We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Role of Osteoporosis as a Systemic Risk Factor for Periodontal Disease

*Silvia Martu, Irina-Georgeta Sufaru,
Sorina-Mihaela Solomon, Ionut Luchian, Ioana Martu,
Liliana Pasarin, Dora-Maria Popescu,
Maria-Alexandra Martu and Monica-Silvia Tatarciuc*

Abstract

Periodontal disease is an infectious and inflammatory disease with a high incidence in the global population and an extremely complex etiopathogenesis. Osteoporosis is one of the systemic diseases that can affect the integrity of periodontal tissues. Osteoporosis, as a skeletal disease, causes a reduction in bone mass and microarchitectural changes in the bone. Discussions about the connection between the two diseases affecting the bone began in 1960, but, contrary to the high number of studies, discoveries are still being made regarding the pathophysiological mechanisms that link the two diseases. The chapter proposes a systematized description of data on the influence of osteoporotic disease on the periodontal structures, therapeutic methods to address the patient with periodontal disease and osteoporosis and data on the potential influence of conventional and adjunctive periodontal treatment on systemic parameters in patients with osteoporosis.

Keywords: periodontal disease, osteoporosis, inflammation, periodontal therapy

1. Introduction

In systemic diseases that can generate periodontal effects, it is worth mentioning osteoporosis as a separate entity in diseases of endocrine origin. Osteoporosis, as a skeletal disease, is characterized by reduced bone mass and micro-architectural changes in the bone that lead to bone fragility and an increased risk of fracture.

Bone tissues are dynamic, with healthy bone undergoing lifelong shaping and reshaping. Modelling is a process by which bone grows linearly in size in response to the stress applied to it. This involves bone neo-formation independent of a previous bone resorption, the skeleton being able to acquire new cortical shapes and thicknesses. On the other hand, remodelling is initiated by resorption and is followed by the formation of new bone tissue at the same resorption site. Bone remodelling repairs skeletal micro-destruction to preserve resistance and provides serum skeletal calcium for mineral homeostasis. Signals from mechanical stress are received by osteocytes and are transmitted to osteoclasts or osteoblasts or their precursors.

Bone resorption reflects the amount of osteoclast recruitment and death, as well as the rate of matrix degradation [1].

Hypothetical patterns linking the two conditions exist: it is assumed that reduced bone density in connection with osteoporosis may accelerate the resorption of alveolar bone caused by periodontitis, facilitating the invasion of pathogenic bacteria. This bacterial invasion affects normal bone homeostasis, increases osteoclastic activity, and reduces bone density both systemically and locally through both the direct effect of releasing toxins and the release of inflammatory mediators [2].

Since periodontal disease is a multifactorial condition, osteoporosis, although it may not be the cause of its onset, may be a factor in further exacerbation. Thus there is a greater predisposition to lose alveolar bone in subjects with osteoporosis, especially against the background of a pre-existing periodontal disease [3].

Oestrogen deficiency leads to the production of several cytokines produced by immune cells (monocytes and macrophages) and osteoblasts. When challenges arise from plaque biofilm products, bone resorption factors such as lipopolysaccharides, and toxins, the host's immune system produces several inflammatory cytokines that activate osteoclasts and cause bone resorption. The accumulation of bacterial plaque made up of periodontal bacteria seems to be necessary for a woman who is deficient in oestrogen to show changes such as loss of attachment and destruction of the alveolar bone.

The inflammatory response of the host to this biofilm starts the inflammatory cascade and can lead to a constant activation of proteinases and enzymes with the role of tissue degradation, leading to destruction of connective tissues, resorption of alveolar bone and finally bone loss, which explains the increased risk of periodontal damage in menopausal women [4].

Oestrogen deficiency-induced osteoporosis, characterized by an imbalance between bone formation and bone resorption, is caused by elevated inflammatory cytokines such as tumour necrosis factor α (TNF α), interleukin 1 (IL-1), IL-6 and gamma interferon (IFN- γ) [5]. Studies have shown that inflammatory cytokines increase osteoclast activity and activate bone resorption. Therefore, anti-resorptive therapy is widely used in the management of osteoporosis. This type of treatment, however, only prevents additional bone loss while barely stimulating bone formation and reversing bone loss. A number of studies have shown that elevated levels of inflammatory cytokines cause deficits in osteogenesis in postmenopausal osteoporosis and in inflammatory diseases such as arthritis and periodontitis [6].

2. Periodontal clinical and radiological status in patients with periodontal disease and osteoporosis

To date, most studies focused on the relationship between periodontal disease and osteoporosis have been performed in small groups, with limited control of bias factors, with significant variations in defining the parameters of periodontal disease and osteoporosis; there are also few longitudinal studies that establish a temporal relationship.

Decreased systemic bone density in patients with osteoporosis, including the jaw bones, may provide circumstances of increased susceptibility of these patients to periodontal damage.

Orthopantomography can be used as a complementary examination of the patient with osteoporosis and periodontal disease, to assess the width of the mandibular cortex, the cortical mandibular angle, the cortical index and the degree of resorption of the alveolar ridge. We conducted a study on a group of 41 subjects, whose aim was to evaluate radiological parameters on digital orthopantomography

in patients with chronic periodontitis and osteoporosis, as well as to establish a correlation between them [7], bone mineral density and periodontal clinical parameters. For radiographic analysis we used digital orthopantomographs. The following determinations were made:

- Mandibular cortex thickness in the chin region (MCT)
- Panoramic mandibular index (PMI) - obtained by dividing the thickness of the mandibular cortex at the distance between the chin hole and the lower mandibular cortex
- Degree of resorption of the alveolar ridge (M/M ratio)
- Morphological classification of the lower mandibular cortex (C classes)

C1: normal bone cortex, with regular endo-osteal margin at both sides;

C2: moderately eroded bone cortex, with endo-osteal margin with semilunar defects;

C3: severely eroded bone cortex, with visibly porous endo-osteal margin.

The mean value of the plaque index was 1.21 ± 0.32 . This index has been closely correlated with a C2 bone cortex class. A positive correlation was demonstrated between this index and the average loss of attachment. The mean value of the gingival index was 0.79 ± 0.21 . This index was correlated with class C2 of bone cortex and with average loss of attachment. The mean value of the bleeding index was 2.3 ± 0.38 , an index also correlated with class C2 of the bone cortex and loss of attachment. The mean value of the periodontal probe performed on all study participants was 4.72 ± 1.02 mm. There was no correlation between probing depth and bone resorption index [7].

The mean value of attachment loss was 4.35 ± 1.01 mm. There was a close positive correlation between the average loss of periodontal attachment and the C2 class of bone cortex. There was a negative correlation between mean attachment loss and bone resorption index. There was a link between the average loss of periodontal attachment and the plaque index, calculus index and gingival index [7].

A total of 58.7% of patients had 15–30 teeth remaining on the arches; 27.9% of the total number of examined patients had up to 15 remaining teeth and 13.4% of them - over 30 remaining teeth on the dental arches. A total of 36.5% of patients had loss of dental-periodontal units due to coronary dental lesions, 30.7% due to periodontal disease and 32.8% due to the association of carious lesions with periodontal disease. Only 3.8% of all patients had intact dental arches. Third molars were not considered in the calculation.

Using a threshold level of 3 mm for cortical thickness, only 2 patients had MCT < 3 mm. We noticed an association between the T score value and MCT; low values of the T score were correlated with low values of cortical thickness ($p < 0.05$) [7].

We noticed that a decrease of MCT by 1 mm increases the risk of osteopenia/osteoporosis by 43%. The p value for MCT was statistically significant ($p = 0.033$). Moreover, when the morphology class is C2 or C3 (moderate and severe erosions), the age is increased and the MCT decreases to a statistically significant level ($p < 0.05$). A decrease of one millimetre of MCT increases the probability of moderately or severely eroded cortex by 96%. In terms of tooth loss, a one-unit increase in the number of missing teeth increases the probability of moderate or severe erosion by 6%.

Given that periodontal examination, along with performing oral radiographs are common procedures [8], the clinical significance for the observation of additional

risk factors for osteoporosis is extremely high, and questions regarding skeletal status may arise. General condition of the patient.

An important result in this study is given by the close correlation between local factors and the loss of periodontal attachment and bone tissue. Moreover, the loss of attachment was closely related to the bone resorption index.

The oral cavity and jaws are examined radiologically more frequently than any other part of the human body. Orthopantomographic radiography can be a useful means of screening in the diagnosis of osteoporosis, providing valuable information on the quality of the maxillary bone.

The radiograph does not allow the visualization of the periodontal infection, nor the migration of the junction epithelium in the initial periodontal lesion. However, the radiographic image reflects the status of the mineralized structures of the periodontium. Thus, radiography is indispensable for assessing bone loss and for establishing residual value.

Clinical measurements by periodontal examination do not always fully and accurately reflect tissue loss, nor is radiography sufficient to establish a positive diagnosis. Radiography provides the image of two-dimensional bone changes, as well as abnormalities of radiopaque structures (carious, endodontic, reconstructive lesions). Radiographic images frequently used in periodontology are given by retro-dental-alveolar radiography, orthopantomography, as well as bite-wing radiography.

The panoramic x-ray represents a complex projection of the maxillary bones and dental arches, with multiple super-positions and distortions that can be exacerbated by image capture errors. Moreover, orthopantomography (OPT) illustrates numerous anatomical structures, in addition to the maxillary bones, which can represent interpretive challenges. In order to obtain a successful interpretation of panoramic radiographs, an understanding of the normal anatomy of the head and neck region and its radiologic aspects is absolutely mandatory.

Analysis of the density of the trabecular pattern of the maxillary bone, seen radiologically, showed that dense trabeculation is a strong indicator of increased mineral density, while thin trabeculation corresponds to low mineral density [9]. It is well known that in patients with osteoporosis the bone loss is not uniform and that the trabecular bone is earlier and more deeply affected than the cortical bone [10].

The mandible has a composition similar to the femoral neck [11], where fractures are mainly caused by a cortical loss rather than a trabecular bone. Given that the jaw consists mainly of trabecular bone, it is possible that the bone density measured at this level is more closely related to osteoporotic disease. However, the lack of fixed reference points in the upper jaw (such as the chin hole in the jaw) makes the assessment of standard points at this level a challenge.

Osteoporosis can be diagnosed by observing tooth loss, thinning of the lower mandibular cortex, and by changes in the morphology of the endo-osteal margin of the cortex and trabecular bone [12].

Mandibular bone mass correlates with systemic skeletal bone mass in numerous studies. Horner and Devlin reported a relationship between mandibular cortical thickness and mandibular bone density [12]. Cortical thickness at the gonial angle was determined by panoramic radiographs on a group of 180 patients; for patients aged 15 to 69 years, this was relatively constant; in subjects over 60 years of age, a decrease was observed, more significant for women than for men [13].

Devlin and Horner [12] reported that a cortical thickness of 3 mm is most appropriate as a threshold value for bone densitometry. White et al. [14] consider that this threshold value is more recommended to be 4 mm. Klemetti et al. [15] reported that the 4 mm threshold is optimal but not sufficient in itself for an

optimal classification of subjects. In the present study we discovered values below 3 mm only on 3 radiographs; thus, we support the opinion of White et al. according to which, if panoramic radiographs are used, the threshold for cortical thickness is more appropriate at 4 mm [14].

As for quantitative computed tomography (QCT), it was first used to study the relationship between oral status and osteoporosis in 1989. Regardless of the technique used, the position of the sections and perspective should be documented by subsequent examinations to avoid the error of precision. The reported accuracy for QCT ranges from 1 to 3% for highly controlled settings and 4–5% for clinical settings [16]. Also, the cost of computed tomography is quite high, so it is not recommended only for a screening system in osteoporotic disease.

It has been demonstrated that mandibular cortex thickness can be a useful parameter to clinically assess metabolic bone loss and that a gonial thickness of less than 1 mm is an indicator of metabolic bone loss [12]. Dissemination of information on the prevention of osteoporosis produces a significant public effect for the implementation of appropriate ways to minimize the process of reducing bone mass.

3. The role of TNF α in patients with periodontal disease and osteoporosis

The main mechanism by which TNF α contributes to the evolution of osteoporosis is by disturbing the balance between bone resorption and bone formation [17]. Previously, TNF α blockade was considered to be an effective method to suppress and prevent bone resorption [18]. TNF α blockade significantly stimulated bone formation in mice.

We conducted a study of 46 postmenopausal female subjects in which we assessed the levels of TNF α in crevicular fluid and serum [19]. Subjects were divided into two groups: the Study Group - patients with osteoporosis and periodontal disease (n = 24) and the Control Group - patients with periodically healthy periodically disease (n = 22).

Probing depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL) had significantly higher values in the study group than in the control group ($p < 0.05$) [19]. We could not observe any significant differences in the values of the plaque index between the groups.

All samples showed detectable levels of TNF α . Significantly high levels of TNF α were detected in both serum and GCF for the study group compared to the control group. Serum TNF α was positively correlated with BOP ($p < 0.01$). There were no significant correlations between probing depth, clinical attachment loss, plaque index, and TNF α levels. Serum TNF α levels were correlated with TNF α levels in crevicular fluid.

Maintaining the balance of proinflammatory and anti-inflammatory cytokines in the body is one of the manifestations of self-regulation [20]. Over the past decade, considerable evidence suggests that oestrogen prevents bone loss by blocking the production of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-10, tumour necrosis factor (TNF) α in the spinal cord and bone cells.

Cytokines are soluble proteins that can initiate, mediate, and control immune and inflammatory responses. It has been proposed that pro and anti-inflammatory cytokines contribute to various bone metabolic diseases, including periodontitis and postmenopausal osteoporosis [21]. Among pro-inflammators, TNF α has been reported to play a key role in periodontal bone destruction [22].

In our study we demonstrated significant differences in TNF α values between the osteoporosis group and the control group. It can be suggested that increased

TNF α values in GCF and serum contribute to the large number of B cells and T cells present in inflammatory periodontal tissues, increasing the destruction of periodontal tissue [19].

The fact that the values in the crevicular fluid were correlated with the serum values clearly indicates the influence that the systemic status generates on the local status (periodontal, in the case of the present study).

Periodontal tissue destruction is closely related to the release of inflammatory mediators, such as TNF α . These mediators are able to aggravate the inflammatory response. It has been shown that the severity of periodontal disease is associated with their concentration in the crevicular fluid.

Some subjects may have a more pronounced inflammatory response to bacterial aggression, a response that depends on the quality and quantity of the bacterial flora, as well as systemic factors (heredity, certain infectious/inflammatory diseases, osteoporosis, etc.).

Inflammatory cytokines can influence this delicate balance by promoting osteoclast differentiation and activation. Bone loss is thus attributed more to increased bone resorption than to reduced bone neo-formation, with osteoclasts being the main culprits.

4. The implications of the IL-1 α and IL-1 β cytokines in patients with periodontal disease and osteoporosis

IL-1 α and IL-1 β are biologically more or less equivalent pleiotropic factors that act locally and systemically. Only a few functional differences between the factors have been described; only IL-1 β appears to be constitutively expressed in the brain. Interleukin-1 is a potent stimulator of bone resorption in vivo; IL-1 β has been shown to be the most potent stimulator of bone resorption in vitro. The mechanism by which IL-1 β stimulates resorption involves the expression of RANKL in osteoblasts and indirect stimulation of osteoclastogenesis and bone resorption [23].

There are also indications that osteoclasts express interleukin-1 receptor I, which is important for osteoclast activity and survival by activating the PI3K/AKT and ERK pathways, a MyD88-dependent response, but not on TRIF [24].

We conducted a study of 38 postmenopausal female subjects with the purpose to investigate differences in IL-1 α and 1 β levels in GCF in patients with chronic periodontitis, with or without associated chronic disease (in the present study - osteoporosis) [25].

IL-1 α was the most prevalent cytokine found in GCF and was detected in all sites studied. We noticed significantly higher differences in interleukin levels for the study group (patients with osteoporosis) compared to systemically healthy patients ($p < 0.05$).

In order to establish the possible clinical relevance of these observations, a correlation analysis was performed between the clinical parameters and the total levels of cytokines in the test sites. Positive correlations were observed between IL-1 α and 1 β levels with PPD and CAL [25].

In periodontal inflammation, IL-1 β is mainly expressed by macrophages and dendritic cells, but gingival fibroblasts, periodontal ligament cells, and osteoblasts can also secrete IL-1 β . Elevated levels of IL-1 β as well as IL-1 α in gingival crevicular fluid have been reported by several groups [26]. There are many clinical studies showing the importance of IL-1 β for inflammation and destruction in rheumatoid arthritis and osteoarthritis, associated with periodontal damage [27].

There are several reasons to believe that IL-1 β could be an important mediator of the destruction of gingival connective tissue and periodontal ligament, as well as the resorption of alveolar bone. IL-1 β is a potent stimulator of matrix metalloproteinase expression in fibroblasts and periodontal ligament cells [28].

The challenge in osteoimmunology is to determine the relative contribution of various components of the immune system to bone loss induced by ovariectomy and senile osteoporosis. These may also involve identical adjustment paths; however, in each system, there will be subtle differences in the net balance of local or systemic regulators, resulting in specific patterns of subsequent bone loss.

5. Influence of hormone replacement therapy on periodontal parameters in patients with osteoporosis

Hormone replacement therapy (HRT) represents an attractive method to counterbalance hormonal changes. The aim of HRT is not only to avoid climacteric signs and symptoms but also to protect the patients from cardiovascular disease and osteoporosis complications [29]. Having in mind that oestrogen deficit is an important risk factor for osteoporosis, it is of high importance to consider the role of oestrogen in the association between periodontal lesions and osteoporosis [30].

We proposed a comparative assessment of periodontal status in postmenopausal patients who were on hormone replacement therapy or not. The study was performed on a group of 23 female subjects, diagnosed with osteoporosis, aged between 50 and 62 years [30]. Subjects were divided into two groups. The first group, the study group, included patients undergoing hormone replacement therapy ($n = 13$); the control group included patients who did not follow this therapy ($n = 10$). The patients underwent periodontal clinical examination.

We noticed that the risk of tooth loss was similar for both groups but this risk shows a slight form of decrease with increasing treatment duration. Regarding the bleeding on probing, its value was approximately twice higher in patients without hormone replacement therapy, compared to the control group. The diagnosis of periodontal disease was higher in patients who did not receive replacement therapy than in those with HRT. In the group without hormone replacement therapy, we noticed more severe periodontal attachment losses than in the study group, with HRT. Moreover, the level of clinical attachment was proportional to the duration of hormone replacement therapy [30].

An important effect of low levels of oestrogen is the decrease in the inhibition of osteoclastogenesis, with a consequent increase in the activity of osteoclasts [31]. The result is a decrease in bone mass and bone resistance.

In vitro experiments indicate that neutrophil chemotaxis is reduced in the presence of low concentrations of estradiol. On the other hand, progesterone increases the chemotaxis of neutrophils, so any change in the balance of these hormones in plasma or gingival tissue can have a significant effect on neutrophil function in vivo [32].

Until recently, hormone replacement therapy was considered the only effective treatment recommended for the prevention of diseases associated with oestrogen deficiency [30]. After the publication of the Women's Health Initiative results in 2002 and 2004 [33], the use of HRT has become a complex debated issue. Women's Health Initiative and other data from dedicated studies suggested that the potential risks associated with HRT (increased risk of breast cancer and severe cardiovascular disease) are in direct relationship with the regimen administered, dose, mode of

administration, age of the patient, associated disease, and duration of treatment [34]. Therefore, based on current data, the purpose, dose and regimen of HRT should be individualized, with a complex evaluation of each case [30].

Oestrogen has two key roles in bone health. First of all, the hormone is essential for the normal maturation of the bone and for ensuring the acquisition of minerals so as to reach an optimal bone mass. Second, oestrogen maintains bone mass throughout adulthood during remodelling processes. Oestrogen deficiency leads to a reduction in bone mass and damage to bone microarchitecture, the two being the main aspects of osteoporosis.

Oestrogen deficiency can cause bone loss by acting directly on bone cells that are involved in bone turnover and by decreasing the influence that oestrogen has on the intestines and kidneys that regulate extra-skeletal calcium levels.

Oestrogen deficiency contributes to the deterioration of bone microarchitecture and to the reduction of bone strength which is determined by bone geometry, cortical thickness, porosity, trabecular morphology. Bone remodelling results in the modification of major determinants of bone strength [35].

Oestrogen has been shown to stimulate osteoprotegerin (OPG) expression in human osteoblasts. The hormone can thus have an effect on bone metabolism through the surrounding soft tissue cells. Oestrogen has been shown to inhibit the formation of osteoclastic cells in cultures of fibroblasts in the periodontal ligaments and mononuclear cells in the peripheral blood. This inhibitory effect was not found in cultures with gingival fibroblasts. This observation suggests that they are not as sensitive to oestrogen without a clear biological mechanism. It is known that oestrogen exerts its effects through intercellular receptors; oestrogen receptor concentration is thus an important determinant of the cellular response to oestrogen.

The inhibitory effect of oestrogen on osteoclastic cell formation corresponds to previous research obtained either by osteoblast-induced osteoclast production or by bone marrow cells used as precursors. In addition, *in vivo* studies using ovariectomized mice have shown that oestrogen deficiency has induced the presence of a large number of osteoclasts in the periodontium. The data thus suggest that oestrogen plays an important role in modulating osteoclast formation.

Another important finding was the increased number of osteoclast-like cells in cultures with periodontal ligament fibroblasts. These observations suggest a difference in the interaction between different osteoblast populations and mononuclear cells in peripheral blood.

Subsequent analysis of molecules that may be involved in the oestrogen inhibitory effect involved the evaluation of mRNA expression of RANKL, OPG, and oestrogen receptors. No effect of oestrogen on the expression of these genes was found in any population of fibroblasts. The data suggest that the oestrogen inhibitory effect on osteoclastic cells can be mediated independently of OPG and RANKL, being mediated by other compounds such as TGF β , TNF α , IL-1, IL-6, IL-7 [30].

There are studies that have shown that the risk of edentulous ridges is lower in patients following HRT [36, 37]; it was observed that HRT reduced the risk of edentulousness by 6% for each year of HRT [38]. The patients with osteoporosis present a significant decrease in alveolar ridge height in edentulous areas and loss of alveolar ridge height is associated with osteoporosis and osteopenia [6]. We demonstrated a higher number of teeth present in patients with hormone replacement therapy [30]. However, teeth loss does not represent an ideal surrogate assessment for periodontal disease, as it can also be caused by carious lesions or traumatic events.

6. Effects of modulating inflammatory response therapy in patients with osteoporosis and chronic periodontitis

Mechanical removal of plaque and calculus from dental surfaces is considered the standard treatment for chronic periodontitis. Contrary to the spectacular evolution in the field of etiopathogenesis of periodontal disease, its treatment has changed very little, in principle. Scaling and root planing remain the “gold standard” of periodontitis treatment.

The importance of the host inflammatory response in periodontal pathogenesis presents the opportunity to explore new therapeutic strategies by means of modulating this response. Modulation therapy can be combined with conventional therapeutic methods to reduce the bacterial load.

To date, the only systemic therapy approved as a modulator of the response in periodontal disease is with sub-antimicrobial doses of doxycycline, which inhibits the activity of matrix metalloproteinases (MMPs) (trade name: Periostat). Doxycycline in sub-antimicrobial doses inhibits MMP activity by synergistic mechanisms, independent of antibiotic properties. Primary studies have shown that the use of tetracycline predominantly inhibits MMPs in excess of periodontitis compared to constitutional MMPs. In vitro studies have shown that MMP-13 is more sensitive to tetracycline inhibitory concentrations than MMP-8 and MMP-1 (fibroblastic collagenase) is the least sensitive.

Doxycycline has been shown to be more effective than other tetracyclines in reducing collagenase activity in crevicular fluid in patients with chronic periodontitis [39]. Doxycycline has a lower inhibitory concentration than minocycline ($IC_{50} = 15 \text{ IM}$ compared to $IC_{50} = 190 \text{ IM}$) or tetracycline ($IC_{50} = 350 \text{ IM}$); thus, a lower dose of doxycycline than minocycline or tetracycline is required to reduce a certain level of collagenase by 50% [40]. Moreover, doxycycline is more effective in blocking the activity of neutrophil collagenase (MMP-8) than the activity of MMP-1 (fibroblastic collagenase), demonstrating that its use is a safe way to reduce pathological collagenase levels without affecting healthy tissues.

We conducted a study in a group of 26 subjects, with the purpose to analyse changes in periodontal clinical parameters that modulation therapy of the host response with sub-antimicrobial doses of doxycycline can exert in patients with periodontal disease and osteoporosis. Patients were randomly divided into two groups: the study group ($n = 17$), which underwent classical debridement therapy (scaling and root planing) plus sub-antimicrobial doses of doxycycline (20 mg twice daily) for 3 months, and control group ($n = 18$), which followed only classical debridement therapy [41].

We analysed the following periodontal parameters: probing depth, level of clinical attachment, PBI and PI index at baseline (pre-therapeutic), on the last day of medication, and 3 months after medication completion (6 months from baseline). The sites were grouped according to the probing depth in: group 1 - superficial (0-3 mm); group 2 - moderate (4-6 mm) and group 3 - deep ($\geq 7 \text{ mm}$) [41].

In the present study, 30 patients were initially enrolled, but 4 of them failed to complete doxycycline therapy. Therefore, the study resulted in the use of two groups: the study group (13 subjects) and the control group (13 subjects). There was no statistically significant difference between groups at baseline in terms of probing depth. No significant differences were observed in the sites with an initial depth of 0-3 mm ($p > 0.05$). Significant reductions in probing depth were observed at sites with an initial depth of 4-6 mm and $\geq 7 \text{ mm}$ ($p < 0.025$) [41].

Although the mean value of pocket reduction for sites with an initial depth of 4-6 mm and $\geq 7 \text{ mm}$ was higher for the study group than for the control group

(1.80 mm versus 1.46 mm for moderate pockets and 3.38 mm versus of 2.57 mm for deep pockets), the difference did not reach the significance threshold ($p > 0.05$). Analysis of sites with an initial depth ≥ 7 mm showed that an increased percentage of sites was reduced by at least 3 mm following doxycycline administration (66.4%), compared to the group without modulation therapy (55.1%) at 3 months, without a statistically significant difference between groups ($p > 0.05$) [41].

However, at 6 months the percentage of sites with an improvement in depth ≥ 3 mm was significantly higher ($p = 0.011$) for the group with modulation therapy (73.4%) compared to the group that followed only classical therapy (49.7%) [41]. At baseline, there were no statistically significant differences in the level of clinical attachment in the sub-grouped sites by probing depth between the main study groups ($p > 0.05$). Sites with moderate depths and deep sites showed significant improvements in attachment level at 3 and 6 months, compared to baseline ($p < 0.025$). Sites with an initial depth of 0–3 mm did not show significant changes in attachment during the study period ($p > 0.05$) [41].

Sites with an initial depth of 0–3 mm in the control group (without doxycycline therapy) showed a slight decrease in attachment (–0.04 mm at 3 months, –0.03 mm at 6 months). On the other hand, the sites with the initial depth of 0–3 mm in the study group showed a slight gain of attachment (0.11 mm at 3 months, 0.14 mm at 6 months), but without significant differences between groups ($p > 0.05$).

Although the average attachment gains for sites with an initial depth of 4–6 mm and ≥ 7 mm was higher for the study group than for the control group (1.12 mm compared to 0.78 mm for sites with moderate depths; 2.15 mm compared to 1.76 mm compared to the deep sites), the statistical analysis did not show a level of significance ($p > 0.05$) [41].

GDP and PI values showed significant improvements between baseline and re-evaluations at 3 and 6 months ($p < 0.025$). The reduction in GDP and IP was similar for both groups ($p > 0.05$).

Periodontal treatment, over time, has focused on reducing the bacterial load and disorganizing the biofilm by mechanical methods. However, recent research has led to a paradigm shift in the evolution of periodontal disease. Thus, it is known today that the lesions that appear at the level of superficial and deep periodontal tissues are a result of the activation of the host's immune-inflammatory defence mechanisms [42].

In addition to the classic periodontal therapy, scaling and root planing, which aims to disorganize the bacterial biofilm and reduce the inflammatory load, new adjunct methods have been postulated, with etiological therapeutic effect in the periodontopathic patient. Among them, the modulation therapy of the host's inflammatory response with pharmacological agents has acquired important dimensions, precisely because of its effectiveness. The success of such therapy is all the more important as it affects a systemically affected area.

Doxycycline has the ability to inhibit the activity of matrix metalloproteinases (MMPs), a capacity confirmed in numerous studies. Minocycline, doxycycline, and tetracycline inhibit collagenolytic activity, while non-tetracycline antibiotics have no effect on collagenase [40]. It was recognized in the mid-1980s that inhibition of collagenolysis by tetracyclines is a new therapeutic method in the management of periodontal disease.

The effects of doxycycline are, in addition to direct inhibition of active matrix metalloproteinases by cationic chelation and inhibition of oxidative activation of latent MMPs, and inhibition of the expression of inflammatory cytokines (IL-1, IL-6, TNF α) and PG-E2; seeks and inhibits the formation of oxygen-reactive species produced by neutrophils; protects the α 1-proteinase inhibitor, thus indirectly

reducing tissue proteinase activity; reduces osteoclastic activity and bone resorption; inhibits osteoclastic MMPs.

Doxycycline contributes to decreased conjunctival lysis by inhibiting pro-inflammatory mediators and cytokines (including IL-1 and TNF α) [43], as well as by increasing collagen production, osteoblast activity and bone formation [41]; this last aspect is of major importance especially for patients with osteoporosis, whose bone capital is affected.

A major concern with long-term administration of doxycycline has been associated with the development of antibiotic resistance. Indeed, when antimicrobial doses of tetracycline were used (250 mg daily, 2–7 years), up to 77% of patients' flora showed resistance to tetracycline [44]. Given this problem, sub-antimicrobial doses (20 mg doxycycline versus 50 or 100 mg) were prepared [41]. One of the preliminary experiments with this new formula clearly demonstrated that such doses (20 mg twice daily), administered 2 weeks, inhibited collagenase activity by 60–80% in gingival tissue in patients with chronic periodontitis [41]. Collagenase activity was significantly reduced in the crevicular fluid collected from these patients. Subsequent studies have indicated that this drug regimen can prevent the progression of periodontitis without the patient developing microorganisms resistant to doxycycline or other types of side effects [42].

The 3-month doxycycline regimen was well tolerated and no adverse reactions (gastrointestinal disorders, etc.) were reported. This may suggest that doxycycline modulation therapy is a safe approach in the long-term treatment of chronic periodontitis.

In the present study we observed improvements in clinical parameters (probing depth, level of clinical attachment, bleeding index, plaque index) both for the study group (with adjunctive modulation therapy) and for the control group (which followed only classical scaling-root planing therapy), improvements that were maintained throughout the study.

Caton et al. [45] established that reductions in probing depth of at least 3 mm represent relevant, clinically significant improvements. In the present study, the percentage of sites of great depth (≥ 7 mm) that showed reductions of at least 3 mm was significantly higher at 6 months for the group with modulation therapy. This result is of special importance, given that sites with such depth are candidates for surgical procedures. Therefore, it can be hypothesized that adjunctive doxycycline therapy may reduce the likelihood of surgical procedures as well as the discomfort caused by them [41].

We also demonstrated that the sites with relatively small depths (0–3 mm) in the study group showed a slight gain of attachment, while these sites in the control group showed a slight loss of attachment. This supports the efficacy of host response modulation therapy by administering sub-antimicrobial doses of doxycycline.

Studies are needed to evaluate the efficacy of very long-term sub-antimicrobial doses of doxycycline in periodontal therapy and in the prevention of loss of dental-periodontal units. The financial benefit derived from adjunctive therapy must also be evaluated (can this minimize costs by avoiding the need for periodontal surgery?).

It is suggested that doxycycline-based products be developed to support plasma concentrations for 24 hours by administering a single dose daily.

Given the increased variability of pathogenic pathways with a role in periodontal destruction (e.g., the cytokine group IL-1 is much more complex today), a more diverse range of host response modulators is also needed [46]. Moreover, most biological responses involve a variety of mechanisms; thus, blocking a single inflammatory pathway may not result in the desired result because receptor-mediated

responses can be activated by alternative pathways. Therefore, a poly-pharmaceutical approach is needed to modify a number of different pathways associated with inflammation and tissue destruction.

Lipoxins are another group of compounds that can alter the inflammatory response in periodontal tissue. These mediators are released during the inflammatory response and have the effect of decreasing inflammation and modulating its disappearance. Lipoxins block the secretion of IL-1 β from neutrophils and block the migration of neutrophils following exposure to *Porphyromonas gingivalis* [47].

Osteotrophic factors such as hormonal or endocrine-related (vitamin D3, parathyroid hormone), cytokines (IL-1, IL-6, IL-11 and IL-17), growth factors (TNF α , morphogenetic protein-2) and others molecules (PG-E2, LyT activator CD40 ligand and glucocorticoids) increase the expression of the RANKL gene in osteoblastic/stromal cells.

Sequentially, RANKL mediates the signal for osteoclastogenesis through RANK or preosteoclastic cells. Thus, the RANKL/RANK interaction is responsible for the differentiation and maturation of osteoclast precursor cells with osteoclast formation. Osteoprotegrin acts by binding to RANKL, inhibiting osteoclastic development.

In periodontal disease the first to investigate the role of RANKL in bone resorption was Teng [48]. It inoculated *Aggregatibacter actinomycetemcomitans* in mice lacking endogenous LyT and LyB and receiving human CD4 cells; Thus, CD4 + activation, RANKL stimulation and bone resorption were initiated, concluding that RANKL expression plays a significant role in bone destruction in periodontitis. Crotti et al. [49] observed a hyper-expression of RANKL in inflamed periodontal tissues, as well as an increased RANKL/osteoprotegrin ratio to healthy subjects.

There are numerous animal studies as well as preliminary human studies demonstrating inhibition of RANKL function by osteoprotegrin treatment, reducing the number of osteoclastic cells and, implicitly, bone resorption from periodontal disease. Of course, more in-depth studies are needed to certify the most effective therapeutic approach to this molecular interaction.

Fatty acids have been proposed to reduce chronic inflammation in arthritis patients by decreasing the release of LTB4 from neutrophils and IL-1 from monocytes. Local application of Omega-3 polyunsaturated fatty acids has been successful in patients with inflammatory diseases such as psoriasis, as well as in models with experimental periodontitis in animals. The mechanism of action is based on decreased leukocyte chemotaxis, expression of molecular adhesion and production of inflammatory cytokines. Offenbacher demonstrated the inhibition of PG-E2 production by eicosapentanoic or docosahexanoic acid administration, with effects similar to ibuprofen in patients with periodontal disease [50].

Vardar evaluated the use of omega-3 fatty acids in order to block arachidonic acid cascade in mice with experimentally induced periodontal disease [51]. This would inhibit the production of cyclooxygenase-derived prostanoids and lipo-oxygenase-derived leukotrienes. The authors relied on two aspects: leukotriene B4 (mediator of arachidonic acid) plays an important role in bone resorption and inhibition of COX with NSAIDs would cause the accumulation of arachidonic acid that is metabolized by lipo-oxygenase, causing continuous bone loss. The authors also administered a combination of omega-3 fatty acids with celecoxib, seeking a synergistic anti-inflammatory effect. Combination therapy resulted in significant decreases in prostaglandin, leukotriene B4 and PAF levels; no effect on bone lysis was observed (this may be due to the short evaluation period).

Elkhoul published the results of a study of 40 patients with at least one grade II furcation defect; patients were divided into two groups: the first group performed allografting, which was associated with therapy with omega-3 polyunsaturated fatty acids and low-dose aspirin; in the second group (control) only allografting

was performed, following a placebo adjunctive therapy. At 3 and 6 months, the clinical parameters (plaque index, gingival index, bleeding index, probing depth, level of clinical attachment) were evaluated, as well as biochemical markers in the crevicular fluid (IL-1b and IL-10) [52]. The results were very good for the test group compared to the control group (reduction of probing depth, gain of clinical attachment, significant modulating effect for IL-1b and IL-10 levels).

There are proven clinical results regarding monounsaturated fatty acid substitution; they influence blood pressure, clotting, endothelial activation, inflammation and thermogenic capacity in cardiac patients, they prevent obesity and other metabolic diseases.

Hasturk demonstrated in a study in rabbits with experimentally induced periodontitis with *Porphyromonas gingivalis* that topical application of the tetradecanol-1 complex (1-TDC: mixture of esterified monounsaturated fatty acids) causes an inhibitory effect on the inflammatory cascade of the host response [53].

Further studies are needed to evaluate the impact of this modulation therapy in patients with periodontal disease and osteoporosis at the molecular level (by examination of the crevicular fluid), on pro-inflammatory cytokines, and at the systemic level, by assessing bone mineral density (correlation with pre- and post-therapeutic T score).

7. Conclusions and perspectives

Chronic periodontitis and osteoporosis are two chronic diseases, with inflammatory etiopathogenesis, whose statistical characteristics are constantly growing worldwide. Studies focused on the association of these diseases in the same subjects are few and with discordant results. These may be due to the variability of the inclusion/exclusion criteria, the research methodology, as well as the small groups of subjects included.

The research supports the role that a routine operation performed in the dental office - panoramic radiography - could play in detecting undiagnosed cases of osteoporosis, given the local changes that occur in such patients. Signs of periodontal tissue destruction are also reflected radiologically, where the thickness of the mandibular cortex and the morphological appearance of the cortex (presence of erosions) have been shown to be closely correlated with the value of the densitometric T Score. Therefore, orthopantomography, a common procedure in the dental office, could be an effective and less expensive method of screening for osteoporosis, with a significant role for the dentist in this procedure.

Despite advances in research methodology and laboratory tests, in order to identify the factors associated with chronic periodontal disease, it is still unclear how to predict the progression of periodontal disease. Extensive research has been done in the area of host biochemical response markers in periodontal disease. It is unlikely that a unitary biomarker will be able to meet the criteria for estimating future destruction from periodontal disease.

Patients with periodontal disease with osteoporosis had higher levels of TNF α , IL-1 α , IL-1 β , IL-6 and RANKL in the crevicular fluid, compared to systemically healthy patients. Also, cytokine values were positively correlated with periodontal parameters. Therefore, it can be stated that these patients are prone to excessive production of this type of cytokine which also activates B cells and promotes B cell activity in periodontal inflammatory sites, aggravating the evolution of periodontal disease.

The conventional systemic hormone replacement therapy was associated with lower indices of gingival inflammation, such as a reduced gingival bleeding index

in subjects who followed hormone replacement therapy when compared to the subjects without HRT [30]. The risk of tooth loss was reduced in the group with HRT, when compared to patients without HRT and, more importantly, the number of present teeth is directly proportional to the duration of the substitution therapy [30]. The diagnosis of periodontal disease was more common in patients without hormone replacement therapy compared to those with HRT. We can conclude that HRT generates a positive effect on periodontal tissues, an effect that is all the better highlighted as hormone replacement therapy has a longer duration; still, this benefit needs to be carefully assessed and compared to the potential risks of such therapy [30]. These aspects bring in a new sphere the ways of complex and interdisciplinary therapeutic approach of patients with osteoporosis and chronic periodontitis.

We also proposed a unique analysis in the context of the association of the two diseases - periodontal disease and osteoporosis - of the effects generated by a deputy form of periodontal therapy, extremely topical, that of modulation therapy by chemotherapeutic agents of the host's inflammatory response. We demonstrated in the study that adjunctive therapy with sub-antimicrobial doses of doxycycline (administration of 20 mg twice daily, 3 months), in combination with classical therapy, generated significant clinical improvements in patients with periodontal disease and osteoporosis - maintained during the study and that could pre-meet the need for surgery. We also demonstrated that sites with relatively small depths (0-3 mm) in the modulation therapy group showed a slight gain in attachment, while these sites in the control group showed a slight loss of attachment. This supports the efficacy of host response modulation therapy by administering sub-antimicrobial doses of doxycycline. Taken together, the data presented should inspire further research, providing epigenetic responses in periodontics, and using this information to develop future therapies.

The set of studies carried out supports in a complex way the importance of the bidirectionality of the periodontal disease - osteoporosis relationship, offering new information and protocols to approach patients, with certain value both for the medical community and, especially, for the entity that is at the centre of its concern - the patient per se.

Studies have shed new light on the link between periodontal disease (particularly in its chronic form) and osteoporosis. The data obtained show observations of a clinical and paraclinical nature, which aim to expand the knowledge related to this complex association, with practical applicability, which supports the concept of interdisciplinary approach to the patient.

Periodontal tissue damage, clinically detectable by measurements of periodontal parameters (probing depth, loss of clinical attachment, probing bleeding), is more severe in patients with osteoporosis than in systemically healthy patients. Research has shown that osteoporosis systemically creates favourable circumstances for the evolution of periodontal disease, but it is also significantly associated with local determinants and factors.

Conflict of interest

The authors declare no conflict of interest.

IntechOpen

Author details

Silvia Martu¹, Irina-Georgeta Sufaru^{1*}, Sorina-Mihaela Solomon¹, Ionut Luchian¹, Ioana Martu¹, Liliana Pasarin¹, Dora-Maria Popescu², Maria-Alexandra Martu¹ and Monica-Silvia Tatarciuc¹

1 “Grigore T. Popa” UMPH, Iasi, Romania

2 UMPH, Craiova, Romania

*Address all correspondence to: irina_ursarescu@yahoo.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Friedlander AH. The physiology, medical management and oral implication of menopause. *Journal of American Dental Association*. 2002;133:73-81. DOI: <https://doi.org/10.14219/jada.archive.2002.0025>
- [2] Guiglia R, Di-Fede O, Lo-Russo L, Sprini D, Rini GB, Campis G. Osteoporosis, jawbones and periodontal disease. *Medicina Oral Patologia Oral Cirurgia Bucal*. 2013;18:93-99. DOI: 10.4317/medoral.18298
- [3] Esfahanian V, Shamami MS, Shamami MS. Relationship between osteoporosis and periodontal disease: Review of the literature. *Journal of Dentistry (Teheran)*. 2012;9:256-264. PMID: PMC3536461
- [4] Buencamino M, Palomo L, Thacker HL. How menopause affects oral health, and what we can do about it. *Cleveland Clinical Journal of Medicine*. 2009;76:467-475. DOI: 10.3949/ccjm.76a.08095
- [5] Brennan RM, Genco RJ, Hovey KM, Trevisan M, Wactawski-Wende J. Clinical attachment loss, systemic bone density, and subgingival calculus in postmenopausal women. *Journal of Periodontology*. 2007;78:2104-2111. DOI: 10.1902/jop.2007.070155
- [6] Tezal M, Wactawski-Wende J, Grossi SG, Ho AW, Dunford R, Genco RJ. The relationship between bone mineral density and periodontitis in postmenopausal women. *Journal of Periodontology*. 2000;71:1492-1498. DOI: 10.1902/jop.2000.71.9.1492
- [7] Ursarescu I, Solomon S, Potarnichie O, Pasarin L, Martu I, Luchian I, martu S. The evaluation of clinical and imagistic parameters on osteoporosis patients. *Romanian Journal of Oral Rehabilitation*. 2012;4:53-57.
- [8] Marques MR, Da Silva MAD, Barros SP. Periodontal Disease and Osteoporosis Association and Mechanisms: a Review of the Literature. *Brasilian Journal of Oral Science*. 2003;2:137-140. DOI: 10.1016/j.anchorbio.2004.08.014
- [9] Jonasson G, Bankvall G, Kiliaridis S. Estimation of skeletal bone mineral density by means of the trabecular pattern of the alveolar bone, its interdental thickness, and the bone mass of the mandible. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology*. 2001;92:346-352. DOI: 10.1067/moe.2001.116494
- [10] Khosla S. Pathogenesis of age-related bone loss in humans. *The Journals of Gerontology Series A Biological Sciences and Medical Sciences*. 2013;68:1226-1235. DOI: 10.1093/gerona/gls163
- [11] Devlin H, Whelton C. Can mandibular bone resorption predict hip fracture in elderly women? A systematic review of diagnostic test accuracy. *Gerodontology*. 2013;32:163-168. DOI: <https://doi.org/10.1111/ger.12077>
- [12] Devlin H, Horner K. Mandibular radiomorphometric indices in the diagnosis of reduced skeletal bone mineral density. *Osteoporosis International*. 2002;13:373-378. DOI: 10.1007/s001980200042
- [13] Bras J, van Ooij CP, Abraham-Inpijn L, Kusen GJ, Wilink JM. Radiographic interpretation of the mandibular angular cortex: A diagnostic tool in metabolic bone loss. Part I. Normal state. *Oral Surgery Oral Medicine Oral Pathology*. 1982;53:541-545. DOI: 10.1016/0030-4220(82)90473-x
- [14] White SC, Taguchi A, Kao D, Wu S, Service SK. Clinical and panoramic

predictors of femur bone mineral density. *Osteoporosis International*. 2005;16:339-346. DOI: 10.1007/s00198-004-1692-4

[15] Klemetti E, Kalmakov S, Kroger H. Pantomography in assessment of the osteoporosis risk group. *Scandinavian Journal of Dental Research*. 1994;102:68-72. DOI: 10.1111/j.1600-0722.1994.tb01156.x

[16] Lang P, Steiger P, Faulkner K, Gluer C, Genant HK. Osteoporosis. Current techniques and recent developments in quantitative bone densitometry. *Radiologic Clinics of North America*. 1991;29:49-76. PMID: 1985329

[17] Nanes MS. Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. *Gene*. 2003;321:1-15. DOI: 10.1016/s0378-1119(03)00841-2

[18] Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. *The Journal of Clinical Investigation*. 2000;106:1229-1237. DOI: 10.1172/JCI11066

[19] Ursarescu I, Pasarin L, Solomon S, Boatca RM, Martu A, Moise G, Martu S. The assessment of serum and GCF proinflammatory cytokines levels in patients with osteoporosis and periodontal disease. *Romanian Journal of Oral Rehabilitation*. 2014;6:45-50.

[20] Garlet GP, Martins WJ, Fonseca BA, Ferreira BR, Silva JS. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. *Journal of Clinical Periodontology*. 2004;31:671-679. DOI: 10.1111/j.1600-051X.2004.00545.x

[21] Miyazaki T, Matsunaga T, Miyazaki S, Hokari S, Komoda T. Changes in receptor activator of nuclear factor kappaB, and its ligand, osteoprotegerin, bone-type alkaline phosphatase, and tartrate-resistant acid phosphatase in ovariectomized rats. *Journal of Cellular Biochemistry*. 2004;93:503-512. DOI: 10.1002/jcb.20201

[22] Graves D. Cytokines that promote periodontal tissue destruction. *Journal of Periodontology*. 2008;79:1585-1591. DOI: 10.1902/jop.2008.080183

[23] Lorenzo J, Horowitz M, Choi Y. Osteoimmunology: Interactions of the bone and immune system. *Endocrine Reviews*. 2008;29:403-440. DOI: 10.1210/er.2007-0038

[24] Sato N, Takahashi N, Suda K, Nakamura M, Yamaki M, Ninomiya T, Kobayashi Y, Takada H, Shibata K, Tamamoto M, Takeda K, Akira S, Noguchi T, Udagawa N. MyD88 but not TRIF is essential for osteoclastogenesis induced by lipopolysaccharide, diacyl lipopeptide, and IL-1a. *Journal of Experimental Medicine*. 2004;200:601-611. DOI: <https://doi.org/10.1902/jop.2008.080183>

[25] Ursarescu IG, Pavai D, Solomon SM, Pasarin L, Boatca M, Nicolaiciuc O, Nitescu DC, Martu S. Study regarding the IL1-A and IL1-B levels in gingival crevicular fluid in patients with chronic periodontitis and osteoporosis. *Romanian Journal of Oral Rehabilitation*. 2016;8:97-102.

[26] Holmlund A, Hanstrom L, Lerner UH. Bone resorbing activity and cytokine levels in gingival crevicular fluid before and after treatment of periodontal disease. *Journal of Clinical Periodontology* 2004; 31: 475-482. DOI: 10.1111/j.1600-051X.2004.00504.x

[27] Daheshia M, Yao JQ. The interleukin-1a pathway in the

- pathogenesis of osteoarthritis. *The Journal of Rheumatology*. 2008;35:2306-2312. DOI: 10.3899/jrheum.080346
- [28] Cox SW, Eley BM, Kiili M, Asikainen A, Tervahartiala T, Sorsa T. Collagen degradation by interleukin-1 α -stimulated gingival fibroblasts is accompanied by release and activation of multiple matrix metalloproteinases and cysteine proteinases. *Oral Diseases* 2006;12:34-40. DOI: 10.1111/j.1601-0825.2005.01153.x
- [29] Grodstein F, Stampfer R. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. *Progress in Cardiovascular Diseases*. 1995;38:199-121. DOI: [https://doi.org/10.1016/S0033-0620\(95\)80012-3](https://doi.org/10.1016/S0033-0620(95)80012-3)
- [30] Laza GM, Sufaru IG, Martu MA, Anton D, Mocanu R, Pasarin L, Surlin P. The role of hormone replacement therapy in patients with osteoporosis and periodontal disease. *Romanian Journal of Medical and Dental Education*. 2020;9:6-12.
- [31] Teitelbaum SL. Bone resorption by osteoclasts. *Science*. 2000;289:1504-1508. DOI: 10.1126/science.289.5484.1504
- [32] Knight ET, Liu J, Seymour GJ, Faggion CM Jr, Cullinan MP. Risk factors that may modify the innate and adaptive immune response in periodontal diseases. *Periodontology* 2000. 2016;71:22-51. DOI: 10.1111/prd.12110
- [33] Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SAA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Morley Kotchen J, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R, Wassertheil-Smoller S, Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *Journal of American Medical Association*. 2004;291:1701-1712. DOI: 10.1001/jama.291.14.1701
- [34] Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, Gower BA, Henderson VW, Jarjour WN, Karas RH, Kleerekoper M, Lobo RA, Manson JE, Marsden J, Martin KA, Martin L, Pinkerton JW, Rubinow DR, Teede H, Thiboutot DM, Utian WH. Postmenopausal hormone therapy: an Endocrine Society scientific statement. *The Journal of Clinical Endocrinology & Metabolism*. 2010;95:s1-s66. DOI: <https://doi.org/10.1210/jc.2009-2509>
- [35] Reid DM. *The Handbook of Osteoporosis*. New York; Springer; 2011. p. 19-22. DOI: 10.1007/978-1-908517-10-4
- [36] Meisel P, Reifemberger J, Haase R, Nauck M, Bandt C, Kocher T. Women are periodontally healthier than men, but why don't they have more teeth than men? *Menopause*. 2008;15:270-275. DOI: 10.1097/gme.0b013e31811ece0a
- [37] Taguchi A, Sanada M, Suei Y, Ohtsuka M, Nakamoto T, Lee K, Tsuda M, Ohama K, Tanimoto K, Bollen AM. Effect of estrogen use on tooth retention, oral bone height, and oral bone porosity in Japanese postmenopausal women. *Menopause*. 2004;11:556-562. DOI: 10.1097/01.gme.0000113845.74462.bf
- [38] Krall EA, Dawson-Hughes B, Hannan MT, Wilson PWF, Kiel DP. Postmenopausal estrogen replacement

and tooth retention. *The American Journal of Medicine*. 1997;102:536-542. DOI: [https://doi.org/10.1016/S0002-9343\(97\)00045-4](https://doi.org/10.1016/S0002-9343(97)00045-4)

[39] Golub LM, Wolff M, Lee HM, McNamara TF, Ramamurthy NS, Zambon J, Ciancio S. Further evidence that tetracyclines inhibit collagenase activity in human crevicular fluid and from other mammalian sources. *Journal of Periodontal Research*. 1985;20:12-23. DOI: 10.1111/j.1600-0765.1985.tb00405.x

[40] Golub LM, Wolff M, Roberts S, Lee HM, Leung M, Payonk GS. Treating periodontal diseases by blocking tissue-destructive enzymes. *The Journal of American Dental Association*. 1994;125:163-169. DOI: 10.14219/jada.archive.1994.0261

[41] Ursarescu I, Solomon S, Rudnic I, Potarnichie O, Martu S. Evaluation of the effects of hormonal substitution therapy upon the periodontal status in female patients during pre-and post-menopause. *International Journal of Medical Dentistry*. 2012;16:300-304.

[42] Offenbacher S, Barros SP, Beck JD. Rethinking periodontal inflammation. *Journal of Periodontology*. 2008;79:1577-1584. DOI: 10.1902/jop.2008.080220

[43] Milano S, Arcoleo F, D'Agostino P, Cillari E. Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. *Antimicrobial Agents and Chemotherapy*. 1997;41:117-121. PMID: 8980766

[44] Kornman KS, Karl EH. The effect of long-term low-dose tetracycline therapy on the subgingival microflora in refractory adult periodontitis. *Journal of Periodontology*. 1982;53: 604-610. DOI: 10.1902/jop.1982.53.10.604

[45] Caton JG, Ciancio SG, Blieden TM, Bradshaw M, Crout RJ, Hefti AF, Massaro JM, Polson AM, Thomas J, Walker C. Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planing in patients with adult periodontitis. *Journal of Periodontology*. 2000;71:521-532. DOI: 10.1034/j.1600-051x.2001.280810.x

[46] Barksby HE, Lea SR, Preshaw PM, Taylor JJ. The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. *Clinical & Experimental Immunology*. 2007;149:217-225. DOI: 10.1111/j.1365-2249.2007.03441.x

[47] Pouliot M, Clish CB, Petasis NA, Van Dyke TE, Serhan CN. Lipoxin A(4) analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry*. 2000;39:4761-4768. DOI: 10.1021/bi992551b

[48] Teng YTA. Mixed periodontal Th1-Th2 cytokine profile in *Actinobacillus actinomycetemcomitans*-specific osteoprotegerin Ligand (or RANK-L)- mediated alveolar bone destruction in vivo. *Infection and Immunity*. 2002;70:5269-5273. DOI: 10.1128/IAI.70.9.5269-5273.2002

[49] Crotti T, Smith MD, Hirsch R, Soukoulis S, Weedon H, Capone M, Ahern MJ, Haynes D. Receptor activator NF kappaB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *Journal of Periodontal Research*. 2003;38:380-387. DOI: 10.1034/j.1600-0765.2003.00615.x

[50] Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE2 secretion as a determinant of periodontal disease expression. *Journal of Periodontology*. 1993;64:432-444. DOI: 10.1902/jop.1993.64.5s.432

[51] Vardar S, Baylas H, Huseyinov A. Effects of selective cyclooxygenase-2 inhibition on gingival tissue levels of prostaglandin E2 and prostaglandin F2a and clinical parameters of chronic periodontitis. *Journal of Periodontology*. 2003;74:57-63. DOI: 10.1902/jop.2003.74.1.57

[52] Elkhoul AM. The efficacy of host response modulation therapy (omega-3 plus low-dose aspirin) as an adjunctive treatment of chronic periodontitis (clinical and biochemical study). *Journal of Periodontal Research*. 2011;46:261-268. DOI: 10.1111/j.1600-0765.2010.01336.x

[53] Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, Petasis NA, Levy BD, Serhan CN, van Dyke TE. RvE1 protects from local inflammation and osteoclast-mediated bone destruction in periodontitis. *The FASEB Journal*. 2006;20:401-403. DOI: 10.1096/fj.05-4724fje