Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 9 (19): 3593-3597, 2009
ISSN 1812-5654

Prevalence of BRAF^{V600E} Mutation in Iranian Patients with Papillary Thyroid Carcinoma: A Single-Center Study

[1]J. Mohammadi-Asl, [2]B. Larijani, [3]Z. Khorgami, [4]S.M. Tavangar, [2]V. Haghpanah and [1]P. Mehdipour
[1]Department of Medical Genetics, School of Medicine,
Tehran University of Medical Sciences, Tehran, Iran
[2]Endocrinology and Metabolism Research Center, School of Medicine,
Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Surgery,
[4]Department of Pathology, Shariati Hospital, School of Medicine,
Tehran University of Medical Sciences, Tehran, Iran

Abstract: The aim of this study was to investigate the frequency of the BARF^{V600E} mutation in Papillary Thyroid Carcinoma (PTC) in Iranian population through PCR-RFLP. Fifty formalin-fixed paraffin-embedded and 26 frozen thyroid tumors including 28 PTCs, two undifferentiated thyroid carcinoma and 46 benign thyroid tumors were evaluated. The BARF^{V600E} mutation was detected in 20 of 28(71.4%) PTCs, but failed to distinguish the mutant allele in benign thyroid tumors. The age, sex, extrathyroid extension and lymph node metastases distribution did not, significantly, differ between the patients with and without the BARF^{V600E} mutation. In conclusion, these findings might pave our way towards considering the BRAF^{V600E} mutation in PTCs in the regions with high prevalence of this alteration as a molecular marker.

Key words: Papillary thyroid carcinoma, mutation, BARF^{V600E}, PCR-RFLP

INTRODUCTION

Thyroid carcinoma is the most common endocrine malignancy. Recently, Kilfoy *et al.* (2009), reported that the world wide incidence rate of thyroid carcinoma varies between 0.7 to 3.4 and 1.8 to 11.8 per 100,000 populations for men and women, respectively in 19 different populations from 1998 to 2002. The thyroid cancer prevalence in Iran has been reported 1.0 and 3.5 per 100,000 population for male and female, respectively and the most frequent morphologic variant is Papillary Thyroid Carcinoma (PTC) with a frequency of 69.9% (Larijani *et al.*, 2005). The BRAF mutations have been found in a variety of human cancers, most notably in melanoma. T1799A transversion (formerly known as T1796A) in exon 15, which is the most common BRAF mutation, accounts for more than 80% of BRAF mutations (Davies *et al.*, 2002). The T1799A mutation results in constitution of Glutamate (E) for Valine (V) (V600E).

This missense mutation alters the conformation of the activation loop in the BRAF kinase domain and activates its kinase activity by simulating phosphorylation. This activation leads to tumorogenesis through the RAS-RAF- MEK-ERK-MAP kinase pathway (Davies *et al.*, 2002). Although, the BRAF^{V600E} mutation is reported to have association with more aggressive tumor phenotype and a higher risk of recurrence and persistent disease in patients with conventional PTC (Kebebew *et al.*, 2007; Xing *et al.*, 2005; Kim *et al.*, 2006; Nikiforova *et al.*, 2003; Xu *et al.*, 2003; Lee *et al.*, 2007), the reports are controversial (Kim *et al.*, 2005; Liu *et al.*, 2005; Ito *et al.*, 2009). Preoperative detection of PTCs by a specific molecular cancer maker, such as BRAF mutation, would specially be helpful for predicting disease aggressiveness while determining the need for and extent of surgery, as well as, the need for adjuvant therapy and follow-up monitoring (Kebebew *et al.*, 2007). As to our knowledge, there is no published data reporting BRAF^{V600E} mutation in Iranian population. The aim of this study was to analyze the prevalence of the BRAF^{V600E} mutation in Iranian PTC patients and its applicability in the diagnosis of PTC.

MATERIALS AND METHODS

Thyroid samples: This study was conducted in Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran from

Corresponding Author: Parvin Mehdipour, Department of Medical Genetics, School of Medicine,
Tehran University of Medical Sciences, Tehran, Islamoic Republic of Iran Tel/Fax: 00982188953005

2007 through 2008. A total of 50 Formalin Fixed Paraffin Embedded (FFPE) samples were obtained from patients who underwent thyroidectomy surgery from 2006 to 2007 in referral Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. The tissue samples of total of 50 patients, consisting of 25 malignant tumors and 25 non-malignant samples were reviewed by a pathologist. Also, a total of 26 fresh thyroid tissues were obtained from the patients who underwent surgery in 2008 in referral Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. Tissue specimens were put in RNAlater (Ambion, Austin, USA) and frozen immediately at -80°C until use. Final Histologic classification was obtained finally from pathologic reports. The samples included 5 malignant and 21 non-malignant tumors. This study was approved by the Ethics and Research Committee of Tehran University of Medical Sciences and was conducted in accordance with the Declaration of Helsinki principles.

Cell line samples, Laser Capture Microdissection (LCM) and DNA extraction: Previously extracted DNA of cell lines, ARO and 8305C (heterozygous for $BRAF^{V600E}$ mutation), 8505C (homozygous for $BRAF^{V600E}$ mutation) and finally TPC-1 (wild type BRAF gene), were used as positive (heterozygous and homozygous mutation) and negative controls, respectively. The 6 μM FFPE tissue sections were deparafinized and stained with Methyl Green (Sigma-Aldrich, St. Louis, MO) and then manually dissected or using laser capture microdissection system (Leica AS LMD, Wetzler, Germany). The DNA from dissected FFPE samples was extracted by using QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA from frozen tissues was extracted by conventional Phenol-Chloroform method. Quality and quantity of the extracted DNAs were determined by spectrophotometry (NanoDrop ND-1000, Wilmington, Delaware USA).

PCR-RFLP: We amplified BRAF exon 15 by PCR using oligonucleotide primer pairs: 5-TCA TGA AGA CCT CAC AGT AAA AAT-3 (forward) and 5-TGG ATC CAG ACA ACT GTT CAA-3 (reverse), as described previously by Takahashi *et al.* (2007). The PCR was carried out with the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec and elongation at 72°C for 30 sec and a final extenstion at 72°C for 5 min. The PCR products were visualized on 8% polyacrylamid gels with Ethidium Bromide staining.

Then, the amplification products were digested by TspRI (New England Biolabs, Ipswich, MA) restriction endonuclease that cuts normal allele (ACA GTG AAA) but not the T1799A mutant sequence (ACA GAG AAA). The wild type BRAF fragment produced two 47 bp and 52 bp bands, while the mutant BRAF fragment was not digested and produced a 98 bp band. For confirmation of the results, we sequenced the PCR product in the 5 cell lines previously mentioned and some positive and negative mutant samples.

Statistical analysis: All the statistical analyses were performed by the SPSS software. Fisher exact test was used for association of $BRAF^{V600E}$ mutation with age and gender. The p-value of less than 0.05 considered to be significant. The True-Positive (TP), True-Negative (TN), False-Positive (FP) and False-Negative (FN) results were defined. Diagnosis sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy were calculated by using the following formulas:

$$\text{Sensitivity}(\%) = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity}(\%) = \frac{TN}{TN + FP} \times 100$$

$$\text{PPV}(\%) = \frac{TP}{TP + FP} \times 100$$

$$\text{NPV}(\%) = \frac{TN}{TN + FN} \times 100$$

$$\text{Accuracy}(\%) = \frac{TP + TN}{TP + TN + FP + FN} \times 100$$

RESULTS

Overall, the prevalence of $BRAF^{V600E}$ mutation was 20 out of 30 (66.7%) in malignant thyroid carcinoma and 71.4% in PTCs. The prevalence of $BRAF^{V600E}$ mutation regarding to histologic classification of tumours was 72, 100 and 0% in Classic-Variant PTCs (CV-PTC), Tall-Cell variant PTCs (TC-PTC) and Follicular Variant PTC (FV-PTC), respectively (Table 1). It is worthy to note that in one TC-PTC patient the $BRAF^{V600E}$ mutation was present as homozygous. No $BRAF^{V600E}$ mutation was detected in the normal thyroid tissues surrounding the malignant tissue.

The age, sex, extrathyroid extension and lymph node metastases distribution of the patients with the malignant thyroid samples regarding to $BRAF^{V600E}$ mutation status are shown in Table 1. All the 46 benign specimens including 24 goiters, 15 follicular adenomas,

Table 1: $BRAF^{V600E}$ mutation analysis of thyroid histology in 30 malignant and 46 benign thyroid tumors

Histology type	No. of cases	$BRAF^{V600E}$ positive No. of cases (%)	p-value
Papillary thyroid carcinoma	28	20 (71.4)	--
Classic variant	25	18 (72)	
Tall-cell variant	2	2 (100)	
Follicular variant	1	-	
Undifferentiated thyroid carcinoma	2	-	---
Multinodular goiter	24	-	
Hashimato's thyroiditis	7	-	
Folicular adenoma	15	-	
Gender distribution among 28 PTCs patients			
Male	6	4 (66.7)	NS
Female	22	16 (72.2)	
Age distribution among 28 PTCs patients			
≤40 years	13	9 (69.2)	NS
>40 years	15	11 (73.3)	
Extrathyroidal extension among 25 PTCs			
Present	19	11 (57.9)	NS
Absent	6	6 (100)	
Lymph node metastases among 25 PTCs			
Present	14	9 (64)	NS
Absent	11	8 (72.2)	

NS: Non significant, p>0.05

Table 2: Correlation between $BRAF^{V600E}$ mutation presence and malignancy detection in study results

Patient groups (n = 76)	Values
TP	20.00
TN	46.00
FP	0.00
FN	10.00
Sn (%)	66.67
Sp (%)	100.00
PPV (%)	100.00
NPV (%)	82.14
Accuracy (%)	86.84

TP: True positive, TN: True negative, FP: False positive, FN: False negative, Sn: Sensitivity, Sp: Specificity, PPV: Positive predicted value, NPV: Negative predicted value

7 Hashimato's thyroiditis, were $BRAF^{V600E}$ mutation negative. Diagnostic sensitivity, specificity, PPV, NPV and accuracy were 66.67, 100, 100, 82.18 and 86.84%, respectively (Table 2).

DISCUSSION

This study showed $BRAF^{V600E}$ mutation in 71.4% of PTC samples and it seems more common in TC-PTC (100%) comparing to CV-PTC (72%). Also there was no $BRAF^{V600E}$ mutation in benign thyroid lesions and in normal cells surrounding malignant tissues and this finding is consistent with other reports (Nikiforova *et al*., 2003; Xu *et al*., 2003; Xing *et al*., 2004).

The PTCs are usually curable with standard surgical and adjuvant radioiodine treatment but unfortunately lymph node metastases are found in 30 to 65% of cases at the time of initial diagnosis and 15% of tumors with lymph node metastases also display very aggressive behavior, characterized by local invasion, distant metastasis, treatment resistance and increased mortality (Mazzaferri and Kloos, 2001). The use of molecular markers for diagnosis and prognosis of thyroid cancers has been explored extensively. The V600E mutation in BRAF kinase appears to be an attractive molecular marker for thyroid cancer diagnosis and prognosis as it has been found to be the most common genetic events and specific for PTCs (Rowe *et al*., 2006). The aim of present study was to determine the prevalence of the $BRAF^{V600E}$ mutation in an Iranian population and its applicability for PTC detection.

This study showed that the $BRAF^{V600E}$mutation was much more common in PTC patients of this study compared to western countries. As meta-analysis by Lee *et al*. (2007) most studies reported that $BRAF^{V600E}$ mutation is common in PTCs but the reported frequencies vary from 30 to 83% (mean 49%). Some investigators have suggested that diagnostic technical methods and geographic factors may account for the differences in reported prevalences for $BRAF^{V600E}$ mutation in PTCs (Kebebew *et al*., 2007).

Guan *et al*. (2009) recently reported that there is an association between $BRAF^{V600E}$ mutation and Iodine intake. According to their report $BRAF^{V600E}$ mutation was found in 69% of PTCs in high iodine content in natural drinking water but it was found only in 53% of PTCs in normal Iodine content in natural drinking water in China (Guan *et al*., 2009). Present results for $BRAF^{V600E}$ mutation prevalence in PTCs is more similar to those published for Asian (Korean and Chinese) populations (Guan *et al*., 2009; Kim *et al*., 2004). The higher prevalence of $BRAF^{V600E}$ mutation in high Iodine intake in China is in agreement with our results. In agreement to this study reports from Korea, a country with very high iodine intake, indicated that $BRAF^{V600E}$ mutation is highly prevalent (52-83%) in PTC (Kim *et al*., 2004, 2005;

Chung *et al*., 2006). In contrast, the prevalence of $BRAF^{V600E}$ mutation in western countries, Such as Spain and Italy showed low prevalence (37-42%) (Lee *et al*., 2007). According to the results we can propose that the high prevalence of $BRAF^{V600E}$ mutation may be due to high Iodine intake in Iran.

The higher frequency of $BRAF^{V600E}$ mutation in the present study might be due to the probable increase in iodine intake after starting a mass programme of iodine supplementation implemented since 1983 in Iran (Larijani *et al*., 2005). However, this proposal requires definition by further studies. Although, we found that $BRAF^{V600E}$ mutation occurred slightly more in females, this was not statistically significant ($p>0.05$). The $BRAF^{V600E}$ mutation was observed in 73.3% of PTC patients older than 40 years old and in 69.2% of PTC patients younger than 40 years old. $BRAF^{V600E}$ mutation prevalence was slightly more common in older patients than in younger patients, but this was not also a significant difference ($p>0.05$). Present results is in agreement with the results of a meta-analysis study on 1168 PTC patients that showed there were not significant association between $BRAF^{V600E}$ mutation and age or gender (Lee *et al*., 2007).

The results of present research showed that there is not significant association between $BRAF^{V600E}$ and extrathyroidal extension or lymph node metastases that is in agreement with some previous reports (Lee *et al*., 2007; Ito *et al*., 2009).

In this study, we confirmed that $BRAF^{V600E}$ mutation was specific for PTC (100%), because no benign thyroid samples were found to harbor $BRAF^{V600E}$ mutation and all the samples that were positive for $BRAF^{V600E}$ mutation were malignant. This result is consistent with previous reports (Rowe *et al*., 2006; Xing, 2005). In the present study, the diagnostic sensitivity and specificity were 66.67 and 100%, respectively. Chung *et al*. (2006) reported the sensitivity of 83.0% and a specificity of 96.0% for direct DNA sequencing method and the sensitivity of 79.6% and a specificity of 80.0% for PCR-RFLP method for detection of PTC in Fine Needle Aspiration Biopsy (FNAB) samples.

High specificity in our study indicates the advantage of $BRAF^{V600E}$ mutation for PTCs detection. However, the method based on PCR-RFLP is enough sensitive to detect $BRAF^{V600E}$ mutation even in the samples of FFPE tissue and when the DNA yield was low (Kumagai *et al*., 2007). The lower sensitivity might be explained by the fact that diagnostic sensitivity of $BRAF^{V600E}$ mutation strictly depends upon its prevalence in PTC patients.

In conclusion, these findings might pave our way towards considering the $BRAF^{V600E}$ mutation in PTCs in the regions with high prevalence of this alteration as a molecular marker.

ACKNOWLEDGMENT

We want to thank K. Hamatani (Hiroshima, Japan) for the donating of cell lines DNA and core facilities to perform microdisection of FFPE samples.

REFERENCES

Chung, K.W., S.K. Yang, G.K. Lee, E.Y. Kim and S. Kwon *et al*., 2006. Detection of BRAFV600E mutation on fine needle aspiration specimens of thyroid nodule refines cyto-pathology diagnosis, especially in BRAF600E mutation-prevalent area. Clin. Endocrinol. (Oxf), 65: 660-666.

Davies, H., G.R. Bignell, C. Cox, P. Stephens and S. Edkins *et al*., 2002. Mutations of the BRAF gene in human cancer. Nature, 417: 949-954.

Guan, H., M. Ji, R. Bao, H. Yu and Y. Wang *et al*., 2009. Association of high iodine intake with the T1799A BRAF mutation in papillary thyroid cancer. J. Clin. Endocrinol. Metab., 94: 1612-1617.

Ito, Y., H. Yoshida, R. Maruo, S. Morita and T. Takano *et al*., 2009. BRAF mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. Endocr J., 56: 89-97.

Kebebew, E., J. Weng, J. Bauer, G. Ranvier and O.H. Clark *et al*., 2007. The prevalence and prognostic value of BRAF mutation in thyroid cancer. Ann. Surg., 246: 466-470.

Kilfoy, B.A., T. Zheng, T.R. Holford, X. Han and M.H. Ward *et al*., 2009. International patterns and trends in thyroid cancer incidence, 1973-2002. Cancer Causes Control, 20: 525-531.

Kim, K.H., D.W. Kang, S.H. Kim, I.O. Seong and D.Y. Kang, 2004. Mutations of the BRAF gene in papillary thyroid carcinoma in a Korean population. Yonsei Med. J., 45: 818-821.

Kim, T.Y., W.B. Kim, J.Y. Song, Y.S. Rhee and G. Gong *et al*., 2005. The BRAF mutation is not associated with poor prognostic factors in Korean patients with conventional papillary thyroid microcarcinoma. Clin. Endocrinol. (Oxf)., 63: 588-593.

Kim, T.Y., W.B. Kim, Y.S. Rhee, J.Y. Song and J.M. Kim *et al*., 2006. The BRAF mutation is useful for prediction of clinical recurrence in low-risk patients with conventional papillary thyroid carcinoma. Clin. Endocrinol. (Oxf)., 65: 364-368.

Kumagai, A., H. Namba, Z. Akanov, V.A. Saenko and S. Meirmanov *et al*., 2007. Clinical implications of pre-operative rapid BRAF analysis for papillary thyroid cancer. Endocr J., 54: 399-405.

Larijani, B., M.A. Mohagheghi, M.H. Bastanhagh, A.R. Mosavi-Jarrahi and V. Haghpanah *et al*., 2005. Primary thyroid malignancies in Tehran, Iran. Med. Princ. Pract., 14: 396-400.

Lee, J.H., E.S. Lee and Y.S. Kim, 2007. Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid: A meta-analysis. Cancer, 110: 38-46.

Liu, R.T., Y.J. Chen, F.F. Chou, C.L. Li and W.L. Wu *et al*., 2005. No correlation between BRAFV600E mutation and clinicopathological features of papillary thyroid carcinomas in Taiwan. Clin. Endocrinol. (Oxf)., 63: 461-466.

Mazzaferri, E.L. and R.T. Kloos, 2001. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. J. Clin. Endocrinol. Metab., 86: 1447-1463.

Nikiforova, M.N., E.T. Kimura, M. Gandhi, P.W. Biddinger and J.A. Knauf *et al*., 2003. BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J. Clin. Endocrinol. Metab., 88: 5399-5404.

Rowe, L.R., B.G. Bentz and J.S. Bentz, 2006. Bentz, Utility of BRAF V600E mutation detection in cytologically indeterminate thyroid nodules. Cytojournal, 3: 10-10.

Takahashi, K., H. Eguchi, K. Arihiro, R. Ito and K. Koyama *et al*., 2007. The presence of BRAF point mutation in adult papillary thyroid carcinomas from atomic bomb survivors correlates with radiation dose. Mol. Carcinog, 46: 242-248.

Xing, M., R.P. Tufano, A.P. Tufaro, S. Basaria and M. Ewertz *et al*., 2004. Detection of BRAF mutation on fine needle aspiration biopsy specimens: A new diagnostic tool for papillary thyroid cancer. J. Clin. Endocrinol. Metab., 89: 2867-2872.

Xing, M., 2005. BRAF mutation in thyroid cancer. Endocr Relat Cancer, 12: 245-262.

Xing, M., W.H. Westra, R.P. Tufano, Y. Cohen and E. Rosenbaum *et al*., 2005. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J. Clin. Endocrinol. Metab., 90: 6373-6379.

Xu, X., R.M. Quiros, P. Gattuso, K.B. Ain and R.A. Prinz, 2003. High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. Cancer Res., 63: 4561-4567.