Original Research Article

A cross sectional study of expression of CK-19 in papillary thyroid carcinoma and its association with prognostic factors

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Abstract

Introduction: Thyroid malignancies are the most common endocrine malignancy. The most common malignancy in thyroid is papillary thyroid carcinoma. About 80% of all thyroid cancers are papillary carcinoma thyroid. Papillary carcinoma typically arises as an irregular, solid or cystic mass that comes from otherwise normal thyroid tissue. Papillary carcinoma has a high cure rate with 10-year survival rates for all patients with papillary carcinoma thyroid estimated at 80% to 90%. **Materials and Methods:** The study was undertaken in the Department of Pathology, Jangaon district hospital, Jangaon. The study was conducted from January 2020 to December 2020. A total of 46 cases including 29 cases of papillary carcinoma thyroid, 17 cases of papillary hyperplasia. Papillary carcinoma and its variants, Beningn lesions with papillary hyperplasia was included. Other malignancy including follicular carcinoma, medullary carcinoma without papillary areas, Benign lesions without papillary hyperplasia were excluded from the study. **Results:** In this study most of the papillary carcinoma of thyroid occur in the age group between 20-50 years. Most of the cases were diagnosed mostly 3 rd to 5th decades. In males, out of 29 cases 5 cases were males, mostly occur above 40 years. The mean age group is 43.9 years. In this study most of the papillary lesions arises from solitary nodular swelling, in our study out of 29 cases of papillary carcinoma 19 cases arises from solitary nodular swelling,5 cases from multinodular swelling , 5 cases from diffuse thyroid lesion. **Conclusion:** In our study most of all papillary carcinoma and its variants shows diffuse and strong positivity against anti ck 19 antibody. Papillary hyperplasia shows negative stain except 2 cases. We conclude diffuse and strong positivity confirms that the diagnosis of papillary carcinoma thyroid. Negative stain indicates it is a benign lesion. Cytokeratin 19 is a useful marker to differntiate papillary carcinoma from other benign lesions shows papillary hyperplasia.

Keywords: Thyroid malignancies, Papillary carcinoma, multinodular swelling, Cytokeratin 19.

Introduction

Thyroid malignancies are the most common endocrine malignancy. The most common malignancy in thyroid are papillary thyroid carcinoma. About 80% of all thyroid cancers are papillary carcinoma thyroid[1]. Papillary carcinoma typically arises as an irregular, solid or cystic mass that comes from otherwise normal thyroid tissue. Papillary carcinoma has a high cure rate with 10-year survival rates for all patients with papillary carcinoma thyroid estimated at 80% to 90%[2].

The presence of lymph node metastasis in these cervical areas causes a higher recurrence rate but not associated with a higher mortality rate. Distant metastasis is rare, but lung and bone are the most common sites if the papillary carcinoma does spread. Tumors that invade or extend beyond the thyroid capsule have a much worse prognosis because of a high local recurrence rate[3].

Peak onset ages are 30 to 50 years old. Papillary thyroid carcinoma is most common in females than in males in a 3:1 ratio. The prognosis directly related to tumor size. That is tumor size is less than 1.5 cm has a good prognosis[4].

The identification of papillary thyroid carcinoma mainly on the presence of papillary architecture. The current accepted diagnosis of this entity is based on nuclear features that include optical clearing, elongation, overlapping and irregular contours with grooves and pseudo inclusions. However, identification of these features remains at times controversial and the distinction of papillary carcinoma from other benign thyroid lesions with papillary features can be difficult[5].

One of these benign lesions is the autoimmune hyperthyroidism (Grave's disease) that is predominantly seen in females. The thyroid in Graves'disease may contain foci showing papillary formation microfollicles, vesicular nuclei, and nuclear grooves, and it may be hard to distinguish these foci from papillary carcinoma depending only on microscopic features. Other condition with difficulty may also occur in papillary formations of Multinodular goiter.

Cytokeratin polypeptide 19 (CK19) is a type I intermediate filament protein and is the smallest known keratin and is remarkable in that, contrary to all other keratins, it does not have a designated partner for the formation of filaments implying that regulation of its expression, so it is different from other keratin encoding genes. Cytokeratin 19 concentrates at sarcomeres of striated muscle and copurify with the dystrophin glycoprotein complex, perhaps through the interaction of the cytokeratin with the actin-binding domain of dystrophin. In vitro studies showed that dystrophin binds directly and specifically to CK19. CK19 is synthesized in simple and stratified epithelia. This study was designed to determine the effectiveness of CK19 to differentiate in distinguishing papillary carcinoma from other papillary hyperplasia[6].

Materials and methods

Study Location

The study was undertaken in the Department of Pathology, Jangaon district hospital, Jangaon.

Study Period

The study was conducted from January 2020 to December 2020.

Samples

A total of 46 cases including 29 cases of papillary carcinoma thyroid, 17 cases of papillary hyperplasia.

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Inclusion Criteria
1. Papillary carcinoma and its variants
2. Beningn lesions with papillary hyperplasia

Exclusion Criteria
1. Other malignancy including follicular carcinoma, medullary carcinoma without papillary areas.
2. Benign lesions without papillary hyperplasia

Materials required
1. Donor blocks which contains formalin fixed paraffin embedded tissue obtained from all the cases of papillary lesions of thyroid.
2. Hematoxylin and eosin stained tissue sections made from the donor blocks.
3. Postively charged slides for holding tissue sections for IHC
4. Chemicals for preparing antigen retrieval solutions and for wash buffers
5. Microwave oven for antigen retrieval.
6. Kit for performing immune histochemistry which includes primary antibodies ck 19 and universal kit.
7. Microscope used for grading of IHC slides

Methodology
Data collection
The data including patients age, clinical status, and other clinical data were obtained from the pathology records.

Processing of specimen
Total thyroidectomy and subtotal thyroidectomy specimens were received with 10% formalin and it is fixed for 24 hrs. Section were taken carefully.

Staining technique
Sections of 4-5µ thickness were cut and stained with Haematoxylin & Eosin. The slides were studied under light microscopy and the data recorded.

Haematoxylin and eosin technique
Prepration of haematoxyline solution
Haematoxyline 2.5 gm
Mercuric oxide 1.25 gm
Potassium alum 50 gm
Absolute ethyl alcohol 125 ml
Sodium iodate 0.5 gm, Distilled water 500 ml

Procedure
Potassium alum ,50 gm is dissolved in 500 ml of distilled water by heating and shaking at 600C. Add solution of 2.5 gm of haematoxyline in 25 ml of absolute ethyl alcohol and bring rapidly to boil. When to begins to boil, remove from flame and add 1.25 gm of mercuric oxide or sodium iodate. Mix by swirling gently.

Prepration of eosin solution
Eosin Y 1gm
95% ethanol 80 ml
Glacial acetic acid 0.2 ml
Distilled water 20 ml

Procedure
Dissolve 1gm of eosin y in 20 ml of distilled water and add 8o ml of ethanol and 0.2 ml of glacial acetic acid.

Staining procedure
1.Xylene 3 changes -2 mins each
2.90%, 80%, 70% alcohol each 5 min
3. Water wash 10 min
4. Harries haematoxyline 10 min
5. water wash 1o min
6. 1% Acid alcohol 2 dip
7. tap water for blueing 10 min
8. 1% eosin 4 dips
9.water wash 5 min
10. Ascending grades of alcohol 3 changes
11. xylene 2 changes
12. Mount in DPX mount
After that screened the slide under light microscope, and papillary carcinoma and its variants and benign lesions with papillary hyperplasia were taken for IHC.

Immunohistochemical evaluation
Immunohistochemistry was performed on 3-4m-thick sections taken on poly-L-lysine-coated slides. Antigen retrieval was performed by heating the sections in tris-EDTA buffer at pH 6.0 using pressure cooker. Mouse Monoclonal antibody was used to bind with the primary antigen and is detected by adding secondary antibody conjugated with horse radish peroxidase polymer and diaminobenzidine substrate. In this study, ck 19 antigens of Pathnsitu laboratory products is used.

Precautions
1.The glassware used should be dry and clean.
2. The buffer used should be prepared fresh and the ph should be adjusted according to preferred PH.
3. The staining procedures are never allowed to dry so they are performed under a humidity chamber.
4. DAB chromogen should be handled and disposed carefully as it is a carcinogen.
5. Primary antibody, DAB chromogen, peroxidase block should be stored at 4-6 degree
6. Then the slides are counterstained with haematoxyline.

Results

Table 1: Age distribution in papillary carcinoma

Age group	Classical papillary carcinoma	Follicular variant of papillary carcinoma	Micro papillary carcinoma	Intracystic papillary carcinoma	Columnar variant Papillary carcinoma
20-30 years	3	3		1	
30-50 years	7	2	2	4	
Above 50 years	3	2		1	1

In this study most of the papillary carcinoma of thyroid occur in the age group between 20-50 years. Most of the cases were diagnosed mostly 3 rd to 5 th decades. In males, out of 29 cases 5 cases were males, mostly occur above 40 years. The mean age group is 43.9 years.

Table 2: Sex Distribution

	Classical papillary	Follicular variant	Micropapillary variant	Intracystic variant	Columnar cell variant
Male	2	1		2	
female	11	6	2	4	1

In this study 29 cases were papillary carcinoma, 17 cases were papillary hyperplasia, in papillary carcinoma out of 29 cases, 24 cases were females and 5 cases were males in the ratio of 1:8.

Table 3: Frequency of Case Presentation

S. No	Clinical presentation	Frequency	Percent
1	Solitary nodular swelling	27	59
2	Multi nodular lesion	9	20
3	Diffuse lesion	10	22
	Total	46	100

		Frequency
Diagnosis	Classical papillary carcinoma	13
	Follicular variant of papillary carcinoma	7
	Papillary microcarcinoma	2
	Intracystic papillary carcinoma	6
	Columnar cell variant	1
	Total	29

	Nodular goitre with papillary hyperplasia	**12**
Diagnosis	Hashimotos thyroiditis with papillary hyperplasia	**4**
	Graves disease with papillary hyperplasia	**1**

In this study most of the papillary lesions arises from solitary nodular swelling, in our study out of 29 cases of papillary carcinoma 19 cases arises from solitary nodular swelling,5 cases from multinodular swelling , 5 cases from diffuse thyroid lesion.

Out of 29 cases of papillary carcinoma 13 cases were classical papillary carcinoma, 7 cases were follicular variant of papillary carcinoma, 2 cases were micropapillary carcinoma, 6 cases were intracystic papillary carcinoma, 1 case were columnar cell variant.

In papillary hyperplasia, 12 cases were multinodular goitre with papillary areas, 4 cases were hashimotos thyroiditis with papillary areas, 1 case graves disease with papillary hyperplasia.

In this study 13 cases were classical papillary carcinoma , Scoring of ck 19 was assesed by according to positivity of cells that is no cells staining graded as 0, <5 % of cells graded as 1, 5-25% graded as 2, 25 -75 % graded as 3, > 75 % of cells positivity graded as 4. In our study, ck 19 expression in out of 13 cases, 11 cases with 4+ positivity that is more than 75 % of cells positivity. 2 cases were 3 + Positivity.

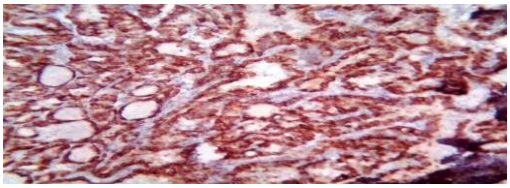

Fig 1: Papillary carcinoma shows diffuse positivity.

Follicular variant of papillary carcinoma

In this study 7 cases were follicular variant of papillary carcinoma, ck 19 expression in out of 7 cases, 4 cases shows 4 + positivity, 2 cases shows 3+ positivity, 1 case with 0. Negativity should take consideration.

Micropapillary carcinoma

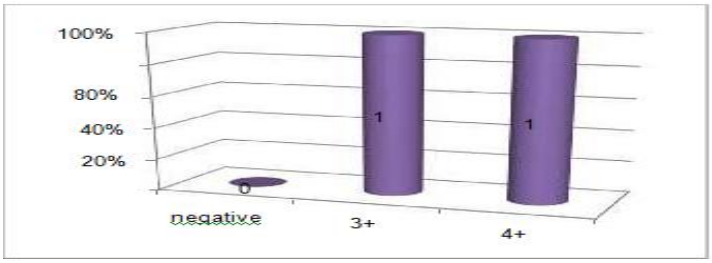

Fig 2: Micropapillary carcinoma

In our study 2 cases were, micropapillary carcinoma both cases show diffuse positivity 1 case with 4+, 1 case with 3+ positivity.

Intracystic papillary carcinoma

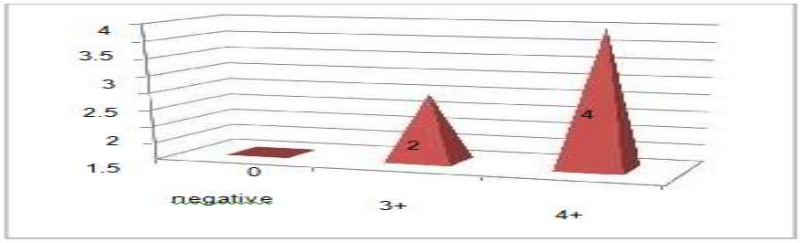

Fig 3: Intracystic papillary carcinoma

In our study 6 cases were intracystic papillary carcinoma, 4 cases shows 4 + positivity, 2 cases with 3+ positivity.

Columnar cell variant of papillary carcinoma

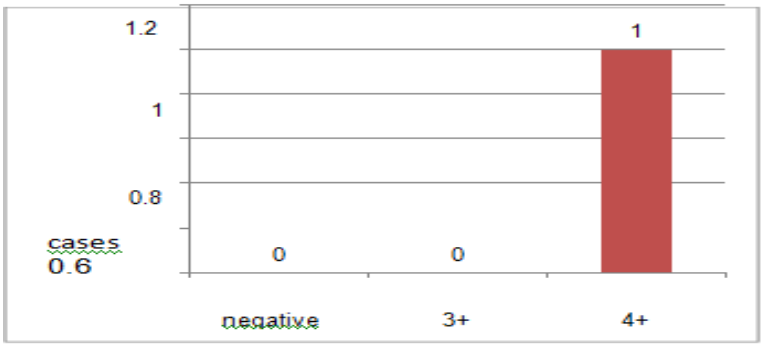

Fig 4: Columnar cell variant of papillary carcinoma

1 Case of columnar cell variant shows also diffuse cytoplasmic 4 + positivity.

Table 4: Columnar cell variant of papillary carcinoma

		CK19			
		negative	3 +	4+	P value
Diagnosis	Classical PTC	0	2	11	0.716
	Follicular variant	1	2	4	
	Micropapillary	0	1	1	
	Intracystic variant	0	2	4	
	Columnar cell variant	0	0	1	

In our study 29 cases were papillary carcinoma, out of 29 cases, 7 cases show 3+ positivity, 21 cases with 4+ positivity, 1 case of follicular variant of papillary carcinoma shows negativity. All variant of papillary carcinoma shows diffuse positivity. There is no significant difference between percentage of ck 19 expression in variants of papillary carcinoma.

Papillary hyperplasia

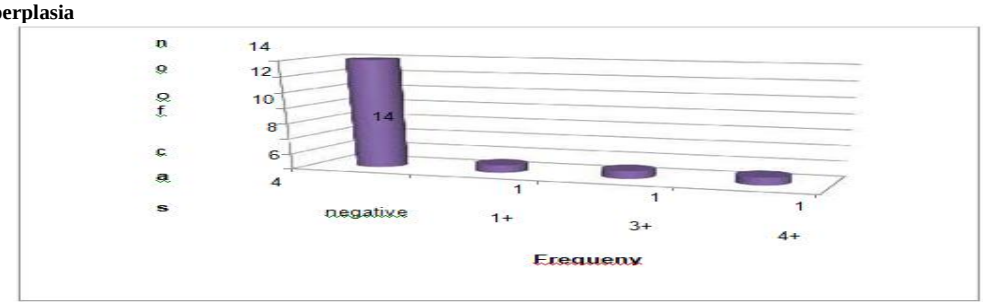

Fig 5: **Papillary hyperplasia**

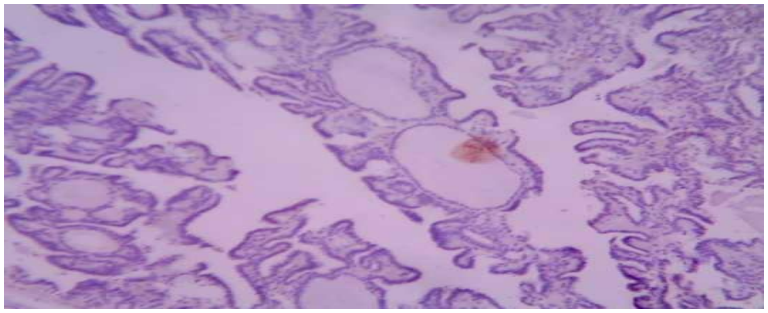

Fig 6: Papillary hyperplasia in Multinodular goitre shows negative stain.

In our study, 17 cases were papillary hyperplasia, 12 cases were multinodular goitre with papillary hyperplasia, 4 cases were hashimotos thyroiditis with papillary hyperplasia, 1 case of graves disease with papillary hyperplasia. In our study ,out of 17 cases, 14 cases show negative, one case with intracystic hyperplasia shows diffuse positivity, 2 cases with hashimotos thyroiditis shows positivity, out of 2 cases, 1 case shows focal positivity in reactive areas but not in papillary areas, few hurthle cells shows positivity. Other case shows diffuse positivity.

Comparison between papillary carcinoma and papillary hyperplasia

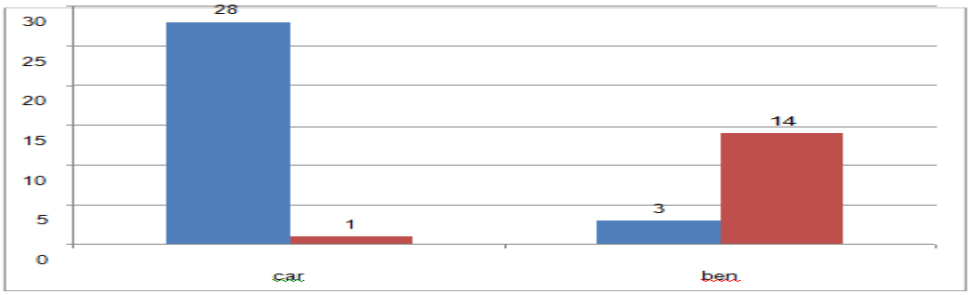

Fig 7: Comparison between papillary carcinoma and papillary hyperplasia

Table 5: Comparison between papillary carcinoma and papillary hyperplasia

	Positive	Negative	P value
Papillary carcinoma	28	1	<0.0001
Papillary hyperplasia	3	14	

In our study 29 cases were papillary carcinoma, 28 cases of papillary carcinoma shows diffuse positivity, 1 case reported as follicular variant of papillary carcinoma, it was negative for ck 19. 17 cases were papillary hyperplasia, 14 case with papillary hyperplasia were negative. 3 cases were positive, 1 case shows focal positivity, that is few hurthle cells in reactive areas only shows positivity but not in papillary areas. One case with intracystic papillary hyperplasia shows difuse positivity, more than 80% of cells show positivity. There is a significant difference between papillary carcinoma and papillary hyperplasia in ck 19 expression.

Sensitivity and specifity of CK 19 expression in diagnostic significance of papillary lesions of thyroid

Senstivity	Specificity	PPV	NPV	Likelihood ratio
93.33%	93.75%	96.55%	88.24%	38.42

In our study there is a high sensitivity and specificity in diagnostic value of ck 19 expression in papillary lesions of thyroid.

Discussion

Thyroid carcinoma (PTC) is the most common form of malignant thyroid neoplasm. Its diagnosis is based on nuclear features such as nuclear clearing, overlapping, grooves and pseudo inclusions. However, identification of these features remains, at times, difficult because of its focal presence and thus the distinction of papillary thyroid carcinoma from other thyroid lesions may not be possible. There are several other thyroid lesions that may contain papillary processes with nuclear features in a focal manner, which pose diagnostic difficulties with papillary thyroid carcinoma. Multinodular goiter (MNG) with delicate papillary budding and focal nuclear clearing may be confused with Papillary thyroid carcinoma. Several immunohistochemical stains have been investigated for their possible role as diagnostic markers for papillary thyroid carcinoma[7]. They are cytokeratin19 (CK19), HBME1, galectin-3 and RET and thyroid transcription factor. Although galectin-3 was initially shown to have utility in the differential diagnosis between benign and malignant thyroid lesions, recent studies suggest that it is not reliable. Several studies have shown conflicting results regarding the usefulness of CK19 as a diagnostic marker in PTC. This study was carried out to investigate the role of CK19 as a possible diagnostic marker of PTC and its utility in differentiating Papillary thyroid carcinoma, from the other benign thyroid lesions with papillary areas mimicking papillary carcinoma.In our study, 29 cases were papillary thyroid carcinoma, mostly occur in age range of 20 to 50 years with an median age is 43.9 years, in bose et al showed a mean age was 34.50 years. In our study it is slightly higher than that. Ikram et al studies showed most of the cases occur 25 to 55 years, in our study also most of the papillary carcinoma diagnosed in the age group between 20 to 50 years. 3 suna and abdullah et al shows mean age is 44.8 years it was similar to our study.In this study male female ratio is 1:8 it is slightly more than already published literature. Bose et al showed the ratio is 1:6.3, slightly lower than our study.Different studies have shown varying percentage distribution of ck 19 positivity in papillary carcinoma thyroid and other lesions of thyroid. In this study 29 cases were papillary carcinoma includes [13 cases were classical papillary carcinoma, 7 cases were follicular variant of papillary carcinoma, 2 cases were micropapillary carcinoma, 6 cases were intracystic papillary carcinoma, 1 case were tall cell variant of papillary carcinoma], 17 cases were other lesions with papillary hyperplasia.

Out of 17 cases of papillary hyperplasia, 11 cases were Multinodular goitre with papillary hyperplasia, 4 cases were hashimotos thyroiditis with papillary hyperplasia, 1 case of graves disease, 1case with intracystic papillary hyperplasia.

Conclusion

In our study most of all papillary carcinoma and its variants shows diffuse and strong positivity against anti ck 19 antibody. Papillary hyperplasia shows negative stain except 2 cases. We conclude diffuse and strong positivity confirms that the diagnosis of papillary carcinoma thyroid. Negative stain indicates it is a benign lesion. Cytokeratin 19 is a useful marker to differntiate papillary carcinoma from other benign lesions shows papillary hyperplasia.

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