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ORIGINAL ARTICLE

Clinical and histopathological profile of BRAF V600E mutation in conventional papillary thyroid carcinoma in a Filipino population

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Abstract

BRAF V600E is a possible biomarker for risk stratification and prognostication in papillary thyroid carcinoma. Studies on its association with aggressive clinicopathological features among East Asian populations are limited. This study examines the clinical and histopathological features of this mutation in Filipinos with conventional papillary thyroid carcinoma. *Methods:* Formalin-fixed, paraffin embedded thyroid tissue blocks of papillary carcinoma for the study period January 2010 to December 2012 were retrieved. Slides were reviewed and described according to tumour size, variant type, sclerosis, multifocality, subcapsular location, extra-thyroidal extension, nodal metastasis, and nodal extracapsular spread. Medical records were reviewed for patient demographics and characteristics. Mutation status was determined using realtime polymerase chain reaction and sequencing. *Results:* Sixty-five patients were included in this study. BRAF V600E mutation prevalence was 38.46%. The mutation positive group was predominantly female, young (mean age 36 years), with tumour size less than 4 cm, and late-stage disease. Extra-thyroidal extension (60%), significant sclerosis (96%), and subcapsular tumour location (72%) were the most frequent findings. Eighty-three percent of patients with nodal metastasis had extracapsular spread. *Conclusions:* Compared to some Asian populations, this study of Filipino patients shows a lower prevalence of BRAF V600E mutation. The clinical and histopathological features of mutation positive patients raise important issues regarding extent of surgical excision and appropriate management of neck metastasis for this group.

Key words: BRAF V600E mutation, clinicopathological features, conventional variant, Filipinos, papillary thyroid carcinoma

INTRODUCTION

Well-differentiated papillary and follicular carcinomas are common endocrine cancers. Of these, papillary thyroid carcinoma (PTC) is the most common type of thyroid malignancy.[1] It is reported to comprise 65% to 88% of all thyroid carcinomas with an incidence that is steadily increasing through the years.[2] Pathogenesis of PTC is associated with mutations in genes that activate the MAP kinase (MAPK) pathway. Among the components of this signaling pathway, RAF kinase is considered to be the strongest activator for downstream MAPK signaling; it is also the most common site of genetic mutation. Specifically for PTC, the BRAF mutation is reported to occur in about 29 to 83% of patients.[3,4] The BRAF V600E mutation, where valine is substituted with a glutamic amino acid at codon 600 of exon 15, results into a constitutive MAPK pathway activation. This leads to uncontrolled and inappropriate cell growth, division, and proliferation that drive tumorigenesis in the thyroid gland. BRAF V600E mutation is found almost exclusively in PTC and PTC-derived anaplastic thyroid cancers; it is absent in normal thyroid tissue, benign thyroid neoplasms, follicular, and medullary carcinomas.[5]

In PTC, the BRAF V600E mutation is reported to be associated with aggressive clinicopathological features such as extra-thyroidal extension, lymph node metastasis, advanced tumour stages, disease recurrence, and patient mortality.[6-10] It is also reported to cause dedifferentiation which results in the

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loss of expression of thyroid genes involved in thyroid iodide concentration. Clinically, this results in failure of radioiodine treatment.[11] The mutation also upregulates several classic angiogenic and tumour-promoting factors and is associated with hypermethylation which results in inactivation of tumour suppressor genes.[12] These associations make the mutation a possible novel and informative prognostic factor for risk stratification and recurrence prediction in PTC patients.

The role of BRAF V600E mutation in the development of PTC, its potential for use as a biomarker, and possible site for therapeutic targets makes this field an area of active research. However, there is limited data on this mutation among Asians, particularly Southeast Asians. To partially bridge this gap, this study examines the clinicopathological features of BRAF V600E mutation in a Filipino population with conventional papillary thyroid carcinoma.

MATERIALS AND METHODS

Clinical and histological data collection

This study was approved by the Institutional Scientific and Ethical Review Boards of St. Luke's Medical Center. Medical records of post-thyroidectomy patients with a final histological diagnosis of papillary thyroid carcinoma for the study period January 2010 to December 2012 were reviewed to obtain the following data: age, gender, nationality, pre-operative laboratory work-up, TNM classification, disease stage, treatment, follow up dates and recurrence.

The haematoxylin & eosin (H&E) slide preparations of the patients were reviewed by a head and neck pathologist member of the group (MVC) and described according to tumour size, variant type, sclerosis, multifocality, subcapsular location, extra-thyroidal extension, nodal metastasis, and nodal extracapsular spread.

The histological diagnosis of PTC was based on the presence of a papillary growth pattern and psammoma bodies plus prototypical nuclear changes. The diagnostic nuclear changes were nuclear enlargement, nuclear membrane irregularities, nuclear crowding, chromatin clearing, nuclear grooves, and nuclear pseudoinclusions. The conventional PTC with tall cell features was diagnosed based on the presence of less than 50% of tumour cells exhibiting increased height of at least three times their width, dense eosinophilic cytoplasm, and a somewhat trabecular growth pattern. The oncocytic variant was diagnosed based on the presence of greater than 50% of tumour cells exhibiting abundant, dense eosinophilic granular cytoplasm with well-developed nuclear changes of PTC.[13]

Significant sclerosis was defined as the presence of fibrotic bands traversing the tumour and its periphery. Multifocality was defined as intraglandular spread in the form of (i) two or more foci of separate tumour, (ii) smaller tumour aggregates with intervening normal thyroid tissue located away from the main tumour mass, (iii) tumour cells within lymphatic vessels, and/or (iv) psammoma bodies in normal thyroid stroma. A subcapsular tumour was defined as a superficially located tumour immediately adjacent to perithyroidal soft tissue.[14]

All data were recorded on Microsoft Office Excel and analyzed.

Molecular techniques

Archived, formalin-fixed and paraffin embedded (FFPE) thyroid tissue blocks were retrieved from the Institute of Pathology of St. Luke's Medical Center, Quezon City. H&E slide preparations of the selected cases were reviewed. Purity and target sampling sites of the tumour were determined. Only FFPE tissues not subjected to decalcification and with adequate tumour size (i.e. >3 mm) were included in the study. Exclusion of specimens processed with decalcifying solutions was done to minimize the extensive DNA fragmentation that has been reported to occur with this technique.[15]

The selected tumour area was marked to delineate site for DNA sampling. Tissue core samples of 0.5 mm were taken from the marked areas using a Harris Micro-Punch®. DNA was extracted using QIAmp DNA FFPE Tissue Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA concentration was determined using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Amplification of BRAF mutation was done using realtime polymerase chain reaction (PCR). The primers used were: Forward: 5'-CAT GAA GAC CTC ACA GTA AAA ATA GGT GAT-3' and Reverse: 5'-GGA TCC AGA CAA CTG TTC AAA CTG A-3'.

Probes used were: VIC/Yakima Yellow/HEX [*BRAF-P*^{wt}] - 5' CCA TCG AGA TTT CAC TGT AG 3' and FAM [*BRAF-P*^{mut}] - 5' CCA TCG AGA TTT CTC TGT AG 3'. Amplification and analysis were performed on Rotor-Gene Q 6000 for 40 cycles (92°C for 15 seconds, 60°C

for 1 minute). The BRAF controls were sourced from gBlocks Gene Fragments (Integrated DNA Technologies, USA).

For sequencing, the following primers were used: Forward - 5'-TCA TAA TGC TTG CTC TGA TAG GA-3', Reverse - 5'-GGC CAA AAA TTT AAT CAG TGG-3' with a resulting amplicon size of 223 bp. PCR products were purified using Quantum Prep® PCR Kleen Spin Columns (Bio-Rad) and sent to First BASE Laboratories Sdn Bhd (Selangor, Malaysia) for sequencing.

RESULTS

Clinical and histological profile of conventional papillary thyroid carcinoma patients

A total of 65 patients with tissue samples of conventional variant papillary thyroid carcinoma (CVPTC) were included in this study. There were 9 males and 56 females with a mean age of 45.18 years and median age of 45 years. In this series, 31 patients (48%) were older than 45 years; three (5%) were pediatric patients. Fine-needle aspiration biopsy (FNAB) was indeterminate in 9 patients (14%) and 22 (34%) were read as "consistent with papillary thyroid carcinoma". It is interesting to note that 16 (25%) specimens were read on cytology as benign nodules. Frozen section was performed in 26 (40%) of the cases. Frozen section was done on 10 patients with cytology readings of "suspicious for papillary thyroid carcinoma" and on 7 patients with "benign nodular hyperplasia". Diagnosis of papillary thyroid carcinoma was correctly made in 23 (88%) specimens including two samples with microcarcinomas (i.e. size less than 1.1 cm). However, three samples were read as "benign nodular hyperplasia".

Forty-nine percent of the patients had Stage I disease (32/65). Total thyroidectomy was done in 42 patients (65%); total thyroidectomy plus some form of neck dissection was done in 16 patients (25%). Fifty-one patients (78%) received radioactive iodine (RAI). Recurrence rate was 8% (5/65). The longest follow up period was 62 months; mean was 25.8 months and median 24.4 months.

Tumour size ranged from 8 to 0.2 cm. Twelve (18%) of the tumours were microcarcinomas; five (8%) measured greater than 4 cm. Of the 65 samples, 44 (68%) were classical/ conventional PTC (Figure 1), 13 (20%) were conventional with tall cell features and 4 (6%) were oncocytic variants. Twenty (31%) cases had multifocal presentation on diagnosis. Thirty two (49%) had extra-thyroidal extension, 61 (94%) had significant sclerosis, and 45 (69%) were subcapsular in location. Of the 19 (29%) cases with nodal metastasis, 11 (58%) had nodal extracapsular spread.

BRAF V600E mutation positive patients:

Twenty-five samples tested positive for BRAF V600E mutation giving a prevalence of 38.46% (Figure 2). There were 21 females in this group. Median age was 36.5 years; mean age was 43 years. Eleven of the patients (44%) were older than 45 years; one was a paediatric patient (14 years old). Sixty percent of patients (15/25) had late stage disease. Total thyroidectomy was done in 19 patients (76%); two patients (8%) had some form of neck dissection in addition to total thyroidectomy. Majority of the patients (18/25) received RAI. None of the patients had recurrence of the disease as of last follow up. Mean duration of follow up was 26.04 months and median was 26 months.

The largest tumour was 5.5 cm; three were microcarcinomas. Six of the samples were conventional papillary thyroid carcinoma with tall cell features. The most frequent histological features present were extra-thyroidal extension (15/25), significant sclerosis (24/25) and subcapsular tumour location (18/25). The clinical profile and most frequent histological characteristics of mutation negative ($BRAF^{wt}$) and mutation positive ($BRAF^{mut}$) patients are summarized in Table 1 and Table 2.

DISCUSSION

Early studies of BRAF V600E mutation in PTC report the prevalence to be highest among Caucasians, followed by Asians.[7] More recent studies on select Asian populations continue to confirm that the mutation is a common genetic event in these populations. Among Chinese patients, studies show a prevalence rate of 68.7% to 80%.[16,17] The Koreans likewise report high prevalence rates of 52% to 83%; these rates are reported to have increased during the last decade.[18-20] Three studies among the Japanese show lower prevalence rates of 29%, 38.4%, and 52%. The study with an intermediate rate of 38.4% involves a large sample size and long follow up period.[21-23] A study involving Indonesians reports a prevalence of 38.6%.[24] These studies examine a heterogeneous group of PTC variants. Among the Asian studies that specifically examine mutation status in CVPTC, prevalence is 79.4% to 85.3% among Korean

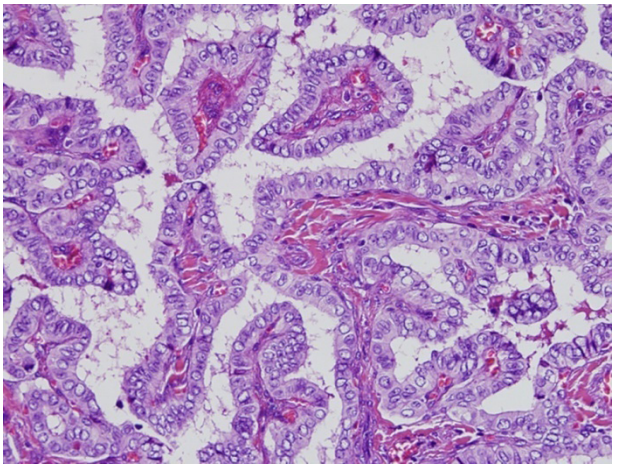

FIG. 1: Conventional papillary thyroid carcinoma (H&E)

FIG. 2: Example of sequencing results of a conventional thyroid carcinoma sample with BRAF V600E mutation

TABLE 1: Comparison of clinical features of BRAF V600E wild type and BRAF V600E mutation patients

	BRAF ^{WT}	BRAF ^{MUT}
Number of Patients	40	25
Sex		
Female	35	21
Male	5	4
Age in Years		
Mean	46.40	43.00
Median	45.50	36.50
Disease Stage		
I – II	22	10
III – IV	18	15
Duration of Follow-Up in Months		
Mean	27.60	26.04
Median	23.10	26.00
RAI Therapy	33	18
Recurrence	5	0

TABLE 2: Comparison of histopathological features of BRAF V600E wild type and BRAF V600E mutation patients

	BRAF ^{WT}	BRAF ^{MUT}
Variant		
Conventional	25	19
With Tall Cell Features	7	6
With Follicular Cell Features	3	0
With Oncocytic Features	4	0
With Oncocytic Warthin's Features	1	0
Tumour Size		
< 1.1 cm	9	3
1.1 – 4.0 cm	28	20
> 4.0 cm	3	2
Nodal Metastasis (%)	13 (33%)	6 (24%)
Nodal Extracapsular Spread (%)	6 (15%)	5 (20%)
Multifocality (%)	12 (30%)	8 (32%)
Extrathyroidal Extension (%)	17 (43%)	15 (60%)
Significant Sclerosis (%)	37 (93%)	24 (96%)
Subcapsular Location (%)	27 (68%)	18 (72%)

patients.[25,26] Our mutation prevalence rate of 38.6% for CVPTC is lower than the average PTC global prevalence range of 45% to 49% but comparable to the Japanese and Indonesian reports.[6,27]

Our patients with CVPTC are predominantly female, with mean and median age of 45 years. Prevalence of female gender in CVPTC is a consistent finding among studies included in a systematic review and meta-analysis of follicular and conventional PTC variants.[28] Among the variants, CVPTC is reported to be second to the oncocytic variant in terms of multifocal presentation, capsular invasion and extra-glandular spill/extra-thyroidal invasion.[29] Extra-thyroidal extension is reported to be significantly greater in CVPTC compared to the follicular variant with overall prevalence of 46.1% versus 15.1% respectively in the 18 studies included in the systematic review and meta-analysis.[28] Our overall rate of 49% extra-thyroidal extension is within the range reported in these studies.

Most of CVPTC cases are also reported to present in tumour stages I-II.[29] In our series, the group is equally divided between those with early and late-stage disease. Multifocal presentation is found in only a third of the patients. Our BRAF^{mut} patients are mostly female, young, with late stage disease, and tumour size less than 4 cm. The most frequent histological features present in this population are extra-thyroidal extension, significant sclerosis, and subcapsular tumour location. Significant sclerosis, subcapsular location, and multifocality are reported to be associated with aggressiveness in PTC tumours regardless of size.[14] A study of a large Asian population with CVPTC reports high rates of extra-thyroidal extension of 64.1% for BRAF mutation positive versus 43.9% for BRAF mutation negative tumours.[30] These findings are consistent with our high rates of 60% for BRAF mutation positive and 43% for BRAF mutation negative tumours.

Previous studies report the association between significant sclerotic type fibrosis in thyroid papillary microcarcinomas and lymph node or distant metastasis.[31,32,14] The high rate of significant sclerosis in our study population suggests the applicability of this association. Tumour desmoplasia is a well-accepted feature of infiltrative neoplastic growth and it is possible that desmoplastic response in the form of sclerotic fibrosis may relate to greater amounts of matrix formation and angiogenesis leading to the greater degree of aggressiveness of tumours. The finding that our mutation positive patients have smaller tumour size but aggressive histological features raises important clinical issues on the extent of the surgical intervention appropriate for these patients.

Although some studies report an association between the BRAF V600E mutation and certain

aggressive clinicopathological features, others fail to demonstrate this relationship.[18,33-36] Among studies on Asian populations, a Japanese series of 631 PTC cases fails to demonstrate a difference in prevalence of the mutation between tumours sized 1.1-4 cm and those measuring greater than 4 cm. This study also fails to find a link with distant metastasis and advanced clinical stage.[21] A series on Korean patients finds no association with age, gender, tumour size, multifocality, extra-thyroidal extension, and lymph node metastasis.[35] A study among Taiwanese patients shows no significant association between the mutation and negative prognostic indicators such as sex, age, multicentricity, thyroid capsule invasion or nodal metastasis.[36] However, another study among Chinese patients shows that the BRAF mutation is significantly associated with extra-thyroidal invasion but not with tumour volume and lymph node metastasis.[37] A sample of studies among Asian populations with divergent results on the association of the mutation with adverse clinicopathological features is presented in Table 3. It is interesting to note that among our $BRAF^{mut}$ patients, only 6 (24%) have lymph node metastasis but 5 of these patients have nodal extracapsular spread (83%). In contrast, of the 13 $BRAF^{wt}$ patients with lymph node metastasis only 6 have nodal extracapsular spread (46%). This finding has important clinical implications in the management of the neck of mutation positive patients.

None of our $BRAF^{mut}$ cases have recurrence. In a multivariable model that adjusted for sex, age at diagnosis, medical center, and various pathological factors, reported recurrence rates are 20.9% for mutation-positive and 11.6% for mutation-negative cases.[38] A retrospective study that demonstrates a direct association of BRAF mutation with high-risk PTC subtypes also shows that the mutation is independently associated with recurrence.[10] In another study with multivariate analysis, the strongest predictor of increased risk for recurrence is cervical lymph node involvement, followed by capsular invasion, vascular invasion, and then histological tumour size.[34] However, for our study, the small sample size and relatively short duration of follow up is a limiting factor in establishing a definite relationship between mutation status and recurrence.

In general low advanced PTC has good prognosis because of its excellent response to therapy and low mortality rates. However, the association of BRAF mutation status in PTC with long term outcome is not established because of inconsistent results among studies. Some studies report mutation status to be a predictor of poor long term outcome[39,40] even in low-risk, early stage PTC.[41,42] In contrast, a retrospective study of a large cohort of 508 patients, with a study population BRAF mutation prevalence of 66.9% and long follow up time (mean of 9.8 years), finds no relationship between BRAF mutation status and recurrence-free survival or disease-specific survival. This finding is consistent across univariate, multivariate, and Kaplan-Meier analyses.[34] Another study of 233 PTC patients, with a BRAF mutation prevalence of 54.5% in the study population, similarly finds no association with disease-free survival. Differences in results among the various studies are attributed to several

TABLE 3: Association between BRAF V600E mutation and adverse clinicopathological features in Asian populations

Population [Ref]	Sample size	BRAF prevalence	Clinicopathological features
	Positive Association		
Chinese [37]	132	60.61%	ETE*
Korean [30]	688	69.2%	ETE*, LNM**, Late stage
Korean [8]	2947	75.3%	ETE*, LNM**, Tumour Size
Japanese [23]	170	29%	Distant metastasis, Late stage
	Negative Association		
Japanese [21]	631	38.4%	none
Korean [35]	107	79.4%	none
Taiwanese [36]	105	47%	none

ETE* - extrathyroidal extension, LNM** – lymph node metastasis

factors such as cohort size, length of follow up, relapse rate, and treatment algorithms.[43]

In conclusion, this study of Filipino patients with CVPTC shows a lower prevalence of BRAF V600E mutation compared to some Asian populations. The mutation positive patients are predominantly female, young, with smaller tumour size, and late stage disease. Extra-thyroidal extension, significant sclerosis, subcapsular location, and nodal extracapsular spread are the most frequent histopathological features present in this population. These findings raise significant clinical issues regarding the extent of surgical excision and appropriate management of neck metastasis for this group. We recommend that additional studies with a larger sample size and longer follow up period be done to confirm these initial findings.

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