® Postepy Hig Med Dosw (online), 2014; 68: 1145-1151
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2014.03.10
Accepted: 2014.08.07
Published: 2014.09.12

Are *KIR* genes associated with clinical parameters in the course of periodontitis?*

Czy geny *KIR* są związane z klinicznymi parametrami w przebiegu choroby przyzębia?

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Małgorzata Mazurek-Mochol[1, A, B, E, F, G], Edyta Majorczyk[2, B, C, D, E, F], Jadwiga Banach[1, E, F], Elżbieta Dembowska[1, F], Andrzej Pawlik[3, D, E, F], Piotr Kuśnierczyk[2, E, F]

[1] Department of Periodontology, Pomeranian Medical University, Szczecin, Poland
[2] Laboratory of Immunogenetics and Tissue Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences Wrocław, Poland
[3] Department of Pharmacology, Pomeranian Medical University, Szczecin, Poland

Summary

Introduction: Periodontitis is a common chronic inflammatory disease. It seems that natural killer (NK) cells play a role in the pathogenesis of periodontitis. KIRs are a family of inhibitory or activating receptors expressed on the surfaces of NK cells and some subpopulations of T lymphocytes. The aim of this study was to evaluate the impact of *KIR* genes on the pocket depth (PD) and clinical attachment loss (CAL) parameter values as markers of disease clinical course.

Materials and methods: The patients in the study were submitted to anamnesis and to clinical and periodontal examination. The subjects (400) were categorized into two groups: periodontitis (250 including 100 with moderate and 140 with severe periodontitis) and controls (150). Both groups were divided into two subgroups: *KIR* gene positive (presence of *KIR* gene in the genome) and *KIR* gene negative (lack of the *KIR* gene in the genome).

Results: The mean value for CAL was more than 5 mm, and the mean value for PD was more than 4 mm in the periodontitis group. The ANOVA test performed for the control group showed that neither PD nor CAL parameters differed between particular *KIR*-positive and *KIR*-negative healthy individuals. Similar results were obtained for all subgroups of chronic periodontitis patients and periodontitis patients (moderate as well as severe): no association between *KIR* genes and PD or CAL parameters was found.

Discussions: The activated immune system is important in pathogenesis of periodontal disease. On the other hand, tissue damage as a response to infection could be due to activation mediated by *KIR*. In our study no association between either *KIR* genes presence or absence and PD and CAL parameters was found. Nevertheless, the impact of *KIR* genes on the clinical course of periodontal disease requires further investigations.

Keywords: **chronic periodontitis • *KIR* genes**

*The research was funded by the Ministry of Science and Higher Education assigned for PAM grant– 9/09/PB, MNiSW N N403 416736.

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1120991

Word count: 2128
Tables: 4
Figures: –
References: 38

Author's address: dr Małgorzata Mazurek-Mochol, Pomeranian Medical University, Department of Periodontology, al. Powst.Wlkp. 72, 71-111 Szczecin, Poland, e-mail: malgorzata.mazurek@poczta.onet.pl

Introduction

Periodontitis is a common chronic inflammatory disease that affects the periodontium (gums, cementum, periodontal ligament and alveolar supporting bone). The most prevalent form of periodontitis is chronic periodontitis. This multifactorial disease, caused by a group of gram-negative bacteria, destroys the tooth-supporting structures and consequently leads to mobility and loss of the teeth [20]. Although the presence of a periodontal pathogen is a prerequisite for periodontitis development, the progression of the disease is dependent on the host innate and adaptive immune responses [36, 38]. In the host immune response many types of cells (leukocytes, macrophages, lymphocytes, fibroblasts) are involved [32]. It seems that natural killer (NK) cells play a role in the pathogenesis of periodontitis.

NK cells are populations of lymphocytes that can be activated to mediate a significant level of cytotoxic activity and produce certain cytokines and chemokines [5]. NK cells contribute to a protective response against a variety of viral infections and cancers [4].

NK cells are a major component of the innate immune system and exert regulatory effects by secreting cytokine [6]. Limited data suggest that NK cells are activated in chronic periodontitis lesions [25] and that NK cell activation is associated with periodontal bone loss in vivo [9]. The mechanisms of activation of NK cells in periodontal tissues are not fully understood [18].

The function of NK cells is controlled by both inhibitory and activating receptors [22]. When the activity of NK cells is balanced towards activation, it leads to enhanced killing, enhanced production of cytokines, or both [9]. Among other receptors, killer cell immunoglobulin-like receptors (KIRs) are used by NK cells for activating signal transfer as well as inhibition of cell activation.

KIRs are a family of inhibitory or activating receptors expressed on the surfaces of natural killer cells and some subpopulations of T lymphocytes. The function of KIR receptors is determined by their structure; thus, inhibitory KIRs possess a long (L) cytoplasmic tail which contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and activating KIRs with a short (S) cytoplasmic tail having no signaling motifs. However, a positively charged arginine residue gives it a possibility to interact with an adapter molecule, DAP12 homodimer, which possesses immunoreceptor tyrosine-based activating motifs (ITAMs).

KIR proteins possess two (KIR2D) or three (KIR3D) immunoglobulin-like domains in their extracellular region, which determine ligand specificity [19]. KIR receptors with two domains recognize HLA-C molecules, and three--domains receptors bind HLA-B molecules.

KIR genes are clustered in the leukocyte receptor complex (LRC) which are located in the 19q13.4 region and are characterized by both allelic (high numbers of variants) and haplotypic polymorphisms defined as different numbers of genes for inhibitory and activating receptors on individual chromosomes [29].

Some *KIR* genes called framework genes (*KIR3DL2*, *KIR3DL3*, *KIR2DL4*, and *KIR3DP1* pseudogene) are present in all haplotypes. Other genes are present only in some of them, in multiple different combinations. Haplotypes consisting mostly of inhibitory genes (called "A" haplotypes) tend to be associated with lower risk of autoimmune diseases, but higher risk of viral infections and neoplasm development than haplotypes ("B" haplotypes) containing several activating *KIR* genes [5,7,16,28]. Interestingly, *KIR* genes appeared also to be associated with the clinical course of particular disorders, e.g. development of extra-articular complications in rheumatoid arthritis [23], the response of patients with non-small cell lung carcinoma to treatment (surgery and/or chemotherapy) [37], disease progression in HIV infection, and protection of HCV-positive patients against hepatocellular cancer [16].

Aim of the study

Therefore, the aim of this study was to evaluate the impact of a distribution of haplotypic polymorphisms within *KIR* genes (presence or absence of particular *KIR* genes in the genome) on the pocket depth (PD) and clinical attachment loss (CAL) parameter values as markers of disease clinical course.

Materials and methods

The protocol of the study was approved by the Committee of Ethical Affairs of the Pomeranian Medical University (protocol number BN-001/93/08).

Patients were included in the study after giving informed consent.

All 400 selected patients were Caucasian (people of European origin), Polish, with an age between 25 and 69 years old. The patients were submitted to anamnesis and to clinical and periodontal examination. The diagnosis of subjects (chronic periodontitis) was established on the basis of clinical criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions [3].

Exclusion criteria included systemic disease, patients who used systemic or sub-gingival antimicrobial agents or made chronic use of anti-inflammatory medication, and non-Caucasian patients. Subjects were also excluded from the study if they had a history of hepatitis, AIDS or HIV, recent radiation therapy, diabetes, uncontrolled hypertension, use of immunosuppressive medications, antibiotic use within the past 6 months, or were pregnant.

All patients were otherwise healthy and were not subjected to periodontal treatment or antibiotics for at least 6 months before the study.

The subjects were categorized into two groups: periodontitis and controls.

Periodontal evaluation included probing pocket depth (PD), clinical attachment loss (CAL), the approximal plaque index (API) [21] and modified sulcus bleeding index (%SBI) [26].

The periodontal subjects comprised 250 patients (104 men, 146 women), aged from 26 to 69 years (mean 50.47±9.09) with chronic periodontitis, diagnosed using the periodontal disease classification system of the American Academy of Periodontology (1999) [3].

Control samples were taken from 150 healthy subjects (56 men, 94 women), aged from 25 to 69 years (mean 42.97± 11.22), free from periodontitis.

DNA isolation and *KIR* genotyping

Genomic DNA was extracted from peripheral blood leukocytes using Invisorb Blood Spin Midi-Kit following the manufacturer's instructions (Invitek, Germany).

Typing of *KIR* genes was performed using polymerase chain reaction with sequence specific primers (PCR-SSP) as described previously [27]. Genotyping was focused on the haplotypic polymorphism of the *KIR* genes (presence or absence of particular KIR genes in the genome of the individual).

Statistical analysis

One-way ANOVA test was used for multiple group comparison, and Tukey range analyses for unequal sample size were performed as post-hoc tests.

All analyses were applied using Statistica package v. 9.0 (StatSoft, OA, U.S.A.), and p values < 0.05 were considered significant.

Results

Clinical data

In the periodontitis group all clinical periodontal parameters (mean PD, mean CAL, mean % API, mean % BOP) were significantly increased compared to the control group ($p<0.001$). The mean value for CAL was more than 5 mm, and the mean value for PD was more than 4 mm in the periodontitis group. There were 140 patients (56%) with severe chronic periodontitis (clinical attachment loss [CAL] greater than 5 mm) and 100 patients (40%) with moderate chronic periodontitis (CAL greater than 3 mm). Detailed subject characteristics are shown in table 1.

Table 1. Subjects characteristics

PARAMETER	CONTROLS (n=150)	PERIODONTITIS (n=250)	p value
GENDER (M/F)	56/94	104/146	0.26
AGE (mean years + SD)	42.97+ 11.22	50.47+ 9.09	< 0.001
API % (mean + SD)	39.33+18.97	80.84+17.54	< 0.0001
SBI % (mean + SD)	6.65+9.87	61.10+24.18	< 0.0001
PD mm (mean years + SD)	1.57+ 0.40	4.31+1.18	< 0.0001
CAL mm (mean years + SD)	1.61+ 4.50	5.27+ 1.87	< 0.0001
CAL mm (n subjects) 1-2 mm 3-4 mm ≥ 5 mm		250 (100%) 10 (4%) 100 (40%) 140 (56%)	

KIR receptor genes in relation to periodontitis

The aim of this study was to evaluate the impact of *KIR* genes' on the clinical course of periodontal lesions. In accordance with this, independently for each *KIR* gene both healthy controls (table 2) and periodontitis patients (table 3) as well as two patient subgroups (with moderate and severe chronic periodontitis) (table 4) were divided into two subgroups, *KIR* gene positive (cases with particular *KIR* gene presence in the genome) and *KIR* gene negative, (cases with particular *KIR* gene absence in the genome) and mean values of PD and CAL parameters were calculated for all obtained subgroups. The ANOVA test performed for the control group shows that neither PD nor CAL parameters differed between particular *KIR*-positive and *KIR*-negative healthy individuals. Similar results were obtained for chronic periodontitis patients and periodontitis patient subgroups

Table 2. *KIR* genes impact on PD and CAL parameters in control group

Control group	2DL1				2DL2				2DL3				2DL5			
	+		-		+		-		+		-		+		-	
PD	1.58±0.40	1-2.62	1.41±0.33	1-2	1.58±0.38	1-2.20	1.54±0.44	1-2.62	1.57±0.40	1-2.63	1.52±0.39	1-2.17	1.55±0.39	1-2.17	1.59±0.42	1-2.63
CAL	0.28±0.77	0-4.67	0		0.24±0.77	0-4.67	0.31±0.73	0-3.33	0.24±0.71	0-4.67	0.52±1.07	0-3.33	0.30±0.82	0-4.67	0.22±0.65	0-3.33
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Control group	2DS1				2DS2				2DS3				2DS4n			
	+		-		+		-		+		-		+		-	
PD	1.53±0.38	1-2.17	1.59±0.41	1-2.63	1.68±0.38	1-2.21	1.55±0.43	1-2.63	1.55±0.40	1-2.17	1.58±0.41	1-2.63	1.54±0.38	21-sty	1.58±0.41	1-2.63
CAL	0.21±0.66	0-4.67	0.32±0.83	0-3.5	0.27±0.8	0-4.67	0.27±0.69	0-3.33	0.37±0.94	0-4.67	0.20±0.59	0-3.33	0.44±1.04	0-4.67	0.19±0.55	0-3.33
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Control group	2DS4d				2DS5				3DL1				3DS1			
	+		-		+		-		+		-		+		-	
PD	1.57±0.40	1-2.63	1.56±0.38	1-2.21	1.56±0.38	1-2.08	1.57±0.41	1-2.63	1.58±0.40	1-2.63	1.33±0.49	1-2.04	1.51±0.39	1-2.17	1.60±0.40	1-2.63
CAL	0.26±0.76	0-4.67	0.29±0.74	0-3.33	0.16±0.34	0-1.83	0.32±0.86	0-4.67	0.28±0.76	0-4.67	0		0.19±0.41	0-1.83	0.32±0.90	0-4.67
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Table 3. *KIR* genes impact on PD and CAL parameters in periodontitis patients

Patient group	2DL1				2DL2				2DL3				2DL5			
	+		-		+		-		+		-		+		-	
PD	4.32±1.20	1.71-7.38	3.38±0.80	2.5-4.13	4.33±1.18	2.25-7.17	4.26±1.19	1.71-7.38	4.30±1.15	1.71-7.38	4.35±1.46	2.42-7.13	4.25±1.14	2.42-7.13	4.37±1.24	1.71-7.38
CAL	5.28±1.87	0.5-11.5	4.67±1.72	2.16-6.00	5.21±1.60	2.0-10.17	5.21±1.68	0.5-11.5	5.19±1.62	0.5-11.5	5.36±1.71	2.17-8.83	5.07±1.49	2-10.17	5.37±1.77	0.5-11.5
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Patient group	2DS1				2DS2				2DS3				2DS4n			
	+		-		+		-		+		-		+		-	
PD	4.29±1.07	2.50-7.13	4.31±1.25	1.71-7.38	4.33±1.16	2.25-7.38	4.27±1.22	1.71-7.04	4.27±1.20	2.42-7.13	4.32±1.18	1.71-7.38	4.24±1.21	2.25-7.38	4.34±1.17	1.71-7.13
CAL	5.17±1.49	2.17-10.17	5.24±1.72	0.5-11.5	5.16±1.46	2-9.17	5.28±1.85	0.5-11.5	5.11±1.51	2-8.83	5.27±1.70	0.5-11.5	5.11±1.48	0.5-9.33	5.27±1.70	2-11.5
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Patient group	2DS4d				2DS5				3DL1				3DS1			
	+		-		+		-		+		-		+		-	
PD	4.33±1.19	1.71-7.38	4.19±1.17	2.25-6.71	4.28±1.10	2.50-7.13	4.32±1.23	1.71-7.38	4.30±1.17	1.71-7.38	4.29±1.40	2.42-7.71	4.34±1.13	2.50-7.13	4.28±1.23	1.71-7.38
CAL	5.21±1.63	2-11.5	5.23±1.64	0.5-9.17	5.18±1.58	2.17-10.17	5.23±1.65	0.5-11.5	5.20±1.64	0.5-11.5	5.37±1.52	3.33-7.38	5.25±1.52	2.5-10.17	5.21±1.71	0.5-11.5
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Table 4. *KIR* genes impact on the CAL and PD parameters in patients' subgroups according to the severity of the periodontitis

	Light + moderate	Severe	Light + moderate	Severe	Light + moderate	Severe	Light + moderate	Severe	Light + moderate	Severe	Light + moderate	Severe	Light + moderate	Severe	Light + moderate	Severe
Patient group	2DL1				2DL2				2DL3				2DL5			
	+		-		+		-		+		-		+		-	
PD	3.46±0.69	4.97±1.03			3.68±0.66	3.49±0.71	4.99±1.00	3.40±0.68	4.89±1.09	3.50±0.70	4.90±1.02	3.03±0.55	5.23±1.17	3.45±0.70	4.93±0.99	3.45±0.71	4.97±1.09
	1.71-5.79	3.00-7.35	2.5		2.92-4.13	2.25-5.79	2.92-7.13	1.71-4.79	3.00-7.38	1.71-5.79	3.00-7.38	2.42-4.25	2.92-7.13	2.42-5.00	2.92-7.13	1.71-5.79	3.00-7.38
CAL	3.84±0.78	6.29±1.27			5.50±0.50	3.84±0.78	6.30±1.20	3.79±0.80	6.23±1.36	3.84±0.79	6.24±1.29	3.71±0.84	6.46±1.13	3.81±0.68	6.14±1.11	3.83±0.92	6.40±1.41
	0.50-4.83	4.67-11.50	2.17		5.00-6.00	2.00-4.83	5.00-10.17	0.50-4.83	4.67-11.50	0.50-4.83	4.67-11.50	2.17-4.83	5.00-8.83	2.00-4.83	5.00-10.17	0.50-4.83	4.67-11.50
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed																

Patient group	2DS1				2DS2				2DS3				2DS4n			
	+		-		+		-		+		-		+		-	
PD	3.50±0.70	4.87±0.92	3.42±0.70	4.99±1.11	3.49±0.70	4.93±1.02	3.39±0.70	4.96±1.07	3.28±0.58	5.09±0.93	3.55±0.74	4.87±1.09	3.44±0.72	4.85±1.10	3.45±0.69	4.99±1.01
	2.50-5.00	3.17-7.13	1.71-5.79	2.92-7.38	2.25-5.79	2.92-7.38	1.71-4.79	3.00-7.04	2.42-4.50	3.21-7.13	1.71-5.79	2.92-7.38	2.25-4.79	3.00-7.38	1.71-5.79	2.92-7.13
CAL	3.89±0.68	6.12±1.18	3.78±0.85	6.36±1.31	3.89±0.77	6.10±1.09	3.72±0.81	6.51±1.46	3.77±0.67	6.23±1.01	3.85±0.85	6.29±1.38	3.88±0.82	6.09±1.10	3.79±0.78	6.35±1.33
	2.17-4.83	5.00-10.17	0.50-4.83	4.67-11.50	2.00-4.83	4.67-9.17	0.50-4.83	5.00-11.50	2.00-4.83	5.00-8.83	0.50-4.83	4.67-11.50	0.50-4.83	5.00-9.33	2.00-4.83	4.67-11.50
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

Patient group	2DS4d				2DS5				3DL1				3DS1			
	+		-		+		-		+		-		+		-	
PD	3.48±0.66	4.96±1.07	3.34±0.83	4.88±0.93	3.55±0.70	4.89±1.01	3.41±0.69	4.97±1.05	3.45±0.68	4.95±1.04	3.37±0.95	4.73±0.94	3.49±0.68	4.98±0.96	3.41±0.71	4.92±1.10
	1.71-5.79	2.92-7.38	2.25-5.00	3.17-6.71	2.50-5.00	2.92-7.13	1.71-5.79	3.00-7.38	1.71-5.79	2.92-7.38	2.42-5.00	3.25-6.04	2.50-5.00	3.17-7.13	1.71-5.79	2.92-7.38
CAL	3.81±0.74	6.23±1.28	3.86±0.98	6.35±1.14	3.87±0.68	6.28±1.25	3.80±0.84	6.26±1.28	3.81±0.80	6.26±1.28	4.06±0.58	6.39±1.11	3.88±0.66	6.23±1.18	3.78±0.87	6.30±1.33
	2.00-4.83	4.67-11.50	0.50-4.83	5.00-9.17	2.17-4.83	5.00-10.17	0.50-4.83	4.67-11.50	0.50-4.83	4.67-11.50	3.33-4.83	5.00-7.83	2.50-4.83	5.00-10.17	0.50-4.83	4.67-11.50
Statistical analysis	ANOVA p> 0.05 post-hoc test was not performed															

(with moderate as well as severe disease): no association between *KIR* genes and PD or CAL parameters was found.

Discussion

Chronic periodontitis is a complex multifactorial inflammatory disease that is caused by pathogenic microflora in the biofilm. Although periodontopathogens are necessary for the initiation of periodontal disease [24], a susceptible host is also needed. The immune-inflammatory response that develops in the periodontal tissues results in destruction of the periodontium [30]. It is interesting that the amount of plaque or the species of bacteria do not necessarily correlate with periodontitis severity. Hence scientists are interested in the role of genes encoding receptors and their polymorphism in host immune responses to periodontitis [24]. NK cells are postulated as potential immune cells involved in both immune and inflammatory responses causing periodontitis. NK cells express direct cytotoxicity against virus-infected cells and neoplasm transformed cells, but their role in immune regulation via cytokine secretion is also observed. NK cells belong to innate immunity but influence adaptive immunity.

Natural killer cells are primarily derived from large granular lymphocytes and display cytotoxic activity against virally infected cells and some tumor cells. NK cells express CD16 (IgG Fc receptor), CD56 or CD57, but not CD3. NK cells are typically CD4-CD8-. Their activity is enhanced by IL-1, IL-2, and interferon gamma, and is inhibited by some prostaglandins. The number of NK cells in peripheral blood has been reportedly increased in patients with periodontitis [1,12,31]. Although few if any NK cells are seen in healthy tissues, their numbers increase from health to gingivitis to periodontitis [10,13,18,25]. Typically, these cells are located subjacent to the epithelium, and commonly in association with foci of B cells or plasma cells [10,11]. Although the number of NK cells increases with disease severity [34], the proportion of these cells relative to the total lymphocyte count actually decreases. NK cells may play a role in the pathogenesis of periodontal disease through their cyto-

toxic activity against host cells. Pre-treatment of gingival fibroblasts with sonic extracts of dental plaque increases NK cell cytotoxicity against these cells. Ultrastructural changes in fibroblasts indicative of NK-mediated cytotoxicity have also been observed histologically [11]. NK cells may also regulate B-cell activity and proliferation, which is a hallmark histological feature of the advanced periodontal lesion. The cytotoxic activity of NK cells is potentiated by IFN-y. Furthermore, periodontal bacteria have been shown to induce NK-mediated cytotoxicity against cells other than fibroblasts. Surface lipopolysaccharides from Gram-negative periodontal bacteria appear to provide the major activation signal for NK-cell-mediated cytotoxicity [10,11,17].

In addition to T and B lymphocytes, natural killer cells also constitute an important part of the cell-mediated immune system. In contrast to CD8+ cytotoxic T lymphocytes, which kill infected cells in an antigen-specific manner, NK cells kill infected target cells or tumor cells in an antigen-non-specific manner. Other important cell types that are involved in cell-mediated immunity are neutrophils and macrophages which phagocytose and destroy opsonized micro-organisms. Phagocytic cells play a critical role in the defense against bacterial infections. All of the above cell types are found in the periodontal lesion, and are important in the pathogenesis of periodontal disease [14]. Several lines of evidence suggest that periodontal diseases may either result from or cause imbalances in regulation of the local immune response. This dysfunction may differentially affect T and B lymphocytes [2]. The peripheral and local immune cell profiles in patients with disease differ substantially, which suggests that the immunological response to periodontal pathogens is primarily a local phenomenon.

NK cells are key effector cells of the innate immune system, and as such are crucial in the antiviral immune response. They are multifunctional, with an ability to interact directly with infectious agents, through pattern recognition receptors, with infected cells, via expressed cell surface receptors, and with cells of the adaptive immune system via cell-cell interactions and through secretion of cytokines. Such cytokines are predominantly proinflammatory such as interferon IFN-γ or tissue growth factor TGF-β, but may in some cases be immunoregulatory. NK cell activation is controlled by a complex balance between activating and inhibitory receptors such that the net signal derived from these receptors is integrated to determine whether or not NK cell effector functions are initiated [15,33]. Many of these receptors are monomorphic and expressed on all cells. The KIR family is more likely to be responsible for generating diversity in the immune response to specific pathogens within the human population [8,35].

As KIR act to inhibit or promote the function of cells that are critical both in regulating the intrinsic and adaptive immune responses as well as acting as effector cells that may result in tissue injury, it is reasonable that they may play a role in the pathophysiology of autoinflammatory and infectious diseases such as periodontal disease. There have been several reasons to evaluate whether *KIR* genes increase the risk for periodontal disease. The activated immune system is important in the pathogenesis of periodontal disease. On the other hand, tissue damage as a response to infection could be due to activation mediated by KIR. In our study we analyzed the impact of *KIR* genes on the pocket depth (PD) and clinical attachment loss (CAL) in patients with chronic periodontitis as markers of disease clinical course. There were no statistically significant associations between *KIR* genes and these parameters in accordance with severity of the disease. Nevertheless, the impact of *KIR* on the clinical course of periodontal disease requires further investigations.

References

[1] Afar B., Engel D., Clark E.A.: Activated lymphocyte subsets in adult periodontitis. J. Periodontal. Res., 1992; 27: 126-133

[2] Amer A., Singh G., Darke C., Dolby A.E.: Spontaneous lymphocyte proliferation in severe periodontal disease: role of T and B cells. J. Oral. Pathol. Med., 1990; 19: 49-52

[3] Armitage G.C.: Development of a classification system for periodontal diseases and conditions. Ann. Periodontol., 1999; 4: 1-6

[4] Biron C.A., Nguyen K.B., Pien G.C., Cousens L.P., Salazar-Mather T.P.: Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu. Rev. Immunol., 1999; 17: 189-220

[5] Boyton R.J., Altmann D.M.: Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease. Clin. Exp. Immunol., 2007; 149: 1-8

[6] Caligiuri M.A.: Human natural killer cells. Blood, 2008; 112: 461-469

[7] Campbell K.S., Purdy A.K.: Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations. Immunology, 2011; 132: 315-325

[8] Carrington M., Martin M.P.: The impact of variation at the KIR gene cluster on human disease. Curr. Top. Microbiol. Immunol., 2006; 298: 225-257

[9] Chaushu S., Wilensky A., Gur C., Shapira L., Elboim M., Halftek G., Polak D., Achdout H., Bachrach G., Mandelboim O.: Direct recognition of Fusobacterium nucleatum by the NK cell natural cytotoxicity receptor NKp46 aggravates periodontal disease. PLoS Pathog., 2012; 8: e1002601

[10] Cobb C.M., Singla O., Feil P.H., Theisen F.C., Schultz R.E.: Comparison of NK-cell (Leu-7+ and Leu-11b+) populations in clinically healthy gingiva, chronic gingivitis and chronic adult periodontitis. J. Periodontal. Res., 1989; 24: 1-7

[11] Cobb C.M., Singla O., Theisen F.C., Shultz R.E.: Ultrastructural evidence of large granular lymphocyte (LGL) activity in lesions of chronic adult periodontitis. J. Clin. Periodontol., 1990; 17: 371-378

[12] Conigliaro P., Scrivo R., Valesini G., Perricone R.: Emerging role for NK cells in the pathogenesis of inflammatory arthropathies. Autoimmun. Rev., 2011; 10: 577-581

[13] Fujita S., Takahashi H., Okabe H., Ozaki Y., Hara Y., Kato I.: Distribution of natural killer cells in periodontal diseases: an immunohistochemical study. J. Periodontol., 1992; 63: 686-689

[14] Garlet G.P., Martins W.Jr., Ferreira B.R., Milanezi C.M., Silva J.S.: Patterns of chemokines and chemokine receptors expression in different forms of human periodontal disease. J. Periodontal. Res., 2003; 38: 210-217

[15] Ham H., Billadeau D.D.: Human immunodeficiency syndromes affecting human natural killer cell cytolytic activity. Front. Immunol., 2014; 5: 2

[16] Khakoo S.I., Carrington M.: KIR and disease: a model system or system of models? Immunol. Rev., 2006; 214, 186-201

[17] Komiyama K., Hirsch H.Z., Moro I., Umemura S., Mestecky J.: HNK-1+ (Leu-7) cells and natural killer cell activity in inflamed human gingival tissue. J. Oral. Pathol., 1988; 17: 118-123

[18] Krämer B., Kebschull M., Nowak M., Demmer R.T., Haupt M., Körner C., Perner S., Jepsen S., Nattermann J., Papapanou P.N.: Role of the NK cell-activating receptor CRACC in periodontitis. Infect Immun., 2013; 81: 690-696

[19] Kuśnierczyk P.: Killer cell immunoglobulin-like receptor gene associations with autoimmune and allergic diseases, recurrent spontaneous abortion, and neoplasms. Front. Immunol., 2013; 4: 8

[20] Laine M.L., Crielaard W., Loos B.G.: Genetic susceptibility to periodontitis. Periodontol. 2000, 2012; 58: 37-68

[21] Lange D.E., Plagmann H.C., Eenboom A., Promesberger A.: Klinische Bewertungsverfahren zur Objectivierung der Mundhygiene. Dtsch. Zahnärztl. Z., 1977; 32: 44-49

[22] Lanier L.L.: NK cell recognition. Annu. Rev. Immunol., 2005; 23: 225-274

[23] Majorczyk E., Pawlik A., Łuszczek W., Nowak I., Wiśniewski A., Jasek M., Kuśnierczyk P.: Associations of *killer cell* immunoglobulin-like receptor genes with complications of rheumatoid arthritis. Genes Immun., 2007; 8: 678-683

[24] Martelli F.S., Mengoni A., Martelli M., Rosati C., Fanti E.: VDR TaqI polymorphism is associated with chronic periodontitis in Italian population. Arch. Oral. Biol., 2011; 56: 1494-1498

[25] Muthukuru M.: Technical advance: decreased helper T cells and increased natural killer cells in chronic periodontitis analyzed by a novel method for isolating resident lymphocytes. J. Leukoc. Biol., 2012; 92: 683-692

[26] Mühlemann H.R., Son S.: Gingival sulcus bleeding - a leading symptom in initial gingivitis. Helv. Odontol. Acta, 1971; 15: 107-113

[27] Niepiekło-Miniewska W., Majorczyk E., Matusiak Ł., Gendzekhadze K., Nowak I., Narbutt J., Lesiak A., Kuna P., Ponińska J., Pietkiewicz-Sworowska A., Samoliński B., Płoski R., Szepietowski J.C., Senitzer D., Kuśnierczyk P.: Protective effect of the *KIR2DS1* gene in atopic dermatitis. Gene, 2013; 527: 594-600

[28] Parham P.: MHC class I molecules and KIRs in human history, health and survival. Nat. Rev. Immunol., 2005; 5: 201-214

[29] Parham P., Norman P.J., Abi-Rached L., Guethlein L.A.: Human-specific evolution of killer cell immunoglobulin-like receptor recognition of major histocompatibility complex class I molecules. Philos. Trans. R. Soc. Lond. B Biol. Sci., 2012; 367: 800-811

[30] Pihlstrom B.L., Michalowicz B.S., Johnson N.W.: Periodontal diseases. Lancet, 2005; 366: 1809-1820

[31] Reichert T., De Bruyère M., Deneys V., Tötterman T., Lydyard P., Yuksel F., Chapel H., Jewell D., Van Hove L., Linden J., et al. Lymphocyte subset reference ranges in adult Caucasians. Clin. Immunol. Immunopathol., 1991; 60: 190-208

[32] Seymour G.J., Gemmell E., Reinhardt R.A., Eastcott J., Taubman M.A.: Immunopathogenesis of chronic inflammatory periodontal disease: cellular and molecular mechanisms. J. Periodontal. Res., 1993; 28: 478-486

[33] Sharma R., Das A.: Organ-specific phenotypic and functional features of NK cells in humans. Immunol. Res., 2014; 58: 125-131

[34] Stelin S., Ramakrishan H., Talwar A., Arun K.V., Kumar T.S.: Immunohistological analysis of $CD1a^+$ langerhans cells and $CD57^+$ natural killer cells in healthy and diseased human gingival tissue: a comparative study. J. Indian. Soc. Periodontol., 2009; 13: 150-154

[35] Tsuchiya N., Kyogoku C., Miyashita R., Kuroki K.: Diversity of human immune system multigene families and its implication in the genetic background of rheumatic diseases. Curr. Med. Chem., 2007; 14: 431-439

[36] Van Dyke T.E., Lester M.A., Shapira L.: The role of the host response in periodontal disease progression: implications for future treatment strategies. J. Periodontol., 1993; 64: 792-806

[37] Wiśniewski A., Jankowska R., Passowicz-Muszyńska E., Wiśniewska E., Majorczyk E., Nowak I., Frydecka I., Kuśnierczyk P.: KIR2DL2/S2 and HLA-C C1C1 genotype is associated with better response to treatment and prolonged survival of patients with non-small cell lung cancer in a Polish Caucasian population. Hum. Immunol., 2012; 73: 927-931

[38] Yamazaki K., Ohsawa Y., Yoshie H.: Elevated proportion of natural killer T cells in periodontitis lesions: a common feature of chronic inflammatory diseases. Am. J. Pathol., 2001; 158: 1391-1398

The authors have no potential conflicts of interest to declare.