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RESEARCH ARTICLE

Angiotensin Converting Enzyme (ACE) Gene I/D Polymorphism Genotypes and ACE Levels of Serum and Synovial Fluid of Patients with Osteoarthritis

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Angiotensin Converting Enzyme (ACE) Gene I/D Polymorphism Genotypes and ACE Levels of Serum and Synovial Fluid of Patients with Osteoarthritis

Summary

This study was conducted to determine the association between angiotensin converting enzyme gene I/D polymorphism genotypes and angiotensin converting enzyme levels of serum and synovial fluid of patients with osteoarthritis. Genomic DNA obtained from 42 persons (27 patients with osteoarthritis and 15 healthy controls) was used in the study. DNA was multiplied by polymerase chain reaction using I and D allele-specific primers. Polymerase chain reaction products were assessed with UV transilluminator by being exposed to 2% agarose gel electrophoresis. Synovial fluid was obtained by needle aspiration from patients with knee effusion and collected during the arthroscopic procedures in cases with osteoarthritis and during the trauma surgery in cases without osteoarthritis. Angiotensin converting enzyme levels of serum and synovial fluid were determined by using ELISA kit. Angiotensin converting enzyme serum and synovial fluid levels were higher in patients with osteoarthritis than controls but the difference was not significant (p>0.05). According to genotypes there was also no significant difference between angiotensin converting enzyme levels of serum and synovial fluid of the groups (p>0.05). In this study, no association was determined between angiotensin converting enzyme gene I/D polymorphism genotypes, and serum and synovial fluid angiotensin converting enzyme levels in osteoarthritis patients.

Key Words: *ACE, synovial fluid, osteoarthritis.*

Osteoartritli Hastalara Ait Serum ve Sinovyal Sivi Örneklerinde Anjiyotensin Dönüştürücü Enzim (ACE) Geni I/D Polimorfizmi Genotipleri ve ACE Düzeyleri

Özet

Bu çalışmada osteoartritli hastalara ait serum ve sinovyal sıvı anjiotensin dönüştürücü enzim düzeyleri ile anjiotensin dönüştürücü enzim geni I/D polimorfizmi genotipleri arasındaki ilişkinin belirlenmesi amaçlanmıştır. Çalışmada 42 bireyden (27 osteoartritli hasta ve 15 sağlıklı control) genomik DNA elde edilmiştir. Elde edilen DNA I ve D alelleri için spesifik olan primerler kullanılarak polimeraz zincir reaksiyonu ile çoğaltılmıştır. Polimeraz zincir reaksiyonu ürünleri %2'lik agaroz jel elektoroforezine maruz bırakılarak UV transillüminatör ile değerlendirilmiştir. Sinovyal sıvı örnekleri osteoartrit vakalarında artroskopik müdahele sırasında, osteoartrit olmayan vakalarda ise travma cerrahisi sırasında diz efüzyonu olan bireylerden iğne aspirasyonu ile elde edilmiştir. Serum ve sinovyal sıvı anjiotensin dönüştürücü enzim düzeyleri ELISA kiti kullanılarak belirlenmiştir. Serum ve sinovyal sıvı anjiotensin dönüştürücü enzim düzeyleri osteoartritli bireylerde sağlıklı kontrollere oranla artış göstermiş ancak bu fark istatistiksel olarak anlamlı bulunmamıştır (p>0.05). Genotipler açısından da serum ve sinovyal sıvı anjiotensin dönüştürücü enzim düzeyleri gruplar arasında anlamlı farklılık göstermemiştir (p>0.05). Çalışmanın sonucunda, osteoartritli hastalarda anjiotensin dönüştürücü enzim geni I/D polimorfizmi genotipleri ile serum ve sinovyal sıvı anjiotensin dönüştürücü enzim düzeyleri arasında bir ilişki olmadığı belirlenmiştir.

Anahtar Kelimeler: *ACE, sinovyal sıvı, osteoartrit.*

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INTRODUCTION

Osteoarthritis (OA) is the major chronic disease leading to musculoskeletal morbidity and functional loss, but is of unknown cause (1-3). It is generally agreed that the cause is multifactorial, involving genetic predetermination, age, gender, acute and chronic joint trauma, metabolic and inflammatory mechanisms and dietary factors (1,4-6).

Angiotensin converting enzyme (ACE) (also known as peptdyl dipeptidase A or kininase II) is a metalloenzyme converts angiotensin I to a potent vasoconstrictor angiotensin II (7,8). It also inactivates bradykinin which is a vasodilator of the kallikrein kinin system and has major implication in inflammatory process including OA (9,10). OA starts developing in synovial joints at twenties and results in clinical symptoms at forties (11,12). ACE levels of synovial fluid (SF) in patients with OA has been found higher than controls (13).

ACE gene (Gene Bank accession number: NM 000789.2) is localized on chromosome 17 and contains a polymorphism based on the presence (insertion, I) or absence (deletion, D) within intron 16, of a 287 base pair ALU repeat sequence; resulting in 3 genotypes: DD and II homozygotes and ID heterozygote. DD genotype is associated with higher concentrations of circulating ACE (14-19). DD genotype of this polymorphism has also been found associated with the development of primary knee OA in Korean and Turkish population (2, 9).

The literature review in this field revealed no study explaining the relationship between ACE gene I/D polymorphism and ACE levels of SF in OA (2,9). Therefore this study aims to determine the association of this polymorphism genotypes, and ACE levels of serum and SF of patients with OA and controls without OA.

MATERIAL AND METHODS

Study Population

This study included 27 OA patients (11 males and 16 females; mean age, 56,6±12,15 years) and 15 controls (7 males and 8 females; mean age, 32,9±13,43 years) recruited from the department of Orthopedic and Traumatology, Private Mus Sifa Hospital in Mus, 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. Inclusion criteria were with any symptom and/or sign of OA, positive finding in radiographs according to Kellgren-Lawrence grading, informed consent, and no evidence of arthritis due to other disease (20). Patient's history taking, physical examination and radiographic finding provided clinical information of the patients. Control subjects were consecutively selected among people without personal and family history of OA.

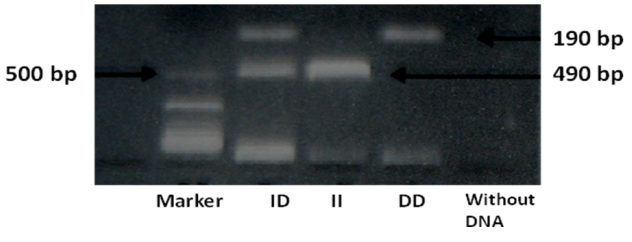
Genotype Determination

DNA was extracted from 2 mL venous blood according to kit procedure (Vivantis, Malesia) and stored at +4^{0}C. Amplification of DNA was performed by PCR in a thermal cycling (Amplitronyx 4, USA) with 0.5 µL of DNA sample according to Bayram et al. (2). The PCR products were separated by electrophoresis on 2% agarose gel containing 4 µL ethidium bromide (50 µg /µL) and were visualized using a UV transilluminator (Illuminyx UVB Transilluminator, USA). PCR method resulted in a 190 bp product (D allele) and in the presence of insertion, produced a 490 bp product (I allele). In heterozygous samples, two bands (490 and 190 bp) were detected (Figure 1).

Measurement of Serum and SF ACE Levels

Serum samples were collected before the surgery. SF was obtained by gauge aspiration from patients with knee effusion and collected during the arthroscopic procedures in cases with OA and during the trauma surgery in cases without OA. Subjects using ACE inhibitors were not included in the study. Serum

Figure 1. Determination of DD, II and ID Genotypes by PCR Using ACE Primer



and SF samples were centrifuged immediately and then stored at -20^{0}C until analysed. Serum and SF ACE levels were determined by using ELISA kit (Boster,China).

Statistical Analysis

Data was analyzed using the MINITAB 14 and statistical package for social sciences (SPSS ver.15). Shapiro Wilk normality test was applied for all variables. The variables which didn't show normal distribution were analyzed by Mann Whitney U test and Kruskal Wallis test. As descriptive statistics, percentages of median (25 % and 75 %) were shown as averages. A p value of less than 0.05 is considered statistically significant.

RESULTS

Table 1 presents the ACE levels of serum and SF of patients and controls. ACE levels of serum and SF of patients were higher than controls but there was no significant difference (p>0.05) between them. Genotype distribution of ACE gene I/D polymorphism and ACE serum and SF levels are shown in Table 2. ACE levels of serum and SF of patients and controls carrying DD genotype were higher than ID and II carriers but this increase was not significantly different (p>0.05).

Table 1. ACE levels of serum and SF of patients and controls

Groups	Serum ACE Levels (pg/ml)	SF ACE Levels (pg/ml)
Patients	630 (325-770)	265 (165-627)
Controls	360 (220-690)	234 (157-350)
p value	p>0.05	p>0.05

DISCUSSION

Within this study, we analyzed the association between ACE gene I/D polymorphism genotypes and ACE levels of serum and SF in OA patients and controls. The present study indicates that the ACE levels of serum and SF of patients with OA were higher than controls, but the increase was not significant. According to the ACE gene I/D polymorphism genotypes, ACE levels of serum and SF of patients and controls carrying DD genotype were higher than ID and II carriers respectively. This increase was also not found significantly different.

In concordance with our results, Blann found increased ACE activity in SF of OA patients compared to controls but it was not significantly different (14). Lowe et al., Matucci-Cerinic et al. and Cobankara et al. also did not found any significant differences in serum and SF ACE activities between OA patients and controls (21-23).

We could not find any findings about the association between serum and SF ACE levels and ACE gene I/D polymorphism genotypes in patients with OA to compare with our results. When we have compared our results with the results studied in various diseases; we see that Cambien et al. found no significant difference between patients with myocardial infarction and controls regarding plasma ACE concentrations and ACE gene I/D polymorphism (24). Nevertheless, Samani et al. and Gunes et al. found that plasma ACE concentrations were higher in DD carriers than ID and II with hypertension and myocardial infarction patients respectively (25,26).

Table 2. Genotype distribution of ACE gene I/D polymorphism and, ACE serum and SF levels of patients and controls

Groups		ACE Gene I/D Polymorphism Genotypes			p value
		DD	ID	II	
Patients	Serum ACE Levels (pg/ml)	955 (632.5-1367.5)	620 (457.5-785)	350 (255-667.5)	p>0.05
	SF ACE Levels (pg/ml)	548,5 (282.5-756.75)	259,5 (161.25-631.25)	165 (110-287.5)	p>0.05
Controls	Serum ACE Levels (pg/ml)	550 (280-750)	340 (300-630)	330 (180-540)	p>0.05
	SF ACE Levels (pg/ml)	300 (245-500)	230 (225-365)	160 (105-257)	p>0.05

CONCLUSION

As a conclusion, in this study, we may assert that serum and SF ACE levels are higher in patients with OA than controls without OA, but this increase is not a significant criteria for the development of OA. ACE gene I/D polymorphism genotypes also did not explain the increased serum and SF ACE levels in OA patients in this study. More comprehensive investigations are required to determine definite effect of serum and SF ACE levels on the development of OA and suppression of serum and SF ACE levels could have an effect on the progression of OA.

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