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Original Article

RELATIONSHIP BETWEEN UNDERCARBOXYLATED OSTEOCALCIN AND OSTEOPROTEGERIN IN KNEE OSTEOARTHRITIS

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ABSTRACT

Objective: The purpose of the present study was to evaluate the possible association between the serum levels of undercarboxylated osteocalcin (ucOC) and osteoprotegerin (OPG) in patients with knee osteoarthritis (OA).

Methods: Twenty patients (10 men and 10 women) diagnosed to have knee OA, and twenty healthy subjects of matching age, sex, and BMI as a control group, were enrolled in this study. Serum levels of ucOC and OPG, were assayed using the corresponding human ELISA kits.

Results: Patients with knee OA, showed a statistically significant elevation in serum levels of ucOC ($P<0.001$), and a statistically significant reduction in that of OPG ($P<0.0001$), as compared to the control group. Also, there is significant negative correlation ($r=-0.554$, $p= 0.0113$) between the serum levels of ucOC and OPG in knee OA patients.

Conclusion: there is possible association between the serum levels of ucOC and OPG in patients with knee OA.

Keywords: Osteoarthritis, Undercarboxylated Osteocalcin, Osteoprotegerin, Bone, Cartilage.

INTRODUCTION

Osteoarthritis (OA) is defined by the American College of Rheumatology as a "heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins." OA is usually classified as primary or idiopathic when there is no obvious predisposing cause, and secondary when there is some clearly defined predisposing pathology [1]

Symptomatic knee OA affects approximately 40% of adults over 70 years in the US, and a quarter of these patients have difficulty carrying out their activities of daily living [2]. Knee OA had been viewed as a disease mostly affecting older persons. However, recent evidence documents increased incidence of two key risk factors for knee OA – traumatic knee injury [3] and obesity [4,5] particularly in younger persons.[6].

The progressive degenerative damage of articular cartilage observed in OA is based on a complex etiology that is still insufficiently clear. Biochemical alterations, genetic and environmental factors are important contributors to the manifestations of OA [7, 8]. Osteoarthritis is associated with an initial increase in subchondral bone resorption and resultant thinning of the subchondral plate, followed by subchondral sclerosis and osteophyte formation [9].

The key regulators of bone metabolism are osteoblasts, which are involved in bone formation, and osteoclasts, which are responsible for bone resorption. Dynamic changes in bone turnover result from increased activity of osteoclasts and osteoblasts, and the osteoprotegerin (OPG)/receptor activator of NF-κB (RANK)/receptor activator of NF-κB ligand (RANKL) system is also critical for this activity [10,11]. Terpos et al. had showed that soluble RANKL (sRANKL)/OPG ratio is increased in serum of patients with malignant diseases and lytic bone disease [12]. Moreover, RANKL/OPG mRNA ratio in the synovial tissue and sRANKL/OPG ratio in the synovial fluid are elevated in patients with rheumatoid arthritis and predicts for disease progression, suggesting a major role of RANKL/OPG pathway in its pathogenesis [13,14]. Gene expression of RANKL and OPG and the association between their mRNA levels indicate their involvement in the pathogenesis of femoral neck osteoarthritis [15].

Osteocalcin (OC) is the most abundant noncollagenous protein of bone matrix. Once transcribed, this protein undergoes posttranslational modifications within osteoblastic cells before its secretion, including the carboxylation of three glutamic residues in glutamic acid, a vitamin K-dependent process, which is essential for hydroxyapatite binding and deposition in the extracellular matrix of bone [16, 17]. The presence of undercarboxylated osteocalcin in human circulation could be the consequence of two separate processes: incomplete carboxylation of osteocalcin due to suboptimal vitamin K intake or decarboxylation during osteoclast resorption, mediated by inhibition of osteoprotegerin release [18]. The aim of this study was to evaluate the possible association between ucOC and OPG in the sera of patients diagnosed with knee OA.

MATERIALS AND METHODS

Forty subjects were enrolled in this study; twenty of whom (10 men &10 women) were diagnosed for the first time, as having knee osteoarthritis by specialist orthopedic at the Rheumatology Clinic of Baghdad Teaching Hospital. The diagnosis was based on medical history, physical examination, and plain x-rays of the patient. The remaining 20 healthy subjects (10 men &10 women) were considered as control group. The two groups were of comparable age and body mass index (BMI), (Table1). The study was approved by ethical committee of Baghdad university/College of pharmacy, and a written consent was obtained from all subjects prior to conducting this study.

Subjects with any of the following criteria were excluded from the study: hyperthyroidism, hyperparathyroidism, diabetes mellitus, hepatic or renal dysfunction, primary painful inflammatory conditions of the knee (e. g., rheumatoid arthritis, Paget's disease, gout and psoriatic arthropathy), and nutritional derangements which might cause changes in bone metabolism. Also those subjects taking any drug or hormone that is known to affect bone metabolism, including sex steroids, glucocorticoids, warfarin, vitamin K, and bisphosphonates.

Five ml blood samples were collected from each participant in the study, then serum was separated and kept frozen at -18^0C until the time of estimation of undercarboxylated osteocalcin (unOC) and osteoprotegerin (OPG) levels.

Serum levels of ucOC and OPG were measured by enzyme linked immunosorbent assay (ELISA), using the corresponding kits purchased from (CUSABIO®, China), according to the manufacturer instructions.

Statistical analysis was performed using Graph Pad Prism® software version 5 for windows. Results were expressed as Mean ± SE. Student's *t*-test was utilized to examine the degree of significance. Pearson's correlation analysis was performed to study the association between variables. *P* value less than 0.05 was considered significant.

RESULTS

Both OA and control groups were of comparable sex, age, and BMI. Patient's characteristics are shown in table 1.

Table 1: Participant's characteristics

Character	Control (n=20)	OA (n=20)
Sex:		
Male	10	10
Female	10	10
Age (Year) (Mean ± SE)	60.55 ± 0.8899	62.95 ± 1.001
BMI (Mean ± SE)	23.96 ± 0.7590	26.17 ± 0.8411

Serum levels of ucOC in OA group showed a statistically significant elevation as compared to the control group (P<0.001), while that of OPG showed a statistically significant reduction (P<0.0001), as shown in table2. And there is significant negative correlation (r=-0.554, p= 0.0113) between the serum levels of ucOC and OPG in knee OA patients, as presented in figure 1.

Table 2: Serum levels of undercarboxylated osteocalcin and osteoprotegerin of the study groups.

	Control (n=20)	OA (n=20)
ucOC (ng/ml)	2.719 ± 0.1509	3.451 ± 0.2029**
OPG (pg/ml)	144.9 ± 2.476	115.0 ± 3.488***

Values are presented as Mean ± SE, P<0.001= **, P<0.0001= ***

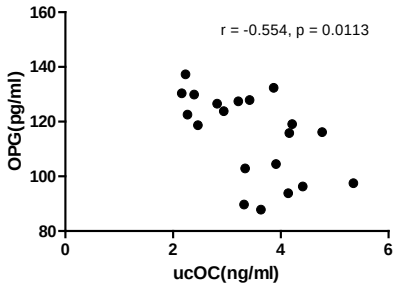

Fig. 1: Pearson's correlation between serum levels of ucOC and OPG in knee OA patients

DISCUSSION

Osteoarthritis is considered to damage the whole joint, involving both bone and synovial tissues, although cartilage degradation is its main feature. Many clinical evidences suggest the role of subchondral bone in the pathogenesis of OA [19,20]. Negative correlations between hip or knee OA and osteoporosis have been reported, and densification of subchondral bone is a common feature in OA progression [21–23]. High bone resorption occurs during the early stages of OA [40], followed by stages characterized by an increase in bone volume, and these later stages occurs when the cartilage has been degraded [24,25]. The mechanism by which bone contributes to OA until now is poorly understood. However, several biomolecules have been proposed to regulate the interaction between bone and cartilage. Osteoprotegerin and RANKL are known to be key molecules in the regulation of bone remodeling. Both factors are produced by osteoblasts/stromal cells, as well as human chondrocytes, while RANK is expressed only in human OA chondrocytes [26]. The RANKL/OPG ratio expression is highly variable within the OA population [26,27]. This ratio was reported to be increased in human OA cartilage compared to normal cartilage [26], Moreover, Celecoxib, a non-steroidal anti-inflammatory drug, decreased RANKL expression in articular cartilage from OA patients, thereby decreasing the RANKL/OPG ratio [28]. As well as, local [29] or systemic [30] administration of recombinant OPG in mice, results in inhibition of RANKL and provides protection against OA.

The mode of action of the OPG/RANKL system in subchondral bone and articular cartilage changes during OA is not well understood. However, in addition to its action on bone cells, OPG is also known to block the interaction of RANKL with TNF-related apoptosis-inducing ligand (TRAIL), thereby inhibiting chondrocyte apoptosis [31]. Also, it is known to be involved in atherosclerosis and angiogenesis [32,33]. These might be potential mechanisms.

When bone turnover is accelerated, osteocalcin precursor is excessively synthesized. To be fully active, osteocalcin precursor undergoes a vitamin K dependent γ-carboxylation [16,17]. Two separate processes might result in the presence of undercarboxylated osteocalcin in human circulation; incomplete carboxylation of osteocalcin due to suboptimal vitamin K intake or decarboxylation during osteoclast resorption, mediated by inhibition of osteoprotegerin release [18]. Since an acidic pH favors protein decarboxylation [34,35]. Positive correlation between serum ucOC levels and urinary type-I collagen cross-linked-N-telopeptide (NTX), and serum levels of bone specific alkaline phosphatase (BAP), which are markers for bone resorption, was demonstrated in clinical studies [36,37].

Recently, synovitis has gained attention as an important feature of OA. Naito et al., had demonstrated a significant correlation between serum levels of ucOC and hyarulonan (HA), a major product of synovial cells and is recognized as a marker of synovitis in patients with knee OA [38].

Taking together, these findings can explain the results of our study regarding the significant reduction in OPG, significant increase in ucOC serum levels, as well as, the negative correlation between OPG and ucOC in patients with OA.

In conclusion, there is possible association between the serum levels of ucOC and OPG in patients with knee OA, and in part, could explain the interaction between the subchondral bone and articular cartilage in the pathogenesis of the disease.

CONFLICT OF INTERESTS

Declared None

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