BRAF *V600E* Mutations in Papillary Thyroid Carcinoma: Their Relation to Clinical Features and Oncologic Outcomes in A Single Cancer Centre Experience

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Abstract

Introduction BRAF *V600E* is one of the most common mutations in Papillary Thyroid Cancer (PTC). Its clinical correlation has been extensively studied with contradictory results. The aim of this study is to evaluate the oncological impact of BRAF *V600E* mutation on a cohort of Middle Eastern PTC patients treated at a single institute.

Methods Patients with histologically confirmed PTC that were treated surgically between 2006 to 2015 were included in the study. Formalin fixed paraffin embedded tumor blocks were sectioned and tested for BRAF *V600E* mutation. Short- and long-term oncological outcomes were collected.

Results 128 patients (68% females) were included with a mean age of 38 years (± 13.8). Median follow-up was 50 months. BRAF *V600E* mutation was found in 71% of patients.

The BRAF negative tumors were significantly larger than the BRAF positive (3.47 cm versus 2.31 cm respectively, $P = 0.009$). All other clinicopathological characteristics were comparable between BRAF *V600E* mutation positive and negative groups. The two groups showed similar 5-year Disease-free ($P = 0.37$) and Overall survival rates ($P = 0.94$).

Conclusion BRAF *V600E* mutation did not affect loco-regional recurrence, distant metastasis, overall and disease-free survival. These results support the diversity of BRAF *V600E* significance among various ethnicities.

Introduction

Thyroid cancer, with its most prevalent papillary subtype, is the most common malignant endocrine tumor worldwide ¹. In Jordan, thyroid cancer ranked ninth among all cancers with an incidence of 2.9% in 2001. This incidence raised to 4.3% in 2013 making thyroid cancer the 4th most common malignancy in Jordan ². This change is also reflected by a similar increase worldwide. Papillary thyroid cancer (PTC) subtype had the lion's share of this increase and accounted for approximately 85 to 88% of all thyroid cancer diagnoses. The incidence of other subtypes remained unchanged ³.

The reason behind this increase in incidence is controversial. A few have attributed this trend towards an abundant clinical surveillance, improved diagnostics such as high resolution thyroid ultrasound, bedside availability of such imaging modalities, an expansion in their indications, along with an increasing use of fine needle aspiration biopsy ⁴.

An increased detection of occult papillary thyroid cancer on pathologic examination of thyroid glands removed for benign conditions and reported as thyroid cancer cases to national cancer registries may also contribute towards the increasing incidence of thyroid cancer ⁵.

Despite the increasing incidence, the mortality rates for well differentiated thyroid cancer remained relatively stable. The 30-year disease-specific survival rates can exceed ninety five percent. In spite of that, a subset of patients with metastatic disease have a glimmer outcome with a 5-year survival as low as 56%⁶. Although treatment is often curative, there is a 15% recurrence rate over the course of 10 years, and approximately 10% of patients die as a result of disease progression^{7,8}.

BRAF mutations, are the most common genetic events in thyroid cancer with the highest incidence in PTC. Various studies reported an incidence ranging between 29 to 88% of all PTC cases⁹. BRAF *V600E* is the most common of BRAF mutations. Its clinical correlation with PTC has been extensively studied with contradictory results¹⁰⁻¹³. The aim of this study is to evaluate the incidence and clinical impact of BRAF *V600E* mutation on a cohort of PTC treated at a single institute with disease free & overall survival as the primary end point.

Methods

This is a retrospective chart review study approved by the Institutional Review Board (IRB) at King Hussein Cancer Center (KHCC). IRB (Ref: 15KHCC101). The KHCC IRB is guided by the principles described in the World Medical Association's Declaration of Helsinki (1964) and its amendments.

Because of the retrospective nature of the study and the lack of personal or clinical details of participants that compromise anonymity, consent was waived and the study was approved by King Hussein Cancer Center Institutional Review Board (IRB). The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Study Cohort And Tumor Samples

Patients with primary thyroid carcinomas were analyzed between the years of 2006 to 2015.

In all cases, curative hemithyroidectomy or total thyroidectomy with or without neck dissection was performed. Radio-active Iodine was given according to institution guidelines.

All patients were regularly followed with physical examinations, thyroid function tests and neck ultrasonography every 6 to 12 month after the initial surgery. If suspicious thyroid nodules or lymph nodes were found, ultrasound-guided fine needle aspiration cytology (US-FNAC) was used for evaluation.

Tumor-node-metastasis (TNM) staging was defined based on the eighth edition of the American Joint Committee on Cancer (AJCC) staging system.

The study was reviewed and approved by the local institution review board at King Hussain Cancer Center KHCC.

Molecular Testing For Somatic Genetic Changes

All the retrieved Hematoxylin and Eosin (H&E) stained sections for the cohort cases were reviewed separately by two experienced histopathologists in endocrine pathology. The pathology diagnoses were reviewed and confirmed to be papillary thyroid carcinoma.

The most appropriate slide for BRAF molecular testing was determined based on the percentage of primary thyroid tumor and lymph node metastasis if present. Ten percent was deemed as minimal accepted tumor percentage on the selected slides. Five sections of approximately 5 to 10 micrometer thickness were sectioned from the formalin fixed paraffin embedded (FFPE) tumor tissue corresponding to the selected slides. Sectioned tissue was collected in Eppendorf tubes with the appropriate labelling. The deoxyribonucleic acid (DNA) was extracted and purified using the QIAamp(r) DNA Mini Kit (Qiagen). Samples were assessed for DNA concentration and purity using the NanoDrop(r) ND-1000 spectrophotometer. BRAF mutation testing was performed using the Therascreen(r) BRAF RGQ PCR Kit on the QIAGEN Rotor-Gene Q MDx instrument, that is designed to detect five somatic mutations in the BRAF gene including: *V600E*, *V600E* complex (*V600Ec*), *V600D*, *V600K*, and *V600R*.

Statistical analysis

Patients characteristics, clinical, pathological findings, and clinical outcomes were collected in a retrospective manner. Data was analysed using the software package SPSS 24 (Chicago, Illinois, USA). Results were expressed as medians and interquartile ranges (IQR) or mean and standard deviation (SD). Comparison between the two groups was performed using the χ^2 test for categorical variables and the T-test for continuous variables. Survival functions were compared using the non-parametric Kaplan-Meier estimator. Clinical and pathological predictors of overall and disease-free survival were analyzed using univariate and multivariate Cox proportional-hazards models. Significance was defined as P value less than 0.05. Statistically significant factors on univariate analysis were included in the multivariate model.

Results

Patient's characteristics

A hundred and twenty-eight patients were included with a mean age of thirty-eight years (± 13.8) at the time of diagnosis. Forty-one (32%) patients were men and eighty-seven (68%) were women. Patients were followed for a median of fifty-month post-surgical resection.

The mean size of the primary tumour was 2.6 cm (± 2.2). Eighty-six percent of the patients were staged as stage I PTC. Of the 128 patients with conventional PTC, BRAF *V600E* mutation was found in 91 (71%) patients. Table 1 lists the characteristics of the 128 patients with conventional PTC included in the study.

Table 1
Clinicopathological characteristics

Variables		Total N = 128 (%)
BRAF gene mutation	Positive	91 (71.1)
	Negative	37 (28.9)
Age	< 55 years	113 (88.3)
	≥55 years	15 (11.7)
Gender	Male	41 (32)
	Female	87 (68)
Extra-thyroidal extension	True	32 (25)
	False	96 (75)
Family history	Positive	9 (7)
Lymph node	Positive	68 (53)
Multi-Nodular goiter	Concomitant	12 (9.4)
Hashimoto Thyroiditis	Concomitant	23 (18)
Pathology stage	I	110 (85.9)
	II	12 (9.4)
	III	1 (0.8)
	IV	5 (3.9)
Extent of surgery (Total Thyroidectomy)	Without Neck Dissection	50 (39.1)
	With neck dissection	77 (60.2)
Subtype of papillary thyroid cancer	Insular	1 (0.8)
	Follicular	4 (3.1)
	Capsular	11 (8.6)
	Classic	112 (87.5)
Tumor size (cm)	Mean (SD)	2.62 (2.17)
	Median (Range)	2 (0–16)
Bilateral	True	26 (20.3)
	False	102 (79.7)

Variables		Total N = 128 (%)
Multifocal	True	54 (42.2)
	False	74 (57.8)

Association Of Braf V600e With Clinicopathological Features In Ptc

The various clinicopathological characteristics were compared between patients with PTC harboring BRAF *V600E* mutation and those without (Table 2). The age & sex were not significantly different between the two groups (with or without BRAF *V600E* mutation). Patients with BRAF *V600E* mutation were similar to BRAF *V600E*-negative patients with regards to lymph node metastasis (57.1% vs. 43.2%, $P = 0.36$), extra-thyroidal extension (34.1% vs. 35%, $P = 0.90$), positive family history (8.8% versus 2.7% respectively, $P = 0.22$), multifocality (44% vs 37.8%, $P = 0.52$), and the extent of neck dissection. The BRAF negative tumors were significantly larger than the BRAF positive (3.47 cm versus 2.31 cm respectively, $P = 0.009$).

Table 2
Patients characteristics based on BRAF status

Variable vs BRAF Status		Total N (%)	Positive N (%)	Negative N (%)	P value
		128(100)	91(71.1)	37(28.9)	
Gender	Male	41 (32)	28 (30.8)	13 (35.1)	0.63
	Female	87 (68)	63 (69.2)	24 (64.9)	
Positive family history		9 (7)	8 (8.8)	1 (2.7)	0.22
Age	Mean (SD)	37.85 (13.75)	38.54 (13.58)	36.16 (14.22)	0.37
Primary tumor size (cm)	Mean (SD)	2.62 (2.17)	2.31 (1.67)	3.47 (3.04)	0.009
Lymph node	Positive	68 (53)	52 (57.1)	16 (43.2)	0.36
	Negative	57 (44.5)	37 (40.7)	2 (5.4)	
Extra-Thyroidal extension		44(34.4)	31(34.1)	13 (35)	0.90
Total thyroidectomy	Without neck dissection	50 (39.1)	35 (38.5)	15 (40.5)	0.27
	With neck dissection	77 (60.2)	56 (51.5)	21 (56.8)	
Concomitant Hashimoto		23 (18)	13 (14.3)	10 (27)	0.08
Hyperthyroidism		11 (8.6)	7 (7.7)	4 (10.8)	0.56
Multifocality		54 (42.2)	40 (44)	14 (37.8)	0.52
Subtype of papillary thyroid cancer	Insular	1 (0.8)	0 (0.0)	1 (2.7)	0.276
	Follicular	4 (3.1)	2 (2.2)	2 (5.4)	
	Capsular	11 (8.6)	7 (7.7)	4 (10.8)	
	Classic	112 (87.5)	82 (90.1)	30 (81.1)	
Multicentricity		26 (20.3)	19 (20.9)	7 (18.9)	0.80
Percentages are calculated out of total per column					

With a median follow-up of fifty months, the two groups showed similar 5-year Disease-free survival (DFS) (P = 0.37) (Fig. 1) and Overall survival (P = 0.94) (Fig. 2).

Risk Factors Affecting The Disease-free And Overall Survival

To assess the effect of several risk factors on survival time in patients with PTC, the disease-free and overall survival analysis was extended using univariate and multivariate Cox regression model. The BRAF status, gender, family history, concomitant multinodular goiter, Hashimoto Thyroiditis, multifocality, multicentricity, and thyroglobulin level post-operatively, and extent of neck dissection were entered into the model. Female gender was the only factor significantly associated with improved disease-free survival on univariate (Hazard Ratio: 0.637, P = 0.05). None was associated with improved survival on multivariate analysis. (Table 3).

Table 3: Factors associated with Disease-Free survival

Factor	Univariate Analysis							
	Hazard Ratio	95% CI		P-Value	Hazard Ratio	95% CI		P-Value
		Lower	Upper			Lower	Upper	
BRAF status	0.731	0.471	1.134	0.162				
Gender - Female	0.637	0.404	1.004	0.052	0.687	0.435	1.087	0.109
Family history - Negative	0.767	0.383	1.535	0.454				
Age	1.124	0.679	1.861	0.650				
Extra-Thyroidal extension - Negative	0.893	0.582	1.370	0.604				
Extent of Neck Dissection	1.975	1.262	3.090	0.003	1.910	1.216	3.000	0.005
Concomitant multinodular goiter	1.833	0.945	3.553	0.073				
Concomitant Hashimoto	0.842	0.513	1.380	0.495				
Multifocality	0.814	0.543	1.221	0.320				
Multicentricity	0.741	0.447	1.229	0.246				
Thyroglobulin Level (Post-Op)	1.001	0.999	1.004	0.303				

As for the overall survival (Table 4); Patients diagnosed below the age of 50 were significantly associated with improved overall survival on univariate (Hazard ratio 0.031, P = 0.001) and multivariate analysis (Hazard ratio 0.037, P = 0.003).

Table 4: Factors associated with overall survival

Factor	Univariate analysis				Multivariate analysis			
	Hazard ratio	95% CI		P-value	Hazard ratio	95% CI		P-value
		Lower	Upper			Lower	Upper	
BRAF status	0.940	0.182	4.866	0.941				
Gender- Female	6.441	1.240	33.449	0.027	7.841	1.195	51.466	.032
Family history - Negative	5.660	1.036	30.934	0.045	2.367	.409	13.705	0.336
Age < 50 years	0.031	0.004	0.261	0.001	0.037	0.004	0.330	0.003
Extra-Thyroidal extension	0.211	0.041	1.089	0.063				
Extent of Neck Dissection	1.100	0.230	5.268	0.905				
Concomitant multinodular goiter	0.617	0.072	5.286	0.659				
Concomitant Hashimoto	0.990	0.116	8.481	0.993				
Multifocality	0.736	0.149	3.649	0.708				
Multicentricity	0.474	0.087	2.591	0.389				
Thyroglobulin Level (Post-Op)	1.002	0.996	1.007	0.556				

Discussion

In this study, we examined the clinicopathological factors associated with BRAF *V600E* mutation. We also explored the relationship between BRAF *V600E* and the oncological outcomes in PTC. The BRAF *V600E* mutation is considered a specific diagnostic and prognostic marker in PTC. *V600E* is a point mutation at codon 600 of BRAF gene. This causes a constitutive activation of the BRAF kinase and an uncontrolled activation of the MAP Kinase signalling pathway.

Previous publications have linked BRAF mutation with worse prognostic features. This includes age at diagnosis, male sex, multifocal tumor, and advanced TNM stage^{10,11,14-18}. Xing et al reported a significant association between BRAF mutation, adjacent structures invasion, and lymph node metastasis¹⁰. Other reports found BRAF mutation to independently predict Central lymph nodes metastasis¹⁸. At multivariate analysis of BRAF *V600E* mutation showed an independent correlation with worst outcome.

Moreover, the survival curves of PTC patients showed a worse survivor in the BRAF *V600E*-mutated group¹⁹.

These reported outcomes are in contradiction with the result of our current study. Other large retrospective studies were consistent with our findings and failed to corroborate the above reported associations^{12,13,20–22}.

Ito et al investigated BRAF *V600E* mutation in 631 patients with papillary carcinoma with a median follow-up of 83 months. The prevalence of BRAF *V600E* mutation was 38.4%. BRAF *V600E* prevalence was not significantly associated with cases demonstrating high-risk biological features such as clinically apparent lymph node metastasis, massive extrathyroid extension, advanced age, distant metastasis at surgery, and advanced stage. The disease-free survival of patients with BRAF *V600E* mutation did not differ from those without mutation²². These findings along with our results indicate that, although BRAF *V600E* mutation may play some roles in local carcinoma development, there is no significant evidence associating it with aggressive characteristics and poor prognosis.

Studies focusing on Asian populations did not find an association between BRAF *V600E* mutation and regional lymph node metastasis. An example of such study by Junliang et al reported the outcome of 150 PTC cases. Eighty percent of the primary tumors contained a BRAF *V600E* mutation. Lymph node metastasis was equally distributed in patients with or without BRAF *V600E* mutation²³. This may suggest a difference in the mutation's behaviour based on the racial background.

The overall significance of BRAF mutation in PTC is open to debate. In addition, the incidence and clinical impact of BRAF *V600E* mutation among PTC patients from Middle Eastern races remain unknown. To our knowledge; this is the first cohort investigating the relation between BRAF mutation & papillary thyroid cancer in the Middle Eastern population. The limitation of our study is its retrospective nature, single institution & small sample size.

Conclusions

Despite the high percentage of PTCs harboring BRAF *V600E*; mutation positivity did not affect lymph node involvement, loco-regional recurrence, distant metastasis, overall and disease-free survival. These results support the diversity of BRAF *V600E* significance among various ethnicities.

Declarations

Acknowledgements: NA

Authors' contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MM, HKN, TS, MM and RI. The first draft of the manuscript was written by TS,

EY, AT, HH, AJ. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate: This study was approved by the Institutional Review Board (IRB) at the King Hussein Cancer Center (Approval #: 15KHCC101).

Consent for publication: Not Applicable, This is a retrospective Study.

Data availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no conflicts of interest.

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Ethics declarations:

This is a retrospective chart review study approved by the Institutional Review Board (IRB) at King Hussein Cancer Center (KHCC). IRB (Ref: 15KHCC101). The KHCC IRB is guided by the principles described in the World Medical Association's Declaration of Helsinki (1964) and its amendments.

Because of the retrospective nature of the study and the lack of personal or clinical details of participants that compromise anonymity, consent was waived and the study was approved by King Hussein Cancer Center Institutional Review Board (IRB). The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1
Kaplan-Meier curve for the Disease-free survival

Figure 2

Kaplan-Meier curve for the overall survival