ORIGINAL ARTICLE

The diagnostic utility of cytokeratin 19 in differentiating malignant from benign thyroid lesions

Wan Syahira WA KAMMAL[1], Azyani YAHAYA[2], Shamsul A SHAH[3], Shahrun Niza ABDULLAH SUHAIMI[4], Mazne MAHASIN[2], Muaatamarulain MUSTANGIN[2], Nurismah MD ISA[2]

[1]*Department of Pathology, Hospital Seberang Jaya, Penang, Malaysia,* [2]*Department of Pathology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia,* [3]*Department of Community Health Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia, and* [4]*Department of Surgery, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*

Abstract

Introduction: Thyroid carcinoma is classically diagnosed based on certain histological criteria. In some cases, definitive diagnoses may be challenging when morphological features are equivocal. This study evaluated the usefulness of Cytokeratin 19 (CK 19) as an immunohistochemical marker to differentiate the different histological types of malignant thyroid neoplasms, particularly papillary thyroid carcinoma (PTC) from benign thyroid lesions. *Materials and Methods:* We collected 54 malignant and 65 benign thyroid lesions diagnosed by histology in Universiti Kebangsaan Malaysia Medical Centre between January 2010 and December 2015. All cases were immunohistochemically stained with CK 19 and evaluated by 3 independent observers. The immunostaining patterns were scored based on the intensity and proportion of staining and finally graded as negative, weak positive, moderate positive or strong positive. In addition, the immunostaining scores of the malignant cases were correlated with their TNM pathological tumour stages. *Results:* Cytokeratin 19 staining expression was higher in malignant than benign thyroid lesions (p < 0.001) which was most prominent among classical PTC. The four PTC cases that showed negative or weak staining were all follicular variant of PTC. Benign conditions were mostly negative or showed weak positivity. There was no correlation between CK 19 expression and TNM primary tumour stage (pT). *Conclusion:* Cytokeratin 19 is a useful marker in differentiating malignant from benign thyroid conditions particularly the classical PTC, provided its interpretation is by correlation with morphology and takes into consideration the intensity and proportion of positive staining.

Keywords: Cytokeratin 19, CK 19, Thyroid, Malignant, Papillary thyroid carcinoma

INTRODUCTION

Thyroid nodule is a relatively common finding in the general population yet it poses a major health concern worldwide. The vast majority of thyroid nodules are benign. Thyroid cancer is the most common malignancy of the endocrine system.[1] In 2008-2012, the average annual incidence of thyroid cancer in the USA was 13.5 cases per 100 000 population.[2] The incidence of thyroid cancer is increasing and by the year 2030, it is estimated that in the USA, thyroid cancer will be the second leading cancer diagnosis in women and the ninth leading cancer diagnosis in men.[3] In Malaysia, thyroid cancer is ranked as ninth most common cancer among females accounting

to 3% of all female cancers.[4]

Papillary thyroid carcinoma is the predominant form of thyroid cancer. It is classically diagnosed with confidence based on well-established, clear-cut, strict morphological and nuclear features such as the presence of papillary structure, nuclear enlargement and overlapping, nuclear grooving and nuclear pseudo-inclusions. However, in some cases definite diagnosis is challenging especially when the histological features are equivocal. Inter-observer discordance in the diagnosis of certain thyroid lesions has been well documented even among experienced pathologists.[5] This is especially so in papillary thyroid carcinoma where the nuclear features can be ambiguous.

Address for correspondence: Nurismah Md Isa, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Cheras 36000 Kuala Lumpur, Malaysia. Tel: +60 19-2687199; Fax: +60 3-91737340 Email: nuris@ppukm.ukm.edu.my

Despite the increasing trend of thyroid cancer, cancer-related deaths remain unchanged or decreased in most countries. This phenomenon is partly related to recent advances in the radiology and laboratory investigation techniques which allow the detection of otherwise clinically silent thyroid lesions.[6,7,8] There is an increased detection of low-grade cancer particularly papillary microcarcinoma, leading to a valid danger of over diagnosis and over treatment of such lesions. An accurate diagnosis of thyroid neoplasm is therefore crucial as it has a major impact on the treatment modality as well as the patient's prognosis.

In view of the difficulty in diagnosing some cases of thyroid cancers, several immunohistochemical markers have been proposed to be used as diagnostic adjuncts. One such marker is Cytokeratin 19, which expression has been acknowledged in many malignancies including liver, pancreatic and gastric carcinoma. The purpose of this study is to evaluate the diagnostic utility of Cytokeratin 19 as a marker to differentiate thyroid carcinomas, particularly papillary thyroid carcinoma from their benign counterparts.

MATERIALS AND METHODS

Tissue specimens
There were a total of 128 thyroidectomy specimens received at the Department of Pathology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) between January 2010 and December 2015; 60 of these were malignant tumours diagnosed based on World Health Organisation (WHO) histopathological criteria while the remaining 68 were benign lesions. Six of the malignant thyroid tumours were removed for various reasons (exhaustion of diagnostic material on paraffin block (3 cases), recurrent tumour from a previously treated cancer (3 cases)) while among the benign conditions, 3 were removed (exhaustion of diagnostic material (1 case), post radio-iodine treatment (1 case), history of papillary thyroid carcinoma (1 case)). Histopathological slides of all the cases were reviewed and one most representative section of the tumour was selected for each case. The corresponding formalin fixed, paraffin embedded tissue blocks were retrieved from the archive for immunohistochemical staining.

Immunohistochemical staining
Immunohistochemical staining was performed on tissue sections using the protocol from EnVision™ FLEX+, Mouse, High pH (Code No. K8012, Dako Denmark). The primary antibody used was monoclonal mouse anti-human Cytokeratin 19 antigen clone RCK108 (Code No. M0888, Dako Denmark). Primary antibodies were diluted to optimal concentration of 1:50 using Antibody Diluent, Dako REAL™ (Code No. S0809, Dako Denmark). Washing steps between each reagent were performed using EnVision™ FLEX Wash Buffer 20x (Code No. DM831, Dako Denmark) diluted to a 1X working solution with deionised water. The 1X DAB-containing Substrate Working Solution was prepared by diluting the 50X concentrated EnVision™ FLEX DAB+ Chromogen (Code No. DM827, Dako Denmark) with Envision™ FLEX ™ Substrate Buffer (Code No. DM823, Dako Denmark).

Tissue blocks were sectioned at $3\,\mu$m thickness and mounted on adhesive glass slide, Platinum Pro White (Product No: PRO-01, Matsunami Japan). The slides were left to be air-dried in room temperature overnight. The tissue slides were then incubated on hot plate at 60°C for 1 hour. An initial deparaffinisation and pre-treatment step was performed in the DakoPTLink (Product No. PT10126, DakoCytomation, USA) using the EnVision™ FLEX Target Retrieval Solution, pH 9 (Code No. DM828, Dako Denmark) at 95°C for 20minutes, followed by cooling at room temperature for 20 minutes and rinsed with running tap water for another 3 minutes. The slides were subsequently incubated with EnVision™ FLEX Peroxidase-Blocking Reagent (Code No. DM821, Dako Denmark) for 5 minutes followed by washing steps.

Slides were then incubated with primary antibody at room temperature for 30minutes followed by incubation with the secondary antibody EnVision™ FLEX/HRP (Code No. DM822, Dako Denmark) for 20 minutes. The sections were then incubated with 1X DAB-containing Substrate Working Solution for 10 minutes. The slides were subsequently counterstained with Hematoxylin 2 (REF 7231, ThermoScientific USA) for 5 seconds after the procedures have been completed followed by dehydration step with increasing alcohol solutions (80%, 90%, 100% and 100%) and 2-times Xylene. Finally, the slides were mounted and coverslipped using DPX mounting medium (Cat. No.: 100579, Merck Millipore Germany). The positive control of normal tonsil tissue was used.

Immunohistochemical evaluation

Two pathologists and one pathology trainee reviewed all the cases independently and were blinded from the initial diagnoses. Whenever there was discordant finding, a consensus was taken. The cases were regarded as positive for Cytokeratin 19 when immuno-reactivity was clearly present at the cytoplasm of the tumour cells at lesional areas. Non-specific staining of the colloid without staining of thyroid epithelial cells was disregarded. Each case was semi-quantitatively evaluated for the proportion score in a 5-point scale as either 0 (less than 10% positivity), 1 (10-25% positivity), 2 (26-50% positivity), 3 (51-75% positivity) or 4 (more than 75% positivity). They were also simultaneously evaluated for the intensity score into 4 groups of as either 0 (negative staining), 1 (weak staining), 2 (moderate staining) or 3 (strong staining). The final immunohistochemical score was obtained by multiplying both the proportion score and intensity score, which was grouped as negative or - (final score 0), weakly positive or + (final score 1-4), moderately positive or ++ (final score 5-8) and strongly positive or +++ (final score 9-12). The evaluation score was performed based on a previously published study[9]. A p value of <0.05 was taken as being statistically significant.

Statistical analysis

Data were managed using the "Statistical Package for the Social Sciences (SPSS) for windows" software (version 18.0). For univariate analysis, the categorical variables were compared using the chi-square or Fisher's exact tests where appropriate, while Mann-Whitney U (MW) and Kruskal-Wallis (KW) tests were used to compare continuous variables with categorical outcome variables. The frequency and the means score within each groups (malignant and benign) were determined as the number of occurrences of an individual patient. The significant test was evaluated and tested to compare among the malignant and benign groups. A two-tailed P-value of <0.05 was considered statistically significant. The Odds ratio (OR) with a 95% confidence interval (CI) was calculated to investigate the strength of the association between the malignant and benign groups. For multivariate analysis, the binary logistic regression analysis was performed to adjust the mutual effect of the significant factors in univariate analysis. The changes of significant factors in each malignant patient relative to benign patient were calculated based on the factor beta coefficients value.

RESULTS

Demographics and clinicopathological data

A total of a 65 benign and 54 malignant cases were studied for Cytokeratin 19 immunohistochemistry expression. The mean age at diagnosis was 49.4 years (range 17 years to 78 years) in the benign group and 47.3 years (range 21 years to 76 years) in the malignant group. Females predominated in both groups, accounting to 73.8% of benign cases and 57.4% of malignant cases. Both groups showed similar ethnicity distribution, with the majority being Malay (more than 50% of cases) followed by Chinese, Indians and others. In the malignant group, 29.6% of tumours were less than 2cm, 33.3% were between 2 cm to 4cm and 35.2% were more than 4cm in diameter. Extra-thyroidal extensions were present in 13% of cases, nodal metastases detected in 33.3% of cases and distant metastases were noted in 5.6% of cases (Table 1).

The malignant thyroid lesions included 32 papillary thyroid carcinomas (26 conventional type, four follicular variant, one papillary microcarcinoma variant and one oncocytic variant), 11 follicular thyroid carcinomas (six widely invasive type and five minimally invasive type), six medullary thyroid carcinomas, one poorly differentiated thyroid carcinoma and four anaplastic thyroid carcinomas. The benign lesions included 39 nodular hyperplasia, 14 follicular adenoma, nine lymphocytic thyroiditis, two benign thyroid cysts and one hyalinising trabecular tumour. The representative final immunohistochemical score of Cytokeratin 19 in various malignant and benign groups were depicted in Fig. 1.

Comparison of Cytokeratin 19 Expression in All Malignant Thyroid Carcinomas versus Benign Lesions.

Cytokeratin 19 staining expression was higher in malignant than benign lesions (p<0.001). Strong positivity was seen in 33 (67.1%) of malignant thyroid neoplasms while only two benign cases (3.1 %) showed strong positivity. The majority of the benign lesions were either negative (41 cases, 63.1%) or weakly positive (20 cases, 30.8 %) (Table 2).

When the malignant lesions were analysed according to histological subtypes, 87.5% (28 out of 32 cases) of papillary thyroid carcinomas and 75% (3 out of 4 cases) of anaplastic carcinomas showed strong positivity. Follicular and medullary carcinomas showed a wide variation in their final immunohistochemical score, ranging

TABLE 1. Demographics and clinical characteristics of study groups

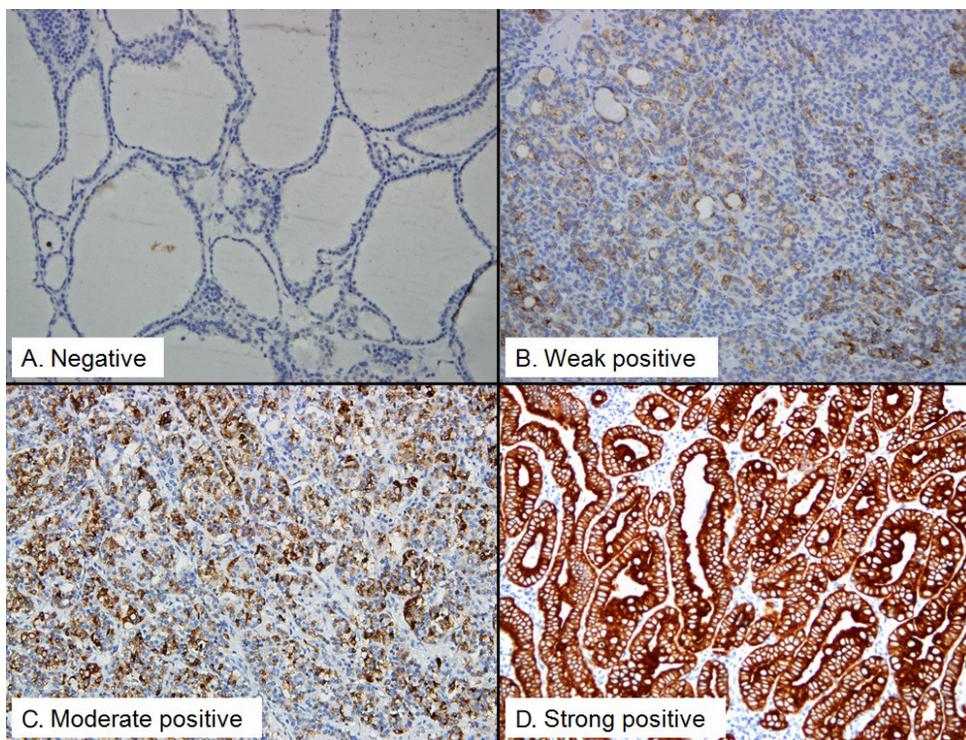| Variables		Malignant cases	Benign cases	P-value
Mean age (SD)		47.3 (14.6)	49.4 (14.7)	<0.001
Sex	Female	31 (57.4%)	48 (73.8%)	0.059
	Male	23 (42.6%)	17 (26.2%)	
Ethnicity				
	Malay	31 (60.8%)	35 (54.7%)	0.864
	Chinese	11 (21.6%)	17 (26.6%)	
	Indian	5 (9.8%)	8 (12.5%)	
	Others	4 (7.8%)	4 (6.3%)	
Tumour Size				
	≤ 2cm	16 (29.6%)	-	
	2- 4 cm	18 (33.3 %)	-	
	> 4cm	19 (35.2%)	-	
	Not available	1 (1.9%)	-	
Nodal metastasis				
	Present	18 (33.3%)	-	
	Absent	36 (66.7%)	-	
Distant metastasis				
	Present	3 (5.6%)	-	
	Absent	51 (94.4%)	-	

FIG. 1: Staining patterns of CK 19: A. Negative (Final score 0) B. Weak positive or 1+ (Final score 1-4) C. Moderate positive or 2+ (Final score 5-8), and D. Strong positive or 3+ (Final score 9-12).

TABLE 2: Comparison of CK19 staining pattern between all malignant and benign cases

CK 19 Staining Pattern	Group	
	Malignant	**Benign**
Negative	10 (18.5%)	41 (63.1%)
Weak Positive	8 (14.8%)	20 (30.8%)
Moderate Positive	3 (5.6%)	2 (3.1%)
Strong Positive	33 (67.1%)	2 (3.1%)

from negative to strongly positive. The only case of poorly differentiated carcinoma in this study was negative (Table 3).

Among the 65 benign cases, four were positively stained, two of these were strongly positive (both cases being follicular adenomas) while the other two were moderately positive (one follicular adenoma and one nodular hyperplasia). The sensitivity of Cytokeratin 19 for the distinction between benign and all malignant cases is 81.48% (95% confidence interval: 68.57% to 90.75%).

Comparison of Cytokeratin 19 Expression in Papillary Thyroid Carcinomas versus Benign Lesions.

When only papillary thyroid carcinomas were compared with the benign lesions, strong Cytokeratin 19 positivity correlated with malignancy while other staining patterns correlated with benign lesions (p value <0.001). A total of 28 out of 32 (87.5%) papillary thyroid carcinomas showed strong positivity. The remaining four cases that were negative and showed weak staining were all follicular variants of papillary thyroid carcinomas. In other words, when only papillary thyroid carcinoma was compared with benign conditions, we observed that 93% of cases that showed strong Cytokeratin 19 staining were papillary thyroid carcinoma while 95% of those that were negative were benign (Fig. 2). The sensitivity of Cytokeratin 19 for the distinction of papillary thyroid carcinoma from benign cases is 93.75% (95% confidence interval: 79.19% to 99.23%).

Correlation of Cytokeratin 19 Expression with pathological tumour stage (pT)

For all the malignant cases, we analysed the correlation between Cytokeratin 19 expression and TNM primary tumour stage (pT) based on 2017 WHO Classification of Tumours of Endocrine Organs (4th edition). We found no correlation between Cytokeratin 19 expression and TNM primary tumour stage (p value = 0.188) (Table 4). A similar finding was noted when only papillary thyroid carcinomas were analysed according to their TNM primary tumour stage (p value = 0.44) (Table 5).

TABLE 3: CK 19 Staining pattern among individual groups of malignant cases

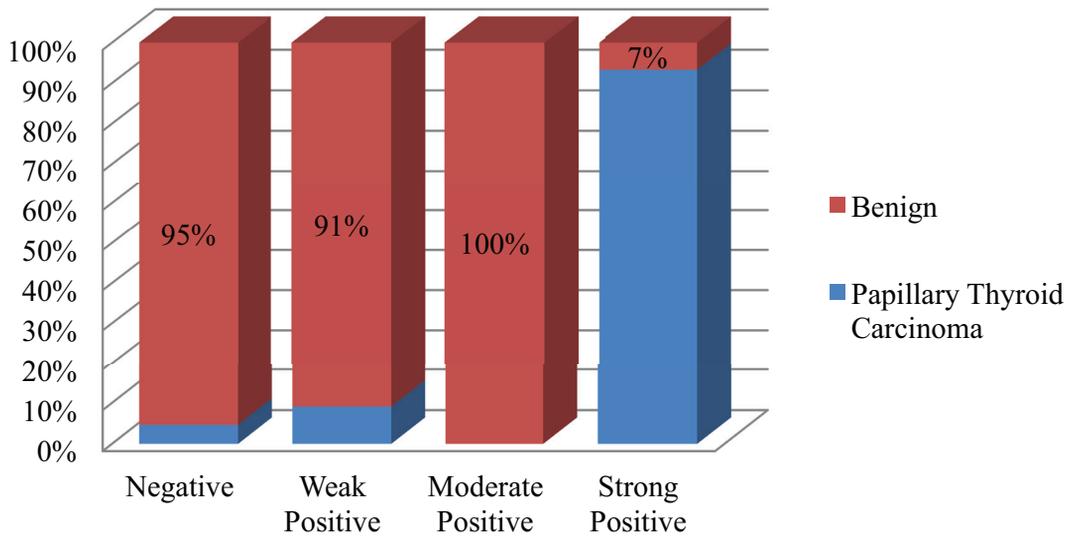| CK 19 Staining Pattern	Diagnosis				
	Papillary carcinoma	**Follicular carcinoma**	**Medullary carcinoma**	**Poorly differentiated carcinoma**	**Anaplastic carcinoma**
Negative	2 (6.25%)	5 (45.5%)	1 (16.7%)	1 (100%)	1 (25%)
Weakly Positive	2 (6.25%)	3 (27.3%)	3 (50%)	0 (0%)	0 (0%)
Moderately Positive	0 (0%)	2 (18.2%)	1 (16.7%)	0 (0%)	0 (0%)
Strongly Positive	28 (87.5%)	1 (9.1%)	1 (16.7%)	0 (0%)	3 (75%)

FIG. 2: Distribution of CK 19 staining patterns among papillary thyroid carcinoma and benign cases.

DISCUSSION

Many immunohistochemical markers have been studied to aid the diagnosis of malignant thyroid lesions, one of which is Cytokeratin 19. Cytokeratin 19 a type I intermediate filament protein which is normally found in simple or glandular epithelium, hair follicles and basal cell compartment of stratified epithelium and the lining of normal pancreatobiliary tract.[10] Cytokeratin 19 expression has been recognised in many malignancies including pancreas, gastric carcinoma and cholangiocarcinoma.[11]

TABLE 4: Distribution of CK 19 staining pattern of malignant cases according to TNM Primary Tumour Stage (pT)

CK 19 Staining Pattern	TNM Primary Tumour Stage (pT)			
	pT1	pT2	pT3	pT4
Negative	4 (30.8%)	4 (25%)	1 (5.3%)	1 (20%)
Weakly Positive	1 (7.7%)	5 (31.3%)	2 (10.5%)	0 (0%)
Moderately Positive	1 (7.7%)	1 (6.3%)	0 (0%)	0 (0%)
Strongly Positive	7 (53.8%)	6 (37.5%)	16 (84.2%)	4 (80%)

TABLE 5: Distribution of CK 19 staining pattern of papillary thyroid carcinoma according to TNM Primary Tumour Stage (pT)

CK 19 Staining Pattern	TNM Primary Tumour Stage (pT)			
	pT1	pT2	pT3	pT4
Negative	1 (14.3%)	1 (14.3%)	0 (0%)	0 (0%)
Weakly Positive	0 (0%)	0 (0%)	2 (11.3%)	0 (0%)
Strongly Positive	6 (85.7%)	6 (85.7%)	15 (88.2%)	1 (100%)

Despite widespread acceptance of its relationship with the aforementioned malignancies, the exact role of Cytokeratin 19 in cancer biology currently remains unknown. It is hypothesised that Cytokeratin 19 expression is a result of de-differentiation of tumour cells during malignant transformation and it has no active role in the steps of carcinogenesis itself.[12]

We studied 119 cases of thyroid conditions diagnosed in our institute within a period of 5 years. The baseline demographics in our study population showed a slight younger median age at diagnosis of malignant lesions (47.3 years) compared to benign conditions (49.4 years) which was statistically significant (p<0.001). Among the malignant group, our study showed slight younger mean age compared to that reported in the USA, where the median age was 51 years. There was a similar pattern of female predominance as in the USA statistics, but our ratio was less striking. The female to male ratio was of 1.3:1 in our study population compared to 3:1 ratio in the USA.[13] Papillary thyroid carcinoma was the predominant form of malignant condition, accounting to 59.2% of thyroid carcinomas. This was comparable to Ireland where it accounted for 65% of cases, but was much lower than the USA (86.2%) as well as Japan and the Republic of Korea (91%).[2,14]

Our results concurred with previously published studies which showed that the expression of Cytokeratin 19 is generally increased in malignant thyroid conditions where the staining pattern is diffuse and strong.[15,16] In our study, the percentage of positivity was highest among papillary thyroid carcinoma. This was in keeping with other studies which reported 45% to 96% positivity.[9,10,17,18,19] The one case of papillary microcarcinoma that we had showed strong immunopositivity. This supported a previously published study that suggested the use of Cytokeratin 19 to differentiate papillary microcarcinoma from benign hyperplastic thyroid lesion[20]. Other forms of thyroid carcinoma showed more heterogonous staining pattern which is also in keeping with previous reports.[10,15]

Our study showed that Cytokeratin 19 is not useful in differentiating follicular variant of papillary thyroid carcinoma from their benign counterparts. The four cases of papillary thyroid carcinomas that were negative and weakly positive in our study were all follicular variant of papillary thyroid carcinoma. We noticed comparable trend in other literatures. In one study, 5 out of 6 (83%) classical papillary thyroid carcinoma showed positivity, while all 5 cases (100%) of follicular variant of papillary thyroid carcinomas were negative.[17] Another study also showed 88% positivity in classical papillary thyroid carcinoma, but only 65% positivity among its follicular variant counterpart.[10] A feasible explanation for this difference in staining is possibly related to the genetic profile of each variant of papillary thyroid carcinoma.[21] As we know, BRAF[V600E] mutation is the most common driver mutation in classical papillary thyroid carcinoma, accounting for 45-80% of genetic alterations in this tumour type.[2] One study showed correlation between BRAF[V600E] mutation and higher CK19 expression among classical papillary thyroid carcinoma.[22] In contrast, follicular variant of papillary thyroid carcinoma mostly harbour RAS mutation.[23,24] Nevertheless, some published studies showed significant Cytokeratin 19 reactivity among follicular variants of papillary thyroid carcinomas.[25,26] Likewise, follicular carcinoma and follicular adenoma which mostly harbour RAS mutation also show heterogeneous staining pattern ranging from negative to moderately positive.[19] Therefore, there may be other mechanisms responsible for this pattern of expression.

Normal or hyperplastic tissues were negative while benign neoplasms were generally negative or expressed weak Cytokeratin 19 staining as reported in earlier studies.[10,27] A few studies reported relatively high percentage of Cytokeratin 19 expression in multinodular goitre and follicular adenoma (between 40 to 56%) although the benign tissues almost always had weak to moderate intensity and focal staining pattern.[28,29] This was in keeping with our finding where 93.8% of benign thyroid conditions were either negative or showed weak positive staining.

It was also speculated that Cytokeratin 19 may carry prognostic implication, having shown correlation with pTNM stage and extra thyroid extension in an earlier study.[10] This allowed the possibility of identifying a subgroup of patients who may benefit from a more aggressive intervention and strict post-operative clinical surveillance. However in our study, there was no correlation between Cytokeratin 19 expression and primary tumour stage. Our finding was easily explained by the heterogeneous expression of Cytokeratin 19 in non-papillary tumours, regardless of the tumour stage.

CONCLUSION

Our study demonstrated that Cytokeratin 19 is a potentially useful marker in differentiating malignant thyroid tumours especially papillary thyroid carcinomas, from benign thyroid condition. The immunohistochemical interpretation needs to be performed in association with morphological features, and should take into consideration the intensity and proportion of positive staining. A strong and diffusely positive Cytokeratin 19 staining strongly supports malignancy, particularly when papillary thyroid carcinoma is suspected. However, the Cytokeratin 19 immunohistochemical staining is not helpful in the cases of follicular variant of papillary thyroid carcinoma. On the other hand, negative or weakly positive Cytokeratin 19 staining suggests a benign condition, provided the morphology supports it.

REFERENCES

1. Vanderpump MPJ. The epidemiology of thyroid disease. Br Med Bull. 2011; 99: 39-51
2. Rosai J, Albores Saavedra J, Asioli S, *et al*. Papillary thyroid carcinoma. In: Lloyd RV, Osamura RY, Klöppel G and Rosai J, editors. WHO Classification of Tumours of Endocrine Organs (4th edition). Lyon: IARC; 2017. p81-91
3. Haugen, BR. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: what is new and what has changed? Cancer. 2917; 123: 372-81
4. Azizah AM, Nor Saleha IT, Noor Hashimah A, Asmah ZA, Mastulu W. Malaysian national cancer registry report 2007-2011. Malaysian cancer statistics, data and figure. National Cancer Institute, Ministry of Health, Putrajaya, Malaysia, 2016. Available from: https://kpkesihatan.com/2016/12/07/the-malaysian-national-cancer-registry-report-mncr-2007-2011
5. Elsheikh TM, Asa SL, Chan JK, *et al*. Interobserver and intraobserver variation among experts in the diagnosis of thyroid follicular lesions with borderline nuclear features of papillary carcinoma. Am J Clin Pathol. 2008; 130(5): 736-44.
6. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneris R. Worldwide increasing of thyroid cancer: update on epidemiology and risk factors. J Cancer Epidemiol. 2013; 2013.
7. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014; 74(11): 2913-21.
8. Vaccarella S, Dal Maso L, Laversanne M, Bray F, Plummer M, Franceschi S. The impact of diagnostic changes on the rise in thyroid cancer incidence; A population-based study in selected high-resource countries. Thyroid. 2015; 25(10): 1127-36.
9. Wu G, Wang J, Zhou Z, Li T, Tang F. Combined staining for immunohistochemical markers in the diagnosis of papillary thyroid carcinoma: Improvement in the sensitivity or specificity? J Int Med Res. 2013; 41(4): 975-83.
10. Dencic TI, Cvejic D, Paunovic I, Tatic S, Havelka M, Savin S. Cytokeratin 19 expression discriminates papillary thyroid carcinoma from other thyroid lesions and predicts its aggressiveness behaviour .Med Oncol. 2013; 30: 362
11. Jain R, Fischer S, Serra S, Chetty R. The Use of Cytokeratin 19 (CK19) immunohistochemistry in lesions of the pancreas, gastrointestinal tract, and liver. Appl Immunohistochem Mol Morphol. 2010; 18(1), 9-15
12. Santos NP, Oliveira PA, Arantes-Rodrigues R, *et al*. Cytokeratin 7/19 expression in n -diethylnitrosamine-induced mouse hepatocellular lesions: Implications for histogenesis. Int. J. Exp. Path. 2014; 95: 191–8
13. Howlader N, Noone AM, Krapcho M, *et al*. (eds). SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda. Available from:http://seer.cancer.gov
14. Forman D, Bray F, Brewster DH, *et al*. Cancer incidence in five continents, Vol. X. IARC Scientíc Publication No. 164. Lyon: International Agency for Research on Cancer. 2014. Available from: https://www.iarc.fr/en/publications/pdfs-online/epi/sp164/CI5volX_Full.pdf
15. de Matos LL, Del Giglio AB, Matsubayashi CO, de Lima Farah M, Del Giglio A, da Silva Pinhal MA. Expression of CK-19, Galectin-3 and HMBE-1 in the differentiation of thyroid lesions: systematic review and diagnostic meta-analysis. Diagn Pathol. 2012; 7(1): 97.
16. Arcolia V, Journe F, Renaud F, *et al*. Combination of galectin-3, CK19 and HBME-1 Immunostaining improves the diagnosis of thyroid cancer. Oncol Lett 2017; 14(4): 4183-4189.
17. Nechifor-Boilă A, Cătană R, Loghin A, Radu TG, Borda A. The diagnostic value of HMBE-1, CD56, Galectin-3 and Cytokeratin-19 in papillary thyroid

carcinomas and thyroid tumors of uncertain malignant potential. Rom J Morphol Embyol. 2014; 55(1): 49-56.

18. Liu Z, Yu P, Xiong Y, *et al*. Significance of CK19, TPO, and HBME-1 Expression for diagnosis of papillary thyroid carcinoma. Int J Clin Exp Med. 2015; 8(3): 4369-4374.

19. Abouhashem NS and Talaat SM. Diagnostic Utility of CK19 and CD56 in the differentiation of thyroid papillary carcinoma from its mimics. Pathol Res Pract 2017; 213(5): 509-17.

20. Zhou Y, Chen ZH, Lu BH, Jiang HG. Diagnostic significance of Ki67, CK19, Galectin-3 and HMBE-1 expression for papillary thyroid microcarcinoma. Biomed Res. 2017; 28 (9): 4002-6.

21. Suh YJ, Moon HJ, Choe JY, and Park HJ. The cancer genome atlas validation of ancillary tests for classifying papillary thyroid carcinoma. Int J Thyroidol. 2017; 10(1): 24-35.

22. Guerra A, Marotta V, Deandrea M, *et al*. BRAFV600E associates with cytoplasmatic localization of p27kip1 and higher cytokeratin 19 expression in papillary thyroid carcinoma. Endocrine. 2013; 44: 165-71.

23. Zhu Z, Gandhi M, Nikiforova MN, Fischer AH and Nikiforov YE. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. Am J Clin Pathol. 2003; 120(1): 71-7.

24. Jeong SH, Hong HS, Kwak JJ and Lee EH. Analysis of RAS mutation and PAX8/PPARγ rearrangements in follicular-derived thyroid neoplasms in a Korean population; frequency and ultrasound findings. J Endocrinol Invest. 2015; 38(8): 849-57.

25. Ma H, Xu S, Yan J, *et al*. The value of tumour markers in the diagnosis of papillary thyroid carcinoma alone and in combination. Pol J Pathol 2014; 65(3): 202-9.

26. Rahman MM, Banu SG, Barua AR, Kamal M, Baqui MN, Parveen S. Follicular variant of papillary thyroid carcinoma on the basis of histopathological and immunohistochemical diagnosis. Bangladesh Med Res Counc. 2016; 42: 21-7.

27. Liu H and Lin F. Application of immunohistochemistry in thyroid pathology. Arch Pathol Lab Med. 2015; 139:67–82.

28. MataracI EM, Özgüven BY and Kabukçuoglu F. Expression of cytokeratin 19, HBME-1 and galectin-3 in neoplastic and non-neoplastic thyroid lesions. Pol J Pathol. 2012; 1: 58-64.

29. Alshenawy HA. Utility of immunohistochemical markers in differential diagnosis of follicular cell-derived thyroid lesions. J Microsc Ultrastruct. 2014; 2: 127-36.