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Research Article

eNOS GENE VNTR POLYMORPHISMS ARE ASSOCIATED WITH ORAL CYST

Esra Duman[1*], Serkan Yildiz[2], Serbülent Yiğit[3],
Nilufer Kuruca[4], Lale Bahrikarehmi[5], Mehmet Kemal Tumer[6],

[1]*Kocaeli University, Medical Faculty, Department of Medical Genetics, Izmit, Turkiye*
[2]*Tokat Gaziosmanpaşa University, Faculty of Dental Medicine, Department of Oral and Maxillofacial Surgery, Tokat, Türkiye*
[3]*Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Genetics, Samsun, Türkiye*
[4]*Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Pathology, Samsun, Türkiye*
[5]*Ondokuz Mayıs University Faculty of Medicine, Department of Biology, Samsun, Türkiye*
[6]*Alanya Alaaddin Keykubat University, Faculty of Dental Medicine, Department of Oral and Maxillofacial Surgery, Alanya Türkiye*

**Corresponding Author:*
E-mail: esra.duman@kocaeli.edu.tr

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ABSTRACT. Odontogenic cysts are originated from maxillary and mandibular teeth bearings odontogenic tissue and pathological cavities which are surrounded by fibrous connective tissue with epithelial layers. Cystic conditions on the jaw would end up bone damage, reabsorption or loss of the related tooth. Odontogenic cysts may occurdevelopmental, or inflammation-related. They are more common in adults than in pediatrics. Reports shows that matrix metalloproteinase synthesis which is activating by Nitric Oxyde (NO) participates bone resorption and cyst enlargement due to high NO production. eNOS gene variants change gene expression and activity due to increasing or over NO production and this situation causes various pathological conditions. The aim of this study is to investigate eNOS gene VNTR 4b/4a variants and oral cysts possible relationships. Genotyping revealed by the PCR method and 31 patients have I/I, 5 patients I/D, 1 patients D/D genotype with a total of 37 patients in the experiment group. According to obtained results, eNOS variantsI/I alleles and genotype frequency patients have more oral cyst possibility than D/D allele and genotype patients. Current results show that eNOS polymorphism may represent possible risk factors for the oral cyst.

Keywords: *eNOS, VNTR, oral cyst, PCR, polymorphism.*

INTRODUCTION

Odontogenic cysts (OC) are common lesions of oral and maxillofacial regions. Odontogenic kerato-cyst (OKC), dentigerous cyst (DC) and radicular cyst (RC) are the most common odontogenic cyst types [1]. In a study conducted on oral biopsies taken from the pathology service of a faculty of dentistry in the United States, the prevalence

of cystic lesions was reported to be 10.7% [2]. Odontogenic cysts are the most common in the maxillary anterior region, followed by the mandibular molar region. Periapical/radicular cysts, dentigerous cysts, residual cysts, and odontogenic keratocysts are the most frequently reported odontogenic cysts [3]. Clinical misdiagnosis is possible due to similar clinical and radiological appearances of these cysts. However, careful understanding and interpretation of clinical and radiological findings aids in the diagnosis of jaw cysts and accurate diagnoses can be obtained through oral and maxillofacial pathology services.

It is still less known about OKC which has more neoplasticism possibility comparing with DC and RC with aggressive clinical behavior and relapse. Biological processes that playing role on cyst development are not fully clear yet [4]. Studies have shown that high NO production due to matrix metalloproteinase which is activating NO may play role on bone resorption or cyst enlargement [5, 6]. NO is synthesis by a complex enzyme family called NO synthetase (NOS).

Nitric oxide (NO) is a biologically active messenger that is crucial for many physiological processes, including the regulation of vascular homeostasis. Nitric oxide (NO) and citrulline are produced from arginine by the endothelial nitric oxide synthase (eNOS) [7]. Genetically three different NOS isoforms defined, are vascular tonus balancing constative from nNOS, playing the role on immune and inflammatory response inducible formiNOS and regulating synaptic shaping and neurotransmission neuronal form nNOS. eNOS regulates vascular tonus and also has effects on thrombocyte aggregation, leukocyte adhesion, smooth muscle cell proliferation suppression by removing superoxide radicals and has a protective role on vessels [8].

eNOS gene is located in chromosome 7q35-36 and includes 26 exons about 21kb size. This gene has only one copy in the haploid human genome and codes a4052 nt mRNA [9]. Various polymorphisms defined on the promoter, exon and intron of eNOS gene. One of the eNOS variants is located in intone 4, four(4a) or five (4b) repeat, 27 base tandem repeat variants. Due to eNOS gene variants changing gene expression and activity, decreasing or over NO production results in various pathological conditions. According to written knowledge about NO in this study, we hypothesised eNOS VNTR 4b/4a variants would be related to oral cysts.

MATERIALS AND METHODS

Study Participants and Sample Collection

The study was performed in accordance with the ethical principles governing medical research and human subjects as in the Declaration of Helsinki and with the approval of the clinical research ethics committee of the Gaziosmanpaşa University (no.19-KAEK-078). The study included 37 patients (18 female, 19 male) with diagnosed oral cyst treated at the Clinic for Maxillofacial Surgery, School of Dental Medicine, Gaziosmanpaşa University, and 100 cyst-free controls (46 female, 54 male) recruited at the same clinic. The age average of the oral cyst patients were found 35.22±14.74, while controls was 34.76±13.46.

2 mL venous blood samples were taken into EDTA containing tubes from both groups. The collected samples were kept in the freezer at -20 °C until DNA extraction.

Genetic Analyze

DNA extraction was performed using the commercial kit (Invitrogen™ PureLink™ Genomic DNA Mini Kit, Catalog no: K182002, USA). Amplification of the interested gene region was performed with the primer pairs listed below [10].

Forward primer; 5'-CTATGGTAGTGCCTTGGCTGGAGG-3'

Reverse primer; 5'-ACCGCCCAGGGAACTCCGCT-3'

25 µl final PCR mix composed from 16.5 µl sterile water, 3.5 µl 5x buffer, 1.6 µl dNTP mix, 0.2 µl Taq polymerase, 1.2 µl each primer and 2 µl DNA for each sample. Amplification performed with Thermo Scientific, Px2 Thermal Cycler, USA. Amplification conditions were the first denaturation at 98 ºC 3 minutes, second denaturation at 98 ºC 30 seconds, annealing at 65Cº 30 seconds, fist extension at 72Cº 45 seconds and last synthesis at 72 ºC 5 minutes with 33 cycles. Amplificated products investigated with 1% agarose gel electrophoresis method and colored with ethidium bromide, finally monitored with Bio-Rad ChemiDocTMMP monitoring system is presented in Figure 1.

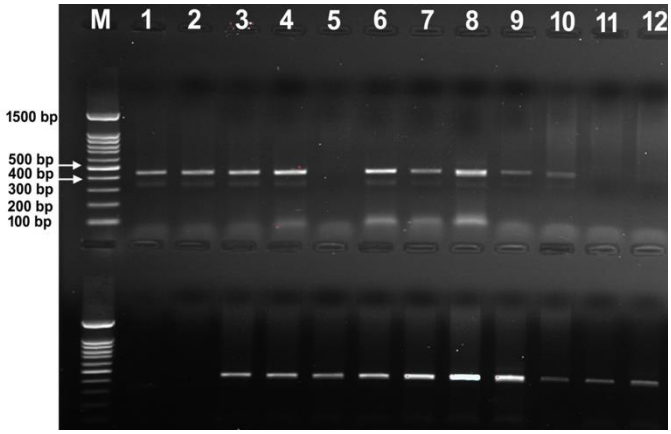

Fig. 1. *Monitored amplification products for VNTR polymorphism.*

Statistical Analysis

All statistical analysis performed with IBM SPSS (version 20) and Open Epi info (www.openepi.com, version 3.01) software's. Allele frequency between groups and genotypic distributions calculated with $\chi 2$ test. Odds ratio (OR) and 95% confidence interval (CI)calculated, $p<0.05$ value accepted for statistically significant.

RESULTS AND DISCUSSION

In this study 37 oral cysts patients and 100 healthy control group included. While OC patient group including 18 (46%) female and 19 male (51.4%) participants, control group included 46 (46%) female and 54 (54%) male participants. The age average was 35.22±14.74 and 34.76±13.46 for OC patients and control groups. Study groups' clinical and demographical characteristics are shown in Table1.

Table 1. *Comparison of Oral Cyst and Normal Patients' Clinical and Demographical characteristics*

	Control Group (n:100)	**OC Patient Group (n:37)**
Gender (female/male)	46/54 (46/54)	18/19 (48.6/51.4)
Age	34.76±13.46	35.22±14.74

Cyst types in patients' group was classified as 30 (81.1%) radicular cysts, 4 (10.8%) odontogenic kerato-cyts and 3(8.1%) dentigerous cysts. The most frequent cyst localization was the posterior mandible (n=21; 56.8%), followed by anterior maxilla (n=8; 21.6%), posterior maxilla (n=5; 13.5%) and anterior mandible (n=3; 8.1%). The most frequent cyst size was between 1-4 mm (n=19; 51.4%), second was 5-9 mm (n=14; 37.8) and only 4 (10.8%) cysts was 10mm and above. Diagnosed oral cyst characteristics are defined and showed at Table 2.

Table 2. *Clinical Characteristics of diagnosed oral cysts in OC patient group*

Characteristics	**OC Patient Group, n (%)**
Cyst Type, n (%)	
RadicularCyst	30 (81.1)
DentigerousCyst	3 (8.1)
OdontogenicKeratocyst	4 (10.8)
Cyst Region, n (%)	
Anterior Maxilla	8 (21.6)
Posterior Maxilla	5 (13.5)
Anterior Mandible	3 (8.1)
Posterior Mandible	21 (56.8)
Cyst size, n (%)	
1-4 mm	19 (51.4)
5-9 mm	14 (37.8)
10 mm and above	4 (10.8)

eNOS VNTR variants allele and genotype frequencies between study grups are listed in Table 3. STRING is a database that analyzes functional interactions between proteins in cell. eNOS protein analysed with STRING database and interacted proteins (CAV1, KNG1, NOSTRIN, CALM1, VEGFA, AKT1, HSP90AA1, KDR, ESR1) are shown in Figure 2. [11].

Table 3. *Oral cytspatientseNOS gene genotype and allell frequency*

	Genotype and Allele	OC Group n=37 (%)	Control Groupn=100 (%)	X2	P	OR (CI 95%)
eNOS	I/I	31 (83.8)	56 (56)			
	I/D	5 (13.5)	43 (43)	10.521	0.005	
	D/D	1 (2.7)	1 (1)			
	I/I+ I/D: D/D	36:01:00	99:01:00	0.2303	0.5443	0.3636 (0.02216-5.967)
	I/I: I/D+ D/D	31:06:00	56:44:00	0.001353	8.995	4.06 (1.556-10.59)
	I	67	155	5.974	0.0145	2.779 (1.192-6.477)

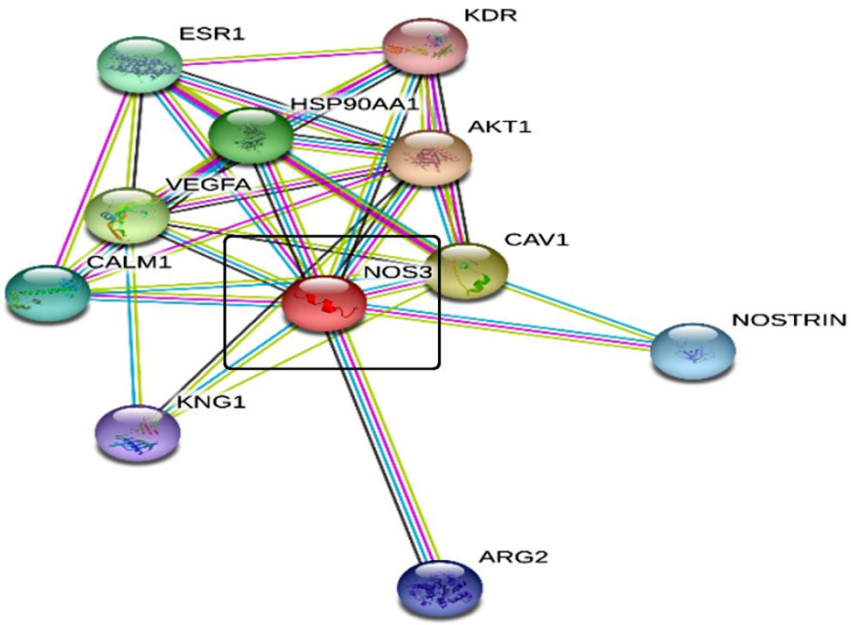

Fig. 2. *STRING software, NOS3 based Protein-protein interaction network* CALM1: calmodulin-1; AKT1: Akt-1; CAV1: caveolin-1; KNG1: kininogen-1; HSP90AA1: Heat Shock Protein90-alfa; NOSTRIN: Nostrin; ESR1: estrogen receptor; KDR: kinaseadding receptor (vascularendothelialgrowth factor receptor 2).

Cysts are pathological caverns that have fluid, semi-solid or solid contents and surrounded by epithelia. Odontogenic cysts originate from serosa cell residuals (dental lamina), mallessez cell residues (Hertwings epithelial residues) and enamel epithelium. [12]. Due to etiological classification odontogenic cysts are divided into two groups as developmental cysts (DC, OKC etc.) and inflammatory odontogenic cysts (RC). Dentigerous cyst, odontogenic kerato-cyst, lateral periodontal and botryoid cyst, gingival cyst, glandular odontogenic cyst, calcified odontogenic cyst and oto keratinized odontogenic cysts are developmental odontogenic cyst while radicular and collateral cysts

are inflammatory originated cysts. Compared with incidence; RC is the most common cyst types followed by DC and OKC [13].

NO is an important precursor molecule that plays essential roles in various pathological and physiological processes like transmission, smooth muscle relaxing, immunity, vasodilatation and carcinogenesis[14]. Previous studies conducted that over NO production inhibits DNA repair mechanism and causes DNA damage[15, 16]. On the contrary some cases reported that NO prevents DNA damage by increasing DNA-PKcs [catalytic subunit of DNA dependent protein kinas complex) activity. However, it is propounded that NO has blood flow increasing, tumor cell eliminating, tumor cells endothelial adhesion activity decreasing and apoptosis regulation abilities [17-20]. NO's anti-tumor effects provided by animal testing studies [21].NO also has an active role on virtual functions for various systems like vasodilatation on cardiovascular system.

NO has an active role on cancer development by toxic effects on cancer cells, T lymphocyte proliferations inhibition, and metastatic cell migration supporting and angiogenesis induction roles of NO reported its cancer developmental effects. NOS (nitric oxide synthase) synthesis NO by oxidating L-Arginin. Three isoforms of NOS is defined as neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). eNOS gene variants effect NO production therefore eNOS variants roles on carcinogenesis studied recently and reported as a modulator on cancer dependent issues angiogenesis, invasion and metastasis[22]. Bayraktutan et al [23] studied eNOS T-786C promoter TT, TC and CC polymorphism and eNOS G894T exon 7 GG, GT, TT polymorphisms in 100 Lung Cancer (LC) patients and 100 control group and reported LC groups plasma ADMA and NO levels statistically higher than control group. They also reported eNOS T-786C and G894T polymorphisms CC and TT genotype patients and control participants have higher plasma ADMA levels than other polymorphic groups. Zhu et al. [24] investigated iNOS and eNOS polymorphisms and stomach cancer risks genetic relationship and reported iNOS rs2297518 and eNOS rs2070744 polymorphism are reprehensive for stomach cancer predisposition. eNOS-786T> C and 894G> T polymorphisms reported in relationship with increasing mammary cancer risk [25]. NOS variants associated with different cancer types and also defined association with oral cancer by Chun-WenSua at al [26]. Their study conducted -786T>C polymorphism plays role on oral cancer progression and eNOS gen polymorphism reported as a susceptive factor for predisposition of oral cancer. Similarly Carkic et al. [27] reported VNTR 4b/a polymorphism increases OSCC risk threefold for heterozygote carriers up to 11-fold for homozygous carriers. They also reported -786 T/C SNP increases OSCC risk 3.6 fold on the contrary 896 G/C SNP has no relationship with OSCC risk. According to literature knowledge discussed above eNOS gene variants also found associated with oral pathologies.

CONCLUSION

According to literature knowledge discussed above it is obvious that eNOS gene variants are associated with various pathological issues including oral pathologies.

Present study is the first report investigating eNOS gene VNTR variants and oral cysts relationship. 37 total participants genotype distribution of I/I, I/D, D/D alles was 3,5 and 1 patients respectively.

As a result current study data proposes eNOS variants I/I carriers' oral cyst predisposition is higher than D/D carriers and eNOS polymorphisms would be a possible

risk factor for investigated population. However, additional studies with larger populations are necessary to validate these findings.

Conflict of Interest. The author declared that there is no conflict of interest.

Authorship Contributions. Concept: E.D., S.Y., S.Y., Design: E.D., S.Y., N.K., L.B., M.K.T., Data Collection or Processing: S.Y., M.K.T., L.B., N.K., Analyses or interpratation: E.D., S.Y., Literature Search: S.Y., S.Y., E.D., M.K.T., N.K, L.B., Writing: E.D., S.Y.

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