

The Relationship Between the BRAFV600E Mutation in Differentiated Thyroid Cancer and Clinicopathologic Factors in Vietnam



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Received:  September 11, 2018; Published:  September 17, 2018

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Abstract

Objective: Among more than 40 mutations identified in the BRAF gene, the BRAF T1799A (V600E) point mutation is the most common and accounts for more than 90% of all the mutations found in the BRAF gene. It has been found to occur frequently in thyroid cancer. While some reports suggest the BRAF V600E mutation is associated with poor prognosis and recurrence or iodine uptake resistance, this remains a controversial issue. The aim of study was to: 1) determine the status of BRAF mutation in differentiated thyroid cancer in Bach Mai hospital; 2) evaluate the association of the BRAF mutation with clinicopathological parameters.

Patients and Methods: Retrospective analysis of the BRAF V600E mutation in differentiated thyroid cancer in 198 patients from November 2015 to March 2017 at Bach Mai hospital using BRAF 600/601 Strip Assay® (ViennaLab, Austria). The results were correlated with clinicopathological factors.

Results: The BRAF V600E mutation was detected in 150 cases (75.8%). BRAF mutation was found in 69/92 (75%) patients having metastasis. Mutation was found in distant metastasis, lymph node metastasis and in both distant-lymph node metastasis: 63/84 (75%), 9/13 (69.2%) and 3/5 (60%), respectively. Among differentiated thyroid cancer, BRAF mutation was detected in follicular-papillary thyroid cancer (80%), papillary thyroid cancer (76.6%) and in follicular thyroid cancer (40%). There was no significant correlation between the presence of BRAF mutation and clinicopathological factors. Conclusions: The BRAF mutation rate in thyroid cancer patients is high, especially for follicular-derived papillary and papillary thyroid cancer. The lack of a statistically significant association of BRAF and clinical characteristics indicates the need of conducting research with larger sample sizes in the future.

Introduction

Thyroid cancer shows the fastest rising incidence among all major human cancers, currently with 44,670 new cases in the United State. Differentiated thyroid cancer is the most common cancer of the endocrine system and the papillary thyroid cancer is the most common type, followed by follicular thyroid carcinoma. In recent decades, the incidence of this type of cancer has increased in Vietnam. According to GLOBOCAN 2012, the prevalence rate is 2.5/100000 people, mortality rate is 1.3/100000 people per

year. Most thyroid cancers generally have a good prognosis after appropriate treatment including surgical procedure and radioiodine therapy [1]. However, the recurrence rate of differentiated thyroid cancer increased up to 30%, and the cancer death rate was 8% after initial treatment at 30 years of follow-up. Studies in the world have shown that the primary cause of recurrence and metastasis in patients treated radioiodine therapy are low absorption and poor iodine intake (Table 1) [1,4].

Table 1: Sociodemographic characteristics of patients (n = 198).

Sociodemographic characteristics of patients		n	Proportion (%)
Age	< 45	96	48.5
	≥ 45	102	
	Median	45.1	51.5
	Range	16-76	
Age group	Under 30	25	12.6

	30 years old and over	173	87.4
Gender	Male	26	13.1
	Female	172	86.9
Sampling method	Surgery	189	95.4
	Biopsy	9	4.6
Sampling locations	Thyroid	169	85.4
	Nodal metastasis	26	13.1
	Distant metastasis	3	1.5
Total		198	100

Comment: Of the 198 patients participated in the study, there were 172 women and 26 men, with an average age of 45.1 years old, in which patients aged 45 years or older accounted for a higher proportion (51.5%) Most patients (189/198 - 95.4%) underwent primary tumor resection and histopathological examination with tissue samples taken from surgery. The primary sampling site was thyroid gland which accounted for 85.4%, 1.5% of sampling site was (3 patients) from distant metastatic tissue.

Activation of MAPK pathway by BRAF V600E was believed to play a dominant role in the development and progression of thyroid cancer[2]. Effectors of MAPK signaling pathway representing an early molecular event in papillary thyroid cancer regulate several genes related to cell proliferation, differential and survival. Gene expression analyses using DNA microarrays showed that different transcriptional profiles were associated with BRAF, RET/PTC and RAS mutation groups in human papillary thyroid cancer [10]. The differential expression profiles between these genetic alternations may explain why BRAF mutation has more close correlations with poor clinicopathological features compared with RET/PTC and RAS mutation[3]. The mutant BRAF can stimulate constitutive signaling which bypasses the need for extracellular growth factors. Subsequently phosphorylation of downstream MER1/2 and ERK1/2 leads to the expression of a few specific genes involved in cell proliferation, differential, survival, tumorigenesis, and even the microenvironment promoting the process of epithelial-mesenchymal transition [3-4].

BRAF V600E mutation in thyroid cancer has been proved to be associated with high-risk clinicopathological characteristics,

Table 2: Clinical characteristics of patients (n = 198).

Characteristics		n	Proportion (%)
Disease staging	I-II	107	59.4
	III-IV	60	33.3
	N/A	13	7.2
T staging	T1	35	17.7
	T2	95	48
	T3/T4	28	14.1
	N/A	40	20.2
Nodal metastasis	N0	70	35.4
	N1	84	40.9
	N2/N3	3	1.5
	N/A	44	22.2
Distant metastasis	M0	143	74.7
	M1	13	6.6
	N/A	37	18.7

tumor recurrence and reduced sensitivity of radioiodine therapy. Conventional factors that demonstrate the high-risk clinicopathological characteristics included increased age, male gender, larger tumor size, extrathyroidal invasion, local lymph node metastasis, distant metastasis, and advanced disease stages. Recent studies have showed that significant correlations of BRAF mutation with the reliable prognostic predictors such as extrathyroidal invasion, lymph nodal metastasis, and advanced TNM stage[6]. In this study, we focus on the association of BRAF mutation with clinicopathological characteristics and BRAF mutation as a therapeutic target of thyroid cancer [5-6].

Patients and Methods

Patients

Retrospective study on 198 patients who had been diagnosed with thyroid cancer at The Nuclear Medicine and Oncology Center of Bach Mai Hospital, from November 2015 to March 2017. Informed consent for the evaluation of BRAF V600E mutation was obtained from all patients (Table 2).

Histopathology	papillary thyroid cancer	188	95
	follicular thyroid cancer	5	2.5
	papillary- follicular thyroid cancer	5	2.5
Total		198	100

Comment: Among 198 participated patients, 107 of them was in early stage (I-II), accounted for 59.4%. Most patients were in the T2 stage (48%), the remaining patients in the T1, T3/T4 and unknown stage with the rate of 17.7%; 14.1% and 20.2%, respectively. The proportion of patients with 1 and 2/3 nodal metastasis were 40.9% and 1.5%, respectively. 143 out of 198 patients did not develop distant metastases, accounting for 74.7%, only 13/198 patients were detected with distant metastatic, accounting for 6.6%. In terms of tumor tissue histology, the majority of 188/198 tumors were papillary, and the rest was follicular (5 patients) and follicular-derived papillary (5 patients).

DNA Extraction and the BRAF V600E Mutation Analysis

We performed the BRAF V600E mutation analysis on paraffin embedded sections of primary tumors obtained after thyroidectomy. Genomic DNA was extracted from 10µm thick sections of formalin-fixed paraffin embedded tumor tissue blocks using the QiAamp DNA FFPE tissue kit (Qiagen, USA). The concentration and purity of the extracted DNA were determined by a Qubit 2.0 spectrophotometer (Thermo Fisher Scientific, USA). The extracted DNA was stocked at -20°C until further use. The assays

for the detection of the BRAFV600E mutation were performed using the BRAF 600/601 StripAssay® (ViennaLab, Austria). All reactions were done in 20-µL volumes using template DNA, and PCR master mix. All needed reagents were included with the kit. The PCR reaction of was performed using a Mastercycler (Eppendorf, USA). PCR cycling conditions were a 10 min hold at 37°C followed by 35 cycles of 94°C for 60sec, 70°C for 50sec, 56°C for 50sec, 60°C for 60sec, and 60°C for 3min (Tables 3 & 4). The PCR products were hybridized with streptavidin-alkaline phosphatase specific probe and results analysis. Data is processed by SPSS 15.0 software [7].

Table 3: Rate of BRAF mutation.

BRAF status	n	Proportion (%)
Mutated	150	75.8
Wild type	48	24.2
Total	198	100

Comment: Of the total 198 patients, the BRAF V600E mutation was found in 150 patients, accounting for 75.8%.

Table 4: BRAF V600E mutation and clinicopathologic factors of differentiated thyroid cancer patients.

Patients clinicopathology		BRAF V600E mutation					
		Yes	No	OR	95% CI	(%)	p
Age	< 45	83	19	0.52	0.27 - 1.02	81.5	0.057
	≥ 45	67	29			69.8	
Gender	Male	19	7	1.18	0.46 - 2.98	73.1	0.732
	Female	131	41			76.2	
Sampling method	Surgery	144	45	0.63	0.15 - 2.60	76.2	0.529
	Biopsy	6	3			66.7	
Sampling locations	Thyroid	127	42	-	-	75.2	0.562
	Nodal metastasis	21	5			80.8	
	Distant metastasis	2	1			66.8	
Histopathology	Papillary	144	44	-	-	76.6	0.243
	Follicular	2	3			40.0	
	Papillary- follicular	4	1			80.0	
Stage	I-II	80	27	1.22	0.57-2.59	74.8	0.605
	III-IV	47	13			78.3	
T staging	T1	29	6	-	-	82.9	0.52
	T2	71	24			74.7	
	T3/T4	20	8			71.4	
N staging	N0	53	17	-	-	75.7	0.8
	N1	60	21			74.1	

	N2/N3	3	0			100	
M staging	M0	113	35	0.68	0.2-2.4	76.4	0.813
	M1	9	4			69.2	

Comment: The results of the study show no correlation between BRAF mutagenicity characteristics such as age, sex, sampling method, sampling location, disease staging, T-stage, Nodal metastasis, distant metastasis and histopathological characteristics ($p > 0.05$).

Result

Patients' Characteristics

Tables 1 & 2

The Status of BRAF Mutation

Table 3

The Relationship between the BRAFV600E Mutation in differentiated Thyroid Cancer and Clinicopathologic Factors

Table 4

Discussion

In our study, the majority of patients were female, accounting for 86.9% (172/198), while the rate of male patients was 13.1% (26/198). Female/male ratio was 6,6/1 (172/26). The average age for both men and women were 45.1 years. These findings are in accordance with some findings to other studies: Nguyen Quang Truong (2013) reported that the proportion of male and female patients were 17.5% and 82.5%, respectively; the rate of female/male was 4.7/1 (113/24)[7]. This study also found that the average age of both men and women is 39.7 years old. In the study conducted by [1-8], 76.3% of patients was female which was 3 times higher than male patients (23.7%), the average age was 40.9 ± 14.1 . The most common age group was from 31 to 45 years old, accounted for 40.1% of total patients while the high-risk age group (under 15 and over 45) accounted for 31.8% [8]. In general, the incidence of thyroid cancer in women is three to five times higher than in men and is more likely to occur in young people. In terms of histopathological classification, our study showed that 95% of thyroid cancer patients was papillary form[9]. Similar studies conducted by Le Van Quang (2002)[4] among 308 cancer patients in the Vietnam nation cancer hospital discovered that 79.5% thyroid cancer was papillary form, only 15.6 % was in follicular form. Our study also like what is reported by Lim L.H và colleagues (2002). Among 198 thyroid cancer patients, 78% of them was papillary form, 19% of them was papillary form, 1% medullary thyroid carcinoma and 2% undifferentiated thyroid cancer [10].

This similarity suggests that papillary form remains a common histopathology of thyroid cancer. The results of many international researches reported the wide range of BRAF mutations frequency which was from 39% to 84%. The frequency of BRAF mutation in our study was 75.8% and all mutations were detected as V600E which like the results of Kim SJ et al. (2012) in Korean patients (71%). In addition, our results showed the high rate of BRAF mutation in the group of under 45 years (81.4%), which is 12% higher in

comparison with the group of over 45 years old. BRAF mutations were more common in women than in men with a mutation rate of 73.1% and 76.2%, respectively. In our study, the BRAF mutations occurred more frequently in patients with papillary and follicular-derived papillary form with mutation rates of 76.60% and 80%, respectively. Patients with disease progression stage (III / IV) had a 4% higher of BRAF mutation rate in comparison with early stage patients (I/II). Regarding the T staging, nodal metastasis and distant metastasis, there was 8-11% difference between T1 stage and other T staging; 100% of patients with 2 or 3 nodal metastases had BRAF mutation. The rate of BRAF mutations in non-metastatic patients was 76.4% and was 5% higher than in patients with distant metastases. In term of sampling method, our results demonstrated that the detection rate of mutations in surgical specimens was 76.19%, 10% higher than that of biopsy specimens.

For the sampling site, the specimen collected at the nodal metastasis had the highest BRAF mutation rate (80.8%), followed by thyroid site and distant metastatic with mutation rate of 75.2% and 66.7%, respectively. Although our study found a difference in BRAF mutations in every clinical characteristic, but the results of the χ^2 test showed no significant difference ($p > 0.05$). These results suggest that there is no significantly association between BRAF mutations and some specific clinical characteristics such as age, sex, stage of disease, histopathological result, sampling sites and sampling methods, which are in accordance with other similar sample sizes research in the world. However, other studies performed on larger sample sizes, resulting in contradictory findings. This difference may be due to insufficient sample sizes and limited follow-up. Therefore, the implementation of future studies with larger sample sizes is critical to assess the prognosis of BRAF mutations in thyroid cancer patients. In addition, our study reported that the sampling site and sampling method did not have any statistically significant association with BRAF mutation ($p > 0.05$). This result has once again confirmed that the BRAF V600E mutation are unaffected by the sampling method and site.

Conclusion

Of the 198 thyroid patients in our study:

- BRAF mutations were detected in 150/198 patients (75.76%), with all mutations at codon 600 exon 15 (V600E).
- BRAF mutation does not depend on age, gender, sampling methods, sampling sites, histopathological features, the stage of disease, T staging, Nodal metastasis and Distant metastasis.
- The implementation of future studies with larger sample sizes is critical to assess the prognostic potential of BRAF mutations in thyroid cancer patients.

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ISSN: 2574-1241

DOI: [10.26717/BJSTR.2018.09.001748](https://doi.org/10.26717/BJSTR.2018.09.001748)

Pham Cam Phuong. Biomed J Sci & Tech Res



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