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Original Article

Angiotensinogen (AGT) gene screening and nutrigenetic approaches in patients diagnosed with hypertension

Hipertansiyon tanısı alan hastalarda angiotensinojen (AGT) gen taraması ve nutrigenetik yaklaşımlar

Simge Becer[1], Didem Ozkan*[2]

[1]Istanbul Okan University, Nutrition and Dietetics Department, Istanbul, Turkey
[2]Istanbul Okan University, Vocational School of Health Services, Istanbul, Turkey

ABSTRACT

Aim: Today, hypertension is one of the most important preventable risk factors for diseases with fatal consequences such as stroke, heart failure, myocardial infarction, peripheral vascular disease, and end-stage renal failure, and it is estimated that there are approximately 15-16 million hypertension patients in Turkey. The aim of the study; to determine the nutritional habits of patients with clinically diagnosed hypertension and to investigate the nutrigenetic approaches of the disease.

Material and Methods: For this purpose, the study is planned as two-stage. In the first stage, it was aimed to examine the nutritional habits by applying a questionnaire to the patients diagnosed with hypertension. In the second stage, it was aimed to reveal genetic changes by a screening of the Angiotensinogen gene which is the disease-related gene in the clinically diagnosed patients..

Results: In the result of the study; p.M268T change in Angiotensinogen gene was defined in one patient and this change was defined in the literature as rs699 /NCBI, which is known as one of the most important polymorphisms associated with hypertension. In addition, it was found that there was no change in nutritional patterns before and after diagnosis of hypertensive individuals and the rates of interviewing with dietitians were very low.

Conclusion: The study is important as it is the first study conducted in terms of nutrigenetics in patients diagnosed with hypertension in the Turkish population.

Keywords: Hypertension; Nutrigenetic; Angiotensinogen gene

Corresponding Author*: Didem Ozkan, Istanbul Okan University Tuzla Campus Akfırat-Tuzla/ İstanbul
Email: didemtorun@gmail.com
ORCID: 0000-0003-4210-5073
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ÖZ

Amaç: Hipertansiyon; günümüzde ölümcül sonuçlar doğuran hastalıkların en önemli önlenebilir risk faktörlerindendir ve Türkiye'de yaklaşık 15-16 milyon hipertansiyon hastasının olduğu öngörülmektedir. Bu araştırmada klinik olarak hipertansiyon tanısı alan hastalarda beslenme alışkanlıkları yanında hastalığa ait nutrigenetik yaklaşımların ortaya konulması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada ilk aşamada hipertansiyon tanısı alan hastalara anket uygulanarak beslenme alışkanlıklarını incelemek, ikinci aşamada ise hastalık ile ilişkili gen olan Angiotensinojen geni (AGT) taraması yapılarak genetik değişimlerin ortaya konulmuştur. Çalışmada gen değişimlerinin risk getirisini saptamak ve beslenme alışkanlıklarını saptamak için uygulanan anket sonuçları ise "IBM SPSS Statistics 21ve Kikare" programları ile elde edilmiştir.

Bulgular: Çalışma sonucunda; bir hastada Angiotensinojen (AGT) geninde p.M268T değişimi tanımlanmış olup bu değişim literatürde hipertansiyonla ilişkili en önemli polimorfizmlerden biri olarak bilinen rs699/ NCBI olarak tanımlanmıştır. Ayrıca, hipertansiyonlu bireylerin tanı öncesi ve sonrası beslenme düzenlerinde bir değişim olmadığı ve diyetisyen ile görüşme oranlarının çok düşük olduğu tespit edilmiştir.

Sonuç: Çalışma, Türk popülasyonunda hipertansiyon tanısı alan hastalarda nutrigenetik açısından ilk kez yapılan bir araştırma olması açısından önem arz etmektedir.

Anahtar Kelimeler: Hipertansiyon; Nütrigenetik; Angiotensinojen geni

Introduction

Hypertension is one of the most important preventable risk factors for fatal diseases such as stroke, heart failure, myocardial infarction, peripheral vascular disease, and end-stage renal disease. It is estimated that there are about 15-16 million hypertensive patients in Turkey. The control of blood pressure in the body occurs through the interaction of endothelial tissue, kidneys, central and peripheral nervous systems [1-4]. High blood pressure: It is caused by genetic factors, environmental factors and their interaction. Thus, there is no single pathophysiological mechanism responsible for hypertension. This type of hypertension, whose cause is not precisely known, is called primary (essential) hypertension. This type of hypertension, which occurs in 90% of the population, is a condition that lasts a lifetime. When the cause of hypertension is another disease, it is called secondary hypertension (6-8%) [5-8]. Symptoms of hypertension may include dizziness, darkening of the eyes, weakness, headache in the neck, and palpitations. Etiologically, hypertension is pulmonary arterial hypertension (PAH), pulmonary hypertension due to left heart disease, pulmonary hypertension due to lung disease and/or hypoxia, chronic thromboembolic pulmonary hypertension, and pulmonary hypertension with uncertain mechanism [9-11]. Factors that trigger these formations include genetics, gender, age, obesity, smoking and alcohol consumption, physical activity, stress, salt consumption, the ratio of calcium, magnesium, and potassium in the diet, and a high- fiber diet. People respond differently to diets and the main reason for this difference is genetic. While the cause of hypertension was not well understood in the past, some genetic studies conducted in the 2000s have shown that genetic factors play a role in the development of hypertension. Among the causes of hypertension, genetic structure accounts for an important part, about 30-60% [12, 13]. Genetic studies have shown that the strongest link in the development of hypertension comes from the genes encoding the renin-angiotensin system and its components (angiotensinogen, angiotensin-converting enzyme, angiotensin- II type-1 receptor, adrenergic beta-2 receptor). Disorders in these genes are associated with renal dysfunction leading to increased salt and water retention. As a result, patients die at a young age from cerebral hemorrhage and sudden myocardial infarction after severe hypertension [14-16]

The Angiotensinogen (AGT) gene, which is in the first row of candidate genes for essential hypertension; It is effective in the formation and functioning of many tissues such as liver, heart, brain, vascular wall, adipose tissue, is a member of the SERPIN gene family, localized at 1q42-q43 and consists of 5 exons [17,18].

The purpose of this study is to investigate the dietary habits of people diagnosed with hypertension and to determine if there is a relationship between diet and genetics in hypertension by screening for the disease-associated gene angiotensinogen.

Material and Methods

Data collection: 30 patients who applied to the Cardiology Department of Pamukkale University Medical School and were diagnosed with hypertension were included in the study. Ethics committee approval for the study was obtained from the

Clinical Research Committee (2018/02/50) of Health Sciences University Kartal Koşuyolu Training and Research Hospital.

Each patient was informed of the potential outcomes of the study, and a voluntary informed consent form was signed. A questionnaire was used to determine the dietary habits of patients diagnosed with hypertension. The questionnaire prepared by the researcher to determine the frequency of food consumption was completed during personal interviews with the patients participating in the study. Participants' height and weight were measured by the researcher, but percentage body composition (fat, water, muscle, etc.) could not be determined. Participants' BMI values were calculated by the researcher using the World Health Organization accepted kg/m2 formula. Physical activity status, change in eating habits, and smoking status of the participants after diagnosis of hypertension were studied under the name of 'lifestyle modification.

Data analysis: Mutational analysis of the study examined angiotensinogen (AGT), a gene associated with hypertension. Polymorphic changes (single nucleotide changes, etc) and mutations were evaluated by comparison with a healthy control group of 50 individuals available in our DNA bank. After DNA was isolated from the patients' blood by the classical phenol/chloroform method, the exons of the gene of interest were amplified by polymerase chain reaction. AGT gene , exon 2 was screened by polymerase chain(PCR) reaction using the primers as Forward primer:"CCGTTTGTGCAGGGCCTGGCTCTCTCT", Reverse primer : "CAGGGTGCTGTCCACACTGGACCC"PCR reaction was carried out in a reaction volume of 50 µl containing 50 ng of genomic DNA, 10 pmol of each primer, 0.5 U Taq polymerase, 200 µM of each dNTP and 2.5 Mm MgCl2. PCR products were separated by electrophoresis through using 2 % agarose gel. The PCR products were analyzed by DNA sequence analysis.

Ethics committee approval for the study was obtained from the Clinical Research Committee (2018/02/50) of Health Sciences University Kartal Koşuyolu Training and Research Hospital.

Results

In the first step of the study, the participants' behaviors after the diagnosis of hypertension were examined according to their gender. Regarding the status of meetings with a dietitian after diagnosis, 6.7% (n: 1) of the female participants reported that they saw them regularly, while 60% (n: 9) reported that they never met with a dietitian. In the same situation, 6.7% of men (n:1) ensured regular contact, while 73.3% (n:11) did not meet at all. Regular medical control after diagnosis was positive in women, 73.3% (n:11), while it was equally distributed in men, 46.7% (n:7). Regular blood tests were found to be positive in both groups. In addition, the majority of participants of both sexes were found to be taking medications regularly (Table.1)

Table 1. Lifestyle changes and general behaviors of the participants after the diagnosis of hypertension by gender (n=30)

		Women		Men	
		n	%	n	%
Meeting with a Dietitian After Diagnosis of Hypertension	Regularly	1	6,7	1	6,7
	One time	4	26,7	3	20
	No	9	60	11	73,3
	No Answer	1	6,7	-	-
	Total	15	100	15	100
Regular Doctor Control Situations After Diagnosing Hypertension	Yes	11	73,3	7	46,7
	No	4	26,7	7	46,7
	No Answer	-	-	1	6,7
	Total	15	100	15	100
Having a Regular Blood Test After Diagnosing Hypertension	Yes	11	73,3	9	60
	No	4	26,7	6	40
	Total	15	100	15	100
Regular Drug Treatment After Diagnosing Hypertension	Yes	13	86,7	10	66,7
	No	2	13,3	5	33,3
	Total	15	100	15	100
Exercising Before Diagnosing Hypertension	Yes	-	-	4	26,7
	No	14	93,3	11	73,3
	No Answer	1	6,7	-	-
	Total	15	100	15	100
Exercising After Diagnosing Hypertension	Yes	2	13,3	4	26,7
	No	12	80	11	73,3
	No Answer	1	6,7	-	-
	Total	15	100	15	100
Changing Nutritional Habits After Diagnosing Hypertension	Yes	7	46,7	2	13,3
	No	7	46,7	13	86,7
	No Answer	1	6,6	-	-
	Total	15	100	15	100
Smoking	Yes	2	13,3	9	60
	No	13	86,7	6	40
	Total	15	100	15	100

Table.2 Genetic predisposition of the participants by gender (n=30)

		Women		Men	
		n	%	n	%
Condition of the Person Having Another Chronic Disease	Yes	5	33,3	4	26,7
	No	10	66,7	11	73,3
	Total	15	100	15	100
Hypertension Status in First Degree Relativesa	Yes	13	86,7	15	100
	No	2	13,3	-	-
	Total	15	100	15	100
Hypertension Status in 2nd Degree Relativesb	Yes	7	46,7	8	53,3
	No	8	53,3	7	46,7
		15	100	15	100

(a): This option includes mothers, fathers, children and siblings.
(b): This option includes grandparents, grandparents, uncles, aunts, etc.

The third step of the study examined the genetic predispositions of the participants. Only 33.3% (n:5) of female participants

reported having a chronic disease other than hypertension, whereas 26.7% (n:4) of men reported having a chronic disease. It was found that 86.7% of female participants had a person diagnosed with hypertension in their first-degree relatives (mother, father, sibling, child), whereas the first-degree relatives of all male participants had hypertension. On the other hand, 2nd-degree relatives (grandfather, grandmother, uncle, aunt, uncle) had hypertension in women at a rate of 46.7% (n:7), whereas this rate was 53.3% (n:8) in men (Table 3).

Table 3 Comparison of the risk of gene change in disease and control group

			Change	Chi-Square Value	%95 CI	P Value
Disease AGT	Control Group	MM	36	0,3613	0.0840-1.5533	0.17
		MT	14			
		TT	-			
	Patient Group	MM	4	0.0307	0.0074-0.1280	P<0.0001
		MT	5			
		TT	21			

In conjunction with the laboratory results of the study, a AGT gene screen was performed in 30 patients clinically diagnosed with hypertension and 50 healthy controls. After the blood samples from the patients and healthy participants were amplified using the polymerase chain reaction, purification processes were first performed and then DNA sequence analysis was performed.

As a result of the analysis, a homozygous change from timine to adenine was detected at position 803 in the 2nd exon of the gene, and this change caused the coding of the amino acid methionine at position 268 in the protein as the amino acid threonine (M268T). (rs699/NCBI) (Figure.1) In contrast, no clinically relevant change was detected in the control group.

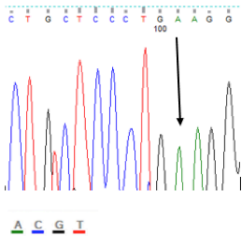

Figure 1. Entire coding exons were amplified by PCR and sequenced. Exon2, c.803 T> A, p. M268T was detected on AGT gene from healthy controls and/or hypertension patients by FinchTV sequence analysis software.

To detect the change in the sequences, a cut was made with the restriction endonuclease enzyme Tth111I (PsyI) cutting the CTGAC sequence to define T/A in this region. After evaluation, the homozygous genotype common in the study population compared with the control group was determined and taken as reference, and the association of hypertension and SNP genotypes was performed. In gene alteration, the M allele was scored as normal and the T allele as disease. The program "IBM SPSS Statistics 20 for Chi-Square" was used to determine the risk of mutations and gene alterations revealed in the gene scans, and the significance level was accepted as $p < 0.05$.

Table 4. Comparison of BMI value> 25 kg / m2 and gene change status

		Yes		No		Total			
		n	%	n	%	n	%		
BMI value >25 kg/m2	Within Group	18	72	7	28	25	100		
	Within the gene change group		85,7		77,8		83,3		
BMI value <25 kg/m2	Within Group	2	50	2	50	4	100	χ 2	P
	Within the gene change group		9,5		22,2		13,3		
No Answer	Within Group	1	100	-	-	1	100	1,238	0,538
	Within the gene change group		4,8		-		3,3		
Total	Within Group	21	70	9	30	30	100		
	Within the gene change group		100		100		100		

When the hypertensive patients group was compared with the control group, a statistically significant difference was found. ($p < 0.0001$). The rs699 polymorphism was associated with hypertension in this study as previously described. The NCBI-SNP database was used for the location of this change. (https://www.ncbi.nlm.nih.gov/clinvar/variation/18068/)

When the BMI value and genetic changes of the cases were examined by chi-square analysis, it was found that BMI value

alone and not directly related to hypertension. (Pearson chi-square ($\chi 2$) (n=30) = 1.238, $p > 0.05$).When the frequency of saturated fat consumption and the results of genetic modification of cases were examined by chi-square analysis, it was found that saturated fat consumption alone, and not directly, was associated with hypertension. (Pearson chi-square ($\chi 2$) (n=30) = 1.591, $p > 0.05$)

Discussion

In this study, the relationship between obesity and saturated fat consumption with the development of hypertension were analyzed statistically. And there was no significant association found between obesity and hypertension (x2=1.238, p>0.05).

In other studies investigating the association between obesity and hypertension, the risk of developing hypertension was shown to increase threefold in individuals with a BMI value of > 27 kg/m2 (6). no significant association was found ($\chi 2$=1.591, p > 0.05). Of the 21 individuals who participated in the study and were found to have gene alterations, 7 reported consuming saturated fat. Because the formation of hypertension increases due to the accumulation of excess saturated fat consumed in the veins, all guidelines assume that the saturated fat content of the diet DASH should be 6% (on a 2100 calorie diet) (10,11). In examining the study's association with lifestyle changes and hypertension, we examined whether or not there was an association between whether or not participants exercised before being diagnosed with hypertension and whether or not there was a gene change. Statistically, there was no significant association between the exercise status of the subjects before diagnosis and genetic alteration. At the same time, genetic changes and status of sport intake after diagnosis were statistically studied. After the diagnosis of hypertension, a total of 5 participants reported taking up sports. It was found that 15 participants did not answer the question. Statistically, a significant association was found between participants' exercise status after diagnosis and the occurrence of genetic changes ($\chi 2$=7.937, $p < 0.05$). As recommended in the DASH diet; A daily 30-minute brisk walk helps lower blood pressure and reduce the need for medication [12].

Today, there are many studies that prove that the response to diet therapy is directly related to the genetic background. As a result of the studies, it was found that many genes that are effective in the development and prevention of hypertension are related to the amount of fat consumed and the amount of sodium. As in many societies around the world, this issue has come to the fore because of the increased prevalence of hypertension and hypertension-related diseases in the Turkish population and because of recent guidelines showing that the relationship between diet and genes plays an important role [8,9].

This study examined the angiotensinogen (AGT) gene, which is thought to be associated with hypertension, and 21 of 30 volunteer patients were found to have a genetic disorder. It was found that 4 out of 9 individuals were genetically healthy and 5 of them were gene carriers.

Considering the small number of patients who participated in the study and other conditions, no statistically significant relationship was found when examining the general relationship between the groups, although there was a significant result between the data of each group. Since this is the first study on this topic in the Turkish population, it should inform future studies, and it was determined that further studies on this topic are needed.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All persons included in the study signed the informed consent form.

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest

Contribution of The Authors

Simge Becer and Didem Ozkan planned the study. Simge Becer contributed to the analysis of the data related to dietary habits and Didem Ozkan contributed to the genetic analysis. This work; contains the results of a master's thesis. It has not been published anywhere else.

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