ORIGINAL ARTICLE

The relation between BRAFV600E mutation and clinicopathological characteristics of papillary thyroid cancer

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ABSTRACT

Aim BRAF mutation inhibits many tumour suppressor genes, increases pro-angiogenic molecules and reduces radioactive iodine uptake of tumour in papillary thyroid cancer (PTC), giving it more aggressive clinical characteristics. In this study, we aimed to evaluate the effect of BRAF V600E mutation on the clinicopathological features in patients with PTC.

Methods The laboratory and clinical findings of 256 PTC patients who were referred to our clinic between 2007 and 2017 were assessed. Subjects involved in the study were divided into two groups depending on the presence of BRAF V600E mutation.

Results BRAF V600E mutation testing gave positive results for 65 (25.4%) out of 256 patients. No significant correlation between BRAF V600E mutation, age and gender was detected. There was no difference between the groups in terms of tumour variant, tumour localization, tumour focality, and perineural invasion. In terms of histopathologic characteristics, presence of tumour capsular invasion (p=0.027), extrathyroidal extension (ETE) (p=0.002), absence of pathologically detected lymphocytic thyroiditis (p=0.006) and radio iodine I-131 treatment (p=0.001) were significantly higher in BRAF V600E (+) patients. During a follow-up period, four patients with BRAF V600E (+) and two patients with BRAF V600E (-) status underwent lateral neck dissection due to lymph node metastasis (p=0.01).

Conclusion The presence of BRAF V600E mutation was proved to be a poor prognostic factor. However, in order to further assess the prognostic effect of BRAF V600E mutation in this group of patients and particularly its effect on mortality, long term follow-up results must be evaluated.

Key words: carcinoma, mutation, prognosis, thyroid neoplasms

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INTRODUCTION

Thyroid cancer is the most common endocrine cancer. In recent years, there has been a significant increase in the incidence of thyroid cancer due to better recognition of nodular thyroid diseases, superior diagnostic techniques, fine needle aspiration biopsy and new definitions in histopathological criteria. Papillary thyroid carcinoma (PTC) is the most common of all types of thyroid cancers, making up to 80-90% of all thyroid carcinomas (1). Some histologic variants of these tumours are known to have potential aggressive behaviour, while PTC progresses slowly and has a good prognosis (2,3). The staging systems consist of Union for International Cancer Control/American Joint Commission on Cancer Tumour (TNM), AGES (age, grade, extent, size), European Organization for Research and Treatment of Cancer (EORTC), MACIS (metastases, age, completeness of resection, invasion locally, size) and AMES (age, metastases, extent, size), which are the most frequently used systems for estimating the prognosis and the risk of recurrences of thyroid carcinomas (4). However, evaluation of prognosis with the help of molecular genetic results, which has recently become popular, is not possible with these staging systems.

Depending on molecular investigations, it is thought that mutations in RAS, BRAF and RET/PTC rearrangement, which take part in mitogen activated protein kinases (MAPK) pathway, cause an aberrant activation and lead to the development of PTC. Studies reveal that the most widespread molecular damage in thyroid cancer is caused by BRAF mutation (29-83%) (5). In this study, we aimed to evaluate if the presence of BRAF V600E mutation had effect on clinicopathologic characteristics and relapse rate of tumour.

PATIENTS AND METHODS

Patients and study design

The study included all patients who underwent surgery for PTC at the Department of Endocrinology and Metabolism of Trakya University between 2007 and 2017. Medical records of the patients were obtained from institutional database system. Participants were classified based on the presence and absence of BRAF V600E mutation, BRAF V600E (+) and BRAF V600E (-). The demographic data (age, gender), histopathologic findings (tumour variant, tumour focality, lymph node metastasis, extrathyroid expansion, tumour diameter, tumour capsule invasion, and lymphovascular invasion), preoperative neck ultrasonography findings, radioiodine (RAI) 131 treatment were evaluated for all patients. The Ethics Committee of the Trakya University approved the study protocol.

Methods

Tumour tissue containing at least 30% of tumour cells was isolated from the sections of patients for BRAF analysis. DNA purification was performed, using the nucleic acid isolation kit for paraffin-embedded tissue (QIAamp® DNA FFPE Tissue Kit, EZ1® DNA Tissue Kit, PAXgene® Tissue Containers, and PAXgene Tissue DNA Kit (50) (QIAGEN, Hilden/Germany). Following polymerase chain reaction (PCR) procedures, pyrosequencing analyses were performed on PyroMarkQ24, using sequencing primers including the Seq Primer BRAF 600 or Seq Primer BRAF 464–469 (QIAGEN) for BRAF. BRAF V 600 mutation (absent or present) and subtype of BRAF V 600 mutation (BRAF 600E, BRAF 600K, BRAF 600R) were noted.

Statistical analysis

The Mann Whitney U test was used to compare numerical independent factors in patients with BRAF V600E (+) and BRAF V600E (-) results. Pearson $\chi 2$ test and Fisher's exact test were used to calculate the bivariate analysis of the relationship between the BRAF V600E mutation status and clinicopathological features. A multivariate analysis was performed using binary logistic regression analysis for variables, which were significant in the bivariate analysis. p-values less than 0.05 were considered statistically significant.

RESULTS

In this study, we retrospectively evaluated clinical, histopathological and genetic characteristics of 256 with PTC who met inclusion criteria. The median follow-up period was 38±19.1 months (min-max=17 to 124 months) for patients. The mean age of the patients at the time of diagnosis was 47.98±11.99 years. The number of female and male was 220 (85.9%) and 36 (14.1%), respectively. Ninety (35.1%) patients were aged below 45 years, while 166 (64.9%) were aged ≥45 years.

According to tumour size, of ≤1 cm, >1-≤2 cm, >2-≤4 cm and >4 cm there were 144 (56.3%), 78 (30.5%), 30 (11.7%) and 4 (1.6%) patients, respectively.

One hundred twenty-one (47.3%) patients had unifocal PTC, while 135 (52.7%) had multifocal PTC. Extrathyroidal invasion was absent in 219 (85.5%), and it was detected in 37 (14.5%). A total of 212 (82.8%) and 44 (17.2%) patients were without and with lymph node metastasis, respectively. Two of 256 (0.7%) patients were found to have distant metastasis (lung) at the time of the diagnosis. BRAF V600E mutation was detected in 65 of 256 (25.4%) patients with PTC (Table 1).

Table1. Clinicopathological features of 256 patients with papillary thyroid cancer

Clinicopathological features		No (%) of patients
Gender	Female	220 (85.9)
	Male	36 (14.1)
Age (years)	Mean ± SD	47.98±11.99
	<45	90 (35.1)
	≥45	166 (64.9)
Tumour size (cm)	≤1	144 (56.3)
	>1-≤2	78 (30.5)
	>2-≤4	30 (11.7)
	>4	4 (1.6)
Focality	Unifocal	121 (47.3)
	Multifocal	135 (52.7)
Pathologically lymphocytic thyroiditis	No	129 (50.4)
	Yes	127 (49.6)
Tumour capsule invasion	No	207 (80.9)
	Yes	49 (19.1)
Perineural invasion	No	250 (97.7)
	Yes	6 (2.3)
Lymphovascular invasion	No	223 (87.1)
	Yes	33 (12.9)
Extrathyroidal extension	No	219 (85.5)
	Minimal	31 (12.1)
	Common	6 (2.4)
Lymph metastasis	No	212 (82.8)
	Yes	44 (17.2)
Radio iodine-131 treatment (+)	No	103 (40.2)
	Yes	153 (59.8)
Distant metastasis	No	254 (99.2)
	Yes	2 (0.7)
BRAF V600E mutation	No	191 (74.6)
	Yes	65 (25.4)

Bivariate analysis of clinicopathological characteristics of patients with and without BRAF V600E mutation demonstrated a correlation between tumour size, the presence of extrathyroidal, capsular and lymphovascular invasion, absence of pathologically detected lymphocytic thyroiditis, the presence of lymph node metastasis, RAI-131 treatment and BRAF V600E (+) mutation (Table 2).

In addition, multivariate analysis (binary logistic regression) revealed a positive statistical relationship between the absence of pathologically detected lymphocytic thyroiditis (p=0.006), the presence of extrathyroidal extension (p=0.002) and capsular invasion (p=0.027), RAI-131 treatment (p=0.001) and the presence of BRAF V600E mutation (Table 3). During the follow-up stage, four patients with BRAF V600E (+) and two patients with BRAF V600E (-) status underwent lateral neck dissection due to lymph node metastasis (p=0.01).

Table 2. Relationship between the BRAF V600E mutation and clinicopathological features in patients with papillary thyroid cancer

Variable		Total	BRAF V600E mutation, No (%)		p
		Negative	Positive		
Gender	Female	220	167 (75.9)	53 (24.1)	0.238
	Male	36	24 (66.7)	12 (33.3)	
Age	<45	90	65 (72.2)	25 (27.8)	0.518
	≥45	166	126 (75.9)	40 (24.1)	
Focality	Unifocal	121	88 (72.7)	33 (27.3)	0.512
	Multifocal	135	103 (76.3)	32 (23.7)	
Tumour size (cm)	≤1	144	117 (81.3)	27 (18.8)	0.006
	>1	112	74 (66.1)	38 (33.9)	
Extrathyroidal extension	No	219	175 (79.9)	44 (20.1)	<0.001
	Minimal	31	13 (41.9)	18 (58.1)	
	Common	6	3 (50)	3 (50%)	
Pathologically lymphocytic thyroiditis	No	129	86 (66.7)	43 (33.3)	0.003
	Yes	127	105 (82.7)	22 (17.3)	
Tumour capsule invasion	No	207	163 (78.7)	44 (21.3)	0.002
	Yes	49	28 (57.1)	21 (42.9%)	
Perineural invasion	No	250	187 (74.8)	63 (25.2)	0.651
	Yes	6	4 (66.7)	2 (33.3)	
Lymphovascular invasion	No	223	172 (77.1)	51 (22.9)	0.016
	Yes	33	19 (57.6)	14 (42.4)	
Lymph metastasis	No	212	168 (79.2)	44 (20.8)	<0.001
	Yes	44	23 (52.3)	21 (47.7)	
Radio iodine-131 treatment(+)	No	103	94 (91.3)	9 (8.7)	<0.001
	Yes	153	97 (63.4)	56 (36.6)	
Distant metastasis	No	254	190 (74.8)	64 (25.2)	0.422
	Yes	2	1 (50)	1 (50)	
Lymph node metastasis after long term (> 12 months) follow-up	No	250	191 (99)	61 (93.8)	0.01
	Yes	6	2 (1)	4 (6.2)	

Table 3. Multivariate analysis of the association between clinicopathological factors and BRAF V600E mutation

Clinicopathological features	Odds ratio	95 % confidence interval		p
		Lower	Upper	
Tumour size (>1 cm)	0.552	0.247	1.232	0.147
Pathologically lymphocytic thyroiditis (-)	0.388	0.199	0.759	0.006
Extrathyroidal extension (+)	3.698	1.641	8.331	0.002
Tumour capsule invasion (+)	2.312	1.101	4.855	0.027
Lymphovascular invasion (+)	0.710	0.246	2.049	0.527
Lymph metastasis (+)	1.788	0.696	4.597	0.228
Radio iodine-131 treatment (+)	5.403	1.959	14.898	0.001

DISCUSSION

Papillary thyroid cancer is the most common endocrine cancer that develops from thyroid follicular epithelial cells. Advanced age, male sex, some histologic subtypes and tumour size greater than 4 cm negatively affect the prognosis (4). Among recent studies investigating the effects of the B-type Raf kinase (BRAF) mutation on PTC progression and prognosis, some reported BRAF V600E mutation to be related to aggressive prognostic outcomes (6,7), while some revealed no negative results (8-10). Mutations in BRAF (29-83%), RET/PTC (10-50%) and RAS (1-10%) that play a role in the pathway of MAPK are thought to cause PTC due to aberrant activation (4-10). Studies have shown that molecular damage in the thyroid cancer genome mostly occurs through BRAF mutation (5). The serine-threonine kinase RAF has three isoforms known as ARAF, BRAF, and CRAF. BRAF, the most common isoform in thyroid follicle epithelium, is located on the 7th chromosome and strongly stimulates MAPK pathway (5). The most common form of BRAF mutation is valine-glutamate substitution at Exon 15 with a resultant Timin → Adenosine transformation at position 1799 → residue 600. This MAPK pathway mutation leads to proliferation in thyroid cells (5). It is claimed that in patients with BRAF mutation, the clinical course of PTC is more aggressive, and they are more prone to invasion. BRAF mutation leads to more aggressive features in patients with PTC (11-13). In our study, BRAF positivity was detected in 25.4%. In a meta-analysis, Tufano et al. (14) observed a 1.32-fold increase in the risk of lymph node metastasis in BRAF (+) patients in 11 of 13 studies, while 2 of 13 studies demonstrated a reduced risk. It has been suggested that this difference among the studies might be due to different treatment protocols used in different institutions, since lymph node metastasis is highly detectable when prophylactic lymph node dissection is performed. In our study, in patients with central lymph node dissection, lymph node metastasis was more frequent in BRAF (+) group. In their study, Yip et al. (15) reported 56 patients with ETE among 106 BRAF (+) patients, while 15 of 100 patients with BRAF (-) disease had ETE. It was emphasized that patients with BRAF positivity had 3.52 times more ETE. In our study, we found a statistically significantly higher ETE rate in BRAF (+) cases. In a study by Fugazzola et al. (16), BRAF V600E was reported to be associated with papillary growth patterns but was not associated with poorly differentiated PTC variants.

A significant association of BRAF mutation was found when the patients were of older age. In addition, they reported that there was no correlation between worse outcomes after 72 months of median follow-up and BRAF positivity. In our study, no significant relationship was found between age and BRAF V600E mutation status. In the study by Kim et al. (17), BRAF V600E mutation was positive in 381 (69.7%) of 547 patients with PTC. BRAFV600E mutation was significantly associated with age (≥45 years), tumour size (>1 cm), extrathyroidal extension and cervical lymph node metastasis. Kim et al. (17) reported that BRAFV600E mutation was significantly associated with male gender, tumour size and extrathyroidal extension. Our study revealed a positive statistical relationship between the absence of pathologically detected lymphocytic thyroiditis, the presence of extrathyroidal extension and capsular invasion, RAI-131 treatment and the presence of BRAF V600E mutation. In a study conducted by Kim WW et al. (18), BRAF mutations were positive in 75.6% with PTC, chronic lymphocytic thyroiditis was present in 27.9% patients. In the same study, BRAF V600E mutation incidence was reported to be significantly higher in the group without chronic lymphocytic thyroiditis suggesting that BRAF V600E mutation is less common in chronic lymphocytic thyroiditis and the development of thyroid cancer is independent of mutation. In our study, BRAF V600E mutation was observed more frequently in patients without pathologic lymphocytic thyroiditis. Chronic lymphocytic thyroiditis and BRAF V600E mutations probably have independent mechanisms for the formation of thyroid cancer. We think that there is a need for more studies to investigate this relationship.

In conclusion, our results showed that BRAF V600E mutation was an important prognostic factor for lymph node metastasis that may develop during long-term follow-up of patients

without lymph node metastasis. BRAF (V600E) mutation has shown to be a poor prognostic factor, independent of other clinical pathologic features in patients with PTC. BRAF (V600E) mutations may be useful for differentiating less or more aggressive treatment requirements.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare

REFERENCES

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016; 26:1-133.
2. Sak SD. Variants of papillary thyroid carcinoma: multiple faces of a familiar tumor. Turk Patoloji Derg 2015; 31:34-47.
3. Pusztaszeri M, Auger M. Update on the cytologic features of papillary thyroid carcinoma variants. Diagn Cytopathol 2017; 45:714-30.
4. Pontius LN, Oyekunle TO, Thomas SM, Stang MT, Scheri RP, Roman SA, Sosa JA. Projecting survival in papillary thyroid cancer: a comparison of the Seventh and Eighth Editions of the American Joint Commission on Cancer/Union for International Cancer Control Staging Systems in two contemporary national patient cohorts. Thyroid 2017; 27:1408-16.
5. Li DD, Zhang YF, Xu HX, Zhang XP. The role of BRAF in the pathogenesis of thyroid carcinoma. Front Biosci (Landmark Ed) 2015; 20:1068-78.
6. Czarniecka A, Oczko-Wojciechowska M, Barczyński M. BRAF V600E mutation in prognostication of papillary thyroid cancer (PTC) recurrence. Gland Surg 2016; 5:495-505.
7. Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, Yip L, Mian C, Vianello F, Tuttle RM, Robenshtok E, Fagin JA, Puxeddu E, Fugazzola L, Czarniecka A, Jarzab B, O'Neill CJ, Sywak MS, Lam AK, Riesco-Eizaguirre G, Santisteban P, Nakayama H, Tufano RP, Pai SI, Zeiger MA, Westra WH, Clark DP, Clifton-Bligh R, Sidransky D, Ladenson PW, Sykorova V. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA 2013; 309:1493-501.
8. Sancisi V, Nicoli D, Ragazzi M, Piana S, Ciarrocchi A. BRAFV600E mutation does not mean distant metastasis in thyroid papillary carcinomas. J Clin Endocrinol Metab 2012; 97:E1745-9.
9. Niederer-Wüst SM, Jochum W, Förbs D, Brändle M, Bilz S, Clerici T, Oettli R, Müller J, Haile SR, Ess S, Stoeckli SJ, Broglie MA. Impact of clinical risk scores and BRAF V600E mutation status on outcome in papillary thyroid cancer. Surgery 2015; 157:119-25
10. Ito Y, Yoshida H, Kihara M, Kobayashi K, Miya A, Miyauchi A. BRAF (V600E) mutation analysis in papillary thyroid carcinoma: is it useful for all patients? World J Surg 2014; 38:679–87.
11. Elisei R, Viola D, Torregrossa L. The BRAF (V600E) mutation is an independent, poor prognostic factor for the outcome of patients with low-risk intrathyroid papillary thyroid carcinoma: single-institution results from a large cohort study. J Clin Endocrinol Metab 2012; 97:4390–8.
12. Tang KT, Lee CH. BRAF mutation in papillary thyroid carcinoma: pathogenic role and clinical implications. J Chin Med Assoc 2010; 73:113-28.
13. Huang FJ, Fang WY, Ye L, Zhang XF, Shen LY, Han RL, Wei Q, Fei XC, Chen X, Wang WQ, Wang S, Ning G. BRAF mutation correlates with recurrent papillary thyroid carcinoma in Chinese patients. Curr Oncol 2014; 21:e740–7.
14. Tufano RP, Teixeira GV, Bishop J, Carson KA, Xing M. BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. Medicine (Baltimore) 2012; 91:274-86.
15. Yip L, Nikiforova MN, Carty SE, Yim JH, Stang MT, Tublin MJ, Lebeau SO, Hodak SP, Ogilvie JB, Nikiforov YE. Optimizing surgical treatment of papillary thyroid carcinoma associated with BRAF mutation. Surgery 2009; 146:1215-23.
16. Fugazzola L, Puxeddu E, Avenia N, Romei C, Cirello V, Cavaliere A, Faviana P, Mannavola D, Moretti S, Rossi S, Sculli M, Bottici V, Beck-Peccoz P, Pacini F, Pinchera A, Santeusanio F, Elisei R. Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: data from a multicentric Italian study and review of the literature. Endocr Relat Cancer 2006; 13:455-64.
17. Kim SJ, Lee KE, Myong JP, Park JH, Jeon YK, Min HS, Park SY, Jung KC, Koo do H, Youn YK. BRAF V600E mutation is associated with tumor aggressiveness in papillary thyroid cancer. World J Surg 2012; 36:310-7.
18. Kim WW, Ha TK, Bae SK. Clinical implications of the BRAF mutation in papillary thyroid carcinoma and chronic lymphocytic thyroiditis. J Otolaryngol Head Neck Surg 2018; 47:4.