Histological and Immunohistochemical Braf V600e Mutation Detection in Papillary Thyroid Carcinoma

Juliana Escobar
Fundación Valle del Lili
Guillermo Edinson Guzmán
Fundación Valle del Lili
Maria Alejandra Urbano
Fundación Valle del Lili
Laura Juliana Ballen (laura.ballen@fvl.org.co)
Fundación Valle del Lili
Veline Martínez
Fundación Valle del Lili
Ana Arrunategui
Fundación Valle del Lili

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Abstract

Background: Differentiated thyroid cancer is the most frequent malignant endocrine neoplasia in the world. Papillary thyroid cancer (PTC) is the most common type and has the best prognosis. BRAF mutations are reported in 40–70% of patients and are associated with worse prognostic factors.

Materials and methods: A bispective diagnostic test study was carried out by real-time PCR, immunohistochemical and histological tests in patients diagnosed with thyroid cancer between 2013–2016 and who participated in a study of the BRAF V600E mutation at the Fundación Valle del Lili University Hospital (FVL) in Cali Colombia.

Results: Samples from 18 patients with papillary thyroid carcinoma were evaluated by a real-time PCR test for the BRAF gene. It was determined that 69% of the samples had histological characteristics suggestive of the mutation; however, when compared with PCR, the performance of the histological observations had a sensitivity of 100% and specificity of 75%. On the other hand, immunohistochemistry showed a sensitivity of 93% and a specificity of 50%.

Conclusion: Immunohistochemistry and histology features can be used as an alternative test to molecular sequencing. Compared with PCR, these tests show great sensitivity and low specificity. Molecular confirmation of the mutation is necessary.

Highlights

- BRAF gene is reported to be mutated in 40–70% of thyroid cancer cases.
- We evaluated the mutation detection performance in papillary thyroid carcinoma.
- Molecular confirmation of the mutation is necessary for making accurate clinical decisions.

Introduction

Differentiated thyroid cancer is the most common endocrine malignancy, accounting for 1.7% of all malignancies in the world[1, 2]. Its incidence continues to increase due to the high rates of diagnosis by imaging and the greater survival of the population [3]. Clinically, it can range from incipient tumors with a low risk of mortality to highly aggressive cancers, which depend mainly on the histopathological classification.

Genetic mutations associated with thyroid cancer are usually mutually exclusive and are related to sustained activation of the MAPK (mitogen-activated protein kinase) pathway. In papillary thyroid cancer (PTC), the BRAF gene is reported to be mutated in 40–70% of cases, with the substitution of glutamic acid for valine at amino acid 600 (V600E) being the most frequent mutation[4, 5]. This mutation is associated with greater recurrence, metastatic lymph nodes, extrathyroid extension and advanced disease stage; therefore, the detection of this mutation becomes a prognostic parameter. On the other

hand, thanks to advances in therapeutics, genotyping directs oncospecific treatment to improve patient outcomes [6].

Different techniques for BRAF mutation detection have been developed based on polymerase chain reaction (PCR). These are expensive and time-consuming methods [7–10]. Immunohistochemistry can be used as a strategy in oncological genotyping and treated as a surrogate for molecular analysis showing an acceptable correlation [11]. However, a confirmatory molecular analysis by PCR should always be conducted [12, 13].

Numerous studies have shown that tall cell and classic histologic variants, as well as the presence of certain histologic features, are more frequently associated with the BRAF mutation. These histological characteristics include the presence of complete nuclear changes (oval nuclei, overlapping, clear nuclei, irregular nuclear membrane, intranuclear fissures and pseudoinclusion), cells with round eosinophilic cytoplasm (“plump pink cells”), psammoma bodies, infiltrating tumor borders, stromal reaction associated with a tumor that includes desmoplasia, fibrosis and sclerosis. It has been reported that the presence of some of these histological characteristics shows a high sensitivity (> 80%) and a lower specificity (41–71%) for the presence of the BRAF mutation (16).

In the present study, we evaluated the mutation detection performance of the BRAFV600E gene in papillary thyroid carcinoma by histology and immunohistochemistry compared to PCR at the Fundación Valle de Lili, level IV care center.

Materials And Methods

A bispective diagnostic test study was carried out at the Fundación Valle del Lili University Hospital (FVL) in Cali Colombia in men and women over 18 years of age with a diagnosis of thyroid cancer and treated during 2013–2016 and who participated in the BRAF V600E mutation study. Patients with anaplastic carcinoma were excluded based on real-time PCR test results. A nonprobabilistic sampling of 20 patients with the aforementioned diagnosis was carried out, and these patients received approval by their insurance carrier for all the diagnostic methods required. The study was approved by Fundación Valle del Lili’s Ethics Committee on their meeting No. 360 in September 22–2021. All methods were performed in accordance with the relevant guidelines and regulations

Histological features

The hematoxylin-eosin slides were reviewed, and the histological characteristics indicating a BRAF mutation were evaluated according to the criteria of the pathologist. The criteria included the presence of complete nuclear changes: oval nuclei, overlap, clear nuclei, irregular nuclear membrane, intranuclear clefts and intranuclear pseudoinclusion. Furthermore, it involved evaluating the presence or absence of cells with round eosinophilic cytoplasm (“plump pink cells”), psammoma bodies, infiltrating tumor borders and stromal reaction.

Parameters associated with prognosis, such as vascular invasion, perineural invasion, microscopic extrathyroid extension, mitosis rate, presence of lymph node metastases and surgical margins, were also evaluated. Likewise, other histological parameters were reviewed, such as the presence of Hashimoto's thyroiditis, adenomas, and nodular or diffuse hyperplasia.

Immunohistochemical study

The immunohistochemical analysis was performed on tissue fixed with 10% formalin. The tissues were cut at a thickness of 3 µm, and the monoclonal antibody BRAFV600E (clone VE-1) was used in the VENTANA BenchMark ULTRA automated processing system. Prior to the automated process, the tissues were dried in a conventional oven at 60°C for 30 minutes and subsequently subjected to deparaffinization using EZ Prep (Ventana Medical Systems). Incubation was carried out with the prediluted antibody for 24 minutes at 37°C. The Ultraview DAB IHC detection kit was used according to the manufacturer's recommendations. Tissue sheets were contrasted with hematoxylin II (Ventana Medical Systems) and Bluing Reagent (Ventana Medical Systems). The sheets were dehydrated in 95% alcohol and xylol and mounted on Consul-mount Resin (Thermo Scientific).

Previously validated positive and negative controls were used for specimens with papillary thyroid carcinoma with mutation and without BRAF mutation, respectively.

Immunohistochemical interpretation

Immunohistochemistry was interpreted by pathologists who were blinded to the molecular results of BRAF. Immunohistochemical results were assessed according to the cytoplasmic staining of the tumor cells, as follows [11, 14]:

Negative

The absence of staining or weak staining intensity in less than 10% was considered negative. Additionally, when nuclear staining, colloid staining, monocytes or macrophages were observed the result was considered negative.

Positive

Cytoplasmic staining observed in more than 10% of the tumor cells was considered positive, and it was graded according to its intensity (mild, moderate, severe).

BRAF sequencing

DNA samples were extracted from the paraffin blocks of the biopsies of each patient by an experienced pathologist using the Cobas DNA Sample Preparation kit (Roche®). The BRAF gene mutation test was performed by real-time polymerase chain reaction (RT–PCR) on the COBAS z480 Platform (Roche®, performing specific amplification of exon 15 of BRAF, analyzing the results for the BRAF V600 mutation

using the Cobas BRAF V600 mutation test kit (Roche®), according to the protocol recommended by the commercial company.

In this study, we evaluated the immunohistochemical and histological characteristics and compared them with the BRAF status as determined by PCR.

Statistical analysis

Initially, a univariate analysis was carried out to determine the behavior of the numerical variables. The normality of the variables was determined through a Shapiro Wilk test. Those with a $p > 0.05$ were considered to have a normal distribution and are presented with means and standard deviation, and those that were not presented as median and interquartile range. Categorical variables were presented as proportions, and the groups were compared with chi2. When 20% of the cells in the 2x2 table were less than 5, Fisher's exact test was used. Sensitivity, specificity, and positive and negative predictive values were calculated through contingency tables. Based on the results of the detection of BRAF by immunohistochemistry, the sensitivity, specificity (false-positives and false-negatives), and predictive values of the positive and negative tests were established. All analyses were performed in Excel and Stata 12.

Results

Information was collected from 18 patients diagnosed and treated between 2013–2016 for papillary thyroid carcinoma who underwent immunohistochemical and PCR tests for the BRAF gene mutation. The average age of the patients was 49.28 years, with a range between 28 and 82 years, of which 11.1% (2/18) were men. In regard to the histological characteristics, the classic variety corresponded to 55.56% (10/18), and tall cells corresponded to 44.44%.

Of the samples studied, 50% showed vascular invasion, and 27.78% showed perineural invasion. Fifty percent of the samples had extrathyroid extension, 1 of which had extensive extension; in the others, the extension was minimal (17/18), and extracapsular extension was observed in 33.33%. The presence of complete nuclear features was reported in 66.67% (12/18) patients, and cells with round eosinophilic cytoplasm were identified with great frequency (86%). On the other hand, psammoma bodies were present in 16.67% of the samples. Finally, it was determined that the histological characteristics suggestive of BRAF were present in 15 of 17 samples (88.23%), and it was not possible to obtain results for 1 of the 18 samples (Table 1).

Regarding the BRAF mutation detection by real-time PCR compared with the BRAF immunohistochemical technique, 18 patient samples that were tested by PCR and immunohistochemistry were analyzed, and it was determined that the latter had a sensitivity of 92.8% and 50% specificity. The Kappa Cohen’s was 0.47 and showed a moderate correlation between the two techniques (Table 2). Regarding the histological characteristics, 17 samples of patients who had CRP and histology were analyzed. It was determined that the presence of histological characteristics suggestive of BRAF V600E had a sensitivity

of 100% and a specificity of 75%, with a Kappa Cohen's of 0.76, which shows a good correlation (Table 3).

Discussion

Differentiated thyroid cancer is the most common malignant endocrine neoplasia, and its incidence continues to increase [1, 2]. In the United States (USA), 50,000 cases of PTC were reported in 2013(4), and it is currently the second most frequent malignancy diagnosed in Latinas in this country [15]. In Colombia, the country with the second largest Latino population, the incidence of PTC has shown a similar trend; it is the third most common cancer in the country and affects 14.5 out of every 100,000 inhabitants. [15]. Although it is considered a cancer with a good prognosis, 10% of patients follow an abnormal course, with aggressive biological behavior [16]. It is therefore important to identify indicators of severity early.

The BRAF V600E mutation is specific for PTC and is associated with a worse prognosis [4, 17, 18]. The frequency of this mutation in the present study was 76.1% (16/21) of the cases. We previously reported a higher frequency in our population of individuals diagnosed with PTC who were older than 45 years[18]. Frequencies of up to 90% are found in the literature worldwide [19, 20].

The BRAF V600E mutation has been controversially linked to different aggressive clinicopathological features [16, 19]. In a previous local study, we reported that this mutation is related to greater extrathyroid extension, lymphatic invasion, vascular invasion and lymph node involvement, but no relationship was found with respect to tumor size, multicentrality, bilaterality, Hashimoto's thyroiditis or the presence of metastasis [18]. In the current patient sample, we were able to observe the presence of histological characteristics that have been associated with the BRAF mutation [16]. In our group of patients, the presence of these histological variables showed sensitivity but was not highly specific. This technique could be translated into clinical practice as an option for an initial screening strategy, with mandatory molecular confirmation by PCR.

Most of the methods for BRAF V600E mutation detection are based on molecular tests and genomic sequencing, constituting the gold standard for detection. On the other hand, immunohistochemistry has emerged as a surrogate for these tests. In our case, the BRAF V600E mutation was detected by immunohistochemistry in 83.3% (15/18) of the cases. However, false negatives constituted 66.7% (2/3) of the cases, a nonnegligible percentage. This shows a high sensitivity with poor specificity, in our case less than histology. Limited studies report a lack of specificity for the detection of BRAF mutations by immunohistochemistry [21].

However, a better specificity of 82.2% has been obtained, much higher than that reported in our study [11]. In both cases, the same antibody (clone VE-1) was used, which in the past was shown to detect BRAF V600E mutations in both PTC and melanomas, with a specificity and sensitivity of 100%, in adequately preserved samples [22]; however, different commercial kits were used.

In an 11-study meta-analysis that evaluated the concordance between immunohistochemical techniques and sequencing with real-time PCR, it was determined that the concordance rate was up to 92% among positive samples and 85.8% among negative samples for the BRAF V600E mutation by immunohistochemistry and by PCR. However, different types of cancer were included in the meta-analysis [23]. In thyroid cancer, according to the limited literature, immunohistochemistry does not replace molecular analysis by PCR. Szymonek et al. confirmed this in their study, where the correlations did not exceed 76.2% [24]. In this manner, our study confirms the low performance of immunohistochemistry in papillary thyroid cancer given its low specificity. However, it is necessary to consider certain limitations, such as the limited sample size and the possible inadequate conservation of the biological samples, as it is a retrospective study.

Conclusion

Although a favorable clinical correlation between immunohistochemistry and molecular techniques for the BRAF V600E mutation has been shown in different studies, it does not appear to be the same in PTC. The histopathological criteria to detect the BRAF V600E mutation has a better performance as a screening test. This result indicates that molecular confirmation of the mutation is necessary for making accurate clinical decisions.

Abbreviations

- PTC: Papillary thyroid cancer
- FVL: Fundación Valle del Lili University Hospital
- PCR: polymerase chain reaction
- RT–PCR: real-time polymerase chain reaction

Declarations

Ethics approval and consent to participate: Protocol No. 01729 registered and approved by Fundación Valle del Lili's Ethics Committee on their meeting No. 360 in September 22 - 2021. Patients informed consent has been obtained; is retained by the authors and made available to researchers who meet the criteria for access to confidential data only on request.

Consent for publication: Not applicable

Availability of data and materials: The datasets generated and/or analysed during the current study are not publicly available due sensitive data and confidential information of the participants but are available from the corresponding author on reasonable request

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Author information: Guillermo Edinson Guzmán, Department of endocrinology. Fundación Valle del Lili. Email: Guillermo.guzman@fvl.org.co

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Tables

Table 1. Characterization of the population

Characteristic	Total patients
n (%)	18
Age in years	
Median (IQR)	
Average ± SD	49,28
Rank	(28–82)
Male Gender, n (%)	2 (11.1)
Histological cancer type, n(%)	
Classic	10 (55,56)
Tall cells	8 (44,44)
Vascular invasion, n(%)	9 (50,0)
Perineural invasion, n(%)	5 (27,78)
Mitotic Rate, n(%)	5 (27,78)
Extrathyroid Extension n (%)	9 (50,0)
Surgical Margins n(%)	9 (50,0)
Lymph Node Metastasis, n(%)	13 (72,22)
Extracapsular Extension, n(%)	6 (33,33)
Hashimoto Disease, n(%)	10 (55,56)
Nuclear Characteristics, n(%)	
Complete	12 (66,67)
Partial	6 (33,33)
Stromal Reaction, n(%)	16 (88,89)

Continuation of Table 1. Characterization of the population

Plump Pink Cells, n(%)	2 (11,11)
Psammoma Bodies, n(%)	3 (16,67)
Suggestive Histological Characteristics of BRAF n(%)	15 (88,23)
BRAF Immunohistochemistry, n(%)	15 (83,33)

Table 2. Comparison of the detection of BRAF by PCR and by immunohistochemistry

	BRAF immunohistochemistry		Total	P value
BRAF PCR Mutation	No	Yes		
NO	2	2	4	0,108
YES	1	13	14	
Total	3	15	18	

Sensitivity 92,80 (13/14)

Specificity 50 (2/4)

Positive predictive value 83,3 (15/18)

Negative predictive value 66,7. (2/3)

Table 3. Comparison of the detection of BRAF by PCR and by histological predictors.

	Histological characteristics		Total	P Value
BRAF PCR mutation	NO	YES		
NO	2	1	3	0,002
YES	0	14	14	
Total	2	15	17	

Sensitivity 100 (14/14)

Specificity 75 (3/4)

Positive predictive value 87,5. (14/15)

Negative predictive value	100. (3/0)