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RESEARCH ARTICLE

Analysis of the Codon 72 Polymorphism of p53 Gene in Patients with Multinodular Goitre: A Risk Factor of Thyroid Cancer

Banu BAYRAM*°, Reşit SÖNMEZ**, Sedat BOZARI*, Harun ÖNLÜ*, Fezan Mutlu ŞAHIN***

Analysis of the Codon 72 Polymorphism of p53 Gene in Patients with Multinodular Goitre: A Risk Factor of Thyroid Cancer

Summary

Aim: In this study, it was aimed to determine whether the p53 gene codon 72 polymorphism is a genetic marker of thyroid cancer development in multinodular goitre patients.

Methods: Genomic DNA was isolated from 90 persons (42 with multinodular goitre and 48 healthy controls) in the study. DNA was amplified with specific primers by PCR and RFLP technique was used to analyze p53 gene codon 72 polymorphism alleles and genotypes. PCR-RFLP products were assessed with UV transilluminator after application of agarose gel electrophoresis.

Results: According to genotype distribution and allele frequencies of p53 gene codon 72 polymorphism, there was no significant difference between groups.

Conclusions: As a result of our study we may assert that p53 gene codon 72 polymorphism should not be considered as a genetic marker to develop thyroid cancer in the studied Turkish population with multinodular goitre.

Key Words: *p53 gene 72 polymorphism, multinodular goitre, thyroid cancer*

Multinodüler Guatrlı Hastalarda p53 Geni Kodon 72 Polimorfizminin Analizi: Tiroid Kanseri İçin Bir Risk Faktörü

Özet

Amaç: Bu çalışmada p53 geni kodon 72 polimorfizminin multinodüler guatrlı hastalarda tiroid kanseri gelişiminde genetik bir marker olup olmadığının belirlenmesi amaçlanmıştır.

Yöntemler: Çalışmada 90 kişiden (42 multinodüler guatrlı hasta ve 48 sağlıklı kontrol) genomik DNA izole edildi. DNA p53 geni kodon 72 polimorfizmi genotip ve alellerinin analizi için PCR ve RFLP teknikleri kullanılarak spesifik primerler ile amplifiye edildi. PCR-RFLP ürünleri agaroz jel elektroforezine tabi tutularak UV translüminatör ile değerlendirildi.

Sonuçlar: p53 geni kodon 72 polimorfizmi genotip dağılımı ve alel frekansları açısından gruplar arasında anlamlı bir farklılık bulunmadı.

Tartışma: Çalışmamızın sonucunda bu çalışmada yer alan multinodüler guatrlı Türk popülasyonunda p53 geni kodon 72 polimorfizminin tiroid kanseri gelişiminde genetik bir marker olarak düşünülemeyeceğini söyleyebiliriz.

Anahtar Kelimeler: *p53 geni 72 polimorfizmi, multinodüler guatr, tiroid kanseri*

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* Mus Alparslan University, Faculty of Arts and Science, Department of Biology, Mus, Turkey
** Private Mus Sifa Hospital, Department of General Surgery, Mus, Turkey
*** Eskisehir Osmangazi University, Faculty of Medicine, Department of Biostatistic, Eskisehir, Turkey
° Corresponding Author E-mail: bayrambanu@hotmail.com

INTRODUCTION

Thyroid carcinoma represents the most frequent form of cancer of the endocrine glands (1, 2). Epidemiologically ascertained risk factors are ionizing radiation, the presence of thyroid adenoma and multinodular goitre (MNG) (2). MNG involves more than one nodule and develops because of hyperplasia of follicular cells which have potential of abnormal growing in different parts of thyroid gland (3). p53, a representative tumour suppressor, involved in cell proliferation and progression of various tumour types (4). p53 gene codon 72 located on chromosome 17p shows a single nucleotide substitution resulting in the presence of either arginine (Arg) or proline (Pro) in amino acid sequence. This change affects biochemical and functional properties of p53: the proline variant is a stronger transcriptional activator, whereas the arginine variant is a stronger apoptosis inducer (5-7). There have been some studies explaining the association between p53 gene codon 72 polymorphism and thyroid cancer, the Pro/Pro genotype has been found as a risk factor for development of thyroid cancer (1, 8, and 9). But the literature review in this field revealed no study explaining the relationship between p53 gene codon 72 polymorphism and MNG in development of thyroid cancer. Therefore this study aims to determine the association of this gene polymorphisms and allele frequencies with MNG which is a risk factor of thyroid cancer.

MATERIAL AND METHODS

Study Population

This study included 42 unrelated nontoxic MNG patients and 48 controls without MNG, recruited from the General Surgery Department of Private Muş Şifa Hospital, Muş, Turkey. Informed consent in accordance with the study protocol, approved by the ethics committee of Medical Faculty, Eskisehir Osmangazi University, Eskisehir, was obtained from each patient.

DNA isolation

Two millilitres of peripheral venous blood samples were withdrawn from all patients and healthy subjects and stored at -20^{0}C until the process of DNA isolation. Genomic DNA was isolated from the blood samples according to the commercially available kit protocol (Vivantis, Malaysia).

p53 codon 72 polymorphism genotype determination

DNA was amplified by Polymerase Chain Reaction (PCR) in a thermal cycler (Amplitronyx 4, USA). Allele-specific primers were used in the PCR. These primers were as follows: forward, 5'-TTG CCG TCC CAA GCA ATG GAT GA-3' and reverse, 5'-TCT GGG AAG GGA CAG AAG ATG AC-3'. 5 µl of DNA sample was amplified for 35 cycles with denaturation at 95^{0}C for 30 s, annealing at 59^{0}C for 30 s, and extension at 72^{0}C for 35 s using a 50 µl PCR mixture containing a 10 pmol each primer, 10X PCR buffer, 2 mM dNTPs, and 5U Taq polymerase. The PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed by a CCD camera (Cleaver, UK). After confirmation of an amplified fragment of the expected size (199 bp), the PCR products were digested with 1 unit of restriction enzyme *BstFN1* (Vivantis, Malaysia) at 60^{0}C for 1 hour. Digested PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using the UV transilluminator and photographed by a CCD camera. In homozygous samples (Pro/Pro) and (Arg/Arg) detected bands were 113 bp and 199 bp respectively. In heterozygous samples (Pro/Arg), both bands (199 and 113 bp) were detected (Figure 1).

Statistical analysis

Statistical procedures were performed using the IBM Statistical Package for Social Sciences

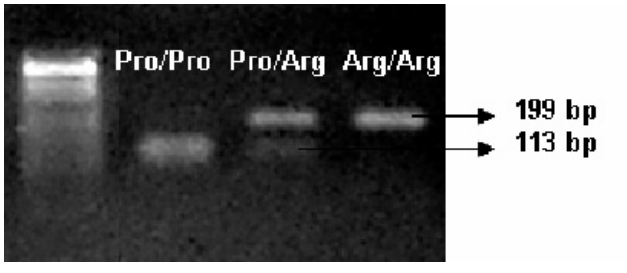

Figure 1. Gel image of p53 codon 72 polymorphism BstU1 products

(IBM SPSS Statistics 20). Shapiro Wilk normality test was performed for numerical variables and measurements with not normally distributed variables were analyzed by using Mann-Whitney U test. As descriptive statistics, median (25-75 percentiles) and mean±SD (standard deviation) were shown as averages. Categorical variables were analyzed using Pearson Exact Chi-Square. P values less than 0.05 ($p<0.05$) were accepted as significant.

Table 1. Gender distribution of the patients and healthy controls

Gender	Patient		Control	
	n	%	n	%
Female	42	100	37	77.1
Male	0	0.0	11	22.9
Probability (P)	<0.05		<0.05	

RESULTS

Gender distributions of the patients and healthy controls presented in Table 1 show predominance of women over men in the patient group. In terms of personal characteristics, although use of non-iodized salt and frequency of family history were significantly higher in the patient group compared to the control group, smoking status did not differ between the two groups (Table 2). Furthermore, age, body mass index (BMI) and TSH levels were not also different between the two groups (Table 3). Table 4 presents the distribution of p53 gene codon 72 genotypes and allele frequencies in the patient and control groups. There was not a statistically significant difference

Table 2. Personel characteristics of the patients and healthy controls

Personel Characteristics		Patient		Control		Probability
		+	-	+	-	
Smoking Status	**n**	5	37	11	37	>0.05
	%	11.9	88.1	22.9	77.1	
Iodized Salt	**n**	22	20	35	13	<0.05
	%	52.4	47.6	72.9	27.1	
Family History	**n**	27	15	15	33	<0.05
	%	64.3	35.7	31.2	68.8	

Table 3. Some parameters of the patients and healthy controls

Parameters	Patient median(%25-%75)	Control median(%25-%75)	Probability
Age (year)	36 (27-47.75)	37.5 (27.25-54.75)	p>0.05
BMI	24.5 (21.89-27)	26 (24-27)	p>0.05
TSH (microU/ml)	1.41 (0.50-3.52)	0.92 (0.64-1.37)	p>0.05

Table 4. Genotype distribution and allele frequencies of codon 72 polymorphism of p53 gene in patients and healthy controls

Groups	Genotype distribution				Allele frequencies	
	Pro/Pro+ Pro/Arg		Arg/Arg		Pro	Arg
	n	%	n	%	n	%
Patient	36+1	88.1	5	11.9	87	13
Control	44+1	93.8	3	6.2	93	7
Probability	>0.05				>0.05	

between the groups with respect to genotype distribution and allele frequencies. According to the p53 gene codon 72 polymorphism genotypes, there was also no association between the genotypes of p53 gene codon 72 polymorphism and the gender, personal characteristics and some parameters of the patients and controls (Table 5 and 6).

DISCUSSION

As a result of this study it has been again put forwarded that goitre is seen more in women than men, supporting the literature (10, 11). Smoking status did not differ between patient and control groups. On the other hand iodine insufficiency, a well-known causative factor for goitre, was also evident in our study (12, 13). Mineral salt consumption was more

Table 5. Gender and genotype distribution of p53 gene codon 72 polymorphism in patients and healthy controls

Groups	Gender	Genotype distribution				Probability
		Pro/Pro+Pro/Arg		Arg/Arg		
		n	%	n	%	
Patient	Female	37	88.1	5	11.9	(Not evaluated)
	Male	0	0	0	0	
Control	Female	34	91.9	3	8.1	>0.05
	Male	11	100	0	0	

Table 6. Personel characteristics and genotype distribution of p53 gene codon 72 polymorphism in patients and healthy controls

Personel Characteristics	Groups	Genotype distribution				Probability
		Pro/Pro+Pro/Arg		Arg/Arg		
		+	-	+	-	
Smoking Status	Patient n	4	33	1	4	p>0.05
	%	80	89.2	20	10.8	
	Control n	10	35	1	2	p>0.05
	%	90.9	94.6	9.1	5.4	
Iodized Salt	Patient n	19	18	3	2	p>0.05
	%	86.4	90	13.6	10	
	Control n	33	12	2	1	p>0.05
	%	94.3	92.3	5.7	7.7	
Family History	Patient n	23	14	4	1	p>0.05
	%	85.2	93.3	14.8	6.7	
	Control n	13	32	2	1	p>0.05
	%	86.7	97	13.3	3	

Table 7. Some parameters and genotype distribution of p53 gene codon 72 polymorphism in patients and healthy control

Parameters		Genotype distribution		Probability
		Pro/Pro+Pro/Arg (mean±SD)	Arg/Arg (mean±SD)	
Age (year)	Patient	38.16±2.388	36.8±3.826	p>0.05
	Control	41.18±2.28	28.67±5.364	p>0.05
		Median(%25-%75)	Median(%25-%75)	
BMI	Patient	25(22.5-28)	21.89(21.89-25.5)	p>0.05
	Control	26(24-27.5)	26(24)	p>0.05
TSH (microU/ml)	Patient	1.33(0.46-3.52)	3.52(0.99-11.375)	p>0.05
	Control	0.91(0.65-1.425)	1(0.62)	p>0.05

Table 8. p53 codon 72 polymorphism genotype distributions on other Caucasian populations with different types of cancer

Cancer type	Genotype distribution (%)	Populations						
		USA	Greece	Spain	France	Germany	Russia	UK
Gastric	Pro/Pro+Pro/Arg	67	-	-	-	-	40	47
	Arg/Arg	33	-	-	-	-	60	53
Colorectal	Pro/Pro+Pro/Arg	-	-	-	55	61	-	-
	Arg/Arg	-	-	-	45	39	-	-
Lung	Pro/Pro+Pro/Arg	56	50	44.6	-	-	-	-
	Arg/Arg	44	50	55.4	-	-	-	-

common than iodized salt consumption in MNG patients compared to the healthy control subjects. Besides, the incidence of goitre was higher in the first-degree relatives of the patients compared to that of healthy controls, supporting the role of genetic factors in the pathogenesis of the disease (14). Age, BMI and serum TSH levels were not different between the two groups. Because the present study had a molecular basis, we paid attention to have a similar mean age between the patient and control groups. Thus, it is not possible to consider the age as an effective parameter for this study. On the other hand, mean serum TSH levels were calculated irrespective of the euthyroid, hypothyroid or hyperthyroid status of the MNG patients. Therefore, it is supposed that serum TSH levels of the healthy controls might be balanced by those of the patient group. BMI also did not increase or decrease in the patient group. The genetic distribution of p53 codon 72 polymophism genotypes did not show a significant difference between MNG patients and healthy controls in this study. According to allele frequencies there was also no significant difference between groups. In recent studies it has been reported that Pro homozygosity is related with a higher risk in thyroid cancer (1, 8, and 9). The present study is the first trial to specify the relationship between p53 codon 72 polymorphism and MNG in adult patients in Turkey. Our findings are consistent with those of previous study conducted on the Turkish population with thyorid and bladder cancer, showing genotype distribution for Pro/Pro+Pro/Arg as 70.7-78%, for Arg/Arg as 29.3-22% (1, 15). However in colorectal, hepatocellular carcinoma and breast cancer patients in Turkish population genotype distribution for Pro/Pro+Pro/Arg as 61-61-50%, for Arg/Arg as 39- 39-50% respectively (16-18). When we consider previous studies conducted on the other Caucasian populations, ratios can be seen on Table 8 (19-21). This inconsistency in the results can be explained by the fact that the diversities in gene pools, lifestyle and gene-environment interactions might not have been reflected to the genotypes (22-24). According to gender, smoking status, iodized salt consumption, familial history, age, BMI and TSH levels, they did not also show an association with p53 gene codon 72 polymorphism genotypes.

CONCLUSION

As a conclusion we did not found any association between p53 codon 72 polymorphism and thyroid cancer development in Turkish population with MNG. In addition, future genetic polymorphism studies with larger numbers of the population may provide more meaningful results. The significance of our result remains to be further investigated in different and even larger populations, combined with other genetic polymorphisms considered as risk factors for thyroid cancer.

Conflict of Interest

The authors declare that they have no conflict of interest.

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