BRAF Gene Mutation (V600E) in Aspiration Cytology of Patients With Suspected Papillary Thyroid Carcinoma

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Abstract- This study was attempted to investigate the prevalence of BRAF gene mutation (V600E) in aspiration cytology of patients with suspected papillary thyroid carcinoma (PTC). Seventy-six patients suspected of having PTC who were referred for fine-needle aspiration (FNA) biopsy were included in this cross-sectional study. Ultrasound-guided FNA was taken from the thyroid masses, and samples were sent for cytologic evaluation. Simultaneously, the samples were sent to a genetic laboratory to check the status of BRAFV600E mutation. Patients with FNA positive for PTC were assigned in one group, and those with FNA negative for PTC were assigned to another group. Cytological and molecular results were compared with those of histopathology and sonography. The results showed that the prevalence of the BRAF gene (V600E) mutation in our study was 21.1% (16 out of 76 patients). In addition, the results showed a significant relationship between gene mutation and pathologic findings so that the highest gene mutation was significantly detected in patients with FNA positive for PTC (P=0.001). Also, our results showed a significant relationship between gene mutation and some sonographic findings (calcification, P=0.004) and no significant relation in the other sonographic findings (hypoechoic changes, P=1.12 and regular changes, P=0.194). According to the results of the present study, BRAF mutation (V600E) can be an effective indicator for definitive diagnosis and primary treatment of PTC in suspected cases.

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Keywords: Papillary thyroid cancer; Fine needle aspiration; B-raf (V600E); Ultrasound

Introduction

Thyroid cancer is one of the most prevalent cancers in many countries around the world (1). Papillary thyroid carcinoma (PTC) is known to be the most common malignancy of endocrine glands, accounting for 85-90% of thyroid cancers. The disease is usually accidentally diagnosed as a thyroid nodule by the patient during medical examinations or by imaging studies done for some reason unrelated to the thyroid. However, many thyroid nodules are benign, and an appropriate diagnosis of thyroid nodules before surgery is one of the main goals of physicians in the field of thyroid disease (2). Genetic changes, including the mitogen-activated protein kinase (MAPK) pathway such as RET/PTC, RAS, and BRAF mutations, have been shown in most cases of PTC (3). More than 90% of BRAF mutations are T1799A, leading to BRAFV600E mutations. BRAFV600E is present in about 50% of PTC cases, but it is rarely present in other sub-types of thyroid cancer, including follicular thyroid cancer. The tumorigenic role of BRAFV600E mutation in the development of PTC has been documented in BRAFV600E transgenic mice, and rat thyroid cells overexpressed with BRAFV600E have indicated that BRAFV600E initiates tumorigenesis and is required for tumor progression in PTC (4). Some clinical studies have suggested the association between BRAFV600E mutation and clinicopathologic characteristics and tumor recurrence (5,6). This association has also been detected in patients with

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papillary thyroid micro carcinoma and low-risk PTC. As a very special and unique mutation in PTC, testing for BRAFV600E mutation in aspiration sample improve the diagnostic accuracy of in indeterminate cytology. Preoperative analysis of BRAFV600E in low-risk patients may provide important value for prognosis, and these patients may benefit from receiving intensive management and repeated follow-up. BRAF-based treatments have been developed to treat various human cancers, including advanced thyroid cancer (7). Overall, the results of the studies on the relation of the BRAFV600E mutation and PTC in different countries are contradictory (8,9). Therefore, considering the importance of this mutation in PTCs and the existence of contradictory results and interpretations in this regard, this study was attempted to estimate BRAFV600E mutation in cytology aspirations of patients with PTC as a diagnostic and prognostic tool.

Materials and Methods

After approval by the local ethics committee and obtaining written informed consent, in this cross-sectional study (2017-2018), 76 patients who were suspected of having PTC and referred to a training hospital for FNA biopsy, were involved. Ultrasound-guided FNA was taken from the thyroid masses and samples were sent for cytologic evaluation. Simultaneously, the samples were sent to a genetic laboratory to check the status of BRAFV600E mutation. The slides containing cells and insufficient DNA or samples were excluded from the study. For DNA extraction we analyzed aspiration samples by QIAquick PCR Purification Kit (Made in Germany). We first treated the samples with sulfate, then PCR reaction was done, and the mutation status of BRAF V600E was investigated. Histological diagnosis and sonographic findings of each patient were obtained from their medical records. Patients with FNA positive for PTC were assigned in one group, and those with FNA negative for PTC were assigned to another group. Cytological and molecular results were compared with histopathology and sonography findings. Patients, who underwent thyroidectomy after FNA, remained in the study, and their histopathological and sonographic results (as gold standard) were compared with cytological and molecular results. The association between clinicopathological and sonographic findings (size>1cm, irregular border, hypoechoic nodule with calcification indicate PTC) and the presence of BRAFV600E mutation was evaluated using an independent t-test and Pearson's chi-square test using SPSS (version 20). $P<0.05$ is considered significant.

Results

A total of 76 patients were evaluated in this cross-sectional study. Primary FNA findings, pathology results, genetic evaluation results, and sonographic findings are shown in (Table 1). The prevalence of the BRAF gene (V600E) mutation in our study was 21.1% (16 out of 76 patients). We found that benign follicular (56.6%) and MNG (56.6%) lesions, lesions with hypoechoic findings (94.7%), and regular changes (78.9%) without calcification (86.8%) and also lesions with 2-3 mm (79.8%) in diameter were the most prevalent lesions. The relation between gene mutation and all the FNA, pathology, and sonographic findings was evaluated, too (Table 2).

Table 1. Different findings in patients (primary FNA findings, pathology results, genetic evaluation results, and sonographic findings)

Different diagnostic approaches	**Different findings in patients**								
FNA	Benign follicular 56.6%	Follicular carcinoma 1.3%		PTC 17.3%			suspected of having PTC 25%		
Pathology	MNG 56.6%	Follicular carcinoma 1.3%			PTC 42.1%				
Genetic test	Positive for mutation 21.1%				Negative for mutation 78.9%				
Sonography	Hyper echo	Hypo echo	Iso echo	Nodule size (mm)		Calcification	No Calcification	Regular	irregular
				2-3	3.5-5				
	1.3%	94.7%	3.9%	79.8%	20.2%	13.2%	86.8%	78.9%	21.1%

Table 2. The relation between gene mutation and the other findings (FNA findings, pathology results, and sonographic findings)

Different diagnostic findings		Gene mutation (number)	*P*
FNA	**Benign follicular**	Positive=2, Negative= 41	0.001
	Follicular carcinoma	Positive=1, Negative= 0	
	PTC	Positive=11, Negative= 2	
	suspected of having PTC	Positive=2, Negative= 17	
	MNG	Positive=1, Negative= 0	
Pathology	**Follicular carcinoma**	Positive=1, Negative=42	0.001
	PTC	Positive=14, Negative= 18	
Sonography	**Hyper echo**	Positive=0, Negative=1	1.12
	Hypo echo	Positive=16, Negative=56	
	Iso echo	Positive=0, Negative=3	
	Calcification	Positive=6, Negative=4	0.004
	No Calcification	Positive=10, Negative=56	
	regular	Positive=9, Negative=51	0.194
	irregular	Positive=8, Negative=8	

The results showed a significant relationship between gene mutation and primary FNA findings so that the highest gene mutation was significantly detected in patients with FNA positive for PTC (*P*=0.001). Also, the results showed a significant relationship between gene mutation and pathology findings so that the highest gene mutation was significantly detected in patients with FNA positive for PTC (*P*=0.001). The relation between gene mutation and sonographic findings showed that the highest gene mutation was detected in patients with hypoechoic findings (*P*=1.12), no calcification (*P*=0.004), and with regular changes (*P*=0.194). According to the pathology findings (as the gold standard test) and considering the prevalence of 4.5 per 100,000 people for the disease, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy indicators were considered for genetic mutation evaluation (Table 3). According to the pathology findings (as the gold standard test) and considering the prevalence of 4.5 per 100,000 people for the disease, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy indicators were considered for FNA evaluation (Table 4).

Table 3. Investigation of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for BRAF mutation

Statistical test	Value
Sensitivity	47.75 %
Specificity	97.45 %
Positive predictive value	93.33 %
Negative predictive value	71.18 %
Accuracy	93.13 %

Table 4. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for FNA

Statistical test	Value
Sensitivity	45.5 %
Specificity	100.00 %
Positive predictive value	100.00 %
Negative predictive value	80.45 %
Accuracy	97.39 %

Discussion

Numerous genetic changes play a key role in thyroid tumor formation, one of which is the T1799A point mutation in BRAF. Mutation in the BRAF-V600E gene occurs in approximately 45% of papillary thyroid

cancers (10). The BRAF-V600E mutation is associated with invasive pathological forms, increased risk of recurrence, lack of desire for radioactive iodine, and lack of response to treatment (11). Most clinical studies have shown an association between the BRAFV600E mutation and the clinical and pathological features of the tumor and tumor recurrence, although the results are controversial. This association has also been observed in patients with thyroid papillary microcarcinoma and low-risk PTC. As a very specific and unique mutation in PTC, testing for the BRAFV600E mutation in the aspiration sample improves the accuracy of PTC detection in unknown cytology (12). Preoperative analysis of BRAFV600E in low-risk patients may provide significant value for prognosis, and these patients may benefit from intensive management and frequent follow-up. BRAF-based treatments have been developed to treat various human cancers, including advanced thyroid cancer. Contradictory results have been reported in various countries regarding the association between the mutation of this gene and PTC (13-15). Therefore, considering the importance of this mutation in PTCs and the existence of contradictory results and interpretations in this regard, we intend to estimate the BRAFV600E mutation in cytology samples of PTC patients as a method of diagnosis and prognosis. The first goal of this study was to determine the frequency distribution of mutations in the FNA group positive for PTC and FNA group negative for PTC. The results showed that the frequency of mutation was 11 in the FNA group positive for PTC, and 5 in the FNA group negative for PTC. We also found that the frequency of mutations in patients with PTC and patients with suspected PTC was higher compared to patients with FNA negative for PTC. In 2012, Marchetti et al, evaluated mutation of the BRAF gene (V600E) in aspiration samples of 95 patients with PTC. They found that 74% of patients with PTC had mutation in the BRAF gene. They also yielded that the combination of cytological diagnosis and molecular analysis was able to detect 82% of PTC cases compared to morphological indicators which could detect only 37% of PTC cases (16). The second goal was to compare the frequency distribution of mutations in the FNA group positive for PTC and FNA group negative for PTC. The results indicated that FNA diagnosis was significantly related to the prevalence of mutation under consideration, so that the frequency of mutation in the FNA group positive for PTC was higher than the other group. Based on these results, it seems that FNA detection has appropriate diagnostic value for PTC and suspected PTC cases. Molecular analysis also confirmed the diagnostic value of FNA detection in this study. The stage at which a penetrating carcinoma creates an invasive genotype cannot be predicted solely based on the size of nodules. In addition, similar studies and clinical experiences have shown numerous examples of cancers with a few millimeters in diameter, but with extensive metastatic spread. Analysis of the molecular characteristics of a lesion can be helpful in describing its behavior. This issue is mostly implemented in PTC cases and suspected PTC cases reported with FNA indicators, which are often multifaceted and associated with extracapsular and metastatic invasion (17-19).

The status of the BRAF gene is very important for determining the cause and pathogenesis of PTC. As a result, its analysis can be a diagnostic and related prognosis tool. In addition, the relationship between BRAF gene mutation status and treatment strategy is a two-way relationship (20). Our study also suggested the diagnostic value of investigation of this gene mutation. Comparison of the data from the pathological study, as the gold standard, showed that 32 patients had PTC; in addition, the molecular study confirmed the prevalence of this gene mutation in this number of patients. It is noteworthy that the data obtained from the analysis of the BRAF gene in PTC strongly support the hypothesis that thyroid carcinomas with a diameter of 1 cm are not harmless. They are real penetrating tumors that demonstrate clear metastatic behaviors (21,22). In our study, it was found that in samples of suspected PTC obtained according to FNA results, the occurrence of mutations indicated the important status of these samples. This finding was later confirmed by the pathology results. The fourth and fifth goals of this study were to compare the frequency distribution of mutations in two groups and study its association with sonographic symptoms. The results showed that all patients in the FNA positive group had hypoechoic mass based on ultrasound findings. The results of the genotypic distribution also showed that in the hypoechoic group, 11 were heterozygotes and four patients were homozygotes for mutations, suggesting a significant relationship between FNA findings, ultrasound data, and genetic distribution. Out of 32 patients who tested positive for FNA, 10 had calcification, and 22 had no calcification. In addition, among patients who tested positive for FNA, 17 patients had a regular ultrasound, and 15 patients had an irregular ultrasound. Kwak *et al.*, showed that BRAF (V600E) mutation could be used as a potential prognostic factor in patients with suspected PTC. In

patients with poor clinical and ultrasound signs, diagnosis based on this mutation can be helpful (22). In our study, it was found that ultrasound findings, such as calcification and ultrasound regularity, had poor diagnostic power for patients suspected of having PTC and those without PTC. However, FNA findings, along with molecular analysis, were well able to detect PTC patients and were consistent with pathological data as the gold standard. Future studies are needed to determine how this data can be used to alter patient care. Several reports have already been published about BRAF gene mutation in PTC. However, these studies include tests performed on paraffin tissues (23). Using this method, the percentage of definitive PTC diagnoses can be increased from 37% to 82%. In fact, 32 patients from PTC suspected group were transferred to PTC final diagnosis group. The combination of classical morphological examination and molecular analysis was able to reduce the number of PTC suspected cases, with a significant percentage of> 76%. Therefore, using FNA technique along with molecular analysis can be a powerful diagnostic tool for diagnosing PTC cases (15,24,25). The prevalence of the BRAF gene (V600E) mutation in our study was 21.1% and 16 out of 76 patients had mutation in this gene. The percentage of cases with BRAF gene (V600E) mutation was higher in similar studies (16,26-28). The percentage of PTC cases with BRAFV600E mutation varies from 20% to 80% (14). This wide range can be explained by considering that different types of PTC can be created through various molecular pathways and, consequently, we can explain the status of different BRAF genes. In our study, there was a high prevalence of this gene mutation in PTC and suspected PTC patients. From a clinical point of view, it seems that the BRAFV600E mutation status in FNA samples could be a good test for potential tumor invasive behavior. Additionally, this finding can be used for preoperative management of patients with PTC.

Based on the results of the present study, investigation of this gene mutation can be effective in determining definitive diagnosis in PTC suspected cases. In this study, patients suspected of having PTC based on FNA data had BRAFV600E gene mutation, confirming the FNA findings through determining the frequency of these gene mutations. Analysis of the BRAF gene mutation status in FNA obtained from papillary carcinoma showed that molecular pathology, along with morphology and molecular biology, is a powerful tool for cytological diagnosis of suspected cases of PTC. Our results also supported the biological relationship of PTC in molecular analysis of cytological cases.

Study Limitations and Recommendations

Due to the small number of patients, the number of cases with the mutation was low, and only two cases of heterozygous mutations were observed in PTC-suspected cases during FNA detection. Therefore, a larger statistical population is suggested for assessing the diagnostic value of BRAFV600E gene mutation in samples suspected of having PTC. It is also recommended that aspirate samples inside the preservative solution be considered for genetic testing.

References

1. Morris LG, Sikora AG, Tosteson TD, Davies L. The increasing incidence of thyroid cancer: the influence of access to care. Thyroid 2013;23:885-91.
2. Grodski S, Delbridge L. An update on papillary microcarcinoma. Curr Opin Oncol 2009;21:1-4.
3. Tang K-T, Lee C-H. BRAF mutation in papillary thyroid carcinoma: pathogenic role and clinical implications. J Chin Med Assoc 2010;73:113-28.
4. Chuang TC, Chuang AY, Poeta L, Koch WM, Califano JA, Tufano RP. Detectable BRAF mutation in serum DNA samples from patients with papillary thyroid carcinomas. Head Neck 2010;32:229-34.
5. 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;33:42-50.
6. Liu S, Zhang B, Zhao Y, Chen P, Ji M, Hou P, et al. Association of BRAFV600E mutation with clinicopathological features of papillary thyroid carcinoma: a study on a Chinese population. Int J Clin Exp Pathol 2014;7:6922-8.
7. Liu C, Chen T, Liu Z. Associations between BRAF V600E and prognostic factors and poor outcomes in papillary thyroid carcinoma: a meta-analysis. World J Surg Oncol 2016;14:241.
8. Kim TY, Kim WB, Song JY, Rhee YS, Gong G, Cho YM, et al. The BRAFV600E mutation is not associated with poor prognostic factors in Korean patients with conventional papillary thyroid microcarcinoma. Clin Endocrinol (Oxf) 2005;63:588-93.
9. Liu RT, Chen YJ, Chou FF, Li CL, Wu WL, Tsai PC, et al. No correlation between BRAFV600E mutation and clinicopathological features of papillary thyroid carcinomas in Taiwan. Clin Endocrinol (Oxf) 2005;63:461-6.
10. Yan C, Huang M, Li X, Wang T, Ling R. Relationship between BRAF V600E and clinical features in papillary

thyroid carcinoma. Endocr Connect 2019;8:988-96.

11. Ayala MD, Rodríguez AE, González AB, Gabiña PM, Rodríguez TG, de Zarate ERO, et al. BRAF V600E mutation in papillary thyroid cancer and its effect on postoperative radioiodine (131I) therapy: should we modify our therapeutic strategy? Cir Esp (Engl Ed) 2018;96:276-82.
12. Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan M. BRAF V600E mutation in papillary thyroid carcinoma: significant association with node metastases and extra thyroidal invasion. Endocr Pathol 2012;23:83-93.
13. Czarniecka A, Oczko-Wojciechowska M, Barczyński M. BRAF V600E mutation in prognostication of papillary thyroid cancer (PTC) recurrence. Gland Surg 2016;5:495-505.
14. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 2007;28:742-62.
15. Moon HJ, Kim E-K, Chung WY, Choi JR, Yoon JH, Kwak JY. Diagnostic value of BRAF V600E mutation analysis of thyroid nodules according to ultrasonographic features and the time of aspiration. Ann Surg Oncol 2011;18:792-9.
16. Marchetti I, Iervasi G, Mazzanti CM, Lessi F, Tomei S, Naccarato AG, et al. Detection of the BRAF V600E mutation in fine needle aspiration cytology of thyroid papillary microcarcinoma cells selected by manual macrodissection: an easy tool to improve the preoperative diagnosis. Thyroid 2012;22:292-8.
17. Pelizzo MR, Boschin IM, Toniato A, Pagetta C, Piotto A, Bernante P, et al. Natural history, diagnosis, treatment and outcome of papillary thyroid microcarcinoma (PTMC): a mono-institutional 12-year experience. Nucl Med Commun 2004;25:547-52.
18. Noguchi S, Yamashita H, Uchino S, Watanabe S. Papillary microcarcinoma. World J Surg 2008;32:747-53.
19. Nucera C, Pontecorvi A. Clinical outcome, role of BRAFV600E, and molecular pathways in papillary thyroid microcarcinoma: is it an indolent cancer or an early stage of papillary thyroid cancer? Front Endocrinol 2012;3:33.
20. Xing M. Prognostic utility of BRAF mutation in papillary thyroid cancer. Molecular and cellular endocrinology. 2010;321:86-93.
21. Xing M. BRAF mutation in papillary thyroid microcarcinoma: the promise of better risk management. Ann Surg Oncol 2009;16:801-3.
22. Kwak JY, Kim EK, Chung WY, Moon HJ, Kim MJ, Choi JR. Association of BRAFV600E mutation with poor clinical prognostic factors and US features in Korean patients with papillary thyroid microcarcinoma. Radiology 2009;253:854-60.
23. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994;97:418-28.
24. Hwang J, Hee Shin J, Han BK, Ko EY, Kang SS, Kim JW, et al. Papillary thyroid carcinoma with BRAF V600E mutation: Sonographic prediction. AJR Am J Roentgenol 2010;194:W425-W30.
25. Park AY, Son EJ, Kim JA, Youk JH, Park YJ, Park CS, et al. Associations of the braf v600e mutation with sonographic features and clinicopathologic characteristics in a large population with conventional papillary thyroid carcinoma. PloS one 2014;9:e110868.
26. 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 2012;91:274-86.
27. Zhang Q, Liu B-J, Ren W-W, He Y-P, Li X-L, Zhao C-K, et al. Association between BRAF V600E mutation and ultrasound features in papillary thyroid carcinoma patients with and without hashimoto's thyroiditis. Sci Rep 2017;7:1-8.
28. Xing M, Clark D, Guan H, Ji M, Dackiw A, Carson KA, et al. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. J Clin Oncol 2009;27:2977-82.