Relationship between BRAF V600E Mutation and Re-arranged in Transformation/Papillary Thyroid Carcinoma Rearrangement and their Clinicopathological Features in Papillary Thyroid Carcinoma

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Abstract—**Background:** Rearranged in transformation/papillary thyroid carcinoma (RET/PTC) rearrangement, Rat Sarcoma gene (RAS) and B isoform of RAF kinase (BRAF) mutations are mutually exclusive in PTC. However, although concomitant mutations of RET/PTC, RAS or BRAF have been reported recently, their significance in tumour progression and survival remains unclear. **Methods:** We performed a retrospective review of 57 patients who underwent surgery for PTC. We studied the prevalence of BRAF V600E mutation and RET/PTC rearrangement, which were determined by fluorescence polymerase chain reaction in a series of 57 conventional PTC. IBM SPSS Statistics computer package v. 24 was used for data analysis, descriptive analysis, Chi-square, T-Test and correlation. Descriptive statistics, percentages and test of significance were obtained to determine the associations between clinicopathological characteristics and mutation status. **Results**: BRAFV600E mutation was observed in 43 patients (75.4%), whereas RET/PTC rearrangement was observed in 5 (8.8%). Statistical analysis revealed no significant correlation between multifocality, tumour size and lymph node metastasis with BRAF V600E mutation and RET/PTC rearrangement except for age and gender. Statistical analysis exposed a highly significant ($P<0.01$) difference in several clinicopathological features and BRAFV600E mutation and RET/PTC rearrangements.**Conclusions:** BRAF V600E mutation and RET/PTC rearrangement showed no significant association with clinicopathological features of PTC characteristics except for age and gender.

Index Terms BRAFV600E mutation, RET/PTC rearrangement, Papillary thyroid carcinoma, lymph node metastases.

1 INTRODUCTION

Thyroid cancer is the most broadly recognised dangerous disease of the endocrine system [1]. This disease is the most rapidly increasing cancer in the USA and has been increasing worldwide, including China, over the past few decades. Such rise is thought to be partly due to increased detection of more sensitive diagnostic procedures, perhaps resulting in overdiagnosis. Lately, full utilisation of thyroid ultrasonography has prompted the expanded location of non-substantial thyroid carcinoma. Papillary thyroid carcinoma (PTC) is the most widely recognised kind of endocrine tumour followed by follicular thyroid carcinoma. The pervasiveness of PTC rapidly expands and presently accounts for >95% of every thyroid carcinoma in Korea [2]. Recent reports from North America and Europe have recorded a yearly increment in the incidence of differentiated thyroid carcinoma [3]. PTC is the most well-known kind of thyroid cancer, and incidence of cervical lymph node metastasis (LNM) resulting from the illness is high [4]. Lymph node dismemberment in patients with PTC is dialectical owing to the excellent prognosis of the disease. Although disagreement continues regarding excision of prophylactic lymph nodes, therapeutic lymph node dissection is suggested in all guidelines for patients with known LNM [5].

The yearly incidence rate of PTC in typical parts of the world reaches 0.5–10 for every 100,000 populations. Clinically, PTC presents as asymptomatic thyroid nodules, is ordinarily single, firm, and generously moveable through swallowing, and not necessarily originates from a specific nodule. A thyroid must be related with carcinoma when detected in children or young people or patients more than 60 years old, especially given a history of the speedily progressive increase in size: the most widely recognised rearrangement concerns RET quality, RAS transformations and BRAF mutation [6]. PTCs are described by two main gene alterations, either a rearrangement of RET gene or a point mutation of the BRAF gene. As the result of a somatic chromosomal event, the RET gene (not expressed in thyroid epithelial cells) undergoes a rearrangement which leads to the

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fusion of its 3′-part encoding the tyrosine kinase domain with the 5′-part of different genes. The expression levels of the resulting chimeric oncoprotein named RET/PTC rely upon the recently gained promoter [7]. The pervasiveness of RET/PTC rearrangements in thyroid cancer differs widely among studies. In an adult population from the United States, the prevalence of rearrangements was around 35% [8]. The most common RET/PTC rearrangements observed in PTC are RET/PTC1 (in combination with H4 gene) and RET/PTC3 (in combination with NCOA4 gene). The prevalence of RET/PTC rearrangements in PTC is the highest in populations exposed to radiation (60%–70%). A prevalence between 3% and 85% has been reported for other regions of V600E mutation, leading to uncontrolled activation of the mitogen-activated protein kinase pathway, which is critical for both tumours start and progression of PTC. BRAF fits in with the RAF group of serine/threonine kinases, which incorporate two other forms, ARAF and CRAF (RAF-1). Essentially, all changes distinguished thus far in PTC influence nucleotide 1799 in exon 15 of BRAF, resulting in a thymine-to-adenine transversion, which interprets valine-to-glutamate substitution at build-up 600 (V600E) [9]. This study aimed to investigate the relationship between clinical and pathological features, such as gender, age, tumour size and tumour location, with BRAFV600E mutation and RET/PTC rearrangements in PTCs.

2 MATERIALS AND METHODS

2.1 Tissue Samples

This study retrospectively collected data from 65 cases of thyroid cancer; data were retrieved from files, whereas all cases that underwent thyroid ectopy and routine central lymph node dissection detected by polymerase chain reaction (PCR), specifically the amplification refractory mutation system in the Department of Pathology of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan. Special care were used to select cases that underwent PCR from October 2014 to October 2015 to analyse BRAFV600E mutation and RET/PTC rearrangements. Histological diagnosis of the cases included papillary thyroid cancer (n = 62), medullary thyroid cancer (n = 2) and follicular thyroid cancer (n = 1), among which eight cases were excluded due to incomplete clinical information or inadequate tumour sampling. Finally, we included 57 formalin-fixed-paraffin-embedded PTC specimens in this study. The rules of tissue collection by the Pathology Department included the informed consent of patients. Clinicopathological features, including gender, age of patient at diagnosis, multifocality, tumour size, tumour location and LNM, were obtained from patient medical histories and pathological reports. Matched paraffin-embedded histological samples of all cases were studied. After surgical resection, tissues were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Sections (4–5 μm thick) were stained with haematoxylin and eosin for histological examination. The International Union Against Cancer/American Joint Committee on Cancer (AJCC) Tumour Node Metastasis classification system was utilised for tumour staging [10]. Table 1 displays all information regarding the samples.

2.2 Statistical Analysis

Raw data were imported into an Excel sheet and categorised according to analysis requirements. The relationship between BRAF V600E mutation and RET/PTC rearrangements in PTC and their association with specific clinicopathological features was determined. IBM SPSS Statistics computer package v. 24 was used for data analysis, descriptive analysis, Chi-square, t-Test and correlation to determine the descriptive statistics, percentages and test of significance. Multivariate logistic regression analyses were also carried out to determine the association between BRAF V600E mutation and RET/PTC rearrangements with clinicopathological features (SPSS Inc, Chicago, IL, USA. under 5% level of significance). Statistical significance was defined as $P < 0.05$.

3 RESULTS

3.1. Clinical Pathological Characteristics of the Cases

PTC was observed in 57/65 cases. The mean age was 43.03 ± 10.78 years, ranging between 21–71 years old. The group comprised 17 men (29.8%) and 40 women (70.2%). A total of 15/57 (26.3%) patients presented with multifocality. The tumours measured 0.30 cm to 6.50 cm, yielding an average size of 1.13 ± 1.04 cm A total of 23/57 (40.4%) of the cases presented in the right gland, whereas 22/57 (38.6%) presented in the left gland, with 1/57 (1.8%) case exposed in the isthmus and 11/57 (19.3%) at both sides. LNM was discovered in 33 cases (57.9%) of PTC patients. For tumour stage, 55 (96.5 %) cases were at stage I, and 2 (3.5%) were at stage II. A total of 9/57 (15.8%) cases were wild type, and 48/57 (84.2%) cases carried either a BRAF mutation (43/57, 75.4%) or a RET/PTC rearrangement (5/57, 8.8%). Table 1 shows the details of clinical data and descriptive statistics.

3.2 Relationship between BRAF V600e Mutation and RET/PTC Rearrangements in the PTC and Its Association with Specific Clinicopathological Features

The details of clinical data and morphological features of the tumours were portrayed elsewhere (Department of Radiology, Department of Surgery and other departments). Out of 57 papillary carcinomas, 43 (75.4%) presented V600E mutation of the BRAF gene, and 5 (8.8%) cases exhibited RET rearrangements involved with RET/PTC1 and RET/PTC3/4. Table 2 provides the results on mutations of these genes in the sample cohort. BRAF (V600E) mutation was detected in 75.4% (43/57) of patients, whereas a RET/PTC rearrangement was detected in 8.8% (5/57) of patients. A statistically non-significant difference was observed in the relationship between BRAF V600E mutation and RET/PTC rearrangements in PTC and their association with certain clinicopathological features in 57 cases.

TABLE 1
CLINICOPATHOLOGICAL FEATURES AND MUTATION FOR PATIENTS CARRYING PTC IN THIS STUDY

Categorical Variable Information					
		Mean ± SD		N	Percent
Factor	AGE	43.03±10.78	<45	34	59.6%
			≥45	23	40.4%
			Total	57	100.0%
	GENDER	1.70±0.46	M	17	29.8%
			F	40	70.2%
			Total	57	100.0%
	TUMOR LOCATION	1.84±0.79	LEFT	22	38.6%
			RIGHT	23	40.4%
			LEFT AND RIGHT	11	19.3%
			ISTHMUS	1	1.8%
			Total	57	100.0%
	TUMOR SIZE	1.10±0.401	T1	53	93.0%
			T2	2	3.5%
			T3	2	3.5%
			Total	57	100.0%
	MUTATION FOUND	0.84±0.36	NO	9	15.8%
			YES	48	84.2%
			Total	57	100.0%
	MUTATION DIFFERENCE		NO	57	100.0%
			Total	57	100.0%
	MUTATION SITES	0.92±0.49	NO	9	15.8%
			BRAE(V600E)	43	75.4%
			RET(NCOA4-RET) (RET-PTC3/4)	5	8.8%
			Total	57	100.0%
	LYMPHO NODE METASTASIS	2.08±0.96	N0	24	42.1%
			N1a	4	7.0%
			N1b	29	50.9%
			Total	57	100.0%
	MULTIFOCALITY	0.26±0.44	NO	42	73.7%
			YES	15	26.3%
			Total	57	100.0%
	STAGE(AJCC)	1.03±0.18	I	55	96.5%
			II	2	3.5%
			Total	57	100.0%

AJCC: American Joint Committee on Cancer, BRAF: B isoform of RAF kinase, RET/PTC: Rearranged in transformation/papillary thyroid carcinoma,

3.4 Relationship between Age and Gender with Specific Clinicopathological Features and Mutation in the PTC

Table 3 displays the results of this study. Statistical analysis of the relationship between age and gender with specific clinicopathological features and mutation in the PTC revealed a highly significant (P<0.01) difference in tumour location, tumour size, detected mutation, mutation type, mutation difference, mutation sites, lymph node metastasis, multifocality and stage (AJCC) based on age, whereas no significant difference was observed in tumour size number. According to gender, a highly significant difference (P<0.01) was identified in tumour size, detected mutation, mutation type, mutation difference, mutation sites, LNM, multifocality and stage (AJCC) with no significant difference obtained in tumour location.

TABLE 2
THE RELATIONSHIP BETWEEN BRAF V600E MUTATION AND RET/PTC REARRANGEMENTS IN THE PTC AND ITS ASSOCIATION WITH SOME CLINICOPATHOLOGICAL FEATURES.

	BRAFV600E mutation		RET/PTC rearrangement		P value
	(+) n (%)	(–) n (%)	(+) n (%)	(–) n (%)	
Age					*P* BRAF =1
<45 years	24(70.6%)	5(14.7%)	5(14.7%)	0(0%)	*P* Combine =1
≥45 years	19(82.6%)	4(17.4%)	0(0.0%)	0(0%)	
Gender					*P* BRAF =0.451
Female	30(75.0%)	5(12.5%)	5(12.5%)	0(0%)	*P* Combine =0.428
male	13(76.5%)	4(23.5%)	0(0.0%)	0(0%)	
Multifocality					*P* BRAF =0.674
NO	33(78.6%)	6(14.3%)	3 (7.1%)	0(0%)	*P* Combine =0.685
YES	10(66.7%)	3(20.0%)	2 (13.3%)	0(0%)	
Tumor size					*P* BRAF =0.717
T1	40(75.5%)	9(17.0%)	4(7.5%)	0(0%)	*P* Combine =0.668
T2	1(50.0%)	0(0.0%)	1(50.0%)	0(0%)	
T3	2(100%)	0(0.0%)	0(0.0%)	0(0%)	
Tumor Location					*P* BRAF =0.791
Left	14(63.6%)	4(18.2%)	4(18.2%)	0(0%)	*P* Combine =0.874
Right	18(78.3%)	4(17.4%)	1(4.3%)	0(0%)	
Left and Right	10(90.9%)	1(9.1%)	0(0.0%)	0(0%)	
Isthmus	1(100%)	0(0%)	0(0.0%)	0(0%)	
LYMPHO NODE METASTASIS					*P* BRAF =0.914
N0	20(83.3%)	4(17.7%)	0(0.0%)	0(0%)	*P* Combine =0.837
N1a	3(75.0%)	1(25.0%)	0(0.0%)	0(0%)	
N1b	20(69.0%)	4(13.8%)	5(17.2%)	0(0%)	
AJCC stage					*P* BRAF =1
I	41(74.5%)	9(16.4%)	5(9.1%)	0(0%)	*P* Combine =1
I I	2(100%)	0(0%)	0(0%)	0(0%)	

*Note: p Combine means the *p* value of combined date of BRAF and RET
AJCC: American Joint Committee on Cancer, BRAF: B isoform of RAF kinase, RET/PTC: Rearranged in transformation/papillary thyroid carcinoma

TABLE 3
THE RELATIONSHIP BETWEEN AGE AND GENDER WITH SOME CLINICOPATHOLOGICAL FEATURES AND MUTATION IN THE PTC.

		Paired Differences					P-value
	AGE with	Mean	Std. Deviation	Std. Error Mean	95% CI of the Difference Lower	Upper	
Pair 1	TUMOR LOCATION	-0.43860	0.98230	0.13011	-0.69924	-0.17796	0.001
Pair 2	TUMOR SIZE	0.29825	0.65370	0.08658	0.12480	0.47169	0.001
Pair 3	TUMOR SIZE NUMBER	0.27193	1.16768	0.15466	-0.03790	0.58176	0.084
Pair 4	MUTATION FOUND	0.56140	0.62728	0.08309	0.39496	0.72784	0.000
Pair 5	MUTATION TYPE	-3.50877	0.68460	0.09068	-3.69042	-3.32712	0.000
Pair 6	MUTATION DIFFERENCE	1.40351	0.49496	0.06556	1.27218	1.53484	0.000
Pair 7	MUTATION SITES	0.47368	0.75841	0.10045	0.27245	0.67492	0.000
Pair 8	LYMPHO NODE METASTASIS	-0.68421	1.16738	0.15462	-0.99396	-0.37446	0.000
Pair 9	MULTIFOCALITY	1.14035	0.66651	0.08828	0.96350	1.31720	0.000
Pair 10	STAGE(AJCC)	0.36842	0.52207	0.06915	0.22990	0.50694	0.000
	GENDER with						
Pair 1	TUMOR LOCATION	-0.14035	0.95316	0.12625	-0.39326	0.11256	0.271
Pair 2	TUMOR SIZE	0.59649	0.65081	0.08620	0.42381	0.76918	0.000
Pair 3	TUMOR SIZE NUMBER	0.57018	1.20889	0.16012	0.24941	0.89094	0.001
Pair 4	MUTATION FOUND	0.85965	0.54898	0.07271	0.71399	1.00531	0.000
Pair 5	MUTATION TYPE	-3.21053	0.74969	0.09930	-3.40944	-3.01161	0.000
Pair 6	MUTATION DIFFERENCE	1.70175	0.46155	0.06113	1.57929	1.82422	0.000
Pair 7	MUTATION SITES	0.77193	0.59814	0.07923	0.61322	0.93064	0.000
Pair 8	LYMPHO NODE METASTASIS	-0.38596	1.03085	0.13654	-0.65949	-0.11244	0.007
Pair 9	MULTIFOCALITY	1.43860	0.62728	0.08309	1.27216	1.60504	0.000

AJCC: American Joint Committee on Cancer

Considering the results in Table (3), tumour location prevailed in the right side of the body (41.2%) for males but with equivalent percentages in the left and right for females (40.16%). Based on age, tumour location predominated the left body side (38.2%) of patients less than 45 years old and in the right (47.8%) of those who were and over 45 years old. The findings were followed by left and right (17.6, 20%) and isthmus (5.9, 0) for males and females along with (23.5, 13%) and (2.9, 0%) for ages < 45 and≥ 45 years old, respectively. Based on age, tumour size was observed in T1 (95%) for females and with lower percentages in T1 for males (88.2%), followed by equal percentages in T2 and T3 (5.9; 2.5%) for males and female. Tumour size predominated T1 (95.7%) for patients 45 years old and above and (91.2%) for those who were less than 45 years old, followed by T2 (5.9; 0%) and T3 (2.9; 0%) for ages < 45 and >= 45 years old, respectively.

The percentage of detected mutation was higher in males than females (87.5% versus 76. 5%), whereas in females, mutation was detected in patients less than 45 years old (85.3%) compared with those who were 45 years old and above (82.6%). Based on mutation type, the BRAF gene prevailed in males (100%) than in males (95%). Based on age, the BRAF gene dominated in patients 45 years old and above (100%) more than patients less than 45 years (94.1%). These results were followed by RET generation in females (2.5., 0%) and males (2.9, 0%) for ages < 45 and > = 45 years old, respectively. Mutation differences were observed in females more than males (40% versus 17%). Mutation difference was also detected in patients less than 45 years old (34%) more than patients 45 years old and above (23%). Considering mutation sites, BRAF (V600E) prevailed in females (30%) versus in males (13%). Based on age, BRAF (V600E) dominated in patients less than 45 years old (34%) more than in patients 45 years old and above (23%), followed by RET (NCOA4-RET) (RET-PTC3/4), which only dominated females and patients less than 45 years old in this study (Table 4).

We observed that LNM prevailed in N0 (47.1%) in males with lower percentages of LNM prevailed in N0 (40%) compared with

females, whereas N1a was observed in males (11.8%) more than females (5%). N1b was observed in females (55%) more than males (41.2%). Based on age, LNM predominated in N0 (52.2%) in patients 45 years old and above and in the right side of those who were less than 45 years old (35.3%), followed by N1b (58.8, 39.1%) for those aged < 45 and ≥ 45 years old and N1a (8.7, 5.9%) for patients ≥ 45 and < 45 years old. Multifocality percentages were more significant in males than females (35.5% versus 27.5%). Based on age, multifocality was higher in patients less than 45 years old (26.5%) more than in patients 45 years old and over (26.1%). AJCC Stage I was observed in males (94.1%) more in than in females (79. 5%). Based on age, AJCC Stage I was detected in patients less than 45 years old (97.1%) compared with patients 45 years old and above (95.7 %). These results were followed by AJCC Stage II with values of 5.9% for males and 2.5% for females and 4.3% and 2.9% for ages > = 45 and < 45 years old respectively (Table 4).

3.5 Association of Age and Gender with Clinicopathological Features and Mutation in PTC

According to multivariate analysis, mutation status was associated with age (P = 0.733) and gender (P = 0.203). Multivariate analyses demonstrated the association of mutation with age and gender and independent association of BRAF V600E mutation with poor prognostic factors (Table 5).

4 DISCUSSIONS

Sanger sequencing, PCR and immunohistochemistry are primary methods for detecting BRAF mutation. PCR is commonly used owing to its high efficiency. Therefore, we only collected data from articles using this method to detect mutations in this study. BRAF V600E occurs as a periodical mutation in thyroid cancer [11-13] and is restricted to papillary and anaplastic or poorly differentiated carcinomas [14]. Prevalence of this mutation is described in 35%–40% of papillary thyroid cancer cases, with a significantly higher prevalence in males than in females [11]. The current study demonstrated a higher significance in both genders when using the t-test. More than 10 types of BRAF mutation alternatives are reported for malignant tumours, for instance, bladder cancer, melanoma and PTC [10]. Here, we studied the relationship between BRAFV600E mutation and RET/PTC rearrangement with PTC. Previous studies showed BRAFV600E mutation, whereas PTC-related mortality in 1849 patients revealed a mortality of 5.3% (mutant) versus 1.1% (wild) (P < 0.001). This research established that BRAFV600E mutation is necessarily connected with extended malignancy-related mortality among patients with PTC [15, 16]. In this study, a significant relationship was observed between BRAFV600E mutation and clinicopathological traits, such as age and gender.

A review of literature has shown that the overall prevalence of BRAF mutation in PTC approximates 45% [17]. However, a propensity for BRAF V600E mutations is more common in female patients with Conventional variant of papillary thyroid cancer (CPTC). Such difference is possibly due to population effect and may be non-replicable for a study with more male subjects. On the other hand, hormonal-based indicate in women might bolster utilizing BRAF to drive cell development in the thyroid, whereas results of the current study showed that detected mutations were observed in males (87.5%) more than in females (76.5%). The present study also identified several relationships among BRAF V600E mutation and RET/PTC rearrangement and their pathological features in PTC. Large tumour size, old age, male gender, multicentricity and LNM are the main determinants of unsatisfactory results in PTC patients. Altogether, these results reinforce the directory that BRAF plays a role in the biology of thyroid cancers.

TABLE 4
THE PERCENTAGES BETWEEN AGE AND GENDER WITH SOME CLINICOPATHOLOGICAL FEATURES AND MUTATION IN THE PTC

		Gender % (N)		Age % (N)	
Tumor location		Male	Female)	< 45	≥ 45
	Left	35.3 (6)	40 (16)	38.2 (13)	39.1 (9)
	Right	41.2 (7)	40 (16)	35.3 (12)	47.8 (11)
	Left and right	17.6 (3)	20 (8)	23.5 (2)	13 (3)
	isthmus	5.9 (1)	0	2.9 (1)	0
Tumor size					
	T1	88.2(15)	95 (38)	91.2 (31)	95.7 (22)
	T2	5.9 (1)	2.5 (1)	5.9 (2)	0
	T3	5.9 (1)	2.5 (1)	2.9 (1)	4.3 (1)
Mutation found					
	Yes	76.5 (13)	87.5 (35)	85.3 (29)	82.6 (19)
	No	23.5 (4)	12.5 (5)	14.7 (5)	17.4 (4)
Mutation type					
	BRAF	100 (17)	95 (38)	94.1(32)	100(23)
	RET	0	2.5 (1)	2.9 (1)	0
	RET, NRAS	0	2.5 (1)	2.9 (1)	0
Mutation difference		100 (17)	100 (40)	100 (34)	100 (23)
Mutation sites					
	NO	23.5 (4)	12.5 (5)	14.7 (5)	17.4 (4)
	BRAE(V600E)	76.5 (13)	75 (30)	70.6 (24)	82.6 (19)
	RET(NCOA4-RET) (RET-PTC3/4)	0	12.5 (5)	14.7 (5)	0
Lympho node metastasis					
	N0	47.1 (8)	40 (16)	35.3 (12)	52.2 (12)
	N1a	11.8 (2)	5 (2)	5.9 (2)	8.7 (2)
	N1b	41.2 (7)	55 (22)	58.8 (20)	39.1 (9)
Multifocality					
	Yes	35.5 (4)	27.5 (11)	26.5 (9)	26.1 (6)
	No	76.5 (13)	72.5 (29)	73.5 (25)	73.9 (17)
AJCC Stage					
	I	94.1 (16)	79.5 (39)	97.1 (33)	95.7 (22)
	II	5.9 (1)	2.5 (1)	2.9 (1)	4.3 (1)

AJCC: American Joint Committee on Cancer BRAF: B isoform of RAF kinase, RET/PTC: Rearranged in transformation/papillary thyroid carcinoma gene, PTC= Papillary thyroid carcinoma RAS: Rat Sarcoma gene

Although the pervasiveness of RET/PTC rearrangement changes extraordinarily as per several reports, it remains less than that of BRAF transformations on average. As opposed to BRAF mutation, which is limited to PTC, RET/PTC rearrangements can be similarly present in favourable conditions, including trabecular adenomas and Hashimoto thyroiditis, as indicated by several studies [18, 19]. This condition may complicate the utilisation of RET/PTC recognition in molecular diagnosis of PTC. Results of the current study support this previous finding. Furthermore, in previous works, examination of 60 specimens for the vicinity of either RET/PTC1 or RET/PTC3 revealed that 18% of PTC tests were RET/PTC-positive, whereas tests detecting RET/PTC were negative for BRAF. Thus, molecular diagnosis of both BRAF mutation and RET/PTC rearrangements in fine-needle aspiration (FNA) refined the findings of 5 (4 BRAF and 1 RET/PTC) of 15 indeterminate/insufficient PTC FNAs [9].

A previous study failed to discover any relationship between BRAF V600E and RET/PTC rearrangements with age and gender, but a robust statistical association has been identified in PTCs with extra-thyroidal invasion, multicentricity, the presence of node metastases and higher tumour class, confirming that BRAF V600E is connected to more aggressive phenotypes [20, 21]. On the contrary, in our study, we noted the association between BRAF V600E and RET/PTC rearrangements and age and gender. However, by contrast, no statistical association has been observed in PTCs and multifocality, tumour size and nodal metastasis. In another study, BRAF mutations were related with older age, extra-thyroidal extension and more frequent presentation at clinical stages III and IV but showed no connection with distant metastases [14]. However, another study [22] revealed no relationship between BRAF mutation and any clinical-pathological characteristics, whereas corresponding significant distant metastases and advanced clinical stage presented no connection with older age and extra-thyroidal extension. Neither of these studies demonstrated any significant relationship between BRAF mutation and sex, tumour size and cervical LNM, whereas in the present study, BRAF mutations and RET/PTC rearrangements were found to be related to young age and male sex, presenting a highly statistical significance. A genetic clinical connotation analysis failed to find any connection between BRAF mutation and age at diagnosis, gender, dimension and local invasiveness of primary cancer, presence of LNM, tumour stage and multifocality of the disease [22], conforming with our results, except for age and gender, which showed a relation with BRAF mutation and RET/PTC rearrangements. As stated by two preceding studies [23, 24], BRAF mutations feature no predictive value in patients with papillary thyroid cancer; similarly, our results revealed no association between BRAF mutation and RET/PTC rearrangements and the examined clinical and pathological characteristics.

In our study, we identified no significant relationship between BRAF V600E mutation and RET/PTC rearrangement and clinicopathological characteristics, such as multifocality, tumour size and nodal metastasis, except for age and gender, which exhibited a significant relationship with BRAF mutation and RET/PTC rearrangements and another clinicopathological characteristic. Furthermore, results of this study confirm a highly significant difference in specific clinicopathological and mutations based on age, whereas no significant difference was associated with tumour size. Statistical analysis exposed a highly significant difference in certain clinicopathological and mutations based on gender but no significant difference obtained in tumour location. Based on these outcomes, BRAF V600E mutation and RET/PTC rearrangements show no connection to prognostic PTC factors that determine the invasiveness of tumours. Also, accordance with our findings, other authors described that presence of BRAF V600E mutation is not an aggressive prognosis on clinical findings in PTC [25].

TABLE 5
MULTIVARIATE ANALYSIS OF THE ASSOCIATION BETWEEN CLINICOPATHOLOGICAL FEATURES AND BRAFV600E MUTATION AND RET/PTC REARRANGEMENT IN PTC.

Features	Odds ratio	95% confidence interval		P value
		Lower bound	Upper bound	
Age	1.320	0.268	6.502	0.733
Gender	2.821	0.571	13.940	0.203
Tumor Location	1.697	0.564	5.108	0.347
Lympho node metastasis	1.282	0.553	2.975	0.562
multifocality	0.434	0.081	2.330	0.330

4 CONCLUSION

According to the findings of this study, despite being the most predominant oncogene in PTCs, BRAFV600E gene mutation and RET/PTC gene rearrangements bear no relation to clinicopathological PTC features, except for age and gender, and thus are inapplicable as possible markers of prognosis in current patients with PTCs.

AUTHORS' CONTRIBUTIONS

Hasan A. M. M. Almansoub: research design, acquisition, and analysis of data, drafting the paper, approval of the submitted and final versions; Maibouge Tanko Mahamane Salissou, and Yusra A. M. Almansob: critical paper revisions; Jamal H. Almansoob: Provide tips and help adjusted tables and figures; Yacoubou Abdoul Razak Mahaman: research design, and author coordination; Nie Xiu approval of the submitted and final versions.

ABBREVIATIONS

AJCC: American Joint Committee on Cancer , ARMS: Amplification refractory mutation system, ATC: Anaplastic thyroid cancer, BRAF: B isoform of RAF kinase, CPTC: Conventional variant of papillary thyroid cancer, CT: computed tomography, DNA: Deoxyribonucleic acid, DTC: Differentiated thyroid cancer, FFPEF: Formalin-fixed-paraffin-embedded, FNAB: Fine-needle aspiration biopsy, FNAC: Fine needle aspiration and cytology, FTC: Follicular thyroid cancer, FVPTC: Follicular variant of papillary thyroid cancer, HCC: Hürthle cell carcinoma, HUST: Huazhong University of Science and Technology, IHC: Immunohistochemistry, LN: Lymph node, MD: distant metastasis, MAPK: Mitogen-activated protein kinases, MEN: Medullary endocrine neoplasia, MTC: Medullary thyroid cancers, NCCN: National Comprehensive Cancer Network, PCR: Polymerase chain reaction, PTC: Papillary thyroid cancer, RAI: Radio-active iodine, RAS: Rat Sarcoma gene, RET/PTC: Rearranged in transformation/papillary thyroid carcinoma, RRA: Radio-active iodine thyroid remnant ablation, T: Primary tumor, T4: Thyroxine, TG: Thyroglobulin, TNM: T-Tumor/N-Node/M-Metastasis, TRH: Thyrotropin-releasing hormone ,TSH: Thyroid-stimulating hormone or thyrotropin, TTE: Total thyroidectomy, UICC: Union International Central Cancer (International Union Against Cancer), US: Ultrasonography, VEGF: Vascular endothelial growth factor, WHO: World Health Organization.

CONFLICT OF INTERESTS

All the authors declare no conflict of interest in the current study.

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