Relationship Between Angiotensin-Converting Enzyme Gene Polymorphism (Insertion/Deletion) and the Clinical Condition of Sepsis in Turkish Children

Türk Çocuklarında Anjiyotensin Konverting Enzim Gen Polimorfizmi (İnsersiyon/Delesyon) ve Sepsis Klinik Durumları Arasındaki İlişki

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ABSTRACT Objective: It has been postulated that genetic predisposition may influence the susceptibility to infection and the disease outcome. The D allele of the angiotensin-converting enzyme (ACE) gene is associated with many diseases. However, there are only few reports available about infection. We have investigated the association between ACE I/D polymorphism and sepsis, its clinical features such as acute respiratory distress syndrome (ARDS), multiorgan dysfunction syndrome (MODS) and mortality. **Material and Methods:** Ninety-eight children who had been diagnosed with sepsis and 100 healthy individiuals were included. The patients were divided into groups based on the presence of ARDS, MODS and survivor or nonsurvivor. The ACE gene polymorphism was analyzed by the polymerase chain reaction. **Results:** There was no statistical difference between the control and patient's genotype (p= 0.29). No evidence emerged regarding the association of ACE I/D polymorphism with MODS, but there was evidence of association with sepsis-related ARDS. It was found that carrying D/D genotypes increased the risk of the having ARDS 4.5 fold (95%CI 1.15-19.6, p= 0.028). On the other hand, there was not statistically significant difference between ACE gene polymorphism and mortality. **Conclusion:** In our study, the deletion polymorphism in angiotensin-converting enzyme gene is associated with increase in the risk of sepsis-related ARDS but not MODS and mortality in Turkish children.

Key Words: Sepsis; polymorphism, genetic; child; multiple organ failure

ÖZET Amaç: Genetik predispozisyonun enfeksiyona duyarlılıkta ve hastalıkların prognozunda rolü olduğu savunulmaktadır. Anjiyotensin-konverting enzim (ACE) geni D alelinin birçok hastalıkla ilişkisi saptanmıştır. Bununla birlikte, enfeksiyonla ilişkili az sayıda rapor mevcuttur. Biz ACE I/D polimorfizimi ile sepsis ve sepsisin klinik özellikleri olan akut respiratuvar distress sendromu (ARDS), multiorgan disfonksiyonu sendromu (MODS) ve mortalite arasındaki ilişkiyi araştırdık. **Gereç ve Yöntemler:** Sepsis tanılı doksan sekiz hasta ile 100 sağlıklı kişi kontrol grubu olarak alındı. Hastalar, ARDS, MODS olup olmadıkları ve yaşayıp yaşamadıklarına göre gruplara ayrıldı. ACE gen polimorfizmi polimeraz zincir reaksiyonu ile analiz edildi. **Bulgular:** Hasta ve kontrol grubunun genotipleri arasında fark yoktu (p= 0.29). ACE I/D polimorfizmi MODS gelişimi riskini artırmazken, sepsis ilişkili ARDS riskini artırmaktaydı. D/D genotipi taşıyor olmanın ARDS riskini 4.5 kat arttırdığı bulundu (95%CI 1.15-19.6, p= 0.028). Öte yandan, ACE gen polimorfizmi ve mortalite arasında istatistiksel olarak fark saptanmadı. **Sonuç:** Çalışmamızda; anjiyotensin-konverting enzim geni delesyon polimorfizmi, sepsis ilişkili ARDS riskinin artışıyla ilişkiliyken, MODS ve mortaliteyi etkilememekteydi.

Anahtar Kelimeler: Sepsis; polimorfizm, genetik; çocuk; multipl organ yetmezliği

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Sepsis is an important cause of mortality in the Pediatric Intensive Care Unit (PICU).[1] The presence of acute respiratory distress syndrome (ARDS), and multiorgan dysfunction syndrome (MODS) may affect

the prognosis of sepsis.[2] The genetic makeup of the host may play an important role in the susceptibility to and the development of sepsis and ARDS, as well as its severity and outcome.[3] The outcome of critical illness may be causally related to the severity of the inflammatory response. Given that tissue angiotensin-converting-enzyme (ACE) regulates such responses and that the deletion (D) variant of the ACE gene is associated with higher tissue ACE levels. Rigat et al. described an insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene that accounted for 40% of the inter-individual variation in serum and cardiac ACE activity.[4] The D allele of the angiotensin-converting enzyme (ACE) gene has been claimed to be associated with many diseases and a poorer outcome.[5-8] However, the results have not been replicated in all studies. Some studies showed that I allele was a risk factor for some disease[9,10] or did not have any effect.[11] However, there are few reports about infection and clinical condition in sepsis.[8,12]

In this study, an investigation was performed to investigate whether or not ACE I/D gene polymorphism had any possible effects on sepsis-related clinical conditions in Turkish children.

MATERIAL AND METHODS

STUDY POPULATION

This study was conducted in the pediatric intensive care unit of Cukurova University Hospital, Adana, Turkey. This unit has 16 beds and serves as a referral center in Southern Turkey for pediatric patients with medical and surgical problems. Written consent was obtained from the family. All patients and controls were Turkish, ethnically homogeneous, and not genetically related.

Eighty-four patients previously admitted to other hospitals and who had been given antibiotics and neonates were excluded from the study. Ninety eight Turkish children with sepsis and 100 healthy subjects were enrolled in this study. The control group consisted of 100 unrelated healthy adult volunteers without renal, metabolic, infectious (with no history of pneumonia or other severe infections), or cardiac disease.

DEFINITIONS

Sepsis was defined as Systemic Inflammatory Response Syndrome *(SIRS)* caused by an infection. SIRS was characterized by the presence of at least two of the following four criteria, one of which should be abnormal temperature or leukocyte count:

- Core temperature of >38.5°C or <36°C.
- Tachycardia, defined as a mean heart rate of >2 SD above normal for age in the absence of an external stimulus, chronic drug intake, or painful stimuli, or otherwise unexplained persistent elevation over a 0.5- to 4-hour time period OR for children <1 yr old; bradycardia, defined as a mean heart rate <10th percentile for age in the absence of an external vagal stimulus, beta-blocker drugs, or congenital heart disease, or otherwise unexplained persistent depression over a 0.5-hour time period.
- Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to an underlying neuromuscular disease, or undergoing general anesthesia.
- Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or >10% immature neutrophils.[13]

Cardiovascular Dysfunction

Despite administration of isotonic intravenous fluid bolus >40 mL/kg in 1 hour;

Decrease in BP (hypotension) <5th percentile for age or systolic BP <2 SD below normal for age, or need for vasoactive drug to maintain BP in normal range (dopamine >5 μg/kg/min or dobutamine, epinephrine, or norepinephrine at any dose), or two of the following: Unexplained metabolic acidosis; base deficit >5.0 mEq/L, increased arterial lactate >2 times the upper normal limit, oliguria; urine output <0.5 mL/kg/hr; prolonged capillary refill: >5 secs; core to peripheral temperature gap >3°C

Respiratory

PaO2/FIO2 <300 in absence of cyanotic heart disease or preexisting lung disease or PaCO2 >65 torr or 20 mmHg over the baseline PaCO2 or proven need, or >50% FIO2 to maintain saturation >92%, or

need for non-elective invasive or non-invasive mechanical ventilation

Neurologic

Glasgow Coma Score <11 or acute change in mental status with a decrease in Glasgow Coma Score >3 points from abnormal baseline

Hematologic

Platelet count <80,000/mm^3 or a decline of 50% in platelet count from the highest value recorded over the past 3 days (for chronic hematology/oncology patients) or International normalized ratio >2

Renal

Serum creatinine >2 times the upper limit of normal for age

Hepatic

Total bilirubin >4 mg/dL (not applicable for newborn) or ALT 2 times the upper limit of normal for age.

The American-European Consensus Conference Committee criteria were used to diagnose *ARDS:*[14] (i) acute onset, (ii) bilateral infiltrates on chest radiography, (iii) PaO_2/FiO_2 of less than 200, (iv) absence of clinical evidence of left-sided heart failure.[14]

ACE GENOTYPING

Blood samples were collected from patients and controls and incorporated into EDTA. Genomic DNA from leukocytes was purified according to the method of Miller.[15] The ACE I/D gene polymorphism was detected by PCR with primer sequences derived from Zee et al.[16] The sequences of the primers were chosen so that they flank the targeted region of the genome on the intron 16 of the ACE gene (17q23). The template DNA (0.4 mcg) was amplified using the following primers: (forward): 5'CTGGAGACCACTCCCATCTTTCT- and 3'reverse 5'GATGTGGCCATCACATT-CGTCAGA T 3'. To avoid mistyping between ID and DD, an I- specific primer pair, 5'- TGGGACCACAGCGCCCGCCAC-TAC-3' and 5'- TCGCCAGCCCTCCCATGCCCA-TAA-3' were also used to analyze all samples showing DD genotype. These primers (10 pmol of each) were added to a mixture containing 5ml of 10X Cetus buffer (pH 8.3), 0.5mM dNTP (dATP, dCTP, dGTP, dTTP), and 1.0 units of Taq DNA polymerase (Perkin Elmer Cetus). The PCR P Program (Perkin Elmer 9600 Thermal Cycler) was initiated in a final total volume of 50 ml.

Thirty PCR amplification cycles consisting of volume with thirty cycles, each made up of denaturation for 1 minute at 94°C, annealing for 1 minute at 58°C and primer extension for 1 minute at 72°C was applied for amplification. PCR products of ACE gene locus were examined by agarose gel electrophoresis (3% agarose) at 150 V for 60 minutes and visualized at room temparature under UV after ethidium bromide staining. The PCR view of ACE gene I/D polymorphism was shown in Figure 1.

Statistical Analysis

Statistical analysis was performed using the SPSS-15.0 for windows. Allelic frequencies were calculated by the gene-counting method. A chi-square test

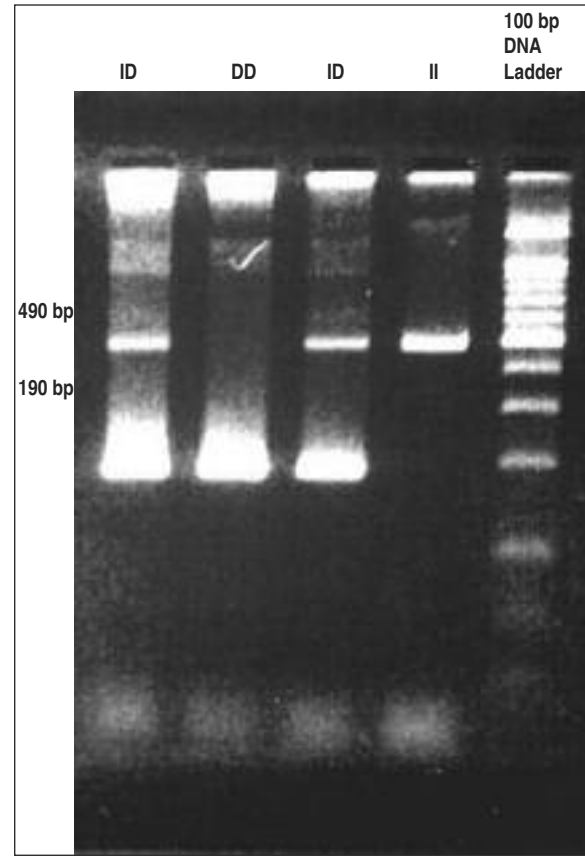

FIGURE 1: The PCR view of ACE gene I/D polymorphism.

was used to test the expected type frequencies. The genotype/allele frequencies in patients were compared with those in the control subjects using the chi-square test. Data were expressed as mean ± SD and count and percentage. For all analyses, a p value of <0.05 was considered statistically significant.

RESULTS

DEMOGRAPHICS

Ninety eight children who had been diagnosed with sepsis were enrolled, with an age range of 1.5-180 months; 70 (68%) were males and 28 (32%) were females.

In 26 patients, sepsis was secondary to bacteremia, the primary focus of which could not be found, in three patients sepsis was secondary to infective endocarditis; in 46 patients sepsis was secondary to pneumonia; in eight it was secondary to an infection of the central nervous system; in three it was secondary to wound infection; in 10 it was secondary to a urinary tract infection; and in two sepsis had originated from a gastrointestinal focus (Table 1).

The patients were divided into groups based on the presence of acute respiratory distress syndrome (ARDS), multiorgan dysfunction syndrome (MODS) and mortality.

GENOTYPING FREQUENCIES OF ACE I/D POLYMORPHISM

The distribution of the ACE genotype in the control group was in the order of I/I: 11; I/D: 70; and D/D: 19 and in the patient group, it was I/I: 17; I/D: 68; D/D: 13. There was no statistical difference between the control and the patient groups (p= 0.29) (Table 2). Thirty-seven (38%) patients had ARDS, and 51 (52%) had MODS. Fifty-four (55%) patients died.

TABLE 1: Primary focus of the sepsis.

Primary focus	n	%
Bacteremia primary focus was not found	26	27
Pneumonia	46	47
Central nervous system	8	8
Infective endocarditis	3	3
Wound infection	3	3
Urinary tract infection	10	10
Gastrointestinal tract	2	2

TABLE 2: Genotypic frequency of ACE I/D polymorphism between control and patients.

Genotypes	Patients		Control	
	(n)	(%)	(n)	(%)
I/I	17	17.4	11	11
I/D	68	69.4	70	70
D/D	13	13.2	19	19

The genotype distributions of all patient groups are displayed in Table 3.

Thirty-seven (37.8%) patients had ARDS. Of these patients, five (13.5%) were of I/I genotype, 23 (62.2%) were of I/D genotype, and nine (24.3%) were of D/D genotype. A statistical difference was found for genotype distribution in patients with or without ARDS (p= 0.04). Having a D/D genotype increased the risk for ARDS 4.5-fold (%95 CI 1.15-19.6, p= 0.028) (Table 4).

Of the total, 17 individuals had the I/I genotype, of whom six (35.3%) had MODS; 68 individuals had the I/D genotype, of whom 35 (51.4%) had MODS; 13 individuals had the D/D genotype, of whom 10 (76.9%) had MODS. Besides, there was no significant difference in the genotyping distributions between patients who had or did not have MODS (p= 0.076) (Table 5).

Fifty four (55.1%) patients died (exitus). Of these patients, 12 (22.2%) were of I/I genotype, 38 (70.4%) were of I/D genotype, and 4 (7.4%) were of D/D genotype. A statistical difference was not found for genotype distribution in patients with or without mortality (p= 0.092). (Table 6).

DISCUSSION

Mortality in sepsis is related to many factors, such as presence of shock, ARDS or MODS, leading to a poor prognosis. It is very difficult to hypothesize in advance which patient will suffer from ARDS or MODS, and the response to infection is variable between different individuals. This variability has been attributed to a number of factors including the virulence or load of the etiological agent, or the length of time between the onset of symptoms and

TABLE 3: Genotypic distribution of ACE I/D polymorphism in clinical features.

Groups	I/I		I/D		D/D		Total	p
	n	%	n	%	n	%	n	
MODS	6	(11.8)	35	(68.6)	10	(19.6)	51	0.076
ARDS	5	(13.5)	23	(62.2)	9	(24.3)	37	0.040
Mortality (non survivor)	12	(22.2)	38	(70.4)	4	(7.4)	54	0.092

TABLE 4: Genotypic frequency of ACE I/D polymorphism in patients who had or had not developed ARDS.

	ARDS	Non-ARDS
	n= 37	n= 61
	Genotypic frequency	
D/D	9 (24.3%)	4 (6.5%)
I/D	23 (62.2%)	45 (73.8%)
I/I	5 (13.5%)	12 (19.7%)
	p= 0.040	

TABLE 5: Genotypic frequency of ACE I/D polymorphism in patients who had or had not developed MODS.

	MODS	Non-MODS
	n= 51	n= 47
	Genotypic frequency	
D/D	10 (19.6%)	3 (6.3%)
I/D	35 (68.7%)	33 (70.3%)
I/I	6 (11.7%)	11 (23.4%)
	p= 0.076	

TABLE 6: Genotypic frequency of ACE I/D polymorphism in patients who died or survived.

	Non survivor	Survivor
	n= 54	n= 44
	Genotypic frequency	
D/D	4 (7.4%)	9 (20.5%)
I/D	38 (70.4%)	30(68.2%)
I/I	12 (22.2%)	5 (11.3%)
	p= 0.092	

initiation of treatment. The renin angiotensin system (RAS) plays a complex role in the pathophysiology of sepsis. In tissues, ACE is expressed by activated macrophages and T lymphocytes.[17] These tissue-derived sources ACE have been shown to upregulate the inflammatory response. Serum ACE activity has been shown to be decreased in vivo in sepsis. It may reflect endothelial cell damage or inhibition of ACE activity by bacterial mediators such as lipopolysaccharide.[18] Because of the important role ACE plays in the inflammatory response, inter-individual genetic variations in ACE activity may alter the outcome in severe infections. A role for genetic variation in ACE activity in both acute and chronic lung disease has recently been suggested.[19-22] Higher intrinsic ACE activity (D/D genotype) is associated with an increased risk of developing ARDS and other lung diseases.[19-22]

Recently, the involvement of angiotensin II in the pathogenesis and evolution of acute lung injury has also gained substantial interest. Activation of the renin angiotensin system can stimulate production of tumor necrosis factor in cardiac fibroblasts.[23] Angiotensin II is an inducer of apoptosis of endothelial cells,[24] and of alveolar epithelial cells.[25,26] Angiotensin II is also considered as a strong fibrogenic factor.[27,28] In vitro, angiotensin II was a potent inducer of procollagen production in human lung fibroblasts via activation of the type 1 receptor and, TGF-beta.[28] Fourrier et al. showed that in patients with ARDS, the circulating ACE was often reduced.[29] Recently, Marshall et al. showed that the ACE I/D polymorphism was associated with susceptibility and outcome in ARDS.[30] The findings here may confirm the effect of ACE polymorphism on the outcome of ARDS. On the other hand, Chan et al. reported that ACE I/D polymorphism was not directly related to increased susceptibility to SARS coronavirus infection, and that it was not associated with poor outcomes after SARS coronavirus infection.[31] Similarly, in our septic patient population, the D/D genotype was found to be a risk factor, and possession of the D/D genotype was found to increase the risk for ARDS 4.5-fold. However, in a study performed in our country, the I/I genotype was considered to be a risk factor for pulmonary disorders in Turkish neonates as the I/I variant was more frequent in neonates with respiratory distress than in the healthy newborns. The ACE I/I genotype is associated with an incre-

ased risk of respiratory disorders among Turkish premature infants, and the D/D genotype is a protective factor for respiratory disorders.[9]

However, the D/D genotype has not been shown to have an effect on mortality in our study. There are no studies in the literature on the direct relationship between sepsis mortality and ACE gene polymorphism. However, a few studies may pave the way as guiding principles. Baier et al. reported that the ACE I/D polymorphism did not have a significant effect on the incidence or outcome of sepsis in ventilated very low birth weight infants.[12] Some studies show that ACE D/D is associated with increased illness severity in diseases such as meningococcemia.[8] Recently a study showed that ACE I/D polymorphism was not associated with severe sepsis susceptibility, mortality or ARDS in Spanish patients.[32] In our study, the D/D genotype was found to increase the risk for ARDS but not directly influenced MODS and mortality. This makes us consider that the blood ACE levels may play different roles in the different stages of sepsis.

We conclude that the D/D genotype may lead to a higher incidence of sepsis-related ARDS, but it has no effect on the incidence of mortality and MODS. In order to support these results, more studies evaluating higher numbers of cases are needed.

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