Clinical Biochemistry 44 (2011) 203–207

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

The serum levels of Receptor Activator of Nuclear Factor-κB Ligand, bone-specific alkaline phosphatase, osteocalcin and osteoprotegerin do not correlate with the radiographically assessed severity of idiopathic hip and knee osteoarthritis

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ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form 24 September 2010
Accepted 29 September 2010
Available online 14 October 2010

Keywords:
Bone turnover markers
Receptor Activator of Nuclear Factor-κB Ligand
Bone-specific alkaline phosphatase
Osteocalcin
Osteoprotegerin
Hip osteoarthritis
Knee osteoarthritis

ABSTRACT

Objectives: Determination of the serum levels of Receptor Activator of Nuclear Factor-Kb Ligand, bone-specific alkaline phosphatase, osteocalcin and osteoprotegerin in patients suffering from osteoarthritis of varying severity and healthy controls and correlation of these results with the patients' age and the radiographically assessed severity of the disease.

Design and methods: Patients suffering from hip ($n=58$) or knee ($n=117$) osteoarthritis and matched controls ($n=19$) were enrolled in this study. Patients underwent physical examination and standard radiographic evaluation before blood sampling.

Results: The serum levels of osteoprotegerin were positively correlated with age in all groups, whereas those of osteocalcin in the 'knee' group only. Osteoarthritis' severity and location did not have a statistically significant impact on the mean serum level of any marker in both groups.

Conclusions: Based on our results, none of the studied markers can serve as a surrogate for radiographic imaging in patients suffering from hip and knee osteoarthritis.

Introduction

Osteoarthritis (OA) is a degenerative joint disease commonly affecting the distal interphalangeal joints, the knees, the hips and the spine of more than 70% of the population aged over 65 years[1]. Despite the high prevalence of OA, its precise etiopathogenesis is not yet completely understood [2]. Most OA joints are characterized by softening and disintegration of articular cartilage, subchondral bone remodelling and increased new bone formation, either in the form of osteophytes or subchondral bone sclerosis [3]. The dynamic morphological changes occurring in the subchondral bone during OA changes are associated with abnormal local biochemical pathways that are related to the altered osteoblast metabolism that is manifested in the OA tissue [4]. Human OA subchondral bone osteoblasts have also demonstrated abnormal phenotypes, including elevated alkaline phosphatase activity and increased release of osteocalcin [5].

Osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor-κB Ligand (RANKL) have been found to be expressed and modulated in OA subchondral bone [6–8]. It was recently demonstrated that during longstanding OA and rheumatoid arthritis, the OPG/RANKL ratio in the synovial fluid is much more elevated in OA compared with rheumatoid arthritis [9]. Gene expression studies in femoral neck have also implicated these molecules in the pathogenesis of hip OA [10]. Moreover, OPG and RANKL have been correlated with the severity of the disease in patients with knee OA [11]. Nevertheless, available information concerning the possible implication of these bone-turnover markers in the etiopathogenesis and severity of OA remains limited.

The aim of this study was to evaluate the serum levels of OPG, RANKL, osteocalcin, and bone-specific alkaline phosphatase (b-ALP) in patients suffering from hip and knee OA of varying severity and matched healthy controls and to correlate these results with the severity of the disease and the age of the patients.

Materials and methods

This cross-sectional comparative study was approved by our Institution's Review Board and was conducted in accordance with the World Medical Association Declaration of Helsinki of 1975, as revised

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0009-9120/$ – see front matter
doi:10.1016/j.clinbiochem.2010.09.029

in 2000. After the patients were fully informed, they consented that data concerning their cases could be submitted for publication and gave informed consent [12].

Male and female patients suffering from idiopathic unilateral knee or hip OA and matched controls, who underwent examination at the OA Clinic of our Department between March 2007 and February 2009, were enrolled in this study. Patients suffering from any endocrine disorder, rheumatoid or other secondary arthritis, metabolic bone disorders or any other disease which could interfere with bone homeostasis as well as patients receiving medication affecting bone metabolism, were excluded. None had suffered any fracture or underwent any orthopaedic operation during the 36 months prior to their enrolment.

Before their enrolment, all patients underwent physical examination and standard radiographic evaluation radiographs (anteroposterior and lateral standing radiograph of the affected knee in patients suffering from knee OA and anteroposterior radiograph of the pelvis with the patient in the standing position and a frog-lateral non-weight-bearing radiograph of the hip under examination in patients suffering from hip OA). All radiographs were reviewed and graded, based on the classification scheme of Kellgren and Lawrence [13], by two independent orthopaedic surgeons who were unaware of the aim of the study.

Following radiographic evaluation and enrolment, blood samples were obtained from each patient and the serum levels of RANKL, OPG, osteocalcin and b-ALP were determined. Whole peripheral blood was collected into Vacutainer tubes (Becton Dickinson, LePont de Claix, France), centrifuged at 400 $\times g$ for 10 min at 4 °C, and was frozen at −70 °C until the assay was performed. All blood samples were collected in the morning (from 8 to 10 a.m.) in order to diminish the effect of diurnal variation of biochemical markers.

The serum level of OPG was assayed by a commercial ELISA sandwich (DRG International Inc., USA). The mean study value with serum samples from young healthy donors has been established by the manufacturer at 4.1 ± 0.33 pmol/L. Observed intra-assay and inter-assay variation Coefficient of Variation (CV, %) was 5% and 6% respectively. The limit of detection was 0.1 pmol/L. An enzyme immunoassay (Demeditec Diagnostics GmbH, Kiel, Germany) was used for the detection of soluble, non-complexed human RANKL directly in biological fluids. Its detection limit was 0.1 pmol/L, the intra-assay CV 4.5–7% and the inter-assay CV 6–8%. Enzyme immunoassay (Ostase BAP EIA, Immunodiagnostic Systems IDS Ltd., Boldon, U.K.) was used for the quantitative measurement of b-ALP with detection limit <1 μg/L and inter and intra-assay CV% lower than 10%. Osteocalcin was assessed by radioimmunoassay (Myria RIA kit, Technogenetics, Milan, Italy) (Range: 0–60 ng/mL (0.172 nmol/L); Sensitivity: 0.30 ng/mL). All samples from the same subject were evaluated in duplicate in the same assay.

Statistical analysis

The determination of the samples' necessary size was made according to the serum levels of biochemical markers as reported in previous studies [11]. Taking into consideration the fact that the mean difference in the serum levels of biochemical markers between patients and controls in previous studies [11] ranged from 0.5 to 1.5 pmol/L, our statistical analysis showed that with a sufficient power of 0.9 and α value of 0.05, in order to see a difference of 0.5 pmol/L between equivalent groups with a standard deviation of 0.5 pmol/L, at least 18 patients had to be enrolled in each of the groups of the study. Standard statistical methods were used for descriptive statistics. The normality of the data distribution among different groups was tested according to the Kolmogorov–Smirnov and Shapiro–Wilk tests. All statistical tests were two-tailed. The alpha level for all analyses was set at 0.05. All analyses were performed with the SPSS statistical software (Version 12, Chicago-IL). Inter-observer reliability was determined by means of k coefficient.

Spearman or Pearson Correlation Coefficient was used to determine the correlation between the age and the serum level of different biochemical markers. The One-Way ANOVA test was used to determine the significance of the differences found in the mean serum level of biochemical markers among healthy controls and patients belonging to each of the four different levels of severity of hip or knee OA according to the Kellgren and Lawrence classification. Post-hoc analyses were used to determine the significance of the differences found in the mean serum level of OPG among patients belonging to each of the four groups of OA severity and healthy control subjects. The One-Way ANOVA test was also used to determine the significance of the differences found in the mean age and x^2 test differences in sex proportion, among healthy controls and patients suffering from different stages of knee or hip OA according to the Kellgren and Lawrence classification system. A two-way between groups' analysis of variance was conducted to explore the impact of the type and severity of OA on the level of each biochemical marker.

Results

A total of 194 patients (control group: 19, knee group: 117, and hip group: 58) were enrolled in the study (Table 1).Healthy controls were age- and sex-matched to the patients belonging to each of the four different levels of severity of hip and knee OA according to the Kellgren and Lawrence classification (Table 1). No significant inter-observer variability was detected between the two Orthopaedic surgeons (k coefficient = 0.77) as far as the grading of OA changes according to Kellgren and Lawrence classification was concerned [13].

The serum level of OPG was highly and positively correlated with the age of the patients in all three groups (Table 2). The serum level of osteocalcin was also positively correlated with the age of the patients

Table 1
Baseline characteristics of the patients that were enrolled in each group.

		Severity of osteoarthritis[a]					
		Control group	Grade 1	Grade 2	Grade 3	Grade 4	p
Number[b]		19 (9.8)	37 (19)	60 (30.9)	61 (31.5)	17 (8.8)	–
Age(years)[c]		70.5(11.1)	70.5(7.2)	70.6(6.6)	71.2(7.0)	69.5(13.1)	0.956[d]
Sex[b]	Women	17	31	46	49	15	0.604[e]
	Men	2	6	14	12	2	
Osteoarthritis group[b]	Knee	–	32(27.2)	49(41.7)	31(26.4)	5(4.7)	
	Hip	–	5(8.6)	11(19)	30(51.7)	12(20.7)	

[a] According to Kellgren–Lawrence classification system [12].
[b] The values are given as raw numbers with the percentages in parentheses.
[c] The values are given as the mean with the standard deviation in parentheses.
[d] Tests performed using One-Way ANOVA test.
[e] Tests performed using Chi-square (x^2) test.