BRAF V600E mutation and correlation with clinicopathologic features in radioactive iodine refractory papillary thyroid carcinoma

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Summary

Objective: To define the rate of *BRAF V600E* mutation in radioactive iodine (RAI) refractory papillary thyroid carcinoma (PTC). The correlation *BRAF V600E* mutation with some clinicopathologic features in RAI refractory PTC. *Subject and method:* 92 patients with RAI refractory PTC were operated recurrent lesions at 108 Military Central Hospital from 9/2009 to 3/2018. Recurrent lesions were evaluated histopathological characteristics, immunohistochemical staining with BRAF V600E (Ventana, Roche), *BRAF V600E* gene test by real-time PCR. Evaluating the status of mutated *BRAF V600E* and correlate with some clinicopathologic features. *Result*: Average age: 47.1 ± 15.6 (12 - 81); the rate of *BRAF V600E* mutation accounted for 80.4%; the average age of unmutated *BRAF V600E* RAI refractory PTC group (40.2 ± 17.2) was lower than the group of *BRAF V600E* mutation (48.5 ± 14.9) (p=0.042); *BRAF V600E* mutation in early-stage disease (I - II) had a higher rate than ones in advanced disease (II-IV); similarly, this gene mutation occurred more frequent in the group received more than 600mCi cumulative dose of RAI compared with the group received less than 600mCi cumulative dose. There was no correlation of *BRAF V600E* mutation with age, tumor size, lymph node, distant metastasis. *BRAF V600E* mutation in tall cell variant of PTC was seen more frequently than other variants and it was also seen in cases of extracapsular lymph node invasion. There was no relationship between the existence of mutated *BRAF V600E* with vessel invasion and calcification. *Conclusion:* The rate of *BRAF V600E* mutation in RAI refractory PTC is high, accounting for 80.4%. It correlates with some clinicopathologic characteristics such as disease stage, cumulative dose of radioactive iodine, tall cell variant of PTC; no correlation of *BRAF V600E* mutation with age, tumor size, lymph node and distant metastasis, extracapsular lymph node invasion.

Keywords: *BRAF V600E*, recurrence, radioactive iodine refractory, thyroid cancer.

1. Background

Papillary thyroid carcinoma (PTC) is the most common cancer of the thyroid gland and the prevalence of PTC is continuously arising, mainly in early detected thyroid cancer with tumor size of less than 1cm [1]. In general, for malignant tumors, relapse and metastasis are closely associated with the rate of mortality but this does not really apply in differentiated thyroid carcinoma including PTC. Especially, young patients with high risk of recurrence have low mortality [2], [3]. The application of suitably surgical procedures, RAI therapy and thyroid hormone therapy has significantly improved the prognosis for patients with RAI refractory PTC. The rate of overall survival over ten years is higher than 90% [2].

RAI therapy has been implemented for over 50 years, considered a targeted therapy for iodine absorption cells. However, the rate of relapse is still

Received: *12 October 2021,* ***Accepted:*** *31 October 2021*

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higher than 20%, the mortality highly increases after 10 years. According to American Thyroid Association identified the concept of RAI refractory as follows [4]: 1. The malignant tissues dose not concentrate ^{131}I outside the thyroid bed at the first therapeutic whole body scintigraphy. 2. Patients with measurable disease without ^{131}I uptake on subsequent diagnostic whole body scintigraphy. 3. ^{131}I uptake is retained in some lesions but not in others. 4. Metastasis disease progress despite significant uptake of ^{131}I (new lesions, progressive growth of lesions, continual rise in serum Tg within months of RAI therapy). In recent years, studies reported that the role of *BRAF V600E* mutation was not only related to pathogenesis of thyroid cancer, recurrence, metastasis and aggressive disease but also related to the process of ^{131}I absorption [8]. Therefore, studying on *BRAF V600E* mutation and relationship with clinicopathologic features of patients with RAI refractory PTC will supply more information about disease status.

2. Subject and method

2.1. Subject

92 patients with recurrent, metastatic PTC who were operated recurrent, metastatic lesions at 108 Military Central Hospital between March 2012 and July 2018.

Inclusion criteria

PTC patients who had total thyroidectomy, ^{131}I treatment, and were periodically monitored.

Presence of recurrent, metastatic lesions on diagnostic immaging (ultrasound, CT, PET/CT) 6 months after the first ^{131}I treatment.

Patients were diagnosed with RAI refractory according to American Thyroid Association 2015.

Availability of clinical data and good quality formalin-fixed paraffin-embbed tissues.

92 patients with full criteria were operated to take out repetitive lesions, including 85/92 (92.4%) of lymph node metastasis, 14/92 (15.2%) of local recurrence at thyroid bed, 4/92 (4.3%) of metastatic lesions to skin, 2/92 (2.1%) of trachea invasion and 1 (1.1%) case of thymic metastasis.

2.2. Method

Study design: A cross-sectional descriptive study.

Data collection tools and method.

Extract clinical data from medical records.

TNM staging was based on AJCC 2010.

Recurrent risk was assessed using America Thyroid Association (ATA) 2009.

Classification of RAI refractory PTC was based on ATA 2015.

Histological studies:

Histopathological types were determined according to WHO 2017.

Evaluation of intravascular tumor cells should be adherent to the vessel walls, either covered by endothelium or in a context of thrombus or fibrin.

Evaluation of extranodal extension when tumor cells extended beyond the lymph-node capsule into the perinodal fibroadipose tissue.

Detection of *BRAF V600E* mutation by two methods:

Immunohistochemistry staining was conducted using Ventana's automated BenchMark Ultra machine, with BRAF V600E (Roche). No staining of tumor cells was considered negative. BRAF V600E was positively evaluated when the cytoplasm membrane of tumor cells expressed brown.

DNA extraction was conducted using the CE-IVD biological kit, Invisorb® Spin Tissue Mini Kit (Invisorb) manufactured by Stratec, Germany. Detection of *BRAF V600E* mutation: By PCR technique using CE-IVD biological kit, Thyroid Cancer Mutation Analysis Kit manufactured by EntroGen, USA.

2.3. Statistical analysis

Using statistical software SPSS, version 20.0. Evaluation: $p<0.05$ difference was statistically significant, $p>0.05$: Difference was not statistically significant.

3. Result

92 patients with RAI refractory PTC were dissected repetitive and metastatic lesions with average age: 47.1 ± 15.6 years, the youngest age was 18 years old, the highest age was 81 years old. The average age of the group without *BRAF V600E* mutation (40.2 ± 17.2) was lower than the *BRAF* carrier group (48.5 ± 14.9) with p=0.042.

The proportion of mutation of BRAF V600E gene in RAI refractory PTC was 80.4%.

Table 1. The patients' clinicopathologic characteristics

Characteristics	Number (%)		
Sex	Female	68	73.9
	Male	24	26.1
Age	< 45	45	48.9
	≥ 45	47	51.1
Stage (n = 86)	Stage I - II	60	70
	Stage III - IV	26	30
Primary tumor size (n = 77)	T1 - T2	52	67.5
	T3 - T4	25	32.5
Lymph node metastasis (n = 88)		57	64.8
Distant metastasis		17	18.5
Recurrent risk (n = 88)	Low to intermediate	15	17
	High	73	83
^{131}I Cumulative dose (n = 64)	< 600µCi	54	84.4
	≥ 600µCi	10	15.6
Histology	Classical PTC	50	54.3
	Tall cell variant	16	17.4
	Other variant	26	28.3
Extranodal extension (n = 85)	No	29	34.1
	Yes	56	64.9
Vessel invasion (n = 92)	No	75	81.5
	Yes	17	18.5
Calcification	No	68	73.9
	Yes	24	26.1

Patients with I-II stage accounted for 70%. T1-T2 stage at the first diagnosis was 67.5%, nearly double when compared with T3-T4 stage (32.5%). Most cases had lymph node metastasis at the first diagnosis (64.8%), belonged to the group of highly recurrent risk (83%). Classical PTC accounted for 54.4%, followed by tall cell variant (17.4%), other variants only were 28.3%. 64.9% of the extranodal extension was quite high while vessels invasive was 18.5

Table 2. Correlation of clinical characteristics with *BRAF V600E* mutation

Characteristics		*BRAF V600E* mutation		p
		Negative n (%)	Positive n (%)	
Age	< 45	12 (66.7)	33 (44.6)	0.093
	≥ 45	6 (33.3)	41 (55.4)	
Sex	Female	13 (19.1)	55(80.9)	1.000

	Male	5 (20.8)	19 (79.2)	

Table 2. Correlation of clinical characteristics with *BRAF V600E* mutation (Next)

Characteristics		*BRAF V600E* mutation		p
		Negative, n (%)	Positive, n (%)	
Stage	I - II	16 (26.7)	44 (73.3)	0.032
	III - IV	1 (3.9)	25 (96.1)	
Tumor size	T1 - T2	8 (15.4)	44 (84.6)	0.167
	T3 - T4	8 (32.0)	17 (68.0)	
Lymph node metastasis	No	8 (25.8)	23 (74.2)	0.521
	Yes	10 (17.5)	47 (82.5)	
Distant metastasis	No	13 (17.3)	62 (82.7)	0.311
	Yes	5 (29.4)	12 (70.6)	
Recurrent risk	Low-intermediate	1 (6.7)	14 (93.3)	0.285
	High	16 (21.9)	57 (78.1)	
Cumulative dose	< 600μCi	6 (11.1)	48 (88.9)	0.01
	≥ 600μCi	5 (50.0)	5 (50.0)	

In RAI refractory PTC, *BRAF V600E* mutation in stage I-II was higher than in stage III-IV ones with p=0.032. The group received ^{131}I cumulative dose less than 600mCi highly carried mutated *BRAF V600E* than those received more than 600 mCi (p=0.01). *BRAF V600E* gene status had no relationship with age, tumor size, lymph node metastasis and distant metastasis.

Table 3. Correlation of pathologic characteristics with *BRAF V600E* mutation

Characteristics		*BRAF V600E* mutation		p
		Negative, n (%)	Positive, n (%)	
Histopathology	Clasical PTC	12 (24)	38 (76)	0.03
	Tall cell variant	0 (0)	16 (100)	
Extranodal extension	No	11 (37.9)	18 (62.1)	0.001
	Yes	4 (7.14)	52 (92.8)	
Vessel invasion	No	15 (20)	60 (80)	1.000
	Yes	3 (17.6)	14 (82.3)	
Calcification	No	8 (15.4)	44 (84.6)	1.000
	Yes	13 (19.1)	55 (80.9)	

BRAF V600E gene was frequently seen in tall cell variant distinguished than in other variants, in the group of extracapsular invasion than the group of non-invasion. The correlation between *BRAF V600E* gene with vessel invasion and calcification feature was not seen.

4. Discussion

Management and treatment for patients with RAI refractory PTC is a big challenge for clinicians because additional RAI treatment does not bring any benefit. ^{131}I should not be additionally used with over 600 mCi cumulative dose in the cases of RAI refractory [3]. Surgery removing repetitive lesions is still a main approach for these cases. Chemotherapy and radiotherapy are palliatives only. Clinical trials with targeted therapy drugs following the mechanism of a MAPK pathway are applying and open new prospects.

Mutated *BRAF V600E* proved to be the most common gene in causing PTC in adults with the frequency from 36% to 69% [5]. The rate of *BRAF V600E* mutation greatly rises in the case of recurrent and distant metastasis without RAI avidity, declining the average survival time. Our study showed 80.4% of *BRAF V600E* mutation. This result was similar to Henderson's one in that the rate of mutated *BRAF V600E* in recurrent PTC was 78% but any way the RAI uptake situation was not mention [6]. Some studies showed that this mutation was not related to age. Additionally, no relationship between *BRAF V600E* with the patient's age was observed in this study. The difference is not significant between the group of younger than 45 years compared with the group age over 45. Anotther study indicated that *BRAF V600E* did not occur in children and young people [7]. In Xing's research, mutated *BRAF* in the recurrent group age over 60 years was higher than the group age over 60. This probably correlated with biological characteristics of PTC between children and adults, partly due to the dependence on the prevalence of *BRAF V600E* mutation [8].

Many authors also mentioned the correlation of tumor progression with B*RAF V600E* mutation but other analyses did not find any correlation. According to our analysis, *BRAF V600E* mutation did not had the association with tumor size, lymph node metastasis at the first diagnosis and distant metastasis. *BRAF V600E* mutation in cases of RAI refractory was related to disease stage and iodine cumulative dose. The patients with RAI refractory received over 600mCi cumulative dose had higher frequency of mutated *BRAF V600E* than in the patients who received less than 600mCi (p=0.01). The proportion of *BRAF V600E* mutation was lower in the I-II stage than the III-IV ones. Mutated *BRAF V600E* was associated with the III-IV stage was reported in Nikiforova and Kebebw' studies [9], [10]. RAI treatment is also a crucial problem for PTC with recurrent disease and metastasis. The effectiveness of RAI therapy is independent of RAI doses divided into tumor tissue and the ability of uptake iodine from tumor cells. Nonetheless, PTC could be lost iodine absorption, mainly causing failure treatment. On the other hand, the change of the *BRAF V600E* gene seemed to be related to the capacity of iodine-avid thyroid tumor cells. The problem was illustrated in clinical practice of our research cases. Even mutated *BRAF V600E* had not been discovered before, recurrent and metastasis lesions were considered removing instead of additional RAI therapy. Several analyses proved that tumors with *BRAF V600E* mutation reduced NIS expression and therefore, node recurrence in the case of *BRAF* positivity limited RAI absorption [11].

When investigating the relationship of *BRAF V600E* mutation with histopathologic features, the study revealed that the characteristics of histological variants, vessel invasion, extranodal and extrathyroid extension correlated with progressiveness of thyroid carcinoma. No definitive evidence of the mutated *BRAF* correlation with vessel invasion was shown in our study. The association of *BRAF V600E* mutation was significant with histological variants and extranodal extension (Table 2). Some observational studies expressed highly mutated *BRAF V600E* in PTC accompanied with lymph node metastasis. Interestingly, increasingly mutated *BRAF V600E* in the case of recurrent lymph nodes did not relate to lymph node involvement at initial diagnosis. The extranodal extension intensively progressed in the group of *BRAF V600E* mutation than in the group of non-mutation [12]. For PTC, histological variants are also a crucial factor for evaluating risk factors. American Thyroid Association rendered aggressive variants such as tall cell variant, columnar cell variant, hobnail variant... to one of criteria promoted recurrent risk [10]. In our collected data, the rate mutated *BRAF V600E* in tall cell variant of PTC accounted for 100% of which was higher than in classical PTC. The differentiation is statistical significance with p=0.03. Our result is similar to Ricarte-Filho's study that *BRAF V600E* mutation occured 100% in tall cell variant of RAI refractory PTC. In the elderly group with PTC, the tall cell variant has higher *BRAF V600E* mutation than the classical PTC and the follicular variant of PTC [13]. Ghossein and his

colleagues indicated that the tall cell variant is more aggresive than the classical PTC but no association with age, sex, tumor size, extranodal thyroid invasion [14]. It could partly explain the independent role of *BRAF V600E* mutation that commonly happened in the tall cell variant.

5. Conclusion

Our researches revealed useful information about mutated *BRAF V600E*. The high proportion of *BRAF V600E* mutation in patients with RAI refractory accounted for 80.4%. *BRAF V600E* mutation correlate with disease stage, RAI cumulative dose, tall cell variant but no evidence of relationship with age, tumor size, lymph node metastasis, distant metastasis, extranodal extension, vessel invasion and calcification.

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