




Relationships of BRAF V600E Gene Mutation With Some Immunohistochemical Markers and Recurrence Rate in Patients With Thyroid Carcinoma

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ABSTRACT

BACKGROUND: The B-type rafkinase (BRAF) V600E gene mutation plays an important role in the pathogenesis, diagnosis, and prognosis of thyroid carcinoma. This study was conducted to investigate the rate of the BRAF V600E mutation, the relationships between the BRAF V600E gene mutation and some immunohistochemical markers, and recurrence rate in patients with differentiated thyroid cancer.

METHOD: The study was conducted by a descriptive and longitudinal follow-up method on 102 thyroid carcinoma patients at 103 Military Hospital, Hanoi, Vietnam. All patients were identified with the BRAF V600E gene mutation by real-time polymerase chain reaction.

RESULTS: The rate of BRAF V600E gene mutation in patients with thyroid cancer was 60.8%. Patients with BRAF V600E gene mutation had a significantly higher rate of positive cyclooxygenase 2 (COX-2) and Ki67 markers than those without the mutation (COX-2: odds ratio [OR] = 2.93; 95% confidence interval [CI] = 1.27-6.74, $P = .011$; Ki67: OR = 3.41; 95% CI = 1.31-8.88, $P = .01$). A statistically significant relationship was identified between the rate of BRAF V600E mutation and the rate of positive Hectort Battifora mesothelial 1 (HBME-1) ($B = -1.040$; $P = .037$) and COX-2 ($B = -1.123$; $P = .023$) markers. The recurrence rate in patients with BRAF V600E gene mutation was significantly higher than that in those without the mutation ($P = .007$). The mean of the recurrence time of patients with BRAF V600E mutation was significantly lower than that in those without the mutation ($P = .011$).

CONCLUSIONS: A high prevalence of BRAF V600E gene mutation was found in thyroid carcinoma patients. The rates of positive HBME-1, COX-2, and Ki67 markers were significantly correlated to BRAF V600E gene mutation. Patients with BRAF V600E gene mutation showed a significantly higher relapse rate and earlier relapse time than those without the mutation.

KEYWORDS: Thyroid carcinoma, BRAF V600E gene mutation, HBME-1, COX-2 and Ki67 markers

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Introduction

Thyroid carcinoma is the most common type of cancer. In 2019, an epidemiological study of thyroid cancer in the United

States based on the American National Cancer Database showed that the incidence of thyroid cancer increased from 7.1 per 100 000 in 2000 to 17.4 per 100 000 in 2013—a 2.5-fold increase.¹ Thyroid carcinoma is divided into two types, including differentiated and undifferentiated types; primarily

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in the differentiated type, including papillary, follicular, and papillary-follicular combined.²⁻⁴ Because differentiated thyroid carcinoma develops slowly and is similar to other benign diseases of the thyroid gland for the clinical manifestations, it is easy to confuse the diagnosis. Thyroid carcinoma usually may develop locally or invade the thyroid cortex, surrounding tissues (esophagus, trachea, larynx, skin infiltrates, and so on), or metastasize to cervical lymph nodes or distant metastases depending on the histopathological type and the time of the disease detection.⁵

Thyroid cancer can be diagnosed based on ultrasound-guided core biopsy and confirmed histopathologically with conventional hematoxylin plus eosin staining.⁶ However, some cases have insufficient laboratory information to distinguish between benign and malignant lesions with conventional hematoxylin plus eosin staining alone.⁷ Although most thyroid carcinomas can be identified preoperatively by cytology, approximately 20% to 30% of cases may not be identifiable by conventional tests.⁸ Many studies have shown that the pathological status of the thyroid gland can be clearly diagnosed using immunohistochemistry with specific antigen-antibody markers.⁹⁻¹¹ In recent years, several studies reported the role of B-type rafkinase (BRAF) V600E mutations in the diagnosis and prognosis of papillary thyroid carcinoma.^{12,13} Position mutation T1799A (V600E) on BRAF gene sequence increases enzyme kinase activity and activates the mitogen-activated protein kinase (MAPK) signaling pathway; somatic BRAF gene mutation was found in tumor cells with different tumor sizes and with detection frequency ranging from 28.8% to 69%.¹² In vitro studies have shown that thyroid carcinoma cell lines with the BRAF V600E gene mutation have a much faster growth rate than wild-type thyroid cell lines,^{14,15} and it is hypothesized that the BRAF V600E mutation acts as an oncogene.¹⁶ The BRAF V600E gene mutation was reported as a very valuable molecular marker in the diagnosis and the following of thyroid carcinoma.¹⁷ The BRAF V600E mutation is only presented in thyroid carcinoma cells but not in benign thyroid cells. Identifying mutations in the BRAF V600E gene will help to limit diagnoses missing of thyroid carcinoma and to improve the quality of medical examination, treatment, following, and patient management.¹⁸ However, previous studies mainly focused on analyzing the value of BRAF V600E mutations in diagnosis and prognosis.^{17,18} Previously, there were some studies investigating the relationships between BRAF V600E mutations and clinical and subclinical characteristics and recurrence status in patients with thyroid carcinoma.¹⁹⁻²¹ From the aforementioned fact, this study was conducted to investigate the rate of BRAF V600E mutation and determine the association of BRAF V600E gene mutation with some clinical, subclinical, and recurrence characteristics in patients with differentiated thyroid cancer.

Materials and Methods

Study population and design

The study subjects included patients who were operated on and diagnosed by postoperative histopathology with thyroid carcinoma at 103 Military Hospital, Hanoi, Vietnam, from July 2013 to December 2016. Exclusion criteria included patients with non-differentiated carcinoma of the thyroid gland, thyroid cancer secondary to metastases from other sites, and extensively invasive differentiated thyroid cancer without total thyroidectomy.²² All patients have explained the study procedure and agreed to participate in the study.

The study was conducted by a descriptive and longitudinal follow-up method. A total of 102 patients were recruited in the present study. All patients who met the inclusion and exclusion criteria were selected during the study period.

Clinical and biochemical assessments

The characteristic information (name, age, gender, address, and occupation), medical history and current diseases, examination of clinical signs and symptoms, laboratory tests, and BRAF gene mutation tests were collected on the patient's record.

The stage of thyroid cancer was identified based on the TNM (Tumor-Node-Metastase) classification of the American Cancer Society (1998).²³ Serum levels of free triiodothyronine (FT3) and free thyroxine (FT4) were quantified by an automatic chemiluminescence system. Tg concentration was quantified by an immunoradiometric assay technique at the Department of Biochemistry, 103 Military Hospital.

All patients were tested for BRAF gene mutation by the real-time polymerase chain reaction technique through candle-molded tumor specimens. The primers and blockers used in the study were ordered from IDT, USA, with the following sequence:

- Forward primers (no.1): 5'-CTGTTTTCTTTACTTA CTACACCTCAGAT-3'
- Reverse primers (no. 2): 5'-CAACTGTTCAAAC TATGGG-3'
- Allele-specific reverse primers (no. 3): 5'-CCCAC TCATCGAGATTTCT-3'
- BRAF-blocker: 5'-CATCGAGATTTCACTGTAG CTAGA-PO4-3'

All postoperative specimens were stained for immunohistochemistry, with markers Hector Battifora mesothelial 1 (HBME-1), cytokeratin 19 (CK19), rearrangement during transfection (RET), P53, Ki67, and cyclooxygenase 2 (COX-2). Immunohistochemical staining using the avidin-biotin complex method²⁴ was conducted at the Department of Pathology, Military Hospital 103 (Supplemental Document).

After surgery, the patient was re-examined at 1 month and received adjuvant treatment with iod-131. The patient was then followed up and evaluated for recurrence status. The time of recurrence was counted from the date of surgery to the date of examination and detection of recurrent disease.

Evaluation of recurrence

After adjuvant treatment with ¹³¹I, patients were followed up every 3 months. At the time of re-examination, the patients were examined with clinical checking, ultrasound, thyroid function measurement, thyroglobulin (Tg) concentration, and thyroid scintigraphy. The results of the assessment of recurrence are carried out according to the guidelines of the Ministry of Health of Vietnam,²⁵ specifically as follows:

- No recurrence: After adjuvant therapy with ¹³¹I, the patient was determined to be disease-free during follow-up visits (complete thyroid tissue, negative whole-body scintigraphy, and Tg concentration < 10 ng/ mL) until the last follow-up visit.
- Relapse: After adjuvant therapy with ¹³¹I, the patient was determined to be free of the disease in the next follow-up (thyroid tissue was gone, whole-body scintigraphy was negative, and Tg concentration < 10 ng/mL) but then re-emergence at a follow-up visit (based on clinical or scintigraphy findings) in the neck or distant metastases, confirmed by fine needle aspiration (FNA) or other appropriate methods.
- Existing disease: After adjuvant therapy with ¹³¹I, it was still determined that the disease has not been cured in follow-up visits (thyroid tissue was still present, whole-body scintigraphy shows lymph node invasion or distant metastases, the concentration of Tg > 10 ng/mL). The disease persisted at the last follow-up.

Ethical statement

All participants provided written informed consent and agreed to join our study. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Review Committee of Vietnam Military Medical University (reference no.: 168/2014/IRB-VMMU).

Statistical analysis

All results are presented as mean (standard deviation [SD]) or, if biased, as median (interquartile range) for continuous variables and as percentages for categorical variables. Differences between groups were tested by *t*-test. The chi-square test or Fisher's exact test was used to assess whether there was a relationship between two categorical variables. The association between gene mutation status and immunological marker test results was determined using multivariable logistic regression,

using the variance inflation factor (VIF) coefficient to assess the presence or absence of multicollinearity between variables. $P < .05$ was considered to be statistically significant. All data were processed using SPSS software version 26 (64-bit) for Windows (SPSS Inc., Chicago, IL).

Results

The demographic and characteristics of the patients were shown in Table 1. The mean \pm SD age of the patients was 45.1 ± 13.4 years. No significant difference in age was observed between men and women ($P > .05$).

The rate of BRAF V600E gene mutation was 60.8%, and there was no difference in the rate of BRAF V600E gene mutation between the sexes ($P > .05$). Fifty-three patients (52% of the total patients) were in the first stage of the cancer; 45 patients (44.1%) were in the second stage; and there were only 4 patients (3.9%) in the third stage. Most patients (84.3%) had the papillary type in histopathology. Increased Tg and FT3 levels were found in 35.3% and 15.6% of the total number of patients, respectively. All patients had the normal FT4 level. No significant differences for the disease stage, histopathology, the concentration of hormones (Tg, FT3, FT4), and immune markers were shown between the sexes. Eleven patients (21.2%) were recrudesced after a treatment. A borderline significant difference in the rate of recurrence was found between men and women (Table 1).

The demographic characteristics were compared between the patients with and without BRAF V600E gene mutation. The results were shown in Table 2. There were no significant differences in age, sex, disease stage, histopathology, or hormone concentration (Tg, FT3, FT4) between the patients with and without BRAF V600E gene mutation ($P > .05$) (Table 2).

The rates of the positive immune markers were compared between the patients with and without BRAF V600E gene mutation and were shown in Table 3. The rates of positive COX-2 and Ki67 markers were significantly higher in the patients with BRAF V600E gene mutation than those in patients without the mutation. Of all, 72.6% of patients with BRAF V600E mutation had a positive COX-2 marker, while the rate of the positive COX-2 marker in the non-mutant group was 47.5% ($P = .011$; odds ratio [OR] = 2.93; 95% confidence interval [CI] = 1.27-6.74), and 41.9% of patients with BRAF V600E mutation had a positive Ki67 marker, while the positive Ki67 marker rate in the non-mutant group was 17.5% ($P = .01$; OR = 3.41; 95% CI = 1.31-8.88) (Table 3).

The relationships between gene mutation and immune markers using a binary logistic regression model were shown in Table 4. The presence of BRAF V600E gene mutation was significantly inversely correlated with the positivity of the HBME-1 marker ($P = .037$; OR = 0.353; 95% CI = 0.133-0.939) and COX-2 marker ($P = .023$; OR = 0.325; 95% CI = 0.124-0.856) (Table 4).

Table 1. The demographic and characteristics of the patients.

VARIABLES, UNIT	Men	WOMEN	ALL	P
Age, years	46.4 ± 15.2	44.9 ± 13.1	45.1 ± 13.4	.665 ^c
BRAF V600E mutation				
Yes	51 (60.7)	11 (61.1)	62 (60.8)	1.0 ^b
No	33 (39.3)	7 (38.9)	40 (39.2)	
Disease stage				
I	45 (53.6)	8 (44.4)	53 (52.0)	.214 ^a
II	37 (44.0)	8 (44.4)	45 (44.1)	
III	2 (2.4)	2 (11.1)	4 (3.9)	
Histopathology				
Papillary type	69 (82.1)	17 (94.4)	86 (84.3)	.292 ^a
Other types	15 (17.9)	1 (5.6)	16 (15.7)	
Tg				
Normal	38 (69.1)	6 (46.2)	44 (64.7)	.195 ^a
Increase	17 (30.9)	7 (53.8)	24 (35.3)	
FT3				
Normal	65 (82.3)	16 (94.1)	81 (84.4)	.295 ^a
Increase	14 (17.7)	1 (5.9)	15 (15.6)	
FT4				
Normal	79 (100.0)	17 (100.0)	96 (100.0)	—
Increase	0 (0)	0 (0)	0 (0)	
HBME-1				
≤3+	49 (58.3)	13 (72.2)	62 (60.8)	.303 ^b
4+	35 (41.7)	5 (27.8)	40 (39.2)	
CK19				
1+ and 2+	14 (16.7)	7 (38.9)	21 (20.6)	.052 ^a
3+ and 4+	70 (83.3)	11 (61.1)	81 (79.4)	
COX-2				
Negative	30 (35.7)	8 (44.4)	38 (37.3)	.593 ^b
Positive	54 (64.3)	10 (55.6)	64 (62.7)	
P53				
Negative	39 (46.4)	9 (50.0)	48 (47.1)	.801 ^b
Positive	45 (53.6)	9 (50.0)	54 (52.9)	
Ki67				
Negative	56 (66.7)	13 (72.2)	69 (67.6)	.785 ^b
Positive	28 (33.3)	5 (27.8)	33 (32.4)	

(Continued)

Table 1. (Continued)

VARIABLES, UNIT	Men	WOMEN	ALL	P
RET				
Negative	10 (11.9)	1 (5.6)	11 (10.8)	.684 ^a
Positive	74 (88.1)	17 (94.4)	91 (89.2)	
Recurrence				
No	37 (84.1)	4 (50.0)	41 (78.8)	.051 ^a
Yes	7 (15.9)	4 (50.0)	11 (21.2)	

Abbreviations: BRAF, B-type rafkinase; CK19, cytokeratin 19; COX-2, cyclooxygenase 2; FT3, free triiodothyronine; FT4, free thyroxine; HBME-1, Hecto Battifora mesothelial 1; RET, rearrangement during transfection; SD, standard deviation; Tg, thyroglobulin.

^aFisher's exact test.

^bChi-square test.

^cStudent's *t*-test.

Table 2. Comparison of demographic characteristics between the patients with and without BRAF V600E gene mutation.

CHARACTERISTICS	BRAF V600E GENE MUTATION				P
	NO		YES		
	N	%	N	%	
Age, years	44.25 ± 12.75		45.71 ± 13.90		.594 ^b
Sex (n=102)					
Female	33	39.3	51	60.7	.975 ^a
Male	7	38.9	11	61.1	
Disease stage (n=102)					
I	22	55.0	31	50.0	0.781 ^a
II	17	42.5	28	45.2	
III	1	2.5	3	4.8	
Histopathology (n=102)					
Papillary type	33	38.4	53	61.6	.686 ^a
Other types	7	43.8	9	56.3	
Tg (n=68)					
Normal	18	40.9	26	59.1	.337 ^a
Increase	7	29.2	17	70.8	
FT3 (n=96)					
Normal	29	35.8	52	64.2	.200 ^a
Increase	8	53.3	7	46.7	
FT4 (n=96)					
Normal	37	38.5	59	61.5	
Increase	0	0.0	0	0.0	

Abbreviations: BRAF, B-type rafkinase; FT3, free triiodothyronine; FT4, free thyroxine; Tg, thyroglobulin.

^aFisher's exact test (Fisher's 2 sides).

^bIndependent-samples *t*-test.

Table 3. Comparisons of the rate of the positive immune markers between the patients with and without BRAF V600E gene mutation.

IMMUNE MARKERS	BRAF V600E GENE MUTATION				OR; 95% CI	P ^a
	NO (N=40)		YES (N=62)			
	N	%	N	%		
HBME-1						
1+ and 2+	29	72.5	33	53.2	2.32; [0.99-5.45]	.052
3+ and 4+	11	27.5	29	46.8		
CK19						
Negative	9	22.5	12	19.4	1.21; [0.46-3.20]	.701
Positive	31	77.5	50	80.6		
COX-2						
Negative	21	52.5	17	27.4	2.93; [1.27-6.74]	.011
Positive	19	47.5	45	72.6		
p53						
Negative	22	55.0	26	41.9	1.69; [0.76-3.77]	.197
Positive	18	45.0	36	58.1		
Ki67						
Negative	33	82.5	36	58.1	3.41; [1.31-8.88]	.010
Positive	7	17.5	26	41.9		
RET						
Negative	6	15.0	5	8.1	2.01; [0.57-7.10]	.270
Positive	34	85.0	57	91.9		

Abbreviations: BRAF, B-type rafkinase; CK19, cytokeratin 19; COX-2, cyclooxygenase 2; HBME-1, Hektor Battifora mesothelial 1; RET, rearrangement during transfection. ^aChi-square Test.

Table 4. Relationships between the BRAF V600E gene mutation and immune markers using a binary logistic regression model.

IMMUNE MARKERS	B	P VALUE	OR; 95% CI	VIF
HBME-1	-1.040	.037	0.353; [0.133-0.939]	1.091
CK19	0.081	.886	1.085; [0.357-3.294]	1.101
COX-2	-1.123	.023	0.325; [0.124-0.856]	1.190
p53	0.266	.630	1.304; [0.443-3.845]	1.550
Ki67	-1.126	.055	0.324; [0.102-1.026]	1.340
RET	-1.188	.117	0.305; [0.069-1.348]	1.092
Constant	2.329	.000	10.269	

Abbreviations: CK19, cytokeratin 19; COX-2, cyclooxygenase 2; HBME-1, Hektor Battifora mesothelial 1; RET, rearrangement during transfection ; VIF, variance inflation factor.

Table 5 showed the comparisons of the recurrence rate and the mean of the recurrence time between the patients with and without the BRAF V600E mutation. The rate of recurrence in the patients with the BRAF V600E gene mutation was significantly higher than that in the patients without

the gene mutation ($P=.007$). The mean of the recurrence time in patients with the BRAF V600E mutation (12.39 ± 7.44 months) was significantly lower than that in the patients without the gene mutation (18.71 ± 7.44 months) ($P=.011$) (Table 5).

Table 5. Comparisons of the recurrence rate and the mean of the recurrence time between the patients with and without the BRAF V600E gene mutation.

CHARACTERISTICS	BRAF V600E GENE MUTATION				P
	NO		YES		
	N	%	N	%	
Recurrence rate					
No (n=41)	23	56.1	18	43.9	.007*
Yes (n=11)	1	9.1	10	90.9	
Time of recurrence (months)	18.71 ± 7.44		12.39 ± 7.44		.011**

Abbreviation: BRAF, B-type rafkinase.

*Fisher's exact test. **Independent-Samples T Test.

Discussion

The BRAF gene mutation is considered to be one of the important mutations related to the invasiveness, advanced stage, recurrence rate, and metastatic degree of thyroid carcinoma.¹⁸ In our study, 60.8% of thyroid carcinoma patients had mutations in BRAF gene at the T1799A (V600E) position. Similarly, Niederer-Wüst et al²⁶ reported that the rate of BRAF gene mutation was 65% in thyroid carcinoma patients with tumor size ≥ 1 cm. Pelizzo et al²⁷ showed 47.8% of BRAF V600E mutations in 226 patients with papillary thyroid carcinoma, stage T1-T2 without lymph node metastasis (N0). In addition, the results from several studies suggest that the BRAF V600E mutation is common in thyroid carcinomas, particularly papillary thyroid carcinomas, with frequency varying from 18% to 87%.^{28,29} This difference in the mutation rate may be due to differences in the selection of research patients and geographical locations, especially in histopathological types. In our study, BRAF V600E mutation was present in 61.6% of conventional papillary thyroid cancers (PTCs) and in 56.3% of other histological types; these results are similar to the results of other previous studies when classified according to histological subtypes.³⁰⁻³²

Our study showed no association between the BRAF V600E gene mutation and the disease stage, which is consistent with the study by Peuxeddu et al.³³ In contrast, other studies reported that BRAF V600E mutations are associated with stage III and IV diseases.³⁴ A multicenter study with a large number of patients showed that patients with stages III and IV had a significantly higher rate of BRAF mutation than the non-mutant group ($P=.007$).³⁰ Overall, almost all studies showed that BRAF V600E mutations occur more frequently in patients with the disease in stages III and IV than in stages I and II.^{12,13,35}

Higher rates of positive COX-2 and Ki67 markers were found in the patients with the BRAF mutation than in those without the mutation. There was an inverse correlation between the positive level of marker COX-2 ($P=.023$) and the occurrence of the BRAF V600E gene mutation, identified using a

binary logistic regression. COX is a group of enzymes required for synthesizing prostaglandin E2 (PGE2).³⁶ Under normal physiological states, COX-2 and PGE2 are upregulated and act as inflammatory factors.³⁷ COX-2 has been observed to be overexpressed in several human cancers.^{38,39} Many studies have described the carcinogenesis-promoting mechanisms by COX-2, including inhibition of apoptosis, cell proliferation, stimulation of angiogenesis, and growth of the pathogenic microenvironment inflammation which promotes tumorigenesis.^{40,41} COX-2 overexpression in different types of thyroid cancer has also been demonstrated.^{42,43} BRAF V600E mutants have been shown to induce oncogenic cell proliferation by constitutive activation of the MAPK pathway,⁴⁴ while BRAF mutations have a role in accelerated production of PGE2 through COX-2.⁴⁵ This might explain the association between BRAF mutation status and the appearance of a COX-2 marker through immunohistochemical staining in the current study.

To evaluate postoperative recurrence and supportive treatment with I¹³¹, we applied the following diagnostic criteria for recurrence: Patients after surgery and adjuvant I¹³¹ therapy were determined to be disease-free at the next follow-up (there was no thyroid tissue, a negative whole-body scintigraphic, and Tg concentration < 10 ng/mL), but at subsequent follow-up visits, there was a recurrence in the thyroid (signs in clinical examination, ultrasound, or scintigraphic), cervical lymph nodes, or distant metastases, and a positive FNA result confirmed the diagnosis. The results of our study showed that the mean of the recurrence time in patients with BRAF V600E gene mutation was earlier than the mean of the recurrence time in the patients without the mutation. Patients with mutations in BRAF V600E gene had a higher recurrence rate than patients without the mutation ($P=.007$), which is consistent with other studies.^{12,13,35} Several studies have shown that the prevalence of BRAF mutations was very high at 80% to 85% of recurrent thyroid carcinomas.^{46,47} Patients with stage I and II diseases usually have a low risk of recurrence, but the risk of recurrence increases markedly in patients with the BRAF gene mutation.³⁰ Compared with BRAF mutation-negative cases,

recurrent thyroid carcinoma patients with the BRAF mutations require more aggressive treatments, such as re-surgery and external beam irradiation, and show a higher rate of treatment failure.³⁰ The cause of the higher recurrence rate in patients with BRAF mutations is the loss of affinity of the follicular cells with radioactive iodine and the consequent PTC unresponsiveness to therapy with radioactive iodine.^{30,48} Abnormal activation of the MAPK signaling pathway is responsible for altering the iodine capture mechanism of thyroid follicular cells.⁴⁹ The BRAF V600E mutation alters the function of the BRAF protein, thereby inhibiting the expression of thyroid function-specific genes and the loss of the iodine tolerance mechanism of thyroid cells through the activation of the MAPK signaling pathway in thyroid carcinomas. Consequently, iodine is only partially tolerated in thyroid cells carrying the BRAF V600E mutation and sparingly accumulated in the lumen of the thyroid follicle.⁴⁹ This is the key to the molecular mechanism of the radioiodide loss induced by BRAF mutations in PTC, leading to the radioiodine therapy failure and increased recurrence rate.

Our study has some limitations. The study design has a limitation of subjects with a small sample size and without a control group. The follow-up time of patients after treatment was not long, so the predictive and prognostic role of immune markers as well as BRAF gene mutations in thyroid carcinoma had not been fully evaluated yet.

Conclusion

The present study found a high prevalence of BRAF gene mutation at the T1799A (V600E) position in thyroid carcinoma patients. The relationships of the positivity rate of HBME-1, COX-2, and Ki67 markers with BRAF V600E gene mutation were identified. Patients with the BRAF V600E gene mutation showed a significantly higher relapse rate and earlier relapse time than patients without the mutation.

Author contributions

TBDM, TND, VPNT, TDL, and MDT contributed to conceptualization. TBDM, VPNT, MDT, THN, ALT, TBT, STN, and HAV carried out data curation. TBDM, TDL, MDT, THN, KXN, TTV, STN, and DNTN performed formal analysis. TND and DNTN acquired funding. TBDM, TND, VPNT, TDL, and BVN carried out the investigation. VPNT, TTV, TBT, STN, HAV, THN, BVN, and DNTN framed the study methodology. TND, TDL, KXN, TBT, STN, BVN, and DNTN contributed to project administration. TBDM and KXN contributed to resources. TBDM, TDL, MDT, THN, ALT, TTV, HAV, and DNTN contributed to software. TBDM, TND, TDL, KXN and DNTN supervised the study processes. TBDM, TTV, HAV, BVN, and DNTN contributed to validation. TBDM, TDL, and DNTN contributed to visualization. TBDM, THN, TND, KXN, TBT, STN, and DNTN reviewed and edited the article. All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.


Ethical Statement

All participants provided written informed consent and agreed to join our study; and the protocol was approved by the Ethical Review Committee of Vietnam Military Medical University (reference no. 168/2014/IRB-VMMU). The study was also conducted using good clinical practice following the Declaration of Helsinki of 1964, as revised in 2013.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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