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BEKİR UÇAN

MUSTAFA ÖZBEK

MUSTAFA ŞAHİN

MUHAMMED KIZILGÜL

ERMAN ÇAKAL

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Cyclooxygenase-2 (COX-2) gene polymorphism in patients with differentiated thyroid carcinomas in the Turkish population

Bekir UÇAN^{1*}, Mustafa ÖZBEK¹, Mustafa ŞAHİN², Muhammed KIZILGÜL¹, Erman ÇAKAL¹

¹Department of Endocrinology and Metabolic Diseases, Dışkapı Training and Research Hospital, Ankara, Turkey

²Department of Endocrinology and Metabolic Diseases, School of Medicine, Ankara University, Ankara, Turkey

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Background/aim: The most common thyroid malignancies are papillary and follicular thyroid carcinomas. They account for approximately 85%–90% of all thyroid cancers. Recent studies have reported the relevance of cyclooxygenase-2 (COX-2) gene polymorphism in human carcinogenesis. The aim of this study was to investigate the relationship between thyroid carcinoma and COX-2 gene polymorphism in the Turkish population.

Materials and methods: We included a total of 96 differentiated thyroid cancer patients (mean age: 46.9 ± 10.3 years; 14 males, 82 females) and 83 healthy control subjects (mean age: 46.0 ± 10.6 years; 39 males, 44 females). The frequency of -765G>C, -8473T>C, and 1195A>G gene polymorphisms in the COX-2 promoter region was investigated in thyroid cancer patients and the control group using the high-resolution melting method.

Results: COX-2-765G>C and COX-2-1195A>G gene polymorphisms were similar between thyroid cancer patients and the control group. There was a statistically significant difference between COX-2-8473T>C gene polymorphism in the thyroid cancer group and the control group (P = 0.012).

Conclusion: The single nucleotide gene polymorphism COX-2-8473T>C might contribute to genetic susceptibility to differentiated thyroid cancer in the Turkish population.

Key words: Cyclooxygenase-2, thyroid cancer, gene polymorphism

1. Introduction

Although thyroid cancer is the most common endocrine malignancy, it is responsible for fewer than 1% of all human malignant tumors. Differentiated thyroid carcinoma (DTC), comprising papillary and follicular forms and their variants, accounts for more than 90% of all thyroid malignancies. Despite increasing incidence of thyroid cancer in many countries over the last decades, mortality has been slowly decreasing (1). Approximately 60% to 80% of thyroid carcinomas detected in thyroid cancer referral centers are micropapillary thyroid carcinomas (<1 cm in size) that have excellent long-term prognosis. The features of the disease have changed dramatically over the last decades (2,3). Genetic factors have been well defined in the pathogenesis of thyroid carcinoma; however, the role of environmental factors is still controversial. Exposure to ionizing radiation, the only established environmental risk factor for thyroid carcinoma, is the strongest known risk factor for the development of thyroid carcinoma (particularly of the papillary type) (4,5). Approximately

50% of papillary thyroid carcinoma (PTC) patients have BRAF gene mutations, which are the most common alterations of PTC (6). Expression of PAX8–PPAR-gamma rearrangement is present in approximately 35% to 45% of follicular thyroid carcinoma cases (7,8).

Cyclooxygenase-2 (COX-2) is an enzyme isoform involved in the conversion of arachidonic acid to prostaglandin H₂, which is the precursor of various molecules, including prostaglandins, thromboxanes, and prostacyclins. COX-2 can be expressed in response to various stimuli such as hormones, mitogens, cytokines, inflammatory mediators, and growth factors via protein kinase C and RAS-mediated signaling (9,10).

COX-2 activity products are believed to be involved in carcinogenesis via promoting angiogenesis, increasing cell invasion, inhibiting apoptosis, and stimulating cell proliferation (11–13). Recent studies have demonstrated increased COX-2 expression in numerous tumors including stomach, breast, and bladder carcinomas (14–16).

* Correspondence: uzm.dr.bekir@hotmail.com

The aim of this study was to investigate the relationship between thyroid carcinoma and COX-2 gene polymorphism in the Turkish population.

2. Materials and methods

A total of 96 histologically confirmed DTC patients and 83 age-matched healthy control subjects were enrolled in the study. This study was approved by the Dışkapı Training and Research Hospital Ethics Committee and was conducted in accordance with the ethical principles described in the Declaration of Helsinki. Written informed consent was obtained from all participants. After an overnight fast, blood samples were taken from all subjects. The control group was composed of volunteers who had no history of chronic disease or thyroid disorders. Individuals who had normal thyroid ultrasonography were selected as controls.

2.1. Genomic DNA isolation

DNA purification from blood or body fluids (spin protocol): This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge. The DNA isolation procedure is ideally suited for the preparation of samples for DNA databasing. The QIAGEN QIAamp Blood Kit was used.

2.2. Genotyping of COX-2-765G>C, -8473T>C, and -1195A>G polymorphisms

COX-2 gene polymorphisms in both groups were detected by the high-resolution melting (HRM) method. The Biorad Sensifast™ HRM kit was used for this method. DNA samples and gene variation analysis were done with an Illumina Eco Real-Time device.

2.3. HRM method

HRM is widely used today since it is a fast, efficient, and cheap technique to find genetic variations including mutation, polymorphism, and methylation. The HRM method is performed after a polymerase chain reaction (PCR), which is based on DNA denaturation by heating. Thus, single nucleotide differences that can be distinguished under fluorescence luminescence vary with even very slight temperature changes.

The Biorad Sensifast™ HRM kit was used for the HRM technique. Real-time PCR master mix containing EvaGreen dye was present in this kit. Template DNA and primer pairs were added to have a direct reaction. Primer pairs were designed to enlarge the gene region containing relevant mutations. These primer pairs were selected between 80 bp and 200 bp to have a higher resolution of the augmented amplicons, and their TMs are around 60 °C. For reaction control, a no template control (NTC) sample containing PCR-grade water instead of DNA appeared in each reaction.

The aim of our study was evaluation of COX-2 associated polymorphisms by HRM technique. Gene variation analyses with obtained DNA samples were performed in the Illumina Eco Real-Time device using HRM software.

2.4. Statistical analysis

Genotypes and alleles of patients and controls were compared using chi-square or Fisher exact tests. Odds ratios (ORs) were calculated with Woolf's method. Comparisons of individual clinical and laboratory variables between genotypes were assessed with one-way ANOVA and chi-square or Fisher exact tests. ORs and 95% confidence intervals (CIs) were calculated using SPSS 20.0 (IBM Corp., Armonk, NY, USA). A two-tailed P-value below 0.05 was considered statistically significant. Data are presented as means ± standard deviations (SDs) or medians with interquartile ranges.

3. Results

Clinical characteristics of patients and control subjects were evaluated according to sex, age, familial thyroid cancer, and radiation exposure history. Clinical and biochemical parameters in patients with DTC and control subjects are shown in Table 1. We studied 96 DTC patients (mean age: 46.9 ± 10.3 years), and 83 age-matched healthy control subjects (mean age: 46 ± 10.6 years). Of the 96 patients with DTC, 86 were diagnosed with papillary thyroid carcinoma, 4 with follicular thyroid carcinoma, 4 with hurtle cell thyroid carcinoma, and 2 with follicular variant of papillary carcinoma.

The mean age was similar between the DTC and control groups (P = 0.739). There were more female patients in the DTC group (P < 0.0001, 82/96 to 39/83).

SNP COX-2-765G>C, COX-2-1195A>G, and COX-2-8473T>C gene polymorphisms in patients with DTC and in the control group were evaluated. There was no statistically significant difference in the COX-2-765G>C gene polymorphism between the DTC and control groups (P = 0.217). Moreover, no statistically significant difference was seen in the COX-2-1195A>G gene polymorphism between the DTC and control groups (P = 0.460). There was a statistically significant difference in the COX-2-8473T>C gene polymorphism between the DTC and control groups (P = 0.012) (Table 2). There was a statistically significant difference in the COX-2-8473CC homozygote genotype between the DTC and control groups (P = 0.018).

The OR for COX-2-8473CC was 2.741 (95% CI: 1.193–6.298). There was no statistically significant difference in sex distribution regarding the COX-2-8473CC homozygote genotype between the DTC and control groups (P = 0.72). The COX-2-8473CC homozygote genotype was not associated with capsule invasion, multifocality, the presence of primary lymph node metastases, or thyroiditis

Table 1. Various clinical characteristics in patients with DTC and control group.

	DTC group (n = 96) Mean ± SD	Control (n = 83) Mean ± SD
Sex, male/female	14/84	39/44
Age (years)	46.9 ± 10.3	46 ± 10.6
Radiation exposure history	None	None
Familial history of thyroid cancer	None	None
Source population	Same	Same
Ethnic differences	None	None
Tumor size in pathology (mm)	13.3 (0.20–46)	-
TSH (mIU/L)	1.91 (0.1–8.5)	1.88 (0.1–7.8)
Capsule invasion (n)	14	-
Multifocality (n)	26	-
Lymphadenopathy (n)	10	-
Thyroiditis (n)	42	-
First stimulated Tg (ng/mL)	5.78 (0.2–13.9)	-

Variables that are not normally distributed are presented as median (min-max) and other variables with normal distribution are represented as mean ± SD.

Table 2. COX-2-765G>C, COX-2-1195A>G, and COX-2- 8473T>C gene polymorphisms between groups.

Genotype	DTC group (n)	Controls (n)	P
COX-2-765G>C			
CC	2	-	0.217
GG	90	75	
CG	4	8	
COX-2-1195A>G			
AA	70	60	0.460
AG	22	15	
GG	4	8	
COX-2- 8473T>C			
CC	24	9	0.012
CT	50	59	
TT	22	15	

in patients with DTC. There was also no statistically significant difference in the proportion of patients with macro- and micropapillary carcinoma regarding the COX-2-8473CC homozygote genotype between the DTC and control groups (P = 0.482).

4. Discussion

In this study, we found that DTC is associated with COX-2-8473T>C gene polymorphism; however, it is not associated with COX-2-765G>C and COX-2-1195A>G polymorphisms.

The cyclooxygenases catalyze the formation of prostaglandins from arachidonic acid (17). COX-1 and COX-2 are the two main cyclooxygenases. COX-1 is a housekeeping gene that is expressed in several human tissues and COX-2 is an inducible gene (18,19). COX-2 is an early expressed gene and is induced by various stimuli including growth factors, oncogenes, and cytokines. Elevated COX-2 expression has been associated with poor prognosis in various malignancies including lung and breast cancer (20–24). Moreover, recent studies have demonstrated that COX-2 plays a role in carcinogenesis and carcinoma progression in many epithelial tumors including thyroid carcinomas (25–27). Several different studies have demonstrated that COX-2 is highly expressed in papillary thyroid malignancies but not in benign thyroid nodules (25,28). Ito et al. demonstrated overexpression of COX-2 in patients with papillary and follicular thyroid cancer (29). In one study, the authors verified that COX-2 overexpression correlates with the pathologic type of thyroid cancer and suggested that overexpression of COX-2 is related to poor prognosis. They also found that COX-2 expression was higher in thyroid adenomas than in normal tissues. They concluded that COX-2 overexpression may be an early event in thyroid neoplasm progression, and COX-2 may promote carcinogenesis by inducing it (30). A recent study reported that COX-2 might be associated with malignant thyroid neoplasia and could be used in the diagnosis of malignant thyroid tumors (31). In vitro studies of thyroid cancer cell lines demonstrated inhibition of proliferation, reduction in cellular migration, and invasion of COX-2 by a selective inhibitor (32,33).

COX-2-765G>C polymorphisms are among the most comprehensive genes studied in cancer risk. Several studies have demonstrated that COX-2-765G>C polymorphism is associated with increased risk of various human cancers (34–36). However, Cox et al. did not find an association between the COX-2-765 C allele and cancer risk (37). To date, a number of case-control studies have been conducted to evaluate the association between COX-2 T8473C polymorphism and susceptibility to lung cancer (38,39). However, a recent metaanalysis suggested that

COX-2 T8473C polymorphism was not associated with lung cancer risk (40). In one study, the authors concluded that the homozygous PTGS2 8473-CC genotype might be associated with increased breast cancer risk. However, they did not find an association between PTGS2 8473T>C polymorphism and tumor size, histologic grade, presence of primary lymph node metastases, progesterone receptor positivity, or age at diagnosis (41). Park et al. suggested that the COX-2-8473T>C polymorphism could be used as a marker for genetic susceptibility to adenocarcinoma of the lung (42). In another study, the authors suggested that the COX-2-8473C allele is a potential genetic marker for susceptibility to esophageal adenocarcinoma (43). Fawzy et al. showed that COX-2-8473T>C polymorphism was associated with breast cancer (44). Kim et al. found a correlation between 8473T>C polymorphism and survival in patients with advanced colorectal cancer (45). Likewise, some studies have demonstrated that the COX-2-1195AA genotype is associated with an increased risk of cancers including colorectal, esophageal, and gastric cancer (46–48). As far as we know, this study was the first study evaluating the association between COX-2-765G>C, -8473T>C, and -1195A>G gene polymorphisms in patients with DTC in the Turkish population and we found an association between DTC and COX-2-8473T>C gene polymorphism; however, no statistically significant difference was observed in COX-2-765G>C and COX-2-1195A>G gene polymorphisms between groups. Although there is a difference in COX-2-8473 CC gene polymorphism between groups in the present study, the COX-2-8473 CC gene polymorphism was not associated with capsular invasion, multifocality, presence of lymph node metastases, or thyroiditis.

The difference regarding sex between the two groups is one of the limitations of our study.

The COX-2- 8473T>C gene polymorphism and the 8473CC homozygote genotype may cause genetic susceptibility to DTC in the Turkish population. Further studies are required to investigate the relation between COX-2-8473T>C gene polymorphism in patients with DTC.

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