Analysis of genetic polymorphisms of the interleukin-1 gene in the implantoprosthetic group of patients – preliminary studies

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Abstract

One of the most frequent causes of failures in the implantoprosthetic treatment, often leading to the loss of implant is bone atrophy around the implant. There are some reports of an inhibitory influence of IL-1 on bone formation process and density of the bone tissue.

The aim of the study was the analysis of genetic polymorphisms of the interleukin-1 gene in patients treated or qualified for the prosthetic treatment with endosseous implants use.

Material for the study was full blood sampled for anticoagulant (EDTA), obtained from 20 patients with full of partial loss of teeth, aged 21-60, who were treated implantologically or were waiting for the treatment with Branemark implants use (Nobel Biocare AB).

Analysis of performed tests showed that in case of patients with IL-1B-511 2/2 genotype marginal bone loss >0.5 mm occurs more frequently than in case of patients with IL-1B-511 1/2 and IL-1B-511 1/1 genotypes.

Key words: *marginal bone loss, gene, polymorphism, interleukin-1, dental implant.*

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Introduction

One of the most frequent causes of failures in the implantoprosthetic treatment, often leading to the loss of implant, is bone atrophy around the implant. Research in recent years has shown that factors as: cigarette smoking, metabolic diseases, poor quality of bone of the alveolar process, improper loading of implant may influence the osseointegration process, impaired healing and increased the marginal bone loss around the implant. Some research projects significantly emphasize the influence of cigarette smoking on the worse prognosis in the implantological treatment. This habit is regarded as one of the most important factors affecting the percentage decrease of successful cases in the implantological treatment [1]. Tobacco addiction influences the increase in a frequency of periimplantitis occurrence, chronic mucosal inflammation and bone resorption around implants. After stage II of surgery in cigarette smokers a greater tendency to lose the marginal bone is observed, particularly in the first years, as compared to non-smoking patients [2]. Bain and Moy proved the correlation between the increase of failures in the implantological treatment and an amount of tobacco provided to the organism. It should be emphasized that not only factors as tobacco smoking or metabolic diseases modifying the immunological potential of the host have an influence on the healing process and implants osseo-integration [3-5]. An equally significant role in regulating the immunological response is more frequently attributed to the genetic factor, because by means of the phenotypic expression, it acts since the connection of parent gametes until death.

A particular attention in recent literature was paid to one of cytokines secreted by macrophages – IL-1. There are some reports of an inhibitory influence of IL-1 on bone

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formation process and density of the bone tissue. At the same time, accompanying inflammation with bacteria residing in the subgingival plaque that stimulate the production of proinflammatory cytokines, among others IL-1, may contribute to the occurrence of chronic parodontosis and inflammation of periimplant tissues in form of peri-implantitis. The basic role in etiopathogenesis of chronic parodontosis is played by Gram-negative anaerobic bacteria, which can directly destroy parodontium tissues by means of the action of enzymes, endo- and exotoxines, as well as the products of metabolism. Moreover, bacteria initiate the mechanisms of degradation of parodontium tissues. Colonization of tooth surface and deepening gingival pocket with increasing number of Gram-negative bacteria causes the increase in the level of bacterial liposaccharides. LPS stimulate the stationary cells in parodontium (keratinocytes, fibroblasts, endothelial cells) and the cells of the inflammatory infiltration (neutrophils, macrophages, PMN leukocytes, lymphocytes) to synthesis and secretion of enzymes, a wide spectrum of pro- and anti-inflammatory cytokines (IL-1β, TNF-α) and lipid mediators of inflammatory condition (prostaglandins, leukotriens and lipoxins). The key role in etiopathogenesis of the parodontium disease is played by the proinflammatory cytokines, and among them, interleukin-1β. Interleukin-1β stimulates synthesis of phospholipase A_2 and production of prostaglandin E_2, which together with interleukin 6, stimulates osteoklasts to destroying alveolar process. Moreover, IL-1β and other pro-inflammatory cytokines (e.g. TNF-α), as well as directly bacterial liposaccharides, stimulate cells of inflammatory infiltration and cells of parodontium to synthesis and secretion of enzymes destroying collagen matrix, called matrix metalloproteinases (MMPs) and decrease the secretion of their tissue inhibitors (TIMPs, tissue inhibitors of metalloproteinases) [6, 7].

Mechanism of reciprocal influence of cytokines and enzymes, as well as the effect of genes polymorphism coding interleukins on the idiopathic marginal bone loss is the subject of many research projects, due to the fact of probability of its significant role in prognosis of treatment outcome in patients qualified for the implantoprothetic therapy.

Aim of the study

The aim of the study was the analysis of genetic polymorphisms of the interleukin-1 gene in patients treated or qualified for the implantoprosthetic treatment with endosseous implants use.

Material and Methods

Material for the study was full blood sampled for anticoagulant (EDTA), obtained from 20 patients with full of partial loss of teeth, aged 21-60, who were treated implantologically or were waiting for the treatment with Branemark implants use (Nobel Biocare AB). 16 patients were subjected to the two-stage implantation procedure, in which the total of 48 implants were placed. The blood samples were collected after half a year from the stage II surgery in this group of patients. Temporary removable prostheses, lined with tissue conditioners material, were given to the patients after two weeks from the stage I surgery resulted elimination of prostheses destructive influence on the healing process. Osseointegration process, loss of marginal bone around the implant and presence – or absence – of chronic peri-implantitis was estimated on the basis of anamnesis, clinical examination, radiological imaging. The depth of gingival pockets was evaluated by Florida probe use.

1 ml of blood was taken from all patients, and from this blood DNA genome was isolated by means of column technique, leaning on the nucleic acids binding by ion-exchangeable substances (kits DNA Blood Midi by DNA Gdańsk). From 1 ml of systemic blood in the viscosity gradient Ficol (1.077) leukocytes were isolated in order to more carefully dispose of the remaining morphotic blood elements. Isolated leukocytes were subjected to the DNA isolation procedure, in accordance with the instruction attached to the kit. Isolated genome DNA was the matrix for the PCR reaction, performed with the use of three different pairs of starters, enabling the amplification of DNA fragments, among which, analysed polymorphic changes were located. The reactions were performed in 50 ul volume. The analysis was performed for three polymorphisms of IL-1α and IL-1β genes (IL-1A-889, IL-1B-511, IL-1B+3954). For each of analysed polymorphisms, the appropriate pairs of starters were used, which made it possible to obtain DNA fragments containing the defined polymorphic place. At the next stage products of the PCR reaction were subjected to digestion by means of the appropriate restrictive enzymes. Fragments of DNA, obtained after digestion, were electrophoretically separated in agarose gel and dyed with ethidine bromide (Table 1). Reagents made by Applied Biosystems were used in all reactions.

Differences were regarded statistically relevant by $P<0.05$.

Results

After stage II surgery the idiopathic marginal bone loss was found in 4 patients around 6 implants >0.5 mm, some symptoms of peri-implantitis appeared in 4 patients. This group was composed of patients with systemic osteoporosis, smokers, denture stomatitis patients in stage II acc. to Newton classification, women in postmenopausal period and subjected to a surgery due to the neoplasm within the digestive tract. All patients with marginal bone loss around the implant <0.5 mm and free from peri-implantitis symptoms were control group. A separate group was created for patients waiting for the implantoprosthetic treatment (Table 2).

Table1. Summary of conditions used for the restriction fragment lenght polymorphism method

Polymorphism	PCR primers	PCR conditions		Restricion fragment product sizes (bp)		
		denaturation	primer annealing	elongation	Allel 1	Allel 2
IL-1A-889	TTACATAATGAGCCTTCCATG AAGCTTGTTCTACCACCTGAACTAGGC	94°C, 7 min 30s	53°C, 30 s	72°C, 30 s	83pz+16pz	99pz
IL-1B-511	GTTTAGGAATCTTCCCACTT TGGCATTGATCTGGTTCATC	94°C, 7 min 30 s	51°C, 30 s	72°C, 30 s	190pz+114pz	z304pz
IL-1B+3954	GCTTTTTTGCTGTGAGTCCCG CTCAGGTGTCCTCGAAGAAATCAA	94°C, 4 min 30 s	63°C, 30 s	72°C, 30 s	108pz+86pz	194pz

Table 2. Characteristics of patients in relation to age, sex, number of implants, marginal bone loss

	Patients with marginal bone loss >0.5 mm (n=4)	Patients with peri-implantitis (n=4)	Control group n=8	Patients waiting for the treatment (n=4)
Age	45.6	52.3	48.3	47.1
Sex female/male	3/1	2/2	5/3	2/2
Number of implants	6	7	35	0

1/1 – homozygotic IL-1 genotype negative; 1/2 – heterozygotic IL-1 genotype positive; 2/2 – homozygotic IL-1 genotype positive.

Table 3. IL-1 genotypes distribution in surveyed group of patients

	Symptoms of peri-implantitis (bleeding, depth of gingival pocket, paradontium tissues status)		Idiopathic marginal bone loss	
	–	+	<0.5	>0.5
Number of patients	12	4	12	4
Dominant genotype	IL-1B-3954 1/2 IL-1B-3954 2/2	IL-1B-3954 1/1	IL-1B-511 1/2 IL-1B-511 1/1	IL-1B-511 2/2

Analysis of performed tests showed that in case of patients with IL-1B -511 2/2 genotype marginal bone loss >0.5 mm occurs more frequently than in case of patients with IL-1B-511 1/2 and IL-1B-511 1/1 genotypes. In the group of people with IL-1B-3954 1/1 genotype more frequent occurrence of peri-implantitis than in patients with IL-1B-3954 1/2 and IL-1B-3954 2/2 genotypes was observed (Table 3). Analysis of IL-1A-889 gene polymorphisms did not show a dependence of idiopathic marginal bone loss occurrence around the implant or per-implantitis in patients with IL-1A-889 1/1, IL-1A-889 1/2, IL-1A-889 2/2 genotypes.

Discussion

The examinations presented in our study proved the relationship between polymorphic changes of the IL-1B gene and more frequent occurrence of idiopathic marginal bone loss around the implant in the surveyed group of patients.

Hitomi Shimpuku et al. (2003) in their study on 39 patients with 251 endosseous implants estimated early marginal bone loss around the implant depending on the patients genotype, taking into consideration age, sex, quality of bones, tobacco addiction and hormonal changes in the postmenopausal period in women. Results of their exprimments suggest the relationship between the occurrence of early marginal bone loss in patients with IL-1B-511 – 2/2 genotype in comperision to patients with IL-1B-511 – 1/1 and IL-1B-511 – 1/2 genotype.

The authors claimed that the presence of IL-1B-511 – 2/2 genotype was a strong risk factor of bone atrophy in patients treated implantologically, irrespectively of other factors, such as the age, sex, quality of bones, tobacco addiction, or hormonal changes in the postmenopausal period

in women [8]. Cattabriga et al. (2001) estimated the role of genetic polymorphism of the IL-1 in the speed of bone loss in 60 non-smoking patients with the perodontium disease (the experiment was carried out on the basis of the 10-year-long observation of cases). The frequency of the positive genotype occurrence in the surveyed group was 38.8%. No relationship was found, however, between the genotype and the number of lost teeth. Moreover, the authors' conclusion was the observation that in the estimation of particular cases, the genotype IL-1 in connection with the initially found loss of bone was useful in predicting the outcome of the implantological treatment [9]. Socransky et al. (2000) after examining 108 generally healthy patients with the perodontium disease found out that in patients with the positive genotype, high titres of bacteria of the "red" or "orange" complex occurred more frequently (Socransky et al. 2000), thus, the bacteria particularly harmful for the periodontium tissues, contributing to bone atrophy around implants in the course of periimplantitis. What is more, those authors suggested that this relationship resulted to a greater extent from the increased number of bacteria, and not from the disturbances of their proportion on the dental plaque [10].

Persson et al. (2003) estimated the outcomes of the periodontological treatment in relation to the polymorphism of the gene IL-1. 224 patients were examined, whose clinical condition of the periodontium was estimated, as well as tobacco addiction, radiological level of the bones, and the genotype. On this basis, the risk of the periodontium disease was assessed (Periodontal Risk Assessment model – PRA). The tested group was observed, and results of this observation claimed that the IL-1 polimorphism significantly influenced the unfavorable change of total PRA, which should be taken into consideration when planning the implantoprosthetic treatment, in particular, in patients with perdiodontium diseases and tobacco addiction. The prediction factors of the unfavorable PRA change in the future were recognised as the initial values of the bleeding indicator, the initial bone loss disproportionate to the patient age, sex and genotype [11].

Tests presented in this thesis, as well as reports from literature, suggest a possible participation of the genetic factor in the occurrence of failures during the treatment with endosseous implants use. Taking into consideration the analysis of polymorphic changes of the IL-1 gene in diagnostic procedures before the planned implantoprostehtic treatment, may make it easier in the future to estimate the course of the osseointegration process, the healing period after implants fixture, as well as predicting of the outcome of the prosthetic rehabilitation in patients qualified for the implantoprosthetic treatment.

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