Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-nahrain.edu.iq

Evaluation of Immunohistochemical Expression of CK19 in Papillary Thyroid Carcinoma and Grave's Disease with Papillary Changes

Ikram A. Hasan[1] *FICMS*, **Methaq M. Najem**[1] *MSc*, **Thair J. Kadem**[2] *FICMS*
[1]Dept. of Pathology, College of Medicine, Al-Mustansirya University, [2]Al-Yarmook Hospital, Baghdad, Iraq.

Abstract

Background Immunohistochemistry seems to be important method for differentiation between papillary thyroid carcinoma which is the commonest thyroid cancer and Grave's disease which may be challenging by light microscopic features only.

Objectives The aim of the study was to evaluate the immunohistochemical expression of CK 19 antibody that is considered a marker of malignancy in papillary thyroid carcinoma and in Grave's disease and to determine whether CK19 is effective in the discrimination between these two pathological conditions.

Methods In this retrospective study paraffin embedded archival materials from 40 cases including 20 papillary thyroid carcinoma and 10 Grave's disease in addition to ten normal thyroid tissue were used as a control group, collected from the department of pathology of Al-Yarmook Teaching Hospital for the period between Jan 2009 to Jan 2011. The immunohistochemical expression of CK19 markers was assessed for intensity and extent of staining in semiquantative method.

Results Sixteen of the 20 papillary carcinomas showed diffuse and intense cytoplasmic staining with CK19 (80%), 4 cases showing diffuse faint staining (20%). Seven of the 10 Grave's cases (70%) are completely negative. The remaining 3 cases showing focal weak staining with CK19 (30%). There was a significant difference in the extent of staining between papillary thyroid carcinoma and Grave's disease and there was highly significant difference in intensity of staining between them.

Conclusions The staining features of CK19 may be helpful in the differential diagnosis between papillary carcinoma and Grave's disease with papillary carcinoma like structures. This immunoreactivity should be evaluated with histopathological findings in order to prevent over diagnosis of papillary carcinoma.

Keywords CK19 immunohistochemical expression, papillary thyroid carcinoma, Grave's disease.

Introduction

Papillary thyroid carcinoma is the commonest thyroid cancer [(1)] and there is a marked increase in its incidence through the recent decades [(2)].

The identification of papillary thyroid carcinoma relied 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 pseudoinclusions [(3)]. 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.

One of these benign lesions is the autoimmune hyperthyroidism (Grave's disease) that is predominantly seen in females. In Grave's

disease, the thyroid is generally diffusely enlarged, but there may be nodules as well [5].

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 [5]. This difficulty may also be encountered in papillary formations of multinodular goiter, where CK19 has been shown to be effective in discrimination [6].

Because of these problems, numerous attempts have been made to apply a variety of techniques to enhance diagnostic reliability like electron microscopy and flow cytometry [7] but the results were disappointing in that the techniques did not yield cheap and rapid diagnostic information that could realistically change the practice of surgical pathology. Over the past decade, however, rapidly expanding techniques available in molecular pathology (like immune-histochemistry) have begun to show real promise to change daily practice and many immunohistochemical markers have been evaluated for their potential in distinguishing Papillary thyroid carcinoma from other benign thyroid lesions, the main ones including cytokeratin (CK) 19, galectin-3 (GAL3), and HBME1 (17).

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 is different from other keratin-encoding genes [8]. Cytokeratin 19 concentrates at sarcomeres of striated muscle and copurify with the dystrophinglycoprotein 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 [9]. CK19 is synthesized in simple and stratified epithelia.

This study was designed to determine the effectiveness of CK19 in distinguishing papillary thyroid carcinoma and papillary carcinoma-like changes in Graves's disease.

Methods

Tissue Sample: In this retrospective study a total of 30 tissue samples of which 20 were of papillary thyroid carcinoma and 10 of Grave's disease. In addition, 10 normal thyroid tissue had been taken as a control. All formalin fixed, paraffin-embedded tissues were retrieved from the archived files of the department" of histopathology of Al-Yarmook Teaching Hospital for the period between Jan 2009- Jan 2011.

Clinicopathological parameters (age, sex, clinical presentation and histopathological diagnosis) were obtained from the available histological reports. For each case, 2 sections of 5μm thickness were taken; one section was stained with (H and E), and the other was stained immunohistochemically for with CK 19 tumor markers.

Immunohistochemical staining was performed by the streptavidin –biotin method.

Interpretation of the results of staining characteristics:

The presence of brown reaction product of more than 10% of tumor cells at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark blue coloration of the cell nuclei. Staining pattern was cytoplasmic or membrane and cytoplasm.

The quantity of the immunostaining was evaluated as follows [10]: Semiquantitative evaluations were made on the basis of intensity and extent of staining for CK-19. The extent of staining by CK-19, were calculated according to the percentage of positive cells as: No staining: 0; < 25% stained cells: 1+; 25% to 75% stained cells: 2+; > 75% stained cells: 3+, whereas the intensity of staining by CK-19 was evaluated as no staining: 0; faint: 1+; and strong: 2+.

The positive result was classified as focal and diffuse. Focal:-tumors in which clusters of positive cells where seen in some areas of the tumor but other region where negative. Diffuse: - tumors in which isolated and/or clusters of

positive cells where seen throughout most areas of the tumor.

Results

The papillary carcinoma group consist of 19 females and 1 male with an age range 25-55 years, Grave's group comprise 8 females and two males with an age range 18-60 years (Table 1).

Table 1. Age distribution of patients with papillary carcinoma and Grave's disease.

Age (years)	Papillary carcinoma		Graves	
	No.	%	No.	%
<50	12	60	8	80
≥50	6	40	2	20

Sixteen out of twenty cases (80%) of papillary carcinomas showed diffuse and intense cytoplasmic staining with CK19 (Fig.1 and 2), four cases (20%) showing diffuse faint staining. Seven of the 10 Grave's cases (70%) are completely negative (Fig.3 and 4). The remaining 3 cases (30%) showing focal weak staining with CK19.

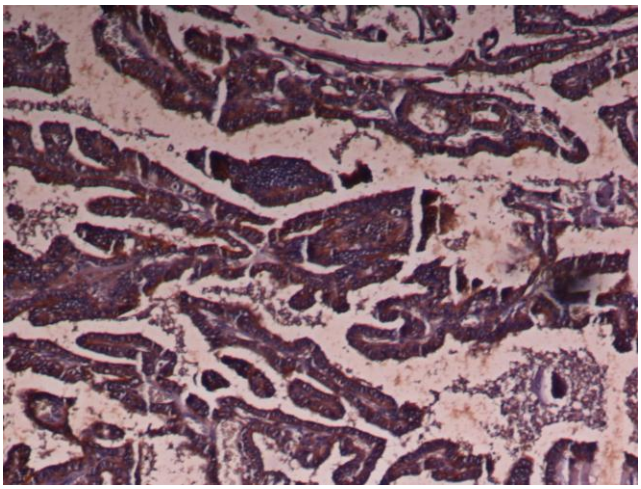

Fig. 1. Diffuse, strong CK19 staining in papillary carcinoma (CK19X100)

There was a significant difference in the extent of staining between papillary thyroid carcinoma and Grave's disease (P = 0.005) by using Chi square test and there was highly significant difference in intensity of staining between them (P = 0.0001) (Table 2 and 3).

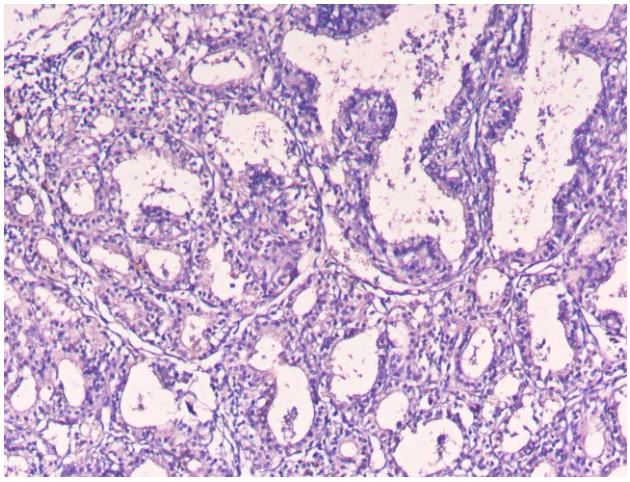

Fig. 2. Diffuse, strong CK19 staining in papillary carcinoma with strong desmoplasia, CK19X100

Discussion

One of the most frequent problems in thyroid pathology is differentiating papillary carcinoma like changes in Grave's disease which may contain vesicular nuclei, nuclear grooves from true papillary carcinoma which may be difficult depending on microscopical features only so there is a need for other methods like immunohistochemistry to solve this problem using different markers like (HMB-1, galectin-3 and CK19).

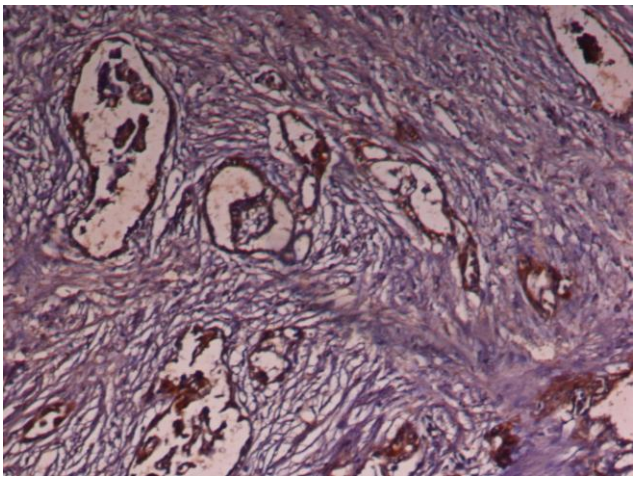

Fig. 3. Faint or absent staining with CK19 in Grave's disease (CK19X100)

CK19 is one of the low molecular weight keratin. It is known to be resistant to denaturation and the preservation of its reactivity has been reported even in necrotic tumor tissue [11].

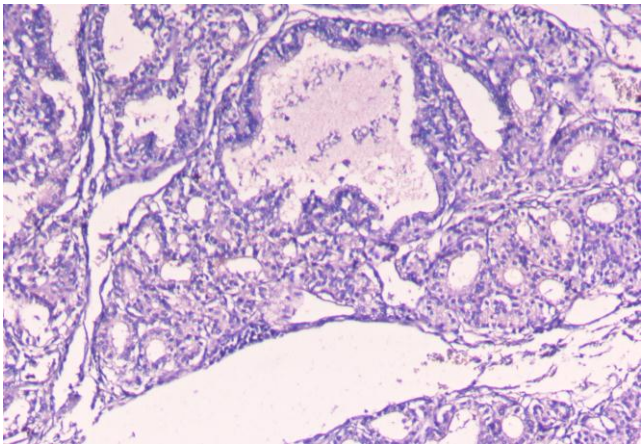

Fig. 4. Another view showing faint or absent staining with CK19 in Grave's disease (CK19, X100)

Diffuse and strong staining with low molecular weight keratins has been reported in thyroid papillary carcinoma [12,13].

In this study, 16 of the papillary carcinoma group exhibit diffuse intense cytoplasmic staining for CK19 and seven of ten Grave's cases are completely negative while the three remaining cases showed diffuse, faint immunoreactivity.

The results of current study agree with other studies like Suna et al [5] that demonstrated that the vast majority of papillary carcinoma exhibit diffuse intense cytoplasmic staining for CK19 whereas majority of Grave's cases are completely negative.

Table 2. Extent of staining of CK19 in papillary carcinoma and Grave's disease.

Extent	Papillary carcinoma		Graves	
	No	%	No	%
0	1	5	6	60
+1	1	5	2	20
+2	3	15	2	20
+3	15	75	0	0

P = 0.005

The weak immunoreactivity in Grave's disease may be similar to the pale staining reported previously by Sahoo et al and Bennet et al in follicular adenoma [1,14].

Table 3. Intensity of staining of CK19 in papillary carcinoma and Grave's disease.

Extent	Papillary carcinoma		Graves	
	No	%	No	%
0	1	5	8	80
+1	22	10	2	20
+2	17	85	0	0

P = 0.0001

The results of this study is in agreement with others like: El Demallowy et al [2] who showed that 85% of cases of papillary carcinoma were positive for CK19 and with Cheung et al [3] who reported diffuse CK19 staining in 80% of papillary thyroid carcinoma and with Baloch and Coworkers [15] who showed that all cases of papillary thyroid carcinoma were positive for CK19 and with Shin et al [16] who showed that 80-90% of cases were positive for CK19 and with Theresa et al [17] who showed that 96% of cases were positive for CK19.

Benign thyroid lesions such as follicular adenomas and multinodular goiter with papillary formations are generally negative for CK19, but may sometimes show faint staining.

This low molecular weight cytokeratin has also proved to be effective in discriminating papillary carcinoma from multinodular goiter exhibiting papillary formation and follicular adenoma [6]. Focal and pale staining with CK19 may be seen in follicular adenoma and multinodular goiter with papillary formation [18]. The vast majority of cases of follicular adenomas exhibit no or focal staining with CK19 [1,5,14]. CK19 immuno-histochemical staining revealed no or focal expression in the majority of cases of papillary hyperplasia [19,20].

In conclusion the staining features of CK19 may be helpful in the differential diagnosis between papillary carcinoma and Grave's disease with papillary carcinoma like structures. This

immunoreactivity should be evaluated with histopathological findings in order to prevent over diagnosis of papillary carcinoma. Other tumor markers had been studied in Iraqi patients with thyroid carcinoma like estradiol, progesterone [21] and tumor suppressor gene P53 [22].

References

1. Sahoo S, Hoda SA, Rosai J, et al. Cytokeratin 19 Immunoreactivity in the Diagnosis of Papillary Thyroid Carcinoma. Am J Clin Pathol. 2001; 116: 696-702.
2. El-Demellawy D, Ahmed N, Alowami S. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. Diagn Pathol 2008; 3: 5-16.
3. Cheung C, Ezzat S, Freeman J, et al. Immunohistochemical Diagnosis of Papillary Thyroid Carcinoma. Mod Pathol. 2001; 14(4): 338-42.
4. Rosai J. Thyroid gland. In Rosai J, (ed). Rosai and Ackerman's surgical pathology. 9th ed. St. Louis, MO: Mosby; 2004. p. 515-94.
5. Eekilic S, Koçer NE. The Role of Cytokeratin 19 in the differential diagnosis of true papillary carcinoma of thyroid and papillary carcinoma-like changes in Grave's disease. Endocr Pathol. 2005; 16(1): 63-6.
6. Erkilic S, Aydin A, Kocer NE. Diagnostic utility of cytokeratin 19 expression in multinodular goiter with papillary areas and papillary carcinoma of thyroid. Endocr Path. 2002; 13(3): 207-11.
7. Raphael SJ. The meanings of markers: ancillary techniques in diagnosis of thyroid neoplasia. Endoc Pathol. 2002; 13(4): 301-11.
8. Stone MR, O'Neill A, Catino D, et al. Specific interaction of the actin-binding domain of dystrophin with intermediate filaments containing keratin 19. Mol Biol Cell. 2005; 16(9): 4280-93.
9. Birchmeier W, Hülsken J, Behrens J. E-cadherin as an invasion suppressor. Ciba Found Symp; 1995; 189: 124-36.
10. Seckin S, Karagece U. Expression of CK-19, cErbB2, galectin-3, and p53 in papillary thyroid carcinomas. Turk J Med Sci. 2010; 40(2): 207-12.
11. Judkins AR, Roberts SA, Livolsi VA. Utility of immunohistochemistry in the evaluation of necrotic thyroid tumors. Hum Pathol. 1999; 30: 1373-6.
12. Raphael SJ, Apel RL, Asa SL. Brief report: detection of high-molecular-weight cytokeratins in neoplastic and non-neoplastic thyroid tumors using microwave antigen retrieval. Mod Pathol. 1995; 8(8): 870-2.
13. Raphael SJ, McKeown-Eyssen G, Asa SL. High-molecular-weight cytokeratin and cytokeratin-19 in the diagnosis of thyroid tumors. Mod Pathol. 1994; 7(3): 295-300.
14. Bennett WP, Bhan AK, Vickery AL Jr. Keratin expression as a diagnostic adjunct in thyroid tumors with papillary architecture. Lab Invest. 1988; 58: 9A.
15. Baloch ZW, Abraham S, Roberts S, et al. Differential expression of cytokeratins in follicular variant of papillary carcinoma: an immunohistochemical study and its diagnostic utility. Hum Pathol. 1999; 30: 1166-71.
16. Shin E, Chung WY,Yang WI, et al. RET/PTC and CK19 Expression in Papillary Thyroid Carcinoma and Its Clinicopathologic Correlation. Med Sci. 2005; 20: 98-104.
17. Scognamiglio T, Hyjek E, Kao J, et al. Diagnostic Usefulness of HBME1, Galectin-3, CK19 and CITED1 and Evaluation of Their Expression in Encapsulated Lesions with Questionable Features of Papillary Thyroid Carcinoma. Am J Clin Pathol. 2006; 126: 700-8.
18. Erkilic S, Aydin A, Kocer NE. Diagnostic utility of cytokeratin 19 expression in multinodular goiter with papillary areas and papillary carcinoma of thyroid. Endocr Pathol 2002; 13(3): 207-11.
19. Casey MB, Lohse CM, BS, and Lloyd RV: Distinction Between Papillary Thyroid Hyperplasia and Papillary Thyroid Carcinoma by Immunohistochemical Staining for Cytokeratin 19, Galectin-3, and HBME-1. Endocr Pathol. 2003; 14: 55-60.
20. Rossi ED, Raffaelli M, Mule' A, et al. Simultaneous immunohistochemical expression of HBME-1 and galectin-3 differentiates papillary carcinomas from hyperfunctioning lesions of the thyroid. Histopathology. 2006; 48: 795-800.
21. Ahmed H. Evaluation of progesterone &estradiol in sera and tissue of thyroid patient. Iraqi J Med Sci. 2011; 9: 206-8.
22. Saeed N, Galoub A. Correlation between tumor suppressor gene P53 in some Iraqi patients with thyroid carcinoma by immunohistochemical assay &insitu hybridization method. Iraqi J Cancer Med Gen. 2012; 5: 168-72.

Correspondence to Dr. Ikram A. Hasan
E-mail: Ikram_alhadithi@yahoo.com
Received 10th Feb. 2013: Accepted 20th may. 2013.