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Gene polymorphism of matrix metalloproteinase-1 in chronic periapical lesions and acute odontogenic infection

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MMP

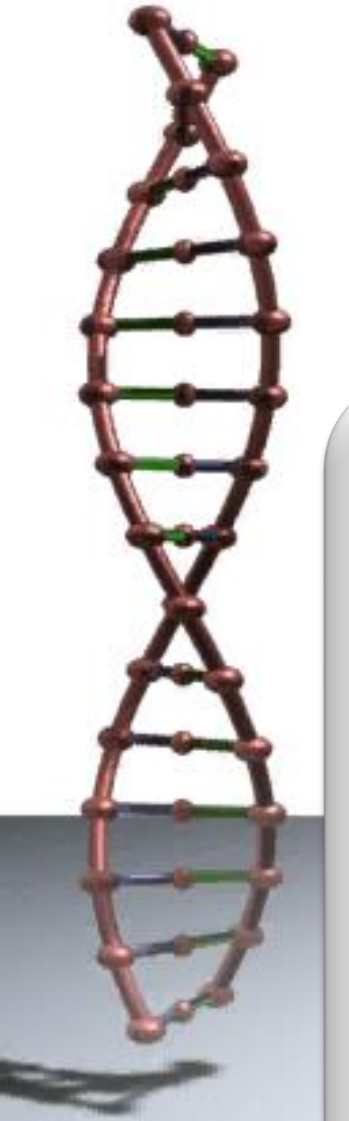
Inflammation involved various genes. Gene polymorphisms are contributing factors in the pathogenesis of inflammation. The promontory region of some matrix metalloproteinase's (MMPs) detected polymorphisms of the DNA (those promontory regions controlled transcription of the gene).

According to the important role of MMPs in inflammation (*Parks WC, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004; 4:617–629*), it has been shown that genetic variation affecting the expression of MMPs influences the susceptibility and progression of various diseases, including chronic periapical lesions and accute odontogenic infection. (*Scherer S, Barboza de Souza T, Juliana de Paoli, Brenol CV, Xavier RM, Brenol JCT, Chies JA, Simon D. Matrix metalloproteinase gene polymorphisms in patients with rheumatoid arthritis. Rheumatol Int 2010; 30:369–373*)

Gen of the MMP-1 is localized on chromosome 11q22 and its expression was shown in various types of healthy cells (stromal fibroblasts, macrophage, endothelial and epithelial cells), as well as in different inflammatory and carcinoma cells. *(Hyong-Suk Oh et al. (2009) MMP-1 promoter polymorphism in Korean with generalized aggressive Periodontitis. J Korean Acad Periodontol, 39:269-278)*

Level of expression of MMP-1 can be under the influence of different single nucleotide polymorphism in promontory region. Insertion or deletion of the guanine from the position -1607 can be identificat in humans in promoter of the gen of MMP-1 developing two different alleles: one have single guanine (1G), and the other two guanine (2G). (*Rutter JL et al. (1998) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res, 58: 5321-5*)

Patients who carried 2G allele have predisposition to develop several types of carcinoma or their rapid progression (*Nishizawa R et al., 2007*), to develop arthritis (*Scherer S et al., 2010*), arteriosclerosis, periodontitisot (*De Souza AP et al., 2003*), unsuccessful osteointegration of the implant (*Leite MF et al., 2008*), coronary hart disease in patients with diabetes mellitus (*Drzewoski J et al., 2008*), as well as in different other pathological conditions.



AIM OF THE STUDY

Identification of genetic factors which are of enormous meaning for establishing of different profile of patients who will develop chronic periapical lesion or acute odontogenic infection, as well as, calculation of total influence of polymorphisms in promontory region of the gen for matrix metalloproteinase-1 (MMP-1) in the patients was aim of this study.

Matherials and methods

Group	Clinical diagnosis	Total	
		Number (n)	%
I Group	Parodontitis chronica periapicalis granulomatosa	40	14,29
	Parodontitis chronica periapicalis progresiva diffusa	40	14,29
	Cystis radicularis	40	14,29
II Group	Accute odontogenic infectio	40	14,29
III Group – controle group		120	42,85

Selection of the patients

- According to internal criteria.

-Excluded those patients who have antibiotic or immuno-suppressive therapy.

-Excluded those patients who have clinical signs of parodontopathia.

In the aseptic condicions, from each patient with vene-punction, sample of vein blood (10mL) was taken in sterile epruvete (Vaccutainer®) in which was anticoagulant ($EDTA{\cdot}Na^2$). Ich sample was frozen at -80ºC as soon as possible and stored until the analysis.

Standard isolation of genomic DNA from the nuclear cells was made with natrium chlorid extraction and afterwords precipitation with ethanol. (*Gemmel NJ and Aniyama S. An efficient method for the extraction of DNA from vertebrate tissues. Trends in Ecology and Evolution 1996; 12(9): 338-339*)

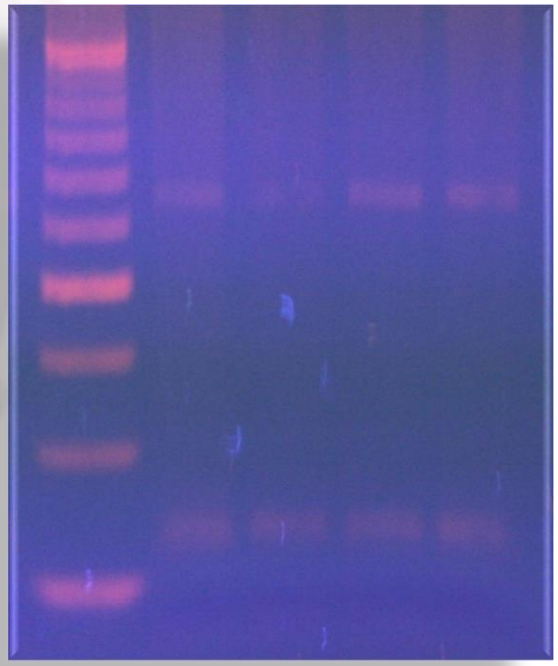
Samples of DNA izolates were aliqvated in few epruvetes, from wich, one was keeped from +4ºC till +8ºC, and that one was used for analysis. The other EDTA tubes were kept as a reserve in the bank of samples at temperature from -18ºC till -20ºC.

Amplification of the isolated DNA

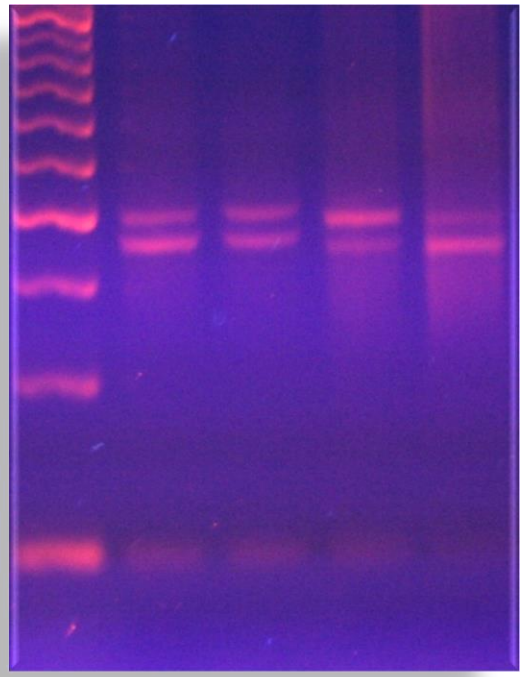
Amplification of the regions of elected genes was made with polymerase chain reaction (*Polymerase Chain Reaction* -PCR) and performed in PCR machine (*Perkin-Elmer Gene Amp System 2400*).

Gene	Polymor-phism	Pramer	PCR conditions	Length of PCR ampli-fication	Restri-ction enzyme	Restri-ction digestion
MMP-1	**-1607 1G/2G**	**(F) 5'-TGA CTT TTA AAA CAT AGT CTA TGT TCA-3' (R) 5'- TCT TGG ATT GAT TTG TTG AGA TAA GTC ATA GC-3'**	**35 ciklusi: 94°C 30s, 54°C 30s, 72°C 30s**	**269 bp**	***Alu*I**	**(241+28) bp**
MMP-1	**-1607 1G/2G**	**(F) 5'-TCG TGA GAA TGT CTT CCC ATT-3' (R) 5'-TCT TGG ATT GAT TTG AGA TAA GTC ATA-3'**	**35 ciklusi: 93°C 30s, 55°C 30s, 72°C 30s, 72°C 5min**	**118 bp**	***Xmn*I**	**(29+89) bp**
MMP-1	**-519 A/G**	**(F) 5'-CAT GGT GCT ATC GCA ATA GGG T-3' (R) 5'-TGC TAC AGG TTT CTC CAC ACA C-3'**	**30 ciklusi: 94°C 30s, 49°C 30s, 72°C 20s, 72°C 4min**	***200* bp**	***Kpn*I**	**(176+24) bp**

Presence or absence of some normal or mutated sequence in PCR product followed by restriction digestion results with different size of fragments. Successful amplification was verified with presence of electrophoresis marker *(PCR Marker, Bio-Rad)* with agrarelectrophoreze *(Bio-Rad, USA)*. Production of digestion were vizualizate with fluoroscence painting of the gel with etidium bromid.



Polymorphism of MMP-1 detected with restriction enzyme *Alu*I



Polymorphism of MMP-1 detected with restricted enzyme *Kpn*I

Conclusions

Polymorphism -1607 1G/2G of the gene for MMP-1 detected with restriction enzyme *Xmn*I and polymorphism -1607 1G/2G for the gene of MMP-1 detected with restriction enzyme *Alu*I are risk factors for developing chronic periapical lesions and acute odontogenic infections, except for the patients with clinical diagnosis Cystis radicularis.

Individuals who are carriers of 2G/2G genotype and individuals with 1G/2G genotype (2G carriers) in this polymorphism show affection for developing chronic periapical lesions and acute odontogenic infections.

Polymorphism -519 A/G of the gene for MMP-1 detected with restriction enzyme *Kpn*I is risk factor for developing chronic periapical lesions and acute odontogenic infections, and individuals who are carriers of G/G genotype and individuals with A/G genotype (G carriers) show affection for developing this inflammatory processes.

MMP

According to investigations in this study we can identification genetic factors which are of enormous meaning for establishment of patients who have risk of developing chronic periapical lesion and acute odontogenic infection in Macedonian population. Variation of the gene alleles of MMP-1 can be markers for monitoring the risk and progression of this inflammatory processes.

This study is the basis for exploring the polymorphisms of the genes for MMPs in Macedonian population as well as pointing mark for fourder investigations of genetic expression of MMPs, with which we can predict the development of the clinical picture of the inflammatory process, indirectly pointing to correct choice of wrights therapeutic method.

THANK YOU FOR YOUR ATEHSOIN