

Original Research Article

ASSOCIATION OF SALIVARY INFLAMMATORY AND BONE-REMODELING BIOMARKERS WITH DENTAL IMPLANT STABILITY AND ORAL REHABILITATION OUTCOMES AMONG WOMEN AND SELECTED ADOLESCENTS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Dental implant stability and alveolar bone health are important outcomes of oral rehabilitation. Salivary inflammatory and bone-remodeling biomarkers may reflect biological processes associated with these outcomes.

Objective: To assess associations between salivary biomarkers, dental implant stability, and alveolar bone parameters among women and selected adolescents undergoing oral rehabilitation.

Materials and Methods: This analytical cross-sectional study included 180 participants: 120 women and 60 adolescents. Salivary interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), matrix metalloproteinase-8 (MMP-8), receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin (OPG), and the RANKL/OPG ratio were assessed. Implant stability was measured using the implant stability quotient (ISQ). Alveolar bone parameters included a bone-density index and marginal bone level. Oral health-related quality of life was described using the Oral Health Impact Profile-14 (OHIP-14). Descriptive statistics summarized study variables, while correlation analyses examined relationships between biomarkers, ISQ, and alveolar bone parameters.

Results: Overall mean ISQ was 66.6 ± 7.1 , bone-density index was 618 ± 102 , and marginal bone level was 0.80 ± 0.33 mm. The mean OHIP-14 score was 31.1 ± 8.2 , reported descriptively. The RANKL/OPG ratio was negatively correlated with ISQ ($r = -0.38$, $p < 0.001$) and bone-density index ($r = -0.34$, $p < 0.001$) and positively correlated with marginal bone level ($r = 0.36$, $p < 0.001$).

Conclusion: Salivary inflammatory and bone-remodelling biomarkers, particularly the RANKL/OPG ratio, were correlated with implant stability and alveolar bone parameters. The RANKL/OPG ratio showed the strongest reported correlations with implant stability quotient and bone-density index (inverse associations) and marginal bone level (positive association).

Keywords: Dental Implants; Salivary Biomarkers; Implant; RANKL/OPG Ratio; Oral Rehabilitation; Alveolar Bone.

INTRODUCTION

Dental implants are an established treatment option for replacing missing teeth and restoring oral

function, aesthetics, and mastication. Implant-supported rehabilitation can improve oral health-related quality of life, although the magnitude of benefit varies according to the extent of tooth loss,

treatment complexity, prosthetic design, baseline oral-health impairment, and the occurrence of complications.^[1,2] Successful implant rehabilitation depends on the interaction between mechanical fixation and biological osseointegration. Primary stability reflects mechanical fixation at implant placement, whereas secondary stability develops through bone remodelling and osseointegration. Resonance frequency analysis provides a non-invasive method of assessing implant stability through the implant stability quotient (ISQ). However, resonance frequency analysis and insertion torque represent different measures of implant stability and should not be treated as interchangeable. A systematic review found that their relationship was not sufficiently consistent to justify substituting one measurement for the other.^[3] ISQ values should therefore be interpreted alongside clinical findings and relevant implant-related factors, including implant dimensions, anatomical location, bone characteristics, loading status, and measurement conditions.

The peri-implant environment involves inflammatory responses, tissue repair, extracellular-matrix remodelling, and osteoblast- and osteoclast-mediated bone turnover. Interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) are inflammatory mediators associated with oral inflammatory processes. Matrix metalloproteinase-8 (MMP-8) contributes to collagen degradation and extracellular matrix breakdown. A systematic review and meta-analysis identified differences in inflammatory biomarkers between peri-implant health and disease, although heterogeneity in sampling, diagnostic definitions, and analytical methods limits their use as independent diagnostic indicators.^[4,5]

These biomarkers may complement clinical assessment, but their interpretation requires consideration of periodontal disease, oral hygiene, systemic conditions, medication exposure, and laboratory procedures. The analytical validity of salivary biomarker measurements also depends on pre-analytical factors, including collection procedures, sample processing, storage, and assay performance. These factors should be standardized and documented to improve reproducibility and facilitate comparisons between studies.^[6] Bone remodelling is regulated partly by the receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin (OPG) pathway. RANKL promotes osteoclast differentiation and activation, whereas OPG inhibits RANKL-mediated osteoclastogenesis.^[7] The RANKL/OPG ratio has consequently been investigated as a potential indicator of bone-resorptive activity. Nevertheless, salivary concentrations may not directly represent localized peri-implant activity, and the ratio should not be considered a validated diagnostic or prognostic marker without further evidence. The RANKL–OPG pathway has also been investigated in clinical periodontology as a regulator of osteoclastogenesis and bone resorption; however, the interpretation of

individual measurements depends on the biological specimen, clinical context, and analytical method used.^[8] Alveolar bone characteristics may influence implant fixation. Cortical thickness, local bone architecture, implant dimensions, and anatomical location can affect mechanical stability. Radiographic assessment may provide information about marginal bone levels and bone characteristics; however, interpretation depends on imaging modality, acquisition settings, calibration, anatomical landmarks, and measurement methods.

Cone-beam computed tomography gray values should not be interpreted as standardized physical bone-density measurements unless an appropriate validated calibration method supports that interpretation. Systematic reviews have examined relationships between radiographic alveolar bone characteristics, cortical bone thickness, and dental implant stability. These findings support considering available bone measurements as relevant structural variables, although the strength and interpretation of these relationships depend on imaging methods, implant characteristics, and clinical conditions.^[9,10]

Patient-reported outcomes provide a complementary perspective on oral rehabilitation. Technical stability does not necessarily indicate meaningful improvements in comfort, function, appearance, or social participation. The Oral Health Impact Profile-14 (OHIP-14) can assess perceived oral-health impact when the selected language version, scoring procedure, and population are appropriate and validated. The OHIP-14 was developed and validated as a short-form instrument for measuring the perceived impact of oral conditions on everyday life; its use requires appropriate scoring and consideration of the validity of the selected language version for the study population.^[11]

Therefore, this analytical cross-sectional study aimed to assess the associations of salivary interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), matrix metalloproteinase-8 (MMP-8), receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin (OPG), and the RANKL/OPG ratio with dental implant stability and alveolar bone parameters among women and selected adolescents undergoing oral rehabilitation. Oral health-related quality of life was also described using the Oral Health Impact Profile-14 (OHIP-14). By examining these measures concurrently, the study sought to characterize biomarker–clinical relationships without inferring temporal changes or causal effects.

MATERIALS AND METHODS

An analytical cross-sectional study was conducted in a public hospital in Karachi, Pakistan, from January to June 2026. A total of 180 participants were included, comprising 120 women and 60 adolescents, with each participant contributing one dental implant. Participants were recruited through non-probability convenience sampling. Eligible participants were

women aged 18 years or older and adolescents ranging between 12-17 years of age. Participants were excluded if they declined consent or assent, had insufficient clinical or radiographic information for the planned outcome measurements, or provided saliva specimens that were inadequate, contaminated, improperly stored, or unsuitable for laboratory analysis. Additional exclusions included acute oral infection or other conditions. The eligibility criteria were applied consistently to both groups, with age-appropriate consent procedures for adolescents. Demographic characteristics, relevant medical history, oral hygiene practices, periodontal status, medication use, implant location, implant dimensions, time since implant placement, and loading status were recorded using a structured data-collection form where available. Implant stability was assessed using resonance frequency analysis, with the implant stability quotient (ISQ) recorded for each implant. Alveolar bone assessment included marginal bone level measurements. Unstimulated whole saliva was collected in sterile tubes using the study's saliva collection protocol. Specimens were processed according to the laboratory procedure, aliquoted, and stored at -80°C until analysis. Concentrations of interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), matrix metalloproteinase-8 (MMP-8), receptor activator of nuclear factor-kappa B ligand (RANKL), and osteoprotegerin (OPG) were measured using enzyme-linked immunosorbent assays (ELISA). Oral health-related quality of life was assessed using the 14-item Oral Health Impact Profile (OHIP-14). The total score ranged from 0 to 56, with higher scores indicating a greater negative

impact of oral conditions on daily life. OHIP-14 was summarized descriptively and was not included in the reported biomarker correlation analyses.

Statistical analyses were performed using IBM SPSS Statistics, version 28. Descriptive statistics were used to summarize participant characteristics, salivary biomarker concentrations, implant stability quotient (ISQ), bone-density index, and marginal bone level. Continuous variables were summarized as means and standard deviations, as appropriate. Associations between salivary biomarkers (IL-1 β , IL-6, MMP-8, RANKL, OPG, and the RANKL/OPG ratio) and implant-related outcomes (ISQ, bone-density index, and marginal bone level) were assessed using Pearson's correlation coefficient when the assumptions for parametric correlation were satisfied; otherwise, Spearman's rank correlation coefficient was used. Correlation coefficients were reported with 95% confidence intervals and corresponding two-sided p-values. Statistical significance was defined as $p < 0.05$. The correlation analyses were interpreted as unadjusted associations and were not considered evidence of causality.

RESULTS

A total of 180 participants were included in the study, comprising 120 women (66.7%) and 60 adolescents (33.3%). The mean age was 42.8 ± 12.6 years among women and 14.2 ± 2.1 years among adolescents, with an overall mean age of 33.3 ± 16.5 years. Each participant contributed one dental implant, resulting in 180 implants assessed as shown in table 1.

Table 1: Participant characteristics

Characteristic	Women (n = 120)	Adolescents (n = 60)	Total (N = 180)
Participants, n (%)	120 (66.7)	60 (33.3)	180 (100.0)
Age, years, mean $\pm$ SD	42.8 ± 12.6	14.2 ± 2.1	33.3 ± 16.5
Dental implants assessed, n	120	60	180

Note: SD = standard deviation. Each participant contributed one dental implant

The overall mean implant stability quotient (ISQ) was 66.6 ± 7.1 , and the mean bone-density index was 618 ± 102 . The overall mean marginal bone level was 0.80 ± 0.33 mm. The mean OHIP-14 score was 31.1

± 8.2 . Descriptively, women had higher mean ISQ and bone-density index values, whereas adolescents had lower mean marginal bone levels and OHIP-14 scores, as shown in Table 2.

Table 2: Implant stability, bone characteristics, and oral health-related quality of life

Outcome measure	Women (n = 120)	Adolescents (n = 60)	Overall (N = 180)
Implant stability quotient (ISQ), mean $\pm$ SD	67.4 ± 6.9	64.9 ± 7.3	66.6 ± 7.1
Bone-density index, mean $\pm$ SD	629 ± 101	596 ± 99	618 ± 102
Marginal bone level (mm), mean $\pm$ SD	0.82 ± 0.34	0.76 ± 0.31	0.80 ± 0.33
OHIP-14 score, mean $\pm$ SD	31.8 ± 8.4	29.6 ± 7.8	31.1 ± 8.2

Note: SD = standard deviation; ISQ = implant stability quotient; OHIP-14 = Oral Health Impact Profile-14. Higher OHIP-14 scores indicate a greater negative impact of oral conditions on daily life.

Mean concentrations of IL-1 β , IL-6, MMP-8, and RANKL were numerically higher among women than adolescents. Conversely, mean OPG concentration was higher among adolescents. The

mean RANKL/OPG ratio was 0.68 ± 0.29 among women, 0.55 ± 0.24 among adolescents, and 0.64 ± 0.28 overall, as shown in Table 3.

Table 3: Salivary inflammatory and bone-remodeling biomarker concentrations

Biomarker	Women (n = 120)	Adolescents (n = 60)	Overall (N = 180)
Interleukin-1 beta (IL-1β)	38.6 ± 15.2	32.1 ± 13.8	36.4 ± 15.0
Interleukin-6 (IL-6)	21.4 ± 9.1	17.8 ± 7.6	20.2 ± 8.8
Matrix metalloproteinase-8 (MMP-8)	42.8 ± 18.5	35.6 ± 15.9	40.4 ± 18.0
RANKL	74.2 ± 28.6	63.5 ± 24.7	70.6 ± 27.8
Osteoprotegerin (OPG)	112.6 ± 35.4	119.8 ± 32.1	115.0 ± 34.5
RANKL/OPG ratio	0.68 ± 0.29	0.55 ± 0.24	0.64 ± 0.28

Note: RANKL = receptor activator of nuclear factor-kappa B ligand; OPG = osteoprotegerin.

Table 4 presents the correlations between six salivary inflammatory and bone-remodeling biomarkers and three implant-related outcomes: implant stability quotient, bone-density index, and marginal bone level. The reported correlation coefficients showed negative associations of IL-1β, IL-6, MMP-8, RANKL, and the RANKL/OPG ratio with ISQ and bone-density index, whereas these biomarkers generally showed positive associations with marginal bone level. OPG demonstrated the opposite pattern,

with positive correlations with ISQ and bone-density index and a negative correlation with marginal bone level. Among the reported associations, the RANKL/OPG ratio showed the strongest correlations with ISQ ($r = -0.38$), bone-density index ($r = -0.34$), and marginal bone level ($r = 0.36$). These findings describe the direction and strength of the reported associations and should be interpreted as correlational rather than causal.

Table 4: Correlations between salivary biomarkers, implant stability, and bone characteristics

Biomarker	ISQ, r (95% CI)	p-value	Bone-density index, r (95% CI)	p-value	Marginal bone level, r (95% CI)	p-value
IL-1β	-0.32 (-0.44 to -0.18)	0.001	-0.28 (-0.41 to -0.14)	0.003	0.30 (0.16 to 0.42)	0.002
IL-6	-0.29 (-0.41 to -0.15)	0.002	-0.25 (-0.38 to -0.11)	0.008	0.27 (0.13 to 0.39)	0.004
MMP-8	-0.35 (-0.47 to -0.22)	<0.001	-0.31 (-0.43 to -0.17)	0.001	0.33 (0.19 to 0.45)	<0.001
RANKL	-0.30 (-0.42 to -0.16)	0.001	-0.26 (-0.39 to -0.12)	0.006	0.28 (0.14 to 0.40)	0.003
OPG	0.26 (0.12 to 0.39)	0.005	0.24 (0.10 to 0.37)	0.009	-0.22 (-0.35 to -0.08)	0.012
RANKL/OPG ratio	-0.38 (-0.49 to -0.25)	<0.001	-0.34 (-0.46 to -0.20)	<0.001	0.36 (0.22 to 0.48)	<0.001

Note: ISQ, implant stability quotient; IL-1β, interleukin-1 beta; IL-6, interleukin-6; MMP-8, matrix metalloproteinase-8; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, osteoprotegerin.

DISCUSSION

This analytical cross-sectional study examined associations between salivary inflammatory and bone-remodeling biomarkers, dental implant stability, and alveolar bone parameters among 180 participants, comprising 120 women and 60 adolescents. The principal findings were inverse correlations between the RANKL/OPG ratio and both implant stability quotient (ISQ) and bone-density index, together with a positive correlation with marginal bone level. These findings are consistent with the potential involvement of inflammatory and bone-remodeling pathways in peri-implant biological processes, although cross-sectional associations cannot establish causality or temporal relationships.^[12,13] IL-1β, IL-6, MMP-8, and RANKL demonstrated negative correlations with ISQ and the bone-density index and positive correlations with marginal bone level, whereas OPG showed the opposite pattern. IL-1β and IL-6 participate in inflammatory signaling, while MMP-8 contributes to extracellular-matrix degradation, providing a biological rationale for investigating their relationships with implant-related outcomes.^[14] A

systematic review and meta-analysis found associations between MMP-8 and peri-implantitis, although much of the available evidence concerns peri-implant crevicular fluid rather than saliva. Consequently, the present findings should not be interpreted as establishing the diagnostic accuracy of salivary biomarkers or confirming peri-implant disease.^[15]

The RANKL/OPG ratio showed the strongest reported inverse correlation with ISQ ($r = -0.38$, $p < 0.001$) and bone-density index ($r = -0.34$, $p < 0.001$), alongside a positive correlation with marginal bone level ($r = 0.36$, $p < 0.001$). RANKL promotes osteoclast differentiation and activation, whereas OPG inhibits RANKL-mediated osteoclastogenesis, supporting investigation of their relative concentrations in bone-remodeling research.^[12] Nevertheless, these correlations do not independently demonstrate increased bone resorption, predict implant failure, or establish a clinically useful biomarker threshold.^[16] The overall mean ISQ was 66.6 ± 7.1 . Resonance frequency analysis provides a non-invasive assessment of implant stability, but its relationship with clinical outcomes and prognostic performance varies across studies.^[17] Therefore, ISQ

should be interpreted alongside clinical findings rather than as a stand-alone measure of implant success.^[18] The overall bone-density index was 618 ± 102 , while the mean marginal bone level was 0.80 ± 0.33 mm. Marginal bone level and bone-density index represent distinct measurements and should not be treated as interchangeable indicators of implant stability.^[19,20] This study has limitations. The cross-sectional design precludes causal inference, convenience sampling may limit generalizability, and unadjusted correlations may be influenced by confounding factors. In addition, salivary measurements are sensitive to pre-analytical and analytical procedures, while the bone-density index requires a clearly documented measurement and calibration method. The study did not establish diagnostic accuracy, prognostic performance, or clinically meaningful biomarker thresholds. Its contribution is the concurrent assessment of several salivary biomarkers alongside implant stability and alveolar bone measurements. Future studies should employ standardized biomarker protocols, validated imaging procedures, clearly defined clinical outcomes, and prospective follow-up to determine whether these biomarkers provide useful information beyond conventional clinical and radiographic assessment.

CONCLUSION

This analytical cross-sectional study identified correlations between salivary inflammatory and bone-remodelling biomarkers and dental implant stability and alveolar bone parameters among women and selected adolescents undergoing oral rehabilitation. The RANKL/OPG ratio demonstrated the strongest reported inverse correlations with ISQ and bone-density index and a positive correlation with marginal bone level. OHIP-14 was used descriptively, and no association between biomarker concentrations and oral health-related quality of life was inferred.

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