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RITA DE CÁSSIA BROKER

Analysis of *RANKL/RANK/OPG* polymorphisms and clinical variables in early dental implant loss: a case-control association study

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Doutora em Odontologia, Área de Concentração Biociências

Orientador: Prof[a]. Dr[a]. Paula Cristina Trevilatto

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TERMO DE APROVAÇÃO

RITA DE CÁSSIA BROKER

ASPECTOS CLÍNICOS E POLIMORFISMOS NOS GENES *RANKL*, *RANK* E *OPG* E A PERDA DE IMPLANTES DENTÁRIOS

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos parciais para a obtenção do Título de **Doutor em Odontologia**, Área de Concentração em **Biociências**.

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SUMÁRIO

ARTIGO EM INGLÊS

Journal: Clinical implant dentistry and related research.

Analysis of *RANKL/RANK/OPG* polymorphisms and clinical variables in early dental implant loss: a case-control association study

Running Title: Factors affecting early dental implant loss

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Abstract

- **Background:** Previous research evaluated the association between clinical and genetic factors and the early loss of dental implants.
- **Objective:** The purpose of this study was to investigate the association between clinical variables, as well as polymorphisms in *RANKL* (rs1054016), *RANK* (rs1805034), and *OPG* (rs2073618, rs3102724) genes, and the loss of osseointegrated dental implants.
- **Materials and methods:** Patients (n=360) were divided into two groups: control group (C, n=238**)**, defined as patients who did not lose any implants, and study group (S, n=122**)**, defined as patients who lost at least one implant of at least 6 months. After the DNA collection and purification, genotypes were determined using real-time PCR. For bivariate and multivariate statistical analyses, *p* values <0.05 were considered.
- **Results:** After multivariate analysis, a higher number of installed implants (*p*=0.000), the shortest implant length (*p*=0.040), the G allele of rs2073618 in the dominant (*p*=0.004) and additive (*p*=0.001) models and the allele G of rs3103724 in the dominant model (*p*=0.015), both in the *OPG* gene, were associated with the loss of dental implants.
- **Conclusion:** A higher number of installed implants, shorter implant length, and the allele G of rs2073618 and allele G of rs3102724 in the *OPG* gene were significantly associated with early dental implant loss.

Key words: dental implant loss, *RANKL, RANK, OPG*, polymorphisms

1 - Introduction

Throughout history, one of the greatest challenges in dentistry was to recover functions associated with the loss of teeth – mastication, pronunciation, and esthetics. Among older adults, tooth loss is one of the parameters that has the highest effect on quality of life, only behind the body aches, affecting self-esteem, social relationships, and general well-being.[1] Therefore, there is an increasing demand for dental implants to replace one or more missing teeth lost due to various circumstances. As a result, it is estimated that around one million of dental implants are placed each year in Brazil.[2]

Even though high success rates (from 83% to 98%) are already established for the implant placement technique[3,4], failures still occur in approximately 2.5% to 6.5% of cases.[5-9] This relatively low rate of implant failure becomes highly relevant, considering the number of people worldwide choosing this technique, as well as the financial investment and high expectations. Isolated factors cannot explain totally the causes of these losses, since implant failure is considered to be a complex condition[6]. Other parameters have also been shown to contribute to the loss of dental implants, including surgical technique, the absence of post-operative care, smoking, oral hygiene, periodontal conditions, systemic diseases, bone quantity and quality, and antidepressant intake.[8-10] Furthermore, several studies have demonstrated that genetic factors, including polymorphisms in *MMP-1*[11], *IL1RN*[12], *IL4*[15], *MMP-8*[17], *LTA*[18], and *VDR* genes[19], could influence osseointegration, resulting in implant loss.[11-16]

Implant failure can occur early, before the osseointegration process, or later, after the load is placed.[8,20] Independent of the timing, multiple factors are involved in implant failure.[6,21] It has been proposed that the disruption of the balance between microbiota and the host immune response is one of the main mechanisms that leads to periodontal disease and the loss of implants.[22] Inflammation plays a fundamental physiological role in tissue repair; however, a dysregulation of the immune response can cause tissue destruction.[23] Several factors that are induced during the immune response, including those involved in bone regulation, may increase individual susceptibility to the disease.[24]

Bone remodeling is a complex network of hormones and cytokines that are secreted in response to stimulation or injury.[25] The RANKL/RANK/OPG system directly regulates osteoclast differentiation, activation, and function, and this regulation is fundamental to the bone remodeling process.[26,27] RANKL is an essential protein that

activates osteoclastogenesis by binding to its cognate receptor RANK; it plays an important role in the maintenance of bone homeostasis, as well as in the development of pathogenic bone resorption. The *RANKL* gene is located on chromosome 13q14.[26,28-30] RANK is a type I transmembrane protein, expressed by dendritic cells, B and T lymphocytes, and osteoclast progenitor cells. The activation of RANK, by binding to its ligand RANKL, triggers a cascade of signaling pathways resulting in osteoclast differentiation and increased bone resorption. The *RANK* gene is located on chromosome 18q22.1.[31,32] OPG is a decoy receptor with a high affinity for RANKL, therefore, preventing binding RANKL to RANK and resulting in decreased osteoclast differentiation and reduced bone resorption. OPG is expressed by osteoblasts, osteocytes, and fibroblasts. The *OPG* gene is located on chromosome 8q24.[29,33]

Single nucleotide polymorphism (SNP) is defined as a change of one nucleotide in the DNA sequence, where the rarest allele has a frequency greater than 1% in the population. SNPs that occur in coding regions can either change the sequence of amino acids (non-synonymous SNPs), or do not affect the amino acid sequence (synonymous SNPs). SPNs in genes responsible for modulating the inflammatory response may be one of the factors that impact the loss of dental implants.[16] The aim of this study was to investigate whether there is a link between the clinical parameters, SNPs in *RANKL, RANK, and OPG* genes, and the loss of dental implants.

2 - Material and Methods

2.1 - Sample selection

Medical records of patients (n=5,734) who received dental implants (NEODENT Osseointegrable Implant, Curitiba-PR, Brazil) at the Latin-American Dental Research Institute (Ilapeo, Curitiba-PR, Brazil) between 1996 and 2017, were examined. Even though it is a cross-sectional observational study with a 21-year interval, the team of teachers at Ilapeo remained cohesive, maintaining standard protocols regarding surgical procedures and types of implants installed. This study was divided into two stages: (1) 1996–2006 and (2) 2007–2017, the later with the aim of increasing the number of the sample. Patients who agreed to participate in the study were informed about the details of the research study, and were asked to sign the free and informed consent form approved by the Institutional Review Board of the Ethical Committee in Research at PUCPR, Brazil, protocol 2.715.180.

Out of 5,734 patients who received dental implants, 263 patients (4.58%) lost at least one (1) implant in less than six months (early loss), as reported in the medical records. Out of these 263 individuals, 122 (122/263, 46.4%) could be contacted, and, therefore, were included into the study group (S). For the control group (C), 238 individuals were selected from the rest of patients who did not lose any implants and whose implants were functional for at least six months. Therefore, the sample size contained a total of 360 patients: 122 in the study group and 238 in the control group.

The variables age, sex, and smoking were matched between groups C and S, in the ratio of 2:1 because they are already known as confounding factors (Table 1).

The exclusion criteria used for this study were as follows: patients with orthodontic appliances, HIV infection, pregnant or lactating women, and the presence of necrotizing periodontal diseases.

Clinical parameters were collected from the medical records which were updated at the time of research. The socio-economic data, systemic disease, rheumatoid disease, diabetes mellitus, osteoporosis, antihypertensive medication, medical treatment, hormone reposition, current medication, brushing daily, dental floss daily, mouth washing daily, number of visits to the dentist, presence or absence of teeth, number of implants installed, were analyzed (Table 2). Periodontal measurements were collected by a single examiner at each stage of this study: 1º stage, 1996-2006 (F.A.P), and 2º stage 2007-2017 (R.C.B). The intra-examiner

calibration was performed for each stage of this study.[34] The instrument used to obtain the periodontal parameters was a Hu-Friedy millimeter periodontal probe (Chicago USA). The analyzed variables were as follows: number of teeth, gingival index (GI)[35], plaque index (PI)[36], calculus index (CI)[37], probing pocket depth (PPD), clinical attachment loss (CAL), and mobility (Table 3).

Among the control and study groups, a total of 1,885 implants were placed. These implants were classified as healthy (N=1,713) and lost (N=187). The following clinical characteristics: position, primary stability, dimension and load were assessed (Table 4).

Table 1. Demographic characteristics of individuals in the sample (n=360).

	Control group (n=238)	**Study group** (n=122)	***p-value***	**OR (CI 95%)**
Age (years)[a]	53.3 ± 11.5	54.9 ± 10.6	0.220*	-
Gender[b]				
Female	160 (67.2)	78 (63.9)	0.533**	0.86 (0.54-1.36)
Male	78 (32.8)	44 (36.1)		
Smoking[b]				
Yes	50 (21.0)	21 (17.2)	0.388**	1.27 (0.72-2.24)
No	188 (79.0)	101 (82.8)		

[a] mean ± standard deviation.
[b] number (frequency).
* Student's t test.
**Chi-Squared test.
Abbreviations: OR: *Odds Ratio*; CI: confidence interval.

Table 2. Patients' clinical findings (n=360).

	Control group (n=238)	**Study group** (n=122)	***p-value***	**OR (CI 95%)**
Social profile[b]				
A1/A2/B1	113 (47.5)	57 (46.7)	0.892**	1.03 (0.66-1.59)
B2/C/D	125 (52.5)	65 (53.3)		
Systemic disease[b]				
Yes	158 (66.4)	92 (75.4)	0.075**	0.64 (0.39-1.05)
No	80 (33.6)	30 (24.6)		
Rheumatoid disease[b]				
Yes	36 (15.1)	26 (21.3)	0.146**	0.65 (0.37-1.15)
No	202 (84.9)	96 (78.7)		

Diabetes[b]				
Yes	12 (5.0)	2 (1.6)	0.090***	3.18 (0.70-14.46)
No	226 (95.0)	120 (98.4)		
Osteoporosis[b]				
Yes	3 (1.3)	2 (1.6)	0.774***	0.76 (0.12-4.64)
No	235 (98.7)	120 (98.4)		
Antihypertensive medication[b]				
Yes	41 (17.2)	35 (28.7)	**0.013****	1.93 (1.15 -3.24)
No	197 (82.8)	87 (71.3)		
Medical treatment[b]				
Yes	94 (39.5)	61 (50.0)	0.057**	0.65 (0.42-1.01)
No	144 (60.5)	61 (50.0)		
Hormone reposition[b]				
Yes	44 (18.5)	27 (22.1)	0.414**	0.79 (0.46-1.36)
No	194 (81.5)	95 (77.9)		
Current medication[b]				
Yes	100 (42.0)	59 (48.4)	0.252**	0.77 (0.49-1.20)
No	138 (58.0)	63 (51.6)		
Brushing daily[b]				
Less than 3 times	212 (89.1)	104 (85.2)	0.300**	1.41 (0.74-2.69)
More than 3 times	26 (10.9)	18 (14.8)		
Dental floss daily[b]				
Yes	179 (75.2)	87 (71.3)	0.428**	1.22 (0.74-1.99)
No	59 (24.8)	35 (28.7)		
Mouth washing daily[b]				
Yes	116 (48.7)	52 (42.6)	0.270**	1.28 (0.82-1.98)
No	122 (51.3)	70 (57.4)		
Clinical appointments[a]	5.69 ± 4.64	5.63 ± 4.27	0.902*	
Edentulous[b]				
Edentulous	45 (18.9)	12 (9.8)	**0.021****	2.13 (1.08-4.21)
Present teeth	193 (81.1)	110 (90.2)		
Placed implants[a]	4.58 ± 3.24	6.24 ± 3.69	**0.000***	

Note: Social profile, the score was defined according to the criteria of economic classification Brazil.
[a] mean ± standard deviation.
[b] number (frequency).
* Student's *t* test.
**Chi-Squared test.
***Fisher's exact test.
Abbreviations: OR: *Odds Ratio*; CI: confidence interval.
The bold values are significant values.

Table 3. Periodontal status of partially edentulous patients (n=303).

Periodontal status	Control group (n=193)	Study group (n=110)	*p-value*	OR (CI 95%)
Number of teeth[a]	16.1 ± 9.80	16.1 ± 8.73	0.997*	-
Gingival index[a]	0.52 ± 0.39	0.54 ± 0.51	0.778*	-
Plaque index[a]	0.14 ± 0.23	0.21 ± 0.38	0.056*	-
Calculus index[a]	0.07± 0.12	0.10 ± 0.21	0.229*	-
PPD (mm)[a]	2.40 ± 0.71	2.23 ± 0.75	**0.049***	-
CAL (mm)[a]	3.01 ± 1.37	3.03 ± 1.56	0.930*	-
Mobility[b]				
Yes	30 (15.5)	19 (17.3)	0.695**	0.88 (0.47-1.65)
No	163 (84.5)	91 (82.7)		

[a] mean ± standard deviation.
[b] number (frequency).
* Student's *t* test.
**Chi-Squared test.
Abbreviations: OR: *Odds Ratio*; CI: confidence interval.
The bold values are significant values.

Table 4. Clinical findings for total implants (n=1,885).

	Healthy implants (n=1701)	Lost implants (n=184)	*p-value*	OR (CI 95%)
Position[b]				
Maxilla	871 (51.2)	76 (41.3)	**0.011****	1.49 (1.09-2.03)
Mandible	830 (48.8)	108 (58.7)		
Anterior region	701 (41.2)	67 (36.4)	0.206**	1.22 (0.89-1.67)
Posterior region	1000 (58.8)	117 (63.6)		
# Primary stability[b]				
> 40 N	870 (65.8)	70 (56.0)	**0.030****	1.51 (1.04-2.19)
≤ 40 N	452 (34.2)	55 (44.0)		
# Dimension[a]				
Diameter (mm)	4 ± 0.41	4 ± 0.47	0.272*	-
Length (mm)	12.65 ± 2.76	12.10 ± 2.84	**0.012***	-
# Immediate load[b]				
Yes	214 (13.8)	16 (9.5)	0.105**	1.52 (0.89-2.60)
No	1342 (86.2)	153 (90.5)		

[a] mean ± standard deviation.
[b] number (frequency).
* Student's *t* test.
**Chi-Squared test.
Abbreviations: OR: *Odds Ratio*; CI: confidence interval.
The bold values are significant values.
Some variables do not correspond to the total number of implants due to the lack of data on medical records.

2.2 - Power calculations

The sample power was calculated using the genetic power calculator, available at http://zzz.bwh.harvard.edu/gpc/cc2.html.[38] (Table 5) Shows the variation in the sample power. The calculation of the sample power is an important tool to identify whether the sample size is sufficient to detect an association between the genetic polymorphism and the studied outcome.[39] The power of this sample, analyzing the 122 patients who lost implants, was greater than 80% for the four polymorphisms selected considering the frequency of the rare allele at 30%.

Table 5. Calculation of the sample power, using the Genetic Power Calculator tool (Purcell et al.,2003), setting as parameters: prevalence of dental implant loss 3.5%, effect size from 1.2 for carrying one copy of the risk allele and 2.4 carrying two copies of the risk allele, D'=1, frequency of the rare allele ranging from 38.2% to 47.6%, 1:2 case and control ratio and level of significance of 5%.

Allele frequency	**Power**	**rs2073618** n cases for 80% power	**Power**	**rs3102724** n cases for 80% power	**Power**	**rs1054016** n cases for 80% power	**Power**	**rs1805034** cases for 80% power
0.05	0.053	33332	0.054	22725	0.054	25426	0.054	23598
0.1	0.116	1706	0.147	1171	0.137	1307	0.144	1215
0.2	0.636	179	0.789	125	0.746	139	0.775	129
0.3	0.819	116	0.994	47	0.989	52	0.998	40

Frequency of the rare allele of the polymorphisms in the studied sample:
rs2073618 – 47.6%
rs3102724 – 38.2%
rs1054016 – 40.9%
rs1805034 – 39.1%

2.3 - DNA collection

Patients were instructed to rinse their mouth with 3% glucose solution for 1 min and then spit the mouth rinse into a suitable container. Next, the cheek mucosa was scraped by the examiner with a sterile wooden spatula to collect the cells.[40] After that, the wooden spatula was stirred inside the container to disperse the cells. The oral epithelial cells were centrifuged at 706 g for 10 min and the supernatant was discarded. Next, 1,300 µL of the extraction solution (10 mM Tris-HCl (pH 7.8), 5 mM EDTA, 0.5% SDS) was added to the pellet. To extract the DNA, 10 µl of proteinase K was added and the samples were incubated in a heated water bath overnight at 65°C. To purify the DNA, 10 M ammonium acetate was added; samples were precipitated with isopropanol, and resuspended in 50 µL of 10 mM Tris (pH 7.6) and 1 mM EDTA.[41]

2.4 - Selection of markers and genotyping

The selection of the *RANKL, RANK*, and *OPG* tag SNPs was carried out using the SNPinfo Web Server platform (https://snpinfo.niehs.nih.gov, 2019). The parameters used were as follows: r^2>0.8, minimum allelic frequency of 0.05, and the "CEU" population (Utah residents with northern and western European ancestry). Based on this information, three SNPs were identified, taking into account the relevance of the functional potential. The rs1054016 of the *RANK*L gene was located in the 3' untranslated region (UTR). The rs1805034 of the *RANK* gene and the rs2073618 of the *OPG* gene were located in exon regions. These functional polymorphisms cause a missense variation, since they encode a different amino acid, and, therefore, could potentially affect protein function. A fourth SNP, rs3102724 of the *OPG* gene, located in an intron region, was included in this study because it was previously associated with external apical root resorption (EARR) in a population very similar to that of this study.[42]

Genotyping was performed using the real-time PCR (Applied Biosystems QuantStudio™ 7 Flex System) and the TaqMan™ Master Mix technology (Applied Biosystems).[43]

2.5 - Genetic models

The following genetic models were tested for this study: additive, dominant, and recessive. Observing the cross-reference table, obtained by IBM SPSS Statistics 20.0, the most frequent allele was established for each polymorphism within the population studied. Groups were compared using binary logistic regression for the additive model and the Chi-square test for the dominant and recessive models.

2.6 - Statistical analysis

Continuous variables are described as means and standard deviations. The association between them was estimated using Student's t-test for independent samples.

Categorical variables were expressed as frequencies and percentages. The comparison between groups regarding categorical variables was performed using the Chi-square test or Fischer's exact test when indicated.

All genetic variable analyses were performed by evaluating the additive, dominant, and recessive models. The groups were compared by binary logistic regression for the additive model and by the Chi-square test for the dominant and

recessive models.

For multivariate analysis, we adopted the criteria of including all variables with a *p*-value <0.20 of the univariate analysis, using the binary logistic regression model, and the backward method. Results with a *p*-value < 0.05 were considered statistically significant.

The software IBM SPSS Statistics 20.0 was used to perform the statistical analysis.

To estimate the Hardy–Weinberg balance, determine the associated allele, and to analyze the linkage disequilibrium (LD) between the two markers of the *OPG* gene, the Haploview 4.2 software was used.

3 - Results

3.1 - Clinical parameters

There was no statistically significant difference (*p*-value > 0.05) between groups C and S for the following variables: age, gender, and smoking (Table 1). Furthermore, the variables, such as socioeconomic status, systemic disease, rheumatoid disease, diabetes mellitus, osteoporosis, medical treatment, hormone reposition, current medication, number of daily brushings, use of dental floss, use of mouthwashes, and clinical appointments, were not associated with the loss of implants. However, the variables: use of antihypertensive medication (*p*=0.013, OR:1.93; CI:1.15-3.24), edentulous (*p*=0.021, OR:2.13; CI:1.08-4.21), and the number of implants placed (*p*=0.000), were associated with the loss of implants (Table 2).

In the analysis of periodontal status performed only among dentulous patients, the number of teeth, GI, PI, CI, CAL, and tooth mobility did not show a statistically significant difference (*p*-value>0.05) between the groups. Only the variable PPD showed a positive association with the loss of implants (*p*=0.049) (Table 3).

A total of 1,885 implants was placed in patients in this study. The following variables were shown to be associated with implant loss: implant position in the mandible (*p*=0.011, OR:1.49; CI:1.09-2.03), torque<40N (*p*=0.030, OR:1.51; CI:1.04-2.19), and the shortest implant length (*p*=0.012) (Table 4).

3.2 - Genetic analysis

The Hardy–Weinberg balance was confirmed in the control population of this study, demonstrating that the expected allele frequency was consistent with the observed allele frequency. LD analysis was performed between rs2073618 and rs3102724 in the *OPG* gene and a disequilibrium of 82% was found, indicating that there is a high disequilibrium between these two polymorphisms (Fig.1).

The univariate analysis did not show statistically significant difference between groups C and S for rs1054016 in the *RANKL* gene and for rs1805034 of the *RANK* gene (*p*>0,05). In relation to the *OPG* gene, for rs2073618, there was a significant association of allele G with implant loss, in the additive and the recessive models for allele G and for rs3102724, there was a significant association of allele G with implant loss, in the additive and dominant models for allele G (Table 6).

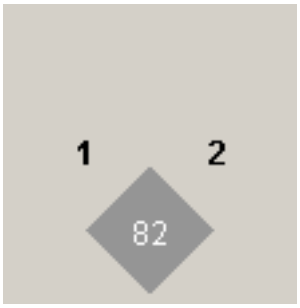

Figure 1 Linkage disequilibrium (LD) analysis between the rs2073618 and rs3102724 (r^2>80) for the *OPG* gene in the CEU population. The number inside the square indicates the amount of LD. The color intensity of the square reflects the strength of binding between the *loci*.

Table 6: Results of *RANKL, RANK*, *OPG* gene SNPs in the additive, dominant, and recessive model

Gene	dbSNP[a]	Genetic Model	Genotype	Groups - n (%)		*p*-value	OR (CI 95%)
				Control	Study		
RANKL	**rs1054016**[b]	Additive	GG	91 (38.6)	39 (32.0)	0.461**	-
			GT	103 (43.6)	60 (49.1)	0.221**	1.35 (0.83 - 2.22)
			TT	42 (17.8)	23 (18.9)	0.447**	1.27 (0.67 - 2.40)
		Dominant G	GG + GT	194 (82.2)	99 (81.1)	0.806**	1.07 (0.61 - 1.88)
			TT	42 (17.8)	23 (18.9)		
		Recessive G	TT + GT	145 (61.4)	83 (68.0)	0.217**	0.74 (0.47 - 1.18)
			GG	91 (38.6)	39 (32.0)		
RANK	**rs1805034**[b]	Additive	TT	83 (35.2)	53 (43.4)	0.929**	0.97 (0.51 - 1.82)
			CT	118 (50.0)	46 (37.7)	0.102**	0.59 (0.31 - 1.11)
			CC	35 (14.8)	23 (18.9)	0.088**	–
		Dominant T	TT + CT	201 (85.2)	99 (81.1)	0.332**	1.33 (0.74 - 2.37)
			CC	35 (14.8)	23 (18.9)		
		Recessive T	CC + CT	153 (64.8)	69 (56.6)	0.128**	1.41 (0.90 - 2.21)
			TT	83 (35.2)	53 (43.4)		
OPG	**rs2073618**[b]	Additive	CC	70 (30.7)	45 (37.5)	0.581**	1.16 (0.67 - 2.01)
			GC	102 (44.7)	33 (27.5)	**0.003****	2.31 (1.32 - 4.06)
			GG	56 (24.6)	42 (35.0)	**0.007****	–
		Dominant G	GG + GC	158 (69.3)	75 (62.5)	0.202**	0.73 (0.46 - 1.17)
			CC	70 (30.7)	45 (37.5)		
		Recessive G	CC + CG	172 (75.4)	78 (65.0)	**0.041****	1.66 (1.03 - 2.70)
			GG	56 (24.6)	42 (35.0)		
OPG	**rs3102724**[b]	Additive	GG	81 (34.8)	49 (41.2)	**0.031****	2.35 (1.08 - 5.14)
			AG	113 (48.5)	60 (50.4)	0.061**	2.07 (0.96 - 4.43)
			AA	39 (16.7)	10 (8.4)	0.096**	–
		Dominant G	GG + AG	194 (83.3)	109 (91.6)	**0.035****	2.19 (1.05 - 4.56)
			AA	39 (16.7)	10 (8.4)		
		Recessive G	AA + AG	152 (65.2)	70 (58.8)	0.240**	1.31 (0.83 - 2.06)
			GG	81 (34.8)	49 (41.2)		

[a] SNP identifier based on the NCBI SNP database.
[b] number (frequency).
**Chi-Squared test.
Abbreviations: OR: *Odds Ratio*; CI: confidence interval.
The bold values are significant values.

3.3 - Multivariate analysis

The variables that remained associated with the loss of dental implants after the multivariate analysis were as follows: higher number of implants placed, the shortest implant length, and the G allele of rs2073618 and allele G for rs3102724 in the *OPG* gene (Table 7).

For a better understanding of the variable length, the installed implants were categorized as short (5-7mm), regular (8-12mm) and long (>13mm), following the manufacturer's catalog (NEODENT Osseointegrable Implant, Curitiba-PR, Brazil).

Table 7. Result of multivariate analysis

	Control group (n=238)	**Study group** (n=122)	***p-value***	**OR (CI 95%)**
Placed implants[a]				
≤5	175 (73.5)	58 (47.5)	**0.000****	3.87 (2.29 - 6.53)
>6	63 (26.5)	64 (52.5)		
# Length				
Short (5-7mm)	1 (0.4)	4 (3.4)	**0.010****	9.87 (1.72 - 56.52)
Regular (8-12mm)	86 (38.2)	69 (58.5)	**0.001***	2.48 (1.48 - 4.15)
Long (>13mm)	138 (61.3)	45 (38.1)	**0.000****	-
# *OPG* rs2073618[b]				
CC+CG	172 (75. 4)	78 (65.0)	**0.015****	2.16 (1.16 - 4.04)
GG	56 (24.6)	42 (35.0)		
# *OPG* rs2073618[b]				
CC	70 (30.7)	45 (37.5)	**0.004****	2.66 (1.37 - 5.14)
GC	102 (44.7)	33 (27.5)	-	-
GG	56 (24.6)	42 (35.0)	**0.004****	-
# *OPG* rs3102724[b]				
GG + AG	194 (83.3)	109 (91.6)	**0.013****	3.01 (1.26 - 7.18)
AA	39 (16.7)	10 (8.4)		

[a] mean ± standard deviation.
[b] number (frequency).
**Chi-Squared test.
Abbreviations: OR: *Odds Ratio*; CI: confidence interval.
The bold values are significant values.
Some variables do not correspond to the total sample due to the lack of data on genetic variables.

4 - Discussion

The goal of this study was to identify possible factors that, together with clinical causes, could help explain the early loss of dental implants. Since dental implant failure is considered to be a complex condition, many factors could be associated with, or could be dependent on, the interaction between the immune response of the host and environmental factors.[6]

Following the surgical procedure, bone repair is initiated, whose process depends on complex cellular interactions[44] and aspects that could affect the success of dental implants osseointegration.[45]

Osseointegration is currently understood as a foreign body reaction in which the healing process triggers inflammation in response to the installation of the implant, to protect against foreign material inserted in the body.[46,47] Numerous host factors have been reported to influence the success or failure of the osseointegration, including bone quantity and quality[6], osteoporosis, smoking, presence of teeth[48], plaque and calculus indexes, tooth mobility[10], compromised general health status[20], and genetic background.[12,14,15,49]

It has been previously demonstrated that antihypertensive medications play a role in bone remodeling.[50] Some of these drugs, such as some antihypertensive medications, could improve osseointegration, as well as implant survival rates.[50-52] In addition, disease control and the use of chronic medications can influence the osseointegration process.[52] However, in our study, we did observe an association between use of antihypertensive medications and the implant loss rate (28.7% S *vs*. 17.2% C). It is worth noting that no information was found about the dosages and frequency of the medications used, which limited our inferences on the outcome.

Presence of teeth was also associated with implant failure (90.2% S *vs*. 81.1% C), with a two times higher risk. Similar observation was made in another study.[53] The significant reduction of bacterial plaque in totally edentulous patients might help explain this association, in addition to the presence of a more complex microbiota.[54,55] At the same time, our results demonstrated that the placement of a higher number of implants was associated with increased average loss (6.24 S *vs*. 4.58 C). This was probably due to the size of the surgical wound necessary for the installation of multiple implants, requiring greater tissue repair.[56]

During the periodontal evaluation of partially edentulous patients, we also observed that the depth of the probe pocket was deeper in the C group (2.23 mm S *vs.* 2.40 mm C); this difference was only 0.17 mm, which is too small to be relevant in clinical practice.

The evaluation of the implant population in our study showed that implants positioned in the mandible (48.8% healthy *vs*. 58.7% lost) had a higher risk of loss compared to the implants placed in the maxilla (51.2% healthy *vs.* 41.3% lost). In addition, denser bone structure of the mandible, may also lead to possible overheating during the implantation surgery, therefore interfering with the blood supply to the area and compromising osseointegration.[57] At the same time, several studies reported that they did not observe any difference between maxillary and mandibular implant positioning.[58,59] However, our findings contradict other studies, reporting that the rate of implant loss in the maxilla is significantly higher compared to the mandible due to the poor bone quality in this region.[4,60-63]

We also observed that increased primary stability >40N ensured decreased implant loss in this study (65.8% healthy *vs*. 56.0% lost). An appropriate torque reduces micromotion of the implant and appears not to damage the bone. Independent of the value of this torque, the healing process is also responsible for providing biological stability to the implant.[64] Some studies, however, showed that low primary stability did not have effect on the clinical results, indicating that lower torque levels could reduce damage to adjacent tissues.[65-68]

Our analysis showed that the average length of the installed implants was lower in the study group (12.65 in C *vs*. 12.10 in S). These data suggest that short and regular have a lower survival rate compared to long implants.[45,69-72]

Several studies reported a possible involvement of genetic factors in the loss of dental implants.[21,62] The regulation of the RANK/RANKL/OPG axis is essential for bone homeostasis[73], while its dysregulation affects several tissues, influencing the progress of various diseases or complex conditions, such as cancer-related osteolysis[74], osteonecrosis[75], rheumatoid arthritis[76,77], breast cancer[78], osteoporosis[79], type 2 diabetes mellitus[80], and vascular diseases.[81] *RANK* rs1805034 polymorphism has been associated with the age of menarche[80,82], osteoporotic fractures[83], susceptibility to adenocarcinoma[84], and angioedema[85], while the *RANKL* rs1054016 polymorphism is involved in the pathogenesis of breast cancer, the progression of

bone metastasis[86], and bone erosions in rheumatoid arthritis.[76] The *OPG* rs2073618 polymorphism has been shown to be associated with temporomandibular ankylosis[87], increased risk of coronary artery calcifications in the presence of the GG genotype[88], increased risk of bilateral osteonecrosis lesions in the femoral head[75], etiology and complications in patients with diabetic foot[89,90], the predisposition to ventricular hypertrophy in patients with thalassemia[91], osteoporotic fractures[92], increased risk for plaque accumulation in the carotid artery[93], musculoskeletal symptoms, and pain in therapy of patients treatment for breast cancer[94], susceptibility to cardiovascular disease for the G allele[95], increased risk factor for calcifications in the aortic artery[19], development of breast cancer associated with the allele C[96], mineral density in postmenopausal osteoporosis influenced by the G allele[97], and the experience of caries in primary dentition[97], increased risk factor for peri-implantitis.[98]

For the population of this study, a high degree of DL (82%) was found between the rs2073618 and the rs3102724 of the *OPG* gene. For future studies, only one SNP might be used.

The loss of dental implants is a complex multifactorial condition and single independent factors cannot provide a whole explanation for implant failure. Therefore, it is important to consider not only the host response, but also all known clinical parameters, to obtain a better understanding of the condition.

The main limitation of this study seems to be the sample size; studies with larger sample sizes increase the statistical power and decrease the chance of false negative results. The lack of some variables in the medical records was a also a limitation of the study.

In conclusion, the higher number of installed implants and shorter implant length were associated with the dental implant loss. Regarding the analysis of the polymorphisms studied in the *RANKL, RANK* and *OPG* genes, there was an association of the allele G of rs2073618 and allele G of rs3102724, both in the *OPG* gene, suggesting that these polymorphisms may be presented as new markers for the early loss of dental implants.

5 - References

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ANEXOS

Comitê de Ética em Pesquisa da PUCPR

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ - PUC/ PR

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ASPECTOS CLÍNICOS E GENÉTICOS ASSOCIADOS COM A PERDA DE IMPLANTES DENTÁRIOS

Pesquisador: Paula Cristina Trevilatto

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 4

CAAE: 87964418.2.1001.0020

Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR

Patrocinador Principal: ASSOCIACAO PARANAENSE DE CULTURA - APC

DADOS DO PARECER

Número do Parecer: 2.715.180

Apresentação do Projeto:

Atualmente está em evidência a valorização da qualidade de vida, da aparência e do sorriso saudável, aliados a uma mastigação mais eficiente e
funcional. Nesse contexto, os implantes dentários têm sido considerados padrão ouro na odontologia, os quais substituem um ou mais elementos
dentários perdidos em diferentes situações clínicas. Com a implementação e o êxito da implantodontia, muitas pessoas aderiram à técnica,
vislumbrando a possibilidade da reabilitação bucal, a qual apresenta uma taxa de sobrevida de 83% a 98% (Simonis et al. 2010; Cakarer et al.
2014). Mesmo com grande porcentagem de sucesso, perdas de implante foram relatadas variando entre 2,5% e 6,5% (Brocard et al. 2000, Montes
et al. 2007; Pedersen et al. 2007; Chrcanovic et al. 2016; Guillaume 2016), tornando-se importante nesse cenário devido ao fato de cada vez mais as
pessoas terem optado por essa forma de tratamento. Efetuada a instalação do implante, o insucesso poderá ocorrer antes da osseointegração
(precoce) ou após a colocação da carga (tardia) (Esposito et al. 1998a; Chrcanovic et al. 2016). Independentemente do tipo de perda, a condição de

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falha apresenta características multifatoriais (Montes et al. 2007; Pereira et al. 2008), tais como: técnica cirúrgica, ausência de cuidados pósoperatórios
e ainda fatores do hospedeiro. Em relação ao hospedeiro, as doenças sistêmicas, tabagismo, higiene oral, condição periodontal,
quantidade óssea, ingestão de antidepressivos, entre outros, podem influenciar a perda de implantes (Doetzer et al. 2014; Chrcanovic et al. 2016).
Autores atribuem a perda sem causa clínica aparente à polimorfismos genéticos, que podem influenciar o processo de osseointegração dos
implantes dentários (Santos et al. 2004; Leite et al. 2008; Montes et al. 2007; Gresser et al. 2013; Costa-Junior et al. 2013; Liao et al. 2014; Pigossi
et al. 2014).Alguns estudos já elucidaram a contribuição genética para a perda de implantes dentários, entre eles estão: MMP1; IL1 RN, IL4 e (Leite
et al. 2008; Montes et al. 2008; Pigossi et al. 2014)

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo da pesquisa será averiguar a associação de variáveis clínicas e genéticas com a perda de implantes dentários osseointegrados.

Avaliação dos Riscos e Benefícios:

Riscos:

Talvez possa ocorrer um leve desconforto no processo de coleta das células epiteliais bucais, que será realizada através de raspagem da mucosa
jugal com espátula de madeira esterilizada.

Benefícios:

Contribuição para um bem maior, auxílio à coletividade.

Comentários e Considerações sobre a Pesquisa:

Sem comentários adiconais

Considerações sobre os Termos de apresentação obrigatória:

Correções realizadas corretamente

Recomendações:

Recomendo colocar o TCLE em primeira pessoa e não em terceira pessoa.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

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Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1051019.pdf	07/06/2018 17:09:39		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	07/06/2018 17:09:04	Paula Cristina Trevilatto	Aceito
Outros	TCUD.docx	04/05/2018 14:58:49	Paula Cristina Trevilatto	Aceito
Folha de Rosto	Folha_rosto.pdf	18/04/2018 14:39:56	Paula Cristina Trevilatto	Aceito
Parecer Anterior	Comite_etica.pdf	16/04/2018 15:04:24	Paula Cristina Trevilatto	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEP.docx	16/04/2018 15:00:23	Paula Cristina Trevilatto	Aceito
Declaração de Instituição e Infraestrutura	Carta_de_autorizacao_de_pesquisa_Ilapeo.pdf	11/04/2018 10:11:45	Paula Cristina Trevilatto	Aceito
Orçamento	Orcamento.docx	11/04/2018 10:08:51	Paula Cristina Trevilatto	Aceito
Cronograma	Cronograma.docx	11/04/2018 10:07:03	Paula Cristina Trevilatto	Aceito
Parecer Anterior	Comite_pag1.pdf	18/01/2018 15:24:18	Paula Cristina Trevilatto	Aceito
Parecer Anterior	Comite_pag2.pdf	18/01/2018 15:23:41	Paula Cristina Trevilatto	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Continuação do Parecer: 2.715.180

CURITIBA, 15 de Junho de 2018

Assinado por:
NAIM AKEL FILHO
(Coordenador)

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Título da Pesquisa: Aspectos clínicos e genéticos associados com a perda de implantes dentários

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O QUE SIGNIFICA CONSENTIMENTO?

Consentimento significa que você concorda em fazer parte de uma pesquisa. Você terá seus direitos respeitados e receberá todas as informações sobre o estudo, por mais simples que possam parecer.
Pode ser que este documento denominado TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO contenha palavras que você não entenda. Por favor, peça ao responsável pela pesquisa ou a equipe do estudo para explicar qualquer palavra ou informação que você não entenda claramente.

PARTICIPAÇÃO NO ESTUDO

Este projeto está sendo desenvolvido por pesquisadores cirurgiões-dentistas empenhados na pesquisa da genética, e que os resultados serão publicados sem a devida identificação dos participantes.

A sua participação no referido estudo será de comparecer à Faculdade Ilapeo para realizar acompanhamento do procedimento de implante dentário já realizado e também passar por exame clínico bucal e depois fazer coleta de saliva. O projeto será desenvolvido por meio do acesso aos dados do prontuário odontológico do paciente.
A saliva coletada será armazenada em freezer –20^{o}C no Laboratório Experimental Multiusuário (LEM) da Pontifícia Universidade Católica do Paraná (PUCPR), segundo as normas da própria instituição.
As despesas necessárias para a realização dessa pesquisa não são de sua responsabilidade. Além disso, você não receberá qualquer valor em dinheiro pela sua participação.

QUE DEVO FAZER SE EU CONCORDAR VOLUNTARIAMENTE EM PARTICIPAR DA PESQUISA?

Caso você aceite participar, será necessário a coleta de saliva com uma duração entre 5 a 10 minutos. Um exame clínico também será necessário. Esse exame, com a finalidade de avaliação inicial de todos os indivíduos que iniciam tratamento nas Clínicas Odontológicas da Ilapeo, será realizado com os devidos cuidados de biossegurança. Esse exame poderá gerar um pequeno desconforto e poderá ser interrompido caso você se manifeste contrário à sua realização, mas esse exame é um procedimento padrão e necessário para o completo exame clínico de rotina realizado pelo Cirurgião-Dentista.

RISCOS E BENEFÍCIOS

Talvez possa ocorrer um leve desconforto no processo de coleta das células epiteliais bucais, que será realizada através de raspagem da mucosa jugal (bochecha) com espátula de madeira esterilizada.

Embora não haja benefício financeiro pela participação, os maiores benefícios serão

Rubrica do sujeito de pesquisa

Rubrica do pesquisador

para o bem da investigação científica futura.

SIGILO E PRIVACIDADE

Nós pesquisadores garantiremos a você que sua privacidade será respeitada, ou seja, seu nome ou qualquer outro dado ou elemento que possa, de qualquer forma, lhe identificar, será mantido em sigilo. Nós pesquisadores nos responsabilizaremos pela guarda e confidencialidade dos dados, bem como a não exposição dos dados de pesquisa.

AUTONOMIA

Nós pesquisadores informamos que você poderá se recusar a participar do estudo, ou retirar seu consentimento a qualquer momento, sem precisar justificar, e de, por desejar sair da pesquisa, não sofrerá qualquer prejuízo à assistência que vem recebendo.

RESSARCIMENTO E INDENIZAÇÃO

No entanto, caso tenha qualquer despesa decorrente da participação nesta pesquisa, tais como transporte, alimentação entre outros, haverá ressarcimento dos valores gastos na forma seguinte: em dinheiro.

De igual maneira, caso ocorra algum dano decorrente de sua participação no estudo, você será devidamente indenizado, conforme determina a lei.

CONTATO

As pesquisadoras envolvidas com o referido projeto são: Profa. Dra. Paula Cristina Trevillatto e Rita de Cássia Broker, doutoranda da PUCPR e com elas você poderá manter contato pelos respectivos telefones (41-3271-1849) e (41-99904-7244).

O Comitê de Ética em Pesquisa em Seres Humanos (CEP) é composto por um grupo de pessoas que estão trabalhando para garantir que seus direitos como participante de pesquisa sejam respeitados. Ele tem a obrigação de avaliar se a pesquisa foi planejada e se está sendo executada de forma ética. Se você achar que a pesquisa não está sendo realizada da forma como você imaginou ou que está sendo prejudicado de alguma forma, você pode entrar em contato com o Comitê de Ética em Pesquisa da PUCPR (CEP) pelo telefone (41) 3271-2292 entre segunda e sexta-feira das 08h00 as 17h30 ou pelo e-mail nep@pucpr.br.

DECLARAÇÃO

Eu, _____declaro que li e entendi todas as informações presentes neste Termo de Consentimento Livre e Esclarecido e tive a oportunidade de discutir as informações deste termo. Todas as minhas perguntas foram respondidas e eu estou satisfeito com as respostas. Entendo que receberei uma via assinada e datada deste documento e que outra via assinada e datada será arquivada pelo pesquisador responsável do estudo.

Enfim, tendo sido orientado quanto ao teor de todo o aqui mencionado e compreendida a natureza e o objetivo do já referido estudo, manifesto meu livre consentimento em participar, estando totalmente ciente de que não há nenhum valor econômico, a receber ou a pagar, por minha participação.

Curitiba, _____ de _____ de _____.

_____	_____
Assinatura do participante da pesquisa	Assinatura do Pesquisador

Rubrica do sujeito de pesquisa

Rubrica do pesquisador