RESEARCH ARTICLE

Surgical Perspective of T1799A BRAF Mutation Diagnostic Value in Papillary Thyroid Carcinoma

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Abstract

Background: Throughout Indonesia, thyroid cancer is one of the ten commonest malignancies, with papillary thyroid carcinoma (PTC) in our hospital accounting for about 60% of all thyroid nodules. Although fine needle aspiration biopsy (FNAB) is the most reliable diagnostic tool, some nodules are diagnosed as indeterminate and second surgery is common for PTC. The aim of this study was to establish the diagnostic value and feasibility of testing the BRAF T1799A mutation on FNA specimens for improving PTC diagnosis. Materials and Methods: This prospective study enrolled 95 patients with thyroid nodules and future surgery planned. Results of mutational status were compared with surgical pathology diagnosis. Results: Of the 70 cases included in the final analysis, 62.8% were PTC and the prevalence of BRAF mutation was 38.6%. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for BRAF mutation analysis were 36%, 100%, 100% and 48%, respectively. With other data findings, nodules with "onset less than 5 year" and "hard consistency" were proven as diagnostic determinants for BRAF mutation with a probability of 62.5%. This mutation was also a significant risk factor for extra-capsular extension. Conclusions: Molecular analysis of the BRAF T1799A mutation in FNAB specimens has high specificity and positive predictive value for PTC. It could be used in the selective patients with clinical characteristics to facilitate PTC diagnosis and for guidance regarding extent of thyroidectomy.

Keywords: Papillary thyroid carcinoma - BRAF mutation - fine needle aspiration - diagnostic value

Asian Pacific J Cancer Prev, **14 (1)**, 31-37

Introduction

Thyroid cancer is the most frequently found endocrine malignancy (SEER, 2010; Wartofsky, 2010). Data analysis from Surveillance, Epidemiology, and End Results (SEER) estimated the incidence rate raised more than two folds with papillary thyroid carcinoma (PTC) is the most common pathological findings with the prevalence between 65-88% (Enewold et al., 2009). Throughout Indonesia, thyroid cancer is one of the ten most commonly found malignancies, comprising about 3.8% of all malignant cases (Tjindarbumi and Mangunkusumo, 2002). Among all thyroid malignancies, our data base showed that PTC prevalence was 83% and it accounts for 61% of all thyroid nodules in our department.

Fine needle aspiration biopsy (FNAB) plays an important role in diagnosing thyroid nodules with sensitivity 90% and specificity 74% (Tee et al., 2007; Cibas, 2010). Although cytology examination is a reliable diagnostic test; however, 10-40% FNAB specimens are diagnosed as indeterminate (Kato and Fahey, 2009; Nikiforov et al., 2009). We often experienced these results, having the fact that approximately 44% of cytology results was indeterminate for malignant diagnosis. This condition caused a difficult surgical decision in determining the initial extent of thyroidectomy. Consequently, about 23% of our cases needed second completion surgery.

In addition to routine cytological examination, nowadays, molecular diagnosis plays an important role in thyroid cancer treatment. The most important oncogenic mechanism found in PTC is BRAF T1799A mutation with a prevalence of approximately 45% (Nikiforov, 2008; Handkiewicz-Junakv et al., 2010). As a stable DNA molecule, BRAF mutation can be detected even in low quantities DNA specimens taken from FNAB (Xing, 2007). The mutation is not only important for PTC diagnosis but in many studies it has been found to have strong associations with aggressive PTC characteristics such as extra thyroidal extension, lymph node metastasis, tumor recurrence, and advanced stage (Nikiforov, 2008; Xing et al., 2009).

On the basis of these data, we conducted a prospective study on molecular diagnostic value of T1799A BRAF mutation in FNAB specimens to improve preoperative

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PTC diagnosis and to establish a clinical characteristic that is feasible to apply it on selected cases. On additional data, we determined clinical characteristics which are associated by means of BRAF mutation aggressive behaviours that might be useful in determining the initial extent of surgery.

Materials and Methods

Participants

In this study, 95 consecutive patients with thyroid nodules were enrolled prospectively either at Cipto Mangunkusumo Hospital Medical Faculty University of Indonesia (79 patients), Dharmais National Cancer Centre (7 patients), and Mitra Kelapa Gading Hospital (9 patients). BRAF mutation analysis was conducted at Eijkman Institute for Molecular Biology. All new patients with nodules measurement 1 cm in diameter or more and physically could undergo a surgery were included. We excluded patients who had pathological results from previous thyroid surgery, with other malignancies or refused to participate. The Institutional Review Board at Medical Faculty of Indonesia approved the study and all patients provided informed consent to participate.

FNA and fresh tissue specimens

The clinical examinations and FNAB procedure were performed by a surgical oncologist (BB). In the outpatients department, FNAB procedure was performed under ultrasound guidance. The FNAB specimens were obtained from the nodule in 2 until 4 passes with a 22 gauge needle attached to a 5 ml syringe. The material from the first needle pass was used for cytology examination. In the same area under ultrasound guidance, the material from second needle pass was collected for BRAF mutation analysis. One cc saline was used to flush the aspiration needle and the extracted cells were together collected in a falcon tube which contains 5 cc solution of saline and penicillin/streptomycin. If patients refused for preoperative FNAB then the procedure was taken intra operatively. After surgical removal of the nodule, it was incised for macroscopic examination. The needle was passed under the same procedure in suspected malignant features, that is, calcification, papillary growth, greyish white fibrotic area, and hard consistency.

Intra operative fresh tissue specimen was also collected for BRAF mutation analysis by a surgical oncologist (BB) based on malignant macroscopic features . Only a small portion of tissue collected for mutation analysis and the rest was sent for histopathology examination. All specimens were kept under temperature -20^{0}C.

BRAF mutation analysis and PCR RFLP

The DNA mutation analysis was performed by the two authors (II and BB) through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genomic DNA was extracted from FNAB specimens and fresh thyroid tissue using the QIAamp DNA minikit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Deoxyribonucleic acid (DNA) template was amplified in exon 15 which contains T1799A BRAF mutation. We used the specific oligonucleotide primers: 5'- GACTCTAAGAGG AAAG ATGAAGTAC-3' (forward) and 5'- CACTGATTTTT-GTGAATACTGGGAC-3' (reverse). The PCR product was reamplified using the specific primer as follows: 5'- TAAAAATAGGTGATTTTGGTCTAGCTCTAG-3' (forward) and 5'-CACTGATTTTTGTG AATACTGGGAC-3'(reverse). BRAF mutation positive control was obtained from one patient after we achieved compatibility with DNA positive control from Department of Surgery and Comprehensive Cancer Centre, University of California, San Francisco, California. Direct sequencing analysis was performed on this patients using the primers: 5'-GACTCTAAGAGGAAAGATGAAGTAC-3' (sequence forward) and 5'- CACTGATTTTTGTGA ATACTGGGAC-3' (sequence reverse) to generate a PCR product which could be sequenced using the primer, 5'-GACTCTAAGAGGAAAGATGAAGTAC-3'. The sample was sequenced in a 3130xl Genetic Analyzer automated capillary DNA Sequencer using the BigDye Terminator v3.1 Sequencing Standard Kit (Applied Biosystems, Foster City, CA). PCRs were performed using the following amplification profile: initial denaturation at 95°C for 5 minutes, followed by 40 cycles for denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and elongation at 72°C for 1 minute. After the last cycle, a final extension at 70°C for 5 minutes was performed. The PCR product size was 219 base pairs (bp) and visualized by electrophoresis on a 2% agarose gel. PCR products were digested with 10U XbaI for 16 hours and electrophoresed in a 10% acylamide gel, followed by ethidium bromide staining. After that the gels were photographed using an ultraviolet light transluminator The mutant DNA PCR RFLP product size was 193 bp and 26 bp.

Cytology and pathology review

All cytology specimens were reviewed at the Department of Anatomical Pathology Universitas Indonesia. Cytological diagnosis categories were benign, atypia, follicular neoplasm, suspicious for malignancy, and malignant. All histopathology examinations were reviewed by the respective hospital's Pathology Department.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) was calculated using CATmaker. Categorical variables were compared using X^2 test, for small values, Fisher's exact test. Bivariate and multivariate logistic regressions analysis were performed to estimate the odds ratio clinical characteristic BRAF mutation positive patients relative to BRAF mutation negative patients. All data were reported with 95% confidence of interval and a P value <0.05 was considered statistically significant. Analysis was performed using SPSS version 11.5.

Results

Patient characteristic

We prospectively enrolled 95 patients from 1 August 2010 until 31 June 2011. Only 70 patients included in

the final analysis. The 25 patients were excluded for the following reasons: 2 patients came with recurrent nodule from previous thyroid surgery with pathological result of PTC, 2 patients with a thyroid nodule and a breast carcinoma, 4 patients refused the FNAB procedure, and 17 patients seek for other hospital after FNAB procedure. These 70 patients underwent DNA analysis as shown in figure 1.

Of the 70 patients, 54 (82.9%) were females and 12 (17.1%) were males. The mean age of the patients was 46±12 years (range 20-71 year). Thirty seven (52.9%) patients had symptom of nodules between 1-5 years, 13 (18.6%) patients >10 years, 11(15.7%) patients below 1 year, and 9 (12.9%) patients between 5 and 10 years.

We had 49 (70%) cases of malignancy and 21 (30%) benign goiter. From all thyroid nodules there were 19 (27.1%) cases of PTC follicular variant (PTCfv), 14 cases (20%) cases classic PTC (PTCcv), 7 (10%) cases PTC solid variant (PTCsv), 3 (4,3%) cases tall cell variant, 2 (2,9%) cases anaplastic carcinoma (AC) in which was one of them was originated from PTC, and the rest was the other type of malignancy.

Patients characteristic according to TNM stage were 17 (34.7%) patients in stage I, 15 (30.6%) patients in stage IV, 11 (22.4%) patients in stage III, and 6 (12.2%) patients in stage II. The most common operative procedure was total thyroidectomy which comprise of 34 (48.6%) cases and isthmolobectomy as the second common procedure that is 18 (27.5%) cases. Fourteen (20%) patients had undergone total thyroidectomy and lymph node surgery. The patient characteristics in this study are shown in Table 1.

FNAB

Of 70 patients undergone FNAB the results were: malignant in 13 (18.6%) patients, suspicious malignant in 10 (14.3) patients, atypical in 8 (11.4%) patients, follicular lesion in 1 (1.4%) patients, and benign in 38 (54.3%) patients. The pathology results for these patients are shown in Figure 2. Not every patients performed FNAB under ultrasound guidance. Only 80% of them were guided under ultrasound and 20% was taken intraoperatively after tissue removal.

PCR RFLP analysis for BRAF mutation

One hundred and forty specimens from FNAB and fresh tissue were taken for DNA mutation analysis. We analyzed specimens after patients had a surgery and correlated with the final pathologic results after DNA mutation analysis were done. BRAF mutation was found in 17 patients and its prevalence in PTC was 38.6%. One mutation was found in a fresh tissue specimen but could not be detected from FNAB specimen from a patient with PTCfv. Fine needle aspiration specimens found 16 patients with BRAF mutation from 12 (85.7%) PTCcv,

Table 1. Patients Characteristics

Characteristic		N (%)
Sex	Male	12 (17.1)
	Female	54 (82.9)
Age (mean)		46±12
On set	<1	11 (15.7)
(years)	1-5	37 (52.9)
	5-10	9 (12.9)
	>10	13 (18.6)
Pathology	Benign goiter	21 (30.0)
	PTCcv	14 (20.0)
	PTCfv	19 (27.1)
	Tall cell	3 (4.3)
	PTCsv	7 (10.0)
	AC	2 (2.9)
	Hurthle cell ca	2 (2.9)
	SCC	1 (1.4)
	Poorly diff ca	1 (1.4)
Stage	I	17 (34.7)
	II	6 (12.2)
	III	11 (22.4)
	IVA	7 (14.3)
	IVB	2 (4.1)
	IVC	6 (12.2)
Surgery	Lobectomy	2 (2.9)
	Isthmolobectomy	18 (25.7)
	Subtotal thyroidectomy	2 (2.9)
	TT	34 (48.6)
	TT+ LN	14 (20.0)

*PTCcv, papillary thyroid carcinoma classic variant; PTCfv, papillary thyroid carcinoma follicular variant; PTCsv, papillary thyroid carcinoma solid variant; AC, anaplastic carcinoma; Hurthle, hurthle cell carcinoma; SCC, squamous cell carcinoma; TT, total thyroidectomy, LN, lymph node surgery

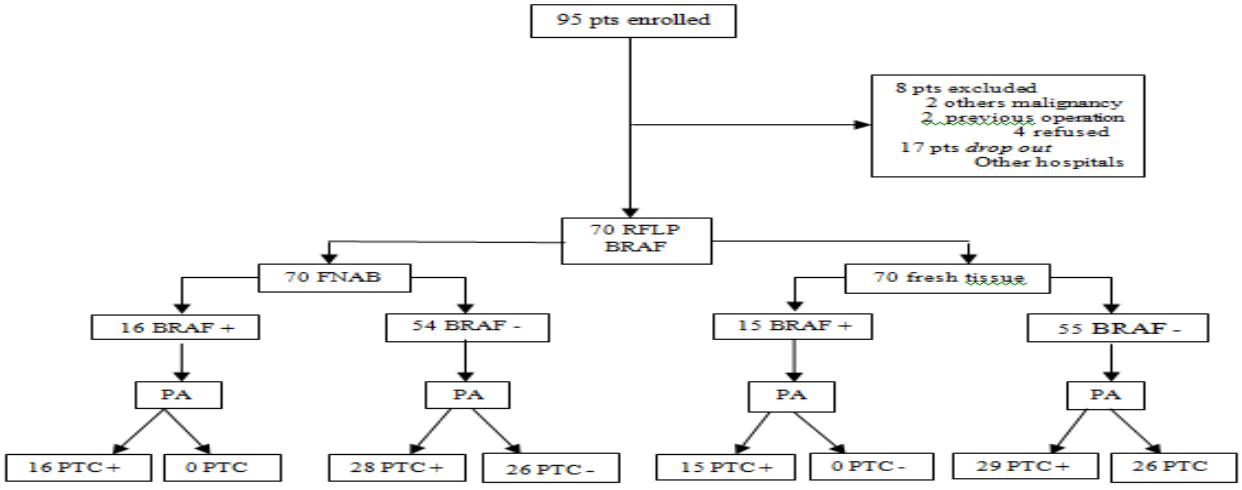

Figure 1. Patient Flowchart for RFLP Mutation BRAF Mutation. PTC, papillary thyroid carcinoma: PCR RFLP, polymerase chain reaction restriction fragment length polymorphism; PA, pathology examination

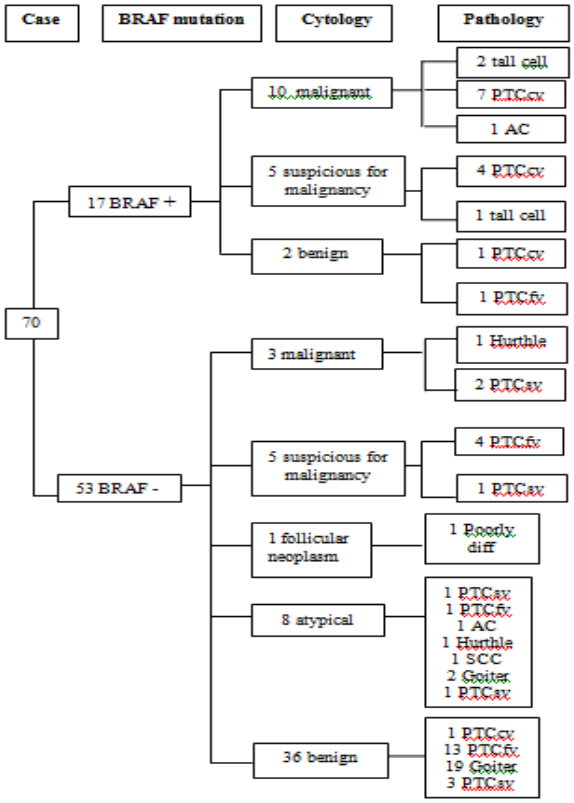

Figure 2. Results of BRAF Mutation, Cytology, and Pathology. PTCcv: papillary thyroid carcinoma classic variant, PTCfv: papillary thyroid carcinoma follicular variant, PTCsv: papillary thyroid carcinoma solid variant, Tall cell, papillary thyroid carcinoma tall cell variant; AC: anaplastic carcinoma, Hurthle : hurthle cell carcinoma, SCC: squamous cell carcinoma

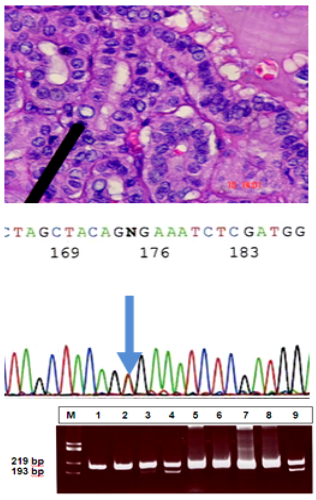

Figure 3. Top: Pathological examination revealed a classic variant of papillary thyroid carcinoma. **Middle:** Sequencing analysis showed T1799A BRAF mutation. **Bottom:** Sample in lane 3 is a positive control from Department of Surgery and Comprehensive Cancer Centre, University of California, San Francisco, California. Lane 4 is our positive control patient and lane 9 is a positive BRAF mutation patient

3 (100%) tall cell variants, and 1 (50%) AC. Table 2 yield the percentage of BRAF mutation for each PTC subtype and anaplastic carcinoma. Figure 3 illustrated our patient who had positive result on BRAF mutation analysis. The histopathological features of this patient described the classic features of PTCcv with papillary growth pattern and ground glass nuclei appearance, and the PCR sequencing analysis revealed T1799A BRAF mutation. The final result of PCR RFLP produced the size of the mutant DNA at 193 bp and 26 bp. This result was in accordance with BRAF mutation positive control patients from University of California using PCR RFLP method.

BRAF mutation diagnostic value

Of the 70 FNAB specimens, BRAF mutation analysis correctly detected PTC in 16 of 44 cases, resulting in a sensitivity of 36% (95%CI, 22%-51%), and correctly identified as negative for PTC in all 26 cases, resulting in a specificity of 100% (95%CI, 100%-100%). The PPV for all 16 cases of PTC was 100% (95%CI, 100%-100%) and the NPV for 26 of 54 cases was 48% (95%CI, 35%-61%). Adding the molecular test to cytology resulted in a sensitivity of 41% (95%CI, 26%-55%), specificity of 96% (95%CI, 89%-100%), PPV of 95% (95%CI, 85%-100%), and NPV 49% (95%CI, 35%-63%). The data is shown in Table 3.

The other findings

BRAF mutation clinical characteristic and aggressive tumor behavior: Because the sensitivity of BRAF mutation analysis is low but it has a high positive predictive value, we tried to indentified patient characteristics who are most likely to have BRAF mutation, so in those patients we estimate that BRAF mutation analysis could be used as a diagnostic tool for PTC. We analyzed clinical characteristics that could be used as diagnostic determinants for BRAF mutation. In a bivariate analysis BRAF mutation was associated with extra thyroidal extension (58.3% vs 17.2%; 95%CI, 1.61-7.08; P=0.003), hard consistency (48% vs 11.1%; 95%CI, 1.71-10.85; P=0.001), and clinically lymph node metastasis (57.1% vs 16.1%; 95%CI, 1.67-7.53; P=0.001) (Table 4). But on

Table 2. BRAF Mutation Analysis for Each Histological Subtypes

	FNA_BRAF		Fresh tissue_BRAF		BRAF_total	
	Positive	Negative	Positive	Negative	Positive	Negative
Histopathology (n)						
PTCcv	12	2	10	4	12	2
PTCfv	0	19	1	18	1	18
Tall Cell	3	0	3	0	3	0
AC	1	1	1	1	1	1
PTCsv	0	6	0	6	0	6
Total	16	28	15	29	17	27
Prevalence					38.6	

*PTCcv, papillary thyroid carcinoma classic variant; PTCfv, papillary thyroid carcinoma follicular variant; Tall cell, papillary thyroid carcinoma tall cell variant; AC, anaplastic carcinoma; PTCsv, papillary thyroid carcinoma solid variant

Table 3. Diagnostic Value of BRAF Mutation in FNAB Specimen

FNAB	SN % (95%CI)	SP % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
BRAF	36 (22-51)	100 (100-100)	100 (100-100)	48 (35-61)
BRAF + CYTOLOGY	41 (26-55)	96 (89-100)	95 (85-100)	49 (35-63)

*SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value

Table 4. Bivariate Analysis of Clinical Characteristics and BRAF Mutation

		BRAF + n (%)	BRAF - n (%)	p	95%CI
Sex	Male	5 (41.7)	7 (58.3)	0.123	0.87-10.16
	Female	12 (20.7)	46 (79.3)		
Age	≥45 y	10 (25)	30 (75)	0.872	0.46-2.48
	<45 y	7 (23.3)	23 (76.7)		
Onset	< 5 y	14 (29.2)	34 (70.8)	0.16	0.68-6.69
	≥ 5 y	3 (13.6)	19 (86.4)		
Tumor size	T4	7 (58.3)	5 (41.7)	0.003	1.61-7.08
	<T4	10 (17.2)	48 (82.8)		
Consistency	Hard	12 (48)	13 (52)	0.001	1.71-10.85
	Not hard	5 (11.1)	40 (88.9)		
Clinical node	Positive	8 (57.1)	6 (42.9)	0.001	1.67-7.53
	Negative	9 (16.1)	47 (83.9)		

Table 5. Multivariate Analysis of Clinical Characteristics and BRAF Mutation

Variabel	Coefisien	p	OR (IK95%)
Onset <5 years	1.56	0.049	4.76(1.00-22.49)
Hard	2.336	0.001	10.33(2.74-38.98)
Constanta	-3.379	0	0.034

Table 6. Bivariate Analysis of BRAF Mutation and Aggressive Tumor Behavior

	BRAF + n (%)	BRAF - n (%)	p	Risk Prevalence	95%CI
Extra thyroidal extension	7 (41.2)	5 (9.4)	0.003	4.36	1.59-11.97
Stage IV	7 (41.2)	8 (25.0)	0.24	1.64	0.72-3.76
Lymph node metastasis	8 (100)	3 (50.0)	0.055	2	0.89-4.45

a multivariate analysis we indentified that a nodule with onset less than 5 year (OR, 4.76; 95%CI, 1.00-22.49; P=0.049) and hard consistency (OR, 10.33; 95%CI, 2.74-38.98; P=0.001) were the only diagnostic determinant for BRAF mutation (Table 5).

The area under the receiver operating characteristic curve (AUC) for it is 0.77 (95%CI, 0.63-0.91; P=0.001). The calculated these clinical variables in the logistic regression equation (P=1/1+e-(a+b1x1+b2x2…+bixi)) will predict a patients to have 62,5% a probability of having BRAF mutation. From the data shown in Table 2, we also analyzed the association of BRAF mutation with clinical tumor behaviors. The mutation was the only risk factor for extra thyroidal extension (PR, 4.36; 95%CI, 1.59-11.97; P=0.003), whereas it was not associated with advanced disease stage and lymph node metastasis. Table 6 showed the analyzed data.

Discussion

Thyroid nodule is a common clinical finding by 4-76% prevalence in different populations and 8-65% prevalance from autopsy data (Kato and Fahey, 2009; Eszlinger and Paschke, 2010). Most of them were a benign condition by 5% prevalence of malignancy (Xing et al., 2004; Eszlinger and Paschke, 2010). Interestingly, our data revealed that 70% of the cases were malignancy and the most common pathology result was PTC (62.8%). It is assumed that our cases were already selected because Cipto Mangunkusumo and Dharmais are national reference hospitals where difficult or malignant cases are referred to. However, we also consider that there is some other factors for this higher incidence. A study confirmed a high iodine intake to be a risk factor for PTC and BRAF mutation (Guan et al., 2009). It is important in the future to conduct a study in our population to answer the fact that our malignant cases are high.

Nowadays, the role of molecular marker in cancer diagnosis and treatment has been established (Nikiforova and Nikiforov, 2009; Handkiewicz-Junakv et al., 2010). In thyroid cancer, molecular test is used to enhanced diagnosis and give an additional information that would affect prognosis. T1799A BRAF mutation is a molecular marker that is specific for PTC (Xing, 2007; 2009; Nikiforova and Nikiforov, 2009). In this study, we tested the role of BRAF mutation to solve our preoperative diagnostic problems. PCR RFLP that was used for mutation analysis in this study is reference to experienced in Japan. This method is considered sensitive in detecting BRAF mutation from FNAB specimen (Hayashida et al., 2004).

In our study, the prevalence of BRAF mutation was 38.6% and it was specific and high in PTCcv, tall cell variant, and AC. Some studies support these findings with a prevalence average of 45% (Xing, 2005; 2009; Nikiforova and Nikiforov, 2009).

On the basis of our mutation analysis, BRAF mutation on FNAB specimen has a high specificity and PPV. The low sensitivity in our study occurred because all PTCfv and PTCsv in FNAB specimen were negative for BRAF mutation whereas these variants accounts for 60% in PTC. The literature that could explain our findings stated that BRAF mutation is common and specific for PTCcv and tall cell variant but less common in PTCfv. Other mutation involving in PTC is RAS mutation which occurs in 10-20% of tumors and this mutation in PTC almost always follicular variant in histology. Solid variant of PTC also has a different type of mutation which is associated with RET/PTC3 rearrangement (Knauf et al., 2005; Nikiforova and Nikiforov, 2009). Although BRAF mutation as a diagnostic test is specific for PTC, there are studies that stated its limitation as a diagnostic tool because of its low sensitivity (Xing, 2007; 2009)

There are some studies evaluated BRAF mutation diagnostic value. Zatelli et al. (2009) found sensitivity of 64% and Sapio et al. (2007) found sensitivity of 37.5% from specimens of inconclusive cytology. A study from Kim et al. (2010), in Korea where the prevalence of BRAF mutation is high on their population revealed a sensitivity of 82.5% and a higher value until 98.5% if BRAF mutation was combined with cytology examination.

Our study also tried to combine between the molecular analysis in conjunction with cytology examination but it could not reach a satisfied result, only increasing 5% of sensitivity. When we analyzed the subgroup data only for PTCcv and tall cell variants, it resulted in increasing diagnostic value with sensitivity of 89% and specificity of 100% (data no shown). But we think it is difficult to use the test based on the above subgroup analysis because in daily practice we hardly predict which patient has a classic variant histology or tall cell variant and we do not recommend using this molecular test on all thyroid nodule.

Therefore in this study we concluded that BRAF mutation has a high specificity and PPV. By looking at this fact, we had questions that are presumably relevant from a surgical point of view. Is it clinically important to use the test in surgical cases and is there any evidence based to support this? And if it is useful, which patient is most likely benefit to be performed the molecular test?

Many studies concluded BRAF mutation is related to aggressive tumor behavior such as extra thyroidal extension, bigger tumor size, lymph node metastasis, advanced disease stage, iodine ablation resistance, and poor disease free survival (Xing et al., 2005; 2009a; 2009b; Kebebew et al., 2007). Xing et al. (2009), showed that analysis of BRAF mutation from FNAB specimens provides a novel tool to identify high risk patients for extensive disease such as extra thyroidal extension and lymph node metastasis and also their study demonstrated a significantly reduced disease free survival in BRAF mutation patients. The other important research was from Kebebew et al. (2007), that demonstrated recurrent and persistent diseases were higher even in TNM stage I or AMES low risk classic PTC harboring BRAF mutation. Two studies conducted by Yip et al. (2009) and Howell et al. (2012) proved that BRAF mutation is related to central lymph node metastasis and they suggest its utility as a guidance to performed the initial extent of surgery. Another interesting study showed that BRAF mutation of PTC significantly have a differential predictive value for lymph node metastasis depend on the tumor size (So et al., 2011).

Our study demonstrated BRAF mutation was a significant risk factor for extra thyroidal extension and although not proven statistically, a tendency for lymph node metastasis. Besides that, this mutation can be used as a marker to predict PTC differentiation into anaplastic carcinoma, as we demonstrated in one of our patient. So, based on our study and the literatures we suggested it is clinically important to use this molecular test because we can get preoperative PTC diagnosis and important information regarding aggressive PTC behavior that needs a different surgical treatment.

As we have mentioned previously, the results of BRAF mutation analysis from this study yield a low sensitivity test but it has high positive predictive value. Therefore we do not recommend for using this molecular test on all thyroid nodules but only in clinically patients who are most likely to have BRAF mutation.

We did search clinical variables that are associated with BRAF mutation. On multivariate analysis we found that only a nodule with onset less than 5 years and hard in consistency are significant risk factors for BRAF mutation and the probability to have PTC harboring BRAF mutation if a patient has those symptoms is 62.5%. These clinical variables are good diagnostic determinants for PTC harboring BRAF mutation as it can rule in about 77% of patients with the mutation. We proposed an algorithm that suggested using the molecular test as an adjunct to routine cytological examination in a selective patient that has a nodule with onset less than 5 years and hard consistency.

This study has several limitations that is 20 percent of specimens taken intraoperatively and the DNA specimens were not checked for cell adequacy with microscopic cytology examination during the procedure make this study has potential measurement biases. But all of the limitations were due to our feasibility on conducting research in our hospital setting.

Although our methods and results are far from perfection, yet we finally can conclude our result that BRAF mutation analysis in FNAB specimens had a high specificity and positive predictive value. It could be used in the selective patients with clinical characteristics of PTC harboring the mutation and it may be used as guidance in the initial extent of thyroidectomy. We need a further study in determining its diagnostic cost effectiveness, the role of BRAF mutation in tailoring the initial extent of thyroidectomy and its association with the risk of lymph node metastasis.

Acknowledgements

The authors thanks Electron Kebebew, MD from the Departments of Surgery, and Comprehensive Cancer Center, University of California, San Francisco, for sharing the DNA positive control of BRAF mutation, and Atsushi Kumagai, MD from Nagasaki University Graduated School of Biomedical Sciences for sharing experiences in the technical aspect of PCR-RFLP. The authors also acknowledges the member of Eijkman Laboratory for their assistance especially Ayang Halim, MD. This work was supported by DRPM University of Indonesia, Jakarta. The authors have no conflict of interest to disclose.

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