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BRAF V600E expression found in aggressive papillary thyroid carcinoma (PTC), lymph node metastasis, and extra-thyroid extension

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ABSTRACT

Purpose: Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy. Most PTC patients have a good prognosis. However, about 10% cases showed aggressive behavior. Its association with BRAF V600E mutation is still controversy. The purpose of this study is to prove the association between BRAF V600E expression and PTC aggressiveness.

Material and methods: This cross-sectional analytic study involving 36 PTC patients. Tumor aggressiveness was determined based on at least one of these categories: tumor size, lymph node metastasis, extra-thyroid extension and vascular invasion. Immunohistochemistry staining VE1 was performed to assess BRAF V600E expression. Chi-square test was used to assess the association between BRAF V600E expression and tumor aggressiveness.

Results: BRAF V600E expression was found in 58.3% of cases of aggressive PTC group. There was a significant difference between BRAF V600E expression in aggressive and non-aggressive PTC group ($p = 0.021$). There was a significant association between BRAF V600E expression and lymph node metastasis ($p = 0.005$) and extra-thyroid extension ($p = 0.011$), but no significant association between BRAF V600E expression and tumor size ($p = 0.252$) and vascular invasion ($p = 0.367$).

Conclusion: BRAF V600E expression has an association with tumor aggressiveness in PTC, as well as with lymph node metastasis and extra-thyroid extension.

Keywords: BRAF, V600E, aggressiveness, papillary thyroid carcinoma.

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INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common thyroid cancer and tends to have indolent biological behavior and with good prognosis.[1] However, about 5-10% of cases recurred.[2] Some clinicopathological features are considered as a poor risk factor: large tumor size, extra-thyroid extension, lymph node and distant metastasis and higher stage.[3] Aggressive clinicopathological features were related to some genetic changes in PTC, the most frequently observed is BRAF mutation that found in 29-83% of cases.[4] BRAF mutation induces Mitogen-Activated Protein Kinase (MAPK) pathway that plays an important role in tumorigenesis.[5] About 90% BRAF mutation in thyroid carcinoma is thymine to adenine transversion that results in valine (V) to glutamic acid (E) substitution in amino acid 600 (V600E).

Various systems have been established to assess thyroid carcinoma prognosis. American Thyroid Association (ATA) risk stratification system uses tumor size, nodal metastasis, extra-thyroid extension, vascular invasion and BRAF V600E mutation as criteria of prognosis in differentiated thyroid carcinoma.[6] Previous study about the association between BRAF V600E mutation with some aggressive clinicopathological parameters in PTC showed inconsistent results. The aim of this study was to determine the association between BRAF V600E expression and tumor aggressiveness in PTC.

MATERIAL AND METHODS

This study was a cross sectional analytic study of 36 PTC patients at Sanglah Hospital from 1 January until 31 December 2017. Diagnosis of PTC was from microscopic evaluation based on a set of distinctive nuclear features: (1) changes in size and shape, (2) irregularities of the membrane, and (3) chromatin characteristics.[1] All PTC variants based on the WHO classification 2017 were included.

Tumor aggressiveness evaluation

Tumor aggressiveness of PTC determined by at least fulfill one of following categories: tumor size, lymph node metastasis, extra-thyroid extension and vascular invasion. The evaluation was from clinical, macroscopic, and/or microscopic data through histopathologic examination using Hematoxylin-Eosin staining. Tumor size >4 cm was considered aggressive tumor.[1, 7] Lymph node metastasis was determined based on clinical, radiological, and/or microscopic data. Metastasis was considered positive if malignant cells were present in at least one

Table 1 BRAF V600E expression and tumor aggressiveness in PTC

Characteristics	BRAF V600E	
	Positive (n = 21; 58.3%)	Negative (n = 15; 41.7%)
Tumor size		
≤ 4 cm	27	75.0
> 4 cm	9	25.0
Lymph node metastasis		
Positive	9	25.0
Negative	27	75.0
Extra-thyroid extension		
Positive	8	22.2
Negative	28	77.8
Vascular invasion		
Positive	6	16.7
Negative	30	83.3

Table 2 Association between BRAF V600E expression and tumor aggressiveness of PTC

		Aggressiveness of PTC				
		Aggressive n (%)	Non Aggressive n (%)	OR	95% CI	p
BRAF V600E Expression	**Positive**	12 (57.1)	9 (42.9)	8.67	1.55 to 48.47	0.021
	Negative	2 (13.3)	13 (86.7)			
Total		14 (38.9)	22 (61.1)			

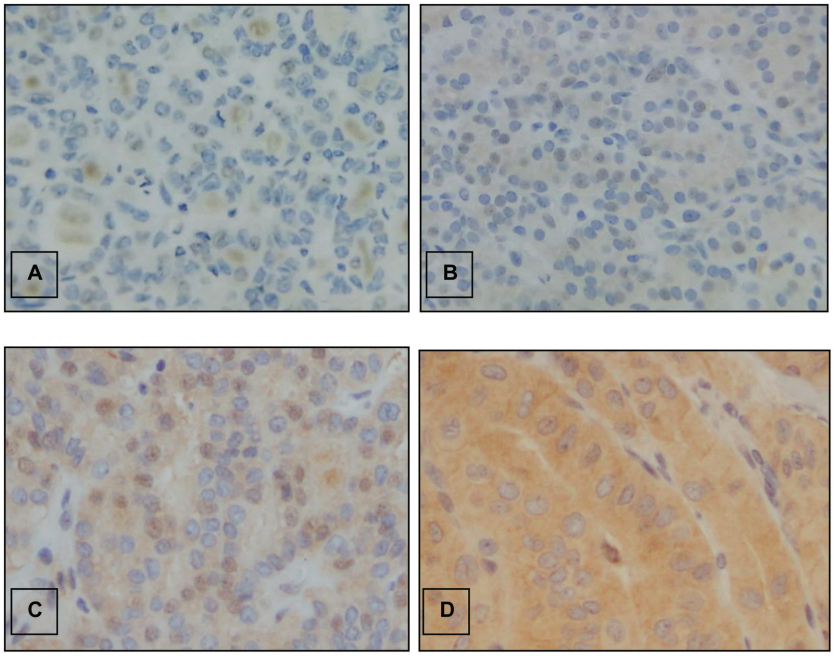

Figure 1 BRAF V600E expression was evaluated by measuring staining intensity and percentage of tumor cells. (A) Absent (0), (B) weak (+1) intensity, (C) moderate (+2) intensity, and (D) strong (+3) intensity

regional lymph node.[6] The extra-thyroid extension was tumor extends beyond the thyroid capsule and invades any of the following: muscle, subcutaneous soft tissue, larynx, trachea, esophagus, recurrent laryngeal nerve, prevertebral fascia, mediastinal vessel, or encase carotid artery.[1] Vascular invasion was tumor cell invasion into the blood vessel within or beyond tumor capsule, or within organ capsule in the un-encapsulated tumor. Intravascular tumor cells should be attached to blood vessel walls, covered endothelial cells or in thrombus / fibrin.[1, 6]

BRAF immunostaining and interpretation

The selected paraffin blocks were sliced into 4 mm thickness for immunostaining. Staining using Ventana BenchMark XT immunostainer with primary antibody mouse anti-human BRAF V600E (VE1, Spring Bioscience, USA) was performed.

Tumor cell with BRAF V600E expression showed cytoplasmic staining. BRAF V600E expression was determined using a semi-quantitative method by calculating the percentage of positive tumor cells and its intensity. The intensity was categorized into strong (+3), moderate (+2), weak (+1), and absent (0).[8] Strong intensity if the immune-reactivity

of tumor cell was stronger or the same as colloid staining, diffuse and clearly seen at 40 times magnification. Moderate intensity if tumor cell showed clear cytoplasmic staining but weaker than colloid staining and easily seen at 100 times magnification. Weak intensity if tumor cell showed vaguely staining or difficult to recognize. Absent if all of the tumor cells were not stained.[8, 9] Percentage of tumor cells was assessed in the range of 0% to 100%. BRAF V600E expression was positive if > 10% of tumor cells showed positive staining with moderate or strong intensity.[9]

Statistical analysis

The association between BRAF V600E expression and tumor aggressiveness was determined by Chi-square test with $p < 0.05$ significance, using SPSS 20.0 software. Association between BRAF V600E and each clinicopathological characteristic was tested with Fisher exact test.

RESULTS

The mean age of patients was a 50.03 ± 12.81 year (range 23-75 year). Women were more frequent than men [11 (30.6%) were men, and 25 (69.4%) were women]. Six PTC variants were identified: 16 (44.4%) cases classic variant, 9 (25.0%) cases follicular variant, 6 (16.7%) cases papillary microcarcinoma variant, 2 (5.6%) cases oncocytic variant, 1 (2.8%) case solid/trabecular variant, and 2 (5.6%) cases tall cell variant. Multifocal PTC was found in 14 (38.9%) cases. Patient distribution based on aggressive clinicopathological parameters was described in table 1.

From 36 patients, 38.9% cases were aggressive, and 61.1% of cases were non-aggressive. BRAF V600E immunostaining showed 21 (58.3%) cases were positive and 15 (41.7%) cases were negative. Chi-square analysis showed a significant difference between BRAF V600E expression in an aggressive and non-aggressive group of PTC ($p = 0.021$). (Table 2) Fisher Exact test showed no significant difference between BRAF V600E expression in tumor size >4 cm and tumor size ≤ 4 cm ($p = 0.252$), significant difference of BRAF V600E expression in PTC with and without lymph nodes metastasis ($p = 0.005$), significant difference of BRAF V600E expression in PTC with and without extra-thyroid extension ($p = 0.011$), no significant difference in BRAF V600E expression in PTC with and without vascular invasion ($p = 0.367$). Patient with positive BRAF V600E expression had 8.67 times higher risk of becoming an aggressive PTC compared with a patient with negative BRAF V600E expression.

DISCUSSION

BRAF V600E mutation is the most common RAF mutation that found in PTC. This mutation has also been widely studied in malignant melanoma, colon, and ovarian cancer. This mutation cause transverse of Thymine into Adenine in exon 15 in nucleotide 1799 (T1799A), which resulted in the substitution of valine to glutamic acid at 600 positions.[10] BRAF V600E mutation is associated with aggressive clinicopathological features.[11] The standard method for BRAF mutation identification is DNA-based molecular examination, but it is expensive and time-consuming.[8] Currently, immunohistochemistry V600E (VE1) antibody to identify BRAF V600E mutation has been developed. This method has good accuracy (sensitivity and specificity of 98% to 100%), with shorter time and lower cost.[12, 13]

The prevalence of BRAF V600E mutation on PTC showed varying numbers in different countries. A meta-analysis study by Li et al. (2012) in the United States revealed a prevalence rate of 50.9%.[2] Some studies in China reported a prevalence rate of 30-80%, while in Japan and North Korea the prevalence rate was 60-90%.[14] Papillary thyroid carcinoma mostly occurs at the age of 31-49 years, with a male to female ratio of 1 : 4.[1] More than half of the patients in this study showed BRAF V600E mutation, with mean age was 50.03 year, and PTC with aggressive behavior showed a slightly higher age. Female was twice than men. The results of this study were similar with the study of Lim et al. (2017) in the United States that showed the dominant patients were female (75%) with a mean age 48 years.[15] The study by Abd Elmageed et al. (2016) in the United States in 130 patients with PTC, the mean age of the patients was 49.3 year, and 73% of the cases were female.[12]

Different aggressiveness parameters have been reported in various studies of PTC. Currently, the BRAF V600E mutation has been known to be used as an additional prognostic factor in PTC. ATA Modified Initial Risk Stratification System 2015 guideline recommendation was used as the basis for this study. The guidelines described the importance of the correlation between BRAF V600E mutation and other prognostic factors, i.e., tumor size, lymph nodes metastasis, extra-thyroid extension, and vascular invasion.[6, 16] Additional of BRAF V600E mutation status can improve the accuracy of prediction related to recurrence risk. This study used at least one of these four categories to determine the aggressiveness of PTC.

Positive BRAF V600E expression was found more frequent in aggressive PTC group. This study

showed that patient with positive BRAF V600E expression had 8.67 times higher risk of becoming an aggressive PTC compared with a patient with negative BRAF V600E expression. Aggressive PTC will affect the prognosis of the patient with decreased survival rate. It has been reported that in aggressive PTC, especially in stage IV, survival rate decline to 41%. Aggressive PTC primarily with lymph nodes metastasis and extra-thyroid extension were also reported to be associated with an increased risk of recurrence. Overall recurrence rates were reported at 15-35%.[1] The addition of BRAF V600E mutation in correlation with the four categories aggressiveness of PTC will increase the prediction value of recurrence. Tumor recurrence will require advanced therapy, such as re-surgery and additional radioiodine therapy that affect the quality of life of the patient. The high recurrence rate in aggressive PTC patients with BRAF V600E mutation has been reported in several studies. A meta-analysis study by Chen et al. and Liu et al. (2016) showed PTC patients with BRAF V600E mutation had an increased risk of recurrence.[17]

This study found that there was a tendency of BRAF V600E expression in PTC with tumor size > 4 cm, although statistically not significant. Previous studies showed inconsistent results.[18,19,20,22] It seems that tumor size is not an independent factor in the prognosis of PTC.

All patients with lymph nodes metastasis in this study showed BRAF V600E expression. Similar results also reported by Na et al. (2015) in South Korea where there was a significant association between the BRAF V600E mutation and lymph nodes metastasis.[8] Lymph nodes metastasis is an important risk factor for recurrence and overall survival. The criteria used by ATA Modified Initial Risk Stratification System 2015 distinguish the risk of recurrence of lymph node metastasis by the size of the node, number of positive lymph node, and extra-nodal extension.

This study showed that all patient with extra-thyroid extension had positive BRAF V600E expression. This was supported by Fraser et al. (2016) that found a significant association between BRAF V600E mutations and extra-thyroid extension.[19] Meta-analysis study by Chen et al. (2016) reported that PTC with extra-thyroid extension had higher rate BRAF V600E mutation.[17] The extra-thyroid extension is an aggressive feature of PTC that associated with an increased risk of recurrence. In such cases, radical surgical treatment is required.

Although there was no significant association between BRAF mutation and vascular invasion, there was a tendency of BRAF V600E expression on PTC with vascular invasion. Several studies showed inconsistent results.[2,8,20,22,23] Vascular invasion is likely not an independent risk factor for aggressive PTC.

CONCLUSION

There is an association between BRAF V600E expression and the aggressiveness of PTC, as well as lymph nodes metastasis and extra-thyroid extension. Molecular testing of BRAF mutation in thyroid carcinoma management should be done in conjunction with classical clinicopathological evaluation. A longitudinal study is needed to determine the role of BRAF V600E expression in PTC prognosis.

DISCLOSURE

The authors report no conflicts of interest in this work.

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