management it is very essential to identify specific diagnosis, since incorrect diagnosis may lead to unnecessary stress for the patients and unwanted healthcare expenditure. Prognosis and follow-up may vary depending upon the thyroid tumors, hence it is necessary to confirm the diagnosis for the better management of the patient.

Follicular lesions of thyroid often pose diagnostic dilemmas due to morphologic resemblance and architectural similarities in benign and malignant lesions. There are studies citing the prevalent inter-observer variability in the diagnosis of thyroid lesions.

With increasing diagnostic perplexity, the focus shifted to use of immunohistochemical markers to delineate benign from malignant lesions ad distinguish the various follicular neoplasms.

Non-invasive Follicular Thyroid neoplasm with papillary like nuclear features – since its first official definition in 2016, the new histopathological entity of non-invasive follicular neoplasm with papillary like nuclear features has attracted much interest among "thyroidologists" worldwide.[2]

Current study focusses on categorization of NIFTP, which is still an evolving diagnosis.

The range of age in this study for NIFTP and PTC includes 18-50 years and 20-69 years respectively. The mean age group of PTC is between 40 to 50 years in the literature, but current study group shows mean age for PTC as 35-36 years and for NIFTP as 31-32 years at the time of diagnosis.

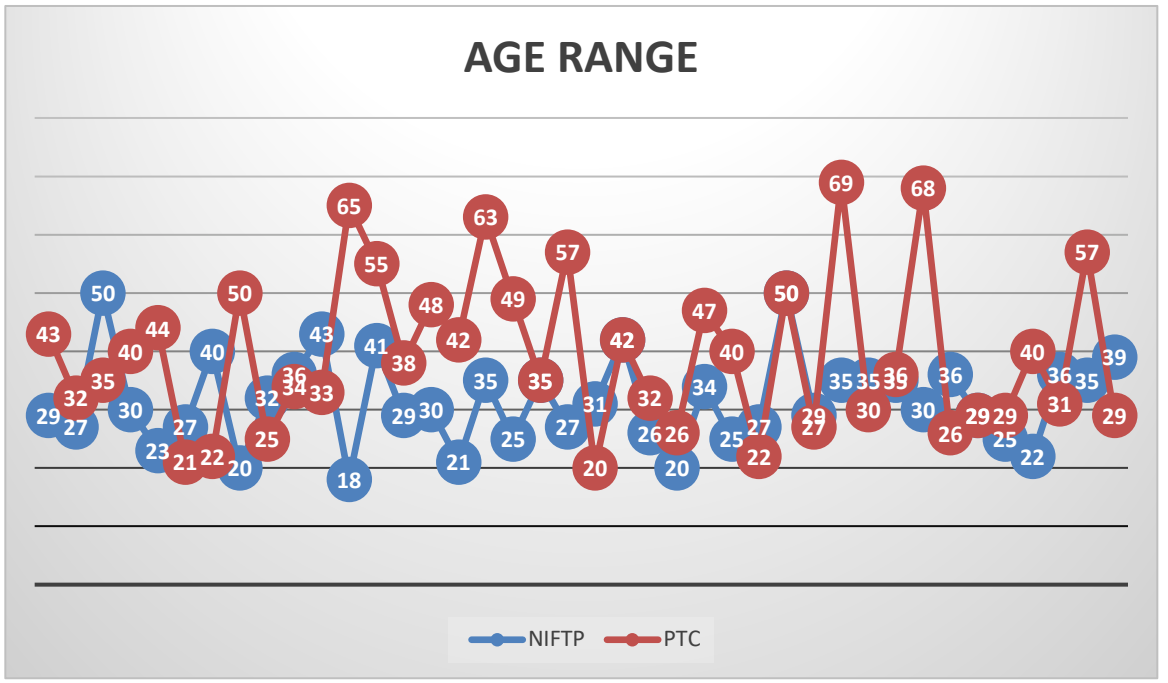

Figure 30: Age range of both the study groups

The above chart shows the minimum age at the diagnosis of NIFTP as 18 years and maximum as 50 years. Similarly, minimum age at the diagnosis of PTC is 20 years and maximum is 69 years.

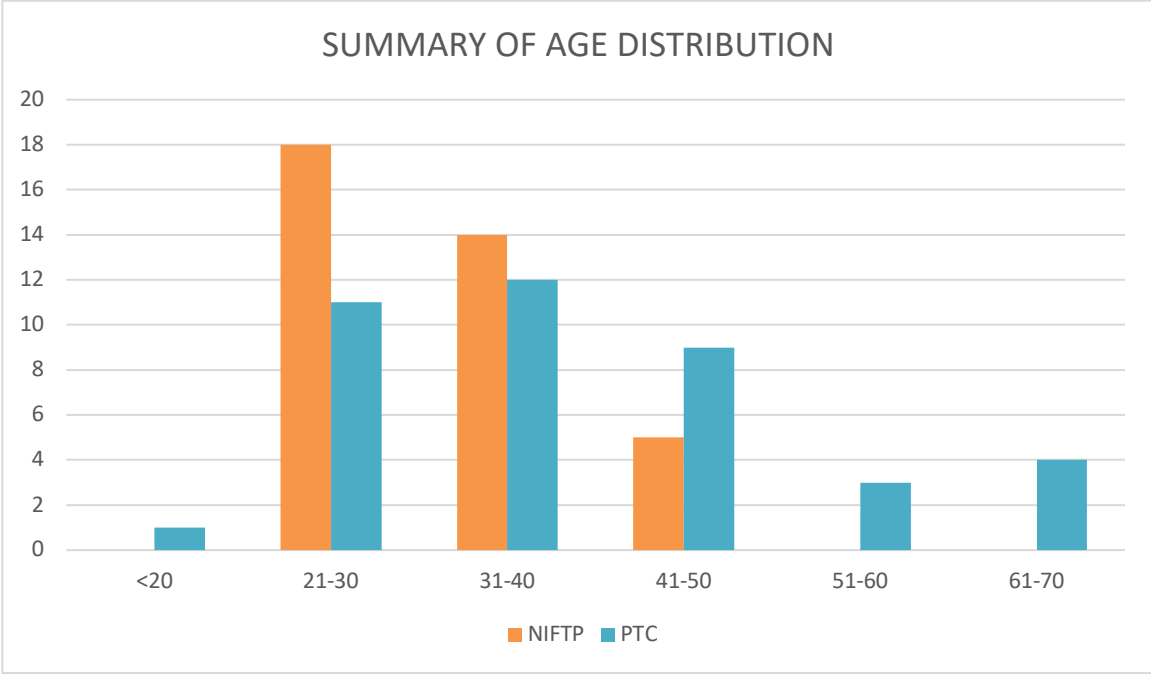

Figure 31: This chart shows that most of the patients in the study group of NIFTP were in their 3rd decade of life whereas those of PTC were in their 4th decade of life.

STUDY	MEAN AGE
Present study	*36 years*
Ambreen Beigh et al	40.2 years
Innocent Emmanuel et al	42.7 years
Ankit A. Shah et al	39 years
Ali S. Alzahrani et al	39 years

Table 09: Age Distribution Correlation with other Studies (PTC)

The findings in this study for PTC are similar to a study conducted by Ankit A. Shah et al thyroid carcinomas were found most commonly in 4th decade (12 out of 42 cases), with mean age of 39 years with youngest case seen in 18 years and oldest in 70 years.[39]

In the study conducted by Ali S. Alzahrani, majority of the cases were seen in 4th (160 out of cases) and 5th decade with mean age 39 years. [40]

In the study conducted by Ambreen Beigh et al peak incidence seen in the age group of 20- 29 years (40 cases out of 140) followed by 2nd highest peak in 30-39 years age group (37 out of 140). Mean age of the patients was found to be 40.2.[41]

In the study conducted by Innocent Emmanuel et al peak incidence was seen in 3rd decade with 32% cases (23 cases out of 70) . The mean age of thyroid carcinoma was 42.7 years, with an age range of 13-80 years.[44]

Non Invasive Follicular Variant with Papillary Like Nuclear features (NIFTP)

The World Health Organization (WHO) classification of tumors serves as an international standard of histopathological diagnosis and the essential basis of clinical practice for neoplastic diseases for all organ systems. The 4th edition WHO Classification of Tumors of Endocrine Organs was published in 2017, in which the new thyroid tumor classification was included.[28]

This included the introduction of borderline tumors (UMP and NIFTP) in thyroid tumor classification. [37]

Introduction of NIFTP into the thyroid tumor classification by the 4th edition WHO classification and risk stratification of differentiated thyroid carcinoma by the 2015 American Thyroid Association (ATA).[43,44]

These guidelines impacted thyroid tumor diagnosis. The upper panel is based on the 3rd edition WHO classification when there were only two choices for diagnosis, benign or malignant, of thyroid tumors. Approximately 20% of thyroid tumor diagnosis had discrepancies (benign vs malignant) with this schema. The lower panel is based on the risk stratification by the ATA recommendation and borderline tumor category by the 4th edition WHO classification. All thyroid tumors have some potential to develop metastasis, and the distinction between benign and malignant is eliminated. Risk stratification of thyroid tumors from very low risk of recurrence to high risk of recurrence is shown as a continuous spectrum, from benign tumor to high-risk cancer. [43,44]

Marc P. Pusztaszeri et al conducted a retrospective study from 2005 to 2015 of all patients with PTCs meeting the histological criteria to be reclassified as NIFTP. Eighty-six cases(13%) of NIFTP were identified in a total of 625 patients harbouring PTC on final pathology.

There were 67 females and 19 males (male to female ratio = 1/3.5), ranging from 17 to 83 years (median: 49.5 years; mean 50.6 years).[45]

Hector Battifora Mesothelial– 1 (HBME-1)

It's a monoclonal antibody known to act against microvillous surface of mesothelial cells and shows membrane positivity in thyroid malignancies, while negative in benign lesions. It's a sensitive and valuable marker in diagnosing lesions with PTC like nuclear features.

Interpretation – membranous stain.

Studies suggested that overexpression of HBME-1 in a thyroid nodule was an indicator of malignancy, especially true for PTC. The overall sensitivity of HBME-1 was 80% and the specificity was 90% for thyroid malignancy.

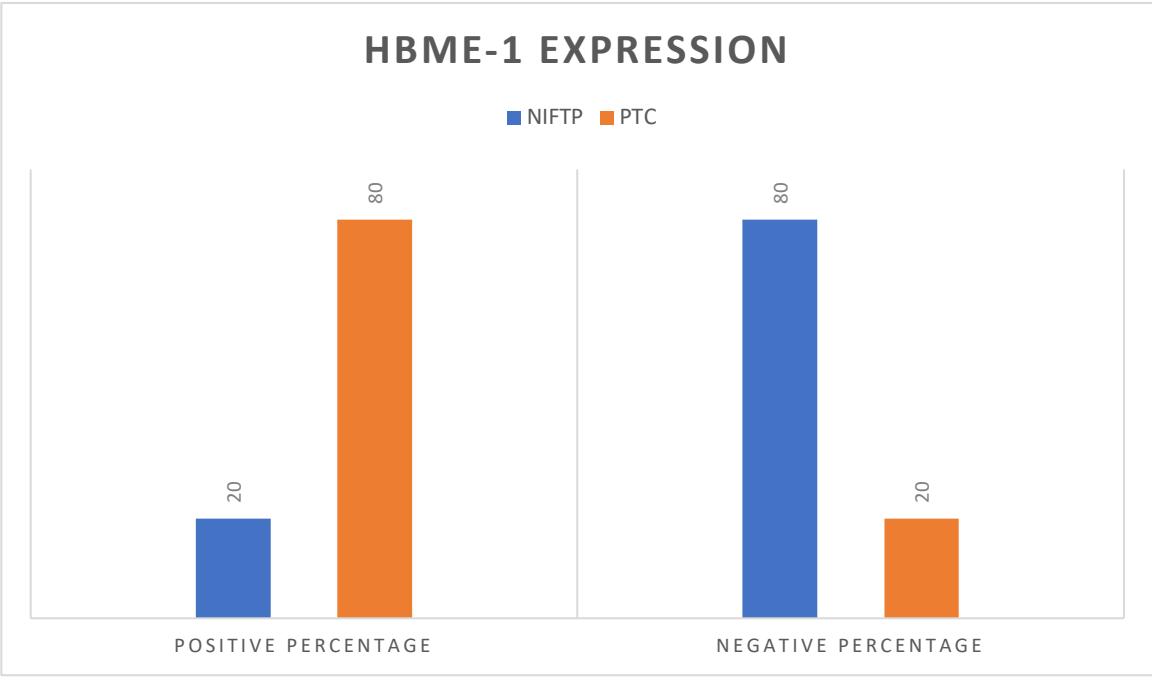

Figure 32: Showing percentage of HBME -1 positive cases in both the study groups

An overall 80% of PTC and 20% of NIFTP cases showed membrane positivity on HBME-1 stain.

STUDY	NIFTP	PTC
Current study	20% +ve	80% +ve
Ebru Tastekin et.al	15% +ve	42% +ve
Haiyan Liu, Fan Lin	Not done	87% +ve
Qandeel Sadiq et.al	88.9% +ve	77.8% +ve

Table 10: COMPARISION OF HBME-1 IN NIFTP AND PTC WITH OTHER STUDIES

NIFTP being a new entity in thyroid lesions, there are very few studies including IHC markers for diagnosing it. Current study showed 20% reactivity wit HBME-1 marker in case of NIFTP which is nearer to the findings of Ebru Tastekin et.al3, which showed 15% positive reaction. But the percentage of PTC showing positive reaction with HBME-1 in our study (80%), differed from Ebru Tastekin et.al (42%)3 but is nearer to the findings of Qandeel Sadiq et.al (77.8%)46.

Cytokeratin -19 (CK-19)

Cytokeratin 19 is a low-molecular-weight cytokeratin found in a variety of simple or glandular epithelia, both normal and their neoplastic counterparts. In the thyroid

gland, normal follicular epithelium usually has shown no detectable CK19 expression however, few reports noted CK19 expression in normal thyroid tissue in a focal staining pattern, especially in inflamed tissue. Many studies reported a strong and diffuse staining pattern of CK19 in PTC.

Interpretation – membranous and cytoplasmic stain.

Membranous expression with or without cytoplasmic staining of the cells qualified the case as positive for CK19. The immunoreactivity was scored as negative, focally positive (+: less than 25%), positive (++:25–50%) or diffusely positive (+++: more than 50%), based on the extent of the reaction.47

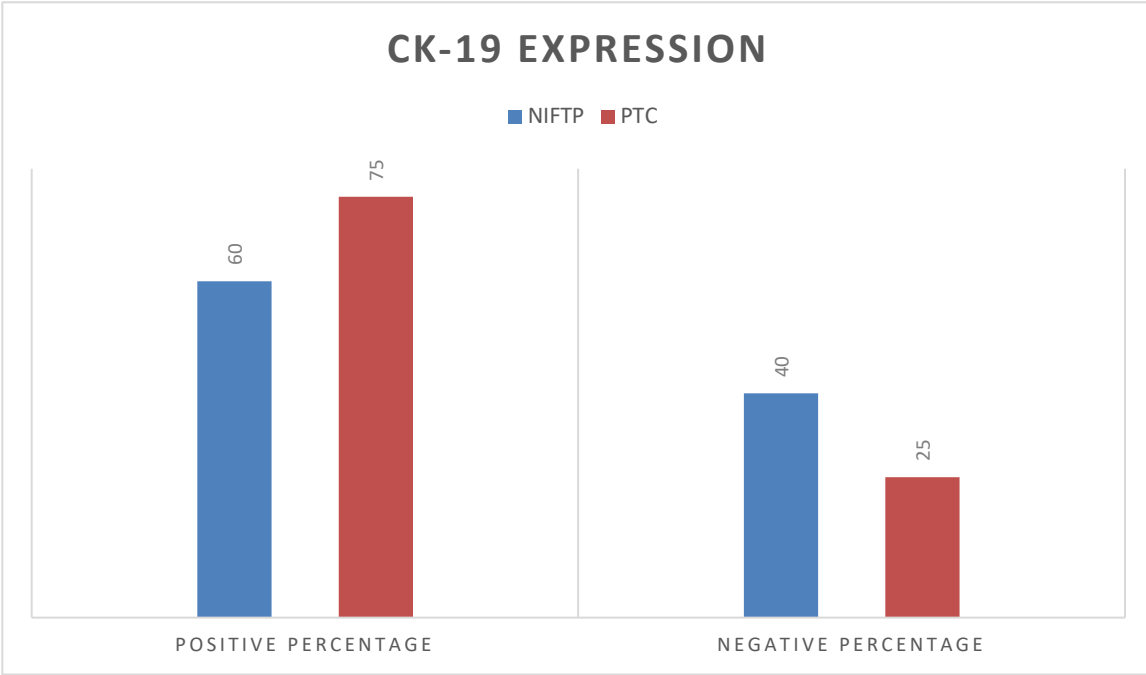

Figure 33: Current study showed 75% of PTC cases with diffuse cytoplasmic and membrane reactivity and around 60% of NIFTP showing focal cytoplasmic and membrane reactivity on CK-19 stain.

STUDY	NIFTP	PTC
Current study	60% +ve	75% +ve
Ebru Tastekin et.al	95% +ve	83% +ve
Qandeel Sadiq et.al	83% +ve	67% +ve

Table 11: COMPARISION OF CK-19 IN NIFTP AND PTC WITH OTHER STUDIES

Our study showed 60% focal positive reaction in case of NIFTP where as it's a bit higher in Ebru Tastekin et.al[3] (95%) and Qandeel Sadiq et.al (83%). Similarly in case of PTC our study showed 75% diffuse positive reaction, which falls in between the values of Ebru Tastekin et.al (83%) and Qandeel Sadiq et.al (67%)[46].

STUDY	RESULTS
Present study	30/40(75%)
Hanan Alsaeid Alshenawy	14/14(100%)
*Debdas Bose et a*l	22/22(100%)
Husain A Saleh	17/20(85%)
park et al	175/181(97%)
Murphy et al	20/20(100%)
Song et al	425/441(96%)
Barroeta et al	10/11(91%)
Prasad et al	48/67(72%)
Liu et al	41/53(78%)
Cheung et al	91/138(66%)
Beesley et al	26/26(100%)
de Matos et al	61/84(73%)

Table 12: COMPARISON OF CK 19 IN PTC WITH OTHER STUDIES

CD56 (Neural Cell Adhesion Molecule)

Neural cell adhesion molecule (NCAM), also called CD56, is a homophilic binding glycoprotein expressed on the surface of neurons, glia and skeletal muscle. Although CD56 is often considered a marker of neural lineage commitment due to its discovery site, CD56 expression is also found in, among others, the hematopoietic system. Here, the expression of CD56 is mostly associated with, but not limited to, natural killer cells. CD56 has been detected on other lymphoid cells, including gamma delta (γδ) T cells and activated CD8+ T cells, as well as on dendritic cells. NCAM has been implicated as having a role in cell–cell adhesion48, neurite outgrowth, synaptic plasticity, and learning and memory.

Few studies suggest positive immunoreactivity with CD56 in certain thyroid tumors. Especially Infiltrative Follicular variant of PTC and Encapsulated papillary oncocytic neoplasms (EPONs)49 of the thyroid apart from follicular carcinoma and certain benign lesions. Other studies suggest that CD56 is typically positive in benign lesions and negative in malignancy.

In this study CD56 is included in the IHC panel to evaluate its expression in NIFTP and PTC that might help us differentiate both the lesions from each other as well as to evaluate the nature of NIFTP. It found that none of the cases in our study group stained positive with this marker ruling out the benign nature of these lesions.

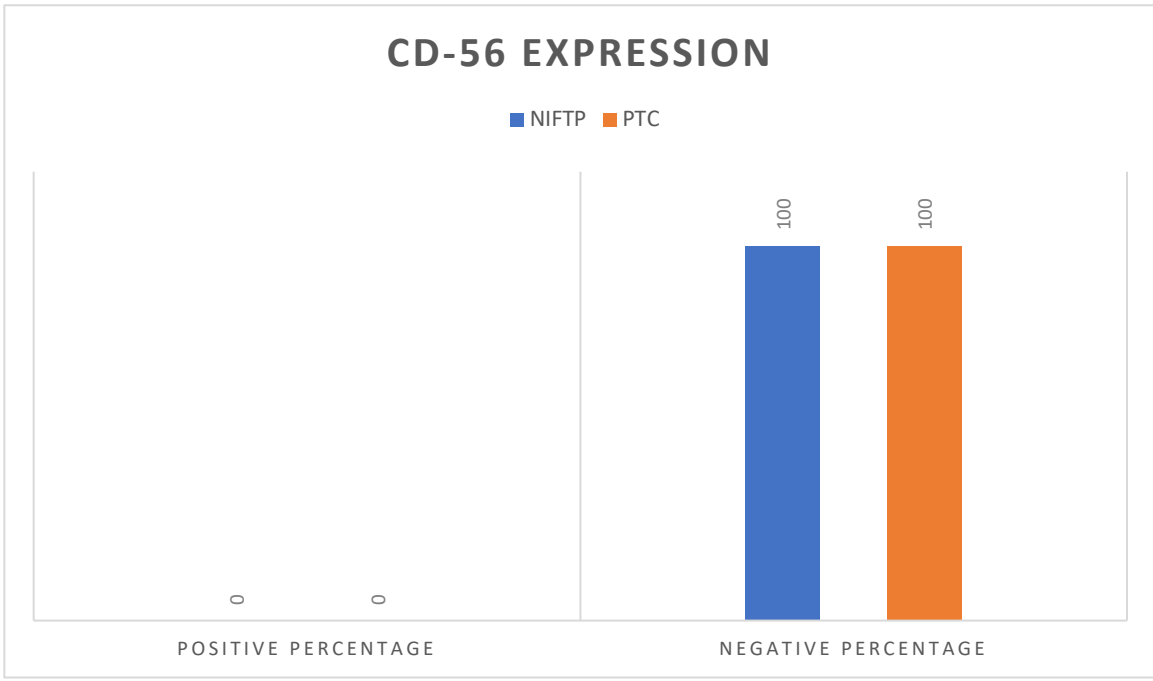

Figure 34: Showing 0% positive expression and 100% negative expression with CD56 in both the study groups

STUDY	NIFTP	PTC
Current study	0% +ve	0% +ve
Ebru Tastekin et.al	15% +ve	35% +ve
Haeyon Cho et.al	60% +ve	56 +ve
Dina El Demellawy et.al	Not done	0% +ve

TABLE 13: COMPARISION OF CD56 IN NIFTP AND PTC WITH OTHER STUDIES

Current study findings matched with a study done by Dina El Demellawy et.al[51] in case of PTC where the expression of CD56 recorded was zero. Other studies done by Ebru et.al[3] and Haeyon Cho[50] et.al showed 15% , 60% positive in NIFTP and 35%, 56% positive in PTC respectively.

p63 (PROTEIN – 63)

Tumor protein p63, typically referred to as p63, also known as transformation-related protein 63 is a protein that in humans is encoded by the TP63 (also known as the p63) gene.[52,53,54,55]

The TP63 gene was discovered 20 years after the discovery of the p53 tumor suppressor gene and along with p73 constitutes the p53 gene family based on their structural similarity.56 Despite being discovered significantly later than p53, phylogenetic analysis of p53, p63 and p73, suggest that p63 was the original member of the family from which p53 and p73 evolved.[57]

Interpretation – typically a nuclear stain.

In this study, p63 stain was used to study its expression in NIFTP and PTC.

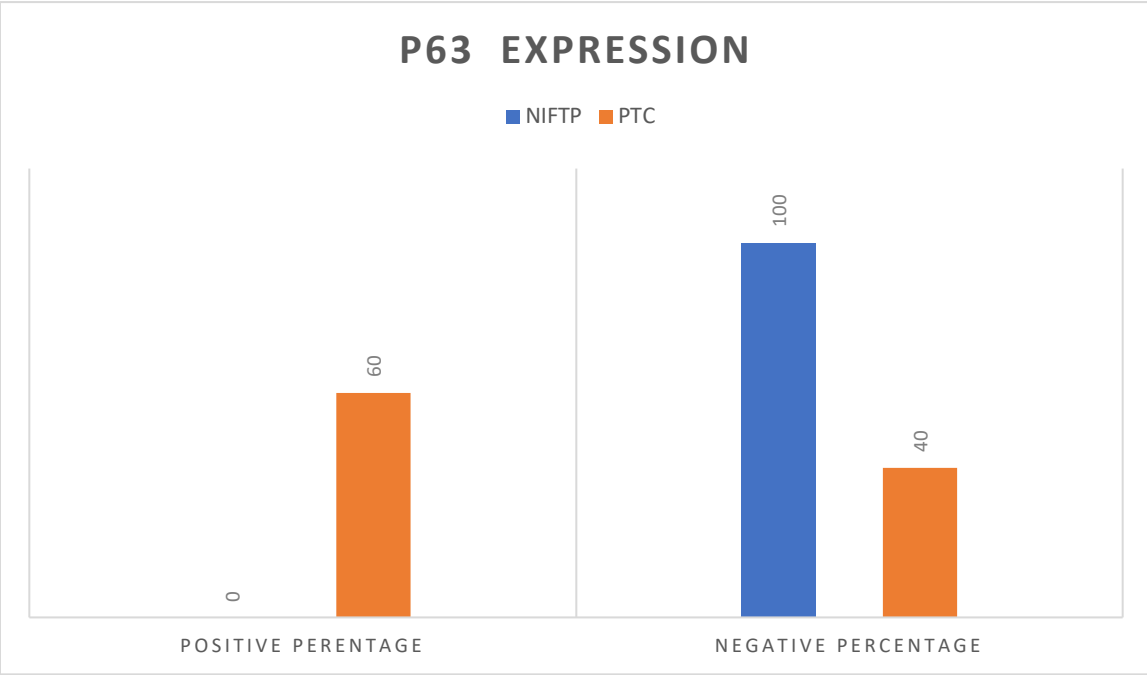

Figure 35: showing no case of NIFTP stained positive on p63 stain, whereas 60% cases of PTC stained showed strong positive nuclear staining, predicting its invasiveness.

STUDY	NIFTP	PTC
Current study	0% +ve	60%+ve
Ebru Tastekin et.al	20% +ve	40% +ve
Dina El Demellawy et.al	Not done	70% +ve

TABLE 14: COMPARISION OF p63 IN NIFTP AND PTC WITH OTHER STUDIES

Current study findings are matching with Dina El Demellawy et.al51 findings in case of PTC and not matching with Ebru Tastekin et.al3 (20%) in case of NIFTP.

Ebru Tas TE kin et.al included 6 markers, which are CD56, CD57, HBME-1, CK19, galectin-3 and p63 in differentiating diagnosis of thyroid Benign/Malignant lesions and NIFTP. This study compared their findings in NIFTP and PTC with 4 out of 6 markers.

	CURRENT STUDY				EBRU TASTEKIN ET.AL			
S.NO.	NIFTP		PTC		NIFTP		PTC	
1.	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE
HBME	20&	80%	80%	20%	15%	85%	42%	58%
2.	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE
CK-19	60%	40%	75%	35%	95%	5%	83%	17%
3.	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE
CD56	0%	100%	0%	100%	15%	85%	35%	65%
4.	+VE	-VE	+VE	-VE	+VE	-VE	+VE	-VE
p63	0%	100%	60%	40%	20%	80%	43%	57%

TABLE 15: COMPARISION WITH OTHER STUDY

The above table shows comparision between current study and a study done in Turkey, comparing our panel of IHC with the four out of six markers included in their study. CD57 and galactin 3 were excluded. Findings of this study matched with their findings in case of HBMe-1 expression and CK-19 expression in case of NIFTP where present study showed 20% positive cases and their study showed 15% positive cases with HBme-1. Our study showed 60% positive NIFTP cases and their study showed 95% positive cases.

This study showed completely negative reaction with CD-56 and p63 stains in the study group of NIFTP whereas their study showed 15% and 20% positive cases respectively.

Comparing the PTC group with their study findings, it matched in case of CK-19 stain where it is 75% and they showed 83% positive cases. Similarly this study showed 60% positive cases on p63 stain and their study showed 43% positive cases.

It differed in case of HBME-1, current study showed 80% and their study showed 42% positive cases only. Current study differed from Ebru Tastekin in case of CD-56, our study showed zero positive cases whereas their study showed 35% positive cases.

S.NO.	IHC MARKERS	NO.OF POSITIVE CASES (NIFTP)
1.	HBME -1	08 (20%)
2.	CK-19	24 (60%)
3.	CD56	00 (0%)
4.	p63	00 (0%)
5.	HBME-1 +CK-19	05 (12.5%)
6.	CK-19+p63	00 (0%)
7.	HBME-1+CK-19+p63	00 (0%)
8.	CK-19+ p63+CD56	00(0%)
9.	HBME-1+p63	00(0%)
10.	CD56+p63	00 (0%)
11.	HBME-1 + CD56 + p63	00 (0%)
12.	HBME-1 + CD56	00 (0%)
12.	HBME-1 + CK-19 + CD56 + p63 (all positive)	00 (0%)
13.	HBME-1 + CK-19 + CD56 + p63 (all negative)	12(30%)

TABLE 16: SUMMARY OF IHC (HBME-1, CK-19, CD56, p63) EXPRESSION IN NIFTP

Current study shows only two out of four markers used in the panel with positive expression HBME-1 (membrane) and CK-19 (both cytoplasm and membrane) in 20% and 60% of total (40) cases of NIFTP. When looked at the combination of above two markers 5 out of 40 cases were positive which accounts to 12.5%. all the other combinations (HBME-1 + CK-19 + CD56 + p63 (all negative), HBME-1 + CK-19 + CD56 + p63 (all positive), CD56+p63, HBME-1+p63, HBME-1 + CD56 + p63, HBME-1 + CK-19 + p63, CK-19 + p63 = CD56, CK-19 + p63) were not seen in any case of NIFTP.

S.NO.	IHC MARKERS	NO.OF POSITIVE CASES (NIFTP)
1.	HBME -1	32 (80%)
2.	CK-19	30 (75%)
3.	CD56	00 (0%)
4.	p63	24 (60%)
5.	HBME-1 +CK-19	24 (60%)
6.	CK-19+p63	17 (63%)
7.	HBME-1+CK-19+p63	15 (40%)
8.	CK-19+ p63+CD56	00(0%)
9.	HBME-1+p63	19(47.5%)
10.	CD56+p63	00 (0%)
11.	HBME-1 + CD56 + p63	00 (0%)
12.	HBME-1 + CD56	00 (0%)
13.	HBME-1 + CK-19 + CD56 + p63 (all positive)	00 (0%)
14.	HBME-1 + CK-19 + CD56 + p63 (all negative)	1(2.5%)

TABLE 17: SUMMARY OF IHC EXPRESSION IN PTC

Current study shows three out of four markers used in the panel with positive expression HBME-1 (membrane) and CK-19 (both cytoplasm and membrane), p63 (nuclear) in 80%, 75% and 60% of total (40) cases of PTC.

When looked at the combination of above three markers 15 out of 40 cases were positive which accounts to 40%. Other combinations like CK-19+p63 (63%), HBME-1+p63 (47.5%), and HBME-1+CK-19 (60%) were also seen. All other combinations (HBME-1 + CK-19 + CD56 + p63 (all negative), HBME-1 + CK-19 + CD56 + p63 (all positive), CD56+p63, HBME-1 + CD56 + p63, HBME-1 + CK-19 + p63, CK-19 + p63 + CD56,) were not seen in any case of PTC.

S.NO.	COMBINATION OF MARKERS	PTC	NIFTP
1.	HBME + CK-19	60%	12.5%
2.	HBME+ CD56	00%	00%
3.	HBME + p63	47.5%	00%
4.	CK-19 + CD56	00%	00%
5.	CK-19 + p63	63%	00%
6.	CD56 + p63	00%	00%
7.	HBME + CK-19 + CD56	00%	00%
8.	HBME + CK-19 + p63	40%	00%
9.	HBME + CD56 + p63	00%	00%
10.	CK-19 + CD56 + p63	00%	00%
11.	ALL POSITIVE	00%	00%
12.	ALL NEGATVE	2.5%	30%

TABLE 18: SUMMARY OF COMBINATION OF MARKERS IN PTC AND NIFTP

The above table concludes:

- The most common combination in PTC is CK-19 + p63 accounting to 63% followed by HBME + CK-19 (60%), HBME + p63 (47.5%) and HBME + CK-19 + p63 (40%) in that order.
- The most common and the only combination found in NIFTP is HBME + CK-19 accounting to 12.5%.
- Remaining all other combinations are found negative.
- The entire panel of markers is negative in 2.5% of PTC and 30% of NIFTP cases.
- The only combination seen both in PTC as well as NIFTP is HBME + CK-19.

Summary

1. The present study was conducted in the department of pathology at SVS Medical college and Hospital, Mahabubnagar from October 2018 to September 2021, for a period of 3 years.
2. Total 80 cases of Thyroid neoplasms were studied, out of which 40 were PTC and its variants, 40 cases were NIFTP.
3. Predominant variant of PTC was classical variant, followed by FVPTC.
4. Lesions were all in females.
5. Peak incidence of PTC and NIFTP was seen in 4th and 3rd decades respectively.
6. Expression of HBME-1, CK-19, CD56 and p63 were studied on all thyroid neoplasms.
7. HBME-1 showed strong and diffuse positivity in 80% PTC and focal positivity in 20% NIFTP cases.
8. CK 19 showed strong and diffuse expression in 75% PTC cases, 60% cases of NIFTP were positive for Ck 19, but the intensity score was less.
9. CD56 expression was negative in all the thyroid neoplasms, ruling out their benign nature.
10. p63 expression was seen in 60% of PTC cases and none of the NIFTP cases, ruling out invasive nature of NIFTP.

Conclusion

The present study concludes that the differential diagnosis of thyroid neoplasms particularly the follicular lesions is sometimes difficult. Though the morphological characteristics hold a major value, immunohistochemical markers contribute to a precise diagnosis.

In present comprehensive immune panel study HBME-1, CK-19 and p63 are found, showing strong expression in PTC and focal to no expression in NIFTP. The most commonly expressed marker among the panel, in both the study groups was HBME-1, accounting to 80% cases of PTC and 20% of NIFTP.

The most common combination in case of PTC is CK-19 + p63 (63%), which might be promising to predict invasiveness. The only combination found in NIFTP was HBME + CK- 19(12.5%). CD56 is found to be negative in all the cases, ruling out the benign nature of the lesions.

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