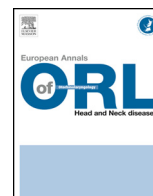




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Original article

The endothelial nitric oxide synthase (eNOS) polymorphism in otitis media with effusion (OME)

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ARTICLE INFO

Keywords:

Otitis media with effusion
eNOS polymorphism
Serous otitis
Turkey

ABSTRACT

Objectives: Otitis media with effusion (OME) is the most common disease after viral infections of upper respiratory tract (URTI) in children. Studies indicate the important role of nitric oxide (NO) in the etiology of hearing loss. However, there is no study that focuses on the role of nitric oxide synthase (eNOS) polymorphisms in the cases with OME. The aim of the present study is to evaluate the eNOS polymorphisms in the pediatric patients with OME.

Materials and methods: Eighty-nine patients who are diagnosed with otitis media with effusion and 85 healthy subjects who are compatible in terms of age and gender were included in the study. All patients in the study were subjected to complete ear, nose, throat (ENT) and audiological examinations. DNA analysis was performed with polymerase chain reaction (PCR) technique from the blood samples. The PCR product was cut by restriction fragment length polymorphism (RFLP) with BanII enzyme and checked by agarose gel electrophoresis.

Results: As a result of genetic analysis, there is no significant difference between patients and the controls in terms of eNOS Glu298Asp polymorphism (G/G, G/T, T/T). When these groups were compared in terms of allele distributions, a significant relationship was found between the patients and the controls ($P=0.037$).

Conclusion: To the best of our knowledge, G allele was identified as predisposing to the development of OME and this is the first report indicates the correlation between the eNOS G894T polymorphism and OME in Turkey.

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1. Introduction

Otitis media (OM) is a disease frequently occurring after viral infections (URTI) of upper respiratory tract in children [1]. Also, it was reported that the preschool children have at least one OM episode in a range of 35% to 70% [2]. Otitis media with effusion [3] is an inflammatory disease characterized by the accumulation of fluid in the middle ear space [4]. The fluid can be hemorrhagic, purulent, mucoid and/or in a combination of these. However, the pathophysiology of OME is not fully clear. Increase in the number of goblet cells in the middle ear is claimed as a common pathophysiological change in OME [5]. Also the increase in the partial pressure of carbon dioxide and negative pressure of ear due to lack of middle ear ventilation are claimed as another main

pathophysiological changes in OME [5]. An increase in a variety of inflammatory mediators and enzymes (histamine, prostaglandins, and platelet activating factor (PAF), proteases, hydrolytic enzymes, leukotrienes, kinins, tumor necrosis factor and gamma interferon) were observed in middle ear of OME patients. This increase is thought to be effective in the pathophysiology of the disease. The PAF which is one of the inflammatory mediators increases in OME. Previous studies showed that the overexpression of PAF has neurotoxic effects on the epithelial cells of middle ear and cochlear [6]. Furthermore, recent animal studies reported that PAF increases the secretion of nitric oxide synthase (eNOS), leading to nitric oxide (NO) release [5]. Rhee reported that PAF causes hearing loss by disturbing the cochlea through NO [5]. There are three different types of NOS isoenzyme: neuronal NOS (nNOS or NOS-I), inducible NOS (iNOS or NOS-II), and endothelial NOS (eNOS or NOS-III) [7]. eNOS is located at the end of the long arm of chromosome 7 (7q35-36). eNOS includes some single nucleotide polymorphism, among them is Glu298Asp polymorphism at codon 298 [8].

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In the literature, there are studies indicating the relationship between NO and various types of ear pathology. Recent studies reported that Glu298Asp polymorphism alters the eNOS enzyme activity and is associated with reduced basal NO production [9]. However, there is no study that highlights the role of eNOS polymorphisms in the cases with OME in Turkey. Accordingly, the aim of the present study is to assess the eNOS polymorphisms in the pediatric patients with OME.

2. Material and methods

2.1. Study population and inclusion criteria

The present study was approved by the local human ethics committee of Mustafa Kemal University, 28/05/2013, n° 2013/102. A power analysis was performed for the determination of appropriate sample size. A total of 89 patients – 45 females and 44 males – who were admitted to Otorhinolaryngology Clinic of Mustafa Kemal University Faculty of Medicine and a total of 85 patients – 51 males and 34 females – who are appropriate in terms of sex and age were included in the study.

All patients in the study were subjected to complete ENT and audiological examinations. Three-milliliter blood samples of the patients and the healthy volunteers were collected into tubes including EDTA.

2.2. DNA extraction and the detection of genetic variants

The blood samples collected for genotypic determination were stored in 4 °C for DNA extraction. Genomic DNA was isolated from whole blood samples using DNA isolation procedure [10]. The detection of the Glu298Asp (894G > T) polymorphism (rs1799983) in the eNOS was achieved by PCR-RFLP. Specific primer pairs were used to amplify a part of the eNOS containing the following flanking intronic primers: (sense) 5-AAGGCAGGAGACAGTGGATGGA-3 and (antisense) 5-CCCAGTCAATCCCTTGGTGCTCA-3. The PCR amplification was performed for 100 ng genomic DNA in 25 µL reaction mixture containing; 2.5 µL 10 X PCR buffer (Fermantas), 200 µM dNTP (Fermantas), 10 pM each of primers, and 5 Unit (U) of Taq DNA polymerase (Fermantas). The PCR procedure set as follows; denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 60 s, repeated for 30 cycles. The PCR product (248 bp) was digested Ban II restriction endonuclease enzyme and was cleaved into two smaller fragments of 163 and 85 bp. Digestion fragments were evaluated by electrophoresis in a 2.0% agarose gel stained with ethidium bromide. The genotypes were assessed as Glu/Glu [11] homozygote genotype (163 and 85 bp), Glu/Asp (GT) heterozygote genotype (248, 163 and 85 bp), Asp/Asp (TT) homozygote genotype (248 bp).

2.3. Statistical analyses

For the statistical analysis, SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) program was used. Chi² test was used to compare the rates between groups. Normal distributions of continuous variables were tested with the Kolmogorov–Smirnov test. For the comparisons of gene polymorphisms, Kruskal–Wallis test and Mann–Whitney U tests were used. $P < 0.05$ was considered statistically significant for statistical analysis.

3. Results

As a result of the genetic analysis, the distribution of eNOS Glu298Asp polymorphism of the patients and the control groups

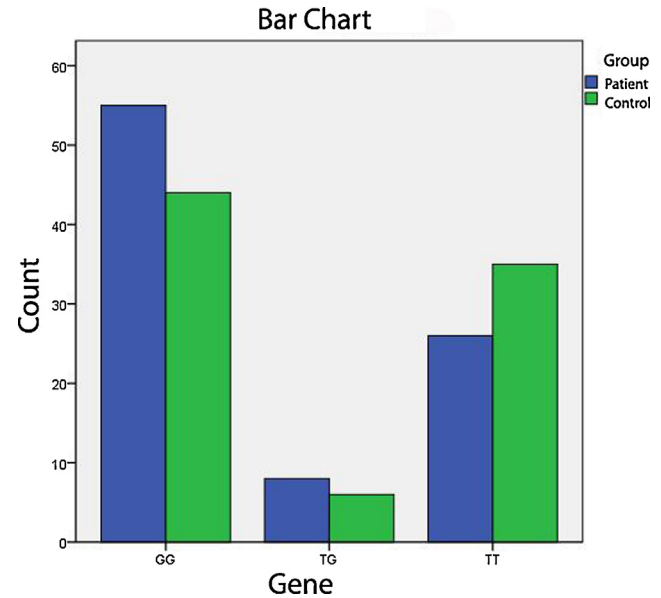


Fig. 1. The distribution of eNOS gene glu298asp polymorphisms between the groups.

were presented in Figs. 1 and 2 and Table 1. eNOS Glu298Asp polymorphism was evaluated in the patients with OME and the healthy volunteers. There was no statistically significant difference in terms of genotypic distributions (Glu/Glu; GG, Glu/Asp; GT, Asp/Asp; TT). When these groups were compared in terms of allele distributions, a significant relationship was found between the groups (Table 1) ($P = 0.037$) and G allele was identified as predisposing factor for the genetic susceptibility to the development of OME. However, there was no significant relationship between the parameters including Glu298Asp polymorphism of eNOS, gender, family history of hearing loss, the presence of surgical history, allergic rhinitis, adenoid vegetation, and presence of systemic disease, the severity and duration of hearing loss in OME patients ($P = 0.369$, $P = 0.550$, $P = 0.390$, $P = 0.805$, $P = 0.606$, $P = 0.857$, $P = 0.989$, $P = 0.432$, respectively).

4. Discussion

Previous studies showed the activity of NO and NOS has the key role in OME. However, the special effects of NO and NOS are unclear. In the present study, we focused eNOS polymorphisms in children with OME. G allele was found to be associated with the susceptibility to OME.

NO plays a role in the pathogenesis of OME by mediating intracellular and intercellular messengers which act as a trigger in

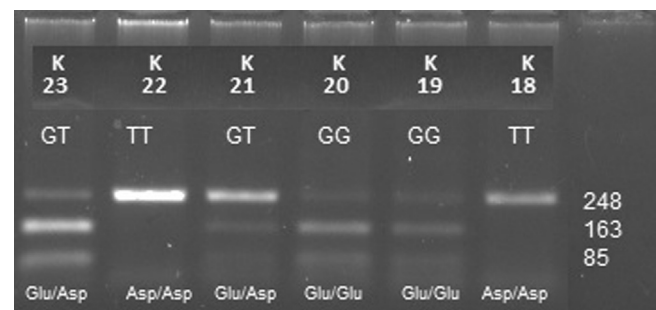


Fig. 2. Genotyping by 2% agarose gel electrophoresis.

Table 1
Genetic analysis of the patient and control groups.

Genotypes/Alleles	Controls (n = 85) n (%)	Cases (n = 89) n (%)	P	OR (95% CI)
G/G	44 (51.8)	55 (61.8)		
G/T	6 (7.1)	8 (8.9)	0.911 ^a	0.93 (0.30–2.90)
T/T	35 (41.2)	26 (29.2)	0.112 ^b	0.59 (0.31–1.13)
G	94 (55.3)	118 (69.4)		
T	76 (44.7)	60 (35.3)	0.037 ^c	1.590 (1.031–2.453)

OR: odds ratio.

^a Chi-square test G/G and G/T.^b Chi-square test G/G and G/T.^c Chi-square test G and T.

several middle ear pathology depending on the amount of NO radicals [12]. In the literature, there are controversial opinions about the effects of NO on inflammation. While some of the investigators reported that NO has anti-inflammatory functions, others reported that NO causes dysfunction in the cells and tissues due to inflammation. Jeon et al. reported that NO is an important inflammatory mediator in OM pathogenesis [13]. After various inflammatory stimuli in the middle ear, nitric oxide synthase [14] is induced in the middle ear mucosa and NO is synthesized. Granath et al. investigated the iNOS, eNOS, IL-1b and TNF-alpha expression levels together with the adenoid vegetation in children with and without OME. They found that iNOS expression is reduced in the patients with OME and adenoid vegetation as compared to the control group. Such a difference is not found in eNOS group. Therefore, they concluded that NO may have protective role against the development of OME in children with adenoid vegetation. Also, they argued that increased expression of NO would result in a reduced ability to respond the microbial infection due to its anti-inflammatory effects in children with OME [15].

Aktan et al. found that NOS activity increases NO synthesis. NO acts as an innate immune response against the pathogens. They concluded that NO has a positive contribution in the elimination of middle ear inflammation [16].

NO derivatives in various pathological conditions like acute inflammation react with reactive oxygen species. Jeon et al. investigated the effects of NO and peroxynitrite on mucociliary activity in experimental animals with OME. They reported that the causative of destructive effects in the middle ear is the reaction of NO with reactive oxygen species [13]. Yilmaz et al. also reported that NOS and NO levels significantly increased in OME group compared to the control group. They have claimed that NO is a double-edged sword in the patients with OME. Long-term high concentration of NO might also cause damage in erythrocytes [17].

Diao et al. reported that the levels of cochlear NOS and NO significantly increased in a rat model of noise induced cochlear damage [18]. While small amounts of NO are effective in regulating the vascular tone and excessive neurotransmission, it can be toxic to the cells. It shows us that NO may be toxic to the cells not only through inflammation but also through its direct toxic effects.

5. Conclusion

In conclusion, G allele was identified as predisposing factor for the genetic susceptibility to the development of OME. Further comprehensive research efforts are needed to examine new treatment modalities in diagnosis and treatment of OME by considering the eNOS polymorphism in pediatric patients with OME.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

This study with 10862 ID number was supported by Mustafa Kemal University Scientific Research Project Coordination.

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