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Evaluation of the rs35996865 polymorphism of the *ROCK1* gene in sepsis

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SUMMARY

OBJECTIVE: Sepsis is a complex and serious medical condition resulting from the activation of an innate host response to infections. The etiology of sepsis is complex and can be influenced by genetic susceptibility. The purpose of the present study was to investigate a possible association of Rho-kinase 1 (*ROCK1)* gene polymorphism with sepsis in a Turkish population.

METHODS: The study group consisted of 100 unrelated patients with sepsis and 100 healthy controls. Genomic DNA was isolated from peripheral leukocytes from EDTA-containing blood using the QIAamp DNA Blood Mini Kit. *ROCK1* gene rs35996865 and rs112130712 (Lys1054Arg) polymorphisms were analyzed in genomic DNA using the LightCycler 480 II real-time polymerase chain reaction.

RESULTS: There were no significant differences in allele and genotype frequencies for *ROCK1* gene rs35996865 polymorphism between the patients with sepsis and control group ($p>0.05$). Additionally, no association was detected between the rs35996865 polymorphism and mortality in the patient group. No polymorphism was detected with *ROCK1* gene rs112130712 (Lys1054Arg) in our study groups.

CONCLUSIONS: Our data showed that there is no marked association between the rs35996865 polymorphism and sepsis. Therefore, these results suggest that *ROCK1* gene rs35996865 polymorphism is not risk factor for the development of sepsis in the Turkish population.

KEYWORDS: ROCK-I protein kinase. Single nucleotide polymorphism. Mortality. Sepsis.

INTRODUCTION

Sepsis is an extremely complex illness with life-threatening organ dysfunction caused by endogenous mediators in response to infection and is one of the leading cause of mortality worldwide[1]. Septic shock and sepsis are widespread medical emergencies and closely linked with an increased rate of mortality, morbidity, and expensive treatment costs[2,3]. Sepsis is also of great challenges in critical care medicine in the intensive care unit (ICU), where it affects approximately 30% of patients, with high variations between different geographical regions[4]. The estimated worldwide incidence of sepsis admissions has been reported to be 31.5 million cases per year, leading to 5.3 million deaths[5]. The prevalence of sepsis, severe sepsis, and septic shock in ICU in Turkey is reported to be 10.9%, 17.3%, and 13.5%, respectively[6]. Despite significant progress having been made in sepsis management in recent decades, sepsis-related mortality remains as high as 30–50%[2,7]. It is the common cause of death in hospitalized patients and associated with long-term disability in survivors[8]. Sepsis is a multifaceted disease, and its management is complex. There are no drugs approved particularly for the treatment of sepsis, and no definitive therapies present to cure this disease. The treatment of sepsis is mainly supportive in nature, involving the administration of antibiotics, vasoactive substances, intravenous fluids, and oxygen. Genetic epidemiologic studies imply that there is a strong genetic influence on the progression and mortality from sepsis[9-12].

Rho-kinase (ROCK) is a serine/threonine kinase regulated by the small GTPase Rho proteins. It has two isoforms, namely, ROCK1 and ROCK2[13]. While ROCK1 isoform is encoded by 18q11, ROCK2 isoform is located in 2p24 on human chromosomes. Rho/ROCK signaling pathway is involved in regulating various important cellular functions, such as cell migration,

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cytoskeleton structure, proliferation, apoptosis, and oxidative stress[13]. Experimental studies suggest that ROCK activity regulates sepsis-induced systemic inflammation and organ injury[14,15]. We hypothesize that *ROCK* gene polymorphisms play a role in the risk of sepsis development. Therefore, the goal of the present study was to assess a possible association between *ROCK1* gene polymorphisms and sepsis in a Turkish population.

METHODS

Study design and patients

A total of 200 individuals, including 100 sepsis patients and 100 healthy volunteers, admitted to the Erciyes University Department of Emergency Medicine were enrolled to this study. This research was approved by the Clinical Research Ethics Committee of the Erciyes University (decision no: 2019/581). The patients' relatives and healthy volunteers were asked to give written informed consent for the study procedures prior to participation in the study. This study was conducted in accordance with the Declaration of Helsinki. All genetic analyses were carried out in the Erciyes University Genome and Stem Cell Center (GENKOK).

All consecutive patients consist of those considered to have septic conditions following consultations, investigations, and interventions pursued in the critical care area of the emergency department and decided to be admitted to the ICU or wards. Quick sequential organ failure assessment (qSOFA) score, based on three criteria describing cardiovascular, neurologic, and respiratory dysfunction, was used clinically for screening to identify patients at increased risk for sepsis[16]. The venous blood samples were collected prior to drug administration. Laboratory and clinical parameters elicited in the emergency department including oxygen saturation, arterial blood gases, mean arterial pressure, pulse rate, complete blood count and other biochemical variables, imaging data, and cultures were recorded and analyzed in the routine evaluation as necessary. Data on the clinical courses, diagnoses, mode of disposition, and mortality were abstracted from hospital information system, patients' electronic reports, and patients' relatives and next of kin. Patients who had known or apparent systemic diseases such as heart failure, terminal-stage malignancies, chronic pulmonary, renal or liver diseases, pregnancy, or breastfeeding were excluded. Age range of the study population was set at 18 and 90 years. Control group composed of the healthy, gender- and age-matched volunteers who had no history of medical illness and/or recent surgery, or a diagnosis of genetic, neurologic, psychiatric, liver, infectious, or chronic inflammatory disease. The volunteers for healthy control group were selected from hospital staff and their families.

DNA isolation and genotyping

Venous blood samples (2 mL) were drawn into EDTA-containing tubes from all individuals and were transferred to GENKOK Genome Unit of the Erciyes University. Genomic DNA was extracted from peripheral leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Germany), according to instructions of the manufacturer. The samples were stored at -20°C until analyses for each polymorphism. The final DNA concentration was assessed with a micro-volume UV-vis spectrophotometer (BioSpec-nano, Shimadzu, Kyoto, Japan)[11].

To identify *ROCK1* gene rs35996865 and rs112130712 (Lys1054Arg) polymorphisms, genotyping was done using commercially synthesized primers and fluorescently labeled probes and the LightCycler 480 II real-time polymerase chain reaction (PCR) system (Roche Diagnostics GmbH, Mannheim, Germany). Gene polymorphism was determined by analyzing the detailed melting curve of the PCR product obtained.

Statistical analysis

Continuous variable are represented as mean±standard deviation (SD), and categorical variables are expressed as frequencies and percentages. Normal distribution of numerical variables was analyzed with Kolmogorov-Smirnov normality test. Unpaired Student's t-test was used for the comparison of the groups for normally distributed data. Mann-Whitney U test was used for data with the abnormal distribution. Categorical data were analyzed with chi-square test with Yate's correction. The chi-square test was also used to examine deviations from Hardy-Weinberg Equilibrium by comparing the observed and expected genotype frequencies. Differences in allele and genotype frequencies among the controls and cases were analyzed by chi-square with Yate's correction or Fisher's exact tests. Statistical analysis was performed by using Graph-Pad Instat version 3.05 (GraphPad Software Inc., San Diego, CA, USA), and the level of significance was set at $p<0.05$.

RESULTS

A total of 100 patients with sepsis and 100 healthy volunteers were recruited into this case-control study. Table 1 shows the demographic, clinical, and laboratory characteristics of the study population. Sepsis was identified on the basis of microbiological blood culture results. Out of 100 sepsis patients, 43 suffered from Gram negative, 28 from Gram positive, 8 from fungal, and 21 patients displayed a mixed pattern of infections. Compared with

Table 1. Demographic, clinical, and laboratory characteristics of the study cases.

Variables	Patients with sepsis (n=100)	Controls (n=100)	p
Age (years)*	65.1±13.1	62.8±11.5	0.1941
Gender, n (%)			
Male	58 (58.0)	60 (60.0)	0.8857
Female	42 (42.0)	40 (40.0)	
MAP (mmHg)*	66.8±12.2	95.5±10.9	<0.0001
Pulse rate (beats/min)*	98.9±16.9	91.8±17.3	0.0037
Respiratory rate (beats/min)*	28.0±5.9	18.2±9.1	<0.0001
Temperature (°C)*	37.8±1.2	36.2±1.9	<0.0001
qSOFA score†	2.2 (2–3)	-	
GCS score†	13.7 (3–15)	-	
Lactate (mmol/L)*	2.6±2.4	1.3±0.7	<0.0001
SaO_2 (%)*	88.9±8.7	-	
Hemoglobin (g/dL)*	11.8±2.5	12.9±1.9	0.0005
WBC (/μL)*	12777.8±8865.9	9250.0±3704.2	0.0003
Platelet (10^3/μL)*	232.3±148.3	274.3±69.9	<0.0001
Neutrophils (10^3/μL)*	10528.3±8296.5	7106.2±3274.3	0.0002
INR*	1.5±1.1	-	
Glucose (mg/dL)*	171.9±93.8	105.9±20.1	<0.0001
Creatinine (mg/dL)*	1.9±1.7	0.9±0.5	<0.0001
CKD-EPI*	54.2±34.5	-	
AST (U/L)*	33.0±29.3	23.7±9.8	0.0546
ALT (U/L)*	24.1±23.3	21.6±8.8	0.0234
Total bilirubin (mg/dL)*	1.3±1.1	0.6±0.5	<0.0001
Uric acid (mg/dL)*	6.8±3.6	6.4±2.5	0.6972
BUN (mg/dL)*	34.8±25.1	16.1±5.9	<0.0001
D-Dimer (μg/L)*	4395.7±3968.1	-	
C-reactive protein (mg/L)*	146.1±119.6	1.15±1.12	<0.0001
Procalcitonin (ng/mL)*	3.1±4.1	-	

*Data are mean±SD; †Scores are given as median (min–max); MAP: mean arterial pressure; GCS: Glasgow Coma Scale; qSOFA: quick sequential organ failure assessment; SaO_2: oxygen saturation; INR: International Normalized Ratio; WBC: white blood cells; CKD-EPI: chronic kidney disease-epidemiology; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen.

the controls, the average age, gender, aspartate aminotransferase, and uric acid in sepsis group were similar. Pulse rate, respiratory rate, body temperature, lactate, white blood cell and neutrophil counts, glucose, creatinine, alanine aminotransferase, total bilirubin, blood urea nitrogen, and C-reactive protein levels were found to be elevated in the sepsis group when compared to the control. We recorded decreases in mean arterial blood pressure, hemoglobin levels, and platelet counts (Table 1).

Both the control (p=0.9923) and patients (p=0.8713) groups were found to be in Hardy-Weinberg Equilibrium. For the *ROCK1* gene rs35996865 polymorphism, no marked differences in both genotype (T/T, 53.0%; T/G, 41.0%; G/G, 6.0%) and allele (T, 73.5%; G, 26.5%) frequencies in the sepsis group were detected when compared to controls (T/T, 63.0%; T/G, 33.0%; G/G, 4.0%; T, 79.5%; G, 20.5%, p>0.05) (Table 2). Mortality distribution according to genotype frequencies of *ROCK1* gene rs35996865 polymorphism in patient group was also examined at the end of 3 months of hospital admission, but no significant change between survival (T/T, 64.2%; T/G, 70.7%; G/G, 50.0%) and exitus rate (T/T, 35.8%; T/G, 29.3%; G/G, 50.0%, p>0.05) was determined (Table 3).

We have also studied *ROCK1* gene rs112130712 (Lys1054Arg) polymorphism, but no polymorphism was found in both patient and control groups, i.e., only T/T genotype was detected.

Table 2. Genotype and allele frequencies of *ROCK1* gene rs35996865 polymorphism among cases and controls.

Genotypes/alleles	Patients (n=100), n (%)	Controls (n=100), n (%)	p	OR (95%CI)
T/T	53 (53.0)	63 (63.0)		
T/G	41 (41.0)	33 (33.0)	0.2471	0.6771 (0.377–1.217)
G/G	6 (6.0)	4 (4.0)	0.5133	0.5608 (0.150–2.094)
T	147 (73.5)	159 (79.5)		
G	53 (26.5)	41 (20.5)	0.1946	0.7152 (0.449–1.139)

Table 3. Mortality distribution according to genotype frequencies of *ROCK1* gene rs35996865 polymorphism among patients.

Genotypes/alleles	Survive, n (%)	Exitus, n (%)	p	OR (95%CI)
T/T (n=53)	34 (64.2)	19 (35.8)		
T/G (n=41)	29 (70.7)	12 (29.3)	0.6514	0.7405 (0.308–1.779)
G/G (n=6)	3 (50.0)	3 (50.0)	0.6614	1.7890 (0.328–9.760)

DISCUSSION

In this case-control study, we showed no significant association between sepsis and *ROCK1* gene rs35996865 polymorphism, and no significant relationship between the rs35996865 polymorphism and mortality in our Turkish population. To the best of our knowledge, this is the first study to investigate the association of the *ROCK1* gene polymorphism with the risk of developing sepsis. Our data indicate that rs35996865 polymorphism is unlikely to play a role in the sepsis development.

The rs35996865 polymorphism located in the *ROCK1* promoter region, about 2 kb upstream of the transcription start site. However, it is not known whether this polymorphism is able to alter the expression level of the *ROCK1* gene[17]. There are only small numbers of studies related to this polymorphism. The *ROCK1* gene rs35996865 polymorphism mapping to the 5′-UTR has been reported to be significantly associated with colorectal cancer[18,19], obesity-related metabolic syndrome[20], renal cell carcinoma[21], respiratory distress syndrome[22], non-syndromic cleft palate[17], and systemic sclerosis[23], but not with Behçet's disease[24] or Alzheimer's disease[25]. Although no association of this polymorphism was noted with primary open-angle glaucoma in a Turkish population[26], this variant is nominally associated with risk of high-tension glaucoma in a Korean population[27].

Experimental studies showed that ROCK activity regulates sepsis-induced systemic inflammation[14,28]. ROCK inhibitors have been shown to exert beneficial effects in models of sepsis as well as endotoxemic injury in the liver[14,28,29]. It has been shown that specific ROCK inhibitor Y-27632 reduces lung injury from septic rats induced by cecal ligation and puncture[30]. Fasudil, another ROCK inhibitor, improves endothelial permeability and inhibits inflammation, oxidative stress, and cellular apoptosis in order to alleviate acute lung injury in septic rats[15]. Taken together, these studies suggest that ROCK is involved in sepsis-induced organ injury.

CONCLUSIONS

This study indicated that *ROCK* gene rs35996865 polymorphism is not associated with sepsis or sepsis-induced mortality in the Turkish population. Thus, this variant may not influence the risk of sepsis. However, analysis of other polymorphisms in this gene for association with sepsis would be helpful in clarifying the involvement of the *ROCK* gene in sepsis pathogenesis. Future genetic expression analysis and studies in larger populations are needed to elucidate the role of the *ROCK* gene in sepsis susceptibility. This is a pilot study with a lower sample size. We believe that further investigations are also needed to verify these results in different ethnic and independent groups.

AUTHORS' CONTRIBUTIONS

AK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **NEG:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **SD:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **EFŞ:** Data curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing. **RT:** Data curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing. **NG:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **ATD:** Formal Analysis, Visualization, Writing – original draft.

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