Analysis of MMP-1 and MMP-9 Promoter Polymorphisms in Early Osseointegrated Implant Failure

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Purpose: *Polymorphisms, such as a guanine inserted at position −1607 in the promoter region of human matrix metalloprotenase 1 (MMP-1) or a C-1562T substitution in the MMP-9 gene, have been shown to increase the transcriptional activity of these MMPs. The objective of this study was to investigate the possible relationship between these polymorphisms and early implant failure.* ***Materials and Methods:*** *Genomic DNA from oral mucosa was amplified by polymerase chain reactions (PCRs) and analyzed by restriction endonucleases. The significance of the differences in observed frequencies of polymorphisms was assessed by the chi-square and Fisher exact tests.* ***Results:*** *The test group comprised patients with early failure of osseointegrated oral implants. In the MMP-1 gene, 2G allele was observed in 25% of the control group and in 50% of the test group (*P = *.013). The genotype 1G/1G was found in 61.5% of the control group, while all patients in the test group had the genotype 1G/2G (*P < *.001). No differences were seen in the allele and genotype frequencies in the MMP-9 gene among the groups (*P = *.15 and* P = *.13, respectively).* ***Discussion and Conclusion:*** *These results suggest that polymorphism in the promoter region of the MMP-1 gene may be associated with early implant failure, while polymorphism in the promoter region of the MMP-9 gene may not have a relationship with implant loss.* INT J ORAL MAXILLOFAC IMPLANTS 2004;19:38–43

Key words: *implant failure, matrix metalloproteinases, polymorphism*

Osseointegrated dental implants have been considered the most esthetic and functional alternative to missing teeth, as they can provide predictable, reproducible, and durable results. Despite the long-term success shown by longitudinal multicenter studies,[1,2] failure is probably inevitable. Implant losses can arbitrarily be divided into early losses, ie, cases in which osseointegration fails to occur, and late losses, ie, those in which the achieved osseointegration is lost after a period of function.[3] Implant loss can be attributed to biologic, microbiologic, or biomechanical factors, but the causes and mechanism of early implant failure are still obscure. The cluster phenomenon, multiple implant failures in the same subject, supports the evidence that individual characteristics play an important role in the early failure process. However, little is known about genetic susceptibility to osseointegration failure.

Gene polymorphisms are genetic variations in some individuals within the range of what is considered biologically normal.[4] Polymorphisms in metalloproteinase genes have been associated with several pathologies.[5–9]

Matrix metalloproteinases (MMPs) represent the major class of enzymes responsible for extracellular matrix metabolism.[10] They are metal-dependent proteolytic enzymes that contribute to the degradation and removal of collagen from damaged tissue. MMPs are secreted by inflammatory cells in response to stimuli such as lipopolysaccharide and cytokines.[11] Previous studies have also shown that

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proteinases (eg, collagenases, gelatinases, elastases) are present in peri-implant sulcular fluid[12–15] and can play a pathologic role in peri-implant bone loss.[16]

Fibroblast-type collagenase (MMP-1) is the interstitial collagenase expressed most widely among tissues and therefore plays a prominent role in collagen degradation.[17] MMP-1 degrades types I, II, III, and IX collagen, which are the most abundant protein components of extracellular matrices.[9,18] Interstitial collagenase allows osteoblasts to initiate bone resorption by generating collagen fragments that activate osteoclasts.[19] Expression of MMP-1 is normally low but is readily induced by phorbol esters, growth factors, and inflammatory cytokines.[20] Overexpression of MMP-1 is associated with several pathologic conditions.[21] The insertion of a guanine at position –1607 of the human MMP-1 gene creates the 2G allele, which has been shown to increase transcriptional activity.[22] The presence of this allele has been associated with the development of ovarian cancer,[5] endometrial carcinomas,[23] changes in bone mineral density,[17] premature rupture of the fetal membranes,[8] and chronic periodontitis severity.[9]

Gelatinase B (MMP-9) is particularly active against gelatins, denatured type I collagen, and type IV collagen—a major component of the basement membrane. It also acts proteolytically against proteoglycan core protein and elastin, which are resistant to degradation by some other MMPs.[24] Expression of MMP-9 is regulated primarily at the level of transcription, where the promoter of the gene responds to different regulators such as interleukin-1 (IL-1), platelet-derived growth factor, tumor necrosis factor α, and epidermal growth factor.[25–27] A polymorphism in the promoter region of the human MMP-9 gene, a C-1562T substitution, has been reported to increase promoter activity by nearly 100%.[28] Franchi and associates[29] reported a correlation between MMP-9 expression and carcinoma of the head and neck.

Finding genetic markers related to early implant failure could allow the identification of individuals susceptible to implant loss. The purpose of this study was to investigate the frequencies of the polymorphism in the promoters of the MMP-1 and MMP-9 genes in individuals with implant failure to verify a possible relationship between these polymorphisms and early failure of osseointegrated oral implants.

MATERIAL AND METHODS

Subject Selection

A sample of 46 nonsmoking subjects more than 18 years of age was recruited for study from the patient pool at the Dental Clinic of the Faculty of Dentistry of Piracicaba, University of Campinas (UNICAMP), São Paulo, Brazil, and the Oral Implantology Clinical Cenior in Salvador, Bahia, Brazil. The rate of implant loss at these 2 centers is less than 5%. All subjects were in good general and oral health and did not have any of the following exclusion criteria: a history of diabetes, osteoporosis, hepatitis, HIV infection, immunosuppressive chemotherapy, or any disease known to severely compromise immune function. The criteria also excluded patients with a precocious prosthesis load; had received regenerative surgery, such as bone grafting; or had had postsurgical complications, such as infection. The baseline clinical parameters for the study population are presented in Table 1. Subjects were divided into 2 groups.

Table 1 Baseline Clinical Parameters of the Study Population (n = 46)

	Control group	Test group
Mean age (y)	43.9	48.6
Age Range	21–71	18–73
Gender		
Female (%)	57.7 (15/26)	40 (8/20)
Male (%)	42.3 (11/26)	60 (12/20)
Implants		
Maxillary (%)	46.0 (29/63)	38.9 (28/72)
Mandibular (%)	54.0 (34/63)	61.1 (44/72)
Implant failures		
Maxillary (%)	—	25.9 (7/27)
Mandibular	—	74.1 (20/27)
Time implants in place (mo)		
Mean	25.1	5.5*
Range	9–72	1–12

*Mean time implants in place after surgery before failure.

- *Control group.* Twenty-six patients with 1 or more healthy implants in place for a minimum of 9 months.
- *Test group.* Twenty patients who had suffered at least 1 early implant failure. Implants were considered early failures if the patient presented with implant mobility or pain before or during the abutment connection necessitating the removal of the implant.

Sampling

The sampling of buccal epithelial cells was performed as described by Trevilatto and Line.[30] Forty-six individuals washed their mouths for 2 minutes with a mouthwash containing 5 mL of 3% glucose, then spat the mouthwash into a receptacle. Following the mouthwash, a sterile wood spatula was used to scrape the oral mucosa. The tip of the

Table 2 Primer Sequences, PCR Conditions, and Restriction Enzymes Used for Genotype for MMP-1 and MMP-9 Polymorphisms

Gene	Primer sequences (5′–3′)	AT	RE
MMP-1	F: TCGTGAGAATGTCTTCCCATT R: TCTTGGATTGATTTGAGATAAGTGAAATC	55°C	XmnI
MMP-9	F: GCCTGGCACATAGTAGGCCC R: CTTCCTAGCCAGCCGGCATC	65°C	PaeI

F = forward; R = reverse; AT = annealing temperature; RE = restriction endonuclease.

spatula was then dipped into the retained mouthwash solution. Buccal epithelial cells were pelleted by centrifugation at 2,000 rpm for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended in 500 µL of extraction buffer (10 mmol/L Tris-HCl [pH 7.8], 5 mol/L EDTA, 0.5% sodium dodecyl sulfate [SDS]). The samples were then frozen at –20°C until they were used for DNA extraction.

DNA Extraction

After defrosting, the samples were incubated overnight with 100 ng/mL proteinase K (Sigma, St Louis, MO) at 52°C with agitation. DNA from the samples was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation. The amount of DNA was estimated by measuring the optical density (OD) at wavelengths of 260 nm and 280 nm and calculating the ratio, which is known as the OD 260/280 ratio.

Polymerase Chain Reaction and Restriction Endonucleases Digestion

Polymerase chain reactions (PCRs) were carried out in a solution with a total volume of 50 µL containing 500 ng genomic DNA; 10 mmol/L Tris-HCl (pH 8.3); 50 mmol/L KCl; 1.5 mmol/L $MgCl_2$; 1 µL of each primer (forward and reverse); 200 mmol/L each of dATP, dCTP, dGTP and dTTP, and 4 units Taq DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden). The PCR products were then digested with 1 unit of the appropriate enzyme at 37°C overnight. The primer sequences, PCR conditions, and restriction enzymes are detailed in Table 2.

Gel Electrophoresis

The entire digest was then mixed with 3 µL of loading buffer and electrophoresed on a 10% vertical nondenaturing polyacrylamide gel (DNA Silver Staining Kit; Amersham Pharmacia Biotech) at 20 mA.

Statistical Analysis

The significance of the differences in observed frequencies of polymorphism in both groups was assessed by the chi-square test or by the Fisher exact test. A finding of $P < .05$ was considered statistically significant.

RESULTS

Two mismatches were introduced into the reverse primer of the MMP-1 gene annealed to the proximity of the polymorphism,[18] creating a recognition sequence (5'-GAANNNNTTC-3') for the restriction endonuclease *XmnI* when the DNA template contained 1G (but not 2G) at the polymorphism site. *XmnI* digested the 1G allele, creating 2 fragments, 89bp and 29bp. In the MMP-9 gene, the *PaeI* enzyme digestion cleaved the PCR products into 2 fragments, 247bp and 188bp, when the polymorphism site contained allele T (but not C).

For the MMP-1 gene, there was a significant difference between the control test groups regarding the presence of the different alleles ($P = .013$). In the control group, the 2G allele was observed with a frequency of 25%, while in the test group (patients with early failure of osseointegrated oral implants) the 2G allele was present at a frequency of 50%. Individuals with the 2G allele appeared to be 3 times more likely to lose an implant ($P = .0239$, odds ratio = 3.0, 95% confidence interval, 1.24 to 7.24). The genotype 1G/1G was found in 61.5% of the control group, while the 1G/2G genotype ($P < .001$) was observed in all test group patients. The distribution of the various alleles and genotypes of the MMP-1 gene is shown in Tables 3a and 3b.

No significant differences were observed in the alleles and genotypes of the MMP-9 gene between the 2 groups ($P = .15$ and $P = .13$, respectively). The allele C was found in 86% of the control group and 95% of the test group. The genotype C/C was observed in 72% of the control group and in 90%

Table 3a Distribution of the MMP-1 Allele in the Control and Test Groups

	Control group		Test group	
Allele	n	%	n	%
1G	39	75	20	50
2G	13	25	20	50

P = .013 (chi-square test); odds ratio = 3.0; 95% confidence interval, 1.24–7.24.

Table 3b Distribution of the MMP-1 Genotype in the Control and Test Groups

	Control group		Test group	
Genotype	n	%	n	%
1G/1G	16	61.5	0	0
2G/2G	3	11.5	0	0
1G/2G	7	26.9	20	100

P < .001 (Fisher exact test).

Table 4a Distribution of the MMP-9 Allele in the Control and Test Groups

	Control group		Test group	
Allele	n	%	n	%
C	45	86.5	38	95
T	7	13.5	2	5

P = .15 (chi-square test).

Table 4b Distribution of the MMP-9 Genotype in the Control and Test Groups

	Control group		Test group	
Genotype	n	%	n	%
C/C	19	73	18	90
C/T	7	27	2	10

P = .13 (chi-square test).

of the test group. The frequencies of different alleles and genotypes of the MMP-9 gene are shown in Tables 4a and 4b.

DISCUSSION

An abnormal immune response involving different cell types such as macrophages, polymorphonuclear neutrophils, T and B lymphocytes, endothelial cells, fibroblasts, keratinocytes, osteoclasts, and osteoblasts can destroy peri-implant tissues.[31–33] If activated, these cells can synthesize and release cytokines[34,35] and lipid mediators,[36] which mediate both the inflammatory and the osteolytic processes. Above all, collagenase is likely to cause increased proteolytic tissue destruction in periprosthetic tissue.[37] Since elevated levels of these mediators are present in diseased implant sites, their analysis may provide effective monitoring of implant-related disease. An accumulation of genetic polymorphisms probably influences the osseointegration process. To understand the importance of the polymorphisms of each allele, it is important to analyze the relative contribution of each polymorphism to the disease phenotype.[38]

Wilson and Nunn[39] assessed the impact of genotype status to IL-1 on implant retention among patients who were smokers or nonsmokers. Thirty-eight patients who experienced no implant or bone loss were compared to 27 patients who either lost implants or experienced 50% bone loss. Wilson and Nunn concluded that increased risk for implant failure could not be attributed to a positive IL-1 genotype. Nevertheless, the presence of smokers in their study group may have masked the genetic influence, since it is known that smoking is a strong risk factor for early implant failure. It has been demonstrated that smokers are 3 times more likely than nonsmokers to lose an implant.[33] Rogers and coworkers,[40] evaluating late implant failures, found no significant association between the composite IL-1 genotype and dental implant loss, but it was not clear whether the implant loss occurred because of biologic or mechanical reasons.

In this study, the 2G polymorphism in the promoter region of MMP-1 gene was associated with early implant failure in nonsmokers. The 2G allele was observed in 25% of the control group, while it was found in 50% in the test group. Patients bearing this allele were 3 times more likely to lose an implant. This allele was shown to augment transcriptional activity and can potentially increase the level of protein expression.[22] This mechanism provides the molecular basis for a more intense degradation of extracellular matrix, which might indicate an increased susceptibility to osseointegration failure. However, the clinical significance of these results must be interpreted with caution, since the biases that affect case-control and cohort studies can produce spurious relative risks of 2 or even 3.[41] The difference between the groups observed in the present study might also be related to a specific genotype (1G/2G), since all patients in the test group were heterozygous.

The sequence variation in the gelatinase B (MMP-9) gene was not associated with early implant failure in this study. One possible explanation could

be the size of the sample. These data, which were derived from 46 patients, provided a moderate power to detect any effective statistical relationship between polymorphism and disease. Experimental evidence obtained in vitro has shown that the C-1562T base substitution in the regulatory region of the gelatinase B gene up-regulates promoter activity. This implies that either the base substitution has no or only minimal effects on gelatinase B gene regulation in vivo, or that the influence of this gelatinase on early implant failure is not highly significant.

CONCLUSION

These results indicate that the polymorphism in the promoter of the MMP-1 gene could be a risk factor for early implant failure. This polymorphism could be used as a genetic marker for unsuccessful implants. Although the findings may suggest that MMP-9 polymorphism marker is not a useful predictor of the pathologic or clinical consequences of osseointegration, more studies are needed to investigate this finding. Perhaps the discovery of several genetic markers related to early implant failure could be of clinical value for a precise and early identification of individuals at high risk of implant loss. Such a discovery could lead to a more strict selection of patients, and in the future, individual therapies could be developed, thereby increasing implant success rates.

ACKNOWLEDGMENT

This work was supported by FAPESP grant 01/11920-6.

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