DOI 10.26724/2079-8334-2023-2-84-7-11
UDC [616.151.5+616.314-089.843]:575.1

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GENETIC PREDISPOSITION TO PERI-IMPLANTITIS AND THROMBOSIS IN DENTAL IMPLANTS

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The study was dedicated to determining the association of interleukin-1 (IL1B C3953T), tumor necrosis factor (TNF G-308A) and platelet fibrinogen receptor (ITGB3 T1565C) polymorphisms with early complications in dental implantation. The study group consisted of 22 patients aged 25–55 years with dental implant complications and loss. The control group consisted of 14 patients with long-term dental implants and no implant complications. The distribution of alleles and genotypes of the single nucleotide polymorphism T1565C of the ITGB3 rs5918 gene was different between the study groups. The minor C allele of the T1565C polymorphism of the ITGB3 rs5918t gene increased the risk of thrombosis in the T<>C allele model. The heterozygous TC genotype was also associated with an increased risk of thrombosis, which is consistent with the dominant inheritance model of CT+CC<>TT.

Key words: dental implantation, oral health, genetic polymorphism, thrombosis, polymerase chain reaction.

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ГЕНЕТИЧНА СХИЛЬНІСТЬ ДО ПЕРІІМПЛАНТИТУ І ТРОМБОУТВОРЕННЯ ПІД ЧАС ДЕНТАЛЬНОЇ ІМПЛАНТАЦІЇ

Дослідження присвячене визначенню зв'язку поліморфізмів генів інтерлейкіну-1 (IL1B C3953T), фактору некрозу пухлини (TNF G-308A) і тромбоцитарного рецептора фібриногену (ITGB3 T1565C) з ранніми ускладненнями при дентальній імплантації. Досліджувана група налічувала 22 пацієнти віку 25–55 років з ускладненнями і втратою дентального імпланта, контрольну групу становили 14 осіб з довгостроковими дентальними імплантами і без ускладнень під час встановлення. Виявлено відмінність між досліджуваними групами за розподілом алелів і генотипів однонуклеотидного поліморфізму T1565C гена ITGB3 rs5918. Мінорний С-алель поліморфізму T1565C гена ITGB3 rs5918т підвищував ризик тромбоутворення в алельній моделі Т <> С. Гетерозиготний генотип ТС також асоціювався з підвищеним ризиком розвитку тромбоутворення, що відповідає домінантній моделі успадкування СТ+СС<>ТТ.

Ключові слова: дентальна імплантація, здоров'я порожнини рота, генетичний поліморфізм, тромбоутворення, полімеразна ланцюгова реакція.

The study is a fragment of the research project "Correction of pathogenetic mechanisms of disorders of carbohydrate and lipid metabolism in the body and tissues of the oral cavity in patients depending on environmental and nutritional factors affecting carbohydrate and lipid metabolism", state registration No. 0118U006966.

Dental implants have become a common and effective method of treatment for patients with total and partial adentia. The 10-year survival rate of successfully placed implants is over 93 % [2].

At the same time, the complication rate ranges from 10 % to 15 %. The importance of the "complication rate" was highlighted in a 10–16-year clinical study that reported an implant biologic complication rate, namely peri-implantitis, of approximately 17 % [3].

Biomaterials implanted into the human body initially interact with blood. Consequently, the exposed surface of the biomaterial will be coated with the proteins of the host plasma. Fibrinogen and its byproduct, fibrin, play a crucial role in the initial phase of wound healing, especially during blood clotting, cell recruitment and angiogenesis. When the vessel wall is damaged, the clotting process is activated. Platelets aggregate (stick together) and close the injured area at the very beginning of the clotting process. Aggregation occurs due to the presence of integrin receptors on the surface of platelets, which are transmembrane heterodimeric complexes that interact with the extracellular matrix and transmit various intercellular signals [14].

Brouwers J.E. et al. data suggest that blood composition and fibrin structure can be critical modulators of implant stability [3]. The fibrin network plays a key role in early wound healing and functions as a framework for cell ingrowth and as a cytokine reservoir [11].

Implant rejection occurs in approximately 1.9–3.6 % of patients with dental implants. Implant rejection can be divided into two types depending on the timing: early and late [15].

Initial tissue damage around the implant causes an inflammatory response mediated by innate immune cells such as macrophages, dendritic cells, mast cells and neutrophils.

Macrophages are the primary cells of the innate immune response to implants. When the body is exposed to implant material, macrophages are the primary phagocytes that are activated in the early stage of inflammation. They play an indispensable role in the osseointegration of implants to the recipient host and determine the fate of the implant. Early rejection occurs before the connection to the abutment. The oral microflora after implant prosthetics changes and the patient is prone to develop inflammatory processes, which negatively affects the functioning of prostheses and implants [6]. Changes in immune status, in particular cytokine production, play a major role in the development of complications such as peri-implantitis and mucositis. Implants can stimulate macrophages to release interleukin-1 (IL-1) and tumor necrosis factor α (TNFα), which are strong proinflammatory cytokines [8]. High cytokine activity in bone metabolism increases the loss of bone mass around the implant, leading to its rejection [4].

Genetic polymorphisms can modify the expression and production of IL-1 and TNFα, ITGB3, affect the immune response and susceptibility to inflammation and thrombosis. Mutations of these genes can cause abnormal inflammatory and resorptive responses that reduce osseointegration of dental implants. Detection of the specific genotypic profile of certain SNPs in dental implant patients will help to assess the level of individual risk and establish appropriate preventive measures.

The purpose of the study was to establish the association of interleukin-1 (IL1B C3953T), tumor necrosis factor (TNF G-308A) and fibrinogen receptor (ITGB3 T1565C) polymorphisms with early complications in dental implants.

Materials and methods. The study group consisted of 22 patients aged 25–55 years with dental implant complications and loss. The control group consisted of 14 patients with long-term dental implants and no implant complications. Dental examination was conducted in the dental office at the Department of Epidemiology and Prevention of Major Dental Diseases, Pediatric Dentistry and Orthodontics of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS").

DNA isolation from buccal epithelial cells was performed according to a modified method using Chelex [12]. Allelic variants of polymorphisms IL1B C3953T rs16944, TNF G-308A rs1800629, ITGB3 T1565C rs5918 were assessed by allele-specific PCR method. Amplification of the studied regions of the genes was carried out in parallel in two test tubes (Eppendorf) for the normal and mutant allele of each gene in 20 μl of a buffer solution with the addition of 100 nM of each pair of allele-specific oligonucleotide primers (sets SNP-express-EF NPF "Litekh"), with the addition of 100–150 ng of DNA. ДНК. Amplification was performed on a "Labcycler" thermal cycler (SensQuest, Germany). Amplicons were visualized by electrophoresis in a 2 % agarose gel, prepared on single tris-acetate buffer (1xTAE) at 100V for 45 min. DNA pUC19: Msp1 was used as a molecular weight marker. The agarose gel was stained with ethidium bromide and visualized in transmitted ultraviolet light.

Statistical processing of the obtained results including the assessment of the association of genotypes and alleles with the risk of periodontitis by the Pearson $\chi 2$ method, was carried out using the DeFinetti genetic statistics program on the website of the Institute of Genetics (Munich, Germany). Associations were characterized by odds ratio (OR) with 95 % confidence interval and Pearson's $\chi 2$ test. The difference was considered to be statistically significant at $p<0.05$ [1].

Results of the study and their discussion. Genotyping of rs16944 IL1B C3953T, rs1800629 TNF G-308A polymorphisms was performed in the group of patients with dental implant complications and loss (experimental group, n=22) and the group of patients with long-term dental implants and without placement complications (control, n=14). Differences between the groups in the distribution of allele and genotype frequencies were analyzed in the study groups (Table 1).

The proinflammatory cytokine interleukin-1 (IL-1) is a key mediator of the inflammatory process. The C3953T polymorphism of the interleukin 1 (IL1B) gene has been studied in dental implant patients with dental implant complications and dental implant loss and in a control group of patients with long-term dental implants and without implant complications.

The study showed that the frequency of C and T alleles of this gene were presented in the ratio of 0.545 and 0.455 in the main group, 0.500 and 0.500 in the control group. Distribution of genotypes of polymorphism C3953T of interleukin 1 gene (IL1B) in the studied sample of patients was the following: functional C/C genotype was 0,455 in the main group and 0,364 in the control group. The frequency of the heterozygous variant of the IL1B gene among the examined patients in the main group is lower than in the

control group 0.272 and 0.364, respectively. The frequency of the minor homozygous variant of the IL1B 3953T gene was the same in the main and control groups – 0.272. Differences between the studied groups in the distribution of genotype and allele frequencies of the rs16944 IL1B C3953T polymorphisms were not statistically reliable.

Table 1

Distribution and comparison of allele and genotype frequencies of rs16944 IL1B C3953T, rs1800629 TNF-α G-308A polymorphisms in patient groups

Polymorphism	rs16944 IL1B C3953T				
Genotype, allele	Alele C	Alele T	CC	CT	TT
Case, frequency	0.545	0.455	0.455	0.272	0.272
Control, frequency	0.500	0.500	0.364	0.364	0.272
Comparison of frequencies	C<>T	–	CT+TT<>CC *DM*	TT<>CC+CT *RM*	–
OR (95 % CI)	0.833 (0.218–3.190)	–	1.313 (0.189–9.102)	0.500 (0.068–3.696)	–
χ^2 p-value	0.071 p>0.05	–	0.076 p>0.05	0.076 p>0.05	–
Polymorphism	rs1800629 TNF-α G-308A				
Genotype, allele	Alele G	Alele A	GG	GA	AA
Case, frequency	0.590	0.410	0.455	0.272	0.272
Control, frequency	0.642	0.358	0.571	0.143	0.285
Comparison of frequencies	G<>A	–	GA+AA<>GG *DM*	AA<>GG+GA *RM*	–
OR (95 % CI)	1.246 (0.312–4.977)	–	1.600 (0.237–10.809	0.938 (0.114–7.729)	–
χ^2 p-value	0.756 p>0.05	–	0.012 p>0.05	0.311 p>0.05	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model. Significant values of the odds ratio (95 % CI) and values of p<0.05 are highlighted in bold.

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that also plays an important role in bone remodeling and homeostasis. Study of the TNF-α rs1800629 gene polymorphism G-308A showed that the frequency of the functional G allele was lower in the group of patients with dental implant complications and loss of a dental implant as compared to the control group 0.590 and 0.642, respectively. The same pattern was observed in the homozygous functional GG genotype: the main group 0.455 and the control group 0.571. At the same time, the frequency of the minor allele A was higher in the main group 0.410 compared to the control group 0.358. There was also an excess in the frequency of the heterozygous GA genotype in the experiment compared to the control: 0.272 and 0.143, respectively. The frequency of the minor homozygous AA genotype was practically the same: 0.272 in the main group and 0.285 in the control group. However, despite the tendency for the prevalence of the functionally deficient allele and genotype in patients with dental implant complications, no reliable difference in the frequencies of alleles and genotypes was detected.

Genotyping of the rs5918 ITGB3 T1565C polymorphism was performed in the group of patients with dental implant complications and loss (n=22) and the group of patients with long-term dental implants and without insertion complications (n=14). Differences between the groups in the distribution of allele and genotype frequencies were analyzed in the studied groups (Table 2).

Table 2

Distribution and comparison of allele and genotype frequencies of the rs5918 ITGB3 T1565C polymorphism in patient groups

Polymorphism	rs5918 ITGB3 T1565C				
Genotype, allele	Alele T	Alele C	TT	TC	CC
Case, frequency	0.636	0.364	0.455	0.364	0.181
Control, frequency	1.000	0.000	1.000	0.000	0.000
Comparison of frequencies	T<>C	–	TC+CC<>TT *DM*	CC<>TT+TC *RM*	–
OR (95 % CI)	16.000 (1.985 –128.988)	–	8.400 (1.756–93.349)	1.556 (0.116–20.855)	–
χ^2 p-value	10.601 p<0.05	–	5.029 p<0.05	0.112 p>0.05	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model. Significant values of the odds ratio (95 % CI) and values of p<0.05 are highlighted in bold.

A difference was revealed between the studied groups according to the distribution of alleles and genotypes of the single nucleotide polymorphism T1565C of the ITGB3 rs5918 gene. The frequency of the functionally complete T rs5918 allele in the control group of patients with long-term implants exceeded that in the main group whose patients had complications and implant loss: 1.00 and 0.636, respectively. The same trend was found in the homozygous TT genotype.

The C allele of the T1565C polymorphism of the ITGB3 gene rs5918t increased the risk of thrombosis in the T<>C allele model, OR=16.000 (95 % CI 1.985 to 128.988) $\chi 2=10.601$ $p<0.05$. The heterozygous TC genotype was also associated with an increased risk of thrombosis, consistent with the dominant CT+CC<>T model of inheritance, OR=8.400 (95 % CI 1.756–93.349) $\chi 2=5.029$ $p<0.05$. The mutant homozygous CC genotype tends to clot, but the differences between the study groups were not statistically significant. OR=1.556 (95 % CI 0.116–20.855).

IL-1 is a key inflammatory cytokine mediating the immune response and bone metabolism in dental implants [7]. It plays a crucial role in the process of osseointegration by stimulating the production of prostaglandins (e.g., prostaglandin E2) associated with enhanced bone resorption. Mutant alleles have been found to increase transcriptional activity, leading to the hyperexpression of proinflammatory cytokines [5]. SNPs in IL1B (c.3953C> T) are associated with increased production of this cytokine, which leads to a more severe course of inflammation and severe periodontitis, and may also be the cause of chronicity of the process. It was shown that the level of IL-1β in the serum was significantly higher in rs16944 TT genotype carriers than in the CC genotype [13]. A proinflammatory cytokine that also plays an important role in bone remodeling and homeostasis is tumor necrosis factor-α (TNF-α), which inhibits osteoblast proliferation and activates osteoclastogenesis at an early stage when bone marrow-derived macrophages are still osteoclast precursor cells [9]. TNF-α is a pleiotropic cytokine that can enhance the host defense mechanism by mediating inflammation and enhancing cellular immune function. At the same time, it can induce various pathological conditions, causing tissue damage (septic shock syndrome, cachexia, autoimmune diseases, rheumatoid arthritis and meningococcal sepsis. There are some studies reporting conflicting results about increased levels of these cytokines. For example, a meta-analysis of combined genetic studies showed no increased risk or protection in dental implant failure due to DNA variations in IL1B, IL10, and TNFα in the study groups compared to control groups [10]. Our studies of IL1B and TNFα cytokine gene polymorphisms showed that differences between the study groups in the distribution of genotype and allele frequencies of the rs16944 IL1B C3953T polymorphisms were not statistically reliable. When comparing studies and analyzing the importance of a particular genetic polymorphism, the ethnicity of the population being studied must be considered, as the prevalence of such polymorphisms in the general population can vary significantly, as well as other factors such as a history of periodontitis, smoking, and oral hygiene. Combined analysis of patient-level data for mixed factors and genotypes will allow us to examine gene-environment interactions and shed light on the independent role of a single polymorphism in the development of peri-implant disease. Altogether, the results of various cytokine and dental implant studies show that titanium particles stimulate the expression and secretion of proinflammatory cytokines not only IL-1, TNF-α, but also IL-17, IL-6, IL-8 and IL-2 [2]. These activations may also be associated with loss of osseointegration and implant rejection. The integrin subunit beta 3 (ITGB3) is a membrane glycoprotein known as platelet glycoprotein IIIa (platelet glycoprotein GPIIIa). On the membrane of platelets, GPIIIa forms a complex with GPIIb, which is a platelet receptor for fibrinogen as well as Willebrand factor and fibronectin and is a receptor that mediates platelet aggregation. In fact, this receptor plays an important role in the regulation of platelet adhesion and aggregation, which is the final process in the formation of platelet mass at the site of vascular injury. The DNA region of the ITGB3 gene in which thymine (T) can be replaced by cytosine (C) at position 1565 is designated as the T1565C genetic marker. Allele C is quite widespread in the European population and occurs in 13 %. As a result, the biochemical properties of GPIIIa protein change, where the amino acid leucine is replaced with proline at position 59 (Leu59Pro). In the case of variant C polymorphism, platelets acquire an increased propensity for aggregation, so carriers of this variant have an increased risk of thrombosis. According to the results of our study, the minor C allele of the T1565C polymorphism of the ITGB3 rs5918t gene increased the risk of thrombosis during dental implantation, which led to complications and implant rejection.

Conclusions

1. Our studies of cytokine gene polymorphisms IL1B and TNFα showed that differences in the distribution of genotype and allele frequencies between the group of patients with dental implant complications and dental implant loss and the control group of patients with long-term dental implants and without dental implant complications are not statistically reliable.

2. According to the results of our study, the minor C allele of the T1565C polymorphism of the ITGB3 gene rs5918t increased the risk of thrombosis during dental implantation, leading to complications and implant rejection. Detection of a specific genotypic profile of certain SNPs in patients undergoing dental implantation will help to assess the level of individual risk and establish appropriate preventive measures.

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Стаття надійшла 24.05.2022 р.