Expression of matrix metalloproteinase-2 in the extracellular matrix of osseointegrated and diseased implants

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ABSTRACT

Background: Recently with improvement of dental implantology science, osseointegrated implants show a considerable durability, however; failures are not completely avoidable. Matrix metalloproteinase-2 (MMP-2) expression is disturbed in many pathological conditions such as peri-implantitis and periodontitis. This study was carried out to investigate the tissue expression of MMP-2 in the extracellular matrix of osseointegrated and diseased implants.

Subjects and methods: Gingival biopsies were collected from six patients having osseointegrated or working implants and twenty with diseased or non osseointegrated implants and (6) controls having no implants. In situ hybridization technique was used to analyze the changes in immunoreactivity of ECM-controlling MMP-2.

Results: The findings of the present study indicate that the expression of MMP2 was significantly elevated in failed implants versus healthy implants ($P<0.01$). In addition, MMP-2 was detected in peri-implant sites with ongoing bone loss, cavitations and inflammatory reaction.

Conclusion: The in situ hybridization technique, showed clear evidence that MMP-2, which is involved in the process of osseointegration and bone remodeling, increase greatly in the presence of bone destruction, cavitations, severe inflammation and fibrous tissue formation. The data link titanium- induced bone remodeling to changes in expression and distribution of MMP-2.

Keywords: Dental implant; Osseointegration; Matrix metalloproteinase-2 (MMP2). (J Bagh Coll Dentistry 2013; 25(3):176-182).

INTRODUCTION

Although implant-supported oral rehabilitation has gained worldwide popularity throughout the last decades due to its efficient clinical success rate and substantiated improvement of individual's quality of life [1,2]. Recent reports on the long-term success of implant therapy have presented surprisingly high prevalence rates of periimplant diseases; perimucositis and peri-implantitis [3] which has been reported to occur in 6–10% of the installed implants and eventually can lead to implant mobility and loss [4,5]. In the initial stage, plaque accumulation can cause perimucositis, a reversible inflammation of the soft tissues surrounding functional implants [6]. Peri-implantitis is defined as an inflammatory process, with microorganisms associated in patterns known from the chronic periodontitis of natural teeth, affecting soft and hard tissues surrounding an osseointegrated implant associated with breakdown of the peri-implant epithelial seal, pocket formation, purulence, and progressive bone loss [7,8]

It is well known that periodontal bacteria are the main causative agents inducing the initiation of periodontitis and peri implantitis. Although dental implant therapy has been considered to have an excellent prognosis, peri-implantitis, subsequent progression and disease severity are also determined by the host immune response [9]

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The degradation of peri-implant and periodontal tissues can be mainly mediated by matrix metalloproteinases (MMPs). Bone matrix turnover is regulated by the extracellular zinc-dependent endopeptidase, family of matrix metalloproteinases (MMPs) comprising collagenases, gelatinases, stromelysins and membrane-type MMPs [10]. Bone development and remodeling requires activity of MMPs for matrix maintenance and repair, bone resorption and the coupling to bone formation [11]

Fibrillar collagens are the major components of periodontal extracellular matrix, during periodontal homeostasis and pathologic conditions they are cleaved into smaller fragments by collagenases (MMPs -1, -8, and -13) and further degraded by active gelatinases (MMPs -2 and -9) and other non specific tissue proteinases [12]. Furthermore both MMP-2 and MMP-9 (gelatinase A&B) have been implicated in bone resorption that results in the loosening of prostheses [13]

Matrix metalloproteinase-2 (also known as gelatinase A or type IV collagenase) is a 72 kDa enzyme in humans encoded by the MMP2 gene [14]. MMP-2 is responsible for the breakdown of type IV collagen of the extracellular matrix, which is a major structural component of a typical basement membrane [15]. In addition, MMP-2 is also able to cleave native type I collagen, which is the abundant component of gingival connective tissue matrix, this protein is widely expressed by a number of normal and transformed cells

[16].MMP-2 plays a critical role in invasion, metastasis, angiogenesis and tissue remodelling [17].

It has been immunolocalized in fibroblasts and macrophages, as well as in epithelial cells of gingival tissues in periodontitis affected patients [18]. Elevated levels of matrix metalloproteinase-2 have also been detected in gingival crevicular fluid [19], peri-implant sulcular fluid [20] and gingival tissues of periodontititis / peri-implantitis patients [21]. Therefore, the present study was performed to investigate the tissue expression of MMP-2 in the extracellular matrix of osseointegrated and diseased implants.

SUBJECTS AND METHODS

A total number of 32 subjects were enrolled in this study. Patients were attending the department of maxillofacial surgery- specialization surgical hospital, Alwasity hospital, Alkarkh hospital, and Al-Mamoon dental center.

Partially edentulous patients of age range (40-60) were grouped in to three groups, (6) with working osseo-integrated implants (2 male and4 female), (20) patients with at least one failed or diseased implants (7 male and 13 female), beside (6) randomly taken healthy control subjects (3 of each sex). Identifying socio demographic information's together with radiographic and clinical evaluation including pain, mobility, bone loss, suppuration, peri-implantitis were recorded for each subject. Nevertheless criteria of failing implants were judged by the maxillofacial surgeon. Flap surgery was performed where gingival biopsy taken, fixed with formaldehyde and paraffin sections performed for in situ hybridization technique as recommended in leaflet with the kit [Maxim biotech. USA Cat No. IH-60001 (IHD-0050).

Statistical analysis: SPSS statistical analysis was used. Semirnov- kolmogorov test was used to find the frequency distribution for selected variables. Non- parametric tests were used to assess the statistical significance for these variables. Mann-Whitney test was used to assess the statistical significance of difference in median of quantitative variable between two groups. Kruskall- Wallis test was used to assess the statistical significance of difference in median of quantitative variable between more than two groups Bonferonni t- test was used to assess further exploration of statistical significance of difference in mean between each pair of groups.

RESULTS

In this study ISH was attempted in order to identify the cellular types expressing MMP-2 cDNA and the changes in distribution of this endoproteinase in gingival tissue biopsies taken from patients post dental implantation. The result reported the changes in MMP2 levels among those patients relative to controls. The histological analysis of titanium bone interface following 8 wks of implant surgery indicates successful osseointegration with minimal inflammatory reaction and minimum expression of MMP2, while the ECM of the implant bone interface showed an increase expression of MMP2 in diseased implants. Figure (1 and 2) show clear evidence of increase in expression of MMP2 in failed implants versus healthy implants

An increase expression of MMP2 was associated with the presence of bone destruction , cavitations , inflammation , granulation tissue in addition to fibrous tissue formation table(3,4,5,6 and7)

The differences in MMP-2 score and intensity among the three study groups is glanced in table 1 figure 1&2.The marker score was clearly but not significantly higher among failure group 10(50%), 6(30%) and 4(20%) at low, intermediate and high grades respectively, compared to osseointegrated group (P-value=0.34), while significant higher scores were seen in failure group compared to controls (P-value=0.04) table 1, Fig 1. Furthermore higher but not significant marker intensities was observed in failure group12 (60%), 4(20%) (x2) at low, intermediate and high grades respectively, compared to osseointegrated group (P-value=1.0) table 1, fig 2.

Talking about the correlation between subjects expressing bone destruction in histopathology compared to those having no evidence of bone loss, higher intermediate scores 4(36%) and intensity 3(2.3%) were seen in the failure group than those in osseointegrated implant group ,although these differences did not reach the statistical significance (P-value=0.2 and 0.19) respectively. (Table 2)

As clearly shown in table 3 the marker values reach the statistical significance, they were increased with increasing severity of cavitations 41.2% and 17.6% at intermediate and high grades respectively compared to negative ones(P-value=0.014)and (23.5%) or both intermediate and high intensities (P-value=0.003). Although gingival tissues reflects different grades of inflammatory reaction, heavy inflammation of 6(46.2%), 4(30.8%), 3(23.1%) were associated with low, intermediate and high scores respectively (P value=0.2 NS)(r=0.34NS), the heaviest inflammatory reaction 9(69.2%) was seen in tissues expressing low marker intensity (P value=0.19 NS) Table 4.

Similar association was seen in table 5 regarding the presence of granulation tissue in which 8(47.1%), 6(35.3%), 3(17.6%) positive values were seen in the three marker scores respectively (P value=0.07) and 11(64.7%), 3(17.6%), 3(17.6%) positivity related to intensities (P value=0.16).

DISCUSSION

Integration of external titanium fixtures into living bone (osseointegration) occurs through active bone remodeling [22], resulting in sensory neuronal changes, these changes were associated with permanent pure titanium implants rather than bone surgery alone [23].

It is well known that the peri-implant diseases are characterized by implant loosening, destruction of collagen fibers and other extracellular matrix components in periodontal tissues that is likely to be mediated, to a significant extent, by the host cells derived MMPs and many studies have establish the relationship between these endoproteinases and periodontal / peri-implant diseases.

Tissue degradation by the matrix metalloproteinase-2 (gelatinase A) is pivotal to inflammation and metastases, however, both MMP-2 and MMP-9 have been implicated in bone resorption that results in the loosening of prostheses [12]. This suggests that matrix metalloproteinases are both effectors and regulators of the inflammatory response [24].

In the present study, ISH was assessed to quantify and localize the expression of MMP-2 in gingival tissues of controls, versus healthy and diseased implant biopsies which showed that the number (score) and intensity of MMP2 signals positive cells varied between the three study groups.

Gelatinase A (MMP-2) cDNA was most frequently found in diseased implants and less in osseointegrated ones. Although this level was not significantly different between the two groups, the observation pointed out that MMP2 signals found mainly in fibroblast cell sites in biopsies of diseased implants specially when there is bone destruction , cavitations and inflammation more extensive than healthy ones and controls, this might explain the process of remodeling which occur during the osseointegration process, however, the presence of bacterial infection in diseased implants might participate in the process of degradation of ECM and activation of fibroblast to produce MMPS.

Dahan et al [25] use ISH and RT-PCR to quantify and localize the expression of mRNA for MMP-1, MMP-2 and MT1-MMP and stated that the mRNA encoding those MMPs are most frequently found in periodontitis affected and healthy patients, and they were expressed in fibroblastic spindle-shaped cells at sites of connective tissue remodeling or chronic inflammation.

Meikle et al [18] stated that the number and distribution of MMP-1,MMP-2 and MT1-MP positive cells varied considerably not only between individual biopsy specimens but also from section to section within the same specimen, and their observation pointed out that fibroblasts are the major cell origin for MMP-2 and MT1-MMP production.

Similarly, Corroti et al [26] and Paula-Silva et al [27] observed the critical role of MMP-2 and MMP-9 in the development of inflammatory peri apical lesions and ECM degradation during the initiation and progression of apical periodontitis

Dale [28] suggests that the host response to microbial infection in periodontal tissues may lead to the altered production of human MMPs and that the human, rather than bacterial proteinases are predominantly responsible for cleavage of the Ln-332 molecule and for pathological changes in the junctional epithelium.

An immunohistochemical examination done by Yokohama et al [29] revealed that expression of MMP-2 and TIMP-1 mRNA in the multinucleated giant cells that are present in fibrous granulation tissue of the membranes obtained from the loose bone-implant interface, was demonstrated by in situ hybridization, where MMP-2 was immunolocalized mainly in the fibroblasts while TIMP-1 was localized in the endothelial cells of the blood vessels and weakly in fibroblasts

Other investigators as Di Nezza et al [30] studied the actions of many extracellular-matrix degrading enzymes, matrix metalloproteinases (MMPs) in tumorigenesis using ISH and in situ zymography and found that MMP-9 and MMP-2 mRNAs were predominantly observed in tumor epithelial cells as well as in the stroma to varying degrees.

As far as many investigators focus on the increased expression and activity of MMP-2 and -9 in tumors which leads to the degradation of basement membranes, an essential step in tumor invasion. In this respect, a correlation between a high expression of MMP-2 and reduced survival in breast cancer patients has been proved by Andrea Köhrmann et al [31]

Vasaturo et al [32] observed a significant and direct correlation between the concentrations of MMPs 2 and 9 and tumor histological grade of breast cancer, and suggests that the quantification of plasma MMP 2 and MMP 9 levels may provide

additional clinical information of the tumor and it is, therefore, a possible prognostic index for breast cancer.

As a conclusion, MMP-2 (gelatinaseA) is an important enzyme envolved in the process of remodeling of ECM of bone tissue interface but there is an imbalance increase in MMP2 in diseased and failed implants.

Furthermore the results of this study suggested the gingival fibroblasts as a major source for MMP-2 and evidenced the fact that the host endoproteinases plays an important role in the degradation of the extra-cellular matrix components. Quantification by in situ hybridization of the DNA-encoding MMP-2 levels, and other degradative enzymes, will help in understanding the molecular mechanisms underlying peri-implant diseases and confirm the possible role of the matrix metaloproteinases as predictors of active periods of peri-implantitis and alveolar bone loss.

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Table 1: The difference in MMP2 score and intensity between the 3 study groups

	Implant Failure		**Osteo-integrated implant**		**Control subjects**	
	N	**%**	**N**	**%**	**N**	**%**
MMP2 score						
Low	10	50	4	66.7	6	100
Intermediate	6	30	2	33.3	0	0
High	4	20	0	0	0	0
P (Mann-Whitney) for difference between:						
Osteo-integrated implant X Control =**0.14**$^{[NS]}$						
Osteo-integrated implant X Implant failure =**0.34**$^{[NS]}$						
Implant failure X Control =0.04						
MMP2 intensity						
Low	12	60	6	100	6	100
Intermediate	4	20	0	0	0	0
High	4	20	0	0	0	0
P (Mann-Whitney) for difference between:						
Osteo-integrated implant X Control =**1**$^{[NS]}$						
Osteo-integrated implant X Implant failure =**0.07**$^{[NS]}$						
Implant failure X Control =**0.07**$^{[NS]}$						
Total	20	100	6	100	6	100

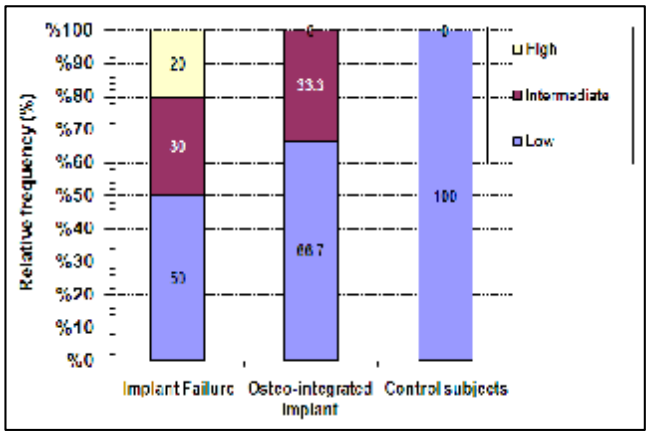

Figure 1: Component bar chart showing the difference in MMP2 score between the 3 study groups

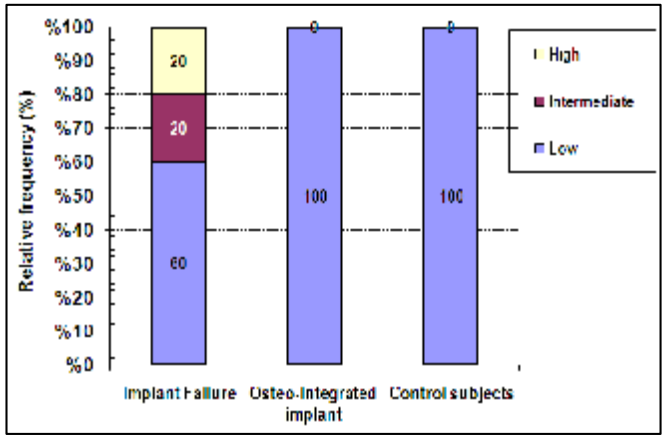

Figure 2: Component bar chart showing the difference in MMP2 intensity between the 3 study groups

Table 2: The difference in MMP2 score and intensity by presence of bone destruction on histo-pathological examination

	Bone destruction				
	Negative		Positive		
	N	%	N	%	P
MMP2 score					
Low	5	55.6	5	45.5	**0.8 [NS]**
Intermediate	2	22.2	4	36.4	
High	2	22.2	2	18.2	
MMP2 intensity					
Low	7	77.8	5	45.5	**0.17 [NS]**
Intermediate	1	11.1	3	27.3	
High	1	11.1	3	27.3	
Total	9	100	11	100	

Table 3: The difference in MMP2 score and intensity by presence of cavitations on histo-pathological examination

	Cavitations				
	Negative		Positive		
	N	%	N	%	P
MMP2 score					
Low	13	86.7	7	41.2	**0.014**
Intermediate	1	6.7	7	41.2	
High	1	6.7	3	17.6	
MMP2 intensity					
Low	15	100	9	52.9	**0.003**
Intermediate	0	0	4	23.5	
High	0	0	4	23.5	
Total	15	100	17	100	

Table 4: The difference in MMP2 score and intensity by severity of inflammatory reaction on histo-pathological examination

	Inflammatory reaction								
	Negative		Mild		Moderate		Heavy		
	N	%	N	%	N	%	N	%	P
MMP2 score									
Low	5	100	5	62.5	4	66.7	6	46.2	**0.2** *[NS]*
Intermediate	0	0	2	25	2	33.3	4	30.8	
High	0	0	1	12.5	0	0	3	23.1	
r = 0.34 *[NS]*									
MMP2 intensity									
Low	5	100	7	87.5	3	50	9	69.2	**0.19** *[NS]*
Intermediate	0	0	1	12.5	1	16.7	2	15.4	
High	0	0	0	0	2	33.3	2	15.4	
r = 0.26 *[NS]*									
Total	5	100	8	100	6	100	13	100	

Table 5: The difference in MMP2 score and intensity by presence of granulation tissue on histo-pathological examination

	Granulation tissue				
	Negative		Positive		
	N	%	N	%	P
MMP2 score					
Low	12	80	8	47.1	**0.07** *[NS]*
Intermediate	2	13.3	6	35.3	
High	1	6.7	3	17.6	
MMP2 intensity					
Low	13	86.7	11	64.7	**0.16** *[NS]*
Intermediate	1	6.7	3	17.6	
High	1	6.7	3	17.6	
Total	15	100	17	100	

Table 6: The difference in MMP2 score and intensity by type of fibrous tissue observed on histo-pathological examination

	Fibrous tissue				
	Delicate bands		Coarse heavy collagen		
	N	%	N	%	P
MMP2 score					
Low	14	77.8	6	42.9	
Intermediate	3	16.7	5	35.7	**0.042**
High	1	5.6	3	21.4	
MMP2 intensity					
Low	16	88.9	8	57.1	
Intermediate	1	5.6	3	21.4	**0.045**
High	1	5.6	3	21.4	
Total	18	100	14	100	