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Original article

Relationship between the BRAF V600E and tumor size, lymph node, and distant metastasis in papillary thyroid carcinoma

Edmond Rukmana Wikanta [1], Yan Wisnu Prajoko [1], Benny Issakh [1], Hermawan Istiadi [2], Dik Puspasari [2]

[1] University of Diponegoro, Semarang, Indonesia
[2] Dr. Kariadi Centra l Hospital, Semarang, Indonesia

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Abstract: *Aim* – This study aimed to analyze the association between B-Raf proto-oncogene, serine/threonine kinase (BRAF) V600E and tumor size, lymph node, and distant metastasis (TNM) status on patients with papillary thyroid carcinoma.
Material and Methods – This research is a cross-sectional study. A total of 40 samples were papillary thyroid carcinoma tissue paraffin blocks from thyroidectomy surgery at State General Hospital (RSUP) Dr. Kariadi Semarang. Assessment of BRAF V600E mutation protein expression through immunohistochemical methods using antibody BRAF V600E was conducted.
Results – The mean age of patients with papillary thyroid carcinoma with a positive BRAF V600E mutation was 49 years. The highest incidence was 71.4% in women. A significant difference between BRAF V600E and tumor size (p=0.03) was observed with as many as 53.2% more cases at size > 4 cm. A significant difference in the expression of BRAF V600E with enlargement of the lymph node (p=0.011) by 60.7% was observed compared to those without enlarged lymph nodes. On the other hand, no significant relationship between BRAF V600E expression and distant metastasis (p=0.652) was observed. The prevalence ratio in the positive BRAF V600E case at tumor size is more than 4 cm, and enlargement of lymph is 9.533 and 7.727.
Conclusion – There is a significant relationship between BRAF V600E expression and tumor size and enlarged lymph nodes, while there is no significant relationship between BRAF V600E expression and distant metastases.

Keywords: BRAF V600E, papillary thyroid carcinoma, tumor size, lymph node, distant metastasis.

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Correspondence to Edmond Rukmana Wikanta. Address: Departement of Surgical Oncologist, Faculty of Medicine, Universitas Diponegoro, Jl. Prof. Soedarto, SH Tembalang, Semarang, Indonesia. Phone: +62247460024, ext 101 or 128. Fax: +62247460027. Email: edmsurg@fk.undip.ac.id.

Introduction

Thyroid carcinoma is a malignancy in the thyroid tissue, of which 9% is derived from follicular epithelial cells. This tumor mostly occurs at the age of 31-49 years, with a 1:4 men to women ratio [1]. In 2010, the American Cancer Society discovered 44,670 new cases of malignancy in the form of thyroid carcinoma. Thyroid carcinoma ranks ninth out of the ten most types of cancer, with a 4.443% prevalence rate in Indonesia [2]. Various risk factors are suspected as causes of papillary thyroid carcinoma, including radiation exposure, obesity, diabetes, smoking habits, alcohol consumption, foods containing nitrate, high iodine intake, and genetic and reproductive factors. However, until now, the relationship between these factors and papillary thyroid carcinoma is not fully understood.

The data found that as much as 80-85% of all thyroid malignancies are papillary thyroid carcinoma. The survival rates for papillary thyroid carcinoma in 5, 10, and 20 years are 96%, 93%, and 90%, respectively, with a high recurrence of 20% [3]. Moreover, as many as 10-15% of papillary thyroid carcinoma was found to have distant metastases [4]. Factors that can affect the recurrence and can worsen a patient's quality of life are estimated to be related to tumor size (T), enlarged lymph nodes (N), and distant metastases (M) [5].

Following the development of genetic science, cell growth that occurs in thyroid cancer is known through several mechanisms, including B-Raf proto-oncogene, serine/threonine kinase (BRAF) mutations (40-45%), Rat Sarcoma (RAS) (10-20%), and rearranged in transformation/papillary thyroid carcinomas (RET/PTC) rearrangement (10-20%). About 90% of BRAF mutations are thymine to adenine transversions at exon 15 in nucleotide 1799 (T1799A), resulting in the replacement of valine to glutamic acid at a position of 600 (BRAF V600E). The next process is the excessive activation of BRAF [6]. BRAF V600E mutations induce mitogen-activated protein kinase (MAPK) pathways, playing a role in tumorigenesis. Activated BRAF mutations play an important role in cancer, especially papillary thyroid carcinoma, showing a high prevalence of mutations [7]. BRAF V600E mutations in papillary thyroid carcinoma have been widely reported in various western countries (America and Europe) with a 40-45% prevalence rate. Some research in China found a prevalence rate of 30-80% and a 60–90% prevalence rate in Japan and North Korea [8].

Research in mice with papillary thyroid carcinoma revealed a significant role for BRAF mutations with aggressive features of

papillary thyroid carcinoma that could be used in predicting the bad prognosis. It is said that the staging system could help provide a prediction of mortality risk in patients with thyroid carcinoma [6]. An American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) research showed that the tumor, node, and metastasis (TNM) staging system and the distant metastasis, patient age, completeness of resection, local invasion, and tumor size (MACIS) system provide the highest proportion when used in research or clinics [8]. However, the staging system can only predict the mortality of thyroid carcinoma in a small proportion of 5-30%. Besides, the staging system is unable to predict the risk of recurrence of thyroid carcinoma [9].

BRAF V600E in another research showed an association with poor clinicopathology in cases of papillary thyroid carcinoma. Another study on BRAF V600E showed that the mutation does not affect the incidence of papillary thyroid carcinoma. BRAF mutations are also mentioned not to be a risk factor for the aggressiveness of papillary thyroid carcinoma [10].

Table 1. Characteristic of patient with papillary thyroid carcinoma

Variable	*F*	*%*
Gender		
Men	5	12.5
Women	35	87.5
Age		
<55 th	27	67.5
≥55 th	13	32.5
Location		
Unilateral	28	70.0
Bilateral	12	30.0
BMI		
Underweight	6	15.0
Normal	17	42.5
Overweight	12	30.0
Obese	5	12.5
Location of sample residences		
Semarang regency	4	10.0
Semarang town	9	22.5
Grobogan	1	2.5
Magelang	1	2.5
Demak	1	2.5
Jepara	9	22.5
Pati	9	22.5
Rembang	1	2.5
Tegal	4	10.0
Outside Java	1	2.5
Size		
≤ 4 cm	26	65.0
> 4 cm	14	35.0
Metastasized lymph nodes (LN)		
N0	21	52.5
N1	19	47.5
Metastasis		
M0	33	82.5
M1	7	17.5
Stage		
I	22	55.0
II	13	32.5
III	0	0.0
IVA	0	0.0
IVB	5	12.5
BRAF		
Negative	12	30.0
Positive	28	70.0

There was inconsistency from published research concerning the association of BRAF V600E mutation. This research can be a new perspective that can change the need to detect the BRAF V600E mutation which will later change the algorithm in the management of papillary thyroid carcinoma. This research aims to analyze the relationship between BRAF V600E expression and tumor size, lymph nodes, and distant metastases in papillary thyroid carcinoma.

Material and Methods

This research is a cross-sectional study conducted from April 2019 to June 2019. As many as 74 medical records data of papillary thyroid carcinoma patients from July 2018 to December 2018 were collected from the State General Hospital (RSUP) Medical Record Unit, Dr. Kariadi Hospital. Histopathological diagnosis and paraffin block data collection were continued in the anatomic pathology laboratory. Immunohistochemistry was carried out at the Anatomical Pathology Laboratory of Diponegoro University, RSUP Dr. Kariadi Semarang. The study sample consisted of 40 paraffin blocks of thyroid tissue collected from patients who showed histopathological features of papillary thyroid carcinoma. Sampling is done through a consecutive non-probability sampling technique. All of the method in this study were approved by Health Research Ethics Committee of RSUP Dr. Kariadi, Semarang, Indonesia with certificate no. 172/EC/KEPK-RSDK/2019.

BARF V600E Expression

The assessment of BRAF V600E mutation protein expression through an immunohistochemical method using antibody anti-BRAF V600E was conducted. The assessment used a semiquantitative system by calculating the positive staining intensity and percentage of tumor cells. Staining intensity assessments of strong, moderate, weak, and absent are recorded in numbers 3+, 2+, 1+, and 0, respectively [7]. Strong staining intensity is scored 3+ if the immunoreactivity of tumor cells is stronger or equal to the surrounding follicular colloid, or diffuse staining, and is seen at 40× magnification. Moderate staining intensity is scored 2+ if viable tumor cells showed clear cytoplasmic smears but their intensity is weaker than the surrounding follicular colloids, easily seen at 100× magnification. Weak staining intensity is scored 1+ if tumor cells showed vague or difficult staining recognition. Absent staining intensity is scored 0 if all tumor cells are not stained [7, 8]. The percentage of tumor cells is assessed in the range of 0-100%. The BRAF V600E expression is positive if > 10% of tumor cells showed positive moderate (2+) or strong (3+) positive staining [8]. The interpretation was carried out by the researcher and two anatomical pathology experts in a blind independent manner without knowing the patient's clinicopathology data, including the patient's name, object number, and BRAF V600E expression.

Tumor Size

The tumor size category is evaluated by measuring macroscopically the tumor tissue using the same iron ruler. The aggressive category limit for tumor size used is > 4 cm in the largest dimension [1, 9]. Tumor size and immune checkpoint inhibitor (CPI) evaluation in papillary thyroid carcinoma are determined from the largest tumor size [10].

Table 2. The proportion of BRAF V600E expressions in papillary thyroid carcinoma

Variable	*BRAF*				*p*
	Negative		Positive		
	N	%	n	%	
Gender					
Men	2	16.7	3	10.7	0.627
Women	10	83.3	25	89.3	
Age					
< 55 th	11	91.7	16	57.1	0.063
≥ 55 th	1	8.3	12	42.9	
Location					
Unilateral	10	83.3	18	64.3	0.285
Bilateral	2	16.7	10	35.7	
BMI					
Underweight	0	0	6	21.4	0.052
Normal	4	33.3	13	46,4	
Overweight	7	58.3	5	17.9	
Obese	1	8.3	4	14.3	
Size					
≤ 4 cm	1	8.3	13	46.4	0.030*
> 4 cm	11	91.7	15	53.2	
Metastasized lymph nodes (LN)					
N0	10	83.3	11	39.3	0.011*
N1	2	16.7	17	60.7	
Metastasis					
M0	11	91.7	22	78.6	0.652
M1	1	8.3	6	21.4	
Stage					
I	10	83.3	11	39.3	0.032*
II	2	16.7	12	42.9	
IVB	0	0	5	17.9	

* – significant.

Table 3. Relationship between BRAF V600E expression and tumor size

Size	*BRAF V600E mutation*		*OR*	*95% CI*	*P*
	Positive n (%)	Negative n (%)			
≤ 4cm	13 (46.4%)	1 (8.3%)	9.533	1.080-84.139	0.030
> 4cm	15 (53.2%)	11 (91.7%)			
Total	28 (100.0%)	12 (100.0%)			

Table 4. Relationship between BRAF V600E expression and lymph nodes

Enlarged lymph nodes	*BRAF V600E mutation*		*OR*	*95% CI*	*P*
	Positive n (%)	Negative n (%)			
N0	11 (39.3%)	10 (83.3%)2	7.727	1.416-42.175	0.011
N1	17 (60.7%)	(16.7%)			
Total	28 (100.0%)	12 (100.0%)			

Table 5. Relationship between BRAF V600E expression and distant metastases

Distant metastasis	*BRAF V600E mutation*		*OR*	*95% CI*	*P*
	Positive n (%)	Negative n (%)			
M0	22 (78.6%)	11 (91.7%)	3.000	0.320-28.104	0.652
M1	6 (21.4%)	1 (8.3%)			
Total	28 (100.0%)	12 (100.0%)			

Enlarged lymph nodes (LN)

This category is determined based on clinical and radiological data from medical records. It is positive if LN is found in at least one regional LN structure (metastases in LN level VI pretracheal, paratracheal, and prelaryngeal/Delphian, superior mediastinal LN, or unilateral, bilateral, or contralateral LN level I, II, III, IV, IV, or V or retropharyngeal LN) [1]. Patients with papillary thyroid carcinoma without any suspicion of metastasis to the LN from clinical, radiological data, and cytological findings or without surgical LN tissue are categorized as negative.

Distant metastasis

This category is determined based on clinical and radiological data on medical records. It is positive if found in the data.

Statistical analysis

Data is processed using the Statistical Package for the Social Sciences SPSS Program 20.0 for Windows. The relationship between BRAF V600E expression and tumor size, LN, and distant metastases was determined using Fisher's exact test. The significance value (α) is determined at the probability (p) < 0.05.

Results

Characteristics of Sample

The study sample consists of all patients with papillary thyroid carcinoma who were examined histopathologically using paraffin blocks in the Anatomical Pathology Laboratory of RSUP Dr. Kariadi Semarang/FK UNDIP, from July 1, 2018, to December 31, 2018. During that period, a total of 74 papillary thyroid carcinoma cases were obtained. A total of 58 samples met the inclusion and exclusion criteria. Based on the calculation of the sample size, 40 samples were obtained for this study. Furthermore, with consecutive sampling, 40 samples were obtained. Papillary thyroid carcinoma patients consisted of 5 men (12.5%) and 35 women (87.5%). This study obtained 35 people in stages I-II and 5 people in stage IVB (*Table* 1).

BRAF V600E Expression on Papillary Thyroid Carcinoma

Furthermore, the expression of BRAF V600E on papillary thyroid carcinoma was analyzed. As shown in *Table* 2, a positive BRAF V600E mutation was found in 25 women (71.4%) and in 3 men (10.7%). The average age of patients with papillary thyroid carcinoma with a positive BRAF V600E mutation was 49 years with a diversity of 15.2 years and an age range of 14 to 75 years.

Figure 1 showed the expression of BRAF V600E on papillary thyroid carcinoma using immunohistochemical staining. *Figure* 1A showed no outward appearance in the cytoplasm, whereas *Figure* 1B showed an outward appearance in the cytoplasm of carcinoma cells with weak intensity. Furthermore, *Figure* 1C showed cytoplasmic appearance of carcinoma cells with moderate intensity. *Figure* 1D showed cytoplasmic appearance of carcinoma cells with strong intensity.

Relationship between BRAF V600E expressions and tumor size

Table 3 showed that the proportion of positive BRAF V600E expression was lower in the ≤ 4 cm tumor group, which is 13 of 28 cases (46.4%), compared to tumors sized > 4 cm in 15 cases (53.2%). The results of data analysis using Fisher's exact test found a significant difference in BRAF V600E expression in tumor groups measuring > 4 cm and tumors sized ≤ 4 cm (p=0.030).

Relationship between BRAF V600E expression and lymph nodes

Moreover, the relationship between BRAF V600E expression and LN was determined. *Table* 4 showed that the proportion of

positive BRAF V600E expression was lower in the papillary thyroid carcinoma without lymph node nodules group in 11 of 28 cases (39.3%) compared to the group with enlargement of LN in 17 of 28 cases (60.7%). The proportion of negative BRAF V600E expression in the papillary thyroid carcinoma with enlargement of the LN group was presented only in two cases (16.7%). The results of data analysis showed a significant difference in the expression of BRAF V600E in the papillary thyroid carcinoma with enlarged LN group and without enlarged LN group (p=0.011).

Relationship between BRAF V600E expression and distant metastasis

A shown in *Table* 5, the proportion of positive BRAF V600E expression is higher in the papillary thyroid carcinoma without distant metastases group with 22 out of 28 cases (78.6%) compared to those with distant metastases group with 6 out of 28 cases (21.4%). The proportion of BRAF V600E expression was negative in the papillary thyroid carcinoma without distant metastases group in all 11 cases (91.7%) compared to those with distant metastases. The results of data analysis showed no significant difference in the expression of BRAF V600E in the group of papillary thyroid carcinoma with distant metastases and those without distant metastases (p=0.652).

The prevalence ratio of BRAF V600E to tumor size, lymph nodes, and distant metastases in papillary thyroid carcinoma

Tumor size more than 4 cm 9.533 (1.080-84.139) is stronger than under 4 cm in positive BRAF cases. Enlargement of LN 7.727 (1.416-42.175) is stronger than non-enlarged LN in positive BRAF cases. Distant metastases did not obtain a prevalence ratio due to a lack of significant association with positive BRAF V600E mutation expression.

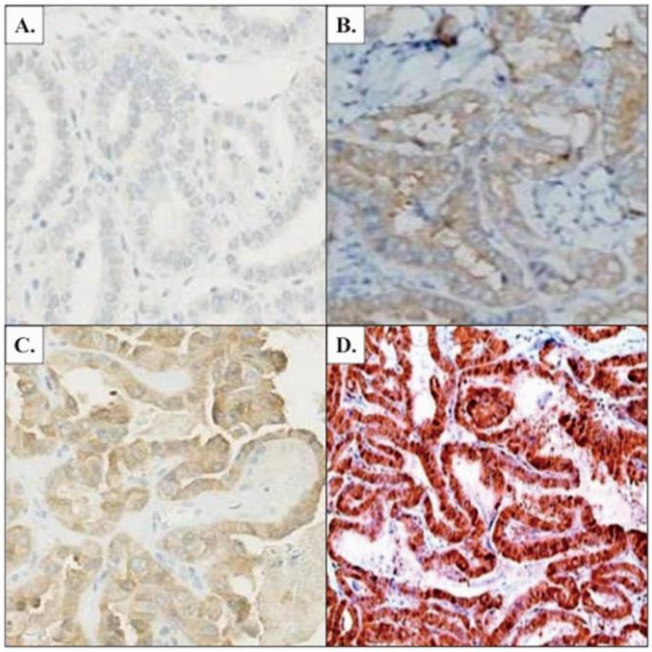

Figure 1. Expression of BRAF V600E. (A) Negative expression, (B) +1 expression, (C) +2 expression, (D) +3 expression.

Discussion

BRAF V600E expression in papillary thyroid carcinoma

The proportion of positive BRAF V600E expression was found more in women in as many as 25 people with a magnitude of 89.3% than in men, with 3 people with a magnitude of 10.7% (*Table* 2). The results of this study are similar to Lim's study in the United States, showing that the dominant patients were women (75%) with an average age of 48 years [11]. As many as 16 people with positive BRAF V600E had an age younger than 55 years (57.1%) than those older than 55 years (42.9%). Papillary thyroid carcinoma mostly occurs at the age of 31-49 years. Kim's study reported a significant relationship between BRAF V600E expression and the age of ≥ 45 years (p=0.02) [12]. A study by Abd Elmageed et al. [1] on 130 papillary thyroid carcinoma (PTC) patients showed an average age of 49.3±13.2 years, with 73% of cases being female. This is supported by several studies reporting similar results by Daliri (p<0.001) [13].

Relationship between BRAF V600E expression and tumor size

The results showed that the proportion of positive BRAF V600E expression was higher in the tumor > 4 cm group compared to tumor sized ≤ 4 cm group and that there was a significant relationship between BRAF V600E expression and tumor size (*Table* 3). This result is supported by Lin-Bo Yang's study whose study revealed a positive relationship between BRAF V600E mutations and tumor size of papillary thyroid cancer [14]. A similar study was reported by Fernandez et al. [15] with a value of p=0.00615. Research by Xing showed a significant relationship with tumor size (p=0.003) [16]. Henke et al. [17] reported a significant relationship with multivariate analysis (p<0.001). The BRAF V600E gene mutation activates the MAPK pathway, resulting in the intensity of cell proliferation, inhibition of differentiation, and apoptosis. In other words, this mutation causes a loss of control over the cellular cycle, leading to the onset of malignant development [6].

Several studies reported different results. In China, research by Sun et al. involving 556 cases reported no significant relationship between BRAF V600E mutation and tumor size (p=0.161) [7]. In Australia, Fraser et al. reported no significant differences in tumor size with positive and negative BRAF V600E in cases of papillary thyroid carcinoma (p=0.23) [18]. The differences in the results of the research can be caused by factors other than the size of the tumor that is also influential, such as metastases to LN resulting in enlargement, as well as the existence of extrathyroidal extension that occurs in cases of papillary thyroid carcinoma. Other factors that can cause differences are different sample sizes, staining techniques, different methods of interpretation of BRAF V600E expressions, and nonuniformity in tumor size measurement techniques, as this study used retrospective data.

Relationship between BRAF V600E Expression and Lymph Nodule Enlargement

The results of data analysis in *Table* 4 showed that there was a significant relationship between BRAF V600E expression and the occurrence of lymph node nodules (p=0.011). Feng's research in China reported that the expression of BRAF V600E was found to be significantly higher in the group with lymph node nodules (p=0.019) [19]. Similar results were reported in a South Korean study where a significant relationship was observed between BRAF

V600E mutations and enlarged lymph node nodules (p=0.001) [7]. Research in Australia showed that lymph node involvement was higher in the group of PTC patients with positive BRAF V600E with a ratio of 44%:29.4% (p=0.0004) [12]. In North Korea, research conducted by Jung et al. [20] reported a significant relationship between BRAF V600E mutation and the enlargement of the LN (p=0.018). Chen's meta-analysis study in China also showed that papillary thyroid carcinoma patients with BRAF V600E mutations had an odds ratio for lymph node involvement of 1.59 (95% CI 1.40-1.81; p<0.0001) [21]. Various studies above support the high incidence of enlarged LN in patients with papillary thyroid carcinoma with BRAF V600E mutations, which is consistent with the literature where thyroid carcinoma tends to spread through the lymphatic vessels [1]. The presentation of lymph node nodules is an important factor for determining the recurrences and overall survival. Evaluation of the results of thyroid surgery with positive expression of the BRAF V600E mutation can be a prognostic factor to the appearance of lymph node nodules in thyroid carcinoma cases. The 2015 American Thyroid Association (ATA) Modified Initial Risk Stratification System includes BRAF mutation expression to assess the high and low risk of recurrence in papillary thyroid carcinoma.

The limitation of this association research is the data used were retrospective; thus, the involvement of lymph node enlargement criteria is only based on the presence or absence of enlarged nodules in regional lymph node structures. In addition, this study did not assess the number of nodules involved, size, and extranodal extension. The identification of lymph node enlargement is done during the physical examination, imaging, and macroscopic appearance of tissue, in which the identification of the level of lymph nodes is more ideal determined during surgery to minimize false-negative results.

Relationship between BRAF V600E Expressions and Distant Metastasis

The results of data analysis showed that there was no significant relationship between BRAF V600E expression and distant metastases (*Table* 5). Several other studies report the same results. The rate of BRAF mutations in nonmetastatic patients is 76.4% and 5% higher than in patients with distant metastases [5]. A meta-analysis study by Li in the United States reported no significant relationship between the BRAF V600E mutation and the vascular invasion that started the distant metastasis (odds ratio=1.23; 95% CI 0.76-2.01) [22]. Similar results also showed in South Korea (p>0.05) [7]. Other studies also reported no association between BRAF V600E mutation and distant metastases [12]. Research by Gandolfi showed that the BRAF V600E frequency was less than 30% in a large group of 47 PTCs with distant metastases, a lower percentage of those detected in the control group of PTCs without distant metastases (44%) compared with the average incidence reported in the literature for this mutation (45%) [23].

A different research by Xing et al. showed a significant relationship between BRAF V600E expression and distant metastases (p=0.045) [16]. Henke's study reported a significant relationship between BRAF V600E expression and distant metastases in multivariate analysis (p<0.001) [24]. A 2016 research reported a significant relationship between BRAF V600E and distant metastases [25]. The difference in the results of the study of the relationship of distant metastases with papillary thyroid carcinoma that contains the BRAF V600E mutation can be caused by earlier treatment received by the patient. Other studies revealed a correlation between BRAF V600E mutations and vascular invasion conditions, leading to the occurrence of distant metastases. Other factors that can cause differences in results are the sample size used, the staining technique, and the interpretation method of BRAF V600E expression that is different. There is a tendency to spread through the lymphatic vessels. Expansion through blood vessels can occur, most often affecting the lung [1]. Initially, no studies showed the relationship of mutations to the presence of distant metastases, that is, the relationship with the strongest factor in the prognosis of papillary thyroid carcinoma, most likely due to the rarity of metastases [21].

The prevalence ratio of BRAF V600E to tumor size, lymph nodes, and distant metastases in papillary thyroid carcinoma

A retrospective analysis that showed a positive BRAF in tumors increases the risk of cancer associated with cancer including papillary thyroid carcinoma. Positive BRAFs are found mostly in severe clinical findings. BRAF V600E mutations in primary lesions can predict PTC lymph node metastases, and their predictive value is higher than other clinical factors such as age, clinical stage, and tumor size [17].

Other important parameters that do not consist of molecular testing, represented by some specific morphological aspects, still play an important role, perhaps still more significant than molecular diagnostics. Mortality per 1000 person-years for adjoining distant metastatic disease and BRAF V600E was 87.72 (95% CI, 62.68-122.77), whereas the ones who were in distant metastases are positive, but BRAF V600E patients are negative with 32.28 (95% CI, 16.14-64.55) and 3.54 (95% CI, 1.96-6.39) in distant metastasis patients but negative BRAF V600E. As much as 20% of fine needle aspiration (FNA) obtained ambiguous or uncertain results, and surgical intervention is becoming the advanced step, although around 80% of this population does not have thyroid cancer on further histological analysis. PTC represents the majority of thyroid carcinoma (~ 80-90%).

Limitation

However, there are limitations in this study that the researchers obtained clinical data from medical records. Researchers did not directly examine the patient's condition.

Conclusion

There is BRAF V600E expression in papillary thyroid carcinoma. There is a significant relationship between BRAF V600E expression and tumor size in papillary thyroid carcinoma and between BRAF V600E expression and lymph node involvement in papillary thyroid carcinoma. On the other hand, there is no significant relationship between BRAF V600E expression and distant metastases in papillary thyroid carcinoma.

Suggestion

Immunohistochemical examination of BRAF V600E should be considered in providing treatment for papillary thyroid carcinoma. Further research of BRAF V600E as a prognostic marker is suggested in papillary thyroid carcinoma. Research in various centers is necessary, supporting meta-analysis of size, stage, and LN enlargement.

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Conflict of interest

The author(s) declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Abd Elmageed ZY, Sholl AB, Tsumagari K, Al-Qurayshi Z, Basolo F, Moroz K, et al. Immunohistochemistry as an accurate tool for evaluating BRAF-V600E mutation in 130 samples of papillary thyroid cancer. *Surgery* 2017; 161(4): 1122-1128. https://doi.org/10.1016/j.surg.2016.06.081.
2. Ellys, Kartika I, Aspitriani, Yuwono. Galectin-3 expression level as a marker of benign and malignant lesions of the thyroid. *Jurnal Kedokteran dan Kesehatan* 2015; 2(3): 311-317. Indonesian. https://media.neliti.com/media/publications/181704-ID-tingkat-ekspresi-galectin-3-sebagai-pena.pdf.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistic. *CA Cancer J Clin* 2011; 61(2): 69-90. https://doi.org/10.3322/caac.20107.
4. Rosai J, Delellis RA, Carcangiu ML, Frable WJ, Tallini G. AFIP Atlas Of Tumor Pathology: Tumors of the Thyroid and Parathyroid Glands. Silver Spring, Maryland: American Registry of Pathology. 2014; 606 p. https://www.worldcat.org/title/tumors-of-the-thyroid-and-parathyroid-glands/oclc/904183558.
5. Enewold L, Zhu K, Ron E, Marrogi AJ, Stojadinovic A, Peoples GE, et al. Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980-2005. *Cancer Epidemiol Biomarkers Prev* 2009; 18(3): 784-791. https://doi.org/10.1158/1055-9965.epi-08-0960.
6. Song YS, Lim JA, Park YJ. Mutation profile of well-differentiated thyroid cancer in Asians. *Endocrinol Metab (Seoul)* 2015; 30(3): 252-262. https://dx.doi.org/10.3803%2FEnM.2015.30.3.252.
7. Sun J, Zhang J, Lu J, Gao J, Lu T, Ren X, et al. Immunohistochemistry is highly sensitive and specific for detecting the BRAF V600E mutation in papillary thyroid carcinoma. *Int J Clin Exp Pathol* 2015; 8(11): 15072-15078. https://pubmed.ncbi.nlm.nih.gov/26823846.
8. Carcangiu ML. Thyroid. In: Mills SE, Eds. Histology for pathologists. 4th Ed. Philadelphia: Lippincott Williams & Wilkins. 2012; 1331 p. https://www.worldcat.org/title/histology-for-pathologists/oclc/810314019.
9. Czarniecka A, Oczko-Wojciechowska M, Barczyński M. *BRAF V600E* mutation in prognostication of papillary thyroid cancer (PTC) recurrence. *Gland Surg* 2016; 5(5): 495-505. https://doi.org/10.21037%2Fgs.2016.09.09.
10. Caronia LM, Phay JE, Shah MH. Role of BRAF in thyroid oncogenesis. *Clin Cancer Res* 2011; 17(24): 7511-7517. https://doi.org/10.1158/1078-0432.ccr-11-1155.
11. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in thyroid cancer incidence and mortality in the united states, 1974-2013. *JAMA* 2017; 317(13): 1338-1348. https://doi.org/10.1001/jama.2017.2719.
12. Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, et al. The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. *Cancer* 2012; 118(7): 1764-1773. https://doi.org/10.1002/cncr.26500.
13. Daliri M, Abbaszadegan MR, Bahar MM, Arabi A, Yadollahi M, Ghafari A, et al. The role of BRAF V600E mutation as a potential marker for prognostic stratification of papillary thyroid carcinoma: a long-term follow-up study. *Endocr Res* 2014; 39(4): 189-193. https://doi.org/10.3109/07435800.2013.879169.
14. Yang LB, Sun LY, Jiang Y, Tang Y, Li ZH, Zhang HY, et al. The Clinicopathological Features of BRAF Mutated Papillary Thyroid Cancers ini Chinese Patients. *Int J Endocrinol* 2015: 2015; 642046. https://doi.org/10.1155/2015/642046.
15. Fernandez IJ, Piccin O, Sciascia S, Cavicchi O, Repaci A, Vicennati V, et al. Clinical significance of BRAF mutation in thyroid papillary cancer. *Otolaryngol Head Neck Surg* 2013; 148(6): 919-925. https://doi.org/10.1177/0194599813481942.
16. Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J Clin Oncol* 2015; 33(1): 42-50. https://doi.org/10.1200/jco.2014.56.8253.
17. Henke LE, Pfeifer JD, Ma C, Perkins SM, DeWees T, El-Mofty S, et al. BRAF mutation is not predictive of long-term outcome in papillary thyroid carcinoma. *Cancer Med* 2015; 4(6): 791-799. https://doi.org/10.1002%2Fcam4.417.
18. Fraser S, Go C, Aniss A, Sidhu S, Delbridge L, Learoyd D, et al. BRAF V600E mutation is associated with decreased disease-free survival in papillary thyroid cancer. *World J Surg* 2016; 40(7): 1618-1624. https://doi.org/10.1007/s00268-016-3534-x.
19. Feng L, Li M, Zhang QP, Piao ZA, Wang ZH, Lv S. Utility of BRAF protein overexpression in predicting the metastasis potential of papillary thyroid carcinoma. *Oncol Lett* 2011; 2(1): 59-63. https://doi.org/10.3892/ol.2010.219.
20. Jung YY, Yoo JH, Park ES, Kim MK, Lee TJ, Cho BY, et al. Clinicopathologic correlations of the BRAF V600E mutation, BRAF V600E immunohistochemistry, and BRAF RNA in situ hybridization in papillary thyroid carcinoma. *Pathol Res Pract* 2014; 211(2): 162-170. https://doi.org/10.1016/j.prp.2014.10.005.
21. Chen Y, Li Y, Zhou X. BRAF mutation in papillary thyroid cancer: a meta-analysis. *Int J Clin Exp Med* 2016; 9(7): 13259-13267. https://e-century.us/files/ijcem/9/7/ijcem0027653.pdf.
22. Li C, Lee KC, Schneider EB, Zeiger MA. BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: a meta-analysis. *J Clin Endocrinol Metab* 2012; 97(12): 4559-4570. https://doi.org/10.1210/jc.2012-2104.
23. Gandolfi G, Sancisi V, Piana S, Ciarrochi A. Time to re-consider the meaning of BRAF V600E mutation in papillary thyroid carcinoma. *Int J Cancer* 2015; 137(5): 1001-1011. https://doi.org/10.1002/ijc.28976.
24. Henke LE, Pfeifer JD, Ma C, Perkins SM, DeWees T, El-Mofty S, et al. BRAF mutation is not predictive of long-term outcome in papillary thyroid carcinoma. *Cancer Med* 2015; 4(6): 791-799. https://doi.org/10.1002/cam4.417.
25. Jiang L, Chu H, Zeng H. B-Raf mutation and papillary thyroid carcinoma patients. *Oncol Lett* 2016; 11(4): 2699-2705. https://doi.org/10.3892/ol.2016.4298.

Authors:

Edmond Rukmana Wikanta – MD, Department of Surgical Oncology, Faculty of Medicine, University of Diponegoro, Semarang, Indonesia. https://orcid.org/0000-0002-3568-4276.

Yan Wisnu Prajoko – MD, Department of Surgical Oncology, Faculty of Medicine, University of Diponegoro, Semarang, Indonesia. https://orcid.org/0000-0003-2659-9923.

Benny Issakh – MD, Department of Surgical Oncology, Faculty of Medicine, University of Diponegoro, Semarang, Indonesia. https://orcid.org/0000-0002-0994-0934.

Hermawan Istiadi – MD, Department of Pathological Anatomy, Dr. Kariadi Centra l Hospital, Semarang, Indonesia. https://orcid.org/0000-0002-6391-3830.

Dik Puspasari – MD, Department of Pathological Anatomy, Dr. Kariadi Centra l Hospital, Semarang, Indonesia. https://orcid.org/0000-0002-0724-1398.