Biochemical markers as predictors of bone remodelling in dental disorders: a narrative description of literature

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Summary

Osteoporosis is a systemic disease in which the skeletal condition is characterized by a decreased mass of normally mineralized bone, due to an augmentation of bone resorption processes. Bone biomarkers serum are used for the diagnosis. On the other hand the main cause of the resorption in the bone jaws are periodontitis, inflammatory cysts, developmental cysts, odontogenic neoplasms. Periodontal diseases can be localized to a single site of the jaws or can affect all the teeth, with a massive bone resorption. The cysts are classified in developmental and inflammatory. They caused a local bone resorption in the jaws. Keratocystic odontogenic tumor produces a large bone resorption for its local aggressive nature. Their diagnosis is clinical and radiological. The aim of our review is to find a correlation between bone biomarkers serum and periodontitis, inflammatory cysts, developmental cysts, odontogenic neoplasms. The RANK/RANKL/OPG system is the most studied not only in osteoporosis but also in the periodontitis, inflammatory cysts, developmental cysts, odontogenic neoplasms. In the last years osteoimmunology was used to study the periodontal disease progression, because the immunity cells start the bone resorption processes. A lot of studies analyze the biomarkers present in the biofluids, as saliva and gingival crevicular fluid, but not the correlation with serum biomarkers. Future studies must be organized to deepen the correlation between bone biomarkers and bone jaws resorption and to allow diagnosis and prognosis of periodontitis, inflammatory cysts, developmental cysts, odontogenic neoplasms.

KEY WORDS: osteoporosis; periodontitis; odontogenic cyst; serum marker; Rankl.

Introduction

The alveolar bone tropism of the jaws differs from skeleton ones. The alveolar bone remodelling depends on whether the teeth are present or not. When teeth erupted jaw bone grows, instead it is reabsorbed with teeth loss (1). Several factors play an important role on the alveolar bone remodelling, as in other skeletal parts (2). These factors are:
- anatomical factors: quality and quantity of available bone;
- metabolic factors: hormonal influence, osteoporosis, systematically disorders;
- mechanical factors: occlusal parameters, bruxism, presence of prosthesis.

Moreover there are some specific diseases that affect jaws and lead to bone resorption processes. These are: periodontal disease (PD), inflammatory cysts, odontogenic neoplasms.

Dentists use radiological and histological findings for the clinical diagnosis of these diseases. However it would be extremely useful to have serum/plasma markers to evaluate these diseases progression and prognosis, because they have phases of clinical worsening and stasis.

Osteoporosis is a systemic diseases that can affect the jaws and influence the jaw bone tropism; so there is a possible correlation between osteoporosis and periodontal disease (PD), inflammatory cysts, developmental cysts, odontogenic neoplasms, that are specific to the jaws.

Aim. The relationships between systemic bone biochemical markers and alveolar bone loss – which is caused by periodontal disease, osteoporosis, odontogenic cysts and neoplasms – are still not well known. The aim of this analysis of literature is to investigate if bone biomarkers, which reflect systemic bone loss and formation, are associated also with alveolar bone resorption.

Periodontitis

Periodontitis is a chronic bacterial infection that affects the gingival and the bone supporting the teeth. Bacterial plaque stimulates the host inflammatory response leading to tissue damage and bone resorption (3). Despite many studies in the past years, focused their attention on bacterial plaque, as a cause of periodontitis, today their attention is mainly about osteoimmunology (4). They try to explain the role and the interaction between the host immune response, the cytokines and the bone biology in development of PD (5). In 1998 the AAP (American Association of Periodontology) proposed this classification (6):
- Gingivitis
  - Plaque associated
  - No plaque associated
- Periodontitis:
  - Type I: Aggressive periodontitis
  - Type II: Chronic periodontitis
  - Type III: Periodontitis as a manifestation of systemic disease
  - Type IV: Necrotizing periodontitis
- Recurrent periodontitis
- Refractory periodontitis

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Patients treated for periodontitis had clinical healing with no restitutio ad integrum because of the bone loss (7). The bone loss range from small resorption when periodontitis affects a single site of the tooth, to large resorption if involves several or all teeth.

The diagnosis of periodontitis is clinical. Outcome measures are: pocket depth (pd), bleed on probing (BOP), clinical attachment level (CAL), plaque index (Pl), but the loss of alveolar bone can determine variations in the bone turnover markers. Due to that, authors have analysed the markers in GCF (Gingival Crevicular Fluid), saliva, serum and plasma.

Buduneli and Kinnane (8) do a systematic review of literature analyzing studies regarding markers of tissue destruction in biofluids, GCF (gingival crevicular fluid, saliva and serum/plasma), in patients affected and/or treated for periodontitis. Authors reported data of studies correlating serum/plasma levels of a marker with clinical measurements and different phase of progression/healing of PD:
- Serum Calcium correlated with PD may be a risk factors for progression (9);
- IL-17: higher in GAGP (generalized aggressive periodontitis) and decrease after SRP (10);
- IL-6: is associated to PD severity and decrease after therapy (SRP, scaling and root planning) (11-15);
- TNF-α: increase with PD and decrease after therapy (SRP) (10, 13);
- CRP: discording report (12, 13, 15-17);
- MMP: level of MMP-3, -9, -8 increases in Chronic Periodontitis and decreased after therapy (12, 18);
- Serum cortisol associated to clinical parameters (BOP, pd and CAL) (19); 
- Serum albumin may be a risk predictor for progression (20);
- Osteocalcin: negative correlation between CAL>6mm and osteocalcin (21).

Cochran (22) describes studies that analyse RANKL and OPG in GCF and saliva from individuals affected by PD and found that usually the RANKL/OPG ratio was higher in individuals with periodontitis than in healthy controls despite concentration of RANKL and OPG varied from study to study.

We found only few studies analysing RANKL/RANKOPG pathway in serum/plasma.

A study analysed serum levels of RANKL and OPG in 35 smokers and 35 non-smokers (23). Similar values were found of serum RANKL in smoker (mean=41.7 pM) and in non-smokers (mean=48.23 pM) instead lower value of OPG in smoker (mean=33.76 pM) than in non-smoker (mean=59.28 pM). The higher ratio RANKL/OPG can explain tendency in smoker to bone loss.

Lappin et al. (24) in a human study on diabetics and non diabetics patients with or without periodontitis found that the ratio of RANKL to OPG depends on periodontal status.

Oçaka et al. (25) analysed plasma levels of patients smoker and non-smoker, systematically healthy, affected or not by chronic periodontitis. They report no difference in RANKL levels for smoker with or without periodontitis and non-smoker with or without periodontitis but higher RANKL/OPG ratio for smoker with chronic periodontitis in comparison to smoker without chronic periodontitis because of reduced OPG levels.

Systemic and maxillary osteoporosis

Osteoporosis is characterized by reductions of bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility, with consequent increase in fracture risk. It is considered the most common metabolic bone disease; in fact it constitutes one of the most important public health problems (26). Different authors analyzed dealings within systemically and maxillary osteoporosis. In a systematic review of literature, Jeffcoat (27) found that thirteen of the 15 studies analyzed showed correlation between oral and systemic osteoporosis. However these findings do not exclude the possibility to have oral osteoporosis without systemic osteoporosis and vice versa.

The diagnosis of osteoporosis is based on blood and urine tests (VES, haemochrome, serum calcium, phosphataseaemia, creatinine, azotemia, calcuria, phosphaturia, pyridinoline, alkaline phosphatases, PTH) and on measures of the bone density through single photon absorptiometry (SPA), dual energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT) and radiographic absorptiometry (RA).

Studies can be found in literature that are focused on the possibility to diagnose osteoporosis through OPT. Klemetti found that a subject with osteoporosis is more likely to show erosion of the inferior border of mandible than control. Klemetti index seems to be useful for screening of skeletal osteoporosis (28, 29). Taguchi (30) demonstrates that the mandibular inferior cortical shape on dental panoramic radiograph may be an indicator of bone turnover and spine BMD in post-menopausal women.

So we can identify postmenopausal women with increased risk of osteopenia and osteoporosis on routine dental panoramic radiographs.

In clinical investigation of osteoporosis we can use biochemical markers. They are different for bone deposition and bone resorption (31). For the bone deposition there are:
- ALP (Alkaline phosphatase)
- Serum Osteocalcin (OC)
- Propeptide pyridinoline cross-link of type 1 collagen.

For bone resorption there are:
- Carboxy-terminal telopeptide pyridinoline cross-link of type 1 collagen (CTX)
- Deoxypyrinolone in urine tests
- Idrobisphosphonate Tartrate resistant in serum
- Bone Sialoprotein serum
- Idrosilsine-Glucosid in urine tests.

New studies demonstrate the correlation between serum bone biomarkers and oral bone loss, and as the oral bone loss can affect the serum bone biomarkers. Deguchi et al. (32) found that a mandibular inferior cortical erosion finding on dental panoramic radiographs is significantly associated with increased biochemical markers of bone turnover. They measured values of serum bone-specific alkaline phosphatase (S-BAP) and urinary N-telopeptide cross-links of type I collagen (U-NTX). To evaluate the jawbone, they used mandibular inferior cortex (MIC) classification on dental panoramic radiographs and found a significant correlation between MIC classification and S-BAP.

Taguchi et al. (30) found correlation between mandibular cortical erosion and N-telopeptide cross-links of type I collagen (NTx) and alkaline phosphatase (ALP).

In another study by Payne (33) it is showed that changes in serum bone biomarkers over the time are associated not only with systemic bone density loss, but also with loss of alveolar bone density and alveolar bone height in post menopausal women with periodontitis and systemic osteopenia. The considered bone biomarkers were osteocalcin and pyridinoline crosslink fragment of type I collagen (ICTP). In this study the authors found a positive correlation between these markers and alveolar bone loss. In particular the first marker was associated with the alveolar bone density and the second with alveolar bone height loss.

Odontogenic cysts

Odontogenic cysts are lesions that affect the jaws. These cysts can be classified as inflammatory and developmental (Table 1).
Keratocystic odontogenic tumor (KCOT) (38).

KCOT seems to have different growth mechanisms from DC and IC. The authors concentrate their attention on studying factors related to epithelium and to fibrous wall of the cyst. Different authors reported high value of Ki 67 and P53 in epithelium of KCOT that indicate proliferation activity (39, 40). Zhang et al. (41) propose that Hedgehog (Hh) signalling can play a role in pathogenesis of KCOT.

Results

The web searching of literature was conducted using the terms: “keratocyst”, “dentinogenic cyst”, “follicular cyst”, “radicular cyst”, “cyst”, “ameloblastoma”, “serum marker”, “marker”, “RANK”, “RANKL.”

We did not find studies that analysed serum marker of bone remodelling in odontogenic cysts disease and/or treatment. Different authors concentrate their attention on histological and immunohistochemical analysis on specimens of the cysts. The RANKL, RANK, OPG signalling pathway is the most analysed by immunohistochemical analysis aimed to find RANK-RANKL-OPG + cells. Data are reported in Tables 2 and 3. All authors find RANKL, OPG and RANK expression in all specimens. The data retrieved are few and discordant.

Discussion

Periodontitis is a major public health problem in Europe because it is quite common and causes tooth loss and disability (42). A diagnostic method for PD should be able to screen susceptible subject, to distinguish active and inactive site, to predict future tissue destruction and to monitor therapy (43).

Today clinical measurements (pd, BOP, CAL, PI) are the best parameters to diagnose PD, however clinical measurements give either poor or no information to screen susceptible subjects and to predict tissue destruction. Because of that, different authors analysed different markers of tissue destruction in GCF, saliva and serum/plasma. The most studied markers in serum/plasma are IL-6 (11-15) and CRP (12, 13, 15-17); some others are TNF-alfa (10, 13), IL-17 (10), Osteocalcin (21), albumin (20), cortisol (9) and MMPs (12, 18).

Despite molecules in biofluids are associated with tissue inflammation and bone loss in PD, the specificity and sensitivity of these molecules to screen susceptible subjects and to predict future destruction are not scientifically demonstrated (8). There are some difficulties to analyse markers of bone turn-over so to find conclusive data. These difficulties include inter-individual variability, different methods of analysis, the nature of samples (GCF, saliva or serum), number of molecules to analyse, influence of other systemic disease, like osteoporosis, on levels of biomarker.

The attention of researchers is now focused on osteoimmunology (4) to understand connections between immune response and bone system and how these interactions lead to bone resorption. Despite molecules in biofluids are associated with tissue inflammation and bone loss in PD, the specificity and sensitivity of these molecules to screen susceptible subjects and to predict future destruction are not scientifically demonstrated (8). There are some difficulties to analyse markers of bone turn-over so to find conclusive data. These difficulties include inter-individual variability, different methods of analysis, the nature of samples (GCF, saliva or serum), number of molecules to analyse, influence of other systemic disease, like osteoporosis, on levels of biomarker.

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Table 2 - Studies analysing RANK-RANKL-OPG pathway in odontogenic cysts.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Specimens</th>
<th>Epithelium</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tay (52)</td>
<td>2004</td>
<td>5 RC</td>
<td>RANKL, positive cells in epithelium and stroma of all specimens. Moreover they demonstrate the presence of osteoclasts with immunohistochemical analysis for TRAP and in situ hybridization for calcepin human receptor.</td>
<td>RANKL + cells higher in KCOT than RC.</td>
</tr>
<tr>
<td>Tekkesin</td>
<td>2011</td>
<td>20 KCOT</td>
<td>20 RC</td>
<td>RANK expression in KCOT higher than in RC.</td>
</tr>
<tr>
<td>De Moraes</td>
<td>2011</td>
<td>20 DC</td>
<td>Similar expression of RANK, RANKL, and OPG in DC and RC. However most cases of RC (55%) and of DC (70%) exhibited a higher content of OPG than RANK.</td>
<td></td>
</tr>
<tr>
<td>Da Silva</td>
<td>2008</td>
<td>9 KCOT, 9</td>
<td>No differences in expression of RANKL, RANK and OPG between specimens.</td>
<td></td>
</tr>
<tr>
<td>Menezes</td>
<td>2006</td>
<td>10 RC</td>
<td>Higher value of RANKL + cells than OPG + cells in RC (cells: polymorphonuclear neutrophils, macrophages, endothelial cells, lymphocytes and epithelial cells).</td>
<td></td>
</tr>
<tr>
<td>Andrade</td>
<td>2008</td>
<td>7 AOT, 5 AF</td>
<td>RANK, RANKL and OPG in all specimens.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 CCOT, 7</td>
<td>No differences.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CEOT, 7 OM</td>
<td>- RANK, RANKL and OPG in all specimens.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Similar value of RANKL and OPG for CCOT, AOT, CEOT, and AF.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Higher content of OPG than RANKL in the majority of AOT and CCOT.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Higher content of RANKL than OPG in CEOT, OM and especially AF.</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 - Studies analysing other markers.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Specimens</th>
<th>Epithelium</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbas Ali</td>
<td>2008</td>
<td>13 KCOT</td>
<td>EMPRIN (extracellular matrix metalloproteinase inducer) more expressed in KCOT, DC and RC than in DF and in KCOT than DC or RC.</td>
<td></td>
</tr>
<tr>
<td>Wang (59)</td>
<td>2010</td>
<td>3 DC, 11 RC</td>
<td>ORN (osteopontin), a molecule related to cancer metastasis and bone destruction, was found in some KCOT (8/20) but not in DC or RC.</td>
<td></td>
</tr>
<tr>
<td>Del Rossa-Tonelli (60)</td>
<td>2010</td>
<td>3 DC</td>
<td>In the cyst fluid they found higher PAI-1 levels in DC while higher u-PA levels in RC.</td>
<td></td>
</tr>
<tr>
<td>Tsai (61)</td>
<td>2004</td>
<td>30 RC</td>
<td>t-PA and PAI-1 in epithelium and stroma.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Higher t-PA levels in epithelial cells while higher PAI-1 levels in stroma cells.</td>
<td></td>
</tr>
</tbody>
</table>


The pro and anti-inflammatory cytokines act on gingival fibroblast, osteoblast, macrophages and periodontal ligament fibroblast, under this stimulation these cells produce RANKL and OPG (46). Evaluating maxillary bone resorption and serum markers authors reported that serum bone-specific alkaline phosphatase is associated to MIC (mandibular inferior cortex) erosion (32), osteocalcin with alveolar bone density and ICTP with alveolar bone height loss (33). Moreover the amount of bone resorption due to periodontitis can be affected by maxillary or systemic osteoporosis. Many authors focus their attention on the possible correlation between periodontitis and osteoporosis, in particular if in osteoporotic patients there is higher risk of periodontitis. A review published by Wactawski-Wende (47) found several similarities and correlations between...
Osteoporosis and periodontal diseases. However a casual nature to this association is not firmly established. Jabbar S. et al. found another correlation (48) between periodontal disease and plasma cytokines, vitamin D and bone mineral density in postmenopausal women with and without osteoporosis. The positive correlation found by these authors suggested that the bone markers of resorption may be affected by periodontal diseases. Other authors obtained results in accordance with this hypothesis. Sultan and Rao (49) found that skeletal BMD (hand-wrist radiograph) is related to interproximal ABL and CAL, but without a statistical significant level. Suresh et al. (50) found that the BMD of lumbar spine (L2) and femur were significantly lower in postmenopausal women with PD than postmenopausal women without PD.

However some problems exist to compare different studies that investigate osteoporosis and periodontitis. Del Rosso and Tonelli (60) found uPAR in epithelium of DC and lium of some specimens of KCOT but not in DC or RC (59). RC and DF (58) and OPN (osteopontin) that was found in epithelium of KCOT, DC, diseases. The scope will be to find therapeutic strategies for the first line treatment for PD. However authors try new treatment on animals basing on acquisitions in the field of osteoorimmunology. The fact that RANKL/RANK/OPG pathway is involved in bone destruction due to PD allows us to think that drugs interfering with this system are therapeutic. In literature there are animal studies: Yang et al. (62) in mice model of periodontitis demonstrate that RANKL antagonist are effective in reducing alveolar bone loss due to PD. In another study 32 rats were administered with human OPG-Fc subcutaneous twice weekly for 6 weeks. Authors found significant preservation in treated animal than control (63). These findings open new horizons in PD treatment, however new studies are necessary to better understand pathogenic mechanisms before starting human studies.

Conclusion

- Markers in serum/plasma are associated with bone loss in PD, but if these molecules are predictor of bone loss is not scientifically demonstrated.
- In literature studies analysing serum markers of bone resorption due to odontogenic cysts were not found.
- RANKL/OPG/RANK pathway plays a role in bone resorption due to PD and odontogenic cysts.
- Higher RANKL/OPG ratio is usually associated to active PD.
- The literature research has demonstrated that it is difficult to find a correlation between the systemic disorder as osteoporosis and specific pathologies that affect the bone jaws as periodontal, inflammatory cysts, developmental cysts, odontogenic neoplasms. So new investigations are necessary to find this correlation.
- Osteoporosis, though not being the initial cause of periodontitis, has been shown to be a risk indicator that may contribute to the progression of PD. However a casual nature to this association is not firmly established.
- Dentists can screen osteoporosis through OPG.
- Drugs that inhibit RANKL promise to be effective in reducing bone loss due to PD.

Future implications

New findings in pathogenic mechanism of PD and odontogenic cysts are now topics of research to try new therapeutic solutions. For many years the treatment of periodontitis consisted in removing bacterial plaque. Today removing bacterial plaque remains the first line treatment for PD. However authors try new treatment on animals basing on acquisitions in the field of osteoorimmunology. The fact that RANKL/RANK/OPG pathway is involved in bone destruction due to PD allows us to think that drugs interfering with this system are therapeutic. In literature there are animal studies: Yang et al. (62) in mice model of periodontitis demonstrate that RANKL antagonist are effective in reducing alveolar bone loss due to PD. In another study 32 rats were administered with human OPG-Fc subcutaneous twice weekly for 6 weeks. Authors found significant preservation in treated animal than control (63). These findings open new horizons in PD treatment, however new studies are necessary to better understand pathogenic mechanisms before starting human studies.

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30. Taguchi A, Sanada M, Kral E, et al. Relationship between dental par-


