Original article:

The Prevalence and Prognostic Value of BRAF^{V600E} Mutation in Papillary Thyroid Cancer

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Abstract

Objective: B-type Raf kinase (BRAF)^{V600E} mutation in papillary thyroid cancer (PTC) has variable prevalence worldwide and it is hypothesized to worsen tumor prognosis. This study was conducted to investigate the prevalence of BRAF^{V600E} mutation among PTC patients and to find out its prognostic impact measured by its association with various clinicopathologic features, recurrence, and mortality. ***Methods:*** This is a retrospective study that included 123 PTC patients who underwent thyroidectomy at Jordan University Hospital between January 2010 and December 2015. They were followed up over a mean of 18 months (range: 4-72). BRAF^{V600E} mutation was analyzed by direct sequencing. A *p* value less than 0.05 was defined as statistically significant. ***Results:*** Twenty three out of 123 (18.7%) PTC patients were BRAF^{V600E} mutation positive. BRAF^{V600E}- mutant patients were more likely to have larger tumor size (1.8 vs 2.5 cm, p=0.040), to present with lymph node metastasis (LNM) (41.2% vs 82.4%, p=0.002), and to develop recurrence (1 vs 3, p=0.0003). Moreover, tumor recurrence which was recorded in 4 patients was significantly associated with LNM (p=0.038). Cancer-specific mortality rate was null. ***Conclusion:*** BRAF^{V600E} mutation rate in PTC was low relative to world-wide prevalence. BRAF^{V600E} mutation has prognostic value for PTC management. However, its cost-effectiveness should be revised. Further larger prospective studies in the region are recommended.

Keywords: Papillary thyroid carcinoma, BRAF kinases, prognosis.

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Introduction

Papillary thyroid cancer (PTC) is the most common thyroid malignancy accounting for 85% of the cases. Fortunately, it carries a favorable prognosis with a 5-year cancer-specific survival rate of 95%[1]. Although most PTC cases can be managed successfully with surgery and adjuvant treatment, recurrence occurs in 20% of patients after approximately 10 years requiring reoperation and extended follow up which accounts for tumor burden[2]. In addition, a small percentage can develop distant metastasis which decreases 10-year survival rate to 40%[3]. So, it is important to stratify patients' risk and apply proper management.

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Clinicopathologic features such as age, gender, tumor size, LNM, and distant metastasis have been used to assess patients' prognosis. Moreover, several genetic alterations have been found to be involved in tumor pathogenesis and aggressiveness[4].Among them, $BRAF^{V600E}$ mutation is the most extensively studied one as it is the most common mutation in PTC with high specificity[5].

B-type Raf kinase (BRAF) is a serine/threonine protein kinase which is a member of mitogen-activated protein kinase (MAPK) pathway, an intracellular cascade chain involved in cell growth and differentiation. In $BRAF^{V600E}$ point mutation, thymidine is replaced by adenosine at exon 15 nucleotide (T1799A) resulting in substitution of valine for glutamic acid at amino acid 600. This leads to constitutive activation of MAPK pathway and subsequent tumorigenesis[6].The prevalence of $BRAF^{V600E}$ mutation among PTC is 45% on average with wide geographic variation being higher in Asia especially in Korea[7]. Such discrepancy can be due to multiple genetic and environmental factors[7,8].

The role of $BRAF^{V600E}$ mutation in predicting the prognosis of PTC is controversial. It was associated significantly with at least one of the poor clinicopathologic features such as advanced age, male gender, larger tumor size, capsular invasion, and advanced TNM stage[9,10]. However, other studies did not find $BRAF^{V600E}$ mutation of prognostic value[4,11-14]. Our aim was to investigate the prevalence of $BRAF^{V600E}$ mutation among PTC patients and to find out whether it has a prognostic impact.

Materials and methods

Patient selection and data collection

This retrospective study included 123 PTC patients who underwent thyroidectomy at Jordan University Hospital between January 2010 and December 2015. Ethics committee approval was taken according to the institution's protocol. Histopathology results were re-read and confirmed by an experienced pathologist.

We reviewed the medical records of the patients and collected relevant clinicopathologic data including patients' age, gender, tumor size, LNM, and TNM staging according to the American Joint Committee on Cancer (AJCC) staging system (7^{th} edition). Tumor recurrence was defined by biochemical and/or radiological evidence confirmed by fine needle aspiration (FNA) and/ or histopathology.

DNA analysis

Samples were generated from formalin-fixed paraffin-embedded (FFPE) tissues, DNA was extracted using the QIAamp® DNA FFPE Tissue (Qiagen, Germany) following the manufacturer's recommendations. Tissue punches were obtained from paraffin blocks using microtome (Leica-biosystems, USA) 5 micron/section. The extracted DNA was quantified using a NanoDrop spectrophotometer ND-2000 (Thermo Fisher Scientific, USA). Polymerase chain reaction (PCR) was performed as illustrated in **(Table 1)**.

Table 1. Genotyping method of BRAFV600E polymorphisms by PCR direct sequencing.

Primer name	Sequence
$BRAF^{V600E}-F^{a}$	5′- TCATGAAGACC TCACAGTAAAAAT -3′
$BRAF^{V600E}-R^{b}$	5′- TGGATCCAAGACAACTGTTCAA -3′
PCR program: start with 95°C for 5 min, followed by 36 cycles of 94°C for 30 sec, 54°C for 1 min and 72°C for 30 sec, ended with 72°C for 7 min and 4°C forever.	
[a]Forward primer. [b]Reverse primer.	

PCR products were analyzed by 3% agarose gel electrophoresis (Cleaver, UK). Automated dideoxy sequencing and purification were performed by Macrogen® (Korea) after PCR amplification using the forward and reverse primer pair.

Statistical analysis

Descriptive statistics were computed as mean±SD for numerical data, count and percent frequencies for categorical data. Fisher-Freeman-Halton exact test was used for the relation between $BRAF^{V600E}$ mutation, recurrence, and categorical variables. The relationships between $BRAF^{V600E}$ mutation, recurrence, age, and tumor size were evaluated by independent samples t-test. A p value less than 0.05 was defined as statistically significant derived from two-tailed tests. All statistical analyses were performed with the SPSS (ver. 18) software.

Results

One hundred and twenty-three patients with PTC who underwent thyroidectomy consisting of 102 females and 21 male patients with a male/female ratio of 1:5 were included. The mean age was 43.7±14 years (range: 16- 80 years).

On histologic examination, the mean tumor size, described by the largest diameter, was 1.92 cm (range: 0.1- 8 cm). One hundred and four out of 123 cases were classic-variant papillary thyroid cancer (CVPTC) (84.6%), 14/123 (11.4%) were follicular-variant papillary thyroid cancer (FVPTC), 2/123 (1.6%) were tall cell variant, and 2/123 (1.6%) were Hürthle cell tumors while 1/123 (0.8%) was detected to be the trabecular type.

Tumor stage was available for 120 patients. According to AJCC staging system, 97/ 120 were diagnosed at Stage I (80.8%), 5/120 at Stage II (4.2%), and another 5/120 at Stage III (4.2%) while 13/120 were found to be at Stage IV (10.8%). Lymph node metastasis was detected in 42/120 (35%). None of the cases had distant metastasis.

Twenty-three out of 123 cases harbored $BRAF^{V600E}$ mutation with a prevalence 18.7% (23/123). All of the 23 cases were found to be of the Classic (conventional) subtype and were heterogeneous mutations. Tumor size (p= 0.040), lymph node involvement at the time of diagnosis (p=0.002), and tumor recurrence (p=0.003) were significantly higher in mutant than wild type tumors. There was no significant association between $BRAF^{V600E}$ mutation and age, gender, histologic subtype, or tumor stage **(Table 2).**

Table2. The association between $BRAF^{V600E}$ mutation and clinicopathologic features.

			BRAF state			P value
		Mutant (n=23)		Wild (n=100)		
		Count	*(%)*	*Count*	*(%)*	
Age (*mean)*		41.6 ±15.7		43.3 ±14.9		0.636
Gender	Female	18	81.8	84	83	0.894
	Male	4	18.2	17	17	
FNA [a]	Non-diagnostic	0	0	3	4.8	0.961
	Benign	3	20.0	12	19.0	
	AUS/FLUS [b]	1	6.7	5	7.9	
	FN/SFN [c]	1	6.7	5	7.9	
	Suspicious for malignancy	3	20.0	14	22.2	
	Malignant	7	46.7	24	38.1	
Mean tumor size[d] *(cm)*		2.5 ± 1.37		1.8 ± 1.38		**0.040***
Histologic Subtype	Classic (conventional)	23	100	81	81	0.270
	Follicular	0	0	14	14	
	Tall variant	0	0	2	2	
	Oncocytic (Hürthle)	0	0	2	2	
	Trabecular	0	0	1	1	
Lymph node involvement	No	3	17.6	40	58.8	**0.002***
	Yes	14	82.4	28	41.2	
Tumor stage	Stage I	7	30.4	59	59.6	0.090
	StageII	10	43.5	24	24.2	
	Stage III	5	21.7	14	14.1	
	Stage IV	1	4.3	2	2.0	
Recurrence	No	13	81.3	69	98.6	**0.003***
	Yes	3	18.8	1	1.4	

[a]FNA,fine needle aspiration. [b]AUS/FLUS, Atypia of undetermined significance/ follicular lesion of undetermined significance; [c] FN/SFN, follicular neoplasm / suspicious for follicular neoplasm.
[d]tumor size measured to the largest diameter.
* p value less than 0.05 is statistically significant.

Due to the available data, only 86 patients could be assessed for recurrence. At the end of the mean follow up period of 18 months (range: 4-72 months), four cases developed locoregional recurrence, three of them were $BRAF^{V600E}$ mutated as shown in (**Table 3)**.

Table 3. Characteristics of the patients who developed recurrence.

Characteristics	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**
Age (year)	61	38	22	32
Gender	Female	Female	Male	Female
Tumor size (cm)	3.2	(N/A)[a]	2.5	1.1
Tumor stage	IVa	I	I	I
Histologic subtype	Classic	Classic	Classic	Classic
Type of initial surgery	Total thyroidectomy	Total thyroidectomy	Total thyroidectomy	Total thyroidectomy
Initial LN[b] dissection	Lateral	(N/A)	Pretracheal	Not performed (T1bNxMx)
Follow up period (months)	36	60	26	12
Site of recurrence	Right cervical LN (levels 2, 5)	Thyroid bed, left deep cervical LN	Thyroid bed, Delphian LN, upper and lower cervical LN	Thyroid bed, cervical LN
$BRAF^{V600E}$ mutation	Wild/ Mutant	Wild/ Mutant	Wild/ Mutant	Wild/Wild

[a]N/A, not available. [b]LN, lymph node.

No distant recurrence was encountered. In addition, none of the patients died due to PTC. Tumor recurrence was significantly correlated with $BRAF^{V600E}$ mutation (p= 0.003) and Lymph node involvement (p= 0.038). On the other hand, no association was found regarding recurrence with age, gender, histologic subtype, tumor size, or stage **(Table 4)**.

Table 4. The association of recurrence with clinicopathologic features.

		Recurrence				**p value**
		No		**Yes**		
		Count	*%*	*Count*	*%*	
Mean age (*years)*		44.5 ± 14.4		41.25 ± 13.4		0.659
Gender	Male	15	18.3	0	0	0.458
	Female	67	81.7	4	100	
Mean tumor size (*cm)*		1.92 ± 1.30		1.96 ± 0.76		0.960
Initial lymph node involvement	Yes	26	47.3	3	100	**0.038***
	No	29	52.7	0	0	
Stage	I	65	79.3	3	75	0.615
	II	4	4.9	0	0	
	III	3	3.7	0	0	
	IV	10	12.2	1	25	
Histologic subtype	Classic	66	80.5	4	100	0.811
	Follicular	12	14.6	0	0	
	Tall variant	2	2.4	0	0	
	Oncocytic	2	2.4	0	0	

Note: A total 86 patients were assessed for recurrence.

*p value less than 0.05 is statistically significant.

Discussion

Although PTC has excellent prognosis, recurrence takes place. In fact, it exposes patients to reoperation(s),[15] higher radioiodine doses, and some cases loose response to therapy[16].

Several scoring systems have been used to stratify patients' risk that include patient age, size of primary tumor, extent of disease, histologic grade, and completeness of resection besides the TNM staging system[4].However, this approach is limited in part by relying predominantly on postoperative results and it is not accurate for intermediate risk patients according to the American Thyroid Association (ATA) guidelines[17].

Recently, multiple molecular markers have been studied for possible impact on patients' assessment and management. Among thyroid carcinomas, $BRAF^{V600E}$ mutation is predominantly found in PTC.[7] It was correlated with advanced tumor stage and more aggressive course than wild type $BRAF^{V600E}$ in PTC[18,19]. However, results are still controversial[20,21].

In our study, the prevalence of $BRAF^{V600E}$ mutation was 18.7% (23/123). This number may seem lower than the global numbers which range between 27.3 - 87.1%[7]. It is noticeable that the prevalence of $BRAF^{V600E}$ mutation differs significantly between countries, where a higher prevalence exists in the Eastern countries especially in Korea while a lower prevalence in Western countries[7]. In a retrospective study that involved 1849 patients, the prevalence of BRAF mutation in PTC was 67.4% in Japan, 45.7% in the United States of America, 48.3% in Italy, and 42.4% in Poland[14].

A retrospective study by Kim et al.[22] on 103 patients diagnosed with PTC found the prevalence of $BRAF^{V600E}$ mutation to be 33%. They used real-time PCR method on FFPE tissues for their study[22]. Another study conducted in Czech Republic reported the overall prevalence of $BRAF^{V600E}$ mutation among different variants of thyroid cancer to be 33.5%[23]. They compared the incidence of $BRAF^{V600E}$ mutation before and after the Chernobyl nuclear disaster and found a higher incidence of $BRAF^{V600E}$ mutation after the accident. Though radiation-induced thyroid cancer usually harbors RET/PTC rearrangement[8, 24]. So they concluded that the increased rate of $BRAF^{V600E}$ mutation was due to older age of the participants.

The variation of $BRAF^{V600E}$ mutation prevalence is attributed to many genetic and environmental factors such as radiation exposure[26], iodine intake[26], Hashimoto's thyroiditis[7],and the histologic subtype as tall cell and classic variants PTC tend to harbor $BRAF^{V600E}$ mutation more than the follicular variant PTC[10], besides the methodology adopted by each study[27]. To decrease such variations, we compared our results with a study conducted in the Arab Region at Kingdom of Saudi Arabia. They reported, however, higher rate of $BRAF^{V600E}$ mutation 63% (72 out of 115) among CVPTC larger than one cm[28].

In the present study, multiple clinicopathologic features were studied with $BRAF^{V600E}$ mutation. Tumor size ($p= 0.040$) and lymph node involvement ($p=0.002$) were significantly associated with $BRAF^{V600E}$ mutation. In addition, $BRAF^{V600E}$ mutation was significantly higher in patients who developed recurrence than who did not ($p= 0.003$). There was no significant correlation between $BRAF^{V600E}$ mutation and age, gender, histologic subtype, or tumor stage.

There is controversy regarding $BRAF^{V600E}$ mutation and tumor size. Although some studies found a significant role for $BRAF^{V600E}$ mutation and larger tumor size[29], a meta-analysis did not find out an association[30]. On the other hand, when micropapillary thyroid cancer was correlated with BRAF status, there was a significant association between tumor size and $BRAF^{V600E}$ mutation[31].

Several studies reported $BRAF^{V600E}$ mutation to be associated with LNM[32-35]. Park et al.[32] who studied 688 CVPTC among Koreans showed that BRAF-positive tumors were associated with male gender, larger tumor size, extrathyroidal extension, lymph node involvement, and advanced stage[32]. On the contrary, another Korean study on 107 CVPTC in which $BRAF^{V600E}$ mutation rate reached 79.4% did not demonstrate any correlation between $BRAF^{V600E}$ mutation and clinicopathologic features[12].Such discrepancy can be explained by the sample size and the performance of therapeutic or prophylactic lymph node dissection that may reveal more LNM[32]. A study from four surgical centers that were more representative for PTC histologic subtypes and performed central lymph node dissection routinely found $BRAF^{V600E}$ mutation to be significantly associated with some clinical and pathological parameters that included LNM, worse tumor stage, and histologic subtype[33]. However, the association lost significance among CVPTC only suggesting the marginal prognostic value for $BRAF^{V600E}$ mutation when CVPTC is

highly prevalent[33].

We are consistent with the literature that most PTCs are diagnosed early at Stage I or Stage II with comparable mean age and female predominance due to the nature of the disease. However, many of our cases are CVPTC and routine lymph node dissection, which has controversial importance, was not routinely recommended[33].

Regarding tumor recurrence, four patients developed recurrence all of which were locoregional recurrences after a mean follow up period of 18 months (range: 4-72 months). Three of them harbored BRAF mutation with significant association (p= 0.003). In addition, tumors with LNM were more likely to recur (p=0.038) while patient's age, gender, tumor size, stage, and histologic subtype were not significantly associated with recurrence. In fact, LNM was found to be the most important risk factor for PTC recurrence especially in young patients[36]. According to Alzahrani et al.,[37] patients who had any form of cervical lymph node dissection were found to have significantly more aggressive tumor behavior like; extrathyroidal and vascular invasion, multifocal tumor, and advanced stage than patients who did not have any form of dissection[37]. Moreover, LNM was significantly associated with decreased disease-free survival, and overall survival in other studies[38].

Data regarding PTC recurrence, and mortality are conflicting taking into consideration the indolent nature of the disease, short follow up period, and/or small sample size in many studies. Higher rates of PTC recurrence among BRAF positive patients was supported by several studies[9,39]. In the study of Xing et al.,[13] which included 2099 cases, patients with $BRAF^{V600E}$ mutation were found to have almost twice the risk of developing recurrence in the future[13]. This relation was explained by BRAF-induced tumorigenesis[9]. However, other studies did not support this association[4,11,20]. Niederer-Würst et al.[4] found that the overall survival, disease-specific survival, and recurrence-free survival rates were better estimated by clinical risk scores rather than $BRAF^{V600E}$ mutation which was neither significantly associated with score parameters, nor adverse clinicopathologic features[4]. Moreover, Czarnieckaet al.[20] reported that the extent of tumor, and LNM were significantly related to PTC recurrence rather than $BRAF^{V600E}$ mutation[20].

Limitations to our study include its retrospective design with small sample size. In addition, prophylactic lymph node dissection was not routinely performed. On the other hand, the mean follow-up period is short which can neither represent tumor recurrence, nor mortality accurately.

Conclusions

Despite the controversy of $BRAF^{V600E}$ mutation prevalence, and prognostic role in PTC, we found a low prevalence of $BRAF^{V600E}$ mutation (18.7%) in PTC which may be explained by multiple genetic, and environmental factors including radiation exposure.

$BRAF^{V600E}$ mutation was significantly associated with larger tumor size, LNM, and tumor recurrence. In addition, patients who develop recurrence were more likely to present with LNM. We suggest that testing for $BRAF^{V600E}$ mutation for PTC risk stratification is of prognostic value. However, its cost-effectiveness is questionable in low-prevalence countries. Further larger prospective studies combining RET/PTC rearrangement in the region are recommended for further evaluation.

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