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Diagnostic Utility of Cytokeratin-19 and Galectin-3 in Papillary Carcinoma Thyroid

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Abstract
Background: *Papillary thyroid carcinoma (PTC)is the most common form of thyroid cancer, however, it is often difficult to differentiate PTC from benign papillary hyperplasia of the thyroid gland based on their morphology. Immunohistochemical markers can decrease the diagnostic dilemma, effectively pointing out cases with more chance of becoming malignant.*
The objective of my study is to understand the utility of cytokeratin-19 and Galectin-3 in the diagnosis of papillary thyroid carcinoma
Method: *A total of 54 samples from benign group and 54 samples from histopathologically proven papillary thyroid carcinoma were collected. Histomorphological features and their immunohistochemical expression of CK-19 and Galectin-3 were studied. Data thus obtained was analysed using software SPSS version17.0. Histopathological diagnosis was considered as the gold standard.*
Results and Discussion: *The 108 thyroid samples were diagnosed as follows; 30 Cases of multinodular goiter, 24 case of follicular adenoma, 19 cases of PTC classic variant, 17cases of follicular variant of papillary carcinoma, 17 cases of other variants of papillary thyroid carcinoma. Sensitivity, specificity and diagnostic accuracy of CK-19 being 94.4 %, 50% and 72.2%. respectively with a kappa value 0.481 .Positive predictive value of CK-19 was found to be 67.1% and negative predictive value of 84.4%. Sensitivity, specificity and diagnostic accuracy of Gal-3 being 72.2% 90.7% and 81.5% respectively with a kappa value 0.630. Positive predictive value was found to be 88.6% and negative predictive value was found to be 76.6%.By combined immunostaining of CK-19 and Gal-3 96.3% malignant nodules could be identified and 51.9% of benign nodules could be differentiated correctly.*
Conclusion: *In this study CK-19 was more sensitive while Galectin-3 was found to be more specific. when combined the sensitivity has increased to 96.3%.So if we use a panel of markers including CK-19 and galectin-3,it would help to pick out more cases of Papillary thyroid carcinoma so that early treatment can be given to such patients*
Keywords: *Immunohistochemistry; Galectin-3; CK-19; follicular adenoma; Multinodular Goiter; papillary thyroid carcinoma.*

Introduction

Thyroid cancer is the most commonly found endocrine malignant tumor in India. It encompasses the most common well-differentiated papillary carcinoma (80% of all thyroid cancers) and follicular carcinoma (15%), as well as poorly differentiated carcinoma (< 1%) and anaplastic carcinoma (< 2%)[1]. The increasing incidence partially reflects the earlier detection of small asymptomatic cancers because of the prevalence of screening. However, the incidence has also increased across all tumor sizes and stages[2]. Most of the thyroid cancers are biologically of an indolent phenotype and they have an excellent prognosis. Survival rates of these tumors are more than 95% at 20 years if diagnosed at stage 1. So early diagnosis is critical in the management of these tumors. However, the recurrence rate is still high[3]. The incidence of thyroid cancer is about three to four times higher among females than among males worldwide. It comes to the rank of the sixth most common malignancy diagnosed in women. Most tumors are diagnosed between the third and the sixth decade of life. Even though papillary thyroid carcinoma (PTC) is the most common form of thyroid cancer; it is often difficult to differentiate PTC from benign papillary hyperplasia of the thyroid gland based on their morphology alone[1].

PTC is one of the major differentiated adenocarcinomas. Most cases have excellent prognosis but approximately 10% of PTC patients undergo recurrences such as lymph node recurrence and lung metastasis Clinicopathologically, age>45 years, large tumor size, extrathyroidal invasion, distant metastasis, vascular invasion, and poorly differentiated histology are the well known detrimental prognostic factors. PTC is usually gray-white in color but also shows a variety of gross appearances such as tumors with a central scar and infiltrative borders, calcification and encapsulation.

Most of the PTCs shows papillary growth pattern; however, nuclear features are the most important diagnostic hallmark. They are common in almost all cases[4]. The nuclear appearances of PTC are clear, ground glass, or Orphan-Annie eyed.

These nuclei are larger than normal follicular nuclei. They overlap each other.

The nuclei contain eosinophilic inclusions and contain longitudinal grooves[9]. These nuclear features are the important characteristics of PTC but not are specific. Indeed, nodular goiter, as well as follicular adenoma, frequently shows similar nuclear features including intranuclear inclusions and nuclear grooves[4].

In the recent years, a large number of molecular alterations in thyroid cancer have been used in the distinction of malignant from benign thyroid lesions.

These biomarkers, such as cytokeratin 19 (CK-19), thyroglobulin (TG), Ki67, Calcitonin, thyroid transcription factor-1(TTF-1), BRAF, RET, Hector Battifora mesothelial-1(HBME-1), and galectin-3(Gal-3), have been translated into clinical practice which offered significant improvement in the preoperative diagnosis of thyroid cancer[8,9].

Cytokeratin-19 (CK-19) is a member of the keratin family. The keratins are intermediate filament proteins which are responsible for the structural integrity of epithelial cells. CK-19 is strongly and diffusely expressed in papillary carcinoma, whereas it is usually absent or focally expressed in benign follicular adenomas [10].Many cytokeratins have been evaluated for the differential diagnosis of thyroidnodules, of which CK19 has been found to be the most useful one. Studies showed that CK19 is strongly and diffusely positive in malignant thyroid tumors including classic PTC, Follicular variant of papillary carcinoma (FVPC) and Follicular carcinoma[11,12].

Galectin-3 (Gal-3) is a carbohydrate binding protein with an affinity for beta galactosides.

This protein is a member of the lectin family. It is involved in cell-cell and cell-matrix interactions and in RNA splicing[13]. Increased levels of Galectin-3 levels have been recently observed in

malignant thyroid tumors. But they are not elevated in adenomas or in normal thyroid tissue. Hence Galectin-3 expression by immunohisto-chemistry is of value in discriminating between benign and malignant thyroid nodules.

In this study, we are assessing two immunohistochemical markers, Cytokeratin-19 and Galectin-3 and are evaluating their diagnostic significance for papillary thyroid carcinoma.

Materials and Methods

A total number of 54 from each group of thyroid samples have been collected from the department of Pathology, Jubilee Mission Medical College & Research Institute for the next one and a half years. The resected specimens were fixed in 10% formol saline overnight. Detailed gross examination was done on the next day. All the gross findings were recorded. Sections were taken from representative areas and were then processed using an automated tissue processor followed by paraffin embedding. Sections were cut at five microns size and then stained using a standard hematoxylin and eosin staining protocol for examining surgical specimens. The sections were studied under light microscopy. For Immunohistochemical study, paraffin blocks were subjected to two markers namely CK 19 and Galectin-3.

Informed consent had been obtained from all patients whose specimens were collected, and all tests had been done only after approval obtained from the institutional's ethics committee.

The cells were regarded as positive for Galectin-3 when immunoreactivity was clearly observed in their nucleus and/or cytoplasm. A positive membranous expression with or without cytoplasmic staining in 10% or more of neoplastic cells were regarded as positive for CK19. For all antibodies, immunoreactivity was considered positive if more than or equal to 10% of follicular epithelial cells are stained. For each antibody, immunoreactivity was scored as negative (-:less than 10%), focally positive (+:less than 25%), positive (++:25-50%) or diffusely positive (+++: more than 50%) based on the extend of reaction.

All categorical variables were expressed as frequency and percentages. To understand the diagnostic utility of CK-19 and Galectin-3, diagnostic test was applied. To obtain the association of study variables (age & sex) with behavior, chisquare test was applied.

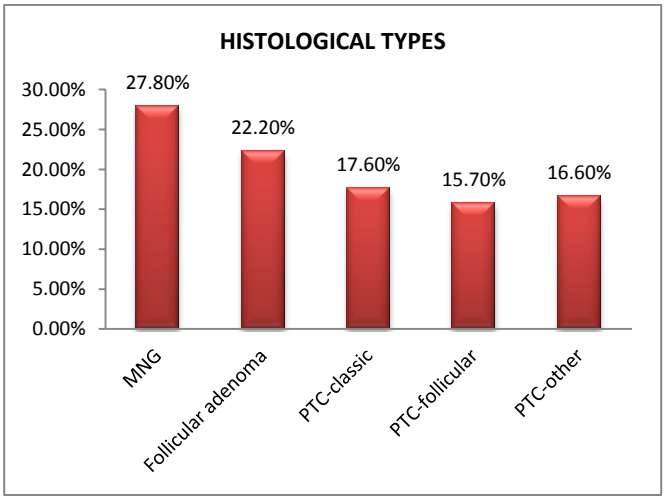
Sensitivity(truepositive/(truepositive+falsenegative)), specificity(truenegativ e/(truenegative +false positive)), falsenegative rate(1-sensitivity), false positive rate(1-specificity), and diagnostic accuracy(true positivity +true negative/all positives +all negatives) of each marker and their combination has been assessed

Results

A total of 108 specimens were studied. Major population of my study come under the age group 21-40 years. 85% of cases include females and 15 % include males. Total thyroidectomy specimens in my study include the major percentage which corresponds to 80.6% and only 5.6% cases include left hemithyroidectomy specimens. Benign as well as malignant lesions were more common in female.

Histologic Types

Figure-1



My study included 27.80% cases of MNG,22.20% cases of follicular adenoma,17.60% cases of classic variant of PTC,15.70% cases of follicular variant of PTC and 16.60% cases of other variants of PTC

Age-Behaviour Association

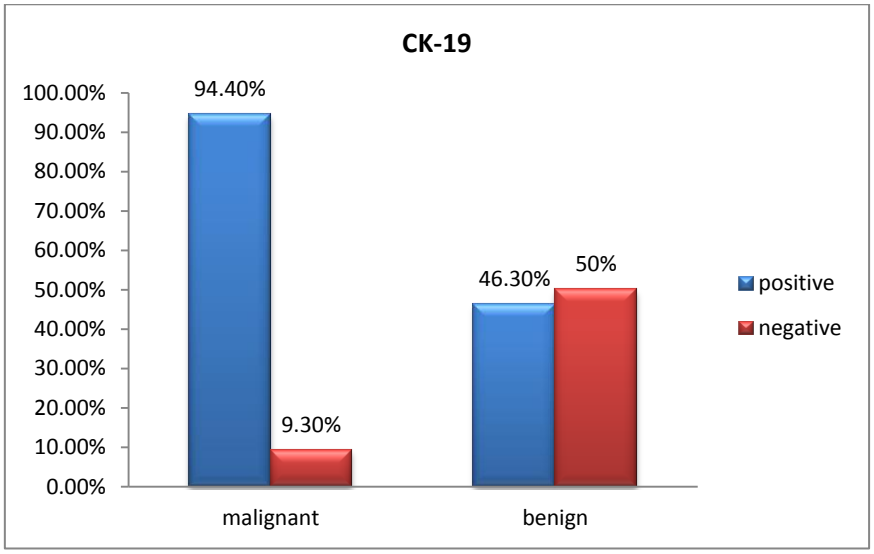
Table-1

AGE GROUP	BENIGN	MALIGNANT
≤40	39%	61%
>40	65.2%	34.8%
TOTAL	54(50%)	54(50%)

39% of benign cases and 61% of malignant cases come under age group ≤40 years whereas 65.2% of benign cases and 34.8% of malignant cases come under the age group >40 years

CK 19 Expression

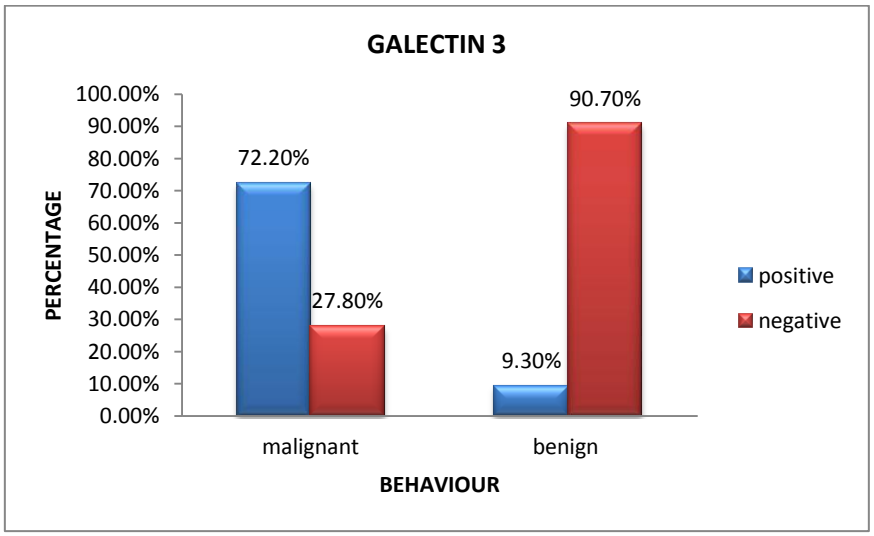
Figure-2



CK-19expression was observed in 94.4% of malignant cases and 46.3%% of benign cases whereas lack of expression was observed in 9.3%% of malignant cases and 50% of benign cases. Hence sensitivity of CK-19 obtained in my study is 94.4%% whereas specificity is 50%. Positive predictive value and negative predictive value of CK-19 obtained are 67.1 % and 84.4% respectively. Diagnostic accuracy of CK19 is 72.2%

Galectin-3 Exression

Figure-3



Galectin-3 expression was observed in72.2% of malignant cases and 9.3% of benign cases whereas lack of expression was observed in 27.8% of malignant cases and 90.7% of benign cases. Hence sensitivity of galectin-3 obtained in my study is 72.2% whereas specificity is 90.7%. Positive predictive value and negative predictive value of galectin-3 obtained are 88.6% and 76.6% respectively. Diagnostic accuracy of galectin-3 is 81.5%

Descriptive Statistics for each Immunocytochemical Marker

Table-2

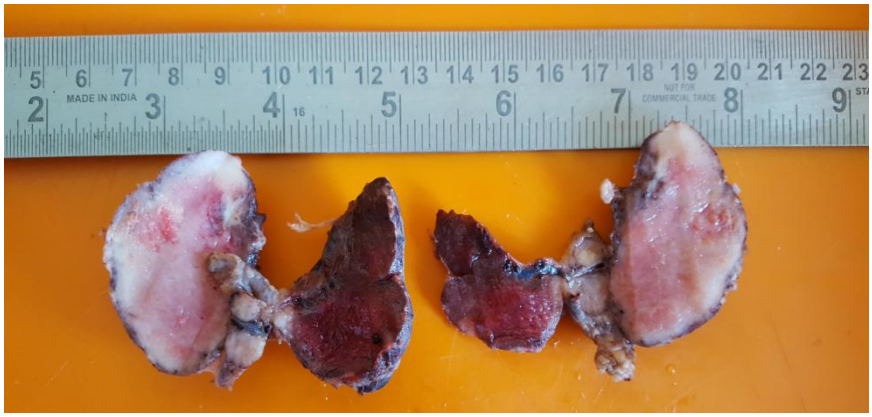
	Sensitivity	Specificity	Diagnostic accuracy	kappavalue	Positive predictive value	Negative predictive value
CK19	94.4%	50%	72.2%	0.481	67.1%	84.4%
Galectin-3	72.2%	90.7%	81.5%	0.630	88.6%	76.6%

Statistics for Combined Ck19 and Galectin-3 Staining

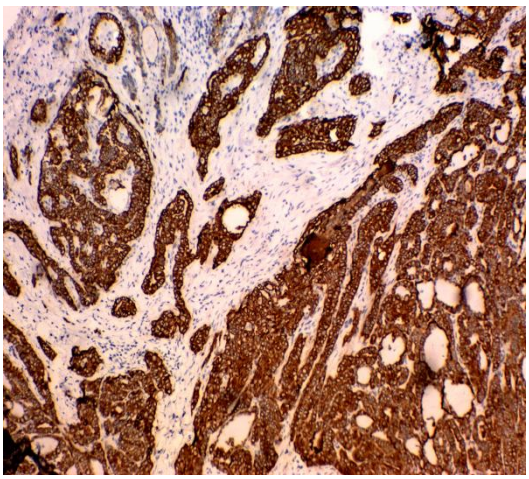
Table-3

	Sensitivity	Specificity	Kappa value	Diagnostic accuracy
CK19/galectin-3	96.3%	51.9%	0.481	74.1%

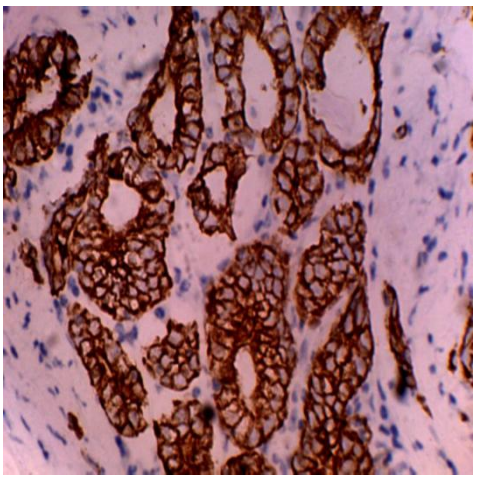
When Galectin-3 and CK-19 are both combined, the sensitivity has become 96.3%, specificity 51.9% and diagnostic accuracy 74.1% at a kappa value of 0.481



Picture 1 Gross picture of Diffuse sclerosing variant of papillary thyroid carcinoma



Picture 2a



Picture 2b

Diffuse membranous and cytoplasmic staining of CK19(2a) cytoplasmic positivity of Galectin3(2b) in Papillary throid carcinoma,classic variant.

Discussion

Thyroid nodules are very common in general population. Although most ofthem are benign, malignant tumors of the thyroid represents the mostcommon endocrine malignancy, which is usually seen more in solitary thyroidnodules.

Malignant tumors exhibit a variety of histopathological features and hence immune markers are gaining more importance in the diagnostic pathology. Papillary thyroid carcinoma exhibits a number of nuclear features including nuclear elongation, crowding, clearing and intranuclear grooving. These features can also be seen in some benign thyroid lesions making the diagnosis of papillary thyroid carcinoma difficult.

Several recent studies have addressed the issue of using immunohistochemical markers in the diagnosis of thyroid neoplasms. Mauro papotti et al[14] and Matos et al[15] investigated the use of panel of immunohistochemical markers like Gal-3, HBME-1 and cytokeratin19 in the diagnosis of thyroid malignancy.

In study conducted by Matos et al,sensitivity of both CK 19 and galectin 3 was found to be 72.6% each. In study conducted by Laco j et al[17], sensitivity of cytokeratin was found to be 94 %

In the present study, the immunohistochemical expression of CK19 and Gal-3 were studied in benign thyroid lesions and papillary carcinoma thyroid and we investigated the possibility of using these to differentiate between benign and malignant thyroid tumours. In this study, a total of 108 thyroid specimens were included in which 54 were benign and 54 were histopathologically proven papillary carcinoma

It was observed that benign as well as malignant tumors were common in females. 85.2% of benign tumours and 85.2% of malignant tumours occurred in females. Findings were similar to those studied by Mazzaferri et al who got 80% of nodules in female[18]. Major population of malignant group occurred in the age range of 21-40 years where as major population of benign group occurred in the age range of 41-60 years.

By histopathological examination which is the gold standard, there were 30cases (27.8%) of multinodular goiter, 24 cases (22.2%) of follicular adenoma, 19 cases (17.6%)of PTC classic variant, 17cases (15.7%) of follicular variant of papillary carcinoma, 10cases (92.6%) of multifocal papillary thyroid carcinoma, 5 cases (4.6%) of encapsulated follicular variant of papillary carcinoma,1 case(0.9%) of diffuse sclerosing variant of PTC,1 case (0.9%)of columnar cell variant of papillary thyroid carcinoma,1 case and 1 Case (0.9%)of papillary microcarcinoma.

In study conducted by Qingbin Song et al[8], CK-19 and Galectin-3 expression was 96.37% (425/441) and 96.82% (427/441) respectively, for the PTC group and the expression of these markers in the benign thyroid lesions group was 25.83% (39/151) and 50.99% (77/151), respectively. The diagnostic efficiency of CK19 and galectin-3 for PTC was 96.37% (537/592) and 84.63% (501/592).In study conducted by Leandro Luongo de Matos et al (24), CK 19 showed sensitivity of 81% and specificity of 73%; Galectin-3 showed sensitivity of 82% and specificity of 81%.

The present study confirmed positive expression of CK19 in 94.4% cases of Papillary thyroid carcinoma and 50% of benign thyroid lesions (follicular adenoma, multinodulargoiter) were characterized by loss of expression of CK19 whereas 46.3% of benign lesions also showed CK19 positivity

Sensitivity, specificity and diagnostic accuracy of CK19 being 94.4 %, 50% and 72.2% respectively at a kappa value of0.481.Positivepredictive value of CK19 was found to be 67.1%and negative predictive value of 84.4% Findings were similar to those by Laco j et al[17] and Scognamiglio T et al [19]

In study conducted by Song Q et al, sensitivity of CK19 and Galectin 3 was found to be 96.37% and 96.82% respectively and specificity of CK19 and galectin 3 was found to be 25.83% and 50.99% respectively[8]

In my study positive expression of Galectin-3 in 72.2% cases of Papillary thyroid carcinoma and 90.7% of benign thyroid lesions (follicular adenoma, multinodulargoiter) were characterized by loss of expression ofGal-3 Sensitivity, specificity and diagnostic accuracy of Gal-3 being 72.2% 90.7% and 81.5% respectively at a kappa value of 0.630.Positivepredictive value of Gal-3 was found to be 88.6%and negative predictive value of 76.6%

By combined immunostaining of CK19 and Galectin3, sensitivity was increased to96.3% and specificity was 51.9%

Though our sample size is limited, this study hereby confirms that CK 19 isa more sensitive marker of papillary thyroid carcinoma whereas Galectin-3is more specific.

Conclusion

By combined immunostaining of CK19 and Gal-3, 96.3% malignant nodules could be identified and 51.9% of benign lesions could be differentiated correctly. We suggest that immunopanel comprising of Ck19 and Galectin-3 can help greatly in distinguishing benign thyroid lesions from true papillary thyroid carcinoma

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