Association of BRAF V600E Mutation with Tumor Recurrence in a Small Sample of Filipino Patients with Papillary Thyroid Cancer in a Single Center

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ABSTRACT

Background and Objective. Epidemiological studies have shown that Filipinos have a higher prevalence of well-differentiated thyroid cancer and a higher rate of recurrence. The BRAF V600E mutation has been proposed as a potential prognostic marker in aggressive papillary thyroid cancers. In this study, we determined whether this mutation is a risk factor for tumor recurrence in papillary thyroid cancer among Filipinos.

Methods. We conducted an age and sex-matched case-control study of patients with papillary thyroid cancer; we had two groups – with and without tumor recurrence – of 14 patients each, with at least a 5-year follow-up. We extracted the DNA samples from the patients' (paraffin-embedded) tumor biopsy tissue blocks from thyroidectomy specimens, then detected the BRAF V600E mutation using polymerase chain reaction. The McNemar's test for difference of proportions in paired data was used to determine the association of BRAF V600E mutation with recurrence.

Results. The BRAF V600E mutation was found in 57.14% of all cases. We found a prevalence of 64.29% among those with recurrence and 50.00% among those without recurrence, with no significant difference between the two groups (p = 0.688).

Conclusion. Our study showed the BRAF V600E mutation was not associated with recurrence. We encountered several limitations: we had limited data regarding molecular methodologies in the Philippine setting, we had a small sample size, and therefore we could not study other parameters (e.g., tumor characteristics, lymph node metastasis, stage of disease). We hope that this paves the way for future studies and collaborations to establish the role of BRAF V600E in Filipinos with papillary thyroid tumor recurrence.

Poster presented in the 2021 Philippine Society of Endocrinology, Diabetes, and Metabolism, Annual Virtual Convention on March 18-20, 2021.

eISSN 2094-9278 (Online)
Published: June 28, 2023
https://doi.org/10.47895/amp.vi0.4972

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Keywords: papillary thyroid cancer, BRAF mutation, molecular diagnostics, tumor recurrence, V600E mutation

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, of which papillary thyroid carcinoma (PTC) is the most common, accounting for more than 80–90% of all thyroid tumors in iodine-sufficient areas globally. It has an overall excellent prognosis owing to advances in screening via imaging and ultrasound-guided fine-needle aspiration biopsy (FNAB), which have facilitated early detection, cytopathologic diagnosis, and surgery, followed by adjuvant radioactive iodine therapy (RAI).[1]

Epidemiological studies across the world have repeatedly shown a higher incidence and prevalence of thyroid cancer

among Filipinos and more aggressive tumor behavior compared with different nationalities.[2-4] A Philippine population-based cancer registry study found that the age-standardized incidence rate of thyroid cancer was increasing among Filipinos living in Metro Manila and Rizal province.[5] Multi-ethnic studies in North America have also shown that Filipinos, particularly females, have higher incidence rates of thyroid cancer compared with other ethnicities.[3,6,7]

Filipinos have also been found to have a higher risk of recurrence (OR 3.20; 95% CI: 1.23-7.49) compared with other ethnicities.[2] Recurrence is positively associated with age more than 45 years old, multifocality, nodal involvement, distant metastasis at presentation, tumor diameter ≥ 2 cm, and a family history of PTC.[8] Conversely, RAI therapy and low initial titers of thyroglobulin and anti-thyroglobulin antibody are protective.[9]

Due to these characteristics, the University of the Philippines Manila Philippine General Hospital (UP-PGH) local practice guidelines[10] recommend a more aggressive approach than the American Thyroid Association 2015 guidelines.[11] Total or near-total thyroidectomy is recommended for all patients with a cytopathologic diagnosis of PTC, even in low-risk patients defined as those with papillary thyroid cancer (with all of the following): no local or distant metastases; all macroscopic tumor has been resected; no tumor invasion of loco-regional tissues or structures; the tumor does not have aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma); if I-131 is given, there are no RAI-avid metastatic foci outside the thyroid bed on the first posttreatment whole-body RAI scan; no vascular invasion; clinical N0 or <5 pathologic N1 micrometastases (<0.2 cm in largest dimension), intrathyroidal, encapsulated follicular variant of PTC; intrathyroidal, well differentiated follicular thyroid cancer with capsular invasion and no or minimal (<4 foci) vascular invasion; intrathyroidal, papillary microcarcinoma, unifocal or multifocal, including BRAF V600E mutated (if known), because of the better outcomes. High risk patients are those with macroscopic invasion of tumor into the perithyroidal soft tissues (gross extrathyroidal extension), incomplete tumor resection, distant metastases, postoperative serum thyroglobulin suggestive of distant metastases, pathologic N1 with any metastatic lymph node >3 cm in largest dimensions, follicular thyroid cancer with extensive vascular invasion (> 4 foci of vascular invasion).[12] A 10-year follow up of 132 patients with stage I or stage II papillary thyroid cancer seen at the Thyroid Clinic of UP-PGH from January 1986 to December 1990 reports more recurrences (28% versus 0%) and metastases (61.7% versus 17.6%, p<0.01) in patients who underwent non-total thyroidectomy compared to those who had total thyroidectomy plus RAI. Risk stratification is thus imperative in decision-making for patients with PTC.

Molecular biologists have identified several genetic alterations in these tumors that could better stratify patients. These are RET rearrangements (10-15%), RAS point mutations (10–15%), and BRAF point mutations (40–60%), found in PTCs more than 1 cm, and 60–75% of those with known mutations.[13] BRAF is a protein kinase – encoded by the BRAF gene located in chromosome 7q34 – which functions as an intermediary in the RAS/MAPK signaling pathway that regulates cell proliferation, differentiation, movement, and apoptosis.[14]

The BRAF V600E is a specific mutation in the BRAF gene resulting in an altered serine/threonine protein kinase activating the MAP kinase/ERK-signaling pathway mutation. It is proposed as a prognostic marker for risk stratification for PTC. It is associated with more aggressive disease due to activation of MAPK and down-regulation of the sodium-iodide symporter, which decreases RAI avidity,[15,16] stimulating tumor growth and invasion.[17,18] It is also associated with higher recurrence rates, higher stage at presentation,[14,19,20] persistent disease, a higher number of RAI courses to obtain disease-free status,[21] and lower overall survival rate,[22] as shown in Western countries. Given its negative predictive value, it may help identify low-risk – i.e., BRAF-negative – patients who will benefit from adjuvant radioactive iodine ablation therapy.[18]

However, other studies have failed to show this association.[23,24] Among patients in Korea[25] and Japan,[26] the BRAF V600E mutation was not found to be a significant prognostic factor for tumor aggressiveness; this suggests the potential role of racial and genetic differences in terms of disease presentation.[27]

Currently, we have limited data on molecular biomarkers in Filipino patients with thyroid cancers. Navarro-Locsin and colleagues (2016) found that 38.5% of Filipino patients with conventional PTC had the BRAF V600E mutation, and none of them developed recurrence.[4]

This study determined the association of BRAF V600E mutations with tumor recurrence in Filipinos with PTC (specifically, in archived paraffin-embedded biopsy tissue blocks).

METHODS

We conducted an age- and sex-matched case-control study with 28 patients: 14 with recurrence and 14 without recurrence. The study was approved by the University of the Philippines Manila Research Ethics Board (UPM REB Code: 2017-304-01).

Inclusion and Exclusion Criteria

We reviewed the list of PTC patients in the Thyroid Cancer Registry. Patients who underwent total or near-total thyroidectomy followed by adjuvant RAI therapy with available paraffin-embedded biopsy tissue block were included. In this study, cases were defined as patients who presented with tumor recurrence at least six months following treatment, as defined by any of the following: (1) having elevated stimulated (>1 ng/mL) or unstimulated

(>0.20 ng/mL) serum thyroglobulin following thyroidectomy and radioactive ablation; (2) having new-onset or recurrent lymphadenopathies; or (3) having recurrent or new-onset distant metastases (latter two as proven by histopathology or I-131 whole body scan). Controls were defined as patients with no tumor recurrence within five years of follow-up.

Patients were excluded on any of the following: (1) having an unretrievable medical chart, (2) having no follow-up at our outpatient department, (3) having less than five years of follow-up, (4) having an insufficient amount of DNA extracted.

Sample Size

We calculated a minimum sample size of 51 cases and 51 controls using a 95% confidence level and 80% power based on the assumptions of a minimum odds ratio of 4.18, with a proportion of BRAF positive among those with no recurrence (exposed controls) at 67.8% based on the study of Riesco-Elzaguirre.[28]

Histopathologic Examination

The Department of Laboratories of the Philippine General Hospital archives its thyroid histopathologic samples obtained from thyroidectomy for ten years. However, despite all efforts, logistical problems only allowed us to retrieve samples from the last five years from 2013 to 2018. Paraffin-embedded PTC specimens were retrieved, along with the official histopathologic report. Two pathologists reviewed the histopathologic diagnosis of each patient. Hematoxylin and eosin-stained sections were re-examined, documenting tumor size, histologic variant, multifocality, extrathyroidal tumor extension, and presence of lymph node metastasis.

Molecular and Genetic Analysis

Two methods were used to detect the BRAF V600E mutation. The first was Sanger sequencing based on the study by Colanta et al.,[14] wherein we amplified (by polymerase chain reaction) and sequenced the 224 base pair fragments encompassing the coding region of exon 15. The second procedure is allele-specific PCR based on the protocol of Kowalik et al.[29] Forward and reverse primers were used to amplify the target with the mutation at the center. A third mutation-specific primer then detected the presence of the mutation.

Demographic and Clinical Characteristics

We obtained each patient's demographic data and clinical characteristics (family history of thyroid cancer, extent of thyroid surgery, post-operative complications, stage of disease at diagnosis, presence of lymph node metastasis, and recurrence of disease).

We then staged the tumors using the American Joint Commission on Cancer (AJCC) and the International Union Against Cancer [Union Internationale Contre le Cancer (UICC)] 7th Edition/TNM Classification System for differentiated thyroid cancer. Recurrence was documented by imaging studies or the presence of detectable or rising thyroid stimulating hormone-stimulated thyroglobulin levels.

Data Analysis

Clinical, histopathologic, and genetic data were merged into one data set for each patient. Descriptive statistics were used to summarize the general and clinical characteristics of the participants. Frequency and proportion were used for categorical variables. Shapiro-Wilk test was used to determine the normality distribution, while Levene's test was used to determine the homogeneity of variance of continuous variables. Continuous quantitative data that met the normality assumption was summarized using mean and standard deviation (SD), while those that do not were described using median and range. The sign-rank test for difference of medians in paired data while the McNemar's test for difference of proportions in paired data was used to determine the association of BRAF V600E mutation with recurrence for this interim analysis. Missing values were neither replaced nor estimated. We used STATA 15.0 software for all data analyses with a level of significance of $p < 0.05$.

RESULTS

We analyzed data from 28 patients, divided equally between cases and controls (Table 1). Unfortunately, we were not able to reach our calculated sample size as most of the paraffin blocks in our patient census who are on long-term follow-up of more than 5 years cannot be retrieved anymore because the archival time of the stored samples has lapsed. Females comprised 86% of both groups. No one was reported to have a history of malignancy in the family. The majority in both groups were younger than 55 years of age.

TNM staging was unavailable for two cases in the younger age bracket (Table 2). The majority of those under 55 years old were PTC stage I: 9/10 of cases and 13/13 of controls. In contrast, the older subgroup was comprised of stage IV in 2/3 of the cases and stage I in the single control.

Table 1. Demographic characteristics of age- and sex-matched patients with PTC by recurrence

	Overall (n=28)	Cases (Recurrence) (n=14)	Controls (No recurrence) (n=14)
	Mean ± SD; Frequency (%)		
***Age** (years)*	38 ± 12	38 ± 12	37 ± 12
<55	25 (89)	12	13
≥55	3 (11)	2	1
Sex			
Male	4 (14)	2	2
Female	24 (86)	12	12
Family history of cancer	0 (0)	0	0

Table 2. Histopathologic results of patients with PTC

	Overall (N=28*)	Recurrence (n=14)	No Recurrence (n=14)
	Frequency (%)		
TNM stage			
Age <55 years	[n=23]	[n=10]	[n=13]
I	22 (96)	9	13
II	1 (4.35)	1	0
Age ≥55 years	[n=3]	[n=2]	[n=1]
I	1 (33)	0	1
IVA	1 (33)	1	0
IVB	1 (33)	1	0
Primary tumor			
Age <55 years	[n=23]	[n=10]	[n=13]
T1a	1 (4)	0	1
T1b	5 (22)	2	3
T2	9 (39)	2	7
T3	3 (13)	2	1
T3a	4 (17)	3	1
T3b	1 (4)	1	0
Age ≥55 years	[n=3]	[n=2]	[n=1]
T2	2	1	1
T4b	1	1	0
Regional lymph nodes			
Age <55 years	[n=23]	[n=10]	[n=13]
NX	1 (4)	1	0
N0	18 (78)	5	13
N1	1 (4)	1	0
N1a	2 (9)	2	0
N1b	1 (4)	1	0
Age ≥55 years	[n=3]	[n=2]	[n=1]
N0	3	2	1
Distant metastasis			
Age <55 years	[n=23]	[n=10]	[n=13]
M0	22 (96)	9	13
M1	1 (4)	1	0
Age ≥55 years	[n=3]	[n=2]	[n=1]
M0	2	1	1
M1	1	1	0
Histologic variant	[n=23]	[n=11]	[n=12]
Conventional	14 (61)	8	6
Conventional with follicular	2 (9)	1	1
With follicular	7 (30)	2	5
Tumor size *(cm)*†	[n=22]	[n=11]	[n=11]
Left	0 (0–3.8)	0 (0–1.5)	0 (0–3.8)
Right	2.3 (0–9.3)	4 (0–9.3)	2 (0–4.4)
Multifocal disease	8/15	5/10	3/5
Extra-thyroidal tumor extension	2/12	2/8	0/4
Nodal metastasis	4/8	4/6	0/2
Lymphovascular invasion	7/17	4/7	3/10

** Not all 28 participants have reported and recorded information regarding the different histopathologic characteristics.*
† Reported in median and minimum to maximum values

The histologic variant was not noted for three cases and two controls. The conventional variant was most common in the remaining sample for both case (8/11) and control (6/12) groups. The control group also had a substantial proportion with follicular PTC (42%). Five patients among the cases (n = 10) and three among the controls (n = 5) had multifocal disease. Among the cases, 4/7 had lymphovascular invasion, and 4/6 had affected adjacent or lymph nodes on the thyroid bed.

Cases have significantly higher median total RAI activity administered compared to controls (Table 3). Whole gland excision with or without lymph nodes dissection was carried out for the majority in both cases (50% and 43%, respectively) and controls (64% and 21%, respectively).

Of the 14 patients with recurrent disease, 9 met one criterion, and 5 met two criteria (for recurrence, Table 3).

BRAF V600E mutation was found in 57% (95% CI, 37, 76) of all patients; 9 out of 14 with recurrence and 7 in 14 without recurrence. All mutations detected involved a T>A transversion at V600E. There was insufficient evidence to prove an association between BRAF V600E mutation and PTC recurrence after thyroidectomy (p = 0.688) (Table 4).

DISCUSSION

PTC is the most common epithelial thyroid tumor, accounting for more than 80 to 90% of all thyroid tumors in the iodine-sufficient areas globally, with Asian women having the highest incidence.[1] The prognosis of PTC is excellent, with 10-year overall survival rates exceeding 90%. However, studies have shown that among Asians, Filipinos have higher rates of thyroid cancer, more aggressive tumors, and a higher risk of recurrence.[8] Our results showed that those who developed recurrence had a higher incidence of local disease spread: 57% with lymphovascular invasion and 67% with lymph node metastases. There were no statistically significant differences in the treatment modalities done for both groups. However, patients with tumor recurrence have received a higher cumulative activity of RAI therapy.

Our results also showed that the overall prevalence of BRAF V600E mutation was 57.14%. Our overall prevalence is higher than another local study (38.5%),[4] but is similar to the Korean[25] and Japanese[26] cohorts. However, it is different from Western cohorts, suggesting that genetic and racial differences may play a role in the discrepant results arising from different ethnicities.[27]

The discrepancies may be due to differences in the treatment approach.[10] Most Western countries continue to advocate and perform lobectomy on low-risk patients (i.e., age less than 45 years, tumor size < 4 cm, papillary in histology, without distant metastasis) with outcomes comparable to those who underwent total thyroidectomy (as supported by retrospective cohort data from the Memorial Sloan-Kettering Cancer Center).[10] Locally, total thyroidectomy with or without extensive lymph node dissection (depending on

Table 3. Clinical characteristics and management among patients with PTC patients

	Overall (n=28)	Recurrence (n=14)	No Recurrence (n=14)	p-value
	Frequency (%)			
***Total RAI activity** (mCi)**	100 (100-500)	250 (100-500)	100 (100-150)	0.001
Surgical extent				0.453
***Total thyroidectomy**†*	18 (64.3)	10	8	
Total thyroidectomy with lymph node dissection	10 (35.7)	4	6	
Post-operative complications, permanent hypoparathyroidism	10 (35.7)	5	5	1.000
Criteria for recurrence				
Elevated TG‡	-	10	-	
Lymphadenopathy¶	-	8	-	
Anterior neck new growth	-	1	-	
Distant metastasis¶	-	0	-	
Met 1 criteria	-	9	-	
Met 2 criteria	-	5	-	

** Reported in median and minimum to maximum values*
† Includes lobectomy and near-total thyroidectomy who underwent completion thyroidectomy.
‡ TG, serum thyroglobulin (stimulated or unstimulated)
¶ New-onset or recurrent
Statistical test used: Sign-rank test for difference of medians in paired data, and McNemar test for difference of proportions in paired data

Table 4. Association between BRAF mutation and PTC recurrence

		Recurrence		P-value
		With recurrence	No recurrence	
Mutation	With BRAF mutation	9	7	0.688
	No BRAF mutation	5	7	

Statistical test used: McNemar's test for difference of proportions in paired data

nodal status) followed by adjuvant RAI has been routinely performed.[10] This strategy of aggressive primary surgery may improve survival for high-risk patients and decrease recurrence rates for low-risk patients.[9,10,12]

However, due to the small sample size and limited data, we cannot make a definitive association between the BRAF V600E mutation and recurrence rates; we also were not able to study its association with clinical parameters, tumor characteristics, presence of lymph node metastasis, and stage of the disease.

First, we fell short of the calculated sample size of 51 patients for each arm because of limited sample retrieval, reducing the statistical power. Second, our data were mainly retrospective; many patients were either missed or excluded due to incomplete or difficult data retrieval. Third, we did not quantify the specific length of follow-up per patient; this could also have been studied in association with rates of recurrence. Fourth, molecular diagnostic studies are costly, and funding is limited. Lastly, this study was conducted as a preliminary and pilot study, and our present results will then be used to plan for a larger prospective study.

If future studies with a larger population and longer follow-up show a positive association between BRAF V600E mutations and tumor recurrence, it will impact our management guidelines, such as in low-risk patients, patients with incongruent ultrasound and biopsy results, and patients with suspected follicular variant.

CONCLUSION

Our study shows no evidence of an association between BRAF V600E mutation and recurrence. However, the statistical power is limited due to the small sample size. Likewise, we cannot determine the association of BRAF V600E mutations with clinical parameters, tumor characteristics, presence of lymph node metastasis, and stage of disease.

Despite the limitations and challenges, we present our results to encourage our country's researchers and institutions to collaborate towards overcoming the challenges of doing molecular studies on thyroid cancer.

Acknowledgments

We would like to express our utmost gratitude to Dr. Tom Edward Lo for providing us with the initial list of patients for data collection and sample retrieval. We would also like to thank Dr. Hydelene Dominguez and Ms. Mary Jane Yapo for their valuable contribution in chart retrieval and data collection.

Statement of Authorship

All authors contributed in the conceptualization of work, acquisition and analysis of data, drafting and revising and approved the final version submitted.

Author Disclosure

All authors declared no conflicts of interest.

Funding Source

The authors received a basic science research grant (₱ 200,000) from the Philippine Society of Endocrinology, Diabetes, and Metabolism for this study.

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