BMC Oral Health

RESEARCH

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Association between salivary soluble RANKL, OPG concentration and mandibular resorption to clinically predict severe residual ridge resorption risk: a cross-sectional study

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Abstract

Background The insidious progression of residual ridge resorption (RRR) following tooth extraction remains a formidable clinical challenge in contemporary dentistry. Understanding its underlying cellular and molecular mechanisms is key to developing both patient-specific risk assessments for preventive care and novel therapies to arrest bone loss. Salivary bone turnover biomarkers show promise as a non-invasive method for evaluating the rate of bone loss. This research aimed to identify the association of salivary receptor activator of nuclear factor-KB ligand (RANKL), and osteoprotegerin (OPG) levels with resorbed mandibular edentulous ridges, proposing a clinical tool for identifying high resorption risk.

Methods The mandibular ridge height was determined radiographically on 140 systematically healthy edentulous participants. Based on the Cawood and Howell system and the American College of Prosthodontists classification, the subjects were categorized as severe or normal resorption. Complete denture clinical quality was assessed using the Chulalongkorn University-modified Kapur criteria. Whole unstimulated salivary was collected, and salivary RANKL and OPG protein levels were quantified by enzyme-linked immunosorbent assay technique. Logistic regression analysis was used to develop a risk assessment model for severe resorption. Nomograms were generated from the final model to estimate the risk of RRR.

Results Excessive RRR were found in a notably higher percentage of women (36.43%) versus men (13.57%). A strong association between severe RRR and RANKL, OPG concentration, denture quality, edentulous duration was found. Elevated RANKL levels were significantly associated with increased alveolar bone loss, with each 10 pg/ml increase corresponding to a 4-fold and 3.5-fold increase in RRR odds for women and men, respectively. Conversely, higher OPG levels showed a protective effect; for every 10 pg/ml increase, the RRR odds decreased by 60% for women and 73% for men. To estimate accelerated bone resorption risk, a nomogram with high predictive accuracy (AUC = 0.94) was developed using edentulous duration and RANKL level.

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Conclusions Salivary RANKL and OPG levels are strongly associated with the severity of RRR, with gender differences observed in resorbed mandibular. Elevated RANKL increases RRR risk, while higher OPG offers a protective effect. This study's novel nomogram offers a practical chairside tool to assess this risk, enabling tailored patient counseling and proactive treatment plans to preserve mandibular bone.

Keywords Osteoprotegerin, Receptor activator of nuclear factor-κB ligand, Residual ridge resorption, Nomogram

Background

Currently, our longer lifespans are associated with a greater incidence of tooth loss in older age. According to the Global Burden of Disease study (2017–2019), 267 million people worldwide have experienced total tooth loss [1], leading to residual ridge resorption (RRR), a progressive and irreversible loss of jawbone volume affecting people of all backgrounds and health statuses [2]. Inter-individual variation in alveolar ridge resorption is substantial. Some individuals experience substantial bone loss [3], resulting in diminished quality of life, nutritional deficiencies [4], and cognitive decline [5]. Additionally, this resorption makes individuals more susceptible to mandibular fractures, with reported complication rates of up to 20% [6]. A complex interplay of local factors, systemic conditions and genetic predispositions drives this irreversible phenomenon [7–10]. Nevertheless, the cellular and molecular mechanisms underlying the persistence of jawbone resorption even after extraction site healing and the risk factors for RRR severity remain subjects of ongoing research [11].

The basic multicellular unit responsible for bone remodeling requires precisely timed and spatially coordinated osteoblast-osteoclast interactions to maintain the localized balance of bone remodeling [12]. However, osteoclast behavior on the buccal and lingual walls of the socket differs significantly. It lacks coupled osteoblastic bone formation, leading to post-extraction alveolar bone reduction over time [13]. Limited research has investigated the abnormal osteoclast activation and influencing factors after tooth loss. Araujo et al., using a canine model, observed numerous osteoclasts on the mature cortical bone during socket recovery, suggesting potential deficiencies in osteoblast-osteoclast communication that may hinder bone regeneration [14].

The receptor activator of nuclear factor-kappa B (RANK), its ligand (RANKL), and osteoprotegerin (OPG) signaling pathway is pivotal in regulating osteoclastogenesis. Discovered in the 1990s, this intricate cellular mechanism has been acknowledged as the primary controller of osteoclast differentiation and activation, playing crucial roles in bone homeostasis [15]. RANKL, a type II transmembrane protein expressed in osteoblasts, osteocytes, and immune cells within bone tissue, undergoes ecto domain cleavage to generate soluble RANKL, which is released into the extracellular environment [16]. RANK, a type I transmembrane protein expressed on osteoclast progenitors, mature osteoclasts, and immune cells, interacts with its ligand RANKL on osteoblast membranes to stimulate osteoclastogenesis, bone remodeling, and calcium homeostasis [17]. OPG, a soluble decoy receptor for RANKL secreted by osteoblasts, inhibits osteoclast formation and bone resorption by preventing RANKL from binding to RANK [18]. The RANKL/OPG ratio serves as an indicator of bone health, determining the bone resorption rate [18, 19]. Notably, significantly elevated salivary RANKL levels and decreased OPG levels have been observed in individuals with periodontitis compared to those with healthy periodontal status [20–22]. These identified biomarkers hold promise as noninvasive diagnostic tools for the prediction, monitoring, and personalized management of alveolar bone tissue diseases.

While research into the molecular mechanisms behind severe RRR continues, several studies highlight the role of these osteoimmunological biomarkers. For example, Canciani et al. conducted gene expression profiling of these biomarkers to monitor bone remodeling dynamics in grafted tissues [23]. Furthermore, Hisamoto et al. demonstrated the therapeutic potential of anti-RANKL antibodies in mitigating bone loss associated with RRR using an ovariectomized mouse model of postmenopausal osteoporosis [11]. These compelling findings underscore the critical importance of elucidating the intricate interplay between RANKL, RANK, and OPG in regulating osteoclast activity during RRR, potentially leading to novel therapeutic targets. Furthermore, despite the burgeoning research on RRR, the development of accurate predictive models for individual risk assessment remains a significant challenge. To address these gaps, this study aimed to determine the association between salivary soluble RANKL and OPG levels and the extent of mandibular resorption as well as to propose a way to clinically predict severe RRR risk.

Materials and methods

Study design and participants

This cross-sectional study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethical Committee Board of the University of Medicine and Pharmacy at Ho Chi Minh City (UMP) (Date: 2021-10–12, No.488/HĐĐĐ-ĐHYD). Recruitment was conducted between November 2021 and April 2024. Informed written consent was obtained

from all participants prior to clinical examinations and saliva collection. The sample size was determined based on data from Abdullameer's study [20].

Subjects were enrolled in this study if they fulfilled the following inclusion criteria: (1) complete edentulism in both the maxilla and mandible for a period of at least 3 years; (2) age greater than 35 years; and (3) absence of any history of alveolar bone augmentation or mandibular defects. Subjects were excluded if they presented with any of the following: xerostomia; systemic diseases known to affect bone metabolism or immune function (e.g., osteoporosis, rheumatoid arthritis, thyroid disorders, diabetes mellitus, HIV infection); a history of malignancy; current treatment with antibiotics or immunosuppressant medications within the 3 months preceding enrollment; or salivary gland disease [20]. The final enrollment count reached one hundred and forty participants. The case registration process is shown in Fig. 1.

Participant categorization

Digital panoramic radiographs were acquired using a Sirona Galileos Digital Panoramic X-ray System (Sirona Dental Systems, Bensheim, Germany) by an experienced radiology technician at the UMP radiology department. Imaging parameters were standardized at 70–80 kVp, 08–10 mA, and an exposure time of 14.1–14.3 s. A trained examiner, employing Xie et al.'s method, determined mandibular height (including body length, midline, premolar, and molar dimensions) via AutoCAD (version 2026.0.1) [24]. Participants were classified into severe (case) and normal (control) RRR groups based on the Cawood and Howell classification system and the American College of Prosthodontists (ACP) classification [25, 26]. The case group was defined as individuals with ridge heights below 25 mm in the anterior region and below 16 mm in the posterior region. Intra-examiner reliability for bone height measurements was assessed using the Intra-class Correlation Coefficient (ICC), demonstrating high reliability with an ICC of 0.86.

Clinical factors

A single calibrated prosthodontist (the Kappa score ranged: 0.90–0.92) evaluated denture-related factors. Complete denture quality, specifically retention and stability, was assessed using the CU-modified Kapur index [27]. Maxillary and mandibular denture retention were acceptable if scores were above 2 and 1, respectively. Acceptable stability was defined as a score of 2 for both maxillary and mandibular dentures. Overall denture quality was defined as clinically acceptable only when both retention and stability criteria were met for both dentures. Patient demographics, including age, sex, duration of edentulism, and prior complete denture experience, were collected via patient or proxy interview.

Saliva sampling and assessment of salivary levels of RANKL and OPG by ELISA

Unstimulated whole saliva samples (~ 5 ml) were collected between 07:00 and 08:00 a.m following a standardized protocol. Participants abstained from food, beverages, smoking, chewing gum, and oral hygiene for a minimum of 30 min prior to sampling [28–31]. The sampling procedure was immediately preceded by denture removal (if applicable), a distilled water oral rinse to clear particulates, and a 10-minute waiting period to prevent dilution. Participants were explicitly instructed to expectorate pure saliva into a sterile polypropylene tube, ensuring the sample was uncontaminated by sputum. Samples were immediately placed in a cool box containing an ice pack and transported to the Center for Molecular Biomedicine. Upon arrival, each saliva sample was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was then transferred to new, sterile micro-cap tubes and stored at −80°C for further analysis.

Concentrations of RANKL and OPG in whole saliva were determined using commercial sandwich ELISA kits (CSBE05125h, Cusabio, China for RANKL; EHTNFRSF11B, Invitrogen, Massachusetts, USA for OPG). In these assays, antigens from saliva samples were captured by plate-bound antibodies and subsequently detected using a biotinylated antibody and streptavidin-HRP conjugate system. Color development was initiated with tetramethylbenzidine substrate and halted with an acidic stop solution. The resulting absorbance at 450 nm (corrected at 540–570 nm), which is proportional to the concentration of bound antigen, was used to interpolate sample concentrations from standard curves (RANKL: 7.8–500 pg/mL; OPG: 1.23–900 pg/mL) via four-parameter logistic regression. The assays demonstrated robust analytical performance, with detection limits of < 1.95 pg/mL for RANKL and ~ 1 pg/mL for OPG, and high precision as shown by low intra-assay (CV < 8–10%) and inter-assay (CV < 10–12%) coefficients of variation.

Statistical analysis

Continuous and categorical data were summarized using descriptive statistics. Independent samples t-tests were used to analyze mean differences in age and mandibular height, while the Mann-Whitney U test compared edentulous period, denture count, salivary RANKL, OPG, and the RANKL/OPG ratio. The Chi-square or Fisher's exact tests assessed the differences in denture quality. Logistic regression analysis was used to develop a risk assessment model for severe resorption. To enhance the interpretability of the odds ratios, concentrations of RANKL and OPG were rescaled and entered into the model per 10 pg/ml increase. The binary variable "denture quality" was included with "acceptable" serving as the reference category. The Bayesian model averaging approach was

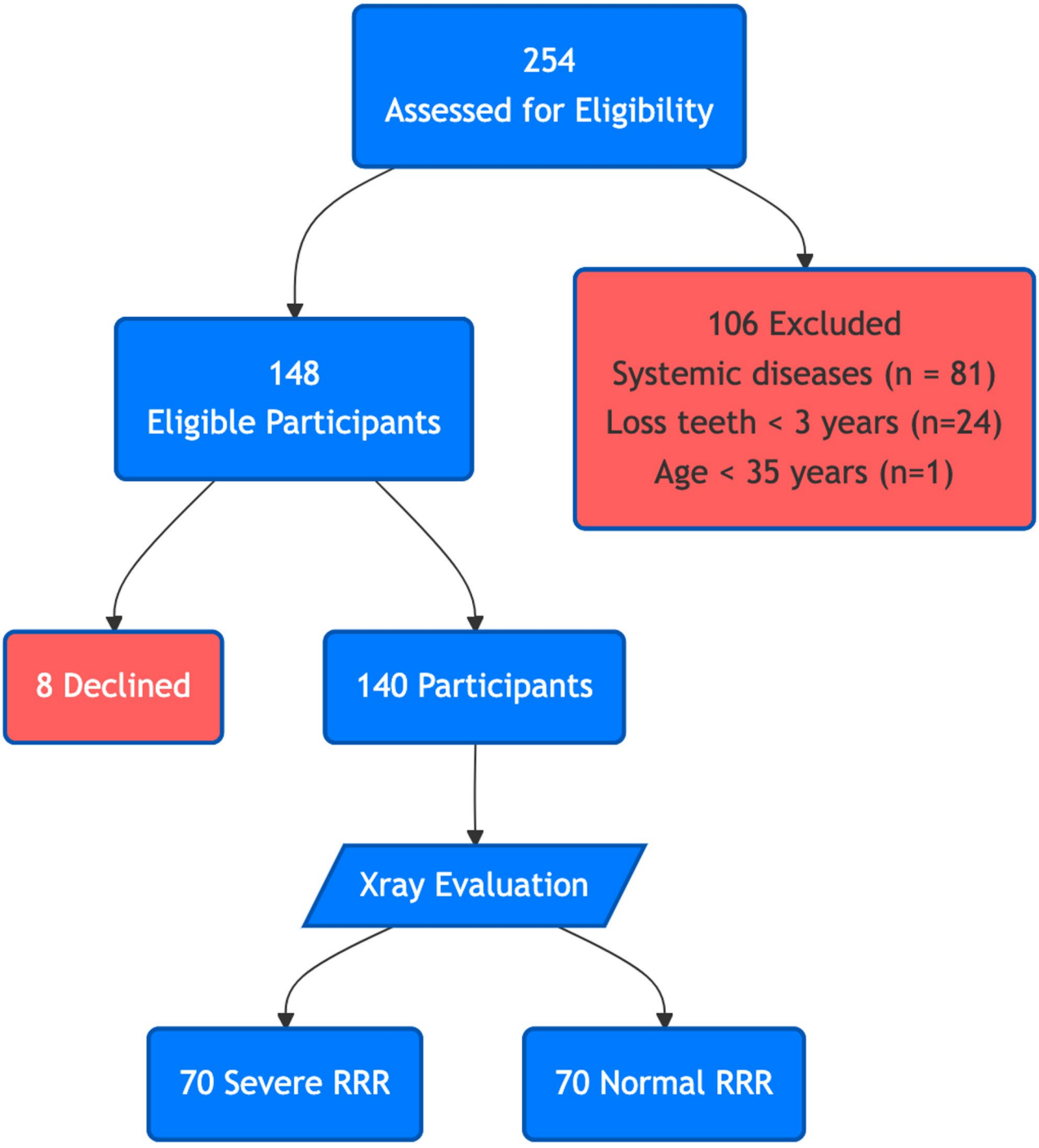

Fig. 1 Flowchart of participants through study

implemented to select the most parsimonious model based on the Bayesian information criterion. Nomograms were generated from the final model to estimate the risk of RRR. The discrimination of the model was evaluated in terms of the receiver operating characteristic curve (ROC) and the area under the curve (AUC). We used the bootstrap method to validate the model's predictive performance internally. All analyzes were performed using the R language version 3.4.0 on the Windows 11 platform.

Results

Participant baseline characteristics

Of the study participants, 61.43% were women. A significantly greater proportion of women presented

with atrophic mandibles (36.43%) compared with men (13.57%), representing a threefold difference ($p < 0.05$). Consequently, demographic and clinical characteristics were classified by both RRR status and sex (Table 1). The participants' mean age was 69.2 ± 8 years. They experienced an overall mean of 14.97 ± 9 years of edentulism.

Table 1 Baseline characteristics stratified by sex and RRR

Variable	RRR status		*p*-value
	Severe	Normal	
Men			
n	19	35	
Age[a] (years)	69 (66–71)	66 (61–72)	0.179
Duration of edentulism[b] (years)	18 (10–20)	10 (8–17)	0.083
Number of denture[b]	2 (1–3)	1 (1–2)	0.061
Denture quality[c]			
Acceptable	1 (12.5%)	7 (87.5%)	0.023*
Unacceptable	15 (55.6%)	12 (44.4%)	
Anterior mandibular height[a] (mm)	19.14 (4.8)	29.42 (4.2)	0.000*
Posterior mandibular height[a] (mm)	12.25 (2.55)	23.65 (4.7)	0.000*
Women			
n	51	35	
Age [a] (years)	70.18 (9.48)	69,29 (7.3)	0.805
Duration of edentulism[b] (years)	16 (8–24)	10 (6–20)	0.041*
Number of denture[b]	2 (1–3)	1 (1–2)	0.049*
Denture quality			
Acceptable	6 (50%)	6 (50%)	0.35
Unacceptable	36 (64.3%)	20 (35.7%)	
Anterior mandibular height[a] (mm)	19.12 (3.47)	24.57 (4.09)	0.000*
Posterior mandibular height[a] (mm)	12.65 (2.27)	20.57 (2.87)	0.000*
Men + Women			
n	70	70	
Age[a] (years)	70.19 (8.79)	68.14 (7.15)	0.134
Duration of edentulism[b] (years)	17 (9.5–20)	10 (7–19.25.25)	0.006*
Number of denture[b]	2 (1–3)	1 (1–2)	0.005*
Denture quality			
Acceptable	7 (35%)	13 (65%)	0.032*
Unacceptable	51 (61.4%)	32 (38.6%)	
Anterior mandibular height[a] (mm)	19.13 (3.84)	27 (4.78)	0.000*
Posterior mandibular height[a] (mm)	12.51 (2.33)	22.1 (4.16)	0.005*

RRR Residual ridge resorption

[a]Mean (SD)

[b]Median (Interquartile Range-IQR),[c]: Frequency (percentage). Statistical comparisons were performed using the Independent samples t-test for age, anterior and posterior mandibular height; the Mann-Whitney U test for duration of edentulism, number of dentures; the Chi-square test and Fisher's exact test for denture quality

*p < 0.05

Within each sex, no significant difference in age was observed between participants with and without severe RRR. As expected, men and women with severe RRR had a prolonged duration of edentulism and a higher number of dentures than those without resorption.

Overall, 80.6% of participants were categorized into unacceptable denture wearers. A statistically significant association was observed between unacceptable denture retention and stability and excessive RRR ($p < 0.05$). Furthermore, participants with severe resorption exhibited a mean posterior mandibular height of less than 13 mm, irrespective of sex (Table 1).

Bone biochemical analyzes

Whole salivary RANKL was significantly higher in the severe resorption group (Median: 39.2, IQR: 34.86–46.69 pg/ml) than the normal mandibular ridges (Median: 12.75, IQR: 8.76–14.77 pg/ml) ($p < 0.000$). Conversely, OPG was significantly lower in the case group (Median: 8.25, IQR: 4.54–18.33 pg/ml) compared to controls (Median: 33.99, IQR: 27.46–55.11 pg/ml) ($p < 0.000$). Consequently, the RANKL/OPG ratio was 12-fold greater in the RRR group, consistent with observed RANKL and OPG differences (Fig. 2).

Risk factors and diagnostic model of severity RRR

To account for potential sex-based differences, an initial analysis of biomarker levels was performed, which confirmed that women had significantly higher RANKL concentrations and RANKL/OPG ratios. Consequently, all univariate analyzes of risk factors associated with bone loss were stratified by sex.

For each sex, univariate logistic regression revealed significant associations of all investigated risk factors with RRR, except for denture quality (Table 2). RANKL emerged as the most significant risk factor. For every 10 pg/ml increase in RANKL concentration, the odds of RRR increased nearly fourfold (95% CI: 2.28–6.47) in women and 3.5-fold in men. In contrast, every 10 pg/ml increase in OPG corresponded to a 60% reduction in the odds of RRR in women and a 73% reduction in men (Table 2).

To identify the most critical predictors of bone resorption from all potential variables, we used Bayesian model averaging (BMA). Bayesian information criteria identified duration of edentulism and salivary RANKL as the most parsimonious diagnostic model:

$$x = -4.78 + 0.08 * \text{duration of edentulism} + 0.13 * \text{salivary RANKL}$$

Each additional year of edentulism was associated with a 1.06-fold (1.02–1.11) increase in RRR odds, while a 10 pg/ml increase in RANKL yielded an odds ratio of

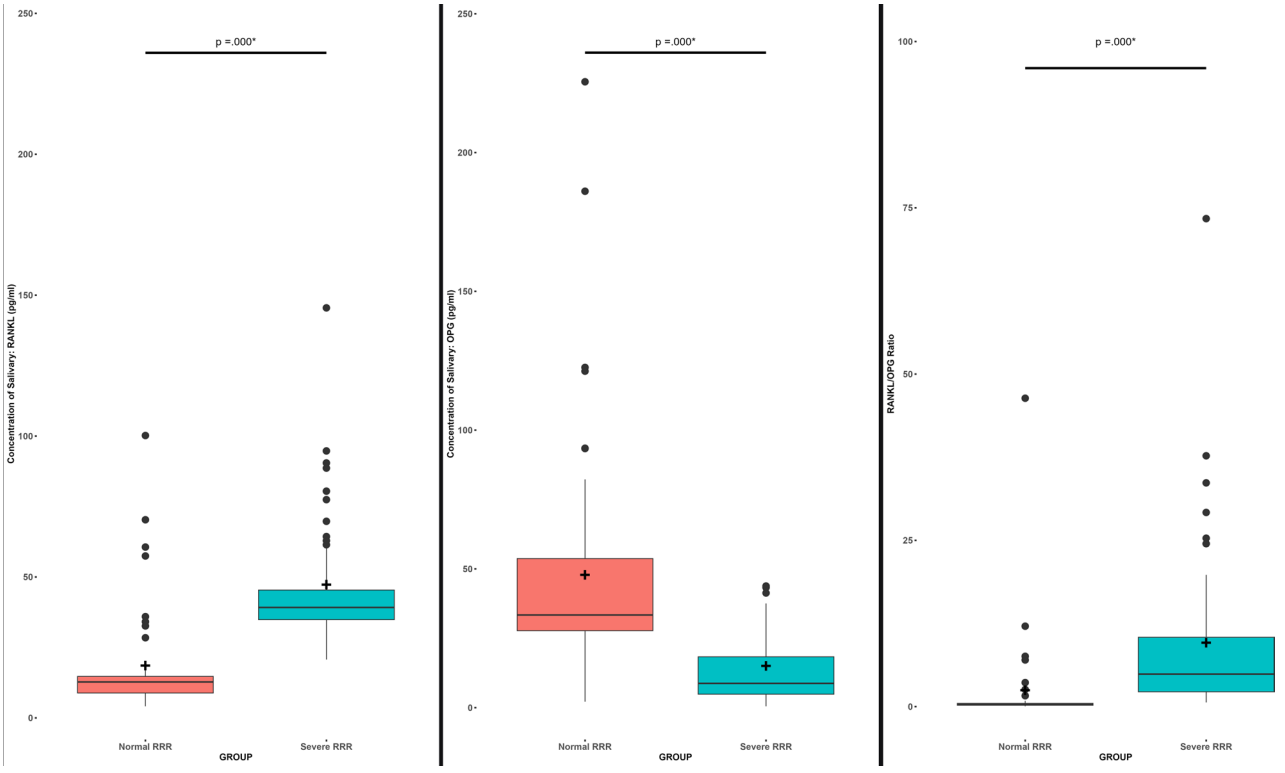

Fig. 2 (**a**, **b**, **c**) The relationship between salivary bone turnover biomarkers and the severity of alveolar ridge resorption Colored boxes within each panel represent the interquartile range (25th to 75th percentiles) for RANKL (**a**), OPG (**b**), and the RANKL/OPG ratio (**c**) across different RRR categories. Solid black dots indicate outliers beyond this range. Black lines and cross symbols denote median and mean values, respectively. Abbreviations: RRR, residual ridge resorption; RANKL, Receptor activator of nuclear factor-κB ligand, Osteoprotegein, OPG; *: Mann-Whitney U test

Table 2 Association between risk factor and RRR: univariate logistic regression analysis

Risk factor	Comparison unit [a]	Women		Men	
		OR (95%CI)	*p*	OR (95%CI)	*p*
Duration of edentulism	+ 1 year	1.06 (1.01–1.1)	0.02	1.08 (0.99–1.7)	0.07
Number of denture	+ 1	1.76 (1.08–2.85)	0.02	1.6 (0.91–2.83)	0.1
Denture quality	-	0.56 (0.16–1.95)	0.36	0.11 (0.01–1.06)	0.056
RANKL	+ 10 pg/ml	3.84 (2.28–6.47)	0.000	3.53 (1.85–6.74)	0.000
OPG	+ 10 pg/ml	0.4 (0.26–0.59)	0.000	0.27 (0.14–0.54)	0.000

RANKL Receptor activator of nuclear factor-κB ligand, *OPG* Osteoprotegein

[a]The clinically meaningful comparison unit was established

3.81 (2.55–5.69). Ultimately, neither sex nor OPG was retained in the final model, as the BMA analysis provided no evidence that their inclusion would enhance the model's predictive efficacy.

Based on this final model's variables, we estimated the risk of RRR according to the following equations:

$$\text{risk} = \frac{1}{(1 + e^{-x})}$$

The connection between edentulous period, RANKL levels, and the risk of severe RRR is illustrated in Fig. 3. The key takeaway is that at the same period of edentulism, the higher the RANKL concentration, the more serious the bone loss.

Nomogram model

Using the estimated variables in the final model, a predictive nomogram was constructed for estimating the risk of advanced resorption mandible (Fig. 4). The magnitude of association between the RANKL factor and RRR was dramatically higher than the time of the edentulous factor.

Clinical applications of the nomograms can be illustrated by the following case scenario: a completely edentulous patient with a salivary RANKL level of 40 pg/ml and an edentulous duration of 5 years. The points for RANKL and Edentulous Duration are approximately 25 points and 2 points respectively. The sum of 27 points is translated to Linear Predictor of 1 and a 70% risk of severe mandibular bone loss.

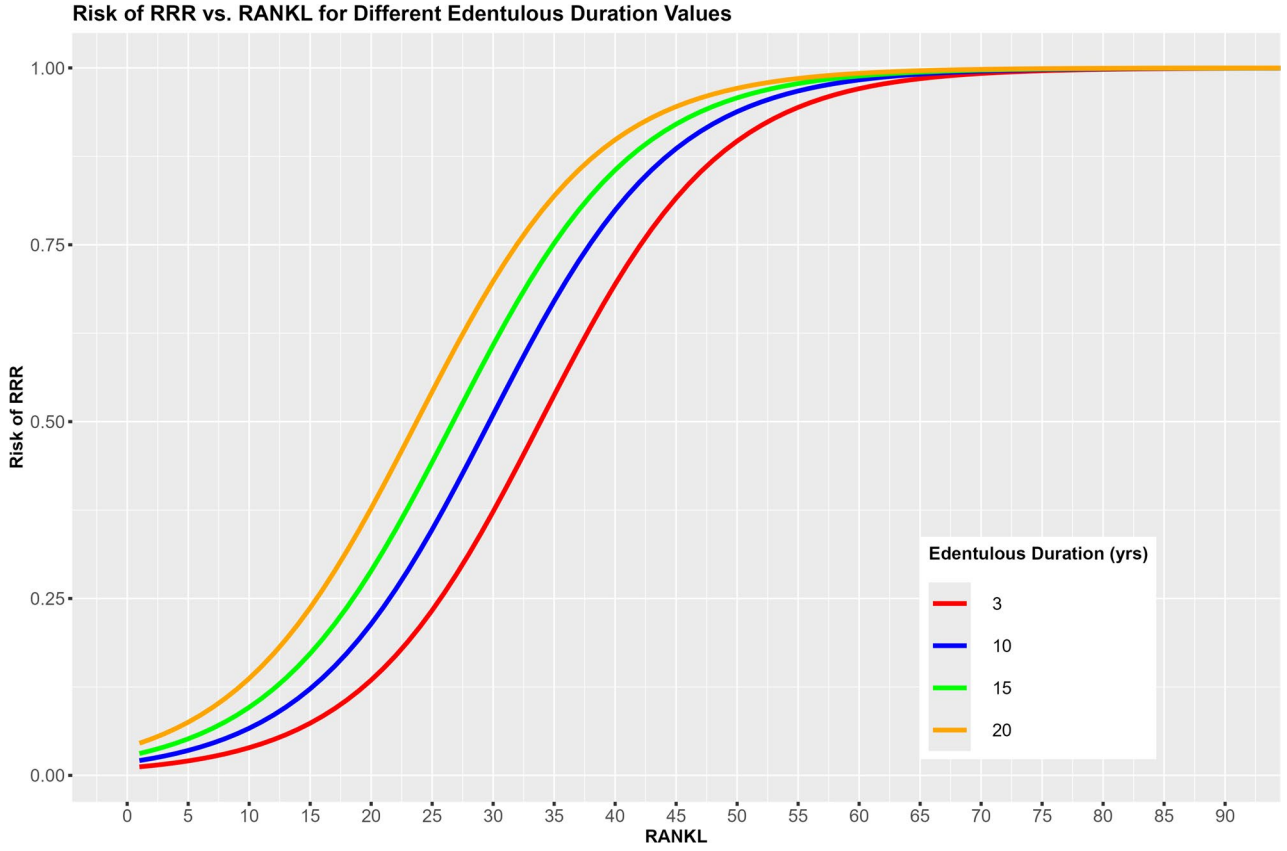

Fig. 3 Predicted risks of severe RRR for a given edentulous duration and whole saliva RANKL level Each line represents the risk trajectory for a specific edentulous duration (3, 10, 15, and 20 years) across a range of RANKL concentrations. The curves demonstrate a clear positive correlation between RANKL concentration and RRR risk. Abbreviations: RRR: residual ridge resorption; RANKL: Receptor activator of nuclear factor-κB ligand

Nomogram validation

To assess the model's predictive performance, we used ROC curves. The ROC analysis yielded an AUC of 0.94 (95% CI: 0.89–0.99), indicating good discrimination (Fig. 5). To mitigate overfitting, we conducted internal validation using bootstrap resampling, which allowed us to evaluate optimism-corrected discrimination and calibration. The calibration curve showed good agreement between observed and predicted probabilities of severe bone loss, with a maximum calibration error of approximately 0.08, underscoring its reliability in assessing the risk of mandibular bone loss (Fig. 6).

Discussion

This study found that salivary RANKL and OPG levels are powerful biomarkers for quantitatively assessing the risk of severe residual ridge resorption, offering a potential paradigm shift in prognostic care for edentulous patients. A 10 pg/ml increase in salivary RANKL concentration led to a 4-fold and 3.5-fold increase in RRR odds in women and men, respectively. Conversely, a 10 pg/ml anti-resorptive cytokine OPG increase was associated with a 60% and 73% RRR odds reduction in women and men, respectively. Moreover, this study also tries to establish a predictive nomogram using osteoclastogenesis biomarkers and clinical parameters to assess alveolar ridge resorption risk in edentulous individuals.

Sample characteristics

The process of alveolar bone resorption following tooth extraction is a complex phenomenon influenced by a multitude of factors, both local and systemic [7, 32, 33]. Additionally, aging contributes to dysregulation of bone remodeling, marked by a shift towards increased osteoclastogenesis and decreased osteoblastogenesis [34]. Thus, the selection has focused on a cohort of elderly edentulous individuals with no underlying systemic conditions known to impact bone health and also has no significant difference in age between the study groups (p >0.05, Table 1). By being carefully tailored to the selection of individuals, the study has effectively eliminated the potential influence of age-related bone remodeling and systemic conditions on the observed outcomes.

We observed that women showed a significantly higher risk of mandibular resorption, experiencing three times more bone loss than men (Table 1). This finding aligns with previous research showing that women experience greater vertical bone loss in their mandibles [2, 33, 35].

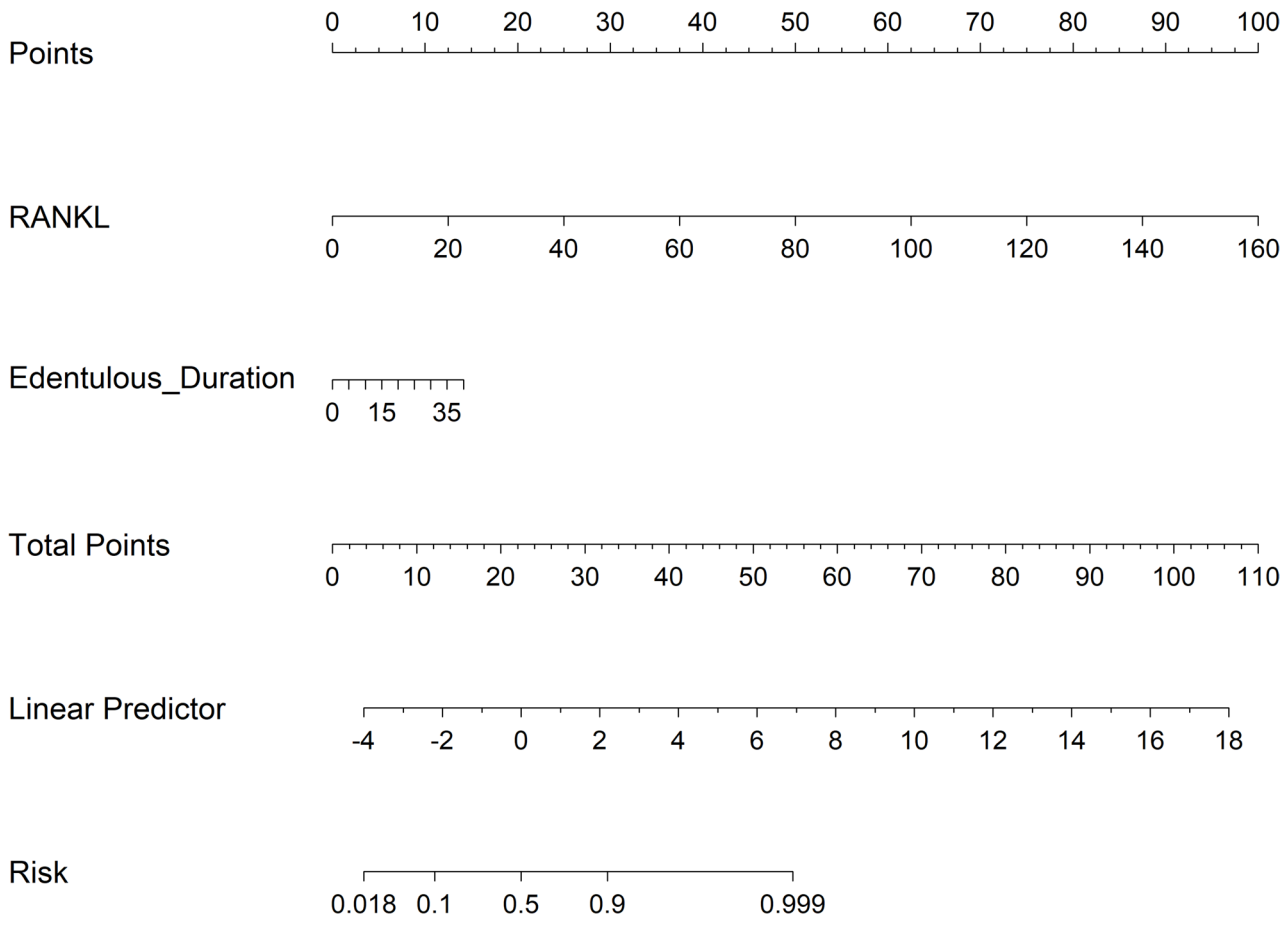

Fig. 4 Nomogram for estimating the risk of mandibular bone loss in the older adult population A point system within the nomogram assesses severe mandibular bone loss risk by summing points from each variable. Aligning total scores to the vertical diagnostic likelihood scale allows for individual risk calculation. Abbreviations: RANKL, Receptor activator of nuclear factor-κB ligand

One plausible explanation is that the estrogen deficiency in menopausal and postmenopausal women increases osteoclast activity, leading to bone loss and cortical porosity [36]. All women in this study were postmenopausal (mean age: 69.81 ± 0.9), further supporting the role of estrogen deficiency in alveolar ridge resorption. Additionally, individuals with significantly greater bone loss had longer histories of edentulism and denture use compared to those with less severe resorption ($p < 0.05$, Table 1). This supports prior research suggesting the process of alveolar atrophy is cumulative and irreversible [33, 35, 37], but the study found no significant correlation between these factors and the amount of RRR in males. This may be because of the small sample size of males with severe RRR (19 subjects), limiting the statistical power to detect a difference.

A substantial majority (80.6%) of study participants presented with clinically unacceptable denture quality. This high prevalence is likely attributable to the study's hospital-based recruitment strategy, which inherently enriched the sample with individuals experiencing severe denture-related problems requiring clinical intervention, rather than reflecting community prevalence. Furthermore, participants with excessive RRR exhibited diminished denture quality in both maxillary and mandibular components (Table 1). These individuals presented with reduced anterior and posterior ridge height, indicative of advanced bone resorption ($p < 0.05$). These findings corroborate previous reports by Huumonen et al. and Marcello-Machado et al., who demonstrated a significant association between severe residual ridge resorption and compromised denture retention and stability [38, 39].

Biochemical analyzes

Bone metabolism's primary regulators, RANKL and OPG, showed significant differences between those with and without an extreme reduction in the height of the alveolar ridge (Fig. 2). Whole salivary RANKL, a key mediator of bone resorption, was markedly upregulated in the severe RRR group (45.56 ± 20.35 pg/mL) compared with the normal group (16.18 ± 10.6 pg/mL). Conversely, salivary OPG, an inhibitor of bone resorption, was significantly lower in the case group ($p < 0.000$). The RANKL/OPG ratio, an indicator of bone remodeling activity, was

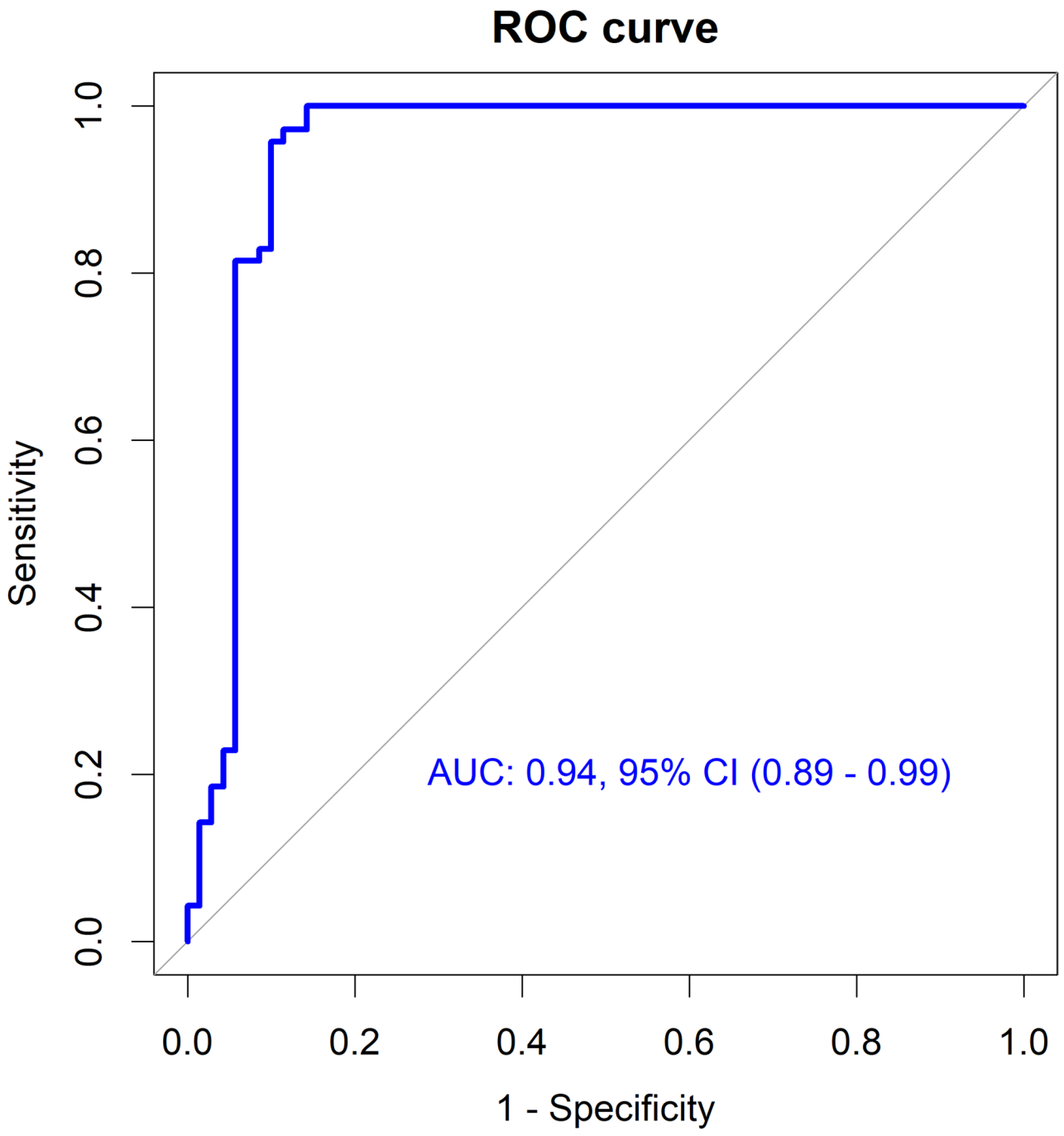

Fig. 5 The ROC Curves Validation Nomogram Discriminative. The AUC was greater than 0.70, reflecting the high performance of the nomogram

>1 in both groups, suggestive of a tendency toward bone resorption. As hypothesized based on individual RANKL and OPG trends, this ratio was strikingly 12-fold higher in individuals exhibiting severe bone degradation (p = 0.000). This observation aligns with findings by Shetty et al., who reported an 11-fold increase in the RANKL/OPG ratio in type 2 diabetic patients with chronic periodontitis compared with normoglycemic individuals [40], thus underscoring the impact of increased osteoclastic activity on resorbed mandibular. This concept is further supported by the correlation between decreased bone volume and increased osteoclast numbers, revealed in a histomorphometric analysis of edentulous mandibular bone samples by Dekker et al. [41]. Collectively, these results highlight the critical role of RANKL/OPG signaling in regulating bone remodeling and its contribution to the pathogenesis of alveolar ridge resorption in edentulous individuals.

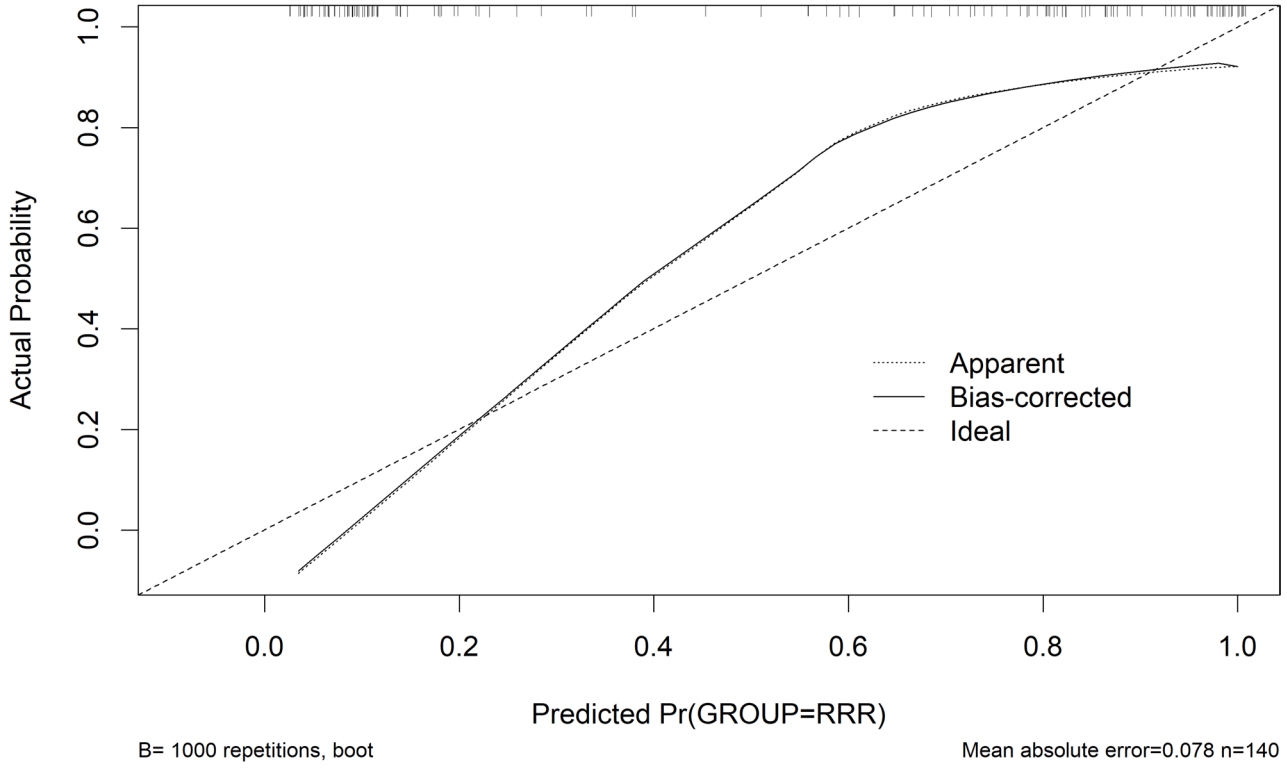

Fig. 6 The calibration curves for the nomogram estimating resorbed mandibular risk The thick dotted line represents an ideal prediction, while the thin dotted line illustrates the nomogram's predictive ability. The closer these lines are aligned, the higher the predictive accuracy will be

The molecular-mechanism that leads to upregulation of RANKL and/or downregulation of OPG in edentulous jawbone turnover has not been identified in the literature [11]. However, some studies have found that osteoimmunological responses in the edentulous oral mucosa can lead to RANKL expression and osteoclast activation. Denture pressure has been shown to induce mucosal ischemia and bone resorption in rat palates [42]. Yamamoto et al. demonstrated that intermittent hydrostatic pressure on mouse mandibular osteoblasts increased inflammatory cytokine production and shifted the RANKL/OPG ratio in favor of RANKL, promoting osteoclastogenesis [43]. Akashi et al. further elucidated this mechanism, revealing that cyclic pressure on human gingival fibroblasts reduced OPG expression, increased inflammatory cytokine production (IL-1β, IL-6, IL-8, MCP-1), and stimulated osteoclast differentiation and activity in vitro [44].

Post-extraction resorption affecting all individuals regardless of demographics or health may advance silently, only becoming noticeable when dentures loosen [7, 33]. Early identification of at-risk people is vital for prevention and prosthodontic treatment. Despite extensive research on RRR, the development of accurate predictive models for individual risk assessment remains limited. Thus, our second objective aimed to develop an estimative model for alveolar ridge resorption in edentulous patients using readily accessible clinical variables, including gender, duration of edentulism, number of dentures worn, and biochemical markers of bone turnover (RANKL, OPG, RANKL/OPG ratio).

Univariate logistic regression analysis was employed to identify potential associated factors. The pro-resorptive ligand RANKL emerged as the most significant risk factor, with a 10 pg/ml increase in RANKL concentration associated with a nearly 4-fold increase in the odds of RRR in women and a 3.5-fold increase in men. Conversely, a 10 pg/ml increase in OPG corresponded to a 60% reduction in the odds of RRR in women and a 73% reduction in men (Table 2). These findings are consistent with previous studies reporting elevated RANKL and decreased OPG levels in conditions associated with bone loss, such as periodontitis [20–22] and osteoporosis [45].

Severe alveolar bone loss nomogram

To develop a parsimonious prognostic model for the amount of jawbone loss, we employed the Bayesian model average approach. Two key risk factors—duration of edentulism and salivary RANKL concentration—were identified as significant predictors of severe RRR. Figure 3 illustrates the correlation between tooth loss time and salivary RANKL concentration, highlighting their combined influence on the risk of severe bone loss. However, using a complex mathematical formula (as mentioned in

the result section above) to calculate RRR risk, as many risk assessment models do, may be unrealistic in clinical practice [46]. To address this, a nomogram was created to visually, pictorial translate the statistical model into a user-friendly tool for clinicians (Fig. 4). This nomogram allows for the calculation of the probability of alveolar atrophy by assigning points to each associated factor and summing them to determine the patient's probability of severe resorption. Our model showed high sensitivity and specificity in discriminating between normal and severe resorbed mandibles (AUC = 0.94, Fig. 5). Internal validation using bootstrapping confirmed the model's robustness, achieving a high accuracy in predicting severe bone loss probability (maximum calibration error ≈ 8%, Fig. 6).

Clinically, our data-driven nomogram offers clinicians a practical risk assessment tool for individual RRR, using easily obtainable clinical data. Notably, our nomogram highlighted the greater influence of salivary RANKL concentration compared to edentulism duration in evaluating alveolar ridge resorption. This observation contrasts with a previous study by Kovacic et al., which found that edentulous duration accounted for up to 48.4% of the variance in RRR in complete denture wearers over a five-year period [35]. The observed discrepancy may stem from the study population's prolonged mean edentulous duration (14.97 ± 9 years). Prior research demonstrated a greater relative reduction rate (RRR) in individuals with shorter edentulous periods (< 10 years) [35], suggesting substantial prior bone loss in the current study and a diminished predictive value of edentulous duration.

Caution is advised when interpreting the nomogram's estimated risk. A high risk suggests an increased likelihood, but not certainty, of severe bone loss. Conversely, low risk does not preclude bone resorption. Additionally, while the model's use of salivary RANKL as a predictor is non-invasive and efficient, it may lack precision in identifying site-specific bone loss patterns [47, 48]. Thus, this predictive nomogram is best used as an initial screening tool for high-risk individuals, with further evaluation needed for a comprehensive assessment. This may enable preventative strategies, like closer monitoring and potential RANKL-targeted interventions, to mitigate alveolar ridge resorption risk and preserve bone health, especially since no definitive preventative solution currently exists. To the authors' knowledge, this is the first study in totally edentulous elders using salivary bone turnover biomarkers to estimate absolute risk of severe mandibular bone loss and explain post-tooth loss physiology.

The use of saliva as a non-invasive diagnostic fluid holds significant potential for clinical applications, primarily because of its simple, low-cost, and point-of-care collection process that does not require specialized personnel [49]. The nomogram developed in this study is designed to harness this potential by translating salivary RANKL/OPG concentrations and clinical parameters into an instantaneous, patient-specific risk score. This tool would enable clinicians to rapidly stratify risk, personalize treatment plans, and enhance patient communication. However, a significant translational gap currently impedes the real-world application of this nomogram. The primary barrier is the reliance on laboratory-based ELISA for biomarker quantification. Therefore, a prerequisite for clinical implementation is the development of a validated, reproducible, and cost-effective chair-side assay with established diagnostic cut-offs. Furthermore, the clinical utility of the nomogram itself is contingent upon its successful replication and independent validation in larger, more diverse patient cohorts before it can be responsibly integrated into routine practice.

Strengths and limitations of study

The interpretation of this study's findings should be considered in the context of several limitations. Primarily, the study's cross-sectional design precludes the inference of causality, establishing only associations. This interpretative constraint is compounded by our hospital-based recruitment, which yielded a sample skewed towards patients with poor denture quality (> 80%), thereby constraining the external generalizability of our findings. To address these fundamental issues, future prospective, longitudinal, and community-based studies are imperative. Such research would be essential not only to establish causality but also to validate our findings in a more representative cohort, specifically by tracking the temporal dynamics of RANKL/OPG levels in relation to RRR progression.

Methodological limitations also warrant discussion. The assessment of alveolar ridge resorption relied on two-dimensional panoramic radiography, a technique unable to capture the three-dimensional volumetric changes inherent to this process. To overcome this limitation, subsequent research should utilize three-dimensional modalities like CBCT to enable an accurate volumetric quantification of post-extraction bone resorption. Furthermore, while saliva collection was standardized to a narrow morning window (07:00–08:00) to mitigate circadian variance, this single time-point approach may not reflect the full diurnal profiles of RANKL and OPG, introducing a potential source of measurement bias.

Finally, we acknowledge that a key limitation of the present study is the lack of external validation. While the nomogram demonstrated robust performance through rigorous internal validation using bootstrapping, its generalizability to other patient populations remains to be established. Therefore, future prospective studies are required to validate our model in larger, independent, and multicenter cohorts. The model will apply to this external dataset, and its predictive performance will be

evaluated by comparing predicted risks with observed outcomes. Such validation is essential to confirm the model's clinical utility and ensure its applicability across diverse populations before it can be recommended for real-world use.

Despite inherent limitations, our study highlights the clinical value of salivary RANKL in estimating residual ridge resorption. The developed nomogram represents a significant step towards personalized risk assessment and treatment planning in this context. Its user-friendly design and reliance on readily available clinical data, such as edentulous duration and salivary RANKL levels, enhance practicality as an estimative tool. By facilitating the early identification of high-risk patients, this model can contribute to improved clinical decision-making and ultimately optimize patient care for those facing alveolar ridge resorption.

Conclusions

In conclusion, this study identifies several clinical and biological factors associated with severe RRR. A significant sex-based disparity was clear, with women being more severely affected. Prolonged edentulism and suboptimal denture quality were also strongly associated with RRR severity. Notably, this is the first human study to associate salivary RANKL/OPG levels with RRR; elevated RANKL markedly increased resorption risk while higher OPG was protective. These findings suggest that RANKL and OPG may play potential roles as biomarkers for screening individuals at risk of this condition, though their clinical utility warrants validation in future longitudinal studies.

Abbreviations

ACP	The American College of Prosthodontists
AUC	The Area Under the Curve
ELISA	Enzyme-Linked Immunosorbent Assay
ICC	Intra-class Correlation Coefficient
OPG	Osteoprotegerin
RANK	Receptor Activator of Nuclear factor-KB
RANKL	Receptor Activator of Nuclear factor-KB Ligand
ROC	The Receiver Operating Characteristic curve
RRR	Residual Ridge Resorption
UMP	University of Medicine and Pharmacy at Ho Chi Minh City

Acknowledgements

We sincerely thank the Center for Molecular Biomedicine, University of Medicine and Pharmacy at Ho Chi Minh City, for supporting this study.

Authors' contributions

The research idea and design came from Thuy Thu Nguyen. Research samples were collected by Thien Lam Lu, under the supervision of Thuy Thu Nguyen. The laboratory process was performed by Hoang Kim Tu Trinh, Kieu-Minh Le and Diem DK Truong. Thien Lam Lu and Nam Cong - Nhat Huynh wrote the main manuscript text, prepared all the figures and tables. All the authors reviewed the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

The datasets that support the findings of this study are available on request from the corresponding author, Thuy Thu Nguyen. To protect research participants' privacy, the data is not publicly accessible.

Declarations

Ethics approval and consent to participate

This study followed the Declaration of Helsinki Ethical Principles for Medical Research involving human subjects. Informed consent was obtained from each participant, and the study was approved by the Ethical Committee Board of the University of Medicine and Pharmacy at Ho Chi Minh City (UMP) (No.488/HĐĐĐ-ĐHYD).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 8 August 2025 / Accepted: 14 November 2025
Published online: 28 November 2025

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