Volume 8
Issue 1
March 2024
ISSN: 3027-5008 (Online)
ISSN: 3027-5016 (Print)

www.kosovajournalofsurgery.net

BRAF (V600E) Mutation in Papillary Thyroid Carcinoma Single Center Experience

Ilir Vela[1], Igor Dzikovski[1], Nexhati Jakupi[1], Goran Kondov[1], Risto Colancevski[1], Argjent Muça[2], Slavica Kostadinova Kunovska[3], Magdalena Bogdanovska Todorovska[3], Sinisa Stojanoski[4]

[1] Clinic for Thoracic and Vascular Surgery,
Clinical Center "Mother Teresa" - Skopje, R.N. Macedonia.
[2] Clinic of Endocrinology, diabetes and metabolic diseases,
Clinical Center Mother Teresa - Skopje, R.N. Macedonia.
[3] Institute of Pathology, Medical Faculty Skopje, R.N. Macedonia.
[4] Institute of Nuclear Medicine and Pathological Physiology, Medical Faculty Skopje, R.N. Macedonia

Corresponding Author:

ILIR VELA

Department of Thoracic Surgery, University Clinic for Thoracic and Vascular Surgery,
Medical Faculty Skopje, Republic of North Macedonia.
E-mail: *ilir.vela@medf.ukim.edu.mk*

Abstract

BRAF has an important role in cellular proliferation, differentiation, and programmed cell death. The BRAF(V600E) gene mutation has recently been found to be present in various types of cancers. Studies on the BRAF(V600E) mutation in thyroid cancer are intensively examined in various countries, showing that the presence of the BRAF(V600E) mutation varies and ranges from 28% to 86% in papillary thyroid carcinoma. Papillary thyroid carcinoma is the most common type of endocrine malignancy, accounting for 85–90% of all thyroid cancers. However, there is a lack of sufficient studies in the Republic of Macedonia regarding the expression of the BRAF(V600E) mutation in papillary thyroid carcinoma. The aim of the study was to analyze the expression of the gene mutation BRAF(V600E) in papillary thyroid carcinoma and the correlation between expression and clinicopathological characteristics. The study population consisted of 35 patients. The cobas4800 BRAFV600E Mutation IVD test was used for detection of mutations in the BRAF gene, which was performed on the Cobas Z 480 IVD Real-Time PCR instrument (Roche Diagnostic). The test kit included appropriate controls (wild type and mutant). Patients with lymph node metastases had a higher risk of BRAF V600E gene mutation compared to patients without lymph node metastasis of the tumor. Greater patient age, invasion of the capsule, multifocality, and male gender were factors associated with increased expression of the BRAF V600E gene mutation.

Key words. papillary thyroid carcinoma, expression, BRAF(V600E) mutation.

Introduction

Papillary thyroid carcinoma, accounting for approximately 80% of all malignant thyroid gland diseases, is a common endocrine malignancy. [1–3] Similar to other carcinomas, the development of thyroid carcinomas requires the initial occurrence of uncontrolled cell division, leading to the accumulation of mutations, the most common of which are BRAF and RAS mutations in thyroid cancer.

The development of thyroid cancer requires interaction between environmental, genetic, and hormonal factors, which together affect cell division. These factors can influence the development of mutations in oncogenes and tumor suppressor genes, leading to uncontrolled cell division. The BRAF gene encodes a protein belonging to the Raf kinase family, which plays an important role in regulating the MAP kinase signaling pathway, which in turn affects cell division, differentiation, and secretion. The most common mutation in the BRAF gene is V600E, where the amino acid valine (V) is replaced by glutamic acid (E) at amino acid 600.[4] , [5] Signaling through proteins produced by the BRAF gene is essential for the normal development of cells before they reach functional maturity. The BRAF gene belongs to a group of genes known as oncogenes. When they mutate, oncogenes have the potential to transform normal cells into cancerous ones. The BRAF mutation is the most common genetic change in thyroid cancer. [6] This mutation is associated with a more aggressive clinicopathological course, including a higher rate of extrathyroidal extension and lymph node metastasis. [7–10]

The BRAF V600E mutation is present in 28% to 86% of patients with thyroid cancer. [9] ,[11] ,[12] It is most commonly found in papillary thyroid carcinoma, but in some cases, it is also present in poorly differentiated thyroid carcinomas and anaplastic carcinomas associated with papillary thyroid carcinoma. [12]

Several well-known health centers worldwide have included the detection of the BRAF V600E mutation in the algorithm for managing patients with thyroid cancer. Specifically patients with BRAF-positive tumors on fine-needle aspiration, who undergo prophylactic central neck dissection.

Several studies have attempted to establish a correlation between BRAF (V600E) and histopathological and clinical prognostic parameters, such as the invasion of the thyroid gland capsule, extrathyroidal invasion, and the presence of lymph node or distant metastasis in papillary thyroid carcinoma, but the results are controversial. [9],[13] In some studies, BRAF (V600E) was associated with a more advanced tumor or a more aggressive phenotype. In this study, we aimed to examine the expression of the BRAFV600E mutation and the established prognostic factors in papillary thyroid carcinoma.

Materials and methods

This study was conducted at the University Clinic for Thoracic and Vascular Surgery at the University "St. Cyril and Methodius" in Skopje, Republic of Macedonia, while immunohistochemical and molecular analyses of surgical specimens will be analyzed at the Institute of Pathological Anatomy. The study population consists of 35 patients who meet all inclusion criteria and no exclusion criteria. The study is planned to be performed as a retro-prospective, cohort study. Prior to surgery, preoperative evaluations such as thyroid gland ultrasound, fine-needle biopsy, and gland scan will be conducted at the Institute of Nuclear Medicine and Pathological Physiology. Patients diagnosed with papillary carcinoma will undergo a neck computed tomography with intravenous contrast at the Radiology Clinic. Laboratory investigations will be carried out at the Institute of Clinical Biochemistry.

During the surgical procedure, total thyroidectomy is performed under general anesthesia and endotracheal intubation in all cases. A 4 to 6 cm incision is made in the lower portion of the neck. The subcutaneous tissue and platysma are then prepared and come to the group of infrahyoid muscles. The thyroid gland is visualized and entirely removed with caution to avoid damage to the parathyroid glands and recurrent laryngeal nerve. Elective neck dissection is performed if enlarged lymph nodes are present

In this retro-prospective study, 35 surgical specimens from thyroidectomies and thyroidectomies with lymph nodes will be analyzed at the Institute of Pathological Anatomy, which will be fixed in formalin and embedded in paraffin. The same will be analyzed by immunohistochemistry. The 8th edition of UICC TNM classification of thyroid cancer will be used for papillary carcinomas classification.

Tissue sample: After completing the macroscopic analysis, a standard number of samples will be taken for routine histological analysis, one representative sample of tumor tissue for immunohistochemical staining, which will also be used to determine the V600E (1799T>A) mutation in the BRAF gene.

Molecular analyses will be performed using tissue sections from paraffin blocks. The cobas4800 BRAFV600E

Mutation IVD test will be used to detect BRAF gene mutations. The Cobas Z 480 IVD Real-Time PCR instrument (Roche Diagnostic) will be utilized for the PCR amplification and detection of target DNA using a complementary primer and two oligonucleotide probes, one for the detection of wild-type BRAF V600 sequence and the other for the detection of V600E mutated sequence. The test kit will also include appropriate controls (wild type and mutant).

Results

During the determination of predictive values for factors such as gender, age, tumor size, capsule invasion, vascular invasion, lymph node metastasis, and multifocality regarding the BRAF gene mutation, the enter method was utilized. The discrimination model was employed to evaluate the predictive performance of the model. The overall accuracy of the model in predicting BRAF gene mutation was found to be 74.30%. Furthermore, the sensitivity and specificity values of the model were determined to be 88.80% and 25.00%, respectively, as presented in Table 19.

Table 19. Predictive values for gender, age, tumor size, capsule invasion, vascular invasion, lymph node metastasis, and multifocality for the BRAF gene mutation / Discrimination model.

Observed			Predicted		
			BRAF mutation		Percentage Correct
			Negative	Positive	
Step 1	BRAF mutation	Negative	2	6	25,0
		Positive	3	24	88,9
	Overall Percentage				74,3

a. The cut value is ,500

When establishing the significance of the contribution to predicting BRAF gene mutation, it was found that tumor size had the greatest influence (Wald = 4.643 / $p < 0.05$ ($p = 0.031$)), followed by lymph node metastasis (Wald = 1.359 / $p > 0.05$ ($p = 0.244$)), age (Wald = 0.785 / $p > 0.05$ ($p = 0.376$)), capsule invasion (Wald = 0.782 / $p > 0.05$ ($p = 0.377$)), and patient gender (Wald = 0.712 / $p > 0.05$ ($p = 0.399$)), while multifocality had a weaker influence (Wald = 0.008 / $p > 0.05$ ($p = 0.930$)) (Table 19.1).

As the size of the tumor increases by one unit (1 cm), the risk of BRAF gene mutation decreases by 59.3% (Exp(B) = 0.407) / 95% C.I.for EXP(B) / 0.180-0.922 /, which is significant for $p < 0.05$ ($p = 0.031$).

Patients with lymph node metastasis (1) are at 14.89 times greater risk for BRAF gene mutation (Exp(B) = 14.892) than patients without lymph node metastasis, 95% C.I.for EXP(B) / 0.159-1396.63 /, which is not significant for $p > 0.05$ ($p = 0.244$).

As the age of the patients increases by one unit (one year), the risk of BRAF gene mutation increases by 3.3% (Exp(B) = 1.033) / 95% C.I.for EXP(B) / 0.962-1.109 /, which is not significant for $p > 0.05$ ($p = 0.376$).

Patients with capsule invasion (1) are at 6.35 times greater risk for BRAF gene mutation (Exp(B) = 6.347) than patients without capsule invasion, 95% C.I.for EXP(B) / 0.106-381.34 /, which is not significant for $p > 0.05$ ($p = 0.377$).

Male patients (1) are at 3.52 times greater risk for BRAF gene mutation (Exp(B) = 3.521) than female patients, 95% C.I.for EXP(B) / 0.198-65.455 /, which is not significant for $p > 0.05$ ($p = 0.399$).

Table 19.1 Binary Logistic Regression Analysis for Prediction of BRAF Gene Mutation.

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Gender(1)	1,259	1,491	,712	1	,399	3,521	,189	65,455
	Age	,032	,036	,785	1	,376	1,033	,962	1,109
	Tumor size	(,898)	,417	4,643	1	**,031**	,407	,180	,922
	Capsule invasion(1)	1,848	2,090	,782	1	,377	6,347	,106	381,339
	Vascular invasion(1)	(,941)	2,080	,205	1	,651	,390	,007	22,991
	Lymph nodes(1)	2,701	2,317	1,359	1	,244	14,892	,159	1396,631
	Multifocoaity(1)	,109	1,243	,008	1	,930	1,116	,098	12,749
	Constant	1,125	1,775	,402	1	,526	3,081		

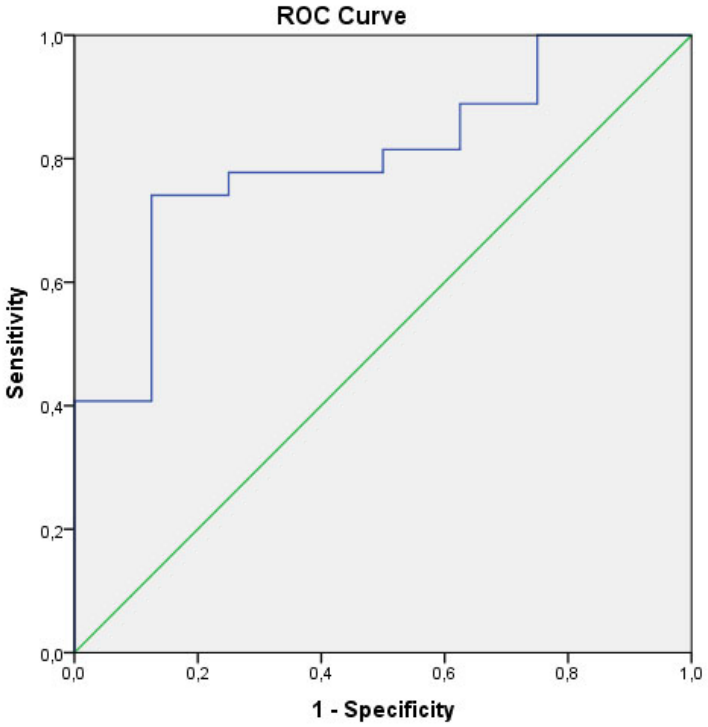
a. Variable(s) entered on step 1: Gender, Age, Tumor Size, Capsule Invasion, Vascular Invasion, Glands, Multifocality.

Patients with vascular invasion (1) are at 0.39 times less risk for BRAF gene mutation (Exp(B) = 0.390) than patients without vascular invasion, 95% C.I.for EXP(B) / 0.007-22.991 /, which is not significant for p > 0.05 (p = 0.651).

Patients with multifocality (1) have a 1.12 times higher risk for BRAF gene mutation compared to patients without multifocality (Exp(B) = 1.116), with a 95% confidence interval for EXP(B) ranging from 0.098 to 12.749, which is not statistically significant with p>0.05 (p=0.377).

The ROC area is 0.801, which means that for 80.10% / 95% CI: 0.641-0.961 / p <0.05 (p = 0.011) / of all possible pairs in which one has a BRAF gene mutation and the other does not have a BRAF gene mutation, this model will determine a higher probability of BRAF gene mutation (Figure 19).

Figure. 1



Discussion

The mutations in BRAF oncogenes have been associated with various types of human carcinomas, but the strongest correlation has been demonstrated in malignant melanoma. [9] The BRAF mutation is one of the most common genetic alterations found in papillary thyroid carcinoma, with the BRAF V600E mutation present in up to 70-80% of these cancers. In contrast, this mutation has not been found in benign thyroid neoplasms, follicular or medullary carcinomas. [13],[14] Consequently, there is a need to analyze the expression of BRAF mutations in papillary thyroid carcinoma, given that the detection of this mutation is associated with an overall poor prognosis. Studies have shown that papillary thyroid carcinomas with the BRAF V600E mutation tend to be larger, have a higher rate of lymph node metastasis, and exhibit increased recurrence rates after treatment. [9]

Furthermore, identifying the BRAF mutation in papillary thyroid carcinoma may impact disease management. For example, patients with this mutation may benefit from more aggressive surgical interventions, such as total thyroidectomy or central lymph node dissection, resulting in better outcomes. These patients may also be candidates for BRAF inhibitor therapy, which has shown great promise. [15]

Furthermore, the BRAF mutation can serve as a biomarker to monitor a patient's response to treatment and to detect potential signs of recurrence. On the one hand, a decrease in BRAF mutation expression after treatment may indicate a favorable treatment response and successful outcome. On the other hand, an increase in expression is often a sign that the tumor is becoming more aggressive and requires further intervention.

In summary, analyzing BRAF mutations and their expression in papillary thyroid carcinoma is a critical component of both diagnosis and treatment, as it provides insight into tumor behavior and helps to determine the most appropriate therapy for each patient.

Conclusion

Patients with lymph node metastases have a higher risk of BRAF V600E gene mutation than those without lymph node metastases. Increasing patient age, capsule invasion, multifocality, and male gender are associated with a greater expression of BRAF V600E gene mutation. As tumor size and vascular invasion increase, the expression of BRAF V600E is reduced. These findings suggest that the presence of lymph node metastases and other clinicopathologic features may be useful in predicting BRAF V600E mutation status and may have implications for treatment decision-making in patients with this mutation.

References

1.Howlader N , Noone Am, Krapcho Met al. , SEER Cancer Statistics Review,1975-2013.Bethesda, MD: National Cancer Institute; 2016.

2. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. "Mutations of the BRAF gene in human cancer" (PDF). Nature. 2012;417(6892): 949–54. doi:10.1038/nature00766. PMID 12068308.

3. Ritterhouse LL, Barletta JA. "BRAF V600E mutation-specific antibody: A review". Seminars in Diagnostic Pathology. 2015;32(5): 400–8.

4. Xing M. BRAF mutation in thyroid cancer. Endocr Relat Cancer. 2005;12:245–262

5. Basolo F, Torregrossa L, Giannini R. Correlation between the BRAF V600E mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. J Clin Endocrinol Metab. 2010; 95:4197–4205

6. Frasca F, Nucera C, Pellegriti G. BRAF(V600E) mutation and the biology of papillary thyroid cancer. Endocr Relat Cancer. 2008;15:191–205

7. Guan H, Ji M, Bao R. Association of high iodine intake with the T1799A BRAF mutation in papillary thyroid cancer. J Clin Endocrinol Metab. 2009;94:1612–1617

8. Kim J, Giuliano AE, Turner RR. Lymphatic mapping establishes the role of BRAF gene mutation in papillary thyroid carcinoma. Ann Surg. 2006;244:799–804

9. Lee JH, Lee ES & Kim YS 2007 Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid: a meta-analysis. Cancer 110

10. Xing M BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocrine Reviews. 2007;28:742–762.

11. Trovisco V, Soares P, Sobrinho-Simoes M. B-RAF mutations in the etiopathogenesis, diagnosis, and prognosis of thyroid carcinomas. Hum Pathol. 2006;37:781–786.

12. Begum S, Rosenbaum E, Henrique R, Cohen Y, Saransk D, Westar WH. BRAF mutations in anaplastic thyroid carcinoma: implications for tumor origin, diagnosis and treatment. Mod Pathol. 2004; 17:1359–1363

13. 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):4559-70. doi: 10.1210/jc.2012-2104. Epub 2012 Oct 9. PMID: 23055546; PMCID: PMC3513529

14. Davies H,Bignell GR,Cox C, et al. Mutations of the BRAF gene in human cancer. Nature. 2002; 417: 949–954

15. Salerno P, De Falco V, Tamburrino A, Cytostatic activity of adenosine triphosphate-competitive kinase inhibitors in BRAF mutant thyroid carcinoma cells. *J Clin Endocrinol Metab* 2010; 95:450–455