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Receptor activator of nuclear factor kappa B (RANK) as a determinant of peri-implantitis

Receptor aktivatora nuklearnog faktora kapa B kao činilac periimplantitisa

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Abstract

Background/Aim. Peri-implantitis presents inflammatory process that affects soft and hard supporting tissues of osseointegrated implant based on inflammatory osteoclastogenesis. The aim of this study was to investigate whether receptor activator of nuclear factor kappa B (RANK) concentrations in peri-implant crevicular fluid could be associated with clinical parameters that reflect inflammatory nature of peri-implantitis. **Methods.** The study included 67 patients, 22 with diagnosed peri-implantitis, 22 persons with healthy peri-implant tissues and 23 patients with periodontitis. Clinical parameters from each patient were recorded and samples of peri-implant/gingival crevicular fluid were collected for the enzyme-linked immunosorbent assay (ELISA) analysis. **Results.** RANK concentration was significantly increased in samples from the patients with peri-implantitis when compared to healthy implants ($p < 0.0001$), where the average levels were 9 times higher. At the same time RANK concentration was significantly higher in peri-implantitis than in periodontitis sites ($p < 0.0001$). In implant patients pocket depths and bleeding on probing values were positively associated with high RANK concentrations ($p < 0.0001$). **Conclusion.** These results revealed association of increased RANK concentration in samples of peri-implant/gingival crevicular fluid with peri-implant inflammation and suggests that RANK could be a pathologic determinant of peri-implantitis, thereby a potential parameter in assessment of peri-implant tissue inflammation and a potential target in designing treatment strategies.

Key words:
receptor activator of nuclear factor-kappa b; sensitivity and specificity; dental implantation, endosseus; periodontitis.

Apstrakt

Uvod/Cilj. Periimplantitis predstavlja inflamatorni proces koji zahvata meko i tvrdo potporno tkivo osteointegrisanog implantata, i zasnovan je na inflamatornoj osteoklastogenezi. Cilj studije bio je da se utvrdi povezanost koncentracije receptora aktivatora nuklearnog faktora kapa-B (RANK), kao glavnog receptora osteoklastnog metabolizma, sa kliničkim parametrima periimplantitisa. **Metode.** Studija je uključila 67 sistemski zdravih pacijenata (22 sa periimplantitisom, 22 sa zdravim implantatima i 23 sa periodontopatijom). Pacijentima su mereni klinički parametri i uziman je uzorak periimplantne/gingivalne tečnosti za određivanje koncentracije RANK-a ELISA metodom. **Rezultati.** Koncentracija RANK-a bila je značajno povišena kod periimplantitisa u odnosu na zdrave implantate ($p < 0,0001$), gde je srednja vrednost koncentracije bila 9 puta veća. Istovremeno, RANK je bio značajno viši kod periimplantitisa nego kod parodontopatije ($p < 0,0001$). U grupi sa implantatima dubina periodontalnog džepa i krvarenje na probu bili su pozitivno udruženi sa visokim vrednostima RANK-a ($p < 0,0001$). **Zaključak.** Rezultati istraživanja pokazuju udruženost povišenosti koncentracije RANK-a sa periimplantnom inflamacijom i navodi na zaključak da bi RANK mogao da bude patološka determinanta periimplantitisa, a time i potencijalni parametar za praćenje inflamacije periimplantnog tkiva i potencijalni cilj za pravljenje terapijskih strategija.

Ključne reči:
receptor, aktivator nuklearnog faktora-kappa-b; osetljivost i specifičnost; stomatološka enosalna implantacija; periodontitis.

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Introduction

Peri-implantitis represents an inflammatory process that affects soft and hard supporting tissues of an osseointegrated implant, where the infection and excessive biomechanical forces are recognized as main etiologic factors [1, 2]. After induction, the peri-implantitis pathogenesis results from the interplay between specific subgingival microorganisms and inflammatory and immune responses, acting in the same way and using the same effector mechanisms as evidenced in periodontal disease (periodontitis) [3, 4]. *Aggregatibacter actinomycetemcomitans*, a gram-negative facultative capnophilic bacteria, is identified as the major etiological pathogen of localized juvenile periodontitis (LJP) until recently known as localized aggressive periodontitis and rapidly progressing periodontitis [5]. Data from experimental model of (NOD)-SCID mice reconstituted with human peripheral blood leukocytes from patients with periodontitis and challenged with *A. actinomycetemcomitans* clearly showed that activated human CD4 T cells are essential effectors of alveolar bone destruction [6]. Maintenance, formation, and remodeling of alveolar bone is an outcome of balanced activity of final effector cells, bone-resorbing osteoclasts and bone-producing osteoblasts. Osteoclastogenesis with consequential bone loss represents the hallmark of peri-implantitis, distinguishing it from previous developmental stage, peri-mucositis, where the process is limited only on soft tissues [7, 8]. In course of such inflammatory bone resorption, receptor activator of nuclear factor kappa B (RANK) and his ligand (RANKL) have been recognized as key regulatory factors in osteoclasts metabolism [9–11], particularly in periodontal disease. Receptor activator of nuclear factor kappa B also known as the osteoclasts differentiation factor receptor is a 11A member of the tumor necrosis factor (TNF) superfamily. The human RANK is a transmembrane receptor of 616 amino-acids expressed primarily on the cells of the monocyte/macrophage lineage including preosteoclasts and osteoclasts, B- and T- lymphocytes, dendritic cells and fibroblasts [12, 13]. Since RANK is localized on the surfaces of preosteoclasts and osteoclasts its ligation by a specific ligand, RANKL, leads to differentiation and maturation of progenitor cells simultaneously with osteoclasts activity enhancement [14–16]. The key signal for this mechanism is the achievement of critical concentrations of pro-inflammatory cytokines whose gene transcription is regulated by nuclear factor kappa B (NF-κB) [17].

Regulation of RANK/RANKL interaction is performed by a receptor-like molecule named osteoprotegerin (OPG) which binds RANKL with high affinity and thereby blocks RANKL/RANK interaction with a consequential inhibition of osteoclasts activity [18, 19]. RANKL could be found in soluble form or expressed by osteoblasts, stromal cells, fibroblasts, B-cells and T-cells [20, 17] under different stimulation such as pro-resorptive hormones (such as parathormone, epinephrine, 17β-estradiol and glucocorticoides), cytokines (such as IL-1, IL-6, IL-8, IL-11, IL-17, TNFα and IFNγ) and bacterial lipopolysaccharide (LPS) [18, 21].

Clinical and radiological parameters of peri-implantitis are conventional tools for determining diagnosis and status in established tissue impairment, but are insensitive for early diagnosis and as a prognostic factors. Peri-implant crevicular fluid (PICF) was found to be reliable in reflecting surrounding tissues status since the volume and composition directly depends on their condition [22]. Considering that, a number of researches were conducted on the topic of RANKL and OPG evaluation in PICF and gingival crevicular fluid (GCF) at different statuses of supporting tissues [23, 24]. However, these results on RANK and its role in peri-implantitis are still non-existent. The aim of this study was to investigate whether RANK concentrations in PICF could be associated with clinical parameters that reflect inflammatory nature of peri-implantitis.

Methods

This was the cross-sectional study conducted in the Clinic of Periodontology and Oral Medicine, School of Dentistry, Belgrade, Serbia, Clinic for Maxillofacial, Oral Surgery and Implantology, Military Medical Academy and Institute for Medical Research, Military Medical Academy from June 2009 until February 2011. The study included 67 patients divided into 3 groups: peri-implantitis (n = 22), healthy implants (n = 22) and periodontitis (n = 23). Peri-implantitis was accepted in the presence of clinical signs (Figure 1) including: peri-implant pocket depth (PPD) ≥ 5 mm or in the presence of gingival recession relative clinical attachment level (rCAL) ≥ 4 mm, with positive bleeding on probing (BOP) and recorded radiographic bone loss involving ≥ 2 threads compared to radiography taken at the time of prosthetic replacement. Intraoral radiographies were performed for radiological evidence of bone loss using paralleling technique, where implant threads were used as referent points. Only peri-implantitis after at least 2 years of loading and without previous peri-implantitis treating were included. As healthy peri-implant tissues were accepted implants without any clinical signs of inflammation including the absence of subjective difficulties, BOP = 0 and PPD ≤ 3mm. Implants included in the study were delayed loaded endosseal implants with the purity level of 2/ASTM (American Society

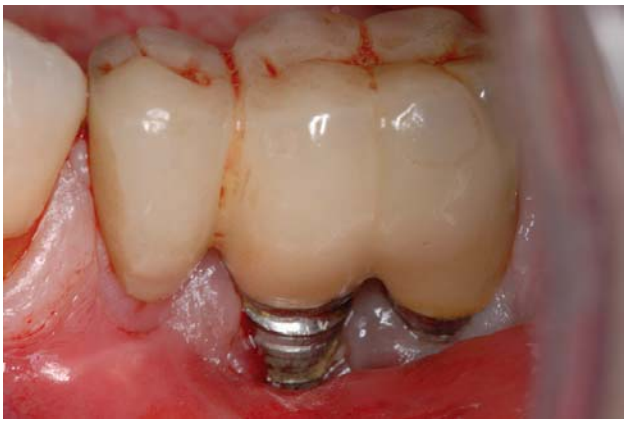

Fig. 1 – Clinical signs of peri-implantitis presented by positive bleeding on probing and a clinically visible loss of soft and hard peri-implant tissues

for Testing and Materials) (99.98%) and a sand-blasted, large-grit, acid (SLA) etched surface inserted. Implants were 4.5 mm in diameter, 3.5 mm long with 4 threads. As periodontitis were accepted the patients with diagnosed severe generalized chronic periodontitis accordingly to the classification of periodontal disease [25].

All the patients were systemically healthy adult non-smokers, and exclusion criteria were received peri-implant/periodontal treatment in the preceding 1 year, usage of antibiotics and anti-inflammatory agents within the preceding 3 months, menstruation, pregnancy and lactation in female patients. The study protocol was approved by the Ethics Committees of both two institutions (Ethics Committee School of Dentistry and Ethics Committee Military Medical Academy), patients were informed on the study protocol and they were obligated to give written consent before procedures.

Clinical examination

The following clinical parameters were measured in 6 points: mesio-bucal, medio-bucal, disto-bucal, mesio-lingual, medio-lingual and disto-lingual (Figure 2): PPD and packet depth (PD) by BOP: presence (1) or absence (0) of bleeding for up to 15 sec after probing, and visible plaque accumulation (PI): presence (1) or absence (0) of plaque along the mucosal margin [26]. All measurements were performed by the one same trained and calibrated examiner using the same type of the graduated periodontal probe (North Carolina–Hu-Friedy, Chicago, IL, USA). Intra-examiner calibration was performed twice, before and during the study, by assessing PPD and with a degree of agreement within ± 1 mm higher than 85%. The implant/tooth site with the deepest probing depth was chosen as a representative for sampling; in case of similar probing depths the anterior point was chosen as a step toward higher precision.

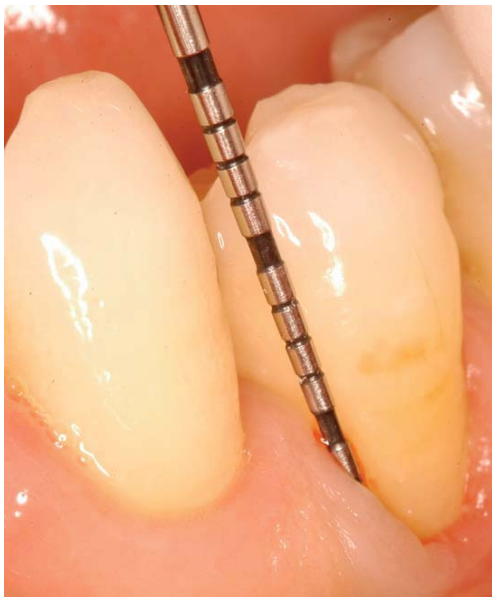

Fig. 2 – Measurement of periodontal pocket depth using a graduated periodontal probe at the tooth with positive bleeding on probing and with a clinically visible loss of soft and hard periodontal tissues

The peri-implant crevicular fluid (PICF) and gingival crevicular fluid (GCF)sampling

PICP and GCF samples were obtained from the patients using the filter paper technique 24 h after the examination. After removing the supragingival biofilm with sterile cotton rolls, the sampling place was isolated with cotton rolls and gently air dried 1 min before sampling in the aim to eliminate any potential contamination with saliva. A paper strip of standard length and height (Periopaper, Pro Flow, Amityville, NY, USA) was inserted into the peri-implant and gingival/periodontal sulcus/pocket until mild resistance was felt and left in place for 30 s. Strips that were visually contaminated with blood or saliva were discarded. Sampled fluid volume was measured with calibrated Periotron 6000 (Interstate Drug Exchange, Amityville, NY, USA). After measurement strips were inserted in microcentrifuge plastic tubes with 100 μL of sterile phosphatebuffered saline. The sampling time method which includes a total amount of RANK in picograms (pg) per site during 30 s was chosen because the method was supported by previous studies as convenient for related researches [27]. Following 10 s of vortexing, eluates were centrifuged 5 min at 3000 g to remove plaque and cellular elements, after that the strips were removed. The samples were stored at −70°C until enzyme-linked immunosorbent assay (ELISA) analysis.

Determination of RANK using ELISA

Concentrations of RANK in PICF and GCF were assessed using a commercially available ELISA kit (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's recommendations. A calibration curve was plotted by regression analysis and the optical density of the sample was used to estimate the concentration of RANK. The intensity of the color was measured using spectrophotometry (450/620 nm, ELISA processor II, Boehring, Germany). The obtained values of RANK were calculated from picomoles into picograms, and the concentration was expressed as RANK (pg) per sample / PICF volume (mL).

Statistical analysis

Analysis of the obtained data was performed using statistical software (SAS Enterprise Guide 4.1, SAS Institute Inc., 2008). After descriptive statistical analysis, data were examined by the Shapiro-Wilk and Kolmogorov-Smirnov test in order to test the normality assumption. Since normality was not achieved for each clinical parameter, further analysis was based upon non-parametric tests. In some cases „Exact" option was applied in order to obtain more precise *p* values. The Wilcoxon test was done to assess the difference in mean for each clinical parameter within groups, whereas another pair wise comparison was done by the Kruskal–Wallis test. The significance level established for all analyses was 5% ($p < 0.05$).

Results

The study population of 67 subjects included 30 females and 37 males, the average age of 38.8 ± 7.73 years (23–60 years).

The volume of collected PICF/GCF was similar in samples of all the investigated groups (Table 1). Mean score values of PPD, BOP and plaque accumulation index (PI) were as expected significantly elevated in the peri-implantitis and periodontitis groups comparing to the controls, but did not differ among each other. Finally, mean RANK levels were highest in the samples of the peri-implantits group and lowest in the control group. Score values of BOP, PI, PPD, volume of collected PICF/GCF and RANK concentrations did not correlate with gender and age of the investigated subjects (Table 2).

The score values of all the clinical parameters were significantly increased in inflamed sites, either in peri-implantitis or periodontitis groups, as expected considering group's characteristics. When we divided patients with peri-implantits according to RANK levels detected in their PICF, mean score levels of peri-implant pocket depth and positive bleeding on probing were significantly higher in those patients that had RANK levels above 1,000 pg/mL, comparing to the group of those patient with lower RANK concentration (Table 4). These findings point out strong association of crucial clinical signs, PPD and BOP with high local RANK concentration.

Table 1

Descriptive statistics of RANK concentration and the measured clinical parameters among the groups

Group of patients	PICF/GCF (uL)	RANK (pg/mL)	BOP (score)	PI (score)	PPD/PD (score)
Control	0.44 ± 0.19	255.36 ± 240.31	0.00 ± 0.00	0.81 ± 0.90	1.72 ± 0.45
Peri-implantitis	0.61 ± 0.23	1514.49 ± 888.01	6.00 ± 0.00	5.04 ± 1.81	5.72 ± 0.88
Periodontitis	0.55 ± 0.39	421.79 ± 266.93	5.30 ± 1.11	5.08 ± 0.79	6.34 ± 1.52

PICF/GCF – peri-implant crevicular fluid/gingival crevicular fluid; RANK – receptor activator of nuclear factor kappa-B; BOP – bleeding on probing; PI – plaque accumulation index; PPD/PD – peri-implant pocket depth/pocket depth

Table 2

Correlation of gender and age with the measured parameters

Patients	PICF/GCF (uL)	RANK (pg/ml)	BOP (score)	PI (score)	PPD/PD (score)
Gender (M/F)	R = -0.049 (p = 0.742)	R = -0.041 (p = 0.786)	R = -0.037 (p = 0.807)	R = 0.015 (p = 0.922)	R = 0,22889 (p = 0,1217)
Age (years)	R = -0.024 (p = 0.631)	R = -0.014 (p = 0.802)	R = -0.042 (p = 0.112)	R = 0.045 (p = 0.622)	R = 0,117 (p = 0,1217)

PICF/GCF – peri-implant crevicular fluid/gingival crevicular fluid; RANK – receptor activator of nuclear factor kappa-B; BOP – bleeding on probing; PI – plaque accumulation index; PPD/PD –peri-implant pocket depth/pocket depth; M – male; F – female

The concentration of RANK was significantly higher in the peri-implantitis than in the control group with healthy implants ($p < 0.0001$). By comparing RANK concentration between the peri-implantitis and the periodontitis group it is observed that RANK concentration was significantly increased in peri-implantitis sites ($p < 0.0001$) (Table 3).

Discussion

A variety of studies were dedicated to resolution of the multifactorial pathogenesis of peri-implantitis, aiming to improve the success of one of the main therapeutic solutions in contemporary dentistry. Still, numerous efforts to identify any

Table 3

Analysis of the differences of clinical and biochemical parameters between the groups (Wilcoxon test)

Groups of patients	RANK (pg/mL)	BOP (score)	PI (score)	PPD/PD (score)
The peri-implantits (P-I) *vs* control (C)	$p < 0.0001$ P-I > C	$p < 0.0001$ P-I > C	$p = 0.033$ P-I > C	$p < 0.0001$ P-I > C
The peri-implantitis (P-I) *vs* periodontitis (P-D)	$p < 0.0001$ P-I > P-D	$p = 0.061$	$p = 0.109$	$p = 0.177$
The periodontitis (P-D) *vs* control (C)	$p = 0.061$	$p < 0.0001$ P-D > C	$p < 0.0001$ P-D > C	$p < 0.0001$ P-D > C

RANK – receptor activator of nuclear factor kappa-B; BOP – bleeding on probing; PI – plaque accumulation index; PPD/PD – peri-implant pocket depth/pocket depth

Table 4

Clinical parameters analysis in implant sites according to RANK concentration

Clinical parameters	RANK < 1,000 pg/mL	RANK > 1,000 pg/mL	p
PPD (mm)	5.78 ± 1.02	3.57 ± 0.52	< 0001
BOP (score)	6.00 ± 0.00	3.73 ± 0.27	< .0001
PI (score)	5.12 ± 0.42	4.98 ± 0.40	= *0.419*

RANK – receptor activator of nuclear factor kappa-B; PPD –peri-implant pocket depth; BOP – bleeding on probing; PI – plaque accumulation index

reliable determinant and disease predictor are far from a usable parameter. To authors' knowledge this is the first study investigating RANK in patients suffering from peri-implantitis, hence direct comparison was limited. In this study, RANK values were assessed as a potential parameter, peri-implantitis for concerning its key role as a receptor mediated bone loss.

The mean RANK concetrations were several times higher in the PICF/GCF samples of peri-implantitis group comparing to the control group of patients with healthy implants, that had no signs of gingival inflammation. The highest individual concentration in healthy implants was lower than the lowest one in the peri-implantitis, indicating clear association of high RANK concentrations with peri-implant inflammation. Furthermore, the significant difference in RANK concentration was evidenced between the peri-implantitis and the periodontitis group, with highest levels in the peri-implantitis group.

From these findings, it could be concluded that a locally increased RANK concentration provided a potential base for more intensive inflammation comparing to periodontitis [28–35].

Our results additionally confirmed the association of clinical parameters as indicators of inflammation (probing depths and BOP) with high RANK values.

RANK is known to activate a cascade of intracellular signaling pathways resulting in rapid nuclear translocation and transcription of the genes coding pro-inflammatory cytokines [35]. The process is based on recruitment of TNF receptor-associated factor (TRAF) proteins that regulate transduction of signals from RANK with consequential activation of NF-κB as well as activation of mitogen-activated protein kinase pathway, where these two are recognized as crucial in regulation of expression and transcription of the genes coding pro-inflammatory cytokines. Moreover, RANK poses specific biological feature to induce osteoclastogenesis ligand independently by self-assembling reported by Kanazawa and Kudo [29], and additionally supported by Otero et al. [30]. They reported spontaneous osteoclastogenesis based on RANK activation that was driven by IkB kinase β (IKKβ) activated by proinflammatory cytokines TNFα and IL-1. In regard to these facts, increase in RANK could proportionally augment osteoclastogenesis independently of his ligand, usually considered as the main factor in inflammatory bone loss, therewith could present a pathologic pattern of enhanced inflammatory response specific to peri-implant inflammation [36–38].

Scores of clinical parameters were in general significantly higher in inflamed sites as expected considering group characteristics, but it was observed that the lowest value of PI in the peri-implantitis group was higher than the highest value in the healthy implants, and findings of increased PI are in correspondence with the previous findings of Duarte et al. [24]. By considering dental plaque as a possible source of of LPS [31, 32] increased PI values in peri-implantitis could suggest increase in RANKL expression in response to LPS stimulation described by Choi et al. [37]. Moreover the association of increased concentration of RANKL with peri-implantitis and their severity is already reported [24]. Local osteoclastogenesis could be also enhanced by augmentation of RANK/RANKL complexes, which are directly proportional to concentration of ligand and receptors found to be increased in peri-implantitis.

Regarding two proposed mechanisms, RANK increase could be a powerful enhancer of peri-implant inflammation by increasing transcriptions of genes coding pro-inflammatory mediators (such as IL-1, IL-2, IL-6, IL-8, IL-12, TNFα, cyclooxygenase-2 and nitric oxid synthase) [36] with consequential elevation of entire cytokine concentration. Local cytokine increment induced by locally produced and expressed RANK could result in osteoclasts differentiation and upregulation of their activity, the same as under increased RANKL expression [17], implicating that RANK could create a vicious circle with its increase.

On the other side, as documented in experimental models and in human samples of patients with periodontitis, activated local CD4 T lymphocytes are principal regulatory cells in alveolar bone destruction. In periodontal disease, gingival production of inflammatory mediators is under strong influence of locally activated Th-17 and Th-1 lymphocytes. Furthermore, Takahashi et al. [38] anticipated that locally activated IL-17 producing T-lymphocytes may be a primary source of RANKL in perodontitis. If we consider that peri-implantits and periodontits could be generated by similar mechanisms, increased RANK levels could have intense influence upon local T-lymphocytes, upregulating their functions, and *vice versa*, resulting in high osteoclasting activity and bone destruction.

This is the first study suggesting that an increased concentration of RANK could be related to peri-implantitis; thereby the special emphasis of forthcoming researches should be on the RANK biology, since the numerous studies were focused on RANKL and OPG [33, 34].

Conclusion

Taking into consideration the obtained results, increased concentration of RANK could be a parameter useful for diagnosis and monitoring peri-implantitis. On the other side, dissolving RANK functioning could provide new therapeutic strategies by bringing new target for therapeutic acting.

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