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The Effect of Inflammation on Axial Bone Mass and Bone Turnover in Premenopausal Egyptian Women with Rheumatoid Arthritis

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ABSTRACT

Generalized bone loss in rheumatoid arthritis (RA) is attributable to several possible factors including inflammatory disease itself. This study was performed to investigate the effect of inflammation on bone mineral density (BMD) and bone turnover in premenopausal women with RA. Thirty two consecutive premenopausal non-steroid user RA female patients without functional impairment and 15 healthy controls were studied. Disease activity was scored using the Activity Score of 28 joints (DAS-28). BMD of lumbar spine and femoral neck was assessed by dual energy x-ray absorptiometry. Urinary excretion rates of bone resorption markers, free pyridinoline (f-PYD) and free deoxypyridinoline (f-DPYD) were assayed. Pro-inflammatory cytokines, IL-1, IL-6, TNF-α and C-reactive protein (CRP) were dosed in blood. Significant lower BMD values in lumbar spine and left femoral neck ($p<0.01$, <0.001, respectively) and significant higher levels of IL1, IL6, TNF-α and CRP were observed in RA patients versus healthy controls. BMD at lumbar spine and left femoral neck was inversely correlated with DAS-28, CRP and TNF-α levels. BMD at femoral neck was inversely correlated with disease duration ($r = -0.463$). Urinary f-PYD was positively correlated with DAS-28, CRP and IL-6 ($r = 0.624$, 0.543, 0.657, respectively) while inversely correlated with BMD at femoral neck ($r = -0.6826$). In conclusion, elevated pro-inflammatory cytokines, high disease activity and less extent disease duration, appear to be critical determinant for much of systemic bone loss in RA. Urinary f-PYD reflects bone resorption more selectively than f-DPYD. Control of biological activity of IL-6 may be a therapeutic approach to control osteoporosis in RA.

Key words: Inflammation, osteoporosis, pro-inflammatory cytokines, bone turnover markers, premenopausal women, rheumatoid arthritis

INTRODUCTION

In rheumatoid arthritis (RA), periarticular bone loss, bone erosions and systemic osteoporosis are observed, with an increased risk of fractures (Roux, 2011). Many indirect factors associated with inflammatory arthritis are implicated in the pathogenesis of generalized bone loss in this disease. They include immobility due to functional impairment, weight loss, use of medications known to promote bone loss, such as glucocorticoids and methotrexate (MTX), advanced age and female gender (Cortet *et al*., 2000). On the other hand, the chronic synovial inflammation in RA also contributes to systemic bone loss (Aizer *et al*., 2009). However, the mechanism by which the inflammatory process affect bone mineral density (BMD) in RA is unclear. High levels of inflammatory cytokines such as TNF-α, IL-1 and IL-6 in the peripheral blood of RA patients have been shown to act on osteoblasts and osteoclasts *in vitro* and *in vivo* resulting in increase in bone resorption (Gough *et al.*, 1998; Geusens and Lems, 2011; Schett *et al.*, 2010). In addition, previous work in animal models of RA has suggested that TNF blockage may result in inhibition of systemic bone loss (Saidenberg-Kermanac'h *et al.*, 2004).

Decreased BMD is commonly seen in RA patients. The International Society for Clinical Densitometry (ISCD) and National Osteoporosis Foundation (NOF) have recommended dual-energy x-ray absorptiometry (DEXA) testing for all adults RA patients. Several characteristics based on DEXA assessment have been identified, including preference for distinct skeletal sites (spine, hip, distal forearm) and the particular intervention of menopause (Leib *et al.*, 2004; Macovei *et al.*, 2011). Despite the many advantages of DEXA in the measurement of BMD, there is still a need for sensitive and specific marker which is able to identify patients who are fast bone losers and to provide rapid indication of any response to therapy (Gough *et al.*, 1994b).

The cross-links pyridinoline (PYD) and deoxypyridinoline (DPYD) have been validated as a useful marker for bone resorption and their urinary excretion rates have been correlated with bone resorption in established osteoporosis. These cross-links form between the two collagen fibrils and on breakdown of collagen are released unchanged in urine. PYD is the main cross-link between type II collagen fibers in cartilage and is also present in bone and vascular connective tissue, whereas DPYD is located in bone and dentine and considered as a marker of type I collagen breakdown (Wada *et al.*, 2009; Yoshimura *et al.*, 2011). Biochemical markers of bone turnover reflect changes in the entire skeleton which may not give information about localized osteoporosis in diseases like RA. Since there are several confounding factors affecting bone turnover as diurnal variation, age, hormonal regulation, nutritional status and sensitivity and specificity of assays, these factors must be taken in consideration when comparing different studies (Szulc *et al.*, 2000).

In view of the fact that the use of either DEXA or biochemical markers has a limited value as a sole tool in prediction of bone loss (Smith and Greer, 2001) and owing to unclear mechanisms of systemic bone loss, the aims of this study were to investigate (1) The value of using DEXA and bone turnover markers in evaluating on-going systemic bone loss and hence potential osteoporosis in the future and (2) The effect of inflammation on bone mass and bone turnover by correlating the serum levels of pro-inflammatory cytokines with both BMD values and bone markers excretion, in premenopausal female patients with RA.

MATERIALS AND METHODS

This prospective study was conducted on 32 white premenopausal female patients with RA who were attending the outpatient clinic of Rheumatology and Rehabilitation, Mansoura University Hospital, Egypt. RA was diagnosed on finding at least four of the 1987 revised classification criteria for RA (Arnett *et al.*, 1988). All patients included in this study were on non steroidal anti-inflammatory drugs (NSAIDs) with hydroxychloroquine (14 patients) or salazopyrin (18 patients). They were on the same dietary calcium intake in the last 3 months. Informed consent was obtained from every subject after approval of this study from local ethical committee. Patients excluded from this study were postmenopausal women, patients with physical incapacity (steinbrocker III and IV), patients with concurrent disease which might affect bone metabolism (e.g., liver and kidney disease, hyperthyroidism, hyper parathyroidism) or treatment which might alter BMD (e.g., corticosteroids, hormone replacement therapy, methotrexate, long term anticonvulsants, bisphosphonates, calcium and Vitamin D). Only RA patients without an associated rheumatic disease were assessed. No intra-articular steroids were allowed in the two months before assessment.

The control group comprised 15 healthy females (hospital staff) matched to RA patients with regard to age and geographical area and without a history of inflammatory rheumatic disease or a condition responsible for bone loss. The exclusion criteria were the same as the patients.

Demographic data and disease characteristics were recorded. Body weight and height were measured to obtain body mass index (BMI). Complete physical examination of patients and controls was performed. The RA activity is estimated using disease activity score of 28 joints (DAS 28 score) (Prevoo *et al*., 1995). DAS 28, consisting of four items, namely the number of swollen and tender joints, the visual analogue scale of patients' assessment of their general health and the Erythrocyte Sedimentation Rate (ESR) in the first hour, gives an absolute number reflecting disease activity. The presence or absence of extra articular manifestations was also determined. Physical disability was measured by Health Assessment Questionnaire (HAQ). Recent radiographs of hands and feet were obtained for all patients. The presence of one erosion was sufficient to fulfill the requirement of an erosive disease.

Blood samples were collected from all patients and controls for determination of complete blood count (CBC), ESR, blood glucose, serum creatinine, serum transaminases, serum calcium, phosphate and alkaline phosphatase, thyroid function, CRP, rheumatoid factors, anti nuclear antibody (ANA), anti double stranded DNA (anti-DsDNA) antibodies and complement components; C3 and C4.

Pro-inflammatory cytokines measurement: Blood samples drawn for determination of cytokines were stored at -70°C before laboratory testing. Cytokines were measured using enzyme linked immmunosorbent assays (Hiss Diagnostics GmbH, Freiburg, Switzerland). IL-1, Il-6 and TNF-α were selected as pro-inflammatory cytokines (Arend, 2001).The intra-assay and inter-assay coefficients of variation (respectively) were 5.1 and 8.6% for IL-1, 3.4 and 5.2% for IL-6 and 6.9 and 7.4% for TNF-α.

Bone mineral density measurement: The BMD of the lumbar spine (L2-L4) and left femoral neck was measured by DEXA (QDR 4500A; Hologic Inc., Walthman, Massachusetts, USA). BMD was defined as bone mineral content, divided by the surface of the projected bone area, expressed in g cm^{-2}. Osteopenia was defined by a T-score between -1 and -2.5 SD and osteoporosis as T-score below-2.5 SD according to the World Health Organization guidelines.

Markers of bone turnover: Early morning eight hours fasting urine specimens were collected for determination of combined free pyridinium cross links (sum of f-PYD+f-DPYD) and free deoxypyridinoline (f-DPYD), as markers of bone absorption. They were extracted from hydrolyzed urine samples separated by high performance liquid chromatography (HPLC) and measured with spectrofluorimetry. The urinary f-PYD levels were obtained by difference between (f-PYD+f-DPYD) and f-DPYD. Creatinine was measured in urine by standard automated technique (Boehringer Mannhiem, Lewes). PYD and DPYD were determined in urine by a reversed phase HPLC method described in details by Muller *et al*. (1996) and expressed as the f-PYD/creatinine ratio (f-PYD/crea) and f-DPYD/ creatinine ratio (f-DPYD/crea).

Serum levels of inflammatory cytokines, CRP, BMD values of the lumbar spine (L2-L4) and left femoral neck and rates of urinary bone markers excretion were compared between RA patients and controls. The correlations of both BMD values and the rates of bone markers excretion, with disease activity (DAS-28 and CRP), disease duration and pro-inflammatory cytokines were assessed in RA patients. The correlation between rates of bone markers excretion and BMD values in RA patients was also assessed.

Statistical analysis: The results are expressed as mean value±Standard Deviation (SD) or percentage. Comparison of two variables between RA patients and controls were calculated using independent sample t-test. Proportional data were compared with χ^2 test. Associations between pairs of factors were assessed using Spearman's rank correlation expressed as a p-value and correlation coefficient (r). A $p<0.05$ was considered to be statistically significant. All calculations were made by SPSS, 17.0.

RESULTS

The demographic data of patients and controls and disease variables are shown in Table 1. The study population comprised 32 premenopausal women with RA and 15 healthy premenopausal women as a control group. The mean±SD disease duration in the patients was 10.6±7.3 years (range 2.5-18 years) and their mean±SD age was 34±11.7 years (range 22-48 years). The mean age of the control group was 37±12.8 years (range 21-49 years). The patients and healthy controls were comparable with respect to age, sex and BMI which was in the overweight range in both groups. Rheumatoid factor was positive in 24 (75%) of RA patients. Nodular, extra-articular and erosive disease were present in 5 (15.6%), 6 (18.7%) and 18 (56.3%) patients, respectively. The patient group was more disabled physically than the healthy group as reflected by higher HAQ scores in the patients.

Table 1: Demographic data and disease variables of RA patients and controls

Variable	RA (n = 32)	Controls (n = 15)	p-value
Age (years)	34±11.7	37±12.8	ns
Female no. (%)	32 (100)	15 (100)	ns
Body mass index (kg m^{-2})	26.4±4.8	25.2±3.6	ns
Disease duration (years)	10.6±7.3		
Activity parameters			
CRP (mg L^{-1})	11.4±2.6	1.8±0.9	<0.001
DAS-28	4.7±0.7		
Severity parameters			
Nodular disease			
Patient No. (%)	5 (15.6)		
Extra articular disease			
Patient No. (%)	6 (18.7)		
IgM-RF positive			
Patient No. (%)	24 (75)		
Erosive disease			
Patient No. (%)	18 (56.3)		
HAQ	0.78±0.6		

ns: Not significant

Table 2: Pro-inflammatory cytokines, CRP, BMD and urinary bone markers in RA patients and controls

Variable	RA (n = 32)	Controls (n = 15)	p-value
CRP (mg L^{-1})	11.40±2.60	1.80±0.90	<0.001
Pro-inflammatory cytokines			
Interleukin-1 (pg mL^{-1})	7.50±3.10	4.70±1.80	<0.010
Interleukin-6 (pg mL^{-1})	20.40±12.5	3.80±1.20	<0.001
Tumor necrosis factor-α (pg mL^{-1})	18.00±4.30	2.70±1.20	<0.001
Bone mineral density (BMD)			
Lumbar spine			
BMD (g cm^{-2})	1.02±0.15	1.18±0.17	<0.010
T-score	-0.84±1.52	0.35±0.87	<0.010
Z-score	-0.68±1.53	0.41±0.98	0.020
Femoral neck			
BMD (g cm^{-2})	0.80±0.14	1.06±0.13	<0.001
T-score	-0.98±1.20	0.38±0.91	<0.001
Z-score	-0.53±1.19	0.45±0.95	<0.020
Urinary bone markers			
f-pyridinoline (nM mM^{-1} crea)	195.00±83.0	138.00±65.00	<0.050
f-Deoxypyridinoline (nM mM^{-1} crea)	70.00±48.0	38.00±12.00	<0.020

Values are Mean±SD

The comparison of serum levels of pro-inflammatory cytokines, CRP, BMD values of the lumbar spine (L2-L4) and left femoral neck and rate of urinary bone markers excretion between RA patients and controls are presented in Table 2. As shown in Table 2, significant higher levels of CRP, IL1, IL6 and TNF-α were observed in RA patients versus healthy controls ($p<0.001$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively). In addition, RA patients had significantly lower mean BMD for lumbar spine (1.02±0.15 g cm^{-2}) and left femoral neck (0.80±0.14 g cm^{-2}) than in healthy controls (1.18±0.17, 1.06±0.13 g cm^{-2}, $p<0.01$, $p<0.001$, respectively). Rates of urinary f-PYD and f-DPYD excretion were significantly higher in patients with RA (Mean±SD: 195±83, 70±48 nmol $mmol^{-1}$ crea) than in controls (138±65, 38±12 nmol $mmol^{-1}$ crea) ($p<0.05$, $p<0.02$, respectively).

The correlations of BMD values and rates of urinary f-PYF and f-DPYD excretion with disease activity (DAS-28 and CRP), disease duration and pro-inflammatory cytokines (IL1, IL-6 and TNF-α) are shown in Table 3. DAS-28 and CRP were inversely correlated with BMD values at the lumbar spine ($r = -0.472$, -0.553, respectively) and at the left formal neck ($r = -0.566$, -0.602, respectively). Disease duration was inversely correlated with BMD values at the left femoral neck ($r = -0.463$) but no correlation was found at the lumbar spine. TNF-α was inversely correlated with BMD values at the left femoral neck ($r = -0.568$) and at lumbar spine ($r = -0.483$). However, no correlation was found between either IL1 or IL-6 and BMD values at all sites. Urinary f-PYD excretion was positively correlated with DAS-28, CRP and IL-6 levels ($r = 0.624$, 0.543, 0.657, respectively) but no correlation was found with disease duration, TNF-α and IL-1 levels. On the other hand, urinary f-DPYD excretion had no correlation with DAS-28, CRP, disease duration, or pro-inflammatory cytokines.

The correlations between BMD values and rates of urinary f-PYD and f-DPYD excretion in patients with RA are presented in Table 4. Urinary f-PYD excretion correlated inversely with BMD

Table 3: Correlations of BMD and urinary bone markers with disease parameters and pro-inflammatory cytokines in RA patients

Disease parameters and proinflammatory cytokines	Lumbar BMD (g cm^{-2})	Femoral BMD (g cm^{-2})	f-PYD (nM mM^{-1} crea)	f-DPYD (nM mM^{-1} crea)
DAS 28				
r	-0.472	-0.566	0.624	-
p	0.027	0.006	0.002	-
CRP (mg L^{-1})				
r	-0.553	-0.602	0.543	-
p	0.008	0.003	0.009	-
Disease duration (years)				
r	-	-0.463	-	-
p	-	0.03	-	-
IL1 (pg mL^{-1})				
r	-	-	-	-
p	-	-	-	-
IL-6 (pg mL^{-1})				
r	-	-	0.657	-
p	-	-	<0.001	-
TNF-α (pg mL^{-1})				
r	-0.483	-0.568	-	-
p	0.023	0.006	-	-

BMD: Bone mineral density, f-PYD: Free pyridinoline, f-DPYD: Free deoxypyridinoline, DAS 28: Disease activity score 28, CRP: C-reactive protein, TNF-α: Tumour necrosis factor-α, Pearson correlation co-efficiency test was nsed, only significant p-values are shown

Table 4: Correlations between BMD values and rates of urinary f-PYD and f-DPYD excretion in patients with RA

	Lumbar BMD (g cm^{-2})	Femoral BMD (g cm^{-2})
f-PYD (nM nM^{-1} crea)		
r	-0.18	-0.6826
p	ns	<0.01
f-DPYD (nM nM^{-1} crea)		
r	0.075	0.065
p	ns	ns

f-PYD: Free pyridinoline, f-DPYD: Free deoxypyridinoline and ns: Not significant

values at the left femoral neck (r = -0.6826) but no correlation, however, was found with BMD values at the lumbar spine. In contrast, no significant correlation was seen between urinary f-DPYD and BMD values at the femoral neck or at the lumbar spine.

DISCUSSION

Both generalized and periarticular bone loss occurs in RA where juxta-articular osteoporosis is one of the earliest radiological abnormalities (Deodhar and Woolf, 1996). There are several possible mechanisms of RA-related reduction of BMD, including reduced mobility, low body weight, corticosteroid therapy, old age, whereas the role of inflammatory process *per se* has been studied less extensively. To ascertain changes in axial bone mineral density and their correlation with inflammatory process in patients with RA, this study was conducted. Because of the well known influence of sex, race and menopausal state on bone metabolism, our patients constituted a high degree of homogeneity; they were all premenopausal females within a narrow age range and BMI in the overweight range. There was no difference in dietary calcium intake among the patients in the last 3 months. Patients with physical incapacity and patients with treatment or disease which might alter the bone mineral content and/or metabolism were excluded. This would seem to strengthen the role of the disease in the determination of BMD.

In the present cohort of 32 Egyptian premenopausal women with RA, BMD in the lumbar spine and left femoral neck was significantly lower than that in matched healthy controls. This result was consistent with several studies which have shown BMD reduction in the lumbar spine (Peel *et al*, 1995), hip (Hall *et al*., 1993; Martin *et al*., 1997) or both (Sambrook *et al*., 1989), in non corticosteroid using (Hall *et al*., 1993; Martin *et al*., 1997), female patients with RA compared with healthy controls. However, these studies have limitations due to selection of patients, being all postmenopausal. Only one previous population based study examined only perimenopausal women and found a BMD reduction of 5.5% in the lumbar spine and 8.7% in the femoral neck (Kroger *et al*.,1994) which in line with our results. In addition, another prospective study examined only premenopausal women with RA and observed significant difference in BMD between patients and controls at femoral neck (Shankar *et al*., 2009). Sambrook *et al*. (1987) in his cross sectional study, also showed reduction of both spinal and femoral neck BMD in female RA patients compared with controls. However, there was no difference in either the spinal or femoral neck BMD between female patients taking corticosteroids and those not taking. In contrast, Laan *et al*. (1993b) in other cross sectional study using Quantitative Computerized Tomography (QCT) have reported significant reduction in trabecular and cortical BMD in the spine of RA patients using long term low dose oral steroids compared with those who did not use them. However, no comparison with controls was available.

Although, cross sectional study design is not suitable to assess the effect of disease activity on bone mass (Deodhar and Woolf , 1996), this cross sectional study was consistent with previous longitudinal studies which have shown that patients with high disease activity lose more bone density than those with inactive disease. The disease activity is however measured differently in these studies. Shenstone *et al*. (1994) used the stoke index (Davis *et al*., 1990) which is a composite algorithm incorporating joint count, duration of early morning stiffness, ESR, Ritchie index and CRP and found that the lumbar spine BMD changes correlated with the initial stoke index but not the mean stoke indices, suggesting that significant bone loss occurs within the first few months of disease in patients with RA. On the other hand, Gough *et al*. (1994a) stratified their patients into those with CRP of <20 and those active with CRP of >20 mg dL^{-1} and demonstrated significantly greater BMD loses at femoral neck and lumbar spine in patients with active disease. Laan *et al*. (1993a) in a longitudinal study, found that the CRP and mean ESR in 6 months before BMD measurement were negatively associated with BMD in the hip and in ward's triangle. Other parameters of disease activity did not show any correlation with BMD.

In this study, there was a positive correlation between BMD reduction in the femoral neck and disease duration. In contrast, Laan *et al*. (1993a) concluded that BMD in the proximal femur may be affected in patients with recent onset RA by disease dependent mechanisms. Other two longitudinal studies using DEXA (Gough *et al*., 1994a; Shenstone *et al*., 1994) showed that patients with RA lose lumbar spine BMD mainly in the early part of their disease. Gough *et al*. (1994a) found no difference in spinal or femoral neck BMD between patients and controls at presentation but over the next 12 months BMD loss was greater in female patients with RA (2.5%) compared with controls. The annual rate of loss of BMD in lumbar spine was 1.3% for females over the next two years of study. The lower sensitivity of dual photon absorptiometry (DPA) technique to measure change in BMD may explain the findings of another longitudinal study (Sambrook *et al.*, 1990) which found no significant reduction in the lumbar spine bone mass over 2 years in female patients with early RA (mean disease duration 1.2 year) as compared with controls.

This study demonstrated that the rates of urinary f-PYD and f-DPYD excretion were significantly increased in patients with RA compared with controls. The increase in bone resorption was associated with high disease activity reflected by positive correlation between the excretion of f-PYD and parameters of disease activity including DAS-28 and CRP. However, f-PYD and f-DPYD levels did not correlate with disease duration. Previous studies that examined urinary excretion of bone resorption markers have shown significantly raised PYD but not DPYD (Black *et al.*, 1989; Gough *et al.*, 1994b; Spector *et al.*, 1993) or raised both of them (Seibel *et al.*, 1989) in RA patients compared with controls. These studies also demonstrated significant correlation of PYD with clinical measures of joint involvement as well as biochemical variables of inflammatory activity (CRP and ESR). Some of these studies, however, have limitations as they included postmenopausal women and some corticosteroids and DMARDs users or they gave no information about menopausal status of the patients, use of corticosteroids, as well as the timing of urine collection. The finding of raised urinary f-PYD but not the bone specific f-DPYD in non steroid using RA cases in this study and previous studies suggests that the increased collagen breakdown does not primarily come from bone but from other sources as cartilage and synovium. f-PYD also correlated with disease activity and may thus reflect diffuse collagen breakdown. In previous studies, no data, however, exist on the correlation between collagen cross link excretion and disease duration.

In the present cohort of RA patients, we found that the patients had higher IL-1, IL-6 and TNF-α concentrations than did control individuals and positively related to disease activity (DAS-28 and CRP) which are consistent with previous studies (Oelzner *et al.*,1999; Vis *et al.*, 2006).

The BMD in the lumbar spine and the femoral neck correlated inversely with the serum level of TNF-α in our patients with RA. These results are consistent with previous studies, in which TNF-α blockade has been shown to arrest spine and hip bone loss, in patients with RA (Vis *et al.*, 2006; Marotte *et al.*, 2007; Seriolo *et al.*, 2006). An earlier smaller study also suggested a beneficial effect of TNF blockage in increasing spine and femoral neck BMD of 2.7 and 13%, respectively in 26 patients with RA (Lange *et al.*, 2005). The results of these studies add some evidence in favor of the hypothesis that bone loss is mainly due to systemic inflammation through direct effects of TNF-α on bone. The bone formation under anti-TNF-α therapy might be the result of recruitment of osteoblasts or their activation.

Moreover, the urinary excretion of f-PYD correlated positively with the serum level of IL-6, indicating that increased secretion of IL-6 is of pathogenic importance in the process of bone resorption in RA. This assumption is in agreement with other *in vivo* and *in vitro* findings demonstrating increased osteoclastogenesis and increased secretion of the pro-inflammatory cytokines IL-6 and Il-8, in the iliac crest bone marrow in patients with RA (Tanabe *et al.*, 1994; Toritsuka *et al.*, 1991).

The mechanism by which inflammatory cytokines from active rheumatoid joints affect bone turnover has recently been studied. IL6 released from inflamed RA synovium may contribute to differentiation and activation of osteoclast either directly by acting on cells of the osteoclast lineage, or indirectly by acting on other cell types to modulate expression of the key osteoclastogenic factor, receptor activator of Nuclear Factor (NF) kappa B ligand (RANKL) and/or its inhibitor osteoprotegerin (OPG) (Hofbauer *et al.*, 2000; Walsh *et al.*, 2005). Osteoblasts express RANKL constitutively on their cell surface. This interacts with its cognate receptor RANK which is expressed on osteoclast precursors and promotes osteoclast differentiation. Interaction of RANKL with RANK on mature osteoclasts results in their activation and prolonged survival. OPG is secreted primarily by osteoblasts and stromal cells. OPG block the interaction of RANKL with RANK and thus acts as a physiological regulator of bone turnover (Boyle *et al.*, 2003; Eghbali-Fatourechi *et al.*, 2003).

The present study has shown that f-PYD excretion was significantly associated with bone density loss at the femoral neck but not at lumbar spine. It was also correlated with disease activity in agreement with the findings of Gough *et al.* (1994b). Another prospective study, however, has shown that the mean of both urinary PYD and DPYD levels were increased in patients with active disease and correlated strongly with BMD change at all sites in early RA (Gough *et al.*, 1998). Other cross sectional studies have shown elevation of resorption markers in active RA, however, no data exist on the correlation of BMD and pyridinium cross links excretion in RA patients (Black *et al.*, 1989; Seibel *et al.*, 1989; Spector *et al.*, 1993).

CONCLUSION

This study showed that premenopausal RA women without functional impairment and without prednisolone treatment have significant amount of generalized skeletal bone loss compared with normal population. The bone loss was more evident at the hip than the spine. The urinary f-PYD/crea ratio reflects bone resorption more selectively than the f-DPYD/crea ratio. The activation of the IL-6 system with high disease activity did appear to be critical determinant for much of the systemic bone loss in RA. Therapeutic strategies targeting TNF-α and IL-1 that activate IL-6 combined with therapies targeting the osteoclast would need to be used to arrest axial bone loss and consequently reduce the risk of future fractures.

Future studies of the specific role of IL-6 in bone metabolism in RA, taking into account the structural properties of bone and therapeutic strategies to neutralize it are warranted. Long-term studies are also needed to determine the role of prophylactic treatment as calcium and vitamin D intake and involvement in exercise programs for osteoporosis to prevent this complication in premenopausal women with RA.

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