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ORIGINAL PAPER

URINE IODINE EXCRETION IN PATIENTS WITH PAPILLARY THYROID CANCER: EVALUATION OF THE RELATIONSHIP WITH THE PRESENCE OF A BRAF MUTATION

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Abstract

Iodine is an essential element for the production of thyroid hormones. In recent years, it has been suggested that excessive consumption of iodine may play a role in the pathogenesis of papillary thyroid cancer (PTC). In addition, studies have suggested that high iodine consumption is an important risk factor for the formation of a BRAF mutation in the thyroid gland. A prospectively designed study included 132 cases scheduled for thyroidectomy for various reasons. Urine iodine levels of all patients were examined before the operation. The iodine excretion levels of the patients were grouped according to the median urinary iodine concentration determined in community screenings (those with <100 μg L^{-1} low iodine excretion, those with 100-199 μg L^{-1} normal iodine excretion, those with 200-299 μg L^{-1} high iodine excretion). Patients were divided into 3 groups according to the post-operative pathology results. As a result of thyroid histopathology, benign (*n*: 44), PTC (*n*: 88) (BRAF (+): 44 and BRAF (-): 44) cases were included in the study. BRAF mutations in patients diagnosed with PTC were evaluated using the "Real Time PCR Melting Curve Analyzer" method. The relationship between urinary iodine excretion levels and clinical, histopathological and BRAF positivity was examined. In our study, no difference was found in urinary iodine excretion between patients with and without PTC. Hashimoto's thyroiditis was observed more frequently in patients with PTC (p=0.023).

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In addition, Hashimoto's thyroiditis was statistically more frequently detected in the BRAF (-) group compared to the BRAF (+) and control group (p=0.034). Despite studies suggesting that high iodine consumption is important in PTC pathogenesis, we did not find a relationship between the mutation and iodine consumption, which plays an important role in the development of PTC.

Keywords: papillary thyroid cancer, BRAF mutation, urine iodine excretion.

INTRODUCTION

Thyroid cancers developing from follicular epithelial cells are the most common endocrine cancers. Papillary thyroid cancer (PTC) accounts for more than 80% of thyroid cancers (Nikiforov, Nikiforova 2011). BRAF, RET / PTC, and RAS, which play a role in mitogen activated proteins kinases (MAPK) pathway as a result of molecular studies, are thought to cause PTC. Studies show that the most common molecular damage in thyroid cancer genetics is through a BRAF mutation (29-83%). The BRAF mutation reduces tumor differentiation in PTC, increases pro-angiogenetic molecules, and reduces the radioactive iodine uptake of the tumor, leading to the clinical course gaining more aggressive features. For this reason, it is suggested that the clinical course of PTC is more aggressive and more prone to invasion in individuals carrying the BRAF mutation (Davies et al. 2002, Fukushima et al. 2003, Xu et al. 2003, Knauf et al. 2005, Xing et al. 2005, Delellis 2006, Patel, Singh 2006). Iodine is an essential molecule required for the thyroid hormone synthesis. It is mainly taken orally and excreted in urine. For this reason, urinary iodine concentrations give an idea about the amount of iodine that people take. According to the median urinary iodine concentration determined in community screenings, 100-199 μg L^{-1} excretion reflects "normal iodine status", 200-299 μg L^{-1} indicates "excessive iodine consumption". Today, people with increased median urinary iodine concentrations tend to have iodine-induced thyrotoxicosis and thyroid autoimmunity. In recent studies, it has been suggested that excessive consumption of iodine may play a role in the pathogenesis of PTC. In addition, studies have found that high iodine consumption is an important risk factor for the formation of a BRAF mutation in the thyroid gland, hence it has been suggested that it may be a risk factor for the development of PTC (Horn-Ross et al. 2001, Teng et al. 2006, Erdoğan et al. 2008, Guan et al. 2009). In our study, the aim was to evaluate urinary iodine excretion in patients planning thyroid surgery, and to investigate the relationship between BRAF mutations and urinary iodine excretion before surgery in patients with PTC detected in pathological examinations after thyroid surgery.

MATERIAL AND METHODS

This study involved patients in need of surgical intervention who were admitted to the Trakya University Medical Faculty, Endocrinology and Metabolic Diseases outpatient clinic for thyroid nodules between 2015-2018. Sociodemographic characteristics, iodized salt consumption and drug habits of the patients who were referred for a surgery were recorded. Patients who used drugs affecting thyroid hormone metabolism, using drugs and substances similar to iodine-containing prenatal vitamins, examined with contrast material containing iodine in the last 6 months, patients with kidney failure and pregnancy were excluded from the study. While thyroid function tests were evaluated in all cases, spot urine samples were taken in the morning. Urine was taken into deiodinized test tubes, sealed with paraffin and stored in deiodinized tubes at +4 C in a light-proof box until iodine analysis. The urine iodine level was measured manually with the Sandell-Kolthoff reaction using the calorimetric seric arsenic acid solution recommended by the International Council for Control of Iodine Deficiency Disorders (ICCIDD). The iodine excretion levels of the patients were grouped according to the median urinary iodine concentration determined in community screenings, i.e. those with low iodine excretion of <100 μg L^{-1}, those with normal iodine excretion of 100-199 μg L^{-1}, those with high iodine excretion of 200-299 μg L^{-1} (Li , Eastman 2012). In cases where PTC was detected after surgery, the areas that best represented the tumor from the paraffin blocks of thyroidectomy material were determined in the Pathology Department of our hospital, and the presence of BRAF mutation was evaluated by transferring the tissue sample to the Genetic Department. The research was presented to the Ethics Committee of Trakya University Faculty of Medicine, and with the approval of the committee we received in 2015, Trakya University was approved as a scientific research project centre by the decision of TÜBAP-2015/134. Informed consent was obtained from all patients.

BRAF mutation analysis

The tissue sample obtained by determining the areas that best represented the tumor from the paraffin blocks of the material from the individuals included in the patient group, using Genomic DNA isolation Qiagen DNA isolation kits (EZ1® DNA Blood 200 μl Kit, Qiagen, Hilden, Germany), and using EZ1 Advanced XL (Qiagen, Hilden, Germany) in the nucleic acid isolator, according to the manufacturer's instructions. Then, the concentration and purity values of genomic DNA samples were measured on a NanoDrop device at wavelengths of 260-280 nm (Nanodrop 2000C, Thermo Scientific, United States). Genomic DNA samples with a wavelength of 1.4-1.8 were included in the study. For the BRAF mutation sites planned to be studied after this measurement, the polymerase chain reaction (PCR) was performed using the PyroMark PCR kits (Qiagen, Hilden, Germany) and PyroMark

Custom Assay kit (Qiagen, Hilden, Germany) for each polymorphism, according to the manufacturer's recommended PCR protocol. After PCR amplification, PCR products obtained from patients were analyzed using the pyrosequence method according to the manufacturer's instructions using the sequence primers available for each polymorphism in the PyroMark Custom Assay kit (PyroMark Q24 System, Qiagen, Hilden, Germany). Then, the data obtained were evaluated in the PyroMark Q24 software system. Following polymerase chain reaction (PCR) procedures, analyses were performed on a PyroMarkQ24 using sequencing primers containing Seq Primer BRAF 600 or Seq Primer BRAF 464-469 (QIAGEN, Hilden, Germany) for BRAF. The BRAF V 600 mutation (absent or present) and BRAF V 600 mutation (BRAF 600E, BRAF 600K, BRAF 600R) were noted.

Statistical analysis

Power analysis was performed using G* Power (v3.1.9) program to determine the sample size (Faul et al. 2007). When a large effect size (d = 0.63) was determined, BRAF (+), BRAF (-) and control groups were planned to be formed from 44 cases in order to provide 90% power at α = 0.05 significance level. Descriptive statistics on histomorphological findings, clinicopathological findings and presence of a BRAF mutation in these tumors and urinary iodine excretion in patients with Papillary Thyroid Cancer were calculated. The Shapiro - Wilk test was carried out to evaluate distribution for all variables. The Mann-Whitney *U* test was used for quantitative data analysis without normal distribution, while the Pearson chi-square test was used for qualitative data analysis. In the BRAF (+), BRAF (-) and control group, the Kruskal-Wallis H test was used in the quantitative data analysis, and the Pearson chi-square test was used in the qualitative data analysis. If 5 <Least theoretical frequency <25, the Yates' Chi-Square Test was used for qualitative data analysis. The one-way analysis of variance ANOVA method and Tamhane's T2 technique was used because the variances did not have normal distribution. The Multivariate Logistic Regression analysis method was used to examine the effect of urine iodine excretion on histomorphological and clinicopathological findings and BRAF mutations in patients with papillary thyroid cancer. $P<0.05$ value was considered statistically significant.

RESULTS

The mean age of the patients included in the study was 48.7±10.4 in those with benign thyroid pathology, and 47.4±11.8 in those with malignant thyroid pathology ($p=0.467$). There was no difference between the groups in terms of median urinary iodine excretion ($p=0.159$). Body weight was higher in patients with malignant thyroid pathology than those with benign

Table 1

Clinicopathological features of the papillary thyroid cancer group and the control group with benign thyroid histopathology

Specification		Benign result after thyroidectomy	Papillary thyroid carcinoma	*p*
Age		48.7±10.4	47.4±11.8	0.467[a]
Gender (female / male)		40(%90.9)/4(%9.1)	73(%83)/15(%17)	0.335[b]
Height		160.3±6.9	162.8±8.3	0.100[a]
Weight		71.9±11.8	77.8±14.5	**0.021**[a]
BMI		28.1±5.3	29.2±4.6	0.123[a]
BMI	18.5-24.9	13(%29.5)	15(%17)	0.224[c]
	25-29.9	17(%38.6)	36(%40.9)	
	≥30	14(%31.8)	37(%42)	
Waist circumference		101.7±12.6	105.9±13.3	0.055[a]
Median urinary iodine		155.2±103.2	181.6±96.1	0.051[a]
MUI	<100	14(%31.8)	19(%21.6)	0.159[c]
	100-199	20(%45.5)	35(%39.8)	
	≥200	10(%22.7)	34(%38.6)	
Fasting blood glucose		100.6±17.5	103.4±21.2	0.290[a]
Diabetes mellitus (+)		7(%15.9)	13(%14.8)	0.864[c]
Hashimoto's thyroiditis (+)		15(%34.1)	50(%56.8)	**0.023**[c]

BMI – body mass index, MUI – median urinary iodine, *a* – Mann-Whitney test, *b* – Yates' chi-square test, *c* – Pearson chi-square test

thyroid pathology (p=0.021). Hashimoto thyroiditis was found to be more common in patients with malignant thyroid pathology (p=0.023) – Table 1. Among the groups with BRAF (+), BRAF (-) and Benign thyroid histopathology, there were statistically fewer cases of Hashimoto's thyroiditis in the BRAF (-) group (p=0.034). In the multivariate analysis, there was an inverse correlation between a BRAF (-) mutation and Hashimoto's thyroiditis (Odds: 0.31; confidence interval: 0.13-0.76, p=0.01). Patients with benign thyroid histopathology results were found to have a lower weight than those with malignant histopathological results, but did not produce statistical difference in multivariate regression analysis (p=0.088). No significant relationship was found between the presence of a BRAF mutation and urine iodine excretion and other demographic, clinical and histopathological results (p>0.05) – Table 2.

Table 2

Clinicopathological features of BRAF (+), BRAF (-) and benign histopathology groups in patients with thyroid surgery

Specification		Benign result after thyroidectomy	BRAF(-) with PTC	BRAF(+) with PTC	*p*
Age		48.7±10.4	47.4±10.1	47.5±13.4	0.766[a]
Gender (female / male)		40(%90.9)/4(%9.1)	39(%88.6)/5(%11.4)	34(%77.3)/10(%22.7)	0.149[b]
Height		160.3±6.9	161.6±7.7	164±8.9	0.098[a]
Weight		71.9±11.8	75.9±13.7	79.3±15.3	0.063[a]
BMI		28.1±5.3	29±4.6	29.4±4.7	0.299[a]
BMI	18.5-24.9	13(%29.5)	8(%18.2)	7(%15.9)	0.545[b]
	25-29.9	17(%38.6)	18(%40.9)	18(%40.9)	
	≥30	14(%31.8)	18(%40.9)	19(%43.2)	
Waist circumference		101.7±12.6	106.1±12.4	105.7±14.2	0.158[a]
Median urinary iodine		155.2±103.2	178.7±94.1	184±99	0.149[a]
MÜİ	<100	14(%31.8)	9(%20.5)	10(%22.7)	0.440[b]
	100-199	20(%45.5)	18(%40.9)	17(%38.6)	
	≥200	10(%22.7)	17(%38.6)	17(%38.6)	
Fasting blood glucose		100.6±17.5	105.3±23.5	101.5±18.7	0.313[a]
Diabetes mellitus (+)		7(%15.9)	9(%20.5)	4(%9.1)	0.326[b]
Hashimoto's thyroiditis (+)		15(%34.1)	27(%61.4)	23(%52.8)	**0.034**[b]

BMI – body mass index, MUI – median urinary iodine, *a* – Kruskal-Wallis H test, *b* – Pearson chi-square test

Table 3

Multivariate analysis with benign pathology as a dependent factor

	BRAF(-) Papiilary thyroid carcinoma					BRAF(+) Papiilary thyroid carcinoma				
Specification	B	SE	OR	CI	*p*	B	SE	OR	CI	*p*
Intercept	-4.17	4.80				-9.35	4.76			
Hashimoto's thyroiditis (+)	-1.16	0.45	0.31	0.13-0.76	**0.01**	-0.85	0.46	0.42	0.17-1.04	0.06
Height	0.02	0,03	1.02	0.96-1.09	0.51	0.04	0.03	1.04	0.98-1.11	0.14
Weight	0.02	0.01	1.02	0.98-1.06	0.27	0.03	0.01	1.03	0.99-1.07	0.09

Hosmer ve Lemshow goodness of fit Deviance = 1.09 (p=0.15), Nagelkerke pseudo-R^2 = 0.126 (SE – standard error, OR – odds ratio, CI – confidence interval)

DISCUSSION

Iodine is a molecule necessary for the synthesis of thyroid hormones and plays a role in regulating thyroid hormones and metabolic functions of many cells. However, iodine deficiency is an important public health problem in most countries. Studies are generally directed towards determining iodine deficiency (Li, Eastman 2012, Sager 2018). In contrast, studies investigating the effects of high iodine consumption are very limited (Horn-Ross et al. 2001, Teng et al. 2006, Erdoğan et al. 2008, Guan et al. 2009). Iodine, a non-metal chemical element, is found in trace amounts in the human body and accumulates mostly in the thyroid gland. Excessive consumption of iodine can have a toxic effect (Teng et al. 2006). In the literature, it has been suggested in some studies that iodine intake is probably a risk factor for thyroid cancer (Feldt-Rasmussen 2001, Guan et al. 2009, Liu et al. 2009). Kim et al. (2016) demonstrated strong correlations with increased risk of thyroid cancer and relatively low iodine intake (iodine concentration in the urine (UIC) <300 μg L^{-1}) and excessive iodine intake (UIC ≥ 2500 μg L^{-1}) in an iodine replacement site. Thyroid nodules and the goiter are common in areas with iodine deficiency (Li, Eastman 2012). Studies have reported a relationship between iodine intake and the formation of benign nodules. However, controversial results have been reported in studies evaluating the relationship between thyroid cancer and iodine intake (Williams et al. 1977, Goodman et al. 1988, Belfiore et al. 1992, Frasca et al. 2008, Kim et al. 2018, Sakafu et al. 2018, Özçelik et al. 2019). In a study conducted in two regions in Italy, iodine showed a two-fold increase in the incidence of thyroid cancer in the region with an iodine sufficient area compared to an iodine deficiency area (Belfiore et al. 1992). Sakafu et al. (2018) reported an increased risk of differentiated thyroid cancer in the region with iodine deficiency in Tanzania. In contrast, increased incidence of thyroid carcinoma has been reported in two areas with high iodine intake, such as Iceland (Williams et al. 1977) and Hawaii (Goodman et al. 1988). In particular, these two regions were areas where volcanic eruptions were intensive. In this case, it is not entirely clear whether radiation exposure after a volcanic eruption or high iodine intake increases the incidence of thyroid cancer. Kim et al. (2018) reported in their study that iodine intake appears to be an important risk factor for the emergence of the BRAF mutation and therefore may be a risk factor for the development of PTC in an iodine replacement region. In China, Guan et al. (2009) reported that high iodine intake in 1032 PTC cases was an important risk factor for the emergence of the BRAF mutation in the thyroid gland. However, Kim et al. (2018) and Guan et al. (2009) retrospectively reviewed cases of patients who were previously under thyroidectomy and under levothyroxine replacement. We think that this may change urinary iodine excretion in patients. In the study conducted in two regions with sufficient iodine and iodine deficiency in southern Italy, the BRAF

mutation was evaluated; however, this study did not look at the individual iodine status of the cases. The prevalence of BRAF positive PTCs was 39.6% in the iodine-sufficient area and 33.9% in the iodine-deficient area, with no statistically significant difference (Frasca et al. 2008). In our prospective study, evaluation was made of the patient and control groups who had been under regular iodine prophylaxis since 1999 and did not receive levothyroxine treatment. The mean of urinary iodine excretion in patients with thyroid cancer and benign thyroid pathology showed adequate iodinization. In our study, no difference was found between urinary iodine excretion between the patient and control groups. In the subgroup analysis, there was no difference between the patient and the control group with low or high iodine excretion. In addition, when the relationship between iodine intake and the BRAF mutation, which plays a role in PTC pathogenesis and negatively affects its clinical course, no statistically significant difference was observed between the groups.

CONCLUSION

Although high iodine intake is an important risk factor in the emergence of the BRAF mutation in thyroid cancer, no relation was found between iodine intake and the BRAF mutation in this study. In this respect, more research needed for evaluation.

Contribution statement

mC, BYB, AT, SA, AK and SG conceived the study design. MC, BYB, NC, SA, ET, AS, FU, SG were involved in data collection. NS, SG, MC, BYB and SA performed the statistical analysis. ET, NC, HG, HT, BD conducted the genetic examination. MC, SG, AT, BYB, SA, AS and ET interpreted data and prepared the manuscript draft. All authors critically reviewed the final version of the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

None declared.

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