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The Association of 4G/5G Polymorphism of PAI-1-675 Gene with Clinicopathologic Features of Thyroid Tumors in Iranian Azeri Turkish Patients

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Abstract

Background: Due to the lack of information about the role of 4G/5G polymorphism of PAI-1 Gene in susceptibility to thyroid tumors, this study was performed to evaluate the potential effects of this polymorphism on clinicopathologic features of thyroid tumors in Iranian Azeri Turkish patients.

Methods: In this case-control study, 90 patients with thyroid tumors who were not blood relatives and their PAI-1 4G/5G polymorphism had been determined in the previous study, were included.

Results: All clinicopathologic features of thyroid tumor related to PAI-1 4G/5G polymorphism that affect the severity of disease were studied. The results show that T1 tumor size (4G/4G, P = 0.019; 4G/5G, P = 0.021) in patients with/without these features was significantly different.

Conclusion: Data showed a protective role for 4G allele versus 5G and 4G/4G versus 5G/5G against the development of thyroid tumors in Azeri Turkish ethnic group.

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Introduction

Thyroid cancer is the most common malignancy of the endocrine system, which in recent decades, has showed an increasing prevalence in many countries (1). Depending on the histological type of tumor, there are wide variations in survival rates. Differentiated thyroid tumors like papillary and follicular thyroid cancers are often treatable and have good prognosis while anaplastic thyroid cancer is aggressive and has poor prognosis (1).

Exposure to ionizing radiation is the only cause of thyroid cancer in humans, although other factors such as dietary iodine deficiency and environmental exposure to various xenobiotics which are the main causes of chromosomal damage have also been related to this pathology (2). Studies have shown that in childhood, thyroid, breast, brain, and bone marrow are more sensitive to radiation and radiation exposure is more harmful for those under 5 years of age (3). In addition, the high frequency of cancer among family members of patients with thyroid cancer supports the hypothesis that genetic factors are involved in the incidence of thyroid cancer.

Plasminogen activator system, too, includes protolithic factors that are distributed by the cancer cells and might decrease the extracellular matrix components, increase invasion, and lead to metastasis (4, 5). This system consists of tissue-type plasminogen activator (TPA), urokinase-type plasminogen activator (UPA), urokinase plasminogen activator receiver (UPAR), and plasminogen activator inhibitors (PAI-1 and PAI-2) (6). PAI-1 is also one of the main inhibitors of fibrinolysis system through inhibiting TPA and UPA (7). PAI-1 gene is located on the long arm of the chromosome 7 and consisted of 9 exons and 8 introns (8). This gene is synthesized in vascular endothelium and its synthesis can be regulated by hormones, cytokines, and growth factors (8,9).

Several polymorphisms (-675 4G/5G, -844G/A, and HindIII C/G) have been described in the PAI-1 gene among which -675 4G/5G polymorphism, due to its location in the promoter region and its possible role in regulating transcription, has been often evaluated (8). It has been found that high level of PAI-1is related to the complications of several types of cancer such as gastric, colorectal, kidney, endometrial, and ovarian cancers (10,11). Due to the lack of information about the role of this polymorphism in susceptibility to thyroid cancer, this case-control study was designed to assess the potential effects of this polymorphism on clinicopathologic features of thyroid tumors in Iranian Azeri Turkish patients.

Methods

In this study, 90 non-relative Iranian Azeri Turkish patients with thyroid cancer who had been diagnosed through screening tests by the specialists and their diagnosis had been confirmed by the pathologist during 2012-2015 and their 4G/5G polymorphism of PAI-1 gene had been determined in the previous study (12), were included. The sample size was determined based on the number of patients referred to the specialists in the past years and other researches that have been performed in this field. From 90 patients, only 80 patients whose clinicopathological information was complete, were included and 10 patients were excluded due to incomplete clinicopathological information.In order to study 4G/5G polymorphism of PAI-1 gene, following steps had been performed in the previous study. First, written and signed informed consent forms had been taken from the patients or their parents for collecting blood samples, and patients' medical records and family history had been collected. Genomic DNA was extracted from peripheral blood leukocytes according to the standard protocols and using salting-out method (13). Each sample was amplified for 4G/5G polymorphisms of PAI-1 gene by using ARMS-PCR method and allele-specific primers (Table 1) (8,9). PCR products were electrophoresed in agarose gel 2% and put under UV light to be observed. Then, their genotypes were determined. The sequencing method also was used for genotypes approval.

Table 1. The primers used for amplification of PAI-1 4G/5G polymorphism by ARMS-PCR method

Primers	Sequence
Upstream control primer	5′-AAGCTTTTACCATGGTAACCCCTGGT-3′
4G Allele specific primer	5′-AGAGTCTGGACACGTGGGGA-3′
5G Allele specific primer	5′-AGAGTCTGGACACGTGGGGG-3′
Common downstream primer	5′-TGCAGCCAGCCACGTGATTGTCTAG-3′

Clinical and pathological features of tumors were determined by a pathologist and recorded for further investigation. According to TNM (tumor, node, and metastasis) staging system recommended by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC), tumor staging was performed. The association of polymorphism with clinical features (age, gender, tumor size, lymph node metastasis, tumor stage, and tumor type) was evaluated by chi-square and Fisher's tests. The odds ratio (OR) and confidence intervals (CI) of

95% were calculated for all data. P-values less than 0.05 were considered significant.

Results

In this study, 90 patients had been selected of whom, only 80 patients whose clinicopathological information was complete, were included. Using patients' genotypes determined in the previous study, in the present study, the association of genotypes with patients' pathological symptoms was evaluated.

The study group included 80 patients (20 males and 60 females) with the average age of 37.98 years. Comparison of age and gender of these patients showed no statistically significant relationship. According to the clinical information, 54 patients (67.5%) were diagnosed with papillary thyroid carcinoma, 4 patients (5%) with follicular thyroid carcinoma, and 22 patients (27.5%) with follicular adenoma. According to the TNM staging system, 67 patients (83.75%) were in stage 1, 6 patients (7.5%) in stage 2, and 6 patients (3.75%) in stage 3.

All clinicopathologic features of thyroid tumor related to PAI-1 4G/5G polymorphism were evaluated. The genotypic and allelic frequencies in patients with/without specific clinical features were compared (Table 2). The results showed that T1 size in patients with/without these features was significantly different (4G/4G; P=0.021, 4G/5G; P=0.019). Also, the genotypic and allelic frequencies in patients with benign thyroid tumors (adenomas) compared to the patients with malignant thyroid tumors (carcinoma) showed no significant difference.

Table 2. Distribution of genotype and allele in patients with thyroid tumors with/without specific clinical features

Symptoms		4G/4G	4G/5G	5G/5G	4G	5G
Tumor Size						
(n=20) T1	+	5(25.00)	10(50.00)	5(25.00)	20(50.00)	20(50.00)
	-	7(11.66)	40(66.66)	13(21.66)	54(45.00)	66(55.00)
	P-value	*0.021	*0.019	0.692	0.571	0.571
(n=36) T2	+	4(11.11)	24(66.66)	8(22.22)	32(44.44)	40(55.55)
	-	8(18.18)	26(59.09)	10(22.72)	42(46.36)	46(52.27)
	P-value	0.208	0.323	0.990	0.654	0.689
(n=7) T3	+	1(14.28)	5(71.42)	1(14.28)	٧(٥٠/٠٠)	7(50.00)
	-	11(15.06)	45(61.64)	17(23.28)	67(45.89)	79(54/10)
	P-value	0.886	0.131	0.120	0.660	0.488
Lymph node metastasis						
(n=31) N0	+	6(19.35)	18(58.06)	7(22.58)	30(48.38)	32(51.61)
	-	٦(12.24)	32(65.30)	11(22.44)	44(44.89)	54(55.10)
	P-value	0.134	0.226	0.997	0.597	0.543
(n=17) N1	+	2(11.76)	10(58.82)	5(29.41)	14(41.17)	20(58.82)
	-	10(15.87)	40(63.49)	13(20.63)	60(47.61)	66(52.38)
	P-value	0.325	0.496	0.143	0.411	0.378
Tumor Stage						
(n=67) Stage I	+	9(13.43)	42(62.68)	16(23.88)	60(44.77)	74(55.22)
	-	3(23.07)	8(61.53)	2(15.38)	14(53.84)	12(46.15)
	P-value	0.081	0.860	0.152	0.174	0.236
(n=6) Stage II	+	1(16.66)	4(66.66)	1(16.66)	6(50.00)	6(50.00)
	-	11(14.86)	46(62.16)	17(22.97)	68(46.62)	80(54.05)
	P-value	0.687	0.573	0.239	0.736	0.484
Type of Tumor						
(n=22) FA	+	4(18.18)	14(63.63)	4(18.18)	22(50.00)	22(50.00)
	-	8(13.79)	36(62.06)	14(24.13)	52(44.82)	65(55.17)
	P-value	0.328	0.920	0.358	0.554	0.409
(n=58) PTC+FTC	+	8(13.79)	36(62.06)	14(24.13)	52(44.82)	65(55.17)
	-	4(18.18)	14(63.63)	4(18.18)	22(50.00)	22(50.00)
	P-value	0.338	0.848	0.233	0.410	0.555

*P-values <0.05

Discussion and Conclusion

In recent years, several studies have shown that PAI-1 belongs to the plasminogen activator system and plays a key role in signal transmission, adherence to the membrane, cell migration, and ultimately promoting the invasion and metastasis (14). In addition, high level of PAI-1 is associated with complications of different types of cancer such as gastric, colorectal, kidney, endometrial, and ovarian cancers (10,11).

Several studies have been performed on the role of PAI-1 4G/5G polymorphism, but the results are inconsistent. Some studies have shown that 4G/4G genotype or 4G allele can be considered as a risk factor for developing cancer (15-18), while others have suggested that 4G/4G genotype or 4G allele has a protective effect against complications of cancer (8, 9, 19, 20). Meta-analysis of this polymorphism and its association with various cancers such as breast, colorectal, and endometrial cancers have shown that the association of this polymorphism with colorectal and endometrial cancers, especially in the Caucasian population, is very significant (11).

Although there have been several studies on the role of plasminogen activator system components in developing many cancers, the effect of PAI-1 in thyroid cancer and its role in the prognosis of thyroid tumors is still unclear. Our previous studies showed that the genotypic and allelic frequencies of PAI-1 4G/5G polymorphism in patients with thyroid and breast tumors compared to the control group have no significant difference (12, 21). However, the association of this polymorphism with clinical features of patients with breast (21) and thyroid cancers in Iranian Azeri Turkish patients has shown significant differences. Analysis of breast tumor samples showed that PAI-1 4G/5G polymorphism is associated with several common factors such as tumor size, lymph node metastasis, and tumor stage (21). The results of this study, as the first study on thyroid cancer, suggest that T1 (4G/4G; P=0.021, 4G/5G; P=0.019) in patients with/without these features is significantly different. Therefore, patients with 5G/5G genotype compared to the patients with 4G/4G genotype are at higher risk, and based on the clinical features of thyroid tumors have high disease severity. In addition, 4G allele compared to 5G allele is likely a risk factor for complications of thyroid tumors in Iranian Azeri Turkish population.

Since no similar study has been performed on the association of PAI-1 4G/5G polymorphism with disease severity of patients with thyroid tumors, the results of this study could not be compared with the other studies. However, further studies with larger sample size are required to

evaluate the main role of this polymorphism in patients with thyroid tumors.

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