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.

Table 2
Correlation of the serum level of osteocalcin, bone-specific alkaline phosphatase, osteoprotegerin, Receptor Activator of Nuclear Factor-κB Ligand (RANKL) and the ratio of osteoprotegerin/RANKL with the age of subjects for each different group of osteoarthritis and control group.

Biochemical marker	Osteoarthritis group	Correlation coefficient (r)	p
Osteocalcin	Knee[a]	0.218	0.02
	Hip[a]	−0.46	0.739
	Control group	0.202	0.407
Bone-specific alkaline phosphatase	Knee[b]	0.131	0.169
	Hip[a]	0.104	0.454
	Control group	0.291	0.213
Osteoprotegerin	Knee[a]	0.376	<0.001
	Hip[a]	0.425	<0.001
	Control group	0.66	0.003
Receptor Activator of Nuclear Factor-κB Ligand	Knee[a]	−0.05	0.636
	Hip[a]	0.015	0.921
	Control group	0.072	0.798
Osteoprotegerin/RANKL	Knee[a]	0.181	0.079
	Hip[b]	0.197	0.176
	Control group[b]	0.181	0.519

[a] Tests performed using the Pearson Correlation Coefficient.
[b] Tests performed using the Spearmen Correlation Coefficient.

in the group of knee OA only ($r = 0.218$, $p = 0.02$). The level of OPG/RANKL ratio showed a tendency towards significance when correlated to the age of the patients of the knee OA group. No other correlation was found between any serum marker and the age of the patients in any group (Table 2).

With the exception of the serum levels of OPG in the group of patients suffering from knee OA, where a tendency towards significance was determined ($F = 2.253$, $p = 0.067$), no statistically significant difference was found in the mean serum level of biochemical markers between the healthy controls and patients belonging to each of the four different levels of (the radiographically assessed) hip and knee OA severity (Table 3). However, post-hoc comparisons using the Tukey HSD test did not indicate the existence of statistically significant differences in the level of serum OPG when we compared the results of any two out of the healthy controls and the four different groups of knee OA severity.

The location of OA (knee or hip) did not have a statistically significant impact on the serum level of any marker either (Fig. 1).

Discussion

The current golden standard when evaluating patients suffering from OA is the plain radiograph [14], which nonetheless has many methodological shortcomings [14,15]. The clinical significance of the serum levels of biochemical bone-turnover markers is well recognized, at least in several diseases affecting the metabolism of bone [16–19]. However, their significance (if any) remains still unknown in the setting of OA diagnosis. Whether the biochemical markers can be accurately used in order to assess the severity of OA of different joints is also not known.

Although the precise aetiology of OA remains yet unknown, certain biochemical factors are likely to play an important role in its pathogenesis [11]. As a result, the idea to use biochemical bone-turnover markers in order to both diagnose and follow the course of OA is tempting and challenging. Recent studies have shown that novel molecules which are implicated in the bone remodelling process such as the Receptor Activator of Nuclear Factor-κB (RANK), its ligand (RANKL) and OPG (the decoy receptor of RANKL) are expressed and modulated in human OA subchondral bone, a region of great importance for the pathogenesis of OA [2]. These molecules have been found to be expressed by other articular cells as well, such as the cartilage cells [6]. Furthermore the RANKL/OPG system is gradually becoming an important marker of the progression of inflammatory joint diseases and mainly of the rheumatoid arthritis [20,21]. The Human OA subchondral bone osteoblasts have also demonstrated abnormal phenotypes, including elevated alkaline phosphatase activity and increased release of osteocalcin [5]. Takemura et al. demonstrated that the concentration of OPG in the synovial fluid of women with knee OA increased with the severity of the disease and it was significantly higher in individuals with OA of grade IV than in those with OA of grade 0 or grade 1. This was considered as a compensatory protective mechanism of the articular cartilage rather than the actual cause of OA [22]. Skoumal et al. reported that in patients with longstanding OA and rheumatoid arthritis, the OPG/RANKL ratio in the synovial fluid is much more elevated in patients with OA than in patients with rheumatoid arthritis [9]. In a study exploring gene expression in patients with OA and osteoporosis, it was demonstrated that the balance between OPG and RANKL is involved in the aitiopathogenesis of hip OA, 'favouring' the serum levels of OPG in hip OA and of RANKL in osteoporosis [10].

In the only published study correlating the serum levels of OPG and RANKL with the radiographic severity of knee OA, Pilichou et al. [11] suggested that patients suffering from OA have increased serum levels of OPG and RANKL in comparison with a healthy group of patients of the same age and reported a correlation of the OPG/RANKL ratio with the severity of the disease, demonstrating a systemic effect of these molecules. However the study group was small and the authors suggested a larger cohort studies for confirmation of their results. Our results confirmed that the serum levels of OPG are highly and positively correlated with the age of the patients in all groups (knee, hip and control) (Table 2). This correlation has been demonstrated before and it was suggested that the production of OPG may rise with age along with an increase in bone-turnover, probably as a homeostatic mechanism to limit bone loss [17]. The serum level of osteocalcin was also positively and significantly correlated with the age of the patients in the group of knee OA (Table 2). To the best of our knowledge this is the first time that this correlation of osteocalcin is reported. No other biochemical marker was correlated with the age of the patients. Pilichou et al. [11] reported increased serum levels of RANKL in patients suffering from knee OA. Furthermore, Uemura et al. in a recent study demonstrated that the serum levels of RANKL had a positive correlation with the age in a group of postmenopausal women [23]. However, in our study the serum levels of RANKL did not significantly correlate with the age and the severity of the osteoarthritic changes in all three groups.

This study tried to correlate the serum levels of four biochemical markers with the radiographically assessed severity of knee and hip OA as well as of healthy individuals. Based on our results, there is no relation between the radiographically assessed severity of knee and

Table 3
One-Way ANOVA test determining the significance of the differences found in the serum level of biochemical markers among patients belonging to the control group and each of the four different levels of hip and knee OA severity according to Kellgren and Lawrence classification [12] for each group of osteoarthritis.

Biochemical marker	Osteoarthritis and control group[a]	F	p
Osteocalcin	Knee/control	1.568	0.187
	Hip/control	0.943	0.444
Bone-specific alkaline phosphatase	Knee/control	0.803	0.526
	Hip/control	1.287	0.287
Osteoprotegerin	Knee/control	2.253	0.067
	Hip/control	1.844	0.130
Receptor Activator of Nuclear Factor-κB Ligand	Knee/control	0.274	0.894
	Hip/control	0.838	0.507
OPG/RANKL	Knee/control	0.893	0.275
	Hip/control	0.077	0.989

[a] Test was performed between five groups each time. These groups were the control and the four groups of severity of osteoarthritis for every location (hip or knee) of osteoarthritis.

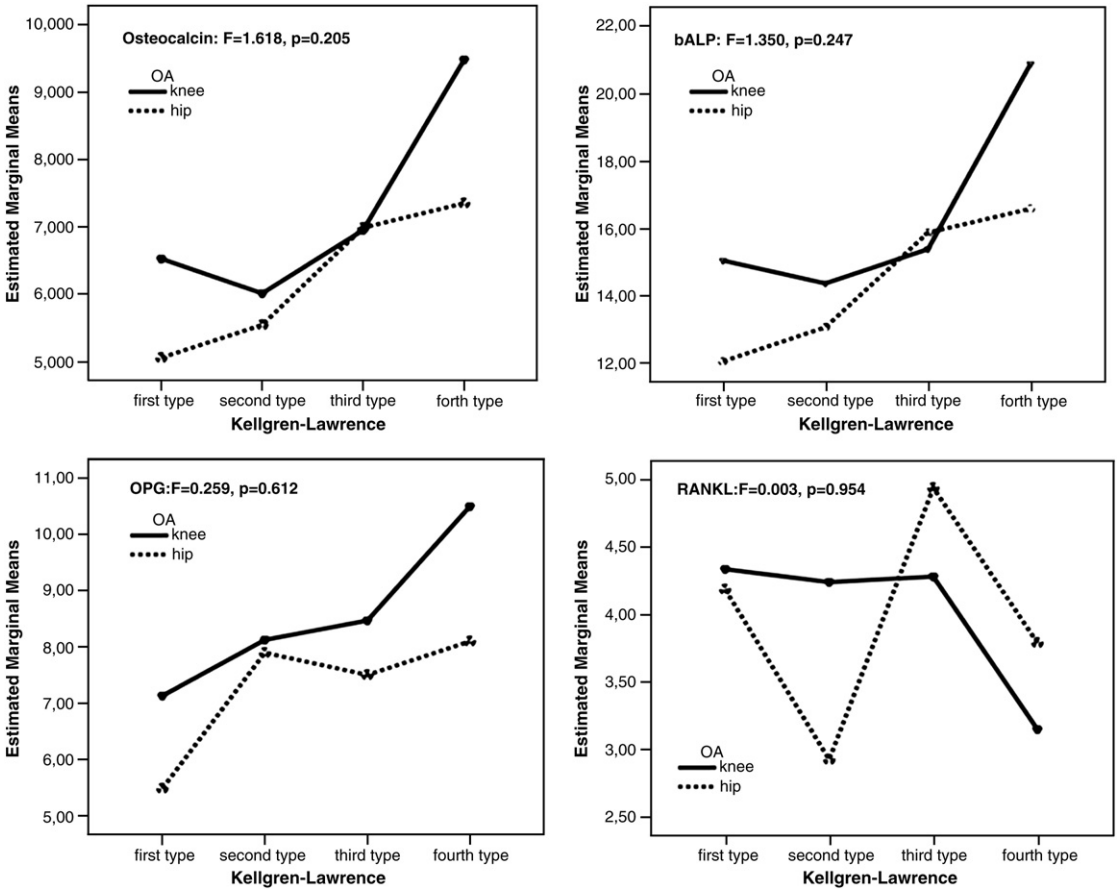

Fig. 1. Comparison of type and severity of osteoarthritis and estimated mean levels of biomarkers.

hip OA and the serum levels of RANKL, OPG, b-ALP and osteocalcin. This was one of the largest (so far) groups of patients suffering from knee OA to whom these four markers were evaluated and correlated with the radiographically assessed severity of the disease. Hunter et al. assessed in a case–control study with twins the relationships of radiographic knee OA with altered bone-turnover and calcium regulation and also reported that the levels of bone-specific alkaline phosphatase, osteocalcin and urinary deoxypyridinoline and calcium regulation had no association with joint space loss [24]. In our study it is interesting that statistical analysis showed that in the group of patients with hip OA there seems to be no doubt that there is no correlation between the serum levels of OPG and the radiographically assessed severity of OA. On the other hand, in the group of patients with knee OA, the serum levels of OPG and also the OPG/RANKL ratio level showed a tendency toward significance as far as their correlation with the radiographically assessed severity of OA was concerned. Further analysis showed that these correlations were probably the result of the fact that patients with more severe osteoarthritic changes were older. Nevertheless, and given the fact that these results are in contrast with the ones reported in the previously published study of Pilichou et al. [11], we believe that there is certainly a need for further investigation.

However, this study has certain limitations as well. An important one is the way that osteoporosis was evaluated. None patient with history of osteoporosis was included. Although every patient included had been screened in the past with DEXA no additional examination was done before the enrolment in our study. However the use of an age-matched control group helps avoiding the problem of osteoporosis evaluation and aging. A second limitation is that patients were not screened for other possible locations of OA, such as spinal OA.

Previous studies have suggested that certain serum and urine biochemical markers and especially combinations of biochemical bone-turnover markers showed significant associations with various imaging features of knee OA [2]. Several reports also support the idea that a combination of biomarkers relate significantly better to the severity of joint damage than individual biomarkers do [25–28]. Based on our results, we cannot support this relationship. Although the synovial levels of biochemical markers seem to be correlated with the severity of the destruction of a joint [10], their systemic values, as measured in the serum, do not show the same sensitivity and do not correlate with the radiographically assessed severity of osteoarthritic changes.

Conclusions

This study provides further evidence for the role of OPG/RANKL pathway in the severity of knee and hip OA. Based on our results, it seems that Receptor Activator of Nuclear Factor-κB Ligand, bone-specific alkaline phosphatase, osteocalcin and OPG cannot be used (either independently or in combination with the others) as surrogates for radiographic imaging in hip and knee OA. More studies are certainly needed in order to better understand the potential role of serum biochemical markers in the assessment of the progression and severity of OA.

Acknowledgments

No benefits or funds (including internal, institutional or departmental support) have been received in support of this study. None of the authors had or has any professional and/or financial affiliation that may have biased this study.

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