



Effect of MMP-1 polymorphism on early term osseointegrated dental implant failure: A pilot study

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Abstract

Osseointegrated dental implants are widely used for replacing missing teeth. Despite of reported high success rates, failures during healing phase of dental implants are still remarkable. Even under strictly controlled cases, sometimes bone tissue does not attach to titanium implant body or the implant body is lost by acute inflammatory reaction during healing phase. Genetic polymorphism may be a promoting factor for this phenomenon. Intravenous blood samples are taken from 11 patients who lost at least one dental implant during healing phase. Genomic DNA was amplified by polymer chain reaction (PCR) and analyzed by endonucleases. Within the limited results of this study it may be concluded that MMP-1 gene polymorphism may be associated with failure during healing time of Osseointegrated dental implants

Key words: polymorphism, implant failure, matrix metalloproteinases, early implant loss.

MMP-1 Polimorfizminin Erken Dönem Osseointegre İmplant Başarısızlığına Etkisi: Pilot Çalışma

Özet

Osseointegre diş implantları diş eksikliğini giderilmesinde yaygın olarak kullanılmaktadır. Bildirililen yüksek başarı oranlarına rağmen, iyileşme dönemindeki kayıplar halen sorgulanmaktadır. Sıkı kontrol altındaki vakalarda bile kimi zaman konak kemik dokusu implant yüzeyine yapışmamakta veya akut inflemasyon sonucu implant kaybedilmektedir. Genetik polimorfizmin bunda rolü olabilir. İyileşme döneminde en az 1 implant kaybeden 11 hastadan intavenöz kan örnekleri alınmıştır. Genomik DNA polimeraz zincir reaksiyonu (PCR) ile büyütülmüş ve endonükleaz enzimleri ile analiz edilmiştir. Bu çalışma sınırlarında MMP-1 gen polimorfizminin osseointegre diş implantlarının iyileşme dönemindeki kayıplar ile ilişkili olabileceği düşünülebilir.

Anahtar Sözcükler: Polimorfizm, implant başarısızlığı, matriks metaloproteinaz, erken implant kaybı

Introduction

Osseointegration is called as direct contact of vital bone to a load bearing titanium implant surface. Branemark et al., (1977) demonstrated the utilization of osseointegrated titanium dental implants for the

rehabilitation of edentulous patients. The results were quite satisfactory for the patients. Afterwards, various kinds of applications had published and dental implant therapy has become a standard therapy for replacing missing teeth.

In this treatment protocol, implants are inserted

surgically in to the alveolar crest and a healing period of 3- 6 month is proposed for the osseointegration to occur. After the healing period implants are functionally loaded by suitable prostheses. Small amount of implants are lost by infection or can not osseointegrate during 3- 6 months of healing. This situation is called as “early implant failure” by some authors. Besides contributing factors as surgical trauma, local and systemic factors, genetic heritages as polymorphisms are investigated at different studies (Dos Santos et al., 2004; Santos et al., 2004; Grucia et al., 2004; Shimpuku et al., 2003).

Today, dental implant therapy has become the ultimate standard for replacing missing teeth. Natural esthetics and optimal function are established with the utilization of dental implants and the patient satisfaction increases as well. Despite of high success rates reported by various authors, mechanisms of implant failure is still questionable. Especially so called early implant failure or implant loss with in the healing time of implants. Surgical trauma, acute infection, lack of stability, insufficient biocompatibility of implant body, smoking and host response are considered as possible factors of early implant failure. Beside these important factors multiple failures seen on same person supports the idea of genetic factors effecting healing mechanisms and probably early implant failure. However, little is known about genetic susceptibility to osseointegrated implant failure (Kronstrom et al., 2001).

Polymorphism is described as minor genetic variations in some individuals that can be considered in the normal biological range. However these variations may increase tendencies or risks to particular diseases such as diabetes, cancer and syndromes. Metalloproteines variations have showed to be associated with several diseases as coronary problems, fetus membrane ruptures and different forms of periodontitis (Birkedal-Hansen., 1993).

Matrix metalloproteins are produced by inflammatory cells and responsible of extra cellular matrix metabolism which associated with collagen

processing. A human study by Golub et. al., (1995) reported elevated levels of MMP at the crevicular fluid around failing implants and teeth affected by periodontitis. Fibroblast type collagenase (MMP-1) is associated with collagen degradation. Collagen types of I, II, III and IX are degraded by MMP-1, hence these are the most common protein components of extracellular matrices (Nomura et al., 2000; Sorsa et al., 2004). Expression of MMP-1 is normally low but induced by phorbol esters, growth factor, and inflammatory cytokinins. If MMP-1 is overexpressed, some pathological problems has associated with this overexpression. The guanine insertion at position 1607 of the human MMP-1 gene creates the 2G allele, which has been shown to increase transcriptional activity (Santos et al., 2004). The presence of this allele has been associated with the development of ovarian cancer and other carcinoma types, changes in bone mineral density, premature rupture of the fetal membranes and chronic periodontitis severity (deSouza et al., 2003; Dos Santos et al., 2004; Engerbretson et al., 1999; Kiili et al., 2002; Kivela et al., 2003).

Objective of our study is to investigate the rate of MMP-1 polymorphism in individuals with early implant loss to verify the relationship between MMP-1 and early implant failure.

Material and methods

Patients

32 patients (14 female, 18 male) are included in the study group. Test group consisted of 11 patients (failure group) who lost at least one implant in the healing time. Control group consisted of 21 patients (successful group) who carry implant supported prosthesis for at least 6 months. All patients were in good general oral and systemic condition, non-smokers and did not have any following diseases: uncontrolled diabetes, hepatitis, immunosuppressive

Table-1: Parameters of failure and successful groups

Implants	Test group (Implant failure)	Control group
Maxillary (%)	12 (%18)	27 (%41)
Mandibular (%)	4 (%6)	22 (%35)

chemotherapy, or any disease known to severely compromise immune function. None of the patients went under extensive bone grafting or regenerative surgery. Mean age for the control group is 37,68 ranged between 65 – 21 and for the test group is 44,36 ranged between 55 – 32. A total of 65 dental implants placed. (39 maxilla and 26 mandibula) Distribution of the implants is shown in Table 1.

DNA extraction

Five ml of venous blood from each subject was drawn in Vacutainer tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within one week after sampling by using proteinase K digestion followed by a Qiagen Blood DNA isolation kit according to manufacturer instructions. The amount of DNA was estimated by measuring the optical density (OD) at wavelengths of 260nm and 280nm. 1% agarose gel containing EtBr was used for DNA bands visualization.

Polymerase chain reaction

The MMP1 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers used for amplifying the MMP1 single nucleotide polymorphism (SNP) were 5'- TGACTTTTAAAACATAGTCTAT GTTCA-3' (forward) and 5'-TCTTGGATTGAT TTGAGATAAGTATAGC-3' (reverse). A mutation from T to G at the second nucleotide close to the 3' end of the reverse primer was made to create an *AluI* (AGTC) recognition site in the case of 1G allele. The PCR reactions were performed in a 25ul volume containing 100ng of DNA template, 2,5ul of 10x PCR buffer, 2.0 mM MgCl₂, 2.5U of Taq DNA polymerase, 0.2 mM dNTPs and 0.2uM forward and reverse primer. The PCR cycling conditions were 5min at 94°C followed by 35 cycles of 30s at 94°C, 30s at 58°C and 30s at 72°C, and with a final step at 72°C for

5 min to allow for the complete extension of all PCR fragments. An 8ul aliquot of PCR product was digested overnight 37°C or 4 hours at 65°C in a 10ul reaction containing 10U of *Alu I* (New England Biolabs). After digestion, the products were subjected to electrophoresis on a 3% agarose gel stained with EtBr. The MMP1 2G alleles were represented by a DNA band with size at 269bp, the 1G alleles were represented by two DNA bands with size 241 and 28bp, whereas the heterozygotes displayed a combination of the both alleles (269, 241 and 28bp).

For negative control, distilled water instead of DNA in the reaction system was used for each panel of PCR. For 10% of samples, the PCR were repeated once for quality control.

Statistical analyses were performed using SPSS 11.0 software package. Hardy Weinberg analysis was performed to compare the observed and expected genotype frequencies using Chi-Square test. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model and adjusted by age and gender accordingly. A probability level of 5% was considered significant for all statistical analyses.

Results

MMP-1 gene allele was found at 8 patients out of total 11 patients (failure group) who lost at least one implant during healing time (p= 0,043). Table – 2 Hardy Weinberg equilibrium demonstrated the present polymorphism (p< 0.05). We have seen a significant difference in the presence of the different alleles when we compared the failure vs. successful group (P = 0.0344). In the implant failure group, the 2G allele was observed with a frequency of 88%, while in healthy subjects the 2G allele was seen in 45%. When we compare the groups, with each other, we observed a difference in the genotypes frequencies between the failure and successful groups (p = 0.0647). The

Table-2: MMP-1 gene polymorphism of failure and successful groups

	Implant Failure	Control group
Number of patients	11	21
1G/1G	3(%27)	16 (%76)
2G/2G	8 (%73)	5 (%24)

Table-3: 1G and 2G allele frequencies of failure and successful groups.

Allele frequency	Implant failure (n=11)	Control group (n=21)
1G (%)	(%12)	(%55)
2G (%)	(%88)	(%45)

genotype 2G/2G was found in 73.15% in the group with implant failure and the frequencies of 24.2% were observed in success group. In a separated analysis, we grouped individuals successful vs. implant failure groups and calculated the risk associated with individual alleles. Individuals with the 2G allele seem to be approximately three times more likely to develop the overall implant failure ($p = 0.046$; $OR = 2.106$, 95% $CI = 1.10$ 4.34). Individuals with the 2G/2G genotype seem to be three times more likely to develop implant failure than individuals who are 1G/1G homozygous ($p = 0.0721$, $OR = 6.10$, 95% $CI = 2.3457$ 24.345) (Table - 3).

Discussion

MMPs are a family of structurally related but genetically distinct enzymes that degrade extracellular matrix (ECM) and basement membrane (BM) components. This group of 23 human enzymes is classified into collagenases, gelatinases, stromelysins, membrane-type MMPs and other MMPs, mainly based on the substrate specificity and molecular structure. MMPs are involved in physiological processes such as tissue development, remodelling and wound healing (Uitto et al., 2003), and play important roles in the regulation of cellular communication, molecular shedding and immune functions by processing bioactive molecules including cell surface receptors, cytokines, hormones, defensins, adhesion molecules and growth factors (Sorsa et al., 2004). MMP activity is controlled by changes in the delicate balance between the expression and synthesis of MMPs and their major endogenous inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) (Nomura et al., 2000). The catalytic competence of MMPs is controlled through the activation of proenzymes, and the inhibition of the activation or activity by TIMPs (Uitto et al., 2003).

As the roles of MMPs in tissue degenerative diseases have become evident, attempts to control their activities by pharmacological means have gained

much attention. Although the exact roles of individual MMPs in various diseases are not fully understood, it is clear that MMPs are often up-regulated in groups forming activation cascades both in the inflammatory and malignant diseases (Uitto et al., 2003).

The story of MMP family members in oral diseases is far from complete it seems that it has only just begun. For example, several molecular forms of MMP-8 isoenzymes and their multiple active forms in periodontitis plaque, GCF and PISF have been identified (Sorsa et al, 1995; Kiili et al., 2002; Kivelä-Rajamäki et al., 2003a,b). Future studies should examine the synthesis, role and inhibition of how these different MMP-8 isoenzymes function in vivo. The possibility to use the local MMP inhibition to prevent dentinal caries progression or loss of adhesive restorations remains to be studied. Matrix metalloproteinase-1 seems to play an important role during the destruction of the extracellular matrix in periodontal disease. Immunoreactivity for MMP-1 was found in granulation tissue of chronic periodontitis patients, while only moderate immunostaining for MMP-8 (neutrophil collagenase) could be detected (Sorsa et al., 2004). Additionally, reverse transcriptase-polymerase chain reaction has shown that mRNA levels of MMP-1 are significantly increased in inflamed gingival tissue, while only very low levels of MMP-8 transcripts were detected in diseased gingival tissue. (Aiba et al., 1996). These results suggest that MMP-1 rather than MMP-8 seems to be the major interstitial collagenase present in inflamed periodontal tissue.

Early term implant loss is still under discussion by authors because of its multi-factoral ethiology. Immunological factors were questioned because of elevated local reaction of immune mediators to implant site seen on some patients. There are few studies regarding implant treatment at patients with immune related diseases like aggressive periodontitis (Yalcin et al., 2001) and Sjogren syndrome (Isidor et al., 1999). No studies conducted with relation to implant treatment and polymorphism for this special patient group. Polymorphisms and different alleles may

also be affecting immune response to dentition and implants in these patients as well. Process of osseointegration may probably being effected by the variations of polymorphic alleles. If activated, cytokines may initiate an inflammatory response wich may lead to ostelysis.

Rogers (2002) searched the link between IL-1 genotype polymorphism and late term implant loss and found no correlation. (Santos et al., 2004) investigated the the MMP-1, MMP-9 and TGF β -1 polymorphisms and their effect on early implant failure. MMP-1 gene, 2G allele was found at 25% of the patients with sucessfull implants and 50% of the patients of early implant failure. In our study we found MMP-1 gene polymorphism in 88% of the the patients in the test group. This may be due to small number of investigated patients of the failure group in this study.

Implants in the maxilla has elevated risks for failure. (Kohavi et al., 2004, Garlini et al., 2003) Decreased bone quality, wide trabeculation and improper angulation are assumed for this failure. In our study 75% of failed implants was installed to maxilla. However our study group is not big enough to exclude the exact risks for prolymorphism or localisation of mandibula versus maxilla.

In this pilot study, patients with polymorphism at the promoter region of MMP-1 gene and 2G allele was found to have increased risk for early term implant failure compared to patients with 1G allele. A larger group of patients have to be investigated in order to obtain a more accurate statistical result about MMP-1 gene polymorphism and early implant failure.

In conclusion, our results indicate that the 2G/2G polymorphism in the promoter of the MMP-1 gene could be a risk factor for implant failure. With in the limits of this pilot study it can be concluded that genetic screening may help to evaluate and discuss considarable risks with patient before the initiation of the implant therapy.

References

- Aiba T, Akeno N, Kawane T, Okamoto H, Horiuchi N. Matrix metalloproteinases-1 and-8 and TIMP-1 mRNA levels in normal and diseased human gingivae. *European Journal of Oral Science*. 104: 562-569, 1996.
- Birkedal-Hansen H. Role of matrix metalloproteinase in human periodontal diseases. *Journal of Periodontology*. 64 (Suppl 5): 474-484, 1993.
- Brånemark P, Hansson B, Adell R, Breine U, Lindström J, Hallén O, Öhman A. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scandinavian Journal of Plastic and Reconstructive Surgery*. 11 (suppl 16): 104-119, 1977.
- de Souza A, Trevilatto P, Scarel-Caminaga R, Brito R & Line S. MMP-1 promoter polymorphism: association with chronic periodontitis severity in a Brazilian population. *Journal of Clinical Periodontology*. 30: 154-158, 2003.
- Dos Santos M, Campos M, Souza A, Scarel-Caminaga R, Mazzonetto R, Line S. Analysis of the transforming growth factor-beta 1 gene promoter polymorphisms in early osseointegrated implant failure. *Implant Dentistry*. 13(3):262-9, 2004.
- Engerbretson S, Lamster I, Herrera-Abreu M, Celenti R, Timms J, Chaudhary A, di Giovine F & Kornman K. The influence of interleukin gene polymorphism on expression of interleukin-1 and tumor necrosis factor-1 in periodontal tissue and gingival crevicular fluid. *Journal of Periodontology*. 70: 567-573, 1999.
- Golub L, Sorsa T, Lee H, Ciancio S, Sorbi D, Ramamurthy N, Gruber B, Salo T, Kontinen Y. Doxycycline inhibits neutrophil (PMN)-type matrix metalloproteinases in human adult periodontitis gingiva. *Journal of Clinical Periodontology*. 22(2):100-9, 1995.
- Gruica B, Wang HY, Lang NP, Buser D. Impact of IL-1 genotype and smoking status on the prognosis of osseointegrated implants. *Clinical Oral Implants Research*. 15(4):393-400, 2004.
- Kiili M, Cox SW, Chen HW. Collagenase-2 (MMP-8) and collagnase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalization in gingival tissue. *Journal of Clinical Periodontology*. 29: 224-232, 2002.
- Kivelä-Rajamäki M, Teronen O, Maisi P. Laminin-5 gamma2-chain and collagenase-2 (MMP-8) in human peri-implant sulcular fluid. *Clinical Oral Implants Research*. 14: 158-165, 2003.
- Kohavi D, Azran G, Shapira L, Casap N. Retrospective clinical review of dental implants placed in a university training program. *Journal of Oral Implantology*. 30(1):23-9, 2004.
- Isidor F, Brøndum K, Hansen HJ, Jensen J, Sindet-Pedersen S. Outcome of treatment with implant-retained dental prostheses in patients with Sjögren syndrome. *International Journal of Oral and Maxillofacial Implants*. 14(5):736-43, 1999.
- Kronstrom M, Svenson B, Hellman M, Persson GR. Early implant failures in patients treated with Branemark System titanium dental implants: a retrospective study. *International Journal of Oral and Maxillofacial Implants*. 16(2):201-7, 2001.
- Rogers MA, Figliomeni L, Baluchova K, Tan AE, Davies G, Henry PJ, Price P. Do interleukin-1 polymorphisms

- predict the development of periodontitis or the success of dental implants? *Journal of Periodontal Research*. 37-41, 2002.
- Santos MC, Campos MI, Souza AP, Trevilatto PC, Line SR. Analysis of MMP-1 and MMP-9 promoter polymorphisms in early osseointegrated implant failure. *International Journal of Oral and Maxillofacial Implants*. 19(1): 38-43, 2004.
- Shimpuku H, Nosaka Y, Kawamura T, Tachi Y, Shinohara M, Ohura K. Bone morphogenetic protein-4 gene polymorphism and early marginal bone loss around endosseous implants. *International Journal of Oral and Maxillofacial Implants*. 18(4): 500-4, 2003.
- Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Journal of Oral Diseases*. 10(6): 311-318, 2004.
- Nomuta T, Ishii A, Shimuzu H, Taguchi N, Yoshie H, Kusakari H, Hara K. Tissue inhibitor of metalloproteinases-1, matrix metalloproteinases-1 and -8, and collagenase activity levels in peri-implant crevicular fluid after implantation. *Clinical Oral Implants Research*. 11(5): 430-440, 2000.
- Uitto VJ, Overall CM, McCulloch C . Proteolytic host enzymes in gingival crevice fluid. *Periodontology 2000* 31: 77-104, 2003.
- Yalcin S, Yalcin F, Gunay Y, Bellaz B, Onal S, Firatli E. Treatment of aggressive periodontitis by osseointegrated dental implants. A case report. *Journal of Periodontol*. 72(3): 411-6, 2001.