The Role of Collagen I A 1 and Vitamin D Receptor Genes Polymorphisms on the Risk of Osteoporotic Fractures in Postmenopausal Turkish Women

Postmenopozal Türk Kadınlarında Osteoporotik Kırık Gelişiminde Kollajen I A 1 ve Vitamin D Reseptör Gen Polimorfizmlerinin Rolü

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ABSTRACT The objective of this study was to investigate the relationship between COLIA1 Sp1 and VDR BsmI polymorphisms and fracture risk in postmenopausal Turkish women. Ninety unrelated females were included in this study. All of these patients were in postmenopausal period. The diagnosis of osteoporosis was done according to WHO criteria. Thirty of them had osteoporosis and also with hip and/or vertebral fracture, 30 of them had osteoporosis but without fracture history and 30 individuals in similar age group had neither osteoporosis nor fracture. COLIA1 gene polymorphisms were defined as SS, Ss, and ss. VDR BsmI gene polymorphisms were defined as BB, Bb, and bb. The relationship between genotypic distribution, BMD, and presence of fracture was evaluated statistically. When distribution of COLIA Sp1 allele was evaluated according to groups, there was no statistically significant difference in frequency of alleles between the groups (p=0.092). Also VDR BsmI allele distribution was evaluated, in all 3 groups frequency of alleles was similar (p=0.563). Relationship between COLIA1 Sp1 and VDR BsmI allele distribution and presence of fracture was evaluated. Data of Group II and Group III was compared with data of Group I. There was no difference in frequency of COLIA 1 and VDR gene polymorphisms between these groups (for COLIA1 p=0.352, for VDR p=0.946). Similarly relationship between presence of osteoporosis and allele distribution was evaluated. Data of Group I +Group II was compared with data of Group III. Allele distribution of both genes was not significantly different in groups (for COLIA1 p=0.436, for VDR p=0.635). In conclusion, COLIA1 and VDR genes are not associated with fracture risk and BMD in postmenopausal Turkish women.

Key Words: Osteoporosis; fracture; COLIA1; Vitamin D Receptor

ÖZET Bu çalışmanın amacı postmenopozal Türk kadınlarında COLIA1 Sp1 ve VDR BsmI polimorfizmi ve kırık riski arasındaki ilişkiyi araştırmaktır. Aralarında akrabalık bulunmayan 90 kadın bu çalışmaya alındı. Tüm hastalar postmenapozal dönemde idi. Osteoporoz tanısı WHO kriterlerine göre koyuldu. Bu hastaların 30' unda osteoporoz mevcuttu ve kalça ve/veya vertebral fraktür gelişmişti, 30 hastada osteoporoz mevcut ancak fraktür öyküsü yoktu, 30 hasta bu hastalarla aynı yaş grubunda idi ve osteoporoz ve fraktür mevcut değildi. COLIA1 gen polimorfizmi SS, Ss and ss olarak, VDR BsmI gen polimorfizmi BB, Bb, and bb olarak tanımlandı. Çalışma grubunun genotipik dağılımı ile BMD ve fraktür varlığı arasındaki ilişki istatistiksel olarak değerlendirildi. Gruplara gore COLIA1 Sp1 allel dağılımı değerlendirildiğinde allellerinin sıklığı açısından gruplar arasında istatistiksel olarak anlamlı farklılığın olmadığı görüldü (p= 0.092). Benzer şekilde VDR BsmI allel dağılımı değerlendirildiğinde, allellerinin sıklığı üç grupta da benzerdi (p= 0.563). COLIA1 Sp1 ve VDR BsmI allel dağılımının kırık varlığı ile ilişkisinin değerlendirilmesinde Grup 2+Grup 3 verileri Grup 1 ile karşılaştırıldı. Buna göre COLIA1 ve VDR gen polimorfizmlerinin sıklığı açısından gruplar arasında farklılık olmadığı izlendi (COLIA1 için, p=0.352; VDR için, p= 0.946). Benzer şekilde osteoporoz varlığı ile allel dağılımının ilişkisini değerlendirmek için Grup1+Grup2 verileri Grup 3 ile karşılaştırıldı. Her iki gene ait allel dağılımları için gruplar arasında anlamlı fark saptanmadı (COLIA1 için, p=0.436; VDR için, p=0.635). Sonuç olarak postmenopozal Türk kadınlarında COLIA1 ve VDR genleri osteoporoz ve kırık riski ile ilişkili bulunmamıştır.

Anahtar Kelimeler: Osteoporoz; fraktür; COLIA1; vitamin D reseptörü

Turkish Medical Journal 2009;3(2):101-5

Osteoporosis is a systemic disease characterized by decreased bone mineral density (BMD) and microarchitectural deterioration of bone tissue, both of which result in an increased bone fragility and fracture.[1] Genetic studies have described several loci and candidate genes in the formation of bone mass and the etiology of osteoporotic fractures.[2]

The genes encoding type I collagen (COLIA1 and COLIA2) are important candidates for the pathogenesis of osteoporosis. Grant et al. described a common polymorphism affecting a binding site for the transcription factor Sp1 in the first intron of COLIA1 that was more prevalent in osteoporotic patients than in controls.[3] Positive associations between the COLIA1 Sp1 polymorphism, bone mass, and osteoporotic fractures were subsequently reported in several populations, and a meta-analysis showed that the COLIA1 genotype conferred differences in BMD of approximately 0,15 Z-score units per copy of the "s" allele and an increase in fracture risk of approximately 62% per copy of the "s" allele.[4]

Vitamin D receptor (VDR) plays a role in regulating calcium homoeostasis through binding and nuclear translocation of 1a25(OH) 2 D3, affecting bone resorption, and increasing calcium absorption.[5] Morrison et al. identified three common polymorphisms in the 3' region of the VDR gene, situated between exons 8 and 9, which are recognized by the restriction enzymes BsmI, ApaI and TaqI.[6] These, more specifically BsmI restriction fragment length polymorphism (RFLP), were found to be associated with circulating levels of the osteoblast- specific protein osteocalcin and bone mass in a twin study and population- based study.

In some studies, polymorphic variants of the COLIA1 and VDR genes are associated with BMD and fracture risk, but some epidemiological studies of several ethnic groups found no correlation.[7-12] The present study was aimed to investigate the relationship between COLIA1 Sp1 and VDR BsmI genes polymorphisms and fracture in postmenopausal Turkish women.

MATERIALS AND METHODS

SUBJECTS

Following the approval of this study by the ethics committee of the Ankara Ataturk Education and Research Hospital, Turkey, a prospective study was conducted in the Department of Endocrinology and Metabolism, Orthopedy and Traumathology, Biochemistry and Radiology.

All of patients included in this study were in postmenopausal period. The diagnosis of osteoporosis was done according to WHO criteria.[13] Among the patients 30 of them had osteoporosis and also hip and/or vertebral fracture, 30 of them had osteoporosis but not history of fracture, and 30 females in similar age group had neither osteoporosis nor fracture history. Totally 90 unrelated Turkish females were included in this study. Written informed consent was obtained from the patients. A comprehensive health questionnaire was given including age, lifestyle, and clinical data (to exclude the possibility of other endocrine diseases, use of corticoids, etc). Height and weight were measured at the initial examination. The body mass index (BMI) was calculated as weight in kg divided by the height (in meters) squared. Hip and vertebral fractures were identified through radiological reports from all sources providing x-ray services in our center. Fractures were only included if the report of fracture was definite.

MEASUREMENT OF BONE MINERAL DENSITY

BMD (grams per square centimeter) at the lumbar spine and femoral neck was measured by dual-energy x-ray absorptiometry using QDR 4500W densitometer (HOLOGIC Inc., Waltham, MA).

DETERMINATION OF POLYMORPHISM IN THE COLIA1 SP1 AND VDR BSMI GENES

Ten mL blood from patients were collected in tubes with EDTA and centrifuged for 5 minutes at 1500 rpm. Genomic DNA from patient bloods was extracted with commercially kit (Invisorbâ Spin Blood Mini Kit, Invitek GmbH, Berlin, Germany). Isolated genomic DNAs were subjected to polymerase chain reaction (PCR) with commercially available primers with specific biotin labeled for

COLIA1 and VDR genes (RDB 2055E- GenIDâ GnbH, Strassberg, Germany). Perkin-Elmer 9600 PCR machine was used. After PCR amplification, samples were evaluated under UV light.

COLIA1 gene polymorphisms were defined as SS, Ss, and ss. VDR BsmI gene polymorphisms were defined as BB, Bb, and bb. The relationship between the genotypic distribution, BMD, and presence of fracture was evaluated statistically.

Statistical Analysis

For the statistical analysis of the study, SPSS 13.0 packet program (SPSS Inc., Chicago, IL) was used. Descriptive statistics were shown as mean ± standard deviation. The frequency distribution of COLIA1 and VDR genotypes in the study group was determined and evaluated using the χ^2- test. Quantitative data of groups were compared by ANOVA. If ANOVA was significant, differences among the subgroups were tested by Tukey's test. Values of $P< 0.05$ were accepted as statistically significant.

RESULTS

Descriptive data to the patients, together with the frequency of the allele with regards to the COLIA1 Sp1 and VDR BsmI genes polymorphisms of the patients divided into tree groups as Group I (osteoporosis and fracture), Group II (osteoporosis and no fracture), and Group III (no osteoporosis and no fracture), and the results of the comparison within the groups has been given in Table 1.

BMI and median ages were compared and no statistically significant difference was found between groups (p= 0.713 and p= 0.191 respectively).

Average values of BMD were compared and no significant difference was found between osteoporotic Group I and Group II (in lumbar region; 0.559 ± 0.173 vs 0.608 ± 0.078, p= 0.584, in femoral region; 0.565 ± 0.135 vs 0.613 ± 0.095, p= 0.282). The values of lumbar and femoral region were compared in Group I and Group III, Group II and Group III. For both regions the difference was statistically significant (p< 0.001).

COLIA1 Sp1 allele distribution was evaluated according to groups. It was found that no statistically significant difference was found in frequency of SS, Ss, and ss alleles (p= 0.092). VDR BsmI allele distribution was also evaluated. The frequency of BB, Bb, and bb alleles was similar in all three groups (p= 0.563).

In evaluation of relationship between COLIA1 Sp1 and VDR BsmI allele distribution and presence of fracture, the data of Group II+ Group III was compared with Group I. Between groups no difference was found in frequency of COLIA1 and VDR gene polymorphisms. (for COLIA1 p= 0.352; for VDR p= 0.946).

TABLE 1: Demographic data and distribution of COLIA1 and VDR BsmI genotypes in groups.

	Group I	Group II	Group III	p
N	30	30	30	
Age (year)	64.1 ± 6.4	62.3 ± 5.9	61.1 ± 7.1	0.191
BMI (kg/m2)	28.9 ± 5.85	27.9 ± 5.6	27.8 ± 5.9	0.713
Femoral neck BMD (gr/cm²)	0.565 ± 0.135	0.613 ± 0.095	0.814 ± 0.130	<0.001
Lumbar spine BMD (gr/cm²)	0.559 ± 0.173	0.608 ± 0.078	0.861 ± 0.272	<0.001
COLIA1 genotypes				
SS	20 (66.7%)	13 (43.3%)	18 (60.0%)	0.092
Ss	9 (30.0%)	16 (53.3%)	8 (26.7%)	
ss	1 (3.3%)	1 (3.3%)	4 (13.3%)	
VDR genotypes				
BB	4 (13.3%)	6 (20.0%)	2 (6.7%)	0.563
Bb	10 (33.3%)	7 (23.3%)	11 (36.7%)	
bb	16 (53.3%)	17 (56.7%)	17 (56.7%)	

The data of Group I +Group II was compared with data of Group III for evaluation of relationship between presence of osteoporosis and distribution of COLIA1 Sp1 and VDR BsmI alleles. No statistically significant difference was found in allele distribution of both genes between groups (for COLIA1 p= 0.436; for VDR, p= 0.635) (Table 2, 3).

DISCUSSION

Multiple researches are found in the literature related to correlation between osteoporosis, fracture risk and COLIA1 gene polymorphism. Although polymorphisms of this gene are frequently seen in Caucasian population, it is also rarely seen in African and Asian race.[14] In a multi-centric study called Genetic Markers of Osteoporosis (GENOMOS) 20786 cases were evaluated from different European countries. In this study it was found that COLIA1 Sp 1 polymorphism was related to BMD loss and also there was increased vertebral fracture risk independent to BMD.[15] Langdahl et al reported that ss genotype owner had 10 fold increased osteoporotic fracture risk compared to control group in Denmark population.[9] Bernad et al evaluated 319 postmenopausal Spanish females. They found COLIA1 Sp 1 "ss" genotype related to increased fracture risk independent to BMD.[16] We found no association between COLIA1 Sp 1 binding site polymorphism and spine and femur BMD or fracture risk. Some authors also described a similar finding.[10,11,17-19]

TABLE 2: Distribution of COLIA1 and VDR BsmI genotypes in groups with presence of fracture.

	Group I	Group I + II	p
COLIA1 genotypes			
SS	20 (66.7%)	31 (51.7%)	
Ss	9 (30.0%)	24 (40.0%)	0.352
ss	1 (3.3%)	5 (8.3%)	
VDR genotypes			
BB	4 (13.3%)	8 (13.3%)	
Bb	10 (33.3%)	18 (30.0%)	0.946
bb	16 (53.3%)	34 (56.7%)	

TABLE 3: COLIA1 and VDRBsmI genotypes of groups with presence of osteoporosis

	Group I + II	Group III	p
COLIA1 genotypes			
SS	33 (55.0%)	18 (60.0%)	
Ss	25 (41.7%)	8 (26.7%)	0.436
ss	2 (3.3)	4 (13.3%)	
VDR genotypes			
BB	10 (16.6%)	2 (6.7%)	
Bb	17 (28.3%)	11 (36.7%)	0.635
bb	33 (55.5%)	17 (56.7%)	

Hubacek et al compared the frequency of COLIA1 Sp 1 allele in 1400 females of Czech population with 218 osteoporotic and 151 postmenopausal non osteoporotic females. At the end of the study they found no relationship between low BMD and polymorphisms of this gene.[17] Banros et al evaluated 220 young Brazilian females and found no association between COLIA1 genotypes, lumbar, and femoral BMD.[18] Linden et al reported no relationship was found between COLIA1 genotypes and BMD in Swedish postmenopausal females.[19]

Common comment is not present about the role of VDR gene polymorphisms in fracture development. Several polymorphisms have been found in the VDR gene and alleles of this gene have been associated with BMD in several studies. Garnero et al followed up 589 postmenopausal females with median age 62 during 11 years. They found that BB genotype of VDR Bsm1 polymorphism was a risk factor for development of vertebral and non vertebral fracture independent to BMD, BMD loss in radius, clinical and biochemical variables.[20] Langdahl et al evaluated 192 osteoporotic patients with fracture and 207 controls in a case-control study. They found that the presence VDR BsmI allele increased the risk of fracture development .[21] Fescanich et al found that presence of BB genotype of VDR Bsm1 increased the hip fracture risk 2 fold in Nurses Health Study.[22] In Rotterdam study, a large comprehensive study, randomly selected 1004 females were followed up during 3.8 years. This study attracted attention to expressive relationship between VDR gene haplotypes and non vertebral fracture risk. This association was independent from age, body mass index, and BMD. Au-

thors determined significant association with the vertebral fracture risk in 7 years follow- up period but did not determine increase in non-vertebral fracture in contrast to first reports.[8,23] The study called "Study of osteoporotic fractures" showed that presences of VDR genotypes were not risk factors for hip, vertebral, and other fractures.[12]

From our country, Dündar et al. investigated the association about VDR gene Apa I polymorphism with bone mineral density in postmenopausal women. They found that postmenopausal women with aa genotype had significantly lower BMD values at lumbar spines compared to persons with AA genotype.[24]

COLIA1 and VDR polymorphisms have been investigated in multiple studies and different results have been found. These results can be explained with expansiveness of samples, design of study, differences of data analysis. Also they can be explained as linkage to genetic features of the population and environmental factors. In our study we did not determine any association between COLIA1 and VDR BsmI gene polymorphisms, BMD, and fracture risk in postmenopausal Turkish females. Our study is different from the other similar studies in two aspects; control group was matched from cases in similar age and sub-control groups were constituted from cases included in the study for determination of fracture and osteoporosis risk. We think that advanced studies including large cases must be done to determine the genetic risk factors in development of osteoporotic fracture in our population.

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