THE ASSESSMENT OF SERUM AND GCF PROINFLAMMATORY CYTOKINES LEVELS IN PATIENTS WITH OSTEOPOROSIS AND PERIODONTAL DISEASE

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ABSTRACT

Chronic periodontitis is related to osteoporosis; TNFα has a potent resorbing activity on bone level, which affects the periodontal status. **Aim of the study** We proposed an evaluation of TNFα serum and gingival crevicular fluid levels in patients with osteoporosis and periodontal disease. **Material and methods** We assessed 64 patients, divided in two groups: osteoporotic patients and systemically healthy subjects. The patients were clinically examined and TNFα levels were measured in GCF and serum samples by ELISA methods. **Results** The clinical parameters presented higher values in the study group; also, TNFα levels were significantly elevated for the osteoporosis patients. **Discussions** It may be suggested that elevated GCF and serum TNFα contributes to high number of B cells and T cells present in the inflammatory periodontal tissues, enhancing the periodontal tissue breakdown. **Conclusions** The osteoporosis patients are prone to overproduce TNFα, which also activates the B cells and promotes the B cells activity in the periodontal inflammatory sites, aggravating the evolution of the periodontal disease.

Keywords: TNFα, osteoporosis, periodontal status, inflammation

INTRODUCTION

Periodontitis is an inflammatory disease characterized by chronic infection and inflammation in the teeth supporting tissues, causing migration of inflammatory cells to adjacent sites of periodontal pocket, resorption of the marginal alveolar bone and collagen fibrils connecting root surface to the connective tissue, apically migration of epithelial attachment.

It is well known that metabolic, hormonal and genetic factors can be risk factors for periodontal diseases, as well as chronic diseases. The alterations of sex hormones aggravate the periodontal tissue breakdown by altering host response against local irritants. Most prominent changes in hormone levels are observed in puberty, pregnancy and menopause. Hormones such as oestrogen and androgens were expressed in periodontal tissues and hormonal imbalances in postmenopausal women have been shown to affect periodontium [1].

Menopause is permanent cessation of

menstrual cycle after 12 consecutive months of amenorrhea and is also characterised with decreasing levels of estradiol (E2) as the principal circulating oestrogen [2]. In addition, osteoporosis, commonly seen in postmenopausal women, is associated with estrogen deficiency and characterized by loss of bone mass and density. Higher incidence of osteoporosis and lower bone mineral density (BMD) were reported in women with early onset of menopause [3].

Osteoporosis (OPR) is a systemic skeletal disease, where there is deterioration of the micro-architectural component of bone with an increase in bone fragility is characterized by low bone mass. OPR is considered to be present when bone mineral density (BMD) is greater than 2.5 standard deviations below the young adult mean value [4].

Epidemiologic research shows that chronic periodontitis is related to osteoporosis. Several studies have already indicated that insufficient estrogen is closely related to periodontitis and osteoporosis. An increasing number of researchers suggest that osteoporosis promotes periodontitis [5]. In a previous study we observed that the clinical and paraclinical parameters suggested a poorer periodontal status in patients with osteoporosis [6].

It has been demonstrated that periodontal bacteria promote the alveolar bone loss in periodontitis. The invasion of periodontal bacteria may reduce bone density and enhance the osteoclastic activity by releasing toxins and/or inflammatory cytokines. These cytokines believed to be involved in alveolar bone remodeling are also highly expressed in osteoporosis [7].

TNFα is a product of mononuclear phagocytes, which was initially found to be cytotoxic to tumor cells. This polypeptide molecule was isolated as a result of efforts to find an endogenous mediator of tumor necrosis. TNF is considered to play a significant role in endotoxic shock, cachexia, and tumor necrosis, In addition to affecting osteoclasts in the presence of osteoblasts, TNF also acts in an autocrine manner to stimulate osteoblast proliferation. Since TNF has a potent bone-resorbing activity and the increased bone resorption is a major factor in postmenopausal osteoporosis, an association between TNF, estrogen, and bone loss has been investigated. Estrogen was shown to inhibit TNF-induced osteoclast development in vitro [8] and TNF release in estrogen-deficient osteoporotic women ex vivo [9].

Studies in animals showed that TNF levels secreted from cultured mouse bone marrow cells, similar to IL-1 levels, increased after ovariectomy, and treatment with cytokine inhibitors, either IL-lra or TNFβp, decreased osteoclast-like cell formation and bone resorption whereas simultaneous administration of IL-lra and TNFβp produced an additive effect in decreasing osteoclast-like cell formation and bone resorption similar to estrogen treatment [10]. This illustrates the complex contribution of the various cytokines acting in concert to affect bone metabolism.

AIM OF THE STUDY

The aim of the present study was to assess the gingival crevicular fluid and serum levels of TNFα in osteoporotic patients in comparison with a systemically healthy control group, all with inflammatory periodontal disease.

MATERIAL AND METHODS

This study was conducted on a number of 46 post-menopausal female subjects, in the Periodontology Clinic of the Faculty of Dental Medicine, "Gr.T.Popa" University of Medicine and Pharmacy, Iasi.

The subjects were divided in two groups: the study group (patients with osteoporosis and periodontal disease) and the control

group (patients with periodontal disease who were systemically healthy). The smokers and patients with any other diseases or drug intake which could affect the periodontal status were excluded from the study. Other exclusion criteria were represented by the periodontal therapy in the last 12 months and antibiotherapy in the last 6 months.

The methodology of the study respected the principles stated in the Declaration of Helsinki; every subject was informed regarding the methods and the purpose of the present study and a signed informed consent was obtained from every patient.

The methodology of our study included two major aspects: clinical methods of investigation and the paraclinical assessment of the TNF-α.

The clinical step of evaluation was represented by the periodontal examination of the patient (probing depths, clinical attachment loss, gingival bleeding on probing); the plaque index was also registered.

The paraclinical step of the study comprised the analysis in gingival crevicular fluid (GCF) and serum of the TNF-α levels.

Gingival crevicular fluid (GCF) samples were obtained from buccal aspects of four interproximal sites (pockets deeper than 5mm); after the supragingival plaque was removed carefully by sterile curettes, the surfaces were dried and isolated by cotton rolls. Filter paper strips were placed in the gingival sulcus/pocket for 30s, taking care to avoid mechanical trauma. The strips contaminated with blood were discarded. Then, the four strips from each patient were placed into two polypropylene tubes, thus pooled to make two samples and immediately frozen at -400C until the laboratory analyses. The Periotron readings were converted to an actual volume of GCF (ml) by reference to the standard curve.

Five millilitres of venous blood were taken from antecubital vein using blood collection tubes. The blood was allowed to clot for 30 min on ice and centrifuged for 10 min at 3000 rpm before removing the serum into polypropylene tubes for freezing in 0.5 ml aliquots and storage at -400C until the biochemical analysis.

For the laboratory assessment of the GCF and serum samples we used the Enzyme-linked Immunosorbent Assay (ELISA) method. The results were expressed in pg/ml and the minimum sensitivity for each assay was of 1.5< TNFα <3pg/ml.

The obtained data were registered and statistically analysed. For the statistical analysis we used the Microsoft Excel and PASW 18 Statistics software.

RESULTS

We examined a number of 46 patients with periodontal disease, divided in two groups: study group – patients with osteoporosis (n=24) and control group – systemically healthy patients (n=22).

The median age and range age of the study group and control group were 58 (50-68 years old) and 51 (41-66), respectively.

Table 1. Clinical parameters in the study and control groups

Parameter	Study Group (n=24)	Control Group (n=22)
PD (mm)	3.17±1.16	2.38±0.47
CAL (mm)	3.95±1.93	2.64±0.67
BOP (%)	84±11	65±35
PI (%)	88±5	89±1

The values are expressed as median value ± Standard Deviation (SD)
PD: probing depth; CAL: clinical attachment loss; BOP: bleeding on probing index; PI: Plaque Index

Table 2. The gingival crevicular fluid and serum TNFα values in the study and control groups

Parameter	Study group (n=24)	Control group (n=22)
Serum TNFα concentration (pg/ml)	113.5±86.8	9.6±4.2
GCF TNFα level (pg)	14.6±3.2	7.2±1.3
GCF TNFα concentration (pg/ml)	25.7±10.3	6.3±3.5

The values are expressed as median value ± Standard Deviation (SD)
GCF: gingival crevicular fluid

Probing depth (PD), bleeding on probing (BOP) and clinical attachment loss (CAL) values were significantly higher in the study group than in the control group ($p<0.05$). We could not observe significant differences regarding the plaque index values between groups (Table 1).

All samples had detectable levels of TNFα. Significant higher levels of TNFα were detected both in serum and GCF for the study group when compared to the control group (Table 2).

Serum TNFα correlated positively with BOP ($p<0.01$). There were no significant correlations between probing depth, clinical attachment loss, plaque index and the TNFα levels. Serum TNFα levels correlated with GCF TNFα levels.

DISCUSSIONS

The possible association between menopause and periodontal bone loss has been discussed in many studies [11, 12]; however the effects of menopause on alveolar bone resorption are still not clarified. Some of the studies suggested that menopause and menopause related systemic conditions may induce increased alveolar bone resorption, periodontal attachment and tooth loss; in contrast some authors suggested that there is no such a relationship [13].

The role of TNF-a has been investigated in numerous studies focusing on periodontal disease [14, 15] but to the best of our knowledge, there are no studies to assess the GCF and serum TNFα levels in patients with osteoporosis and periodontal disease.

The mechanisms involved in the influence that osteoporosis exerts on the periodontal status are still unclear. Since estrogen inhibits the expression of the inflammatory cytokines, it might be that larger amounts of these cytokines are presented in an inflammatory alveolar bone with estrogen deficiency. Therefore, estrogen deficiency may contribute to the alveolar bone absorption in periodontal disease, either by reducing the bone mass of alveolar bone or by causing increased expression of inflammatory cytokines. We have demonstrated in a previous study that, moreover, the substitution therapy with estrogen in menopausal women determined an improved periodontal status [16].

Maintaining the balance of proinflammatory and anti-inflammatory cytokines in the body is one of the manifestations of self-regulation [17]. During the past decade, considerable evidence suggests that estrogen prevents bone loss by blocking the production of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-10, tumor necrosis factor- (TNF-) α in bone marrow and bone cells. Cytokines are soluble proteins which 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 [18]. Among the proinflammatory, TNF-α HAS been reported to present fundamental role in periodontal bone destruction [19].

Elevated proinflammatory cytokines in the periodontal microenvironment increase the number of osteoclasts by promoting osteoclast precursors to differentiate into osteoclasts and extending the lifespan of osteoclasts. Estrogen blocks bone loss by blocking the production of proinflammatory cytokines in the bone marrow, bone cells, and periodontal ligaments. IL-1β and TNF-α are potent promoters of bone resorption and inhibitors of bone formation, and IL-6 promotes the differentiation of osteoclast precursors into osteoclast and MMP production [20].

TNF-α is among the upstream cytokines that are key factors that induce the production and secretion of downstream cytokines, and their slight upregulation leads to significantly higher expression of downstream cytokines such as IL-6.

The main consequence of increased cytokine production in the bone microenvironment is expansion of the osteoclastic pool because of increased osteoclast formation and their extended lifespan.

In our study TNFα clearly showed great differences between the osteoporosis group and the control group. It may be suggested that elevated GCF and serum TNFα contributes to high number of B cells and T cells present in the inflammatory periodontal tissues, enhancing the periodontal tissue breakdown.

Further studies on other cytokine types are necessary to establish a clear inflammatory status in patients with osteoporosis and its effect on the periodontal tissues.

CONCLUSIONS

The periodontal disease patients with osteoporosis exerted higher levels of TNFα in the gingival crevicular fluid and serum when compared to the systemically healthy patients. Our data suggest that these patients are prone to overproduce this type of cytokine which also activates the B cells and promotes the B cells activity in the periodontal inflammatory sites, aggravating the evolution of the periodontal disease.

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REFERENCES

1 Mascarenhas P, Gapski R, Al-Shammari K, Wang HL. Influence of sex hormones on the periodontium. Journal of Clinical Periodontology 2003, 30:671–681.
2 Liu JM, Zhao HY, Ning G, Zhao YJ, Chen Y, Zhang Z, et al. Relationships between the changes of serum levels of OPG and RANKL with age, menopause, bone biochemical markers and bone mineral density in Chinese women aged 20–75. Calcified Tissue International 2005, 76:1–6.
3 Basu S, Michaelsson K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. Biochemical and Biophysical Research Communications, 2001, 288:275–279.
4 Lofman O, Larsson L, Toss G. Bone mineral density in diagnosis of osteoporosis: reference population, definition of peak bone mass, and measured site determine prevalence. Journal of Clinical Densitometry, 2000, 3:2:177–186.
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, 78:11:2104–2111.
6 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 Reabilitation, 2012, 4:4:53-57.
7 Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. The Lancet, 2005, 366:9499:1809–1820.
8 Girasole G, Jilka RL, Passed G, Boswell S, Boder G, Williams DC, Manolagas SC. Np-estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts in vitro: a potential mechanism for the antiosteoporotic effect of estrogens. J Clin Invest, 1992, 89:883-891.
9 Ralston SH, Russell RGG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. J Bone Min Res, 1990, 5:983-988.
10 Kimble RB, Vannice JL, Bleodow DC, Thompson RC, Hopfer W, Kung VT, Brownfield C, Pacifici, R. Interleukin-1 receptor antagonist decreases bone loss and bone resorption in ovariectomized rats. J Clin Invest, 1994, 93:1959-1967.
11 Bullon P, Goberna B, Guerrero JM, Segura JJ, Perez-Cano R, Martinez-Sahuquillo A. Serum, saliva, and gingival crevicular fluid osteocalcin: their relation to periodontal status and bone mineral density in postmenopausal women. Journal of Periodontology, 2005, 76:513–9.
12 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.
13 Kribbs PJ. Comparison of mandibular bone in normal and osteoporotic women. The Journal of Prosthetic Dentistry, 1990, 63:218–222.
14 Tervahartiala T, Koski H, Xu JW, et al. Tumor necrosis factor-alpha and its receptors, p55 and p75, in gingiva of adult periodontitis. Journal of Dental Research, 2001, 80:7:1535–1539.
15 Bostrom L, Linder LE, Bergstrom J. Smoking and crevicular fluid levels of IL-6 and TNF-a in periodontal disease. Journal of Clinical Periodontology, 1999, 26:8:352–357.
16 Ursarescu I, Solomon S, Potarnichie O, Rudnic I, 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, 16:4:300-304.
17 Garlet GP, Martins W Jr., Fonseca BAL, 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, 31:8:671–679.
18 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, 93:3:503–512.
19 Graves D. Cytokines that promote periodontal tissue destruction. Journal of Periodontology, 2008, 79:8:1585–1591.
20 McLean RR. Proinflammatory cytokines and osteoporosis. Current Osteoporosis Reports, 2009, 7:4:134–139.