DISCUSSION

The study consisted of 43 premenopausal rheumatoid arthritis (RA) patients that were further categorized into 2 groups according to anti-cyclic-citrullinated peptide antibodies (ACPA); 31 (72%) ACPA positive patients and 12 (28%) ACPA negative patients and a control group of 30 healthy premenopausal females. Patients were included randomly if they fulfilled the inclusion criteria and had no reason for exclusion. The categorization of the patients into 2 groups according to ACPA levels was done at the end of the study, after data collection in the form of history taking, clinical examination, laboratory testing and radiological study, before statistical analysis was done.

The percentage of ACPA positive (72%) to negative (28%) patients found in this study was consistent with Handa[17] and Ursum et al[213] who found 71% of RA patients to be ACPA positive in their studies.

The 3 groups showed no statistical significant difference as regards demographic characteristics; as the age of the participants (p=0.192), their marital status (p=0.671), parity (p=0.213) and occupation (p=0.065). There was also no statistical significant difference as regards personal history; as the age of menarche (p=0.213), their smoking history (p=0.505), coffee intake (p=0.778), dietary history (p=0.295) and calcium supplementation (p=0.713). Body mass index (BMI) also showed no statistical significant difference between the 3 groups (p=0.222).

This matching of the studied groups as regards to their demographic, personal and physical characteristics helps the aim of the study, which is to study the effect of the disease (duration, severity, drugs and other factors) on bone loss in RA patients, by limiting some of the variables that affect bone loss in the general population and in RA.

In the current study the age of menarche showed no statistical significant difference among the patients' groups. This is inconsistent with the findings by Pedersen et al,[214] who showed that women with a higher age of menarche had twice the risk for development of RA in both serological groups.

In the same previous study patients with higher BMI had a higher risk of ACPA negative RA,[214] this was in contradiction to the findings in the current study where there was no statistical significant difference as regards BMI in the patients' groups.

However there was no statistically significant correlation between parity in either of the serological groups of RA, which was consistent with the same study by Pederson et al.[214]

There was no statistical significant difference between the patients' groups regarding the disease onset and duration (p=0.775 & p=0.477 respectively), this also eliminated some of the confounding factors that may affect bone loss when comparing it in the ACPA positive and negative groups.

The duration of morning stiffness was significantly higher in the ACPA positive group compared to the ACPA negative group (p=0.022). This was in contrary to the findings of Van der Helm et al,[18] who found no significant difference in morning stiffness in 228 ACPA positive and 226 ACPA negative RA patients at onset of disease. This could be explained by the similarity in presentation in both serological groups early in the course of the disease, while later ACPA positive patients run a more aggressive course.[18] However in the current study most patients had moderate disease duration in both the ACPA positive, 4 years (5 months to 20 years), and negative groups, 4.5 years (4 months to 15 years).

Disability as assessed by the health assessment questionnaire (HAQ) was significantly higher in the ACPA positive group with 77% of patients with moderate to severe disability compared to only 50% of the ACPA negative patients had moderate to severe disability (p=0.007). This is in contrast to the findings by Ursum et al [215] and Kastbom et al[216] they showed no difference in the HAQ score between the ACPA positive and negative groups, although there was a significant difference in the disease activity markers ESR, CRP and, DAS28 between the 2 groups. This could be explained by the fact that both studies assessed early RA patients (< 3 years of disease duration), and disease activity takes some time to affect the patients' quality of life and the HAQ score. Also laboratory findings precede the overt clinical manifestation.

Disease activity, as assessed by the disease activity score based on 28 joint-erythrocyte sedimentation rate and C-reactive protein (DAS 28-ESR and –CRP), was significantly higher in the ACPA positive group (p<0.001 & p=0.02 respectively). These findings are also consistent with those by Van der Helm et al,[18] who found significant higher number of swollen joints in the ACPA positive patients after 4 years follow up.

It was noticed in this study that the DAS 28-ESR classified 90.3% of the ACPA positive and 33.3% of the ACPA negative patients as having high disease activity. While the DAS 28-CRP classified 61.3% of the ACPA positive and 25% of the ACPA negative patients as having high disease activity, showing the tendency of the DAS28-ESR to classify patients with higher disease activity than the DAS 28-CRP.

This was similar to finding by Wells et al,[217] who found a tendency for the DAS 28-ESR to classify patients as having a higher score. This could be explained by the sensitivity of CRP levels to short-term changes in disease activity, while a number of factors unrelated to disease activity can influence ESR levels such as age, gender or plasma proteins.[218]

There was statistically significant higher number of patients with feet involvement in the ACPA positive group (48.4%) compared to the ACPA negative group (41.6%) (p=0.019), in the form of pain, tenderness and swelling in the small joints of the feet. This was consistent with studies by Van der Helm et al[18] and Seegobin et al[219] who showed ACPA positive RA to be a more aggressive disease subtype and needs more aggressive treatment strategies.

The median level of serum total procollagen type 1 amino-terminal propeptide (TP1NP), a bone formation marker, was similar for the 3 studied groups. A study by Hall et al [220] also showed similar serum levels for bone formation markers, serum bone specific alkaline phosphatase (BAP), in RA patients and controls. This was inconsistent with findings by Cortet et al[221] where serum bone formation markers, serum P1NP and procollagen type I C-terminal propeptide (PICP), were significantly increased compared with controls. While another study by Harre et al[222] showed similar levels for bone formation markers, BAP, in both ACPA positive and negative patients, but BAP levels tended to be lower when compared to controls.

These conflicting results can be explained by (1) the different markers studied as well as the different techniques in testing of these markers, (2) the circadian variation as well as day to day variations in bone marker levels (3) the effect of menstrual cycle on bone markers, which is not usually an issue as they are usually tested in postmenopausal females but the current study was done on premenopausal females, and (4) prolonged periods of bed rest and immobility can also affect bone marker levels, which may occur frequently in RA patients due to increased disease activity.[223]

The median level of serum β-CrossLaps (β-CTx), a bone resorption marker, was statistically higher in the ACPA positive group compared to the control group. This indicates higher levels of bone loss in ACPA positive RA patients.

This was consistent with a study by Harre et al[222] that found higher levels of serum CTx in ACPA positive patients compared to ACPA negative patients and controls. Also in a study by Cortet et al[221] serum CTx were significantly increased in RA patients compared with controls. The influence of ACPA on bone resorption in RA patients was evident in a study by Haugburg et al[185] where ACPA titers were correlated with CTx levels in ACPA positive patients.

Similarly, a study by Hall et al[220] showed significantly elevated levels of urinary bone resorption markers, urinary CTx and deoxypyridinoline (DPyr), in RA patients receiving corticosteroid (Cs) compared to controls. In another study by Gheita et al[224] there was remarkable increase in the N-terminal cross-linked telopeptides of type I collagen (NTx) in RA patients compared to controls indicating high rate of bone resorption.

The median level of serum N-MID Osteocalcin (OCN), a bone turnover marker, was statistically lower in the ACPA negative patients compared to control, indicating a lower level of bone turnover in ACPA negative patients. This was similar to the findings in a study by Hall et al[220] where serum OCN was significantly lower in RA patients compared to controls.

On the other hand, ACPA positive patients showed no statistically significant difference as regards serum OCN levels compared to ACPA negative patients and controls. This is consistent with a study by Pietschmann et al[225] who showed no significant difference in serum OCN between RA patients and control.

Radiological joint damage in the form of joint space narrowing (JSN) and erosions (E) were assessed quantitatively by the modified Sharp score (mTSS) in the patients' groups, the ACPA positive group had a significantly higher score than the ACPA negative group ($p=0.002$).

These findings are consistent with a study by Van der Helm et al,[18] in which ACPA positive patients had significantly higher mTSS scores than ACPA negative patients, at 2 years and 4 years follow-up. Similarly, Forslind et al[226] showed that ACPA positive patients compared to ACPA negative patients had significantly higher radiological damage as detected by Larsen scores at baseline and at 2 years follow up.

The effect of ACPA as well as RF on joint damage was demonstrated in a study by Hecht et al[227] and van Steenbergen et al. [228] They showed an additive effect on the number and size of erosions by high-resolution peripheral quantitative CT (HR-pQCT) scan of the metacarpophalengeal (MCP) joints, where the higher the ACPA or RF the more aggressive the erosions.

Bone mineral density (BMD) and bone loss in the axial (lumbar spine) and appendicular skeleton (forearm and hip) was assessed by dual energy X-ray absorptiometry (DXA) scan. The 1^{st}- 4^{th} lumbar vertebra (L1-L4) spine BMD in the ACPA positive group was significantly lower than that in ACPA negative and control groups ($p=0.035$ and $p<0.001$ respectively).The left (Lt) total femur BMD in the ACPA positive group and the ACPA negative group were similar to each other, but both were significantly lower than the control group ($p=0.001$ and $p=0.019$ respectively). The Lt total radius BMD in the ACPA positive group showed no statistical significant difference to that in the ACPA negative group but was significantly lower than the control group ($p=0.001$).

These findings show that the ACPA positive group has significantly higher bone loss in the 3 examined regions compared to the control group and a significantly higher bone loss in the lumbar spine compared to the ACPA negative group.

The generalized bone loss at the forearm, hip and lumbar spine in the ACPA positive group is consistent with a study of 32 premenopausal Egyptian RA patients by Mohsen et al[229] who had lower hip and lumbar spinal BMD compared to healthy controls. Similarly in a study of postmenopausal Egyptian RA patients by Gheita et al[224] BMD at the forearm, hip and lumbar spine was significantly lower in RA patients than in controls.

Other studies by Rass et al[157] and Gough et al[162] showed similar results of lower BMD at the hip and lumbar spine compared to controls. Another study by Hansen et al[230] showed lower BMD in the forearm of RA patients compared to age and sex matched healthy controls.

This is also supported by a study by Kocijan et al[20] that demonstrated that the degree of autoantibody positivity is an important indicators of skeletal damage in RA. Recently ACPA has been found to bind osteoclast precursor cells and directly promote their differentiation into bone-resorbing osteoclasts.[71]

In the ACPA negative group there was significantly higher bone loss only in the hip compared to the control group. This is consistent with a study by Haugeberg et al[118] that showed a significant higher bone loss only in the hip in the older RF negative patients. Considering ACPA negative and RF negative patients as the same phenotype can be supported by a study that showed both the ACPA negative RA subset and the RF negative RA subset showed a clearly distinct pattern of association with shared epitopes from the ACPA positive and RF positive RA considering them a similar genetic form.[231]

In the lumbar spine, compared to the control group, there was a statistically significant higher frequency of patients in the ACPA positive group with osteopenia (42%), osteoporosis (OP) (16%), ($p<0.001$) and reduced bone mass (61%), ($p<0.001$).

The frequency of patients with OP and reduced bone mass in the hip, in the current study, were similar to that in a study by Lodder et al.[167] While higher frequency of patients with OP and reduced bone mass in the spine were seen in the current study compared to that by Lodder et al.[167] This could be explained by the fact that most patients in the former study had moderate disease duration, low disease activity or were even in remission, with little radiological damage. This is supported by the fact that bone loss correlates with disease duration, disease activity[185] and radiological damage.[232]

While in the current study of premenopausal RA female patients there was no statistically significant difference in the frequency of osteopenia, OP or reduced bone mass in the hip and forearm between the 3 studied groups. A higher frequency of OP and reduced mass was demonstrated in the spine than in the hip and forearm. This is in contrary to the finding by Lodder et al[121] and Haugeberg et al[118] that demonstrated higher frequencies of OP in both the spine and hip in RA patients, mainly postmenopausal.

These contradictory findings could be explained by (1) the longer disease duration, (2) the older age group, and (3) the postmenopausal status of the studied patients in the other studies. In which osteoarthritis of the spine and increased fracture risk, decrease the reliability of the spinal DXA.[118, 121]

In this work we assessed bone loss in premenopausal RA female patients either generalized, localized or on the micro-architectural level. This was done by assessment of BMD of the spine, hip and forearm, assessment of mTSS and assessment of bone turnover markers (serum TP1NP, β-CTx and OCN). These measurements were then correlated with the patients' age, disease parameters and each other.

In the ACPA positive group, age was an important determinate for serum β-CrossLaps (β-CTx) level, where the older the patient, the higher the serum β-CTx level, indicating higher levels of bone resorption with increasing age.

This is consistent with the fact that bone loss and osteoporosis (OP) are considered diseases of aging.[150] However age didn't correlate with any other form of bone loss as detected with mTSS and DXA, this is in contrast to a the findings by Lodder et al[121] where higher age was associated with higher bone loss at the hip. This could be explained by middle age and premenopausal characteristic of the studied groups.

While age showed no statistically significant correlation with bone resorption in the ACPA negative group, which needs more elaborative work up investigation.

Disease duration in the ACPA negative group, showed a statistical significant correlation with bone turnover, as assessed by serum OCN (p=0.021), where the longer the disease duration the higher the bone turnover. This is consistent with several study by Als et al[233] and Laan et al[65] in which disease duration independently affected bone mass. Also a 10 years longitudinal follow up study of RA patients by Haugeberg et al[185] showed evidence of annual BMD loss. As higher bone turnover is indicative of predominate bone loss.[234] While disease duration showed no statistically significant correlation with serum OCN in the ACPA positive group.

Disease activity in the ACPA positive group, as assessed by DAS 28-ESR and -CRP, showed a statistical significant correlation with bone formation, as assessed by serum TP1NP level (p=0.016 and p=0.049 respectively). While disease activity as assessed by DAS 28-CRP only showed a statistical significant relation with bone resorption, as assessed by serum β-CTx level (p=0.037). While disease activity as assessed by both DAS 28-ESR and -CRP showed no statistically significant correlation with bone formation and resorption in the ACPA negative group.

Which demonstrates that the higher the disease activity the lower the bone formation and the higher the bone resorption. This is consistent with a review article by Deodhar et al[148] in which increase in disease activity was associated with decrease in some bone formation and increase in most bone resorption markers. Also the fact that bone resorption only correlated with DAS 28-CRP favors it as a more accurate disease index.

Bone turnover, as measured by serum OCN, had a positive correlation with bone formation, as measured by serum TP1NP (p<0.001), in both ACPA positive and negative patients. While it had a negative correlation with bone resorption, as measured by serum β-CTx (p=0.041), in ACPA positive patients. Indicating that the higher the level of bone turnover the higher the level of bone formation and the lower the levels of bone resorption.

This could be explained by the fact that OCN found in the circulation mainly results from its synthesis from osteoblasts. While only a small portion of the circulating OCN comes from degradation product of OCN found in the mineralized bone matrix.[134] From this it could be understood that serum OCN levels are proportionate to bone formation markers and inversely proportionate to resorption markers.

While bone turnover showed no statistically significant correlation with bone formation and resorption in the ACPA negative group. This emphasizes the fact that ACPA positive and negative RA are two different disease entities.[18,19]

BMI in ACPA positive group showed a statistically significant correlation with radiological damage in the hands and feet, as assessed by mTSS (p=0.044), where the lower the BMI the greater the radiological damage in the hands and feet.

This is similar to the findings by a study by Hashimoto et al[235] who found low BMI to be independently associated with 1-year progression of joint destruction under conventional DMARDs treatment. Similarly in two studies by Baker et al[236,237] higher BMI was associated with lower risk of joint destruction by both plain X-ray and magnetic resonance imaging (MRI) over 2 years.

The mechanism by which BMI is associated with radiological joint destruction is unknown. It was suggested that severe disease leads to weight loss and cachexia reflecting the burden of disease.[236] On the other hand others suggested that the endocrine function of adipose tissue could modulate the disease through adipokines, which is lowered with increase in BMI [238] and higher in RA.[239] Ebina et al[239] also demonstrated higher levels of adiponectin to be associated with radiographic joint destruction.

Disease duration in the ACPA positive group, showed a statistically significant correlation with radiological joint damage (p<0.001), where the longer the disease duration the greater the radiological damage. This is consistent with several longitudinal studies by Scott[100] and Drossaers et al[101] who showed continuous progressive radiological damage over the years.

Disease activity in the ACPA positive group, as assessed by DAS 28-CRP, showed a statistically significant correlation with radiological joint damage (p=0.042), where the higher the disease activity the greater the radiological damage. This is consistent with a study by Kuper et al[240] that showed a statistically significant correlation between disease activity and joint damage.

The cumulative corticosteroid (Cs) dose in the ACPA positive group, showed a statistically significant correlation with radiological damage (p=0.027), where the higher the cumulative Cs dose the greater the radiological damage. This could be explained by the fact that the more aggressive the RA the more aggressive the treatment (higher Cs intake) and thus more radiological damage.

Serum OCN, a bone turnover marker, showed a negative correlation with radiological damage, as assessed by mTSS, (p=0.03) in ACPA negative patients where the higher the serum OCN the lower the radiological damage. This could be explained based on the other finding in this study, where OCN was more correlated with bone formation.

While BMI, disease duration, disease activity, cumulative Cs dose and bone turnover showed no statistically significant correlation with radiological damage in the ACPA negative group.

Disease activity in the ACPA positive group, as assessed by DAS 28-CRP showed a statistically significant correlation with bone loss at the radius and spine, as assessed by DXA scan (p=0.05 and p=0.012 respectively), where the higher the disease activity the higher the bone loss at the radius and spine. While disease activity showed no correlation with bone loss in the hip in ACPA positive patients. This was consistent with a study by

Gough et al[162] in which disease activity was significantly associated with this spinal BMD loss. In another study by Haugburg et al[185] disease activity, as assessed by DAS-28ESR and –CRP, was associated with spinal and femoral bone loss only in early RA patients.

Disability, as assessed by the health assessment questionnaire (HAQ), showed statistically significant correlation with both bone loss at the forearm and lumbar spine (p=0.05 and p=0.012 respectively) in the ACPA positive patients, where the greater the disability the higher the bone loss. A study by Haugburg et al[185] showed some similarities with the current study, where disability was associated with spinal and femoral bone loss only in early RA patients. This is consistent with the fact that disability can lead to physical inactivity, which is a potent bone mass diminishing factor.[159]

Similarly the cumulative Cs dose in the ACPA positive group, showed a statistically significant correlation with bone loss at the femur and spine, as assessed by DXA scan (p=0.013 and p=0.015 respectively), where the higher the cumulative Cs dose the higher the bone loss. This is consistent with a study by Haugburg et al[185] and Lodder et al[121] in which cumulative Cs does was associated with bone loss but only at the femur.

In a study that assessed effect of prolonged low dose Cs in RA patients on bone by Hajiroussou et al,[174] the Cs treated patients had more severe OP than the Cs naïve RA patients, though the difference was not statistically significant. The same study showed that in the female RA patients the spine was more sensitive than the peripheral skeleton to the osteoporotic effect of Cs.

Bone turnover markers showed no significant correlation with BMD in the current study. This is consistent with a systemic review by Biver et al[241] which demonstrated that in premenopausal women, bone markers are rarely significantly correlated with BMD. High bone turnover showed some moderate correlations with BMD in postmenopausal OP patients compared to controls, which was more marked with bone alkaline phosphatase (BAP), OCN and CTx. Form this they concluded that higher levels of bone turnover markers may suggest screening for OP, especially when associated to high bone remodeling diseases.

In the ACPA positive group, radiological joint damage showed a positive correlation with BMD at the forearm and hip (p=0.008 and p=0.046 respectively), where the higher the joint damage the higher the bone loss at the forearm and hip. This is consistent with a study by Lodder et al[121] where the higher the radiological joint damage as assessed by Larson score the higher the bone loss at the hip. While no statistically significant correlation was found between joint damage and spinal BMD.

However, BMD at the forearm showed a positive correlation with bone loss at the hip (p=0.022) and spine (p=0.015), only in the ACPA positive group, where the higher the bone loss at the forearm, the higher the bone loss at the hip and spine. Also, BMD at the hip showed a positive correlation with bone loss at the spine (p=0.002), only in the ACPA positive group, where the higher the bone loss at the hip, the higher the bone loss at the spine.

These results show that there was a correlation between bone loss in the axial and appendicular skeleton in ACPA positive RA patients. This is consistent with a study by Zhu et al[242] in which BMD and microarchitectural parameters at the second metacarpal head and distal radius was moderately associated with BMD at the axial skeleton in 100 female RA patients.

The correlation between erosions (a localized form of bone loss), generalized bone loss at the appendicular and axial skeleton could be explained by the fact that bone loss anywhere in the body share common pathways through cytokines' activation of osteoclasts, which is supported by several studies.[69,94,95] This is also supported by the fact that denosumab, a monoclonal antibody against RANKL, showed reduced erosive joint damage in RA patients[96] and anti-TNF therapy reduced bone loss in RA patients.[97,184]

Disease activity, disability, cumulative Cs dose and radiological damage showed no correlation with bone loss in the spine, hip and radius in ACPA negative patients. As well as bone loss at the three sites did not correlate with each other in the ACPA negative group. This emphasizes the fact that ACPA positive and negative RA are two different disease entities.[18,19]

SUMMARY

Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory arthritis it is characterized by swelling, tenderness and destruction of the synovial joints. Although considered a "joint disease" RA is associated with involvement of extra articular manifestations (EAM) including secondary fibromyalgia (FM) and rheumatoid vasculitis. There are also important disease-associated comorbidities, including non-Hodgkin's lymphoma, ischaemic heart disease and osteoporosis (OP).

Similarly OP is the most common bone disease in humans, and it represents a major public health problem. It is characterized by low bone mass, deterioration of bone tissue and disruption of bone architecture, leading to compromised bone strength and an increase in the risk of fracture.

The prevalence of OP in RA increases about twofold compared with the general population. There is a decrease in bone mineral density (BMD) plus an increase in the risk of both hip and vertebral fractures in patients with RA compared with patients without RA.

The aim of this study was to assess OP in premenopausal RA patients and its correlation with other disease parameters.

Thus forty-three RA patients (31 anti-cyclic-citrullinated peptide antibodies (ACPA) positive patients and 12 ACPA negative patients) fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for the diagnosis of RA were enrolled in the study.

Detailed history was obtained from each patient followed by thorough clinical assessment with stress on musculoskeletal examination, disease activity and functional ability assessment. Bone turnover markers including serum total procollagen type 1 amino-terminal propeptide (TP1NP), β- CrossLaps (β-CTx) and N-MID Osteocalcin (OCN) were measured to assess bone turnover. Radiological assessment was done to evaluate erosions and joint space narrowing in both hands and feet by modified Sharp score (mTSS) and BMD of spine, hip, and forearm was measured by dual energy X ray absorptiometry (DXA).

Thirty apparently healthy individuals of matching age and sex to the patients were enrolled as a control group.

The ACPA positive group showed higher disease activity and disability than the ACPA negative group. ACPA positive group also showed a higher rate of bone turnover as assessed by serum β-CTx than the control group. More aggressive joint damage, as demonstrated by markedly higher mTSS, was found in the ACPA positive group than in the ACPA negative group.

The ACPA positive group also had a higher rate of bone loss, as demonstrated by markedly lower BMD, in the hip and forearm compared to ACPA negative group and higher rate of bone loss in the spine compared to ACPA negative and control groups. The ACPA negative group also has a higher rate of bone loss, only in the hip compared to the control groups.

There is a statistically significant correlation between bone turnover and both the patients' age and disease activity only in the ACPA positive group. There is a statistically significant correlation between joint damage and disease duration, body mass index (BMI), cumulative corticosteroid (Cs) dose and bone loss in the hip and forearm only in the ACPA positive group.

There is a statistically significant correlation between bone loss in the spine and disease activity, disability, cumulative Cs dose and bone loss in the hip and forearm in ACPA positive group.

CONCLUSION

Based on the results of this study the following can be concluded:

1. DAS 28-CRP is a more accurate disease activity index than DAS 28-ESR for assessment of short-term changes in disease activity.
2. There is higher risk of feet involvement in the ACPA positive patients compared to ACPA negative patients.
3. ACPA positive RA patients have higher disease activity, higher disability scores and more aggressive joint damage compared to the ACPA negative patients.
4. ACPA positive RA patients also have a higher rate of bone loss in the appendicular and axial skeleton and higher rate of bone turnover compared to the normal healthy controls.
5. The hip is the most common site for bone loss in ACPA negative patients compared to the normal healthy controls.
6. Risk factors for joint damage in ACPA positive RA patients are lower BMI, longer disease duration and higher disease activity.
7. Risk factors for higher rate of bone loss and bone turnover in ACPA positive RA patients are older RA patients and patients with higher disease activity, higher disability, higher levels of Cs intake and more aggressive joint damage.