Evaluation of serum undercarboxylated osteocalcin in premenopausal rheumatoid arthritis patients: its correlation with disease activity and bone mineral density

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Background
There is a definite role of vitamin K and undercarboxylated osteocalcin (ucOC) on bone mineral density (BMD) in rheumatoid arthritis (RA). Up to our knowledge, no other work has discussed the relationship between ucOC and BMD in premenopausal RA patients and its correlation with disease activity.
Patients and methods
Sixty premenopausal RA female patients and 30 healthy premenopausal controls of matched age were included. All were subjected to clinical examination, laboratory investigations including serum level of ucOC, disease activity measurement using DAS-28 score, and BMD measurement using dual-energy X-ray absorptiometry.
Results
The level of ucOC was significantly higher in patients with RA than in controls ($P<0.001$). BMD in patients was found to be significantly lower compared with controls in the spine, femoral neck, and distal radius areas. The most frequent osteoporotic site according to *Z*-score was the spine (16.7%), followed by the femoral neck (8.3%) and distal radius (6.7%). The most common commonest osteopenic site according to *Z*-score was the spine (31.7%), followed by the femoral neck (21.7%) and the distal radius (16.7%). Our work showed that ucOC level was found to be high in premenopausal RA patients with higher DAS values than in those with lower DAS value ($P<0.001$). In our work, BMD measured by means of dual-energy X-ray absorptiometry scan was found to be lower with higher DAS values and vice versa.
Conclusion
Serum level of ucOC (which is a mirror of vitamin K deficiency) was found to be higher in premenopausal RA patients than controls and correlated positively with disease activity and inversely with BMD measurement.

Keywords:
disease activity, osteoporosis, premenopause, rheumatoid arthritis, undercarboxylated osteocalcin

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that causes chronic inflammation of the synovium with subsequent destruction and deformity of joints [1].

Osteoporosis results from a loss of bone mass (measured as bone density) as well as from a change in bone structure. Many factors will raise the risk of developing osteoporosis and breaking of the bone. Some risk factors can be changed, but others cannot be changed. Recognizing these risk factors is important to prevent this condition or treat it before it becomes worse [2].

Osteoporosis is more common in RA patients than in the general population. The prevalence of concurrent osteoporosis is 50%. Osteoporosis can cause pain, loss of height, and increases the risk for fractures after falling [3].

The chronic synovial inflammation in RA can promote osteoclastogenesis, leading directly to both focal and generalized bone loss and increased risk for fractures [4].

Vitamin K is a cofactor of γ-carboxylase, which converts three glutamic acid (Glu) residues in osteocalcin (OC) to γ-carboxyglutamic acid (Gla), and is thus essential for γ-carboxylation of OC. Without this modification, OC becomes

undercarboxylated (ucOC), which lacks structural integrity and the ability to bind to the mineral hydroxyapatite. Vitamin K deficiency impairs γ-carboxylation of OC, resulting in high serum concentrations of ucOC [5].

Patients and methods

Subjects

Patients

Sixty premenopausal patients diagnosed as having RA according the ACR/EULAR 2010 criteria [6] with age more than 16 years and disease duration more than 2 years were recruited from outpatient clinic of Physical Medicine and Rehabilitation Department, Faculty of Medicine, Menoufia University.

Exclusion criteria

Postmenopausal RA female patients, RA male patients, patients suffering from serious cardiovascular and/or pulmonary disease, patients having an abnormal thyroid function or a serious infection, patients with hyperparathyroidism, patients with hepatic or renal dysfunction, those taking any drugs or hormones that affect bone metabolism (e.g. sex steroids, etc.), and those taking steroids during the last 6 months prior the study.

Controls

Thirty healthy premenopausal females of matched age who were free from earlier fractures, chronic diseases, and medications influencing bone metabolism (e.g. corticosteroids, anticonvulsants, thyroxine, etc.) were included as controls.

Methods

All patients were subjected to the following:

(1) Demographic data recording.
(2) Clinical assessment: Medical history (menstrual history, disease duration, information about medications such as disease modifying anti rheumatic drugs (DMARDs), antiresorptive drugs, corticosteroids, etc.). General examination (including chest, heart, and abdomen). Locomotor system examination.
(3) Disease activity Measurement: Disease Activity Score (DAS-28) with three variables (erythrocyte sedimentation rate, the number of swollen joints, and number of tender joins) was used [7].
(4) The level of disease activity was interpreted as follows: low: DAS-28≤3.2; moderate: 3.2<DAS-28≤5.1; high: DAS-28>5.1.
(5) A DAS<2.6 corresponds to being in remission according to the American Rheumatism Association (ARA) criteria [8].
(6) Laboratory investigations: Complete blood count [9], erythrocyte sedimentation rate [10], rheumatoid factor [11], anti-CCP level, C-reactive protein [12], and serum levels of ucOC.
(7) Radiographic assessment: Bone mineral density (BMD) was measured at the lumbar spine L2–L4, hip, and distal radius using a dual-energy X-ray absorptiometry (DEXA) equipment [9]. BMD was expressed in SD from the mean of healthy age-matched and sex-matched people (the Z-score) and as the number of SD from the mean of healthy, young, sex-matched individuals (the T-score) using the WHO classification and the 2005 International Society for Clinical Densitometry (ISCD). A T-score of −2.5 or lower was defined as 'osteoporosis'; osteopenia was defined as T-score less than −1 but greater than −2.5; normal was defined as T-score of at least −1 and a Z-score of −2.0 or lower in female patients before menopause was defined as 'below the expected range for age' [13].

Statistical analysis

The data collected were tabulated and analyzed using SPSS (Statistical Package for the Social Science software) statistical package version 20 on IBM compatible computer. Two types of statistics were performed. Descriptive statistics included percentage (%), range, mean (x), and SD, and for analytical statistics Student's t-test, the Mann–Whitney test, and Fisher's exact test were used. The difference was considered nonsignificant if P value was greater than 0.05, significant if P value was less than 0.05, and highly significant if P value was less than 0.001.

Results

In this work, the level of ucOC was significantly higher in RA patients than in controls ($P<0.001$) (Table 1) and higher in patients with osteoporosis than in those with osteopenia and normal BMD patients (Table 2 and Fig. 1). BMD in patients was found to be significantly lower than that in controls at all sites (the spine, femoral neck, and distal radius areas). The most frequent osteoporotic site according to Z-score was the spine (16.7%), followed by the femoral neck (8.3%) and distal radius (6.7%). According to T-score, the most common osteoporotic site was the spine (13.3%). The most common osteopenic site according to Z-score of −1 or less was the spine (31.7%), followed by the femoral neck (21.7%) and the distal radius (16.7%) (Table 3). Level of

Table 1 Comparison between the studied groups of patients and controls as regards undercarboxylated osteocalcin (ng/ml)

	Studied groups			
ucOC (ng/ml)	RA patient group (n=60)	Control group (n=30)	Mann–Whitney U-test	P value
Range	5.00–24.90	2.00–4.00		<0.001
Mean±SD	14.17±5.59	3.00±0.90	7.72	HS

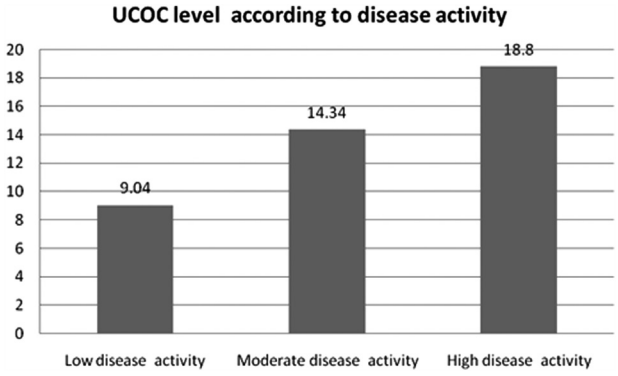
RA, rheumatoid arthritis; ucOC, undercarboxylated osteocalcin.

Table 2 Comparison between osteoporotic female patients, osteopenic female patients, and individuals with normal bone mineral density as regards undercarboxylated osteocalcin (ng/ml)

	DEXA					
ucOC (ng/ml)	Osteoporotic female patients	Osteopenic female patients	Individuals with normal DEXA scan	Kruskal–Wallis test	P value	Post-hoc test
Range	8.00–24.90	5.00–23.20	8.00–9.29	6.68	0.03	P_1=0.03 (S)P_2≤0.001 (HS) P_3=0.006 (S)
Mean±SD	16.98±5.99	13.55±5.69	8.64±0.91		S	

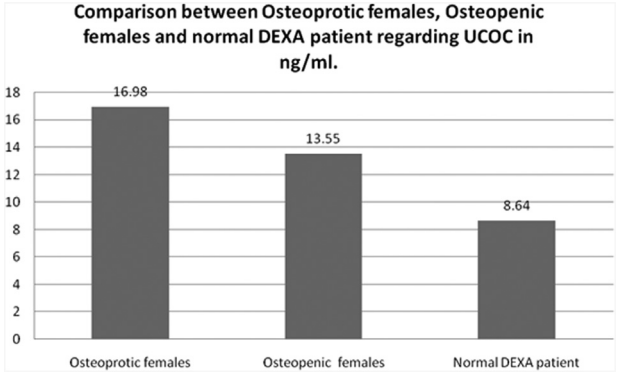
P_1: comparison between osteoporotic female patients and osteopenic female patients is statistically significant. P_2: comparison between osteoporotic female patients and individuals with normal DEXA scan, which is highly significant. P_3: comparison between osteopenic female patients and individuals with normal DEXA scan which is significant. DEXA, dual-energy X-ray absorptiometry; ucOC, undercarboxylated osteocalcin.

Figure 1



Undercarboxylated osteocalcin (ucOC) levels in osteoporotic female patients, osteopenic female patients, and in individuals with normal dual-energy X-ray absorptiometry (DEXA) scan.

Figure 2



Undercarboxylated osteocalcin (ucOC) level according to disease activity.

ucOC was found to be positively correlated with DAS score (P<0.001) (Table 4 and Fig. 2). BMD measured using DEXA scan was found to be lower, with higher DAS values and vice versa, as shown in Table 5.

Discussion

RA is a chronic inflammatory autoimmune disease that causes chronic inflammation of the synovium, with subsequent destruction and deformity of the joints. Osteoporosis is more common in patients with RA than in the general population. Vitamin K is a cofactor of γ-carboxylase, which is essential for γ-carboxylation of OC. Without this modification, OC becomes ucOC, which lacks structural integrity and the ability to bind to the mineral hydroxyapatite, and the secreted OC can no longer be carboxylated. Vitamin K deficiency impairs γ-carboxylation of OC, resulting in high serum concentrations of ucOC.

This study revealed that the level of ucOC was significantly higher in patients with RA than in controls. This is in accordance with the findings of Sakai *et al.* [14], who showed that serum levels of ucOC were increased in higher state of bone turnover as indicated by biochemical markers, and vice versa.

No other researchers discussed ucOC in adult patients with RA. Van Summeren *et al.* [15] found that children with juvenile idiopathic arthritis (JIA) have a high ratio of ucOC/cOC, indicating low vitamin K status that was associated

Table 3 Comparison between the studied groups of patients and controls as regards dual-energy X-ray absorptiometry parameters including number and percent

	Studied groups			
DEXA	RA patient group (*n*=60) [*n* (%)]	Control group (*n*=30) [*n* (%)]	χ^2-Test	*P* value
L2–L4 spine				
T-score				
Osteoporotic	8 (13.3)	0 (0.0)	17.31	<0.001 (HS)
Osteopenic	17 (28.3)	0 (0.0)		
Normal DEXA	35 (58.4)	30 (100)		
Z-score				
Osteoporotic	10 (16.7)	0 (0.0)	18.30	<0.001 (HS)
Osteopenic	19 (31.7)	1 (3.3)		
Normal DEXA	31 (51.6)	29 (96.7)		
Femoral neck				
T-score				
Osteoporotic	4 (6.7)	0 (0.0)	9.00	0.01 (S)
Osteopenic	11 (18.3)	0 (0.0)		
Normal DEXA	45 (75.0)	30 (100)		
Z-score				
Osteoporotic	5 (8.3)	0 (0.0)	11.25	0.003 (S)
Osteopenic	13 (21.7)	0 (0.0)		
Normal DEXA	42 (70.0)	30 (100)		
Distal radius				
T-score				
Osteoporotic	3 (5.0)	0 (0.0)	6.27	0.04 (S)
Osteopenic	8 (13.3)	0 (0.0)		
Normal DEXA	49 (81.7)	30 (100)		
Z-score				
Osteoporotic	4 (6.7)	0 (0.0)	8.29	0.01 (S)
Osteopenic	10 (16.7)	0 (0.0)		
Normal DEXA	46 (76.6)	30 (100)		

DEXA, dual-energy X-ray absorptiometry; RA, rheumatoid arthritis.

Table 4 Relationship between disease activity and undercarboxylated osteocalcin level (ng/ml) in studied group of patients (*n*=60)

	DAS-28					
ucOC (ng/ml)	Patients with low disease activity (DAS<3.2) (n=10)	Patients with moderate disease activity (DAS=3.2–5.1) (*n*=24)	Patients with high disease activity (DAS>5.1) (n=26)	Kruskal–Wallis test	P value	Post–hoc test
Mean ±SD	9.04±4.39	14.34±6.29	18.80±5.87	18.12	<0.001 (HS)	P_1=0.02 (S) P_2≤0.001 (HS) P_3=0.03 (S)

P_1: comparison between patients with low disease activity and patients with moderate disease activity; significant correlation. P_2: comparison between patients with low disease activity and patients with high disease activity; highly significant correlation. P_3: comparison between patients with moderate disease activity and patients with high disease activity; significant correlation. ucOC, undercarboxylated osteocalcin.

with low bone ultrasound parameters, whereas children with a high vitamin K status had markedly higher bone properties.

Moreover, Iwamoto *et al*. [16] found that serum ucOC is an index of vitamin K nutritional status in treating naïve postmenopausal osteoporotic women, and concluded that a high level of ucOC is a risk factor for osteoporotic fracture, which decreased with vitamin K intake.

Moreover, in this work, ucOC level was found to be positively correlated with disease activity in premenopausal RA patients.

To our knowledge, no other studies discussed the relationship between ucOC in premenopausal RA patients and DAS score.

In this work, BMD in patients was found to be significantly lower than that in controls in the spine,

Table 5 Relationship between disease activity and dual-energy X-ray absorptiometry parameters in the studied group of patients (*n*=60)

DEXA	DAS-28			Kruskal–Wallis test	*P* value	Post-hoc test
	Patients with low disease activity (DAS<3.2) (*n*=10) Mean±SD	Patients with moderate disease activity (DAS=3.2–5.1) (*n*=24) Mean±SD	Patients with high disease activity (DAS>5.1) (*n*=26) Mean±SD			
L2–L4 spine						
T-score	−0.55±1.08	−1.50±0.62	−2.55±1.44	19.06	<0.001 (HS)	P_1=0.02P_2≤0.001P_3=0.002
Z-score	−1.01±0.76	−1.57±0.52	−2.11±0.71	15.47	<0.001 (HS)	P_1=0.02P_2≤0.001P_3=0.005
Femoral neck						
T-score	−0.11±0.90	−0.76±0.89	−1.45±1.44	8.70	0.01 (S)	P_1=0.05P_2=0.001P_3=0.04
Z-score	−0.26±0.68	−0.95±0.61	−1.73±1.13	14.82	0.001 (HS)	P_1=0.04P_2≤0.001P_3=0.003
Distal radius						
T-score	−0.26±0.08	−1.01±1.03	−1.71±1.18	17.24	<0.001 (HS)	P_1=0.004P_2≤0.001P_3=0.01
Z-score	−0.60±0.68	−1.15±0.64	−1.95±0.86	18.97	0.001 (HS)	P_1=0.05P_2≤0.001P_3=0.001

P_1: comparison between patients with low disease activity and patients with moderate disease activity. P_2: comparison between patients with low disease activity and patients with high disease activity. P_3: comparison between patients with moderate disease activity and patients with high disease activity. DEXA, dual-energy X-ray absorptiometry.

the femoral neck, and distal radius areas. The most frequent osteoporotic site according to *Z*-score was the spine (16.7%), followed by the femoral neck (8.3%) and distal radius (6.7%). The most common osteopenic site according to *Z*-score of −1 or less was the spine (31.7%), followed by the femoral neck (21.7%) and the distal radius (16.7%).

This is in accordance with the findings of Lee *et al.* [17], who found that the prevalence of osteoporosis in RA patients was 1.9 times higher than that in healthy individuals.

Moreover, Lodder and colleagues found that the frequencies of osteoporosis (*T*-score≤−2.5 SD) is 12.6 and 6.5% in the spine and femoral neck, respectively. Reduced bone mass or osteopenia was 20.7 and 18.9% in the spine and the femoral neck, respectively [18].

Laan and colleagues (2000) examined BMD in 97 patients with recent-onset RA and a mean disease duration of 30 months. Low bone mass (*Z*-score≤−1.0) was found in 32.0% of the patients in the lumbar spine and in 24.2% in the hip. They confirmed that low bone mass in comparison with healthy age-matched and sex-matched controls occurs frequently in patients with RA [19].

In another research, Haugeberg and colleagues (2000) studied a representative sample of 394 female RA patients with a mean disease duration of 13 years, using both *T*-scores and *Z*-scores. The prevalence of osteoporosis (*T*-scores≤−2.5) was 16.8% in the spine, 14.7% in the femoral neck, and 14.7% in the total hip. The prevalence of reduced bone mass (*Z*-scores≤−1.0) was greater than expected (15.9%) in the femoral neck (27.6%), the total hip (31.6), and the spine (19.6%) [20].

However, Hamalainen *et al.* [21] concluded that, according to bone mineral concentration, premenopausal RA women both with and without prednisolone treatment and controls lost bone statistically similarly.

Hui *et al.* [22] had followed up 130 healthy premenopausal white women for 1–9 years and found a significant decrease in femoral neck BMD over time.

In our work, BMD measured by means of DEXA scan was found to be lower with higher DAS values and vice versa.

This is in accordance with the findings of Lodder *et al.* [18], Shenstone *et al.* [23], and Gough *et al.* [24]; they concluded that patients with active disease lost more bone at the spine and hip compared with patients with inactive disease.

Conclusion

From the present study we concluded the following:

(1) Patients with RA are more susceptible to develop generalized osteoporosis compared with other population even if they are premenopausal.
(2) Serum level of ucOC is an indicator of BMD in premenopausal RA patients.
(3) Patients with high serum level of ucOC are more susceptible to osteoporosis compared with other patients.
(4) Patients with active RA disease are associated with higher levels of ucOC and lower BMD values.
(5) The most common sites of osteoporosis in premenopausal RA patients were in the lumbar spine (16.7%), followed by hip (8.3%) and distal radius (6.7%) according to Z-score.

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Conflicts of interest

There are no conflicts of interest..

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