Original Article

EXPRESSION OF CD56, CK19 AND HBME-1 IN FOLLICULAR VARIANT OF PAPILLARY THYROID CARCINOMA AND BENIGN MIMICKERS

Hassan Tariq[1], Amna Iqbal[2], Hafeez Ud Din[1], Syed Raza Jaffar[1], Sardar Waleed Babar[1], Asma Gul[1]

[1]Armed Forces Institute of Pathology (National Institute of Medical Sciences), Rawalpindi Pakistan
[2]Federal Medical and Dental College, Islamabad Pakistan

ABSTRACT

Objective: To assess the diagnostic utility of a set of 3 markers i.e. CD56, CK-19 and HBME-1 to differentiate follicular variant of papillary thyroid carcinoma (FVPTC) from its benign mimickers.

Material and Methods: This is a prospective cross-sectional study conducted at Histopathology department of Armed Force Institute of Pathology (AFIP), Rawalpindi, Pakistan from May 2020 to Aug 2020. A cross-sectional study in which samples of 40 thyroid lesions that included 20 FVPTC and 20 hyperplasia/adenomas were retrieved from the database of the Department of Histopathology of Armed Forces Institute of Pathology, Rawalpindi. Immunohistochemistry was done to assess the expression of CD56, CK19 and HBME-1 in FVPTC (n=20) and hyperplasia/adenoma (n=20). Results were analyzed using SPSS 21.

Results: Strongly positive (2+) staining with CK19 and HBME-1 was observed in 95% and 80% of the FVPTC patients respectively. Expression of CD56 was negative in 80% of FVPTC. A significant statistical difference was observed in the expression of HBME-1, CK19 and CD56 in FVPTC and benign lesions ($P<0.05$).

Conclusion: Negative staining for CD56 in a thyroid lesion while at the same time co-expression of diffuse and strong staining with CK19 and HBME-1 in a follicular pattern lesion posing a diagnostic challenge is highly suggestive of FVPTC provided it is interpreted in the right context.

Key Words: Immunohistochemistry, CD56, HBME-1, CK19, Follicular variant of papillary carcinoma.

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INTRODUCTION

Thyroid carcinomas represent about 1.5% of all malignancies and are the most recurring type of cancers of the endocrine system. Papillary Thyroid Carcinoma (PTC) makes up 80% of these thyroid malignancies [1]. While, a subtype of PTC called Follicular variant of papillary thyroid carcinoma (FVPTC) account for 9% to 22.5% of all PTCs making it the most prevalent among the PTC cases [2].

"Gold Standard" for diagnosing thyroid nodules and tumors is histopathologic assessment with the help of hematoxylin and eosin staining [3,4]. There are many morphological features that are common amongst the follicular nodular thyroid lesions, which can pose a diagnostic dilemma while attempting to make a diagnosis on H&E slides. Even so, amongst some trained and more experienced endocrine pathologists an inter-observer variability is quite often seen [5,6]. As a result, patients with such undetermined tumors often have to undergo thyroidectomy even though the rate of incidence for this condition is very low [6]. Hence, in order to prevent such needless surgical procedures from being carried out, proper diagnosis is very necessary. Diagnosis is often impossible only on the basis of morphological features alone thus immunohistochemistry is employed in order to look for markers that can help differentiate follicular patterned tumors [7].

However, there is not a single marker which is sensitive enough and provides a clear diagnosis. Hence, in order to obtain more accurate diagnosis different panels of combined immunomarkers are generally tested [8]. Cytokeratin 19 (CK19), CD56 and Hector Battifora mesothelial cell 1 (HBME-1) are some of the immunohistochemical markers that are being constantly tested in hopes of finding any probable contribution to differential diagnosis of FVPTC and other suspected follicular neoplasia [9].

Thus, in this study, the role of CD56, HBME-1, and CK19 was investigated in order to discriminate the FVPTC from other lesions of the thyroid. Although, similar studies are available on this subject in international literature, no study was performed in Pakistan in this subset of population. We aim to make a contribution to the routine practice by further supporting the literature through our results.

MATERIAL AND METHODS

A cross-sectional prospective study approved by the Ethical Committee at AFIP, Rawalpindi. WHO calculator was used to calculate the sample size with a confidence interval of 95%. The samples collected

Correspondence: Maj Hassan Tariq, Consultant Histopathologist, Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

Email: **hassantariqamc@gmail.com**

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from the 40 patients under study were paraffin embedded blocks of tissue that included the 20 FVPTC and 20 Adenomas/ Hyperplasia. These samples were collected from the Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi. Irrespective of the histological type as well as the grade of the tumor, all specimens of FVPTC and adenoma/hyperplasia diagnosed on histopathology were included. All the specimens with scanty tumor tissue and those that were poorly fixed were excluded

Following manufacturer's guidelines, Bios kit was used to perform immunohistochemical assay of CK19, HBME-1 and CD56. A second opinion was employed in order to curtail bias. Later, data was analyzed by standard statistical method and expressed in percentages. During data analysis P-value < 0.05, calculated through Chi-square test, was considered significant. For routine histological and ensuing immunohistochemical examinations, the blocks of tissue that were earlier embedded in paraffin were cut into 3µm sections and then examined under microscope. With the help of high-power field objective on a light microscope, the results were interpreted for the immunohisto-chemistry. The results of the stained specimens were considered positive or negative in accordance with the percentage of cells stained in the specimens. And, the extent of percentage staining of cells was scored as per Table-I.

RESULTS

This study included 40 patients in total to be investigated. 20 of the cases were diagnosed as FVPTC and the other 20 as thyroid Adenoma/Hyperplasia. The diagnosis was made histologically with proper clinical information including standard testing through H&E staining as well as interpretation by experienced pathologists.

The tests were considered either positive or negative for each of the immunohistochemical markers (i.e. CK19, HBME-1 and CD56) on account of stained cells percentage in each of the tested sample as shown in Table-I.

Table-I: Scoring of Expression of IHC markers in terms of intensity of cytoplasmic staining and percentage of cells stained.

Intensity of Cytoplasmic staining	Percentage of Stained Cells	Score	Inference/Result
Negative/ Weak	Less than 20	0	Negative/Weak
Weak/ Moderate	20-50	1+	Moderate positive
Moderate/ Strong	More than 50	2+	Strong positive

CK19 showed positive expression in 19 (95%) cases of FVPTC in comparison to 4 (20%) cases of Adenoma/ Hyperplasia. On the other hand, negative expression comprised of 1 (5%) of FVPTC and 16 (80%) of Adenomas. (Figure-I and Figure-II)

An HBME-1 positive expression was seen in 16 (80%) of FVPTC and 4 (20%) in Adenomas. On the contrary 4 (20%) of the FVPTC and 16 (80%) of Adenomas showed a negative expression.

CD56 showed negative expression in 16 (80%) cases of the FVPTC and 3 (15%) cases of Adenoma/Hyperplasia. Conversely, positive expression was seen in only 4 (20%) of the cases of FVPTC as compared to 17 (85%) of thyroid Adenomas as shown in Figure-II.

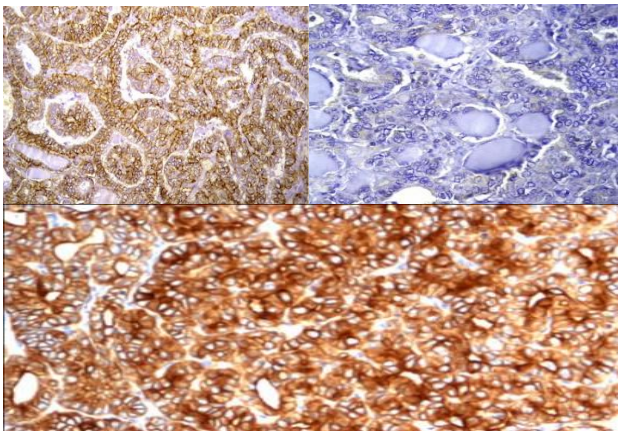

Figure-I: IHC Staining pattern of CK19, HBME-1 and CD56 in FVPTC showing diffuse membranous staining for CK19 and HBME-1 while loss of staining in case of CD56.

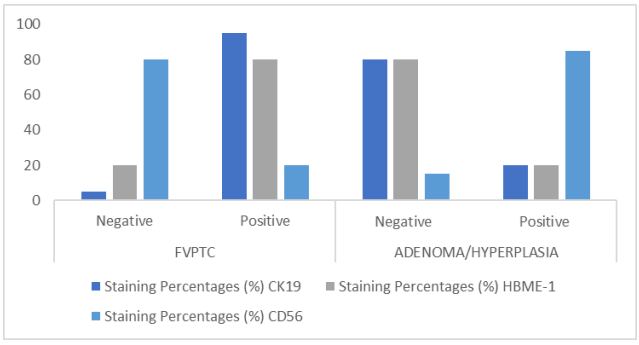

Figure-II: Staining percentages of CK19, HBME-1 and CD56 in FVPTC and Thyroid hyperplasia.

It was observed that the diagnosis of FVPTC was significantly associated with strong diffuse CK19 and HBME-1 expression and negative CD56 expression in relation to the other studied benign thyroid lesions (i.e. Adenomas or Hyperplasia). Between FVPTC and benign groups, a significant difference for percentages of HBME-1, CK19 and CD56 staining was established through Chi- square test (P < 0.05) as shown in Table-II.

Table-II: Distribution of the percentages and p-values of IHC.

CK19	**NEGATIVE**	**5**	**80**	**<0.05**
	POSITIVE	95	20	
HBME-1	NEGATIVE	20	80	<0.05
	POSITIVE	80	20	
CD56	NEGATIVE	80	15	<0.05
	POSITIVE	20	85	

DISCUSSION

Amongst the thyroid neoplasia, the lesions with follicular features particularly adenomas and the follicular variant of papillary thyroid carcinoma (FVPTC) raise the most controversies due to morphological similarity. Therefore, many studies have been directed to search for additional markers besides the routine H&E staining, which is a standard procedure for diagnosing thyroid lesions [17]. However, there is not a single marker which is sensitive enough and provides a clear diagnosis. In order to obtain more accurate diagnosis, different panels of combined immunomarkers are generally tested. Three of such markers were tested in this study i.e. CD56, CK19 and HBME-1.

CD56 is usually found in brain tissue, large granular lymphocytes and NK cells [10]. Specific endocrine cells as well as normal thyroid follicular epithelial cells also show CD56 expression [10,14]. Reduced expression of CD56 has a significant correlation with metastatic potentials as well as poor prognostic outcome in some of the malignancies. Papillary carcinoma of thyroid as well as its variant FVPTC show a minimal or absent expression of CD56 to quite a large extent [14,15].

Cytokeratin-19 (CK19), a low-molecular-weight type 1 intermediate filament protein is found in a varying range of simple and glandular epithelium which includes normal as well as neoplastic tissue [9,10,15]. CK19 expression is not generally seen in normal follicular epithelium of the thyroid [11,16]. However, most of the studies have shown that PTC including FVPTC present with a strong CK19 stain [16].

HBME-1 (Hector Battifora mesothelial cell-1) is a membrane antigen reacting with antibodies in microvilli of mesothelioma cells [4,16]. It is also found in the tumour cells of the thyroid especially those that are follicular type [13]. Normal tissues of the thyroid gland practically have been studied to show weak or negative staining with HBME-1 [16]. Malignant neoplasia of the thyroid, particularly PTCs with the exception of Hurthle cell carcinomas, show an increased expression of HBME-1 [17].

Papillary carcinoma has been known to show an excellent long-term prognosis if proper treatment and clinical management is implemented. The survival rates over a period of 10 years for patients suffering from PTCs and follicular carcinomas (FCs) were observed to be 93% and 85% respectively in the NCDB (National Cancer Data Base, USA) study. [8] Correct diagnosis is highly crucial in order to prevent patients from undergoing unnecessary and aggressive surgical procedures as well as radioactive iodine therapy. At the same time, morbidity and financial costs related to these procedures, may be greatly reduced.

Some of the studies demonstrated a 100% rate of expression of CK19 in PTC (including FVPTC). Though, these results were perceived as controversial, nevertheless it was proved that high percentage expression of CK19 has a close relation with malignancy [18]. In another study conducted by Saleh et al. on immunohistochemical analysis of CK19, HBME-1, galectin-3 and Ret oncoprotein in 98 benign lesions and 54 malignant lesions, it was observed that 83.3% of the FVPTC cases and 31.6% of the benign lesions positively expressed CK-19 [12]. Noroozinia et el. conducted an experiment on CK19 and also showed consistent results [19] similar to our results.

In a study conducted on immunohisto-chemistry of CD56, galectin-3 and CK19 in both benign and malignant lesions of the thyroid by Park *et al*., 92.5% of the PTC (including FVPTC) did not stain with CD56 marker [14]. Another study that also performed immunohistochemical analysis of claudin-1 and CD56 on thyroid lesions, benign lesions showed positive staining in 89.4% with CD56. While weak or negative staining was seen in 82.2% of PTC including FVPTC (amongst which only 3 out of 16 (18.75%) of FVPTC cases stained positively) [20].

CONCLUSION

Hence, it is concluded from our study that HBME-1 and CK-19 were found to be specific for FVPTC, while loss of CD56 is suggestive of any malignancy in the thyroid. Thereby, combination of these markers enables the pathologist to make a more accurate diagnosis.

AUTHOR CONTRIBUTION

Hassan Tariq: Jointly conceived the study
Amina Iqbal: Designed and executed the project
Hafeez Ud Din, Syed Raza Jaffar: Supervised the project

Sardar Waleed Babar: Selected the cases and worked in wet lab

Asma Gul: Assisted in statistical analysis

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