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Original article

THE PRESENCE OF IL-8 GENE POLYMORPHISM AT (-251A/T) AND (-396T/G) POSITION IS RELATED WITH SUSCEPTIBILITY TO PERIODONTITIS DEVELOPMENT.

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disruption of the supportive periodontal tissue. It is known that the chronic periodontitis is initiated by the increased level of specific bacteria, but the progression of the disease is defined by characteristics of the host response to the bacterial load. There is evidence that individual genetic, systemic and environmental risk factors can influence the course of the disease. Some genes and intergenic relations are in connection with the modification of the periodontal disease, and so they change the clinical course, the severity and the prognosis of the decease.

Purpose: The aim of the current investigation is to establish the presence of IL-8 gene polymorphism and serum levels to IL- 8 in patients with chronic periodontitis.

Material and methods: In the recent study, 35 patients (24 females and 11 males) are included with diagnosis moderate to severe periodontitis. Periodontal important parameters such as PD, CAL and Bone loss are included. The level of IL- 8 in a patient's serum is tested by ELISA (enzyme-linked immunosorbent assay). The presence of IL- 8 polymorphism in loci rs4073 (-251A/T) and rs2227307 (-396T/G) is established by polymerase chain reaction (PCR).

Results: The analysis of the current assay recognized a statistically significant correlation between: HI and PBI-severity and HI and BOP; a significant correlation between PBI- severity and PD average and Bone loss; the strong correlation between BOP and Bone loss; the significant correlation between PD 3- 5mm and PD ≥7mm and CAL average, also a strong correlation between PD ≥7mm and Bone loss. The results have shown that the biggest interrelationship observed between the presence of GT/AT genotype and TT/TT genotype and distribution of PD 5-7 mm and PD ≥ 7 mm ($p<0.09$).

Conclusions: In the recent study have shown that by reason of the heterogeneous results achieved in the group investigated by us we can definitely support the hypothesis that the presence of allele A for rs407 (-251A/T) and allele G for rs2227307 (-396T/G) is strongly related with expression of severe periodontitis.

Keywords: gene polymorphism, interleukin-8, susceptibility, periodontitis, enzyme-linked immunosorbent assay, polymerase chain reaction.

INTRODUCTION:

At present, it is known that pathogenic bacteria are the key factors for the initiation of periodontal disease, but the host response and the severity of clinical expression are largely determined by genetic susceptibility and environmental factors [1-5]. The first study of cytokines' gene polymorphisms was reported by Kornman who found a significant association between severe adult periodontitis and composite genotype, namely allele 2 of a single nucleotide polymorphism (SNP)of IL-1A+4845 and IL-1B+3954 located on chromosome2q13[6]. Following this, several studies have been conducted exploring the role of **IL-1 gene polymorphisms** as a severity factor in periodontitis in various population and ethnic groups [7-11]. However, there is evidence that genetic variations affect the host response by means of receptor expression and secretion of proinflammatory cytokines and chemokines measured in the crevicular fluid, such as: **IL- 1β**, **IL- 8, IL- 10**, **IL-13 and IL-17**that are associated with a certain periodontal status [12-19]. There is data that genetic factors are likely to be important determinants of the risk of periodontal diseases by suggesting that these diseases are polygenic rather than monogenic [20-23].

AIM:

To determine the presence of **IL-8 gene polymorphism and serum levels to IL- 8** in patients with periodontitis.

MATERIAL AND METHODS:

In the recent study, 35 patients (24 females and 11 males) were included with diagnosis moderate to severe periodontitis. Periodontal important parameters such as PD, CAL and bone loss were included. The level of IL- 8 in the patient's serum was tested by ELISA (enzyme-linked immunosorbent assay). The present of IL- 8 polymorphism in loci rs4073 (-251A/T) and rs2227307 (-396T/G) was established by PCR.

RESULTS:

The results of the current study are presented in Tables 1, 2 and 3, and Box plots 1 and 2.

Table 1. Spearman rank order correlations (R- coefficients and p-values) among 12 markers in the studied patient group (n=35).

Correlations

		Age	HI	PBI	BoP	PD_ Mean	PD <3 mm	PD = 3-5 mm	PD = 5-7 mm	PD >7 mm	CAL, mm	Bone loss, mm	IL 8
Age	Pearson Correlation	1	,051	,039	-,057	-,060	,027	,009	-,076	,080	,034	,054	-.337*
	Sig. (2-tailed)		,769	,825	,744	,732	,876	,957	,664	,649	,845	,757	,048
	N	35	35	35	35	35	35	35	35	35	35	35	35
HI	Pearson Correlation	,051	1	-.589**	-.853**	-,262	,264	,101	-,189	-,212	-,271	-,034	-,098
	Sig. (2-tailed)	,769		,000	,000	,129	,125	,563	,276	,222	,116	,846	,577
	N	35	35	35	35	35	35	35	35	35	35	35	35
PBI	Pearson Correlation	,039	-.589**	1	.515**	.532**	-,309	-,175	,213	.349*	.426*	.542**	,155
	Sig. (2-tailed)	,825	,000		,002	,001	,071	,315	,219	,040	,011	,001	,375
	N	35	35	35	35	35	35	35	35	35	35	35	35
BoP	Pearson Correlation	-,057	-.853**	.515**	1	,211	-,181	-,028	,099	,135	,222	,037	,214
	Sig. (2-tailed)	,744	,000	,002		,223	,299	,875	,572	,438	,201	,834	,218
	N	35	35	35	35	35	35	35	35	35	35	35	35
PD_Mean	Pearson Correlation	-,060	-,262	.532**	,211	1	-.552**	,022	,231	.376*	.572**	.567**	,136
	Sig. (2-tailed)	,732	,129	,001	,223		,001	,899	,182	,026	,000	,000	,436
	N	35	35	35	35	35	35	35	35	35	35	35	35
PD < 3mm	Pearson Correlation	,027	,264	-,309	-,181	-.552**	1	,142	-.665**	-.524**	-.576**	-,276	-,059
	Sig. (2-tailed)	,876	,125	,071	,299	,001		,415	,000	,001	,000	,108	,736
	N	35	35	35	35	35	35	35	35	35	35	35	35
PD = 3-5mm	Pearson Correlation	,009	,101	-,175	-,028	,022	,142	1	-.707**	-.491**	-,093	-,109	,100
	Sig. (2-tailed)	,957	,563	,315	,875	,899	,415		,000	,003	,596	,532	,570
	N	35	35	35	35	35	35	35	35	35	35	35	35
PD=5-7mm	Pearson Correlation	-,076	-,189	,213	,099	,231	-.665**	-.707**	1	,299	,262	,137	,019
	Sig. (2-tailed)	,664	,276	,219	,572	,182	,000	,000		,081	,129	,433	,915
	N	35	35	35	35	35	35	35	35	35	35	35	35
PD>7mm	Pearson Correlation	,080	-,212	.349*	,135	.376*	-.524**	-.491**	,299	1	.535**	.335*	-,098
	Sig. (2-tailed)	,649	,222	,040	,438	,026	,001	,003	,081		,001	,049	,577
	N	35	35	35	35	35	35	35	35	35	35	35	35

CAL, mm	Pearson Correlation	,034	-,271	.426*	,222	.572**	-.576**	-,093	,262	.535**	1	.711**	,209
	Sig. (2-tailed)	,845	,116	,011	,201	,000	,000	,596	,129	,001		,000	,229
	N	35	35	35	35	35	35	35	35	35	35	35	35
Bone loss, mm	Pearson Correlation	,054	-,034	.542**	,037	.567**	-,276	-,109	,137	.335*	.711**	1	,325
	Sig. (2-tailed)	,757	,846	,001	,834	,000	,108	,532	,433	,049	,000		,056
	N	35	35	35	35	35	35	35	35	35	35	35	35
IL8	Pearson Correlation	-.337*	-,098	,155	,214	,136	-,059	,100	,019	-,098	,209	,325	1
	Sig. (2-tailed)	,048	,577	,375	,218	,436	,736	,570	,915	,577	,229	,056	
	N	35	35	35	35	35	35	35	35	35	35	35	35

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

The analysis of the current assay established statistically significant correlation between:

1. HI and PBI - severity and **HI and BOP-**which shows that the reduction of free from plaque surfaces leads to increased bleeding on probing;

2. A significant correlation between PBI**-** severity and PD **-** average and Bone loss;

3. A strong correlation between BOP and Bone loss

4. The significant correlation between PD 3**-**5mm and PD ≥ 7mm and CAL average, also a strong correlation between PD ≥ 7mm and bone loss.

5. Establishes a very high positive correlation between the CAL and Bone loss (R=0.71);

Established differences between the two most common genotype – GT/TT and TT/TT

Table 2. Statistically reliable differences found in patients with genotype GT/TT and TT /TT.

Variable	Genotype GT/AT	Genotype TT/TT	P value
	Mean	Mean	/ Student t-test /
Patients, N	23	8	
Age	47	43	0.24
HI	41365	12540	0.65
PBI	30713	29252	0.80
BOP distribution	97.12	94.81	0.44
PD mm	47209	41365	0.54
PD ≤3mm %	27.67	31.90	0.09*
PD = 3 ÷ 5mm %	36.23	36.18	0.99
PD = 5 ÷ 7mm %	26.77	25.91	0.83
PD ≥ 7mm %	12298	43836	0.09*
CAL mm	44109	43895	0.63
Bone loss mm	44046	44077	0.63
IL-8 pg/ml	0.97	44044	0.96

Fig. 1. Box plots representing the percentage of (a) PD ≤ 3 mm and (b) PD ≥ 7 mm in patients according to the different 6 genotypes (GT/AT, TT/TT, TT/AT, GG/AA, GG/TT, TT/AA). The upper and lower limits of the boxes represent the 75th and 25th percentiles respectively (the horizontal bar across the box indicates the median and the end of the vertical lines indicates the minimum and maximum data values). The results obtained can be interpreted that in both the genotype GT/TT and TT/ AT predominate moderately to deep periodontal pockets.

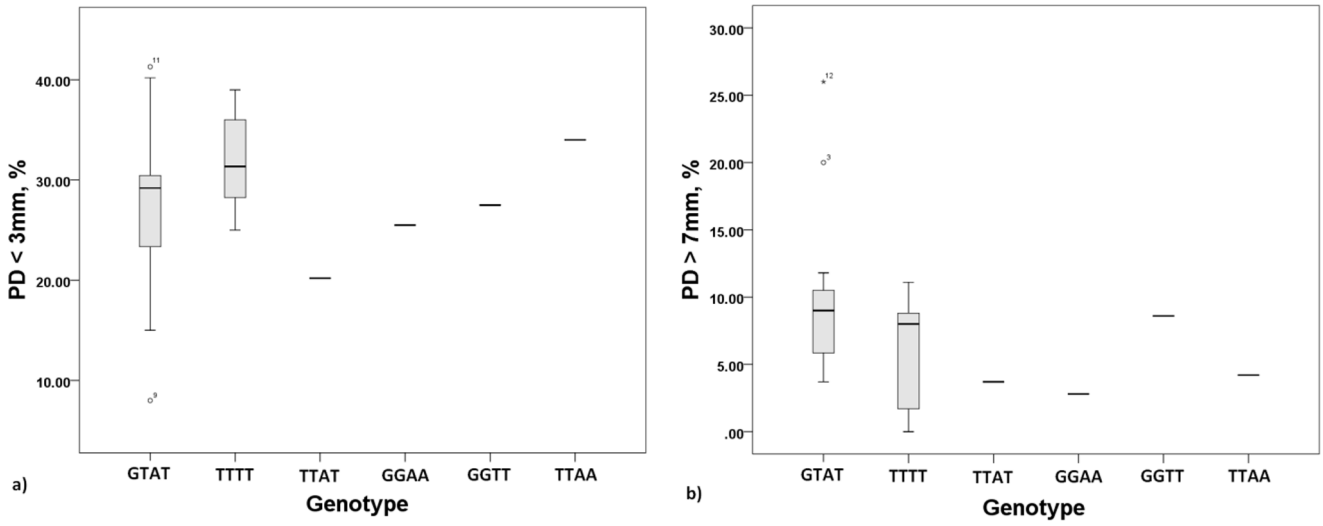
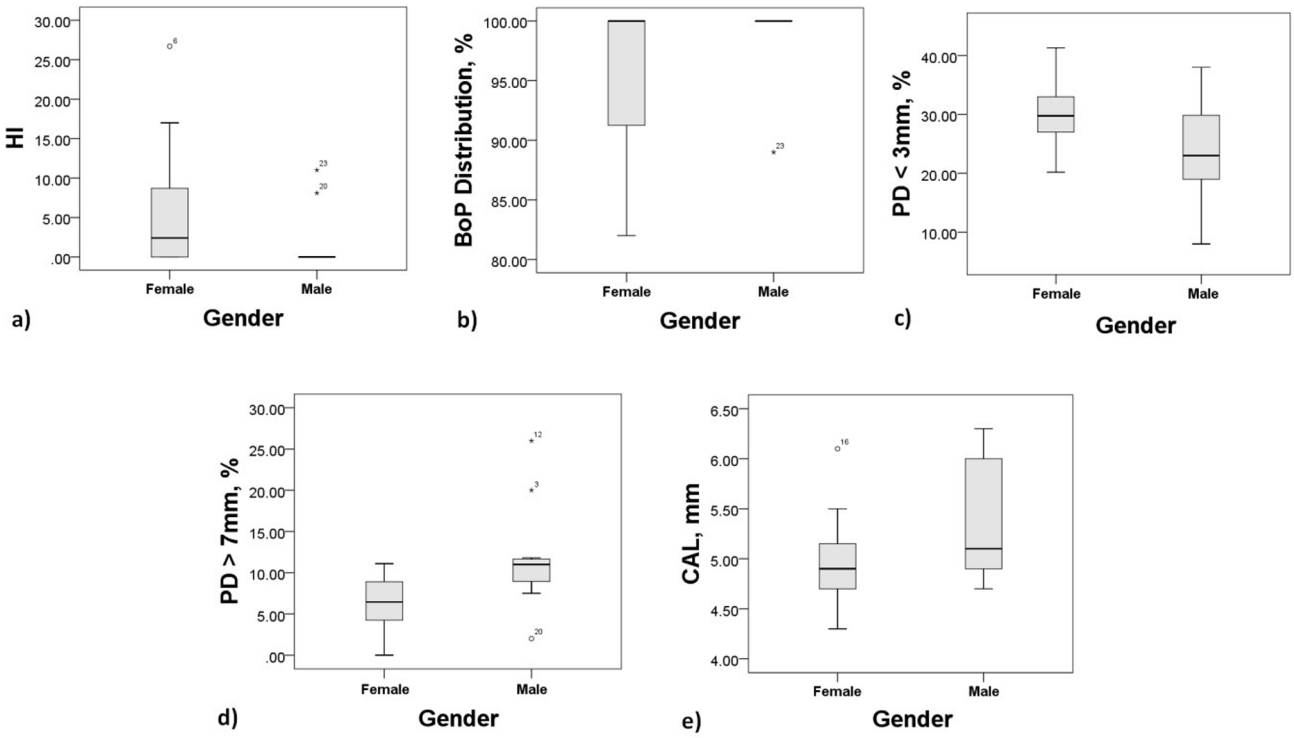


Table 3. Statistically reliable differences identified between the clinical indications for patients depending on gender.

VARIABLE	FEMALE	MALE	P value
	MEAN	MEAN	/ Student t-test /
PATIENTS N	23	8	
AGE	46	46	0,85
HI	5,39	1,74	0.06*
PBI	2,77	3,06	0,11
BOP- distribution	95,52	99	0.04*
PD mm	4,15	4,5	0,16
PD ≤ 3mm, %	30,56	24,14	0.04*
PD = 3 ÷ 5mm, %	39,03	34,53	0,08
PD = 5 ÷ 7mm, %	24,02	29,65	0,11
PD ≥ 7mm, %	6,39	11,69	0.02*
CAL mm	4,93	5,42	0.03*
Bone loss mm	2,98	3,29	0,09
IL-8 pg/ml	1,03	0,95	0,53

Fig. 2. Box plots representing the (a) HI, (b) BOP distribution (c) percentage of PD ≤3 mm, (d) percentage of PD ≥7 mm, and (d) CAL in patients according to the different gender (male and female). The upper and lower limits of the boxes represent the 75th and 25th percentiles respectively; (the horizontal bar across the box indicates the median and the end of the vertical lines indicates the minimum and maximum data values).



DISCUSSION OF THE RESULTS:

In our study, we found a close relationship between the parameters of the periodontal status. The results have shown that the biggest relationship is observed between the presence of GT/AT and TT/TT genotype and distribution of PD 5-7 mm and PD ≥7 mm (**p<0.09**). It has been established that the distribution of BOP is higher in male patients (**p<0.04**). The higher distribution of shallow pockets PD ≤ 3mm is encountered in female patients (**p<0.04**). The bigger distribution of deep pockets PD ≥7 mm is found in male patients (**p<0.02**).

Significant positive correlations were established between the loss of attachment as the main criterion of the severity of the periodontitis (CAL) and bone loss (**p = 0.0001**), and also between the loss of attachment and the deep periodontal pockets ≥7mm (**p=0.001**).

In the present research, the main goal was to establish the distribution of IL-8 gene polymorphism in locirs 4073 (-251A/T) and rs2227307 (-396T/G)in patients with chronic periodontitis. In the scientific literature, there's a discussion about this problem based on the insufficient number of investigations with opposing results [24-27]. In the group of patients investigated by us increased serum levels of IL-8average 169, 91pg/ml were established in most of all, analysed blood samples. Compared to other investigations, our study showed expressively higher values of the levels of the investigated IL-8 [28-30]. We could explain these results by analysing the objective clinical and paraclinical database, which characterize the investigated group as patients with severe chronic periodontitis and advanced tissue destructive processes [31]. For this reason, we think that the results achieved by us correctly reflect the severity of the clinical manifestation of the periodontal disease.

In this research 6 predominant genotypes of IL-8 for rs4073 (-251A/T) and rs2227307(-396T/G) were established: GT/AT, TT/TT, TT/AT, GG/AA, GG/TT и TT/AA, with the highest prevalence of GT/ AT genotype – **65.71 %** and TT/TT genotype – **22.86%.**In compare to patients with **GT/AT genotype**, in patients with **TT/TT** genotype we established a strong correlation between the presence of IL-8 in serum and the values of **HI%** (**p=0.028**), and strong correlation between the presence of **IL- 8** and BOP distribution. Statistically reliable differences in the predominant genotypes GT/AT and TT/TT and the percentage of distribution of PD 5-7 mm and PD ≥7mm (**p<0.09**) were established.

The determination of present genetic factors and the biomarkers specifically related to them could help the clinician to choose the correct approach for prevention and control of periodontal disease in susceptible patients. It is suggested that the establishment of the dynamic interactions between the different cytokines and their level of expression in gingival fluid and blood serum could be a major factor for initiation of periodontal disease as a determinant for its severity and progression [11, 32-34].

CONCLUSION:

According to the achieved results, we could suggest that the monitoring of the cytokine production could be helpful for diagnostics of individuals with periodontal disease or susceptible to it. Because of the heterogeneous results achieved in the group investigated by us, we can definitely support the hypothesis that the presence of allele A for rs407 (-251A/T) and allele G for rs2227307 (-396T/G) is strongly related with expression of severe periodontitis. It is important to be emphasized that except for gene polymorphisms, the severity of periodontitis is determined by other environmental factors and mainly by the dysbalance between the host response and the presence of periodontal pathogens.

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