BRAF GENE MUTATION AND CD56 IMMUNOEXPRESSION IN PAPILLARY THYROID CARCINOMA IN DUHOK-IRAQ

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ABSTRACT

Background

As much as the distinction of papillary carcinoma versus endocrine thyroid lesions may result in some relevant pitfalls, different immunohistochemical panels have addressed this task. The concept of CD56 deletion has been accepted to be specific for papillary carcinoma.

Objectives

This study was an attempt to test CD56 immunoexpression in papillary carcinoma in this locality and to study the relative association of CD56 immunoexpression with BRAFV600 gene mutation on papillary carcinoma.

Methods

Paraffin embedded, thyroid biopsy specimens containing papillary carcinoma were available for 70 patients. The immunohistochemical technique applied was streptavidin-biotin, using CD56 mouse monoclonal antibodies manufactured by Ventana Corporation (Ventana, Rocklin, Calif). BRAFV600 gene mutation was tested on 48 cases using Real-Time PCR, the target (BRAFV600) DNA was amplified with the mutation-specific primers.

Results

Out of 70 papillary carcinoma cases, 7.1% showed CD56 immunopositivity and out of 48 cases, 27.1% were positive for BRAFV600 gene mutation. Morphologically, the five CD56 positive cases included 2 follicular variants, 2 anaplastic and 1 conventional papillary carcinoma. On the other hand, the thirteen BRAFV600 positive cases encompassed 8 conventional, 2 microcarcinoma, 2 columnar variant and 1 anaplastic thyroid carcinoma. The frequency of BRAF mutation was statistically highest among the conventional papillary carcinoma. No any association was detected between CD56 immunostaining and BRAFV600 gene mutations.

Conclusions

Our findings suggest that although CD56 negativity helps but can not role out papillary thyroid cancer. There is no any association between CD56 expression and BRAF gene mutation at least among our cases.

Keywords: *Papillary thyroid carcinoma, BRAF, CD56*

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INTRODUCTION

Thyroid gland enlargement is common in the general population and usually discovered during a routine medical examination.Thyroidtumours comprise about 1–24 % of cancers [1-3]. In Iraq, the frequency of thyroid cancer is 2.5% [4]. In Kurdistan Region-Iraq, the reported crude rates of thyroid cancer at 2007-2009 ranged from 0.28-0.57/100,000 [5]. Papillary thyroid carcinoma (PTC) is by far the most common type of thyroid malignancy (85%-95%) [2]. Independent reports have observed inexplicably increasing incidence of PTC in the last few decades and the mystery behind this ostensible rising frequency has become one of the confounding current medical puzzles [1-3]. The diagnostic dilemmas have important consequences for management and prognosis of patients with PTC [6].

In the vast majority of cases, pathologic diagnosis of surgically removed thyroid tumour is possible by the means of morphological examination of the routine Hematoxylin/Eosin (H&E) stained sections [7-9]. However, in some cases the pathological criteria do not allow definite differentiation of PTC from neoplastic and non-neoplastic endocrine thyroid lesions [9]. Inasmuch as the distinction between PTCs versus endocrine thyroid lesions may result in some relevant pitfalls, a huge number of studies have pointed out the invaluable role of ancillary techniques including both immunohistochemistry and molecular panels in refining and empowering the diagnostic accuracy of morphological specimens especially in difficult cases [3, 6, 7, 10]. Immunohistochemical panels, like CD56, Hector Battifora and Mesothelioma (HBME), Cytokeratin19 and Galectin3 have been tried and used for the diagnosis of difficult cases of thyroid cancer [6, 7, 10, 11]. A significant progress has been made in understanding of the B-type Raf kinase (BRAF) mutation, the commonest genetic mutation in PTC [12, 13]. To present date, literature data are few and inconsistent about studying CD56 immunoexpression and BRAF gene mutations in PTC particularly in our locality, Duhok/Kurdistan-Iraq. The aims of this study are to find out the frequency of $BRAF^{V600}$ mutation, number of CD56-positive PTC in this locality and assessment of the relative association of these two parameters in PTC.

MATERIALS AND METHODS

The study was conducted in the Central Laboratory/ Directorate of Healthand Research Center, Duhok-Iraq. Specimens were retrieved from Duhok histopathologic laboratories (Central laboratory, Duhok Private Medical laboratory and Vin Private Medical laboratory) during the period from May 2011 to August 2015. All patients were newly diagnosed and have received no prior therapy. Formalin-fixed paraffin-embedded thyroid specimens containing papillary carcinoma were available for 70 patients. Four micron-thick tissue sections were taken from the tumour and stained with Hematoxylin and Eosin (H&E) to confirm the diagnosis.

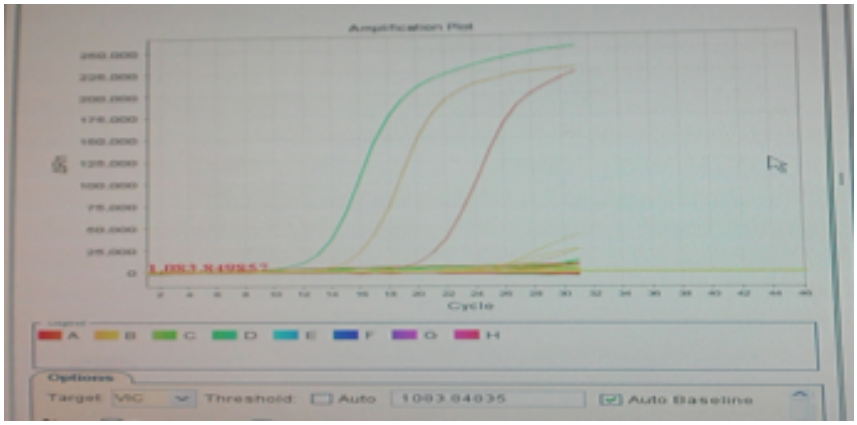
DNA extraction was performed according to the protocol described by Maniatis [14] with measurement of its concentrations by Nanodrop spectrophotometer. The ABI-7500 fast Real-time polymerase chain reaction (RT-PCR)was carried out according to the manufacturer's instruction provided by the kit (AmoyDx) company. *BRAF* gene mutation kit (AmoyDx) was used and designed to detect five types of *BRAF* gene mutation (V600E, K, D, R and M) using the cycling conditions described in table (1). Finally, data analysis was conducted using Bioneerdata analysis system (software), When the FAM Ct value was $\geq$ 28, the sample was considered negative or below the detection limit of the kit while when the FAM Ct value $<$ 28, the sample was considered mutation positive as described by the manufacturer's recommendations, as described by Pity previously [15].

Amplification plot Real-time PCR for BRAF mutation (VIC target) illustrates two positive cases of papillary carcinoma (red and yellow lines).

The immunohistochemical technique used was streptavidin-biotin reaction. The primary antibodies used were mouse monoclonal antibodies for CD56 (NCAM) (NovocastraTM Liquid Mouse Monoclonal Antibody RTU-CD56-1B6, Leica, Germany),and 3-3'-diaminobenzidine tetrahydrochloride (DAB) as a chromogen (DAB detection kit; Ventana) according to the manufacturer's instructions (Ventana). These were described previously by other studies [16, 17, 18].

Table 1. Real-time cycling stages.

Temperature	Time	Cycles
Stage 1		1
95°C	5 min	
Stage 2		15
95°C	25s	
64°C	20s	
72°C	20s	
Stage 3		31
93°C	25s	
60°C	35s*	
72°C	20s	



RESULTS

A total of 70 patients with papillary thyroid (n= 67) and anaplastic (n= 3) carcinoma were enrolled in this study. The mean age was 41.6 years (range: 19-79; median age: 40.3). From these, 50 (71.4%) were females and 20 were (28.6%) males with 2.5: 1 female to male ratio (Table 2).

Correlation of CD56 and BRAF^{V600} Gene Mutation with Age and Gender

Out of seventy cases, only five (7.1%) showed CD56 immunoreactivity while the remaining 65 (92.9%) cases were negative for CD56. From the 48 cases tested for *BRAF* gene mutation, 13 (27.1%) were positive and 35 (72.9%) were negative for *BRAF* gene mutation. No significant differences were demonstrated between CD56 and *BRAF*gene mutation and patient's ages (p= 0.3 and 0.6, respectively) and between these parameters and gender(P= 0.1 and 0.8, respectively).

Correlation of CD56 and BRAF^{V600} Mutation with Papillary Thyroid Carcinoma Variants

From five CD56 positive cases, 3 patients had conventional PTC and 2 had anaplastic carcinoma. The remaining 65 cases (51 conventional, 6 microscopical, 5 follicular, 2 columnar and 1 anaplastic) were negative for CD56.In addition,from13positive BRAF^{V600} gene mutation cases, 12 (92.3%) were papillary carcinoma and 1 (7.7%) anaplastic carcinoma. The frequency of mutation was statistically highest (p= 0.048) among the conventional papillary carcinoma where 8 (61.5%) cases were positive for this mutation. The remaining 4 cases included 2 (15.4%) microscopical and 2 (15.4%) columnar cancers (Figure 1).

Association between CD56 Immunoexpression and BRAF^{V600} Gene Mutation

Positive *BRAF* gene mutation constituted 44.4% (n= 13) of CD56 negative cases (Figure 2). The remaining CD56 negative cases 32 (55.6%) were negative for *BRAF* gene mutation. No *BRAF* gene mutation was detected in any of the CD56 positive cases. No significant association was observed between CD56 immunostaining and *BRAF* gene mutations (p= 0.2).

Table 2. Distribution of thyroid cancer cases according toage and gender.

Cancer	Number (%)	Age (years)		Gender	
		Range	Mean	Female	Male
				Number (%)	Number (%)
		Papillary			
Variant					
Conventional	54 (77.1)	19-79	38.9	39 (55.7)	15 (21.4)
Microscopical	6 (8.6)	21-32	26.5	5 (7.1)	1 (1.4)
Follicular	5 (7.1)	34-50	44.6	3 (4.3)	2 (2.9)
Columnar	2 (2.9)	24-35	29.5	1 (1.4)	1 (1.4)
		Anaplastic			
	3 (4.3)	50-65	60	2 (2.9)	1 (1.4)
		Total			
All cases	70	19-79	39.6	50 (71.4)	20 (28.6)

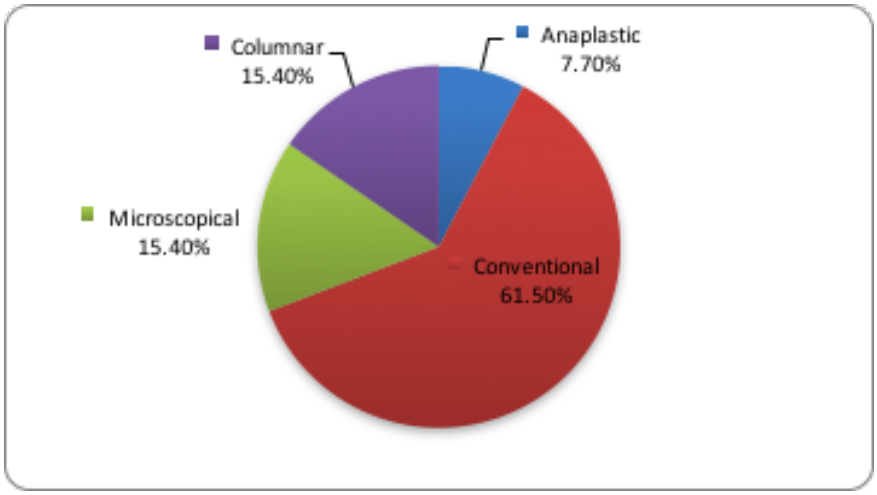


Figure 1. Positive BRAF^{V600}gene mutation and papillary carcinoma variants (χ^2=7.8; P= 0.048).

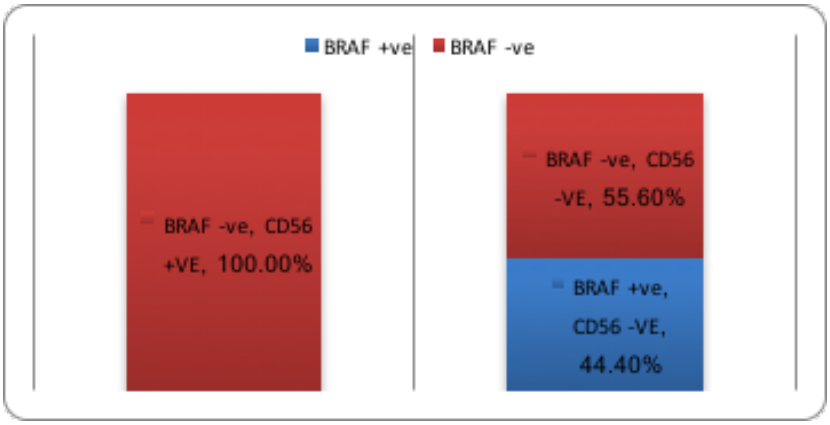


Figure 2. Association between CD56 expression and *BRAF* gene mutation.

DISCUSSION

In this study, $BRAF^{V600E}$gene mutation was detected in tissue specimens of patients diagnosed with thyroid cancer because it has been hypothesized that this gene mutation in thyroid gland is exclusive for papillary carcinoma [19]. Similar studies have also demonstrated correlation between *BRAF* gene mutation and medullary thyroid carcinoma in a prior research in the same particular locality by Pity et al among 54 women and by Cerrato et al [15, 20]. An important finding in the current study was that histologic differences were apparent between papillary thyroid cancers harboring $BRAF^{V600}$ gene mutation. There was a significantly higher positivity among the conventional histology. This result is consistent with what have been conducted in Korea, Greece, US, Italy and Spain [12, 21]. Furthermore, the association between *BRAF* gene mutation and the cases of columnar variant-PTC was significant. Studies conducted in this regard imply that this histologic type may represent an aggressive variant of papillary carcinoma [9,22]. In a study analysis of positive *BRAF*gene mutation in columnar variant of papillary thyroid carcinoma postulated that this mutation may influence the outcome of columnar PTC [23].

Similarly, in our study$BRAF^{V600}$ gene mutation was also positive in two out of four cases of papillary microcarcinoma. Deceivingly, this variant is known to have an excellent clinical course [2, 24, 25]. Large studies described papillary microcarcinoma harbouring $BRAF^{V600}$gene mutation associated with increased aggressiveness [26-28].

On the other hand, $BRAF^{V600}$ gene mutation wastotally absent among the follicular variant PTC. Consistent with our results, an exceedingly low, if not absent, *BRAF* gene mutations have been demonstrated by studies performed among American, European and Asian populations [13, 21]. It has been presumed that the follicular variant PTC develops through a distinct set of molecular abnormalities and may have unique biological properties [29, 30]. The follicular variantsof papillary carcinoma is different from the conventional PTC and shows molecular alterations that areintermediate between follicular neoplasms and papillary carcinomas [9, 26, 27, 29, 30].This, together with the positive *RAS* gene mutation in the follicular variant and its absence in conventional papillary carcinoma, suggest that at least some encapsulated follicular PTC could actually be regarded as follicular cancers when invasive and adenomas when non-invasive [30-32].

A growing number of immunomarkersin thyroid cancershave been used with considerable reproducible results [6, 10, 33]. In this study, we investigated CD56 expression in tissue sections of different papillary carcinoma cases by means of immunohistochemistry. From these, 92.9% of cases did not stain with this antibody. Similar results was observed by Park et al among 67 Korean people with PTC (92.5%) and a higher value of negative cases (96%) has been demonstrated by Bizzarro et al among 50 American patients [10]. Meanwhile, Boila et al, in their study on 55 Romanian patients with PTC reported that CD56 sensitivity in ruling out PTC has reached 81.1% [6].

Interestingly, 5 (7.1 %) cases were found to be positive among our series. Consistent with our experience, Bizzarro et al and Boila et al demonstrated CD56 positivity in some PTC cases [6, 10]. Park et al and El Demellawy et al, who used a <10% cut-off value for recording a positive CD56 expression, have speculated

CD56 negativity with 100% sensitivity and 100% specificity to rule out PTC [8, 34]. Consequently, authors concluded that absence or reduced CD56 expression in PTC can be used as a constant, specific and extremely sensitive diagnostic marker in ruling out papillary thyroid carcinoma with its variants and hence referred to as PTC profile [6, 8, 11, 33, 34]. Based on Park et al speculation, the occasional positive PTC cases reported have to be considered as false negative results [6, 10]. In the present study out of 5 positive cases, 2 were of follicular variant PTC, a finding that has been also documented by Bizzarro et al and Boila et al experiments [6, 10]. The follicular variant PTC has been found to harbor many follicular tumour morphologic characteristics which perhaps include common genetic alterations [15, 29, 31, 32]. Such characteristics may include retained thyroid endocrine features and hence CD56 expression. Two other positive cases among the cases in our study were anaplastic carcinoma. These high grade malignant cases may represent dedifferentiated follicular carcinoma rather than dedifferentiated papillary carcinoma and thus retained their endocrine and CD56 expression [9, 13, 30, 35, 36]. In other studies, a reduced CD56 expression was reported with specificity in ruling out the diagnosis of PTC and its variants to 63.6%. Thus, subsequently denied any concept considering CD56 negativity as a constant "PTC profile", despite the fact that CD56 has been elected by the same authors as the most sensitive marker for the same task [6, 10].

Another parameter added in this study was the association between CD56 immunoexpression and the $BRAF^{V600E}$ gene mutation tested on 48 common PTC cases. Despite no previous document declaring any sort of participation of CD56-related genes in tumorogenesis pathway, there are evidences that *BRAF* gene mutation is involved in PTC initiation [19].

In the present study, we did not find any cases to be positive for both CD56 expression and $BRAF^{V600E}$gene mutation. Most cases investigated were double negative for both CD56 expression and $BRAF^{V600E}$gene mutation. The remaining cases were either CD56 positive or *BRAF* gene mutated. This indicates that both are independent parameters at least among the thyroid cases examined in this study.

In view of the preliminary results obtained in the present study, we can conclude that CD56 may be a reliable marker for ruling out PTCs and its variants but its use as a single independent marker has to be further evaluated. In addition, it is difficult to proof any possible association between $BRAF^{V600E}$ gene mutation and CD56 immunoreactivity in papillary and anaplastic thyroid carcinomas from this limited study.

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