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Short communication

Inflammatory cytokines in Paget's disease of bone

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ABSTRACT

This study was undertaken to evaluate the expression of inflammatory cytokines in patients with Paget's disease of bone (PDB). Serum levels of tumoral necrosis factor-α, interleukin 1β, interleukin-6 and interleukin-17 were measured in 51 patients with PDB and in 24 controls with primary osteoarthritis. Compared to controls, patients with Paget's disease of bone presented higher levels of interleukin 6 and reduced interleukin 17, but levels of tumoral necrosis factor α and interleukin 1 β did not differ significantly. We found no significant differences when patients were compared according to disease activity or current treatment. There were no correlations between cytokine levels and bone-specific alkaline phosphatase or extension of Paget's disease of bone on bone scintigraphs. In conclusion, patients with PDB present significant differences on levels of certain cytokines in comparison to primary osteoarthritis patients, but these alterations did not appear to have a clear correlation with parameters of disease activity or severity.

1. Introduction

Paget's disease of bone (PDB) is a common osteometabolic disease characterized by increased and disorganized bone turnover. It is usually asymptomatic, but some patients may present bone pain, fractures, deformities, secondary osteoarthritis, neurologic and cardiac complications and, in rare cases, neoplasm. It is believed that PDB is caused by alterations in osteoclasts behavior, since pagetic bone is rich in overactive osteoclasts and medications that act in these cells, as bisphosphonates, are very effective in PDB [1,2].

PDB has a multifactorial etiology, genetic factors are clearly important, about 15% of patients have a familial history, but environmental factors, like viral infections [1,3], are also believed to play a role. The most frequent genetic mutation associated with this disease is located on sequestosome 1 (p62) gene, that is carried by 10–50% of patients with familial PDB and by 5–30% of those with sporadic disease[1]. Sequestosome 1 is involved in signal transduction of many cytokines, including receptor activator of nuclear factor kappa-B ligand (RANKL), tumoral necrosis factor (TNF) α, nerve growth factor (NGF) and interleukin(IL) 1 β, besides being involved in autophagy and apoptosis. Other single nucleotide polymorphisms have been described to be associated to PDB, most of them in proteins involved in osteoclast differentiation and activation (but also important in other cells), such as stimulating factor 1 (CSF-1) and tumor necrosis factor receptor superfamily, member 11a (TNFRSF11A). [4,3,5].

As genetic mutations related to PDB occur in proteins that are also important in immune cells and in signaling cascades of inflammatory cytokines, alterations in cytokine levels would be expected to occur in PDB patients. Moreover, osteoblasts and osteoclasts are also able to secrete inflammatory cytokines, such as IL-6. In fact, high IL-6 levels were reported in plasma and in affected bone from PDB patients [6,7], although these findings were not replicated by some small studies [8,9]. High IL-1 levels were also reported in osteoblasts from pagetic bone [10] and in bone marrow monoclonal cells from PDB patients [11].

This study was undertaken to compare serum levels of the inflammatory cytokines TNF α, IL-1 β, IL-6 and IL-17 in PDB patients and in patients without systemic inflammatory diseases and to identify factors associated with any detected differences.

2. Methods

Consecutive patients with PDB followed by rheumatologists in Florianopolis, Brazil, were included after signing an informed consent term. Patients with primary osteoarthritis followed at the Rheumatology Outpatient Clinic of Hospital Governador Celso Ramos from the same age group (within up to 5 year difference from a PDB patient) and matched for sex were recruited as controls. Patients with osteoarthritis were chosen as controls because of the high prevalence of this disease in the age group affected by PDB; most patients with PDB have concomitant primary osteoarthritis. For both, patients and controls, exclusion criteria were: systemic inflammatory diseases, active infections, neoplasms, plurimetabolic syndrome, erosive osteoarthritis and current

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use of any of the following drugs: non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, immunosuppressants, anti-cytokine agents and any medication for osteoporosis, with exception of bisphosphonates, calcitonin, calcium and vitamin D.

PDB and osteoarthritis were diagnosed by typical findings on X-rays. Disease activity was evaluated by 99mTc MDP bone scintigraphs; a patient was considered to have active disease when bone scintigraph shows high uptake suggestive of PDB and other possible diagnosis was excluded by X-rays or other image techniques. All high uptake areas were evaluated by X-rays or computed tomography. Disease extension was determined by previous and recent X-rays and bone scintigraphs. The method described by Meunier et al. [12] was used to calculate disease extension on bone scintigraphs. A patient was considered to be at current treatment if he had used oral bisphosphonates (alendronate, risedronate or ibandronate) in the past 6 months or zoledronic acid in the past 12 months. No patient has taken calcitonin, intravenous ibandronate or pamidronate in the past 12 months.

Fasting blood samples were collected from patients and controls with primary osteoarthritis for determination, by enzyme-linked immunosorbent assay, of serum levels of: TNF α (BD Biosciences, San Jose, CA, ref. 550610), and IL- 1 β (BD Biosciences, San Jose, CA, ref. 557966), IL-6 (BD Biosciences, San Jose, CA, ref. 550799) and IL-17(Raybiotech Inc., Norcross, GA, ref. ELH-IL17-001). Bone specific alkaline phosphatase (BAP) (Mybiosource, San Diego, CA, ref. MBS724100) was also measured in serum of PDB patients. Intra- and inter-assays coefficients of variation were: TNF-α: 4.90 ± 3.67% and 8.83 ± 6.13%, IL-1β = 2.01 ± 2.80% and 4.00 ± 4.5, IL-6 = 7.7 ± 6.70% and 7.70 ± 9.37%, IL-17 <10% and <12% and BAP <9% and <10%. For these analyses, serum levels of cytokines in PDB patients were compared to the results of controls with primary osteoarthritis. In order to identify factors possibly associated to cytokine alterations, PDB patients were further subdivided according to disease activity and treatment status. We also searched for correlations between serum cytokines and disease extension and BAP levels. Patients who received treatment with zoledronic acid during the study period were submitted to a new blood collection 3 months after the infusion.

Results are presented as mean (standard deviation - SD) or percentage (95% confidence interval). Comparisons between groups were done with two-tailed Student's *t*-test. Correlations between linear variables were analyzed by linear regression. Repeated measures were analyzed with paired samples *t*-test. Statistical analysis was performed with SPSS 18.0, with a level of significance of 0.05.

Study protocol was approved by local ethical committee (protocol number 353461). This study was conducted in accordance to the principles of the Declaration of Helsinki ("WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects," n.d.).

3. Results

Fifty-one patients (mean age 66.9 ± 9.59 years), 75% females (CI 95% 63.3–87.8) and 24 subjects with primary osteoarthritis (mean age 62.42 ± 6.97 years), 83.3% females (CI 95% 62.7–86.3) were included. Patients had a mean time from diagnosis of 10.45 ± 15.86 years; 55.1% (CI 95% 40.8–69.4) had a history of symptoms related to PDB, 28.6% (CI 95% 16.3–42.9) had monostotic disease and 44.9% (CI 95% 40.8–69.3) were considered to be in current treatment for PDB. Familial history of PDB and presence of sequestosome 1 gene mutations were both detected in 26.5% (CI 95% 14.3 – 39.2) of patients, 38.5% of patients with familial PDB carried sequestosome 1 gene mutations.

There were no statistically significant differences between patients and controls with primary osteoarthritis in serum levels of TNF α (85.68 ± 34.41 pg/ml vs 75.60 ± 34.04 pg/ml, $p = 0.31$) and IL-1 (5.05 ± 2.94 pg/ml vs 4.43 ± 2.80 pg/ml, $p = 0.391$). Patients with PDB presented higher serum levels of IL-6 (108.06 ± 71.07 pg/ml vs 72.03 ± 36.33 pg/ml, $p = 0.024$), while controls with primary osteoarthritis controls presented higher levels of IL-17 (78.56 ± 59.85 pg/ml vs 115.97 ± 56.70 pg/ml $p = 0.012$) (Fig. 1). Because cytokine levels could differ between patients with active and inactive disease, we compared these groups with controls, but this approach revealed a similar pattern to the comparison between controls and the whole group of patients (Table 1).

In order to investigate if cytokine levels were influenced by PDB activity, we compared subgroups of patients divided according to disease activity and to current treatment. We also searched for correlations between cytokine levels and disease extension and BAP levels. When patients were compared according to the presence of areas of high uptake on bone scintigraphs, the only statistically significant difference between those with active (n 23) and inactive disease (n 28) was BAP levels ($p = 0.017$) (Table 2). There were no statistically significant differences in cytokine levels between patients in current treatment (n 23) and patients without current treatment (n 28), although levels of IL-6 were lower in patients in current treatment, this difference did not achieve statistical significance ($p = 0.053$) (Table 2). There were no significant correlations between BAP and cytokine levels (TNF α: $r = 0.053$ p 0.713, IL-1 β: $r = 0.134$ p 0.349, IL-6: $r = 0.09$ p 0.532, IL-17: $r = 0.209$ p 0.142) or between disease extension and cytokine levels (TNF α: $r = 0.179$ p 0.204, IL-1 β: $r = 0.308$ p 0.027, IL-6: $r = 0.152$ p 0.283, IL-17: $r = 0.265$ p 0.058), but BAP levels were significantly correlated to disease extension ($r = 0.515$, $p < 0.001$).

During the study, seven patients with active PDB were treated with zoledronic acid 5 mg and had their cytokines and BAP levels measured before and 3 months after treatment. This interval was chosen to avoid acute inflammatory reactions that can occur soon after infusions of zoledronic acid. After the treatment, there were reductions in values of TNF α, IL-1 β, IL-6 and BAP and increasing of IL-17 levels (TNF α: 71.00 ± 31.42 pg/ml vs 51.33 ± 31.49 pg/ml, IL-1 β: 6.73 ± 3.6 pg/ml vs 3.39 ± 2.94 pg/ml, IL-6: 159.97 ± 56.80 pg/ml vs 78.04 ± 47.11 pg/ml, IL-17: 89.60 ± 83.62 pg/ml vs 200.51 ± 54.70 pg/ml, BAP 37.06 ± 17.34 U/l vs 16.71 ± .98 U/l) (Fig. 2).

4. Discussion

Our results demonstrated that patients with PDB present higher levels of IL-6, but lower levels of IL-17 in comparison to subjects with primary osteoarthritis. There were no significant differences in levels of TNF α or IL-1 β. These results are not correlated to disease activity determined by bone scintigraphs and there were no correlations between cytokine levels and BAP or extent of areas of high uptake on bone scintigraph. Interestingly, treatment of PDB with zoledronic acid resulted in reductions in serum levels of TNF α, IL-1 β, IL-6 and in increate in IL-17 levels.

Literature regarding inflammatory cytokines in PDB is scarce. There is one published study that reported higher levels of serum IL-6 in pagetic patients [6] and two other studies with opposite results, but their samples were smaller than ours [8,9]. Besides the present one, only one other study has reported serum levels of IL-1 β and TNF α [13], in a small number of PDB patients they were not able to demonstrate differences in serum levels of these cytokines in comparison to controls. To the best of our knowledge, there are no previous studies of serum levels of IL-17 in this population.

Increased serum IL-6 in PDB patients could be produced by the pagetic bone itself, since IL-6 was demonstrated to be overexpressed in osteoblasts and osteoclasts from involved bone, or by inflammatory cells, which function could be altered by the same genetic mutations that predispose to PDB. The last hypothesis seems attractive, as serum levels of IL-6 or IL-17 were not correlated to either BAP levels or extent of affected bone. However, treatment with zoledronic acid resulted in marked reduction in serum levels of IL-6, IL-1 β and TNF α and in increase in IL-17 levels. This signalizes that the source of cytokine alterations should be bony tissue, the main target of bisphosphonates. A possible hypothesis to explain these apparently contradictory findings