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A role of the endothelial nitric oxide system in acute renal colic caused by ureteral stone☆

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ABSTRACT

Background and aims: Endothelial nitric oxide synthase gene polymorphisms play a role in some pathophysiological processes. In this study, the possible effects of endothelial nitric oxide synthase gene polymorphisms on ureteral stone disease in patients who were admitted to the emergency department with severe pain due to renal colic are examined.
Materials and methods: The study groups were designed as controls and patients. The control group was formed from the healthy volunteers who applied to the blood center next to the emergency service. The patient group comprised patients who were diagnosed with ureteral stone disease with severe pain. All of the genetic studies were based on extracted peripheral blood samples using the necessary procedures from the Genome and Stem Cell Center at Erciyes University (GENKOK). The data were analyzed with SPSS (IBM, ver 20, United Sate).
Results: The study group comprised 62 females and 138 males, and the control group comprised 64 females and 136 males. All of the stones that caused renal colic were found to be localized in the ureters and the ureterovesical junction. The genotypes of the intron 4 polymorphism were found to be as follows: 4a/4a in 10 people, 4b/4a in 115, and 4b/4b in 275 people. The GG genotype of the *eNOS*-G894T polymorphism was found in 108 patients in the study group and in117 of the healthy individuals. There was no statistically significant difference between the two groups regarding these data.
Conclusion: Although this study is the first in the literature to examine the relationship between renal colic and endothelial nitric oxide synthase gene polymorphisms, our study demonstrated that no relation was found.

1. Introduction

The severe pain of renal colic (RC) caused by urinary stone disease has an occurrence rate of 1–10% for each person per their lives and accounts for 2% of all admittances to the emergency department [1–3]. The stone passing into the ureter produces high intraluminal pressure, which may cause contractions of the smooth muscles of the ureter and induce irritation and obstruction, which hereby produces spasms that lead to unbearable pain [4,5].

Nitric oxide (NO), which is synthesized from L-arginine, has a role in the physiopathology of RC. Because axons and neuronal endings that are positive for neuronal nitric oxide synthase (nNOS) reside in the human ureter, NOS, which participates in the synthesis of NO may be related to RC [6]. The hypothetical relationship between NO and RC is expressed in the hypothesis that NO can cause the progression of a stone to be easier and stiffer with adilatation effect on the ureter where the smooth muscle is located. The possible relationship between RC and endothelial NOS (eNOS), which is a different isoenzyme that is involved in the synthesis of NO that functions in smooth muscle organs such as the ureter, has been left mostly unexplored to this day. Endothelial NOS, which is coded for by a gene that is located on the long arm of the 7th chromosome, has a role in the synthesis of NO, which relaxes the smooth muscles of the gastrointestinal and cardiovascular systems and acts in other pathophysiological processes [7,8]. The few clinical studies of RC and NO

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in the literature have mostly focused on the medical treatment of pain caused by RC [9]. However, there are no studies of the possible role of the eNOS isoenzyme in severe pain. We believe that analyzing the role of eNOS, which has a well-known role in inflammation [10], in RC processes will facilitate a better understanding of the pathophysiology of RC. In recent years, many polymorphisms and several mutations located in the exons, introns and promoter regions of the gene that codes the eNOS enzyme have been described. These mutations are known to cause some structural and functional changes in gene expression and the eNOS enzyme and therefore lead to changes in NO levels These *eNOS* gene polymorphisms have roles in pathophysiological processes, such as essential hypertension, coronary heart disease, polycystic kidney disease, and cerebrovascular disease [11,12].

In this study, the possible effects of *eNOS* gene polymorphisms in patients who were admitted to the emergency department with severe pain due to RC were examined.

2. Materials and methods

2.1. Patient selection and assessment of the study groups

Two groups were formed to conduct the study. First, the patient group consisted of patients who were diagnosed with RC in the emergency department. Second, the control group was established from healthy breeders and blood donors from a blood center associated with the emergency department. This study was conducted with the collaboration of the Department of Emergency Medicine and the Department of Medical Biology of Erciyes University Medical Faculty. The study was approved by the local ethical committee (date May 9th, 2014. No: 2014/286) and supported by a project (TTU-2015-5634) from the Scientific Research Projects Department of Erciyes University, Kayseri, Turkey. The main inclusion criterion was an age over 18 years and a proven diagnosis of RC. Patients under the age of 18 were not included in the study, mainly because the pediatric population, other than the trauma patients, is not referred to our emergency department. RC patients with systemic diseases, chronic use of medication, and acute renal failure were excluded. Moreover, pregnancy and evident infection were other reasons for exclusion. All of the RC patients were treated for their RC and underlying pathology by an attending physician in emergency medicine. Moreover, a senior resident in emergency medicine informed the subjects and obtained their verbal and written consent. All patients admitted to the emergency department with complaints that were possibly related to RC, such as pain radiating from the groin, testicular or vaginal complaints without side pain, nausea/vomiting accompanied by colic pain, or complaints related to the urinary tract, were evaluated for inclusion [13]. After physical examinations, this population was examined with basic urine testing, plain radiograms of the urinary systems, and ultrasonography. RC was diagnosed according to the presence of a stone on ultrasonographic testing with or without pelvicalyceal ectasia and the presence of no other signs that caused suspicion of different pathologies [14]. Even in cases in which a stone was observed on a plain radiogram, all patients were further tested with ultrasonography because we use plain radiograms only for differential diagnosis. Computed tomography was used in cases with suspected aortic pathologies and in female patients with obstetric/gynecologic pathologies if ultrasonography produced unsatisfactory results [15]. This algorithm for the diagnosis of RC is the routine workup in our emergency department, and no new laboratory or radiological testing was applied. Age, sex, medical history, family history, complaints secondary to pain, and the presence of fever, blood pressure, cardiac pulse, and respiratory rate abnormalities of the RC patients were recorded. Additionally, serum levels based on laboratory testing of these patients were kept in the patients' charts, and the names, dosages, and administration routes of drugs used to treat RC were recorded. Of these criteria, kidney transplantation, postrenal acute kidney failure and severe infections were reasons for the exclusion of patient from present study. However, their treatment was continued in accordance with the most recent guidelines. The same procedures were followed while obtaining samples from the control group. Subjects with histories of major trauma or operations, systemic disease and the chronic use of medications were excluded.

2.2. Sample preparation and genotyping studies of the eNOS gene polymorphisms

All of the genetic studies were performed at the Genome and Stem Cell Center at Erciyes University (GENKOK). Two-milliliter blood samples with EDTA were obtained from each patient and control. Genomic DNA was extracted from the peripheral blood samples using the standard procedures of High Pure PCR Template Preparation Kit (Roche, Germany). The final DNA concentrations were determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific). Amplifications of the *eNOS* intron 4 polymorphism (rs61722009) were performed using standard polymerase chain reaction (PCR). The PCR products were visualized on a 2% agarose gel stained with ethidium bromide [16]. The *eNOS*Glu298Asp (894G>T, rs1799983) polymorphism was identified using a PCR-restriction fragment length polymorphism (RFLP)-based protocol. The PCR products were checked on 2.5% agarose gel, and the 206-bp *eNOS* product was digested overnight at 37 °C with *Mbo I* (Thermo Scientific, USA) restriction endonuclease enzyme [17,18]. The genotyping of the *eNOS*-786 T/C (rs2070744) polymorphism was determined by the PCR-RFLP method using the *Msp I* restriction enzyme at 37 °C for 16 h (Thermo Scientific, USA). The restriction fragments were separated by electrophoresis on a gel composed of 3% agarose. All of the PCR methods are summarized in Table 1.

2.3. Statistical analysis

The data obtained in our study were analyzed with SPSS (IBM, ver 20, United State). Categorical variables were tested with the chi-square test. Numerical variables were analyzed with the Shapiro-Wilk's test. Multiple comparisons were performed with ANOVA analysis. The strengths of the relationships between the numerical variables were tested with Pearson correlation analyses. Statistical significance was accepted at $p < 0.05$.

Table 1
Primer sequences and PCR Program for genotyping *eNOS* gene polymorphisms.

eNOS gene polymorphisms	PCR primers	Annealing TEMPERATURE (°C)	PCR product (bp)	Restriction enzyme
Intron 4 (4a/4b) (rs61722009)	F: 5′- AGGCCCTATGGTAGTGCCTT-3′ R: 5′- TCTCTTAGTGCTGTGGTCAC-3′	56	420	–
894G>T (rs1799983)	F: 5′- CATGAGGCTCAGCCCCAGAAC- 3′ R: 5′- AGTCAATCCCTTTGGTGCTCAC- 3′	60	*206*	*MboI*
−786 T>C (rs2070744)	F: 5′- AGGCCCTATGGTAGTGCCTT-3′ R: 5′- TCTCTTAGTGCTGTGGTCAC-3′	60	*180*	*Msp I*

F: Forward; R: Reverse; bp: base pairs.

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