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The (-174) G/C polymorphism in the interleukin-6 gene is associated with risk of papillary thyroid carcinoma in Turkish patients

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ABSTRACT. ***Introduction:*** **Interleukins and cytokines play an important role in the pathogenesis of many cancers. We aimed to evaluate the interleukin (IL)-6 gene polymorphisms in patients with papillary thyroid carcinoma (PTC) and control subjects.** ***Material and methods:*** **In this study, 42 patients with PTC and 340 healthy controls were included. Peripheral blood samples were taken from control group and patients, and blood samples were preserved at –80 C in tubes containing Na-EDTA.** ***Results:*** **We also found a statistically significant difference between patients with PTC and the control group with respect to IL-6 genotype ($p<0.05$). IL-6 gene polymorphism in patients with PTC patients did not reveal statistically significant difference between the 2 groups (size of tumor >1 cm and <1 cm), multicentricity, RET-PTC types and capsule invasion ($p>0.05$). We also did not find a statistically significant difference between patients with PTC and the control group with respect to IL-6-gene allele frequency ($p>0.05$).** ***Discussion:*** **Our data suggest that the IL-6 G-174 C polymorphism could play a role in thyroid cancer risk, but there is no effective role as a prognostic factor.**

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INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most prevalent endocrine malignancy in humans (1), representing up to 80% of all thyroid cancers (2, 3). Interleukins (IL) and cytokines play an important role in the pathogenesis of many cancers. Several single nucleotide polymorphisms (SNP) identified in cytokine genes are thought to influence the expression or function of these proteins and many have been evaluated for their role in inflammatory disease and cancer predisposition (4). Genetic variations in proinflammatory and anti-inflammatory cytokine genes influence individual response to carcinogenic exposures (5).

These genetic factors have not been thoroughly defined, however, analysis of functional variants in candidate genes offers a plausible approach for identifying them. IL-6 is a pleiotropic growth factor that is involved in inflammation and carcinogenesis (6, 7), acting as a regulator in many malignant tumors (8-10). The IL-6 gene is located in chromosome 1q21.3 and a well-known polymorphism located in the promoter region at position 174 has been associated with levels of circulating IL-6, where a G>C substitution decreases protein expression by reducing promoter activity (11).

Our aim was to evaluate the relation between the genotypic and allelic frequencies of the IL6 G/C gene polymorphism and their association with the prognostic factors and risk of developing PTC in the Turkish population.

Key-words: (-174) G/C polymorphismin, interleukin-6 gene, papillary thyroid carcinoma.

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MATERIALS AND METHODS

Patients and controls

Forty-two patients with PTC and 340 healthy controls were included in this study. Informed consent was obtained from all patients and healthy controls included in this study. The study was carried out in the Department of Endocrinology and Metabolism diseases at Ege University Faculty of Medicine, the main referral center in the Aegean region of Turkey. Initial carcinoma treatment was total or subtotal thyroidectomy in all patients. Of 42 patients with PTC, 17 were diagnosed with papillary thyroid microcarcinoma and 25 with papillary thyroid macrocarcinoma (follicular variant, no.=10; classic variant, no.=11). The clinical histories of patients with PTC and control group were investigated with respect to age, sex, presence of familial thyroid cancer and exposure to radiation (Table 1). Peripheral blood samples were taken from the control group and patients with PTC, and were preserved in fridges at –80 C in tubes containing Na-EDTA.

Genetic analysis

Restriction fragment length polymorphism (RFLP)-IL-6 gene analysis DNA was extracted from cellular blood components by salting-out method. The PCR was used to detect the IL-6 SfaNI RFLP by the method of Fernandez Real et al. (12). The SfaNI polymorphism is due to a replacement of G by C at position 174, and primers were designed to amplify the 5-promoter region of IL-6 gene. The primers used in the PCR were: forward -5′-TGACTTCAGCTTTACTCTTTGT-3′ and reverse -5′-CTGATTGGAAACCTTATTAAG-3′ (TIB MOLBIOL Syntheselabor, Berlin-Germany). The reaction was carried out in a final volume of 25 µl containing 1.5 mmol/l of $MgCl_2$, 0.2 mmol/l each dNTP

Table 1 - *Clinical features in patients with papillary thyroid cancer (PTC) and control group.*

	PTC (no.=42) mean±SD	Control (no.=113) mean±SD
Age (yr)	43.1±9	43.8±5
Sex (male/female %)	19.0/81.0	18.7/81.9
Exposure to radiation history	None	None
Presence of familial thyroid cancer	None	None
Source population	Same	Same
Ethnic differences	None	None
Nodul size (mm)	20.3±10.2	-
Tumor size (mm)	11.2±8.3	-
Free-T4 (0.9-1.7) ng/dl	1.3±0.2	1.22±0.3
Free-T3 (1.8-4.6) pg/ml	3.0±1.2	2.8±0.9
TSH (0.2-4.2) µIU/ml	2.1±1.5	2.2±1.3
Anti-T (0-40) IU/ml	342±112**	25±14
Anti-TPO (0-35) IU/ml	106±46*	23±09

*Statistically significant difference [anti-thyroperoxidase (Anti-TPO)] was determined between two (PTC and control) groups ($p<0.05$). **Statistically significant difference [anti-thyroglobulin (Anti-T)] was determined between the two (PTC and control) groups ($p<0.01$).

(Promega, Madison, WI, USA), 0.2 mmol/l each primer, and 1.0 U Ampli *Taq* Polymerase (PE Applied Biosystems, Foster City, CA), DNA was amplified during 35 cycles with an initial denaturation of 10 min at 94 C and a final extension of 10 min at 72 C. The cycle program consisted of a 1-min denaturation at 94 C, a 1-min, 35-sec annealing at 55 C, and a 1-min extension at 72 C. PCR products were digested with SfaNI restriction enzyme (New England BioLabs, Beverly, MA, USA) at 37 C overnight and electrophoresed on a 2% agarose gel. SfaNI RFLP was detected by ethidium bromide staining. The identified genotypes were named according to the presence or absence of the enzyme restriction sites, thus SfaNI (G/G), (G/C), and (C/C) are homozygous for the presence of the site (140/58 bp), heterozygous for the presence and absence of the site (198/140/58 bp), and homozygous for the absence of the site (198 bp), respectively.

Statistical analysis

Statistical analysis was performed using Mann-Whitney test to examine the age difference between PTC patients and the healthy control group, whereas chi-square test was used for sex comparisons. Fisher's Exact test was used for IL-6 haplotypes between the 2 groups; chi-square test was used for genotype analysis. IL-6 haplotypes, as well as the size of nodule or tumor were also examined with the classified variable, and Fisher's Exact and chi-square tests were used for the haplotype. Kruskal-Wallis test was used for the average of classified variables, such as the size of nodule or tumor in IL-6 genotype/haplotype. SPSS 14.0 for Windows (SPSS Inc. Chicago USA) was used for statistical analysis of results. $p<0.05$ values were accepted as statistically significant.

RESULTS

There were a total of 170 post-menopausal women included in the current study; 160 (94%) of them gave DNA samples. One hundred and fifty-seven (92%) of the latter were successfully genotyped.

Various clinical features of the study population (patients with PTC and control group) are shown in Table 1. No statistically significant difference was determined for various clinical features (age, sex, and exposure to radiation history, presence of familial thyroid cancer, source population, and ethnic differences, free T_3, free T_4, and TSH) between the groups. Statistically significant difference was determined between the 2 groups for anti-thyroperoxidase ($p<0.05$) and anti-thyroglobulin ($p<0.01$). IL-6 gene polymorphism in patients with PTC and control group were examined, and then genotype and gene allele frequency analysis were evaluated between the 2 groups.

Statistically significant difference related to IL-6 gene polymorphism was determined between the 2 groups ($p<0.05$) (Table 2). We also found a statistically significant difference between the patients with PTC and the control group with respect to IL-6 genotype ($p<0.05$) (Table 2).

In addition, patients with PTC were divided into 2 groups according to tumor size >1 cm and <1 cm, and IL-6 gene polymorphism was also analyzed for these groups (Table 3). IL-6 gene polymorphism in patients with PTC patients did not reveal a statistically significant difference between the 2 groups (tumor size >1 cm and <1 cm) ($p>0.05$).

IL-6 gene polymorphism in PTC patients did not reveal a statistically significant difference according to multicentricity, RET-PTC types, and capsule invasion ($p>0.05$) (Table 3). The evaluation of IL-6 gene polymorphism in PTC patients did not reveal statistically significant difference between the 2 groups of classical type and follicular variant ($p>0.05$).

We also did not find a statistically significant difference between the patients with PTC and control group with respect to IL-6 gene allele frequency ($p>0.05$) (Table 2).

DISCUSSION

Tumors of thyroid follicular cells provide a very interesting model to understand the development of human cancer. It becomes apparent that distinct molecular events are associated with specific stages in a multistep tumorigenic process with good genotype/phenotype connection. Genetic factors play an important role in cancer etiology. Several inflammatory IL have been linked with tu-

Table 2 - *Genotype distribution and allele frequency of the interleukin (IL)-6 -174 G>C polymorphism for the thyroid cancer and healthy controls.*

Genotype	Patients		Healtly group	
	no.	%	no.	%
CC	7	16.7*	26	7.6
CG	14	33.3*	171	50.3
GG	21	50.0*	143	42.1
Allele				
C	28	33.3	223	32.8
G	56	66.7	457	67.2

Data were compared between groups by χ^2 test. The mutation rate of -174 G>C in IL-6 is shown in Table 2. *Statistically significant difference -174 G>C in IL-6 genotype and gene allele frequency was determined between the 2 (thyroid cancer and control) groups ($p<0.05$).

Table 3 - *The relation between prognostic factors and interleukin-6 genotypes of the patients with papillary thyroid cancer (PTC).*

Cytokine gene polymorphisms	CC genotype	GC genotype	GG genotype	*p*
Multicentricity	1 (12.5%)	4 (50.0%)	3 (37.5%)	>0.05
Capsule invasion	0 (0%)	2 (50.0%)	2 (50.0%)	>0.05
PTC tumor size				
≥10 mm	4 (16.0 %)	9 (36.0%)	12 (48.0%)	>0.05
<10 mm	3 (17.6%)	5 (29.1%)	9 (52.9%)	>0.05
RET-PTC				
Type 1	3 (23.1%)	5 (38.5%)	5 (38.5%)	>0.05
Type 1+3	1 (20.0%)	2 (40.0%)	2 (40.0%)	>0.05
Type 3	1 (10.0%)	2 (20.0%)	7 (70.0%)	>0.05
Negative	2 (14.3%)	5 (35.7%)	7 (50.0%)	>0.05

morigenesis, which suggests that inflammation is related with cancer development. IL mediate different steps in the pathway leading to tumorigenesis (13, 14).

As in all cancer types, genetic factors in the pathogenesis of PTC may also change between different populations. This study aims at exploring the influence of IL-6 gene polymorphism in PTC on thyroid cancer among Turkish people.

IL-6 produced by transformed epithelial cells or infiltrating leukocytes are known to influence tumor development positively by affecting angiogenesis, growth, survival, immune suppression, DNA damage, tumor suppression, and metastasis (15).

In contrast, tumor-infiltrating leukocytes negatively control tumor progression by producing cytokines or cytotoxic molecules; and stimulate the death of targeted cells. IL-6 expression is related with aggressiveness in both PTC and medullary thyroid cancers (16). IL-6 gene polymorphism and frequencies in PTC and healthy control patients were investigated in our study. Statistically significant difference was determined between the 2 groups (PTC and control) according to IL-6 G->C gene polymorphism. No difference was determined with respect to IL-6 allele frequencies of participating between the control group and the patients with PTC ($p>0.05$). In addition, no statistically significant difference was determined between the 2 groups of PTC patients with respect to the tumor size (>10 mm or <10 mm), and also no statistically significant difference was determined between the control group and the PTC patients with respect to histological variant (classical type and follicular variant). In our study, the polymorphisms of IL-6 genes were significantly associated with the occurrence of PTC. This study adds important information to our understanding concerning IL-6 gene polymorphism in thyroid cancer.

The -174 G/C IL-6 polymorphism is known to reduce gene transcription rate subsequently reducing protein levels. IL-6 is known to inhibit tumor cell growth.

Several studies have shown that the variant C allele is associated with lower IL-6 levels (17-19). It is possible that attenuated IL-6 expressions following an injury may modify the host immune response in a manner that creates a survival advantage for transformed or malignant cells.

The IL-6 variant genotype (C/C) frequency varies dramatically in the literature in different ethnic groups; for example, it is very low in African Americans, Native Americans, and Asian Americans. However, in white subjects, the frequency has been fairly consistent with different studies in Europe and the United States. Most studies with relatively large sample sizes have listed the frequency between 15 and 20%. The largest study of white control subjects in the United Kingdom (2751 subjects) gave a frequency of "CC variant" of 18% (19). In control subjects of our study, CC genotype frequency was 7.6%.

Rearrangement of the RET gene, also known as RET/PTC rearrangement, is the most common genetic alteration identified to date in PTC (20).

RET/PTC3 is associated with poorer prognosis and more aggressive tumor behavior and may well be a marker for radiation-induced carcinoma, particularly in children (21). RET/PTC1 may coexist with RET/PTC3 in the same tumor; in particular, RET/PTC3 may develop with age or become more prevalent in patients with previous RET/PTC1 activation, and determine a more aggressive behavior (22). We also analyzed RET/PTC rearrangement in our study. IL-6 gene polymorphisms in patients with PTC patients did not reveal a statistically significant difference according to RET-PTC types. IL-6 may act as a prognostic factor in PTC. We therefore evaluated prognostic factors (metastasis, capsule invasion, tumor types *etc.*) in PTC patients. IL-6 gene polymorphism in patients with PTC did not reveal a statistically significant difference according to multicentricity, RET-PTC types, and capsule invasion. The evaluation of IL-6 gene polymorphism in patients with PTC showed no statistically significant difference between the 2 groups of classical type and follicular variant.

There is no study on IL-6 G>C polymorphism in papillary thyroid cancer risk. Our data suggest that the IL-6 174 G>C polymorphism is seen to play a role in thyroid cancer risk, but there is no effective role as a prognostic factor. We describe new data with respect to a significant association between the IL-6 polymorphism and papillary thyroid carcinomas.

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