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Application of HMWCK, CK19 and Galectin-3 as Immuno Histochemical Markers in the differential diagnosis of thyroid lesions

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Abstract
Introduction: Thyroid neoplasms constitute the most common of all endocrine neoplasms occurring worldwide and more than 95% arise from follicular epithelial cells. Thyroid tumors are more common in developed countries. The incidence of thyroid tumors has increased in past two decades.
Aim: to assess the role of Galectin-3 in differentiating benign and malignant thyroid lesions along and combined use of CK-19 and HMW-CK in the differential diagnosis between papillary carcinoma and follicular carcinoma.
Material and methods: The present study was conducted in 50 specimens of thyroid tissue received in the Department of Pathology, Government Medical College, Amritsar, after approval from the Institutional Ethics Committee. IHC staining was done using Galectin-3, CK-19 and HMW-CK immuno Histochemical markers. The IHC score was calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score).
Results: Incidence of thyroid nodules most common in the age group between 20-40 years comprising about 66% of the total cases followed by 41-60 years. Females constituted about 74% (37 cases) in contrast to males with 13 cases comprising 26%. Prevalence of benign Lesions of thyroid was slightly higher 29 (58%) in comparison to malignant lesions which comprised of 21 (42%) of the study sample. Galactein 3 is found to be the most sensitive marker while HMWCK is found to be the most specific marker in distinguishing malignant thyroid lesions from benign thyroid lesions in present study. CK-19 and HMW CK when combined together in absence of Galactein 3 shows 100% sensitivity and high specificity in diagnosing malignant thyroid lesions. These combinations show increased specificity and positive predictive value compared to Galactein 3 when used alone.
Conclusion: Therefore we can say that these immuno histochemical panels can be a useful means to increases the likelihood of detecting malignant tumors. These markers along with histopathological diagnosis can aid us in correct diagnosis and thus further help to optimize the management of patients with thyroid nodules and reduce unnecessary surgical resection of benign nodules.

Keywords: Thyroid Neoplasm, Papillary Carcinoma, Follicular Carcinoma, HMWCK, Cytokeratin 19, Galectin 3

Introduction

Thyroid neoplasms constitute the most common of all endocrine neoplasms occurring worldwide and more than 95% arise from follicular epithelial cells. They encompass a wide variety of benign and malignant tumours. About 4% to 8% of adult women and 1% to 2% of adult men present with thyroid nodules that can be identified by physical examination [1]. Majority of the thyroid nodules are benign with malignant nodules comprising only 10% [2, 3]. Thyroid tumors are the most common endocrine tumors. The estimated age standardized annual incidence is 1.0 to 2.9 cases per 1, 00,000 men and 3.4 to 9.1 cases per 1, 00, 000 women according to GLOBACON 2008. Thyroid tumors are more common in developed countries [4]. The incidence of thyroid tumors has increased in past two decades. GLOBACON (2018) reported that an estimated 567,233 new cases have appeared and 41,071 deaths occurred due to thyroid malignancy worldwide [5].
Histopathology remains the gold standard in diagnosing thyroid lesions, but those cases with nodular architecture and follicular growth pattern may show difficulties in accurate diagnosis. The prognosis and management of thyroid nodules depends on their diagnoses.

However, morphologic similarities between benign and malignant lesions are frequent, and follicular and papillary architectures may be seen in both benign and malignant lesions. The diagnosis of follicular neoplasm on cytology or of follicular tumour of uncertain malignant potential on histology is likely to cause confusion and delay the effective management of these lesions.

Recent studies have focused on identifying IHC markers that can help in differentiating benign from malignant lesions, and follicular variant of papillary carcinoma from follicular carcinoma or adenoma. The findings were generally encouraging and promising. Therefore, several immuno Histochemical markers using different antibodies, alone or combined in panels, have been postulated to improve diagnostic accuracy of thyroid lesions.

The present study aims to assess the role of Galectin -3 in differentiating benign and malignant thyroid lesions along and combined use of CK-19 and HMW-CK in the differential diagnosis between papillary carcinoma and follicular carcinoma.

Material and methods

The present study was conducted in 50 specimens of thyroid tissue received in the Department of Pathology, Government Medical College, Amritsar, after approval from the Institutional Ethics Committee. Informed consent of the patient was taken (if required in vernacular language). Relevant history of the patient was taken as per the proforma attached along with. All specimens of thyroid tissue, including benign and malignant lesions, diagnosed histologically as neoplastic or non-neoplastic lesions of thyroid were included irrespective of age. While all the patients with congenital thyroid lesions were excluded from the study.

Paraffin embedded tissue sections were stained with routine haematoxylin and eosin (H&E) and the diagnosis was made on the basis of the histopathological findings. Then IHC staining was done using Galectin-3, CK-19 and HMW-CK immuno Histochemical markers. The IHC score was calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score).

RESULTS:

In the present study a total of 50 samples of thyroid neoplasms were included, with an incidence of thyroid nodules most common in the age group between 20-40 years comprising about 66% of the total cases followed by 41- 60 years. Females constituted about 74% (37 cases) in contrast to males with 13 cases comprising 26%.

The total study sample was categorized as benign and malignant lesions. Prevalence of benign Lesions of thyroid was slightly higher 29 (58%) in comparison to malignant lesions which comprised of 21 (42%) of the study sample. Benign lesions included Colloid Goitre, Adenomatous Goitre, Nodular Goitre, Multinodular Goitre, Chronic lymphocytic thyroiditis & Hashimoto's thyroiditis and follicular adenoma. While malignant thyroid neoplasms comprised of papillary thyroid carcinomas comprised (55.2%), follicular carcinoma with (13.7%) and only one case (3.4%) of medullary carcinoma.

Immunoexpression of HMW-CK

We observed no HMWCK staining in 79.3% cases, weak in 17.2% cases and moderate in 3.4% cases in benign thyroid lesions. Complete absence of strong and diffuse staining intensity (score +3). Goiter showed overall negative staining (0) in 85.7% (n=18) cases and weak staining (+1) in 14.2% (n=3) of the cases. Follicular adenoma showed 62.5% (n=5) cases with weak HMWCK staining intensity, 25% (n=2) showed mild staining intensity and 12.5% (n=1) showed moderate staining intensity. (TABLE: 1).

HMWCK in Malignant thyroid lesions showed overall strongly positive expressions in cytoplasm with membranous accentuation in 66.6% (n=14) of the samples, moderate staining in 19.1% (n=4) of the cases, weak staining in 9.5% (n=2) cases while no staining in only 4.7% cases. Thus, depicting that overall, malignant thyroid lesions showed predominantly a strongly positive diffuse staining intensity with HMWCK. (Table 1) Hence, HMWCK was found to be 80.95% sensitive and highly specific marker (100%) in diagnosing malignant thyroid lesions.

Table 1: Overal Hmw-Ck Staining in thyroid lesions in present study

HMW-CK Staining Interpretation		Benign	Malignant	P Value
Cases showing moderate and strong cases	Positive	0/29 (0)	17/21 (80.95%)	<0.05*
Cases showing weak positivity	Negative	29/29 (100%)	4/21 (19.1%)	

Immunoexpression of CK 19

In the resent study, we observed no CK 19 staining in 72.4% cases, weak in 24.1% cases and strong in 2.4% cases in benign thyroid lesions. While in Malignant thyroid lesions showed overall strongly positive expressions in cytoplasm with membranous accentuation in 80.9% (n=17) of the samples, moderate staining and weak in 4.7% (n=1) of the cases each, while no staining in only 9.5% cases. Thus, overall, malignant thyroid lesions showed predominantly a strongly positive diffuse staining intensity with CK-19. (Table 2). CK 19 staining is found to be a sensitive and specific marker in diagnosing malignant thyroid lesions.

Table 2: Overall Ck-19 Staining in thyroid lesions in present study

Ck-19 Staining Interpretation		Benign	Malignant	P value
Cases showing moderate and strong cases	Positive	1/29 (3.4%)	18/21 (85.7%)	<0.05*
Cases showing weak positivity	Negative	28/29 (96.5%)	3/21 (14.2%)	

Immunoexpression of galactein-3

We observed weak galactein-3 stainnig (62.1%) in benign thyroid lesions and complete absence of strong and diffuse staining intensity (score +3). Staining intensity of Galactein-3 in Malignant thyroid lesions showed overall strongly positive diffuse staining in the cytoplasm of cells in 71.4% (n=15) of the samples and moderate staining in 19.1% (n=4) of the cases and only 9.5% (n=2) cases with weak staining. Thus, depicting that overall, malignant thyroid lesions showed predominantly a strongly positive diffuse staining intensity with Galactein-3 (Table 3).

Table 3: Overal Galactein 3 Staining in thyroid lesions in present study

Galectin-3 Staining Interpretation		Benign	Malignant	P Value
Cases showing moderate and strong cases	Positive	8/29 (27.5%)	19/21 (90.4%)	<0.05*
Cases showing weak positivity	Negative	21/29 (72.4%)	2/21 (9.5%)	

On comparison, Galactein 3 is found to be the most sensitive marker while HMWCK is found to be the most specific marker in distinguishing malignant thyroid lesions from benign thyroid lesions in present study. (Table 4).

Further it was observed that 16/21 malignant thyroid lesions were correctly diagnosed with both galactein-3 and HMWCK staining.3 /21 cases negative on galactein 3 staining were found to be positive with HMWCK. Also we observed that 18/21 malignant thyroid lesions were correctly diagnosed with both galactein 3 and CK-19 staining. 2/21 cases negative on galactein 3 staining were found to be positive with CK19.

Table 4: Comparison of diagnostic accuracy of Galactein-3, CK -19 And HMW-CK in diagnosing malignant thyroid lesions in the present study

Immuno-Histochemical Marker	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Galactein 3	90.4%	72.4%	73.07%	91.3%
Ck-19	85.7%	96.5%	94.7%	90.3%
HMW-CK	80.95%	100%	80.95%	87.8%

To improve the diagnostic accuracy in diagnosing malignant thyroid lesions, we analyzed the combined effect of all the three sensitive markers Galactein 3, HMWCK and CK19. Our results showed that Galactein 3 still remains the most sensitive marker when used alone in comparison to be used in combination with HMWCK and CK19. But these combinations show increased specificity and positive predictive value compared to Galactein 3 when used alone. (Table 5) CK 19 and HMW CK when combined together in absence of Galactein 3 shows 100% sensitivity and high specificity in diagnosing malignant thyroid lesions.

Table 5: Diagnostic accuracy of Galactein-3, CK -19 and HMW-CK When combined together in diagnosing malignant thyroid lesions in the present study

Combination of Immuno-Histochemical marker	Sensitivity	Specificity	Positive predictive value	Negative predictive value
GALACTEIN-3 + CK-19	85.7%	96.5%	94.7%	90.3%
GALACTEIN-3 + HMW-CK	80.95%	100%	80.95%	87.8%
CK-19 + HMW-CK	100%	96.5%	100%	100%

In the present, HMWCK also proves to the 100% sensitive marker in distinguishing papillary carcinoma from other malignant thyroid tumors namely follicular carcinoma (50% sensitive) and medullary carcinoma (Figure 1).

Discussion

In the present study a total of 50 samples of thyroid neoplasms were included, with an incidence of thyroid nodules most common in the age group between 20-40 years comprising about 66% of the total cases followed by 41- 60 years with female predominance.

HMWCK

Complete absence of strong and diffuse staining intensity (score +3) was observed in benign thyroid lesions. While malignant thyroid lesions showed predominantly a strongly positive diffuse staining intensity with HMWCK. Taghreed Abd El-Samee, *et al.* reported that all examined adenomas in their study showed negative HM-WCK (100%), atypical nodules showed diffuse HMWCK positivity in 22% cases and malignant cases showed diffuse HMWCK positivity in 40.8% cases examined. Hence it was interpreted that HMWCK diffuse positivity is not seen in benign cases while was present in atypical and malignant cases [6].

In similarity other authors like Scognamiglio *et al.* [7], and Nasr *et al.* [8], and Liu *et al.* [9], stated that HMWCK is a marker of malignancy detection in thyroid tumors and can separate benign cases from atypical nodules and malignant thyroid tumors but can't separate atypical nodules from malignant cases.

Further in Papillary carcinoma, HMWCK was strongly positive in 81.2% cases, in follicular carcinoma, 25% cases showed strong positivity and moderate staining intensity in 50% cases. In medullary carcinoma 100% cases showed no staining. Hence HMWCK proves to the 100% sensitive marker in distinguishing papillary carcinoma from other malignant thyroid tumors namely follicular carcinoma (50% sensitive) and medullary carcinoma. Taghreed Abd El-Samee, *et al.* reported that PTC cases showed diffuse HM-WCK positivity in 91%, while FTC showed diffuse

HMWCK positivity in no case [6].

In the present study sensitivity and specificity of HMW-CK in distinguishing benign and malignant thyroid lesions came to be 80.95% and 100% (Table 6).

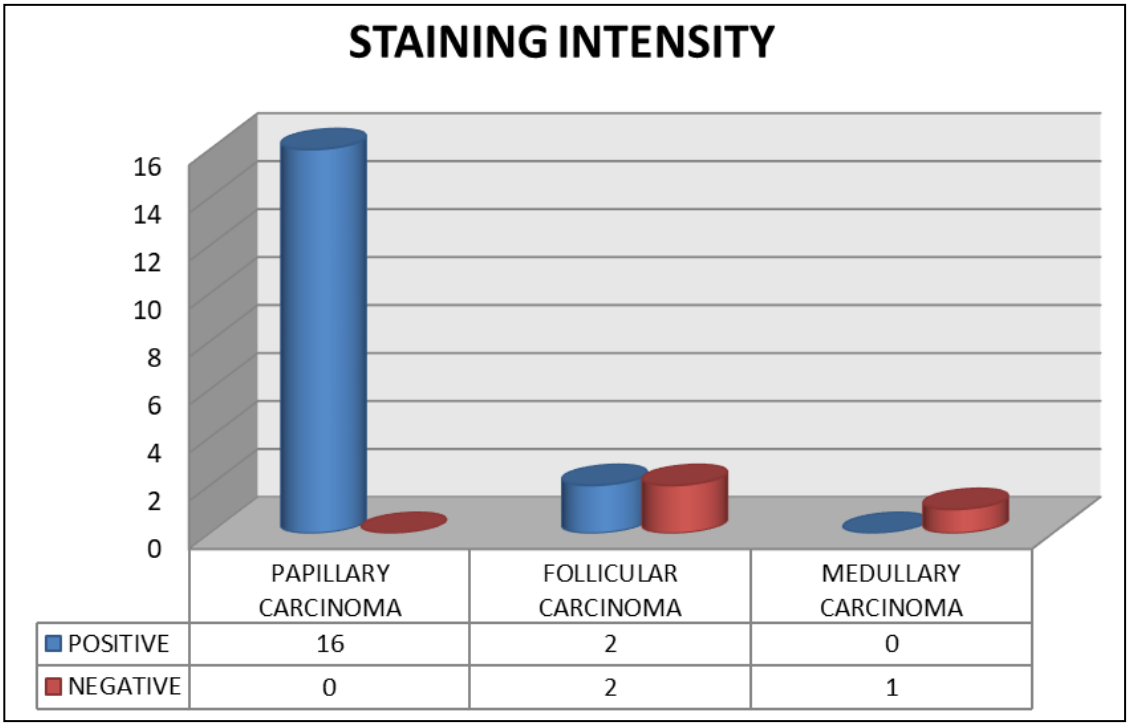

Fig 1: HMW-CK Expression in differentiating papillary carcinoma from other malignant thyroid tumours

Table 6: Senstivity and specificity of HMWCK 1 as reported by various authors

Author	Sensitivity and specificity
PARK YJ *et al.* [14]	52.4% and 100%
Shin kyung M. *et al.* [15]	92.5% and 90.9%
Present study	80.95% and 100%

CK-19

In benign thyroid nodule overall, malignant thyroid lesions showed predominantly a strongly positive diffuse staining intensity with CK-19.

Hanan Alsaeid Alshenawy studied the usefulness of cytokeratin19 in thyroid nodules during the year 2009-2013 and observed cytokeratin19 positivity in 57% of follicular adenoma i.e. 4 cases out of total 7 cases Also they found out that cytokeratin19 showed positivity in 53% of cases of follicular carcinoma [10].

Various other authors like Choi *et al.* [11] and Shelis *et al.* [12] stated that diffuse positive CK-19 Immunostaining is not seen in follicular adenomas or in atypical encapsulated nodules but were evident in 86% of follicular carcinoma cases.

Our results showed that in papillary carcinoma, CK-19 is predominantly strongly positive in 93.7%. Similarly, Husain a Saleh *et al.* reported 85% positivity of classic papillary carcinoma cases with cytokeratin19 and 83.3% in follicular variant of papillary carcinoma [13].

PTC cases showed negative CK19 expression in 27.3%, focal positivity in 31.6% and diffuse CK19 positivity in 41%, while FTC cases showed diffuse CK19 positivity in 82.4% cases, and focal positivity in 17.6%. These results were supported by that done by many authors as Arora *et al.* [14], Kosem *et al.* [15], and Liu *et al.* [16].

In our study sensitivity and specificity of CK-19 in distinguishing benign and malignant thyroid lesions came to be 85.7% and 96.5% respectively (Table 7). Hence, CK19 proves to be sensitive diagnostic marker for malignant thyroid tumors originating from follicular epithelium and successfully can separate them from follicular adenomas in which CK19 diffuse positivity is completely absent.

Table 7: Sensitivity and Specificity of CK 19 as reported by various authors

Authors	Sensitivity	Specificity
Carol C Cheung *et al.* [17]	65.94%	80.43 %
Manju L Prasad *et al.* [18]	71.64%	85.48 %
Dina El Demellawy *et al.* [19]	84.72 %	73.85 %
Mustafa Kosem *et al.* [15]	100 %	57.69 %
Park YJ [20]	90.35	83.1%
Present study	85.7%	96.5%

Galactein-3 Immunostaining

Our results show an overall weak galactein-3 staining in 62.1% of the benign thyroid lesions with complete absence of strong and diffuse staining intensity in all benign thyroid nodules. Malignant thyroid lesions showed predominantly a strongly positive diffuse staining intensity with Galactein-3. Further, in papillary carcinoma, galactein-3 was strongly positive in 68.7% cases where as in follicular carcinoma and medullary carcinoma 100% cases showed strong positivity staining intensity.

Thus our results depicted that neoplastic thyroid lesions showed predominantly a strongly positive diffuse staining intensity with Galactein-3 while non-neoplastic thyroid lesions exhibited predominantly weak Galactein-3 staining. In similarity Bartolazzi *et al.* [21] and Muzafar A *et al.* [22] also reported similar positivities in their studies.

In our study further for papillary carcinoma, galactin -3 is

strongly positive in 68.7% cases, in follicular carcinoma 100% cases showed strong positivity staining intensity. And even Also for medullary carcinoma 100% case showed only moderate staining intensity. Majority of authors like Chiu CG *et al.* [23], Bartolazzi A *et al.* [21], Prasad ML *et al.* [24] and Park YJ *et al.* [20], reported Gal-3 positivity in 80% to 100% of papillary carcinoma cases. In a recent study by BS Sumana *et al.* [25] Gal-3 expression was also reported to be significantly higher (91.3%) in papillary carcinoma.

On statistical analysis, Galactein 3 staining is 90.4% sensitive and 72.4% specific marker in diagnosing malignant thyroid lesions. From these results, we concluded that galectin-3 is the most useful marker for the detection of thyroid carcinomas (Table 8).

Table 8: Sensitivity and specificity of Galactein 3 as reported by various authors

Author	Sensitivity and specificity
Bartolazzi *et al.* [21]	99% and 98%
Prasad *et al.* [24]	92 % and 98%
Qingbin Song *et al.* [26]	49.01% (less specificity)
Park YJ [20]	94.7% and 95.6%

Diagnostic accuracy of HMW-CK, CK-19 and Galactein-3, when combined together in diagnosing malignant thyroid lesions

Our results showed that on comparison, Galactein 3 is found to be the most sensitive marker while HMWCK is found to be the most specific marker in distinguishing malignant thyroid lesions from benign thyroid lesions in present study.

CK-19 and HMW CK when combined together in absence of Galactein 3 shows 100% sensitivity and high specificity in diagnosing malignant thyroid lesions. Hence, Galactein 3 still remains the most sensitive marker when used alone in comparison to be used in combination with HMWCK and CK19. But these combinations show increased specificity and positive predictive value compared to Galactein 3 when used alone.

Conclusion

Hence we can conclude from our study that Immuno expression of HMWCK, CK-19 and galectin-3 is an important supplementary test in the diagnosis of thyroid neoplasms, though it does not replace the conventional histomorphological examination. HMWCK is found to be the most specific marker while Galactein 3 is found to be the most sensitive marker in distinguishing malignant thyroid lesions from benign thyroid lesions in present study. When CK-19 and HMW CK combined together in absence of Galactein 3 shows 100% sensitivity and high specificity in diagnosing malignant thyroid lesions. Therefore we can say that these immuno Histochemical panels can be a useful means to increases the likelihood of detecting malignant tumors. These markers along with histopathological diagnosis can aid us in correct diagnosis and thus further help to optimize the management of patients with thyroid nodules and reduce unnecessary surgical resection of benign nodules.

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