Endokrynologia Polska
DOI: 10.5603/EP.a2019.0025
Volume/Tom 70; Number/Numer 5/2019
ISSN 0423–104X

BRAF V600E mutation in papillary thyroid cancer is correlated with adverse clinicopathological features but not with iodine exposure

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Abstract

Introduction: $BRAF^{V600E}$ activating mutation is the most frequent genetic abnormality in the pathogenesis of papillary thyroid carcinoma. We aimed to evaluate the association between $BRAF^{V600E}$ mutation and well-established prognostic clinicopathological characteristics as well as iodine exposure.

Material and methods: From 2000 to 2012, the data of PTC patients admitted to Dr. Lutfi Kirdar Kartal Education and Research Hospital in Turkey were reviewed retrospectively. Clinicopathological parameters were collected. $BRAF^{V600E}$ mutation was analysed by DNA sequencing method in tumour specimens. We hypothesised that $BRAF^{V600E}$ mutation prevalence is positively correlated with prolonged iodine exposure and expected to be higher in the second half of the recruitment period due to the increment in time spent from the iodisation process of the table salt in our country. Thus, iodine exposure was categorised as short-term (2000–2006) and long-term (2006–2012).

Results: A total of 197 patients were accrued. The study population predominantly consisted of conventional variant. A statistically significant relationship was observed between $BRAF^{V600E}$ mutation presence and age ($p = 0.03$), conventional variant PTC ($p = 0.00002$), T4 stage ($p = 0.002$), vascular invasion ($p = 0.036$), thyroid capsule invasion ($p < 0.00001$), extrathyroidal tissue invasion ($p < 0.00001$), and lymph node metastasis ($p < 0.00001$). When categorised as long-term and short-term, iodine exposure was not statistically significantly related with $BRAF^{V600E}$ mutation; however, there were far more PTC cases in the long-term group (86.3% *vs.* 13.7%).

Conclusion: We revealed that $BRAF^{V600E}$ mutation is associated with adverse clinicopathological parameters. There appeared to be no relation between long-term iodine exposure and $BRAF^{V600E}$. **(Endokrynol Pol 2019; 70 (5): 401–408)**

Key words: *papillary thyroid cancer; BRAF V600E; iodine*

Introduction

Thyroid carcinoma is the most frequent type of endocrine-borne malignancies. Papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), which are derived from follicular cells and C-cell-derived medullary thyroid carcinoma, are the common subtypes. Anaplastic type is the rarest variant and is highly aggressive [1].

Differentiated thyroid carcinomas, PTC and FTC, are slow-growing cancers. Early-stage disease can be treated successfully with surgical excision. Patients with disseminated disease eventually die from their cancer, although the majority survive for years, which is an uncommon circumstance for many other advanced-stage malignancies.

An understanding of thyroid cancer pathogenesis remains critical for the prevention of the disease occurrence and for the development of targeted therapies directed against the causative pathway in the care of patients with advanced disease. Contributory genetic abnormalities have been defined to cause different subtypes of thyroid cancer. Frequent genetic abnormalities associated with PTC are the *BRAF* activating mutations, fusion oncogene *RET/PTC*, and *NTRK* rearrangements. $BRAF^{V600E}$ activating mutation, which occurs in 29-83% of tumours, is the most common mutation in PTC. *BRAF* is the downstream target of *RAS* in the mitogen-activated protein kinase (*MAPK*) signalling pathway. Following activation, *RAF* interacts with MEK and initiates phosphorylation of ERK kinase leading its activation. Activated *ERK* mediates the transcription

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of many genes, which promotes cellular growth and survival [2–5].

Radiation exposure and iodine excess are the best-known predisposing factors for the development of PTC [6, 7]. There is some evidence that high iodine exposure may be a driver event for transforming *BRAF* to a constitutively active state [7]. In Turkey, iodisation of table salt was practised in 1999 and fully carried out in 2002 [8–10]. Duration of time from the iodisation process may be an indirect measure of iodine exposure.

Extensive data suggest that $BRAF^{V600E}$ mutation is associated with a poorer prognosis compared to PTC without harbouring the $BRAF^{V600E}$ mutation. It appears to be associated with increased risk of extrathyroidal tumour extension, lymph node metastases, and recurrence [7]. In this study, we aimed to describe the relation of iodine exposure, if any, as well as the clinicopathological factors with $BRAF^{V600E}$ mutation.

Material and methods

Study oversight

This study was conducted in compliance with the ethical principles according to the Declaration of Helsinki, and it was approved by the local Institutional Review Board (April 8, 2014). Data of PTC patients admitted to Dr. Lutfi Kirdar Kartal Education and Research Hospital were reviewed retrospectively. A total of 197 patients (159 females and 38 males; median age 46, range 17–86 years) with PTC were screened for $BRAF^{V600E}$ mutation in this study from October 2000 to October 2012. Other clinicopathological features including PTC variant, tumour size, T stage, necrosis, calcification, vascular invasion, tumour capsule status and invasion, extrathyroidal invasion, multicentricity, and concomitant pathology in thyroid tissue were collected.

Trials conducted in an effort to define the iodine status in the Turkish population, with the use of urinary iodine excretion, are presented in Table I. Urinary iodine excretion was not evaluated in our study. Using the national studies as the basis for the iodine status of the study population, we principally aimed to look for the concept of *relative increment in iodine exposure* [8-11]. We hypothesised that $BRAF^{V600E}$ mutation prevalence is positively correlated with increasing iodine exposure and is expected to be higher in the second half of the recruitment period (2006 to 2012) due to the increment in exposure time spent from the iodisation process of table salt in our country. Thus, iodine exposure was categorised as short-term (2000–2006) and long-term (2006–2012).

Method

DNA Isolation

Genomic DNA was extracted from 8-10 μm sections of formalin-fixed and paraffin-embedded (FFPE) PTC tissue samples, starting with deparaffinisation using conventional xylene/ethanol treatment, one-hour incubation with proteinase K, and subsequent DNA purification utilising the QIAampDNA FFPE tissue kit (Qiagen, USA) according to the manufacturer's instructions. Following the DNA isolation, DNA were archived at –20°C in a freezer until the start of the study.

PCR and DNA sequencing

In order to detect the mutations at exon 15 of the *BRAF* gene, PCR was performed with the following forward and reverse primers as described by Qu K. et al. (2013): *BRAF* 15F, 5'-CCTAAACTCTTCATAATGCTTGCT-3'; and *BRAF*15R, 5'-AGTAACTCAGCAGCATCTCAGG-3' [12].

Briefly, PCR was performed in a 50 mL volume containing 50 to 100 ng of genomic DNA; 20 pmol/L forward and 20 pmol/L reverse primers; HotStarTaq Master Mix (Qiagen, USA) including HotStarTaq DNA Polymerase (2.5 U), PCR Buffer (with 1.5 mM $MgCl_2$), and 200 μM each dNTP in final reaction volume. The PCR amplification was carried out in a Proflex thermocycler (ABI, USA) under the following conditions: one cycle at 95°C for 15 minutes; 40 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds; and a final extension step at 72°C for 10 minutes. Amplified PCR products were purified by using the PEG precipitation method as described by Rosenthal et al. (1993) [13]. The purified PCR products were sequenced utilising DTCS quick start kit (Beckman Coulter, USA). The sequencing reaction was carried out in a Proflex thermocycler at 96°C for 20 s, 50°C for 20 s, and 60°C for four minutes, according to the manufacturer's manual. Sequence analysis was performed on the automatic DNA sequencer (Beckman Coulter Genome Lab GeXP Genetic Analysis System, USA). DNA sequences and chromatograms obtained were examined by using the Genome Lab GeXP Genetic Analysis System Version 10.2 DNA sequencing program (Beckman Coulter, USA).

Statistical analysis

All statistical analyses were carried out using SPSS 17.0 version (IBM Corp., Armonk, NY, USA). Characteristics of patients were evaluated with descriptive analysis. Chi-squared test and Fisher's exact test were used in order to compare the clinicopathological features as well as the iodine exposure status, between $BRAF^{V600E}$-positive and -negative subgroups. P values below 0.05 were accepted as statistically significant.

Table I. *An overview of the iodine status screening trials in the Turkish population*

	Screening size	Study population (number)	Median UIC [μg/L]
1997–1999	National (20 regions)	5.948	36 μg/L
2002*	National (20 regions)	4.128	53 μg/L
2002**	National (10 regions)	7.006	87,5 μg/L
2002***	National (30 regions)	11.134	75 μg/L
2007****	National (30 regions)	2.280	130 μg/L

*Follow-up studies of 1997–99 screening in 2002 (same 20 regions); **10 new regions screened in 2002 with unknown previous status; ***Total results of all 2002 screening (30 regions); ****Follow-up studies of 2002 screening in 2007; UIC — urinary iodine concentrations

Results

Patient characteristics

A total of 197 patients with PTC were screened for *BRAF*^{V600E} mutation in this study. Characteristics of patients are summarised in Table II. Conventional variant (57.4%) was the predominant variant, followed by follicular (33.5%) and oncocytic (9.1%) variants. Tumour size ranged from 0.1 to 8 cm with a median size of 0.9 cm. Microcarcinoma slightly dominated the samples with a ratio of 53.6%. According to the eighth pTNM staging, 118 patients (60.1%) had T1, 31 patients (15.7%) T2, 27 patients (13.7%) T3, and 21 patients (10.5%) had T4 tumours. Extratumoural thyroid tissue consisted of diffuse hyperplasia (30.4%), nodular adenomatous hyperplasia (24.3%), nodular hyperplasia (19.8%), lymphocytic thyroiditis (17.8%), and multinodular adenomatous hyperplasia (14.7%) with decreasing frequency. One or more condition/s might have been seen in one extratumoural tissue sample. Most of the tumours were limited to thyroid tissue, with only 17.7% having extrathyroidal tissue invasion and 7.6% exhibiting lymph node metastasis. Of 35 patients with extrathyroidal tissue invasion, 14 (40%) had minor and 21 (60%) had gross spread. While calcification status (45.2% present, 54.8% absent) was almost evenly shared by samples, the presence of necrosis (1.5%) and vascular invasion (5.1%) were considerably low.

BRAF^{V600E} *mutation*

BRAF^{V600E} mutation frequency was found to be 22.8% (45/197) in this study. Association of *BRAF*^{V600E} mutation with clinical and pathological parameters is detailed in Table III. A statistically significant relationship was observed between *BRAF*^{V600E} mutation presence and conventional variant PTC ($p = 0.00002$), T4 stage ($p = 0.002$), vascular invasion ($p = 0.036$), thyroid capsule invasion ($p < 0.00001$), extrathyroidal tissue invasion ($p < 0.00001$), and lymph node metastasis ($p < 0.00001$). Age, for the cut-off level, 45 (7th TNM), was not significantly associated with *BRAF*^{V600E} mutation whereas age, for the cut-off level of 55 (8th TMN), was significantly associated with mutation status ($p = 0.03$). The extratumoural thyroid background, tumour necrosis and capsule formation were not significantly associated with *BRAF*^{V600E} mutation. When grouped in two, iodine exposure, was not statistically significantly related with *BRAF*^{V600E} mutation; however, there were more PTC cases in the long-term group compared to short-term (86.3% *vs.* 13.7%).

Rare BRAF mutations

Along with *BRAF*^{V600E} mutation, *BRAF*^{F583Y}, *BRAF*^{F595L}, and *BRAF*^{V600V} were also detected in the study group.

Table II. *Clinicopathological features of patients*

		N (%)
Age	≤ 55	147 (74.6%)
	> 55	50 (25.4%)
Gender	Female	159 (80.7%)
	Male	38 (19.3%)
PTC variants	Conventional	113 (57.4%)
	Follicular	66 (33.5%)
	Oncocytic	18 (9.1%)
T stage	T1	118 (60.1%)
	T2	31 (15.7%)
	T3	27 (13.7%)
	T4	21 (10.5%)
Tumour size	Microcarcinoma	109 (55.3%)
	Macrocarcinoma	88 (44.7%)
Necrosis	Present	3 (1.5%)
	Absent	194 (98.5%)
Calcification	Present	89 (45.2%)
	Absent	108 (54.8%)
Vascular invasion	Present	10 (5.1%)
	Absent	187 (94.9%)
Capsule formation	Present	89 (45.2%)
	Absent	108 (54.8%)
Tumour capsule invasion	Present	44 (22.3%)
	Absent	153 (77.7%)
Thyroid capsule invasion	Present	40 (20.3%)
	Absent	157 (79.7%)
Extrathyroidal tissue invasion	Present	35 (17.7%)
	Absent	162 (82.3%)
Multicentricity	Present	33 (16.8%)
	Absent	164 (83.2%)
Lymph node metastasis	Present	15 (7.6%)
	Absent	182 (92.4%)
Extratumoural thyroid tissue*	Lymphocytic thyroiditis	35 (17.8%)
	Diffuse hyperplasia	60 (30.4%)
	Nodular hyperplasia	39 (19.8%)
	Nodular adenomatous hyperplasia	48 (24.3%)
	Multinodular adenomatous hyperplasia	29 (14.7%)
***BRAF*^{V600E}**	Wild	152 (77,2%)
	Mutant	45 (22.8%)
Iodine exposure	Short-term	27 (13.7%)
	Long-term	170 (86.3%)

*One or more condition might have been seen in one extratumoural tissue sample; PTC — papillary thyroid carcinoma

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Table III. *Association of BRAF^{V600E} mutation with other clinicopathological characteristics*

	Wild n (%)	Mutant n (%)	p
Gender			0.318
Female	125 (78.6%)	34 (21.4%)	
Male	27 (71.1%)	11 (28.9%)	
PTC variants			0.00002
Conventional	74 (65.5%)	39 (34.5%)	
Follicular	63 (95.5%)	3 (4.5%)	
Oncocytic	15 (83.3%)	3 (16.7%)	
Tumour size			0.183
Microcarcinoma	88 (80.7%)	21 (19.3%)	
Macrocarcinoma	64 (72.7%)	24 (27.3%)	
T stage			0.002
T1	100 (84.7%)	18 (15.3%)	
T2	27 (87.1%)	4 (12.9%)	
T3	14 (51.8%)	13 (48.2%)	
T4	11 (52.3%)	10 (47.7%)	
Vascular invasion			0.036
Present	5 (50.0%)	5 (50.0%)	
Absent	147 (78.6%)	40 (21.4)	
Multicentricity			0.506
Present	24 (72.7%)	9 (23.7%)	
Absent	128 (78%)	36 (22%)	
Extrathyroidal extension			< 0.00001
Present	14 (40.0%)	21 (60.0%)	
Absent	138 (85.2%)	24 (14.8%)	
Calcification			0.211
Present	65 (73.0%)	24 (27.0%)	
Absent	87 (80.6%)	21 (19.4%)	
Thyroid capsule invasion			< 0.00001
Present	16 (40%)	24 (60%)	
Absent	136 (86.6%)	21 (13.4%)	
Lymph node metastasis			< 0.00001
Present	2 (13.3%)	13 (86.7%)	
Absent	150 (82.4%)	32 (17.6%)	
Age			0.03
≤ 55	119 (81.0%)	28 (19.0%)	
> 55	33 (66.0%)	17 (34.0%)	
Thyroid background			0.217
Lymphocytic thyroiditis	28 (80.0%)	7 (20.0%)	
Diffuse hyperplasia	41 (68.3%)	19 (31.7%)	
Nodular hyperplasia	30 (76.9%)	9 (21.3%)	
Nodular adenomatous hyperplasia	42 (87.5%)	6 (12.5%)	
Multinodular adenomatous hyperplasia	22 (75.9%)	7 (24.1%)	
Iodine exposure			0.059
Short-term	17 (63.0%)	10 (37.0%)	
Long-term	135 (79.4%)	35 (20.6%)	

PTC — papillary thyroid carcinoma

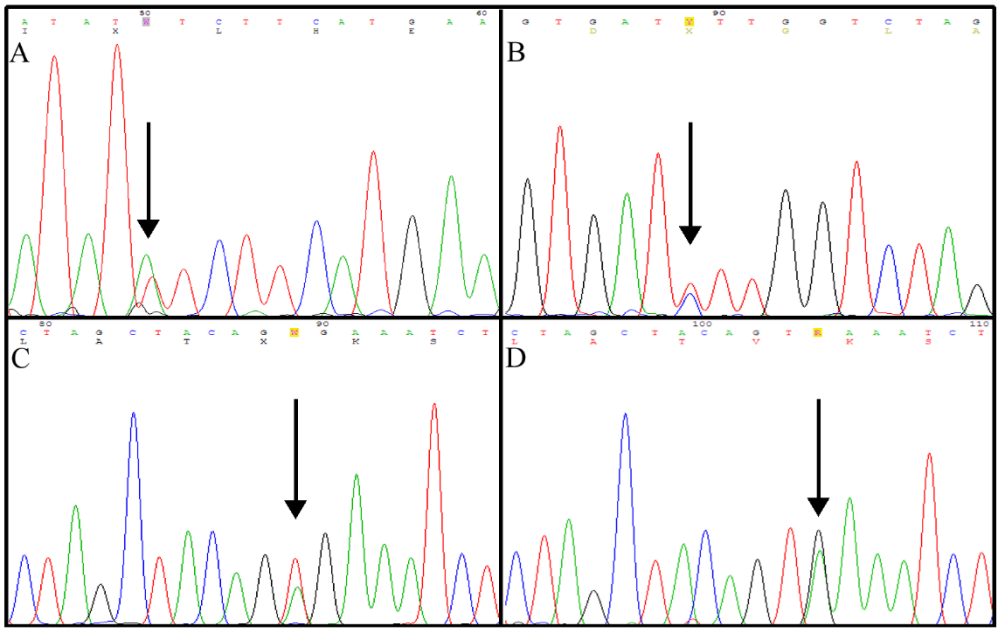

Figure 1. *Detected mutations at exon 15 of the BRAF gene.* ***A.*** *ttt→tat exchange at codon 583 (BRAF^{F583Y} mutation);* ***B.*** *ttt→ctt exchange at codon 595 (BRAF^{F595L} mutation);* ***C.*** *gtg→gag exchange at codon 600 (BRAF^{V600E} mutation);* ***D.*** *gtg→gag exchange at codon 600 (BRAF^{V600V} mutation). Arrows indicate localisation of the mutations*

Table 4. ***Rare*** *BRAF* ***mutations***

BRAF^{F583Y}	**Variants of PTC**	**Tumour size [cm]**	**Thyroid capsule invasion**	**Soft tissue invasion**	**Lymph node metastasis**
Patient 1	Conventional	1.5	+	+	+
Patient 2	Conventional	1.4	+	+	+
Patient 3	Conventional	1.1	+	+	+
Patient 4	Conventional	2.5	+	+	+
BRAF^{F595L}					
Patient 1	Oncocytic	3.5	+	+	–
BRAF^{V600V}					
Patient 1	Conventional	0.6	–	–	–
Patient 2	Follicular	3.0	–	–	–
Patient 3	Oncocytic	3.5	+	+	–

PTC — papillary thyroid carcinoma

The *BRAF^{F583Y}* was found in four conventional variants of PTC. Tumours with *BRAF^{F83Y}* mutation were macrocarcinomas, all of which had thyroid capsule invasion, soft tissue invasion, and lymph node metastasis. The identified *BRAF^{F595L}* mutation in the oncocytic variant of PTC was a macrocarcinoma with thyroid capsule and soft tissue invasion. Lastly, the *BRAF ^{V600V}* mutation was estimated as one in each of the conventional, follicular, and oncocytic variants (Fig. 1). Regarding this mutation arising from three different PTC variants, the conventional one was microcarcinoma whereas follicular and oncocytic variants were noted as macrocarcinoma (Tab. IV). All these mutations were previously reported, and they were not directly associated with the constitutive activation of the BRAF protein. But it is reported that *BRAF ^{F595L}* mutation is a gain-of-function variant with intermediate activity that does not act paradoxically, but cooperates with mutant *RAS* to promote oncogenic signalling [14, 15].

Discussion

Papillary thyroid carcinoma is the most frequently encountered malignant thyroid tumour. Although cu-

rative treatment options for advanced disease are still lacking, rapid progress has been made over the years in understanding the molecular mechanisms underlying PTC tumorigenesis and progression. Among the genetic abnormalities, activating $BRAF^{V600E}$ in the mitogen activated protein kinase pathway is the most commonly observed mutation, with a prevalence of 29–83% [2–5]. In the current study, we evaluated the $BRAF^{V600E}$ mutation prevalence and its association with clinicopathological characteristics and a potential environmental causative agent of BRAF V600E, iodine exposure.

The presence of $BRAF^{V600E}$ mutation portends a worse prognosis in many series [16–18]. In an analysis of 314 patients, those with a $BRAF^{V600E}$ mutation had a significantly worse outcome than did those with a wild-type *BRAF* [19]. Higher rates of recurrent and persistent disease were observed. Similarly, a recent metanalysis [16] involving 2247 patients found a higher likelihood of recurrent disease in $BRAF^{V600E}$-positive patients. A retrospective multicentre study by Xing et al. revealed poorer recurrence-free survival in mutation-positive patients [20]. The impact of $BRAF^{V600E}$ mutation on survival could not be addressed in the present study because most of the patients were lost to follow-up.

Despite the scant evidence on survival due to the necessity of longer follow-up time, abundant data is available regarding clinicopathological factors and $BRAF^{V600E}$ relation. Xing et al., in their review, reported that $BRAF^{V600E}$ was associated with extrathyroidal invasion, lymph node metastasis, and advanced surgical stage [21]. Our findings, in addition to these three parameters that have been confirmed to be poor prognostic by almost all studies, indicate that conventional variant and vascular invasion were positively associated with $BRAF^{V600E}$ mutation. The specific histological variant being a high-risk feature was compatible with the results of Lee et al., who reported that $BRAF^{V600E}$ mutation was most frequent in tall-cell followed by conventional variant [22]. There were no tall cell variants in the present study. However, follicular and oncocytic variants existed and had lower frequencies of mutation than the conventional variant. In accordance with our findings, Smith et al., in their analysis investigating whether mutation rates differ between conventional versus follicular variant, found that $BRAF^{V600E}$ mutation is significantly more common in conventional variant PTC [23]**.** Another study by Nikiforova et al. came to same conclusion, finding an impact of $BRAF^{V600E}$ on the incidence of unfavourable prognostic factors including classic and tall cell variant histology and advanced stage [24]. The authors also noted that older age was correlated with mutation positivity. Likewise, > 45 years of age at diagnosis was shown to have close association with $BRAF^{V600E}$ in the study by Lu et al. [25]. On the other hand, the current study failed to show the same, but, adjusting the cut-off level to 55 years according to the current TNM staging, our results were also consistent with the previous studies. Older age at diagnosis (> 55) was correlated with $BRAF^{V600E}$ mutation presence.

In a recent study from China, 1032 patients were evaluated. The authors ended up with 54.6% $BRAF^{V600E}$ mutation, which was significantly associated with extrathyroidal extension and advanced TNM stage [7]. They also concluded that thyroid background of Hashimoto thyroiditis (HT) and lymphocytic thyroiditis (LT) were negatively correlated with mutation presence. Lim et al., by a single-centre experience with 3130 cases, reported a similar result, observing that PTC with a background of LT was significantly lower in those with the $BRAF^{V600E}$ mutation compared with those with wild-type *BRAF* [26]. In another study exploring $BRAF^{V600E}$ mutation as a predictor for central nodal metastasis, HT emerged as an independent protective factor. In the current study we found no significant association with thyroid background and $BRAF^{V600E}$ mutation. However, the frequency of $BRAF^{V600E}$ mutation was remarkably low (22.8%) compared to both previous reports [27] and the above-mentioned data, in which mutation rates were 54.6%, 74.3%, and 75.3%, respectively. The difference might be attributable to the diverse histological variant composition across the studies. The current study involved 34.5% of follicular variant PTC. A study by Navarro et al., involving a much lower percentage of follicular variants (4.6%) than our study, identified a relatively low ratio of $BRAF^{V600E}$ mutations (38.4%) compared to existing literature [28]. Another possible explanation for this could be the heterogeneity in selected populations and the mutation analysis techniques.

In a study conducted in the Irish population, the prevalence of $BRAF^{V600E}$ mutation, compared to the *RET/PTC* mutation in PTC, was higher than previously, and the authors suspected that this might be due to an environmental factor [29]. At least some data suggest potential association with high iodine intake and $BRAF^{V600E}$. The available data are conflicting, with some suggesting positive and others negative or no relation.

In the first study analysing iodine status in Turkey conducted from 1997 through 1999, median urinary iodine concentration of the various regions was noted as 36 μg/L; the follow-up study, in 2007, evaluated the performance of the iodisation of table salt and showed that the median urinary iodine amount increased to 130 μg/L. Considering these data as a guide, our study, undertaken between 2000 and 2012, did not address the urinary iodine concentration, but aimed at looking for the impact of "relative increment in iodine exposure" on *BRAF* mutation. We defined the exposure as the time spent from the iodisation process of table salt in

our country and dichotomised into short-term and long-term, according to whether the PTC was diagnosed in the first or second half of the study period. The trials had different designs on defining iodine exposure. In one study, the authors compared the prevalence of the $BRAF^{V600E}$ mutation in classical PTC of 1032 patients from five regions in China that harbour different iodine content in natural drinking water [7]. They argued that high iodine intake may be a risk factor for PTC because the prevalence of $BRAF^{V600E}$ mutation was significantly higher in regions with high iodine content than any of the regions with normal iodine content. Contrary to this finding, in the present study, there was no significant association between long-term iodine exposure and $BRAF^{V600E}$. However, the iodine content of drinking water in the previous trial was far above the amount used for prophylaxis in table salt. Additionally, the authors speculated that the worldwide increment in the frequency of PTC is the consequence of rising iodine support, but this should be interpreted cautiously. More extensive utility of thyroid ultrasonography and fine-needle aspiration biopsy compared to previous years may have an effect on the rise in incidence. In support of this, we experienced far more PTC cases in the second half of our study period.

On the other hand, investigators at Harvard Medical School noted that, on exposure with excess iodine, rat thyroid follicular cells that conditionally express $BRAF^{V600E}$ showed a decrease in $BRAF^{V600E}$-induced up-regulation of miR-17-92, blocking NOTCH signalling, which confers proliferative advantage. Overall, this study shows that high iodine exerts a protective influence over$BRAF^{V600E}$-activated thyroid cells. Iodine might reduce acute $BRAF^{V600E}$ oncogene induction and activity [30]. Frasca et al. recently examined the relationship of $BRAF^{V600E}$ mutation in PTC with iodine intake in some regions in Italy. The authors found $BRAF^{V600E}$mutation in 107 of 270 cases in an iodine-sufficient region (40%) *vs.* 18 of 53 cases of PTC in an iodine-deficient region (34%), which was, similarly to our study, statistically insignificant [31].

This study had some features that might be viewed as potential weaknesses. First, selection bias might have been introduced due to the retrospective nature of the study. Second, follow-up data do not exist, thus we are unable to comment on whether the findings translate into outcome measures. Lastly, the sample size is small.

Conclusion

The results of our retrospective study provide evidence suggesting that $BRAF^{V600E}$ mutation is correlated with unfavourable prognostic features. The results also challenge existing assumptions about the high iodine exposure and *BRAF* mutation incidence, demonstrating no association.

Acknowledgements

This work was supported by a grant of the Research Fund of the Tekirdağ Namık Kemal University (Project number: NKUBAP.01.YL.16.021).

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