An In-Vivo Comparative Evaluation and Role of MMP-1 (Matrix Metalloproteinase) and RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) Biomarkers in Peri-Implant Crevicular Fluid (PICF) For Bone Remodelling & Peri-Implantitis with their Direct Correlations with Primary Stability and Success of Implants in Flapless Implant Surgeries: An (Clinical) Original Research Study

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Abstract

Aim: This study aims to evaluate the role of MMP-1 (matrix metalloproteinase) and RANKL (receptor activator of nuclear factor kappa-b ligand) biomarkers in peri-implant crevicular fluid (PICF) for bone remodelling & peri-implantitis with their direct correlations with primary stability and success of implants in flapless implant surgeries

Materials and Methods: Forty patients with a missing right mandibular first molar sought replacement. Thirty patients chose implants and implant-supported prostheses, aged 25 to 50, after screening for mental issues, smoking, and systemic diseases. Following informed consent and CBCT analysis, implants were placed under anaesthesia, with healing abutments added after two months. Sutures were removed a week later, and oral hygiene instructions were provided. An implant-supported prosthesis was placed in the third month, and peri-implant crevicular fluid samples were collected by the fifth month. The patients were divided into two groups: Group 1 analysed MMP-1 levels, and Group 2 assessed RANKL levels using the ELISA test, with primary stability measured by the Perio test system. Group 1 focused on MMP-1 in inflammation and tissue remodelling, while Group 2 examined RANKL in osteoclast genesis and bone resorption.

Statistical Analysis and Results: This study, analysed using SPSS software, involved 30 patients (16 males, 14 females and aged 25-50) seeking dental implants for a missing maxillary right central incisor. Participants underwent a Cone Beam Computed Tomography (CBCT) scan, followed by immediate implant placement and a two-month healing period. By the third month, implant-supported prostheses were completed. Five months post-implantation, peri-implant crevicular fluid (PICF) samples were analysed for inflammation markers using ELISA. In Group 1 (n=15), MMP-1 levels were 0.1-1.0 pmol/L, with 9 showing elevated levels. Group 2 (n=15) had RANKL levels of 450-800 pg/mL, with 10 elevated. Stability assessment revealed that 5 patients in Group 1 and 4 in Group 2 had stability under 1. A one-way ANOVA was used for comparative analysis among the groups.

Conclusion: This study concluded that RANKL and MMP-1 are essential biomarkers for assessing bone remodeling and peri-implantitis, with RANKL showing a stronger correlation and potential as a more reliable indicator. Both biomarkers provide insights into disease progression, though their predictive effectiveness may vary by context. Future research is needed to refine their clinical applications.

Keywords: Matrix Metalloproteinase, Receptor Activator of Nuclear Factor Kappa-B Ligand, Bone Remodelling, Peri-implantitis

Introduction

Dental implant-supported prostheses effectively restore function and appearance for patients missing teeth, but their increased use has led to a rise in peri-implant diseases. Peri-implant infections cause inflammation around implants and can lead to bone loss (peri-implantitis) or just inflammation (peri-implant mucositis). Symptoms of peri-implantitis may include redness, swelling, deeper probing depths, bleeding, and pus formation, sometimes with no pain.[1,2] Early diagnosis of peri-implantitis is crucial, but there is no standard treatment protocol. Current clinical signs and bone level changes may not

sufficiently predict a person's risk or treatment outcomes. Therefore, detecting early signs of bone loss and identifying biomarkers, such as chemokines, cytokines, and enzymes from surrounding tissue, is essential for diagnosis.[3,4] Biomarkers such as pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-17) initiate the inflammation process. Other important markers for bone tissue include osteoprotegerin (OPG) and soluble RANKL. Inflammation-related molecules like G-CSF, MMP-8, and MCP-1 provide insights into the body's response to peri-implant diseases.[5,6] Peri-implant crevicular fluid (PICF) can be easily collected from the tissues surrounding dental implants and serves as a valuable resource for investigating various immune markers. Notably, the volume of PICF tends to increase significantly in cases of peri-implantitis, a condition characterized by inflammation around dental implants.[7-9] Matrix metalloproteinases (MMPs), specifically MMP-1, MMP-8, and MMP-13, play a critical role in the degradation of periodontal tissues, which can ultimately lead to tooth loss in individuals suffering from periodontitis. Additionally, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) is a key player in the complex process of bone remodelling and is linked to numerous pathological conditions. The levels of RANKL in the body can serve as important indicators of disease activity or the efficacy of treatment interventions.[10,11]To investigate these vital biomarkers, we employ advanced laboratory techniques such as Enzyme-Linked Immunosorbent Assay (ELISA), Luminex multiplexing, and flow cytometry. ELISA, in particular, is a widely adopted method known for its precision in detecting and quantifying various biomarkers. This technique is essential for the early diagnosis of diseases, monitoring the effectiveness of treatment regimens, and evaluating the severity of conditions, thereby playing a crucial role in improving patient outcomes.[12,13]This study aims to evaluate the role of MMP-1 (matrix metalloproteinase) and RANKL (receptor activator of nuclear factor kappa-b ligand) biomarkers in peri-implant crevicular fluid (PICF) for bone remodelling & peri-implantitis with their direct correlations with primary stability and success of implants in flapless implant surgeries

Materials and Methods

A total of 40 patients with a missing right mandibular first molar sought replacement options were included initially. Among these, 30 patients expressed interest in replacing the missing tooth with implant placement and an implant-supported prosthesis. The inclusion criteria for the study allowed individuals aged 25 to 50 years, encompassing both males and females with a missing right mandibular first molar. The exclusion criteria included patients who were mentally unstable, smokers, or had systemic diseases. Informed consent was obtained from all patients willing to participate. A cone beam computed tomography (CBCT) analysis was conducted for precise implant placement. Patients received a chlorhexidine mouthwash rinse pre-operatively, followed by an inferior alveolar nerve block for anesthesia. An implant was placed in the right mandibular first molar position. After two months, healing abutments were added, and the gingival tissue was sutured. Sutures were removed a week later, and patients received oral hygiene instructions. In the third month following the procedure, an implant-supported prosthesis was meticulously positioned to ensure optimal integration with the surrounding tissue. By the fifth month, a critical step was taken as peri-implant crevicular fluid (PICF) samples were carefully collected from the implant site. This was done with precision using calibrated microcapillary pipettes, after securing the area with cotton rolls to maintain a sterile environment. The collected samples were promptly placed in a phosphate-buffered solution to preserve their integrity and then frozen at an ultra-low temperature of -70°C. Any samples that showed signs of contamination were rigorously discarded to ensure the validity of the findings. The experimental procedures began with the application of an ELISA test kit, a sensitive method for measuring levels of MMP1 (matrix metalloproteinase) and RANKL (receptor activator of nuclear factor kappa-B ligand). A total of 30 patients who underwent dental implant placement and received implant-supported prostheses after a three-month healing period were enrolled in the study. These patients were divided into two groups for comparison. In this study, we analyzed two distinct groups of patients, each consisting of 15 individuals, to examine specific biomarkers in their conditioned saliva, known as peri-implant crevicular fluid (PICF). Group 1 focused on the evaluation of matrix metalloproteinase-1 (MMP-1) levels, measured using the highly sensitive enzyme-linked immunosorbent assay (ELISA) technique, within a defined range of 0.1 to 1.0 pg/mol/L. Meanwhile, Group 2 concentrated on the assessment of receptor activator of nuclear factor kappa-B ligand (RANKL), also analyzed via ELISA within the same concentration parameters. To determine the primary stability of the dental implants in both groups, we utilized the Periotest system, which provides a quantitative measure of implant mobility. While Group 1 centered on the MMP-1 biomarker, indicating a role in inflammatory processes and tissue remodeling, Group 2's investigation into RANKL aimed to understand its influence on osteoclastogenesis and bone resorption. Both groups underwent a thorough assessment of primary stability, allowing us to draw meaningful comparisons regarding their respective biopsies and stability metrics.

Statistical Analysis and Results

In this study, we used SPSS software for statistical analyses. To assess the significance of our findings, we employed the chi-square test, which is effective for comparing proportions across groups. This method enabled a rigorous comparison of categorical data, accurately reflecting underlying trends and relationships within the dataset.

Results

This study involved 30 patients, aged 25 to 50 years, who sought to replace a missing maxillary right central incisor with dental implants and implant-supported prostheses. The group included 16 males and 14 females. Initially, each participant underwent a Cone Beam Computed Tomography (CBCT) scan to assess their bone structure and determine the optimal location for the implants. The implants were placed immediately after this assessment. Following placement, there was a two-month healing period to allow the implants to bond with the bone. After healing, abutments were attached to prepare for the next stage of treatment. By the third month, the implant-supported prostheses were completed, restoring the patients' dental function and appearance. In the fifth month after implantation, we collected peri-implant crevicular fluid (PICF) samples from the participants. We analyzed these samples using an Enzyme-Linked Immunosorbent Assay (ELISA) to measure inflammation markers and evaluate the body's response around the implants. Table 1 presents age and gender-based statistical descriptions of the participating patients, while Graph 1 illustrates the demographic distribution and associated details, showing that 16 males and 14 females were included in the study. Table 2 displays data for Group 1 (n=15) where implants were inserted. After five months, we assessed the levels of MMP-1 (Matrix Metalloproteinase [pg/ml]) to evaluate bone remodeling and peri-implantitis using the ELISA technique, with levels ranging from 0.1-1.0 pmol/L from PICF. Statistical analysis using the Pearson Chi-Square test indicated that elevated levels were found in 9 patients. Table 3 contains information for Group 2 (n=15), where implants were also inserted. After five months, we measured the levels of RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) to assess bone remodeling and peri-implantitis, with levels ranging from 450-800 pg/mL from PICF. Statistical analysis using the Pearson Chi-Square test revealed elevated levels in 10 patients. Table 4 evaluates primary stability in Group 1 (n=15) using the Perio test in patients with the MMP-1 biomarker. A statistical analysis conducted with the Pearson Chi-Square test found that 5 patients showed stability levels of less than 1, while 14 patients showed levels greater than 9.Table 5 assesses primary stability in Group 2 (n=15) using the Perio test for patients with the RANKL biomarker. The statistical analysis using the Pearson Chi-Square test indicated that 4 patients had stability levels of less than 1, while 9 patients showed levels greater than 1.Table 6 presents a comprehensive analysis of the estimation across all studied groups, utilizing one-way ANOVA to compare the variables effectively. This statistical method enables us to draw meaningful conclusions about the differences and similarities among the groups under investigation.

Table 1: Age & gender based statistical description of contributing patients

Age Group (Yrs)	Male	Female	Total	P value
25-30	4	3	7	0.01*
31-35	3	4	7	0.20
36-40	4	3	7	0.01*
41-45	3	2	5	0.70
46-50	2	2	4	0.40
Total	16	14	30	*Significant
*p<0.05 significant				

Graph 1: Patients demographic distribution and associated details

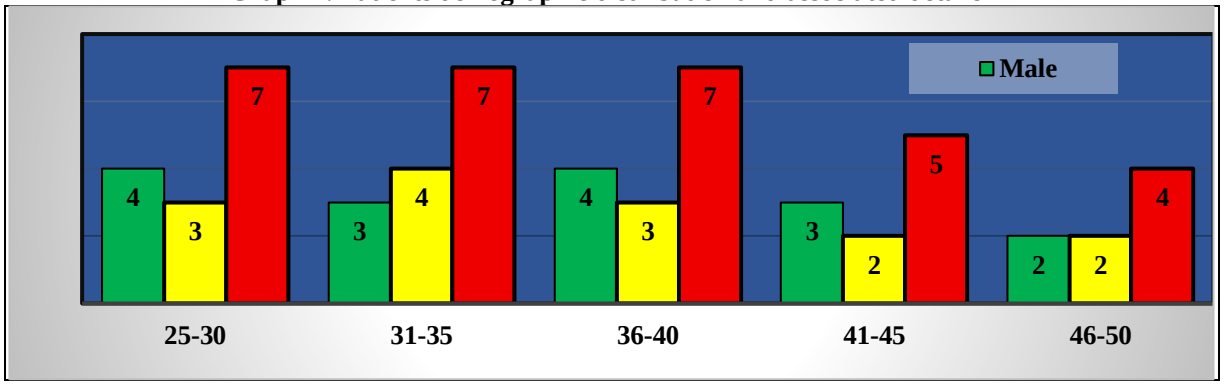

Table 2: Group 1(n=15) Implants were inserted, and after a period of 5 months, the levels of MMP-1 (Matrix Metalloproteinase [pg/ml]) were used to assess the bone remodelling and peri-implantitis using the Enzyme-Linked Immunosorbent Assay (ELISA) technique within the range of 0.1-1.0 pmol/L from Peri-Implant Crevicular Fluid (PICF). Following this, a statistical analysis was performed utilizing the Pearson Chi-Square test to determine the significance of the findings

Biomarker	Ranges	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
MMP-1 (Matrix Metalloproteinase)	Elevated levels	9	1.13	2.12	2.109	2.103	2.19	2.104	1.0
	Within normal range	2	1.12	2.10	1.106	1.103	2.09	2.094	1.0
	Below Normal range	1	1.10	2.08	2.012	1.038	1.23	1.108	0.02*
*p<0.05 significant									

Table 3: Group 2(n=15) Implants were inserted, and after a period of 5 months, the levels of RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) were used to assess the bone remodelling and periimplantitis using the Enzyme-Linked Immunosorbent Assay (ELISA) technique within the range of [450-800 pg/mL]from Peri-Implant Crevicular Fluid (PICF). Following this, a statistical analysis was performed utilising the Pearson Chi-Square test to determine the significance of the findings

Biomarker	Ranges	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand)	Elevated levels	10	1.16	2.24	2.156	2.125	2.36	2.126	1.0
	Within normal range	2	1.10	2.08	2.012	1.038	1.23	1.108	0.02*
	Below Normal range	2	1.09	1.09	1.112	1.134	1.24	1.128	0.03*
*p<0.05 significant									

Table 4: Group 1 (n=15) evaluated primary stability using the Perio test in patients with the MMP-1 (Matrix Metalloproteinase) biomarker. A statistical analysis was conducted utilising the Pearson Chi-Square test to determine the significance of the findings

Biomarker	Values of Perio test (-8 to +5)	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
MMP-1 (Matrix Metalloproteinase)	Less than+1	5	1.09	1.01	1.09	1.013	1.02	1.004	0.02*
	More than +1	9	1.12	1.13	2.102	2.090	2.12	2.112	1.0
*p<0.05 significant									

Table 5: Group 2 (n=15) evaluated primary stability using the Perio test in patients with the RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) biomarker. A statistical analysis was conducted utilising the Pearson Chi-Square test to determine the significance of the findings

Biomarker	Values of the Perio test (-8 to +5)	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand)	Less than+1	4	1.09	1.09	1.080	1.02	1.07	1.024	0.02*
	More than +1	9	1.16	2.24	2.156	2.125	2.36	2.126	1.0
*p<0.05 significant									

Table 6: Estimation amongst all studied groups using one-way ANOVA

Variables	Degree of Freedom	Sum of Squares ∑	Mean Sum of Squares m∑	F	Level of Sig. (p)
Between Groups	4	1.340	2.064	1.5	0.001*****
Within Groups	17	2.214	1.045	_	
Cumulative	123.03	1.096	*p<0.05 significant		

Discussion

Renvert S reviewed in their study that a dental implant is a common option for replacing missing teeth and helping people who have lost some or all of their teeth. Recent research shows that dental implant diseases, such as peri-implantitis (PI) and peri-implant mucositis (PIM), are quite common. However, diagnosing these diseases can be difficult. Standard methods like probing and X-rays may not give accurate results since they only show existing damage and not current disease activity. There is currently no reliable way to predict how peri-implant diseases will progress.[14-16] Delucchi F et al included in their study that while we lack a predictive model, certain biomarkers have the potential to improve diagnosis. Biomarkers help doctors objectively evaluate disease states or how patients respond to treatment. In periodontitis, biomarkers found in gingival crevicular fluid (GCF) have shown moderate success in diagnosis. Similarly, biomarkers in peri-implant crevicular fluid (PICF) also show promise for diagnosis and prognosis. Detecting these biomarkers in different fluid samples can be a dependable alternative to traditional tissue biopsies for inflammatory disease assessment. Research indicates that analysing PICF from the implant area can effectively evaluate peri-implant disease.[17,18] Bas et al. showed in their study that, in recent years, many studies have found host-derived biochemical markers in PICF. The levels of these inflammatory markers can indicate active peri-implantitis. By studying biomarkers in PICF, we may identify specific markers that contribute to the onset and progression of peri-implantitis, even in its early, harder-to-detect stages.[19] Gürsoy UK et al reviewed in their study that one significant advantage of utilizing peri-implant crevicular fluid (PICF) is its straightforward collection process, as it originates directly from the specific area of concern around dental implants. This non-invasive technique can be easily repeated over time, which is particularly beneficial given the association between peri-implantitis and an elevated presence of PICF.[20] Ono, T et al included in their study that several biomarkers play crucial roles in understanding the underlying processes. Matrix metalloproteinases (MMPs) are a class of enzymes that require calcium and zinc for their activity and play a crucial role in various physiological functions within the body. These enzymes are closely linked to inflammatory responses, autoimmune disorders, cancer progression, and infectious diseases. MMPs can exert both beneficial and detrimental effects throughout the phases of disease development and healing.[21,22] Chaudhary A et al reviewed in their study that the receptor activator of nuclear factor-kappa B ligand (RANKL), along with its receptor RANK and the decoy receptor osteoprotegerin (OPG), were initially characterized within the contexts of the immune and skeletal systems. Innovations such as plasmonic enzyme-linked immunosorbent assays (ELISAs) have revolutionized biomarker detection by integrating metal nanostructures with traditional immunoassays. This cutting-edge methodology enables rapid, highly sensitive detection of multiple biomarkers simultaneously, thereby enhancing the capacity for research and clinical applications.[23]

Conclusion

Within the limitations of this study, the findings of the study revealed that both biomarkers, RANKL and

MMP-1, play a vital role in the evaluation of bone remodeling processes and the condition of peri-implantitis. Notably, RANKL exhibited a moderately stronger correlation than MMP-1, highlighting its potential as a more reliable indicator in certain contexts. Both biomarkers are not only significant but also serve as promising prognostic tools, capable of providing valuable insights into disease progression. However, their predictive efficacy may vary considerably depending on the specific disease in question and the unique circumstances surrounding it. To fully unravel the complexities and implications of these biomarkers, future research that is both comprehensive and extended will be crucial. Such studies will deepen our understanding and potentially refine their application in clinical settings.

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