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Bone Mineral Density in Female Patients with Rheumatoid Arthritis in Relation to Anti-Cyclic Citrullinated Peptide Antibody and Rheumatoid Factor

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune disorder associated with a chronic inflammation, which can cause joint damage and extra-articular complications. Anti-citrullinated protein antibody (ACPA) positivity is accompanied by poor prognosis in RA, and testing for ACPA has become traditional practice in the context of RA diagnosis.

Objective: The aim of the study was to estimate the prevalence of low bone mineral density (BMD) among female patients with RA using dual energy x-ray absorptiometry (DEXA), and to study the correlation between BMD & levels of anti-cyclic citrullinated peptide (anti-CCP) and RF in the studied cases.

Patients and methods: The present study was a cross-section descriptive study that was conducted on eighty female patients with RA. All patients were subjected to assessment to the levels of anti-CCP and RF using ELISA. BMD was measured by DEXA.

Results: Osteopenia and Osteoporosis at lumbar spine, femoral neck and distal end of radius showed statistically significant increase in +ve anti-CCP patients when compared to –ve anti-CCP patients. BMD at the lumbar spine, femoral neck and lower end of radius showed statistically significant decrease in +ve anti-CCP patients when compared to –ve anti-CCP patients. History of fragility fracture showed statistically significant increase in RA patients with +ve RF when compared to -ve RF patients.

Conclusion: Bone loss and fragility fracture are common in RA and increased in seropositive RA, post-menopausal state and corticosteroids use. The presence of ACPA and RF are strongly predictive for the development of osteoporosis and erosions in RA patients. It would be a more appropriate approach to carefully monitor osteoporosis in seropositive RA.

Keywords: Anti-CCP, Rheumatoid factor, Rheumatoid arthritis, Dual energy X-ray absorptiometry.

INTRODUCTION

RA is a chronic immune-inflammatory disease accompanied by bone loss, erosions, and osteoporosis. Erosive RA and osteoporosis have a comparable cellular pathway, that comprises inflammatory stimulation of osteoclasts and reduced osteoblast stimulation [1]. Minimal BMD in cases with RA has been demonstrated to be associated with a higher possibility of fractures and mortality, particularly among postmenopausal females [2].

The best approach for measurement of systemic BMD is DEXA. ACPA positivity is accompanied by poor prognosis in RA, and evaluating ACPA has become traditional practice in the context of RA diagnosis [3]. Novel study recommended that ACPA could trigger bone loss and serum receptor activator of nuclear factor kappa beta (RANKL) is recorded to be elevated in ACPA-positive cases regardless of acute phase reactants and proinflammatory cytokines [4].

Cases with positive anti-cyclic citrullinated peptide-II antibodies were recorded to be accompanied by bone damage, which initiates before the onset of RA [5]. Increase in anti-CCP2 antibody values revealed to be a predictor of localized hand digital X-ray radiogrammetry (DXR) BMD loss in cases with early RA [6]. Data analysis showed that anti-CCP2 antibodies and RF were accompanied by bone loss in cases with early, untreated RA [7].

This work was done to estimate the prevalence of low BMD among female patients with RA using DEXA and to study the correlation between BMD & levels of anti-CCP and RF in the studied cases.

PATIENTS AND METHODS

This was a cross-section descriptive study performed on eighty (80) female cases with RA who were diagnosed based on ACR/EULAR [8] classification criteria for RA. The patients were selected from Outpatient Clinics of Rheumatology & Rehabilitation Department, Mansoura University Hospitals through the period from January 2021 to October 2021.

Exclusion criteria: Patients with other immune–mediated diseases as systemic lupus, scleroderma, Behcet disease and ankylosing spondylitis. Patients with medical conditions as celiac disease, inflammatory bowel disease, renal or hepatic diseases, malignant tumours and multiple myeloma. Patients with hormonal disorders as thyroid problems, hyperparathyroidism, hypogonadism and patients on medications that can cause bone loss as anti-depressants, anti-epileptics, loop diuretics, heparin and warfarin.

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American College of Rheumatology/European League Against Rheumatism (EULAR) Criteria for AR Diagnosis (Figure 1):

Criteria		Points
Joint disease	1 large	0
	2–10 large	1
	1–3 small	2
	4–10 small	3
	>10 (at least one small)	5
Serology	Negative RF and ACPA	0
	Low positive RF/ACPA	2
	High positive RF/ACPA	3
Acute phase reactants	Normal CRP/ESR	0
	Abnormal CRP/ESR	1
Symptom duration	< 6 weeks	0
	> 6 weeks	1

Figure (1): ACR-EULAR criteria.

The 2010 ACR-EULAR criteria are utilized for RA diagnosis. RA diagnosis is established if the total score is > 6 and other causes of synovitis were ruled out [8].

Assessment of Disease activity

The most frequently used measures are the disease activity score (DAS) 28, simple disease activity index (SDAI), and the clinical disease activity index (CDAI). DAS28 its calculation can be done either with ESR, CRP, or both, depending on their availability [9].

Methods

All cases were subjected to complete history taking which included age, duration of disease, medical diseases. The clinical examination included general examination (general appearance, body built, mental & neurological status, complexion, vital signs & skin and eye & thyroid examination) and musculoskeletal examination (inspection, palpation of affected joints, movement assessment, para-articular structures, spine, sacroiliac joints and muscle examination).

Laboratory tests included complete blood count, antinuclear anti-bodies, RF, anti-CCP, ESR, CRP, serum creatinine, urine analysis and liver function tests. BMD was measured by DEXA. Osteoporosis and low BMD were described by utilizing the WHO criteria. Osteoporosis was described as a T-score ≤ –2.5 at the femoral neck or the lumbar spine. Among those without osteoporosis, low BMD was described as those with T-scores between –1.0 and –2.5 at either skeletal site.

Radiological investigations included x-ray for the affected joints, chest x-ray (CXR) to exclude pleural and pericardial effusion, pulmonary infiltration, pneumonitis, pulmonary infarction, pulmonary fibrosis & cardiac enlargement and abdominal ultrasound. ECG was done to detect pericardial effusion & myocardial infarctions when needed.

Specific Investigations

All patients were subjected to assessment to the values of anti-CCP and RF using ELISA and nephlometry techniques (Inova Diagnostics Company, Catalog No. 704535, China) (MD PACIFIC BIOTECHNOLOGY CO. Catalog No. MD219.SL, China) retrospectively.

Assay of the Anti-CCP

The antigen used in the test was a synthetic CCP, which was bound to the surface of a microwell plate. Pre-diluted control samples and diluted patients' samples were added to separate wells, to allow binding of any CCP IgG antibodies to antigen. Unbound sample was removed and the enzyme labeled anti-human IgG conjugate was added to wells. Then, incubation was performed to allow binding of the enzyme labeled anti-human IgG to any patient antibodies, which are attached to microwells. Following washing of unbound enzyme labeled anti-human IgG, the residual enzyme activity was evaluated by adding a chromogen substrate and measurement of the intensity of developing color. The assay was measured spectrophotometrically by measuring colour intensity in the patient wells and comparing it with that in control wells.

Procedure

The reagents were brought to 22 °C before the assay. The required strips were placed in the holder. 100µl of prediluted CCP3 IgG ELISA low positive was added, the CCP3 IgG ELISA high positive, calibrators B to E, ELISA negative control and diluted patients' samples to wells, which were covered and underwent incubation over 30 min on a level surface. Thoroughly the contents were aspirated of all wells. 200-300 µl of diluted HRP was added and washed buffer to each well, the aspirate was repeated twice more for 3 washes. The plate and tap were inverted and were tapped on absorbent material to wash any remaining fluid. 100 µl of the HRP CCP3 IgG was added and conjugated to all wells. The volume of conjugate from the bottle required for the assay was removed. The wells were incubated for 30 minutes on a level surface at 22 °C. 100 µl of TMB chromogen was added to wells and incubated for 30 minutes at 22 °C. Then 100 ml of HRP stop solution was added to each well. Identical sequence and timing of HRP stop solution addition was maintained as was utilized for the TMB chromogen. The optical densities

(OD) of all wells were read at 450 nm within 60 minutes after adding stop solution. The mean value was determined for all duplicate measurements. The mean optical density of samples was plotted in standard curve against their values in units. A linear/linear cubic spline was used for drawing of the curve. The CCP3 value of a sample was determined from "Y" axis after reading its corresponding OD on "X" axis. The human anti-CCP of the samples can be inserted from the standard bend.

Assay of rheumatoid factor

Latex-enhanced immunonephelomtric was applied. Latex particles coated with human IgG aggregate in the presence of RF forming immune complexes. The aggregation causes an increase in turbidity, which is proportional to the concentration of RF in the specimen. Concentration was measured in an automated manner by reference to a calibration curve stored in the instrument.

Assay Procedure

The frozen reagents should stand a while until they get to 22 °C (18-25 °C). R1-reagent can be stored at temperature only in case of short time storage within 1 week. Switch imagine on, insert the IC card after warming up finished, click on the analysis icon on the screen, then the test name RF showed on screen and dilution degree is 11. We added 240 ul RF R1 into the cup, put it into incubating position of analyzer and incubate it for 5 minutes or above. Sample was diluted with sample diluent as proportion of 1:11 (e.g. 400 ul sample diluent + 40 ul sample).

Pipette was used to add 40 ul diluted sample into the cup. When the analyzer showed, we added R1+ sample, put the cup into testing position straightly. The analyzer sensed the cup and started mixing sample and R1 reagent automatically. After the mix finished, it turned to a short-time incubation. After the incubation, the analyzer showed "Add R2", we used pipette to add 50 ul RF reagent, the analyzer started mixing and analyzing-countdown automatically. After countdown finished, test results were shown on screen directly. We removed reagent cup, pressed the back key to start a next RF test. After all RF tests were finished, we changed the other item's IC card, pressed back key, analyzer showed new item name and lot NO. Then test of other kit could be performed. After the use of kit, the remaining R2 reagent should be stored in 2-8 °C with the top of bottle screwed tightly. R1 reagent can be placed in room temperature and stored for short time (1 week), but please cover the foam box properly to avoid contamination. 2-8°C refrigerated if long term store was wanted. Diluent can be stored in room temperature with sealed tightly, 0-30 IU/ml was considered within the normal range, this range was given for orientation only. Each laboratory established its own reference values.

Ethical approval:

The Ethics Committee of Faculty of Medicine, Mansoura University granted the study approval. All participants signed informed consents after a thorough explanation of the goals of the study and submitted to detailed personal and past history questions including previous medications and periods of activity. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

Data were analyzed by SPSS for windows version 20.0 (SPSS, Chicago, IL). Continuous data were described as means ± standard deviations (SDs), whereas categorical data were described in numbers and percents. The differences between the groups were detected by independent sample Student's t test for continuous data and chi-square test for categorical data. Correlation co-efficient test was used to evaluate the correlation between serum levels of anti-CCP & RF and BMD parameters. Statistical significance was set at $p \leq 0.05$.

RESULTS

The Mean age was 46.3 ±11.8 ranging from 28 to 80 years, there were 51 (63.8%) premenopausal patients and 29 (36.2%) postmenopausal patients. Mean duration of postmenopausal state was 10.6 ±7.4 ranging from 1 to 27 years. Mean duration of RA (years) was 14.8 ± 5.1. Duration of morning stiffness (minutes) was 138.7 ± 64.6, TJC was 13.9 ± 6.5 and SJC was 15.2 ± 6.7. Patient global health assessment (PGA) was 49.7 ± 19.7, DAS28-ESR was 4.79 ± 1.12 and DAS28-CRP was 4.24 ±1.03. Concerning the laboratory and radiological findings of RA patients; Mean Hb concentration was 10.6 ± 1.2 (gm/dl), RBCs count was 3.9 ± 0.5($x10^6/mm^3$), WBCs count was 6.6 ±1.4 ($x10^3/mm^3$), platelets count was 257.4 ± 62.7 ($x10^3/mm^3$), ESR level (1st hour) was 42.6 ± 10.4, CRP level was 24.8 ± 6.1 (mg/dl) and the mean modified sharp score was 53.9 ± 13.2. RF (IU/L) was + ve in 62 (77.5%) with Mean of 116.3 ± 27.8 and Anti-CCP (IU/L) was +ve in 57 (71.3%) with Mean of 157.6 ± 63.9 (Table 1).

Table (1): Age, menopausal state, clinical features, DAS28-ESR, laboratory, radiological findings and AAbs serum level of the RA patients

	Range	**Mean ±SD**
Age	28 – 80	46.3 ±11.8
Duration of Post-menopausal state (years) (n=29)	1 – 27	10.6 ±7.4
Duration of RA (years)	6 – 22	14.8 ±5.1
Duration of morning stiffness (minutes)	30 – 240	138.7 ±64.6
TJC (tender joint count)	3 – 27	13.9 ±6.5
SJC (swollen joint count)	3 – 26	15.2 ±6.7
Patient global health assessment (mm)	12 – 80	49.7 ±19.7
DAS28-ESR	2.18 – 7.96	4.79 ±1.12
DAS28-CRP	2.15 – 6.25	4.24 ±1.03
Blood Picture		
• **Hb concentration (gm/dl)**	10.6 ±1.2	
• **RBCs count (x106 / mm^3)**	3.9 ±0.5	
• **WBCs count (x103 / mm^3)**	6.6 ±1.4	
• **Platelets count (x103 / mm^3)**	257.4 ±62.7	
Acute Phase Reactants		
• **ESR level (1st hour)**	42.6 ±10.4	
• **CRP level (mg/dl)**	24.8 ±5.1	
• **Radiological assessment Modified Sharp score**	53.9 ±11.2	
• **RF (IU/L)**	116.3 ±27.8	
• **Anti-CCP (IU/L)**	157.6 ±6.9	

There was no statistically significant difference between RA patients with -ve anti-CCP and patients with +ve anti-CCP regarding age, post-menopausal state and duration of menopause ($p > 0.05$). There was no statistically significant difference between RA patients with -ve anti-CCP and patients with +ve anti-CCP regarding duration of RA (years), duration of morning stiffness (minutes), TJC, SJC, patient global health assessment (mm), DAS28-ESR and DAS28-CRP ($p > 0.05$). There was no statistically significant difference in Hb concentration, the RBCs count, WBCs count, platelets count, ESR level (1st hour), CRP level and Modified Sharp score between RA patients with -ve versus +ve anti-CCP. There was no statistically significant difference between RA patients with -ve anti-CCP and patients with +ve anti-CCP regarding frequency of drug intake ($p > 0.05$) (Table 2).

Table (2): Comparison of the age, postmenopausal state, and duration of menopause between RA patients with +ve versus -ve anti-CCP

	RA patients with					
	-ve anti-CCP		**+ve anti-CCP**		**Student's t test**	
	Mean ±SD		**Mean ±SD**		**t**	**p**
Age (years)	45.3 ±12.3		46.5 ±11.3		0.433	0.666
Postmenopausal state (n, %)						
Pre-menopausal	16, 69.6%		35, 61.4%			
Post-menopausal	7, 30.4%		22, 38.6%		0.472*	0.492
Duration of menopause	12.1 ±5.7		10.0 ±4.6		0.995	0.329
Duration of RA (years)	15.3 ±5.2		14.6 ±5.2		0.569	0.571
Duration of morning stiffness (minutes)	127.1 ±59.3		143.4 ±66.6		1.020	0.311
TJC	12.3 ±6.1		14.5 ±6.2		0.821	0.419
SJC	15.2 ±6.4		15.2 ±6.8		0.043	0.966
Patient global health assessment (mm)	47.1 ±20.6		50.7 ±19.4		0.741	0.461
DAS28-ESR	4.5 ±1.1		4.9 ±1.2		1.196	0.236
DAS28-CRP	4.0 ±1.0		4.4 ±1.1		1.293	0.200
Blood Picture						
Hb concentration (gm/dl)	10.8 ±1.0		10.6 ±1.3		0.798	0.427
RBCs count ($x10^6$ / mm^3)	3.9 ±0.6		3.8 ±0.94		0.600	0.550
WBCs count ($x10^3$ / mm^3)	7.0 ±1.74		6.4 ±1.5		1.169	0.246
Platelets count ($x10^3$ / mm^3)	253.7 ±61.2		258.9 ±63.1		0.276	0.784
Acute Phase Reactants						
ESR level (1st hour)	40.2 ±9.9		43.6 ±10.5		0.661	0.511
CRP level (mg/dl)	24.9± 6.1		25.7±6.3			0.538*
Radiological assessment Modified Sharp score	43.8±21.5		54.1±21.6		1.524	0.132
Corticosteroids	**n**	**%**	**n**	**%**	**X^2**	**P**
No	14	60.9	39	68.4		
Yes	9	39.1	18	31.6	0.418	0.518
Methtrexate (MTX)						
No	8	34.8	18	31.6		
Yes	15	65.2	39	68.4	0.077	0.782
Hydroquine (HCQ)						
No	13	56.5	29	50.9		
Yes	10	43.5	28	49.1	0.209	0.647
Leflunomide						
No	14	60.9	38	66.7		
Yes	9	39.1	19	33.3	0.242	0.623
Biologicals						
No	18	78.3	45	78.9		
Yes	5	21.7	12	21.1	0.005	0.946
Bisphosphonates						
No	20	87.0	53	93.0		
Yes	3	13.0	4	7.0	0.745	0.388
Calcium						
No	10	43.5	17	29.8		
Yes	13	56.5	40	70.2	1.366	0.242
Vitamin D						
No	10	43.5	18	31.6		
Yes	13	56.5	39	68.4	1.020	0.313

X^2 value: Chi square test, *p value: Mann-Whitney U test

Osteopenia and osteoporosis at lumbar spine, neck of femur and distal end of radius showed statistically significant increase in +ve anti-CCP patients when compared to –ve anti-CCP patients (p=0.008- 0.008 and 0.024 respectively)

(Table 3). BMD at lumbar spine, neck of the femur and distal end of radius showed statistically significant decrease in +ve anti-CCP patients when compared to –ve anti-CCP patients (p=0.006-0.002 and 0.008 respectively) **(Table 4)**.

History of fragility fracture showed statistically significant increase in +ve anti-CCP patients when compared to –ve anti-CCP patients (p=0.012) **(Table 5)**. There was no statistically significant difference was found regarding age, postmenopausal state, and duration of menopause between RA patients with +ve versus –ve RF (p>0.05). There was no statistically significant difference between RA patients with –ve RF and patients with +ve RF regarding duration of RA (years), duration of morning stiffness (minutes), TJC, SJC, patient global health assessment (mm), DAS28-ESR and DAS28-CRP (p >0.05).

There was no significant difference between RA patients with –ve RF and patients with +ve RF regarding frequency of drug intake (p >0.05). There was no statistically significant difference in Hb concentration, the RBCs count, WBCs count, platelets count, ESR level (1st hour), CRP level and Modified Sharp score between RA patients with -ve versus +ve RF.

Table (3): Association between anti-CCP and the bone quality and BMD in patients with RA

	RA patients with					
	-ve anti-CCP		**+ve anti-CCP**		**Chi square test**	
	n	%	n	%	X^2	p
Bone quality at lumbar spine						
Normal	9	39.1	6	10.5		
Osteopenia	10	43.5	29	50.9		
Osteoporosis	4	17.4	22	38.6	9.602	0.008
Bone quality at neck of the femur						
Normal	12	52.2	11	19.3		
Osteopenia	8	34.8	25	43.9		
Osteoporosis	3	13.0	21	36.8	9.582	0.008
Bone quality at distal end of radius						
Normal	13	56.5	14	24.6		
Osteopenia	6	26.1	26	45.6		
Osteoporosis	4	17.4	17	29.8	7.487	0.024

Table (4): Comparison of the BMD between RA patients with +ve versus –ve anti-CCP

	RA patients with		
	-ve anti-CCP	**+ve anti-CCP**	**Mann-Whitney U test**
	Median [IQR]	Median [IQR]	p
BMD at the:			
Lumbar spine	-1.30 [1.50]	-2.10 [1.65]	0.006
Neck of the femur	-0.50 [2.70]	-1.90 [1.60]	0.002
Distal end of radius	-0.50 [1.70]	-1.70 [1.85]	0.008

Table (5): Association between anti-CCP and history of fragility fracture in patients with RA

	RA patients with					
	-ve anti-CCP		**+ve anti-CCP**		**Chi square test**	
History of fragility fracture	n	%	n	%	X^2	p
No	21	91.3	36	63.2		
Yes	2	8.7	21	36.8	6.338	0.012

There was statistically significant difference between with +ve RF when compared to RA patients with -ve RF. Osteoporosis showed significant increase in RA patients with +ve RF when compared to RA patients with -ve RF. History of fragility fracture showed statistically significant increase in RA patients with +ve RF when compared to -ve RF patients. BMD at the, Lumbar spine, Neck of the femur and Distal end of radius showed statistically significant decrease in RA patients with +ve RF and patients with -ve RF (Table 6).

BMD at the lumbar spine, neck of the femur and distal end of radius showed statistically significant decrease in postmenopausal RA patients when compared to premenopausal RA cases. BMD at the lumbar spine, neck of the femur and distal end of radius showed statistically significant decrease in RA patients with current utilization of corticosteroids in comparison with RA cases without current use of corticosteroids (Table 6).

Linear regression analysis was conducted to assess factors that can predict the T score at the lumbar spine in RA patients. Current use of steroids, DAS28.ESR, DAS28.CRP and Anti-CCP titer were statistically significant predictors of T score at the lumbar spine in RA patients. Linear regression was conducted to assess factors that can predict the T score at the neck of femur in RA patients. Current use of steroids, DAS28.ESR, DAS28.CRP and anti-CCP titer were statistically significant predictors of T score at the neck of femur in RA patients (Table 6).

Linear regression analysis was conducted to assess factors that can predict the T score at the distal end of radius in RA patients. Current use of steroids, DAS28.ESR, DAS28.CRP and anti-CCP titer showed statistically significant predictors of T score at the distal end of radius in RA patients (Table 6).

Table (6): Association between RF and the bone quality and BMD, and history of fragility fracture and Comparison of the BMD between RA patients with +ve versus -ve RF

	RA patients with					
	-ve RF		**+ve RF**		**Chi square test**	
	n	%	n	%	X^2	p
Bone quality at lumbar spine						
Normal	7	38.9	8	12.9		
Osteopenia	9	50.0	30	48.4		
Osteoporosis	2	11.1	24	38.7	8.301	0.016
Bone quality at neck of the femur						
Normal	9	50.0	14	22.6		
Osteopenia	8	44.4	25	40.3		
Osteoporosis	1	5.6	23	37.1	8.331	0.016
Bone quality at distal end of radius						
Normal	11	61.1	16	25.8		
Osteopenia	6	33.3	26	41.9		
Osteoporosis	1	5.6	20	32.3	9.199	0.010
History of fragility fracture						
No	17	94.4	40	64.5		
Yes	1	5.6	22	35.5	6.100	0.014
BMD at:	Median [IQR]		Median [IQR]		Mann-Whitney U test (P value)	
Lumbar spine	-1.35 [1.28]		-2.00 [1.75]		0.009	
Neck of the femur	-1.10 [2.88]		-1.85 [1.75]		0.012	
Distal end of radius	-0.95 [2.25]		-1.55 [2.35]		0.037	

DISCUSSION

RA is an autoimmune disorder associated with a chronic inflammation, which can cause joint damage and extra-articular complications, comprising the cardiac, renal, cerebral, and ocular tissues [10]. Osteoporosis (OP) is an extra-articular complication in RA. It might be induced by inflammation or glucocorticoid therapy. It is well-known that bone resorption is increased in RA because of upregulation of inflammatory cytokines. Moreover, bone formation is troubled in RA cases [11].

Recent study proposed that antibodies to citrullinated proteins (ACPAs) might enhance osteoclasts' differentiation and thus bone degradation. ACPAs enhance IL-8-mediated osteoclasts' differentiation from monocyte-derived or circulating CD1c+ DCs. ACPAs binding to DCs may be linked to activation and differentiation toward osteoclast lineage, enhancing the risk of bone erosions in ACPA-positive RA [12].

About 68% of the cases were negative for IgM-RF and ACPA, 3% were low positive, and 29% were high positive for IgM-RF and/or ACPA. The present study revealed that the frequency of normal BMD in the lumbar spine of the RA patients was 18.8% (n=15), osteopenia was 48.8 % (n=39) and osteoporosis was 32.5 % (n=26). The frequency of normal BMD of the neck femur of the RA patients was 28.8% (n=23), osteopenia was 41.3% (n=33) and osteoporosis was 30.0 % (n=24). The frequency of normal BMD of the distal end of radius of the RA patients was 33.8% (n=27), osteopenia was 40.0% (n=32) and osteoporosis was 26.3 % (n=21). Osteopenia occurred most commonly in lumbar spine (39.48%), followed by left neck of femur (33.41%) and left distal end of radius (32.4%). Osteoporosis occurred most frequently in lumbar spine (26.32%), followed by left neck of femur (24.3%) and left distal end of radius (21.26%). **Hafez *et al.*** [13], demonstrated that, 13.3% of RA patients had OP, 50% had osteopenia, and 36.7% had normal BMD. The commonest site of OP was lumbar spine (13.3% of cases), then the femur (6.6% of cases), and the forearm (3.3% of cases).

The present study revealed that Rheumatoid Factor (IU/L) was + ve in 62 (77.5%) with mean of 116.3 ± 27.8. Anti-CCP (IU/L) was +ve in 57 (71.3%) with mean of 157.6 ± 63.9. **Naredo *et al.*** [14], found that RF was positive in 30 (71.4%) RA cases and negative in 12 (28.6%) patients with a mean of 135±160 IU/ml.

The present study revealed no significant difference between RA patients with -ve RF and patients with +ve RF regarding age, post-menopausal state, duration of menopause, duration of RA (years), duration of morning stiffness (minutes), TJC, SJC, PGA (mm), DAS28-ESR, DAS28-CRP, laboratory finding and frequency of drug intake. In line with our finding, **Cader *et al.*** [15] showed that the anti-CCP positive and negative patients were comparable as regards demographics, inflammation biomarkers, joint number and 1987 ACR classification criteria, however the more anti-CCP positive cases had significantly more positive rheumatoid factor (83.3% versus 11.4%, $p < 0.01$). No significant difference was reported in the pattern of joint affection with exception of the high prevalence of knee joint swelling in anti-CCP positive cases (42.9% versus 22.2%, $p = 0.03$). **Serdaroğlu *et al.*** [16] revealed that there was no significant association between anti-CCP antibody and ESR, CRP, VAS and DAS 28 or radiologic evaluation. However, significant correlation was demonstrated between RF and anti-CCP antibody. Also, **Youssef *et al.*** [17] studied 110 anti-CCP positive RA cases and 45 anti-CCP negative RA cases. No significant differences were found regarding demographics (age, sex, disease duration and therapy, etc.) amongst both groups. Contrarily to our results, **Madan *et al.*** [18] showed that there was a significant association in the titers of anti-CCP and disease activity indices-SDAI and CDAI. Also, **Soha *et al.*** [19] have demonstrated that serum anti-CCP levels were significantly greater in RA cases than in controls. Anti-CCP levels demonstrated a positive significant association with RF, CRP, and disease activity score.

The present study revealed that the RA cases with positive anti-CCP had significantly higher frequency of osteopenia and osteoporosis at lumbar spine, femoral neck and lower end of radius, significantly lower BMD at the lumbar spine, neck of the femur and distal end of radius and significantly higher frequency of fragility fracture in comparison with -ve anti-CCP patients ($p=0.012$). The basis for lower BMD in ACPA-positive disease is not known, however anti-CCP positivity has been associated with reduced BMD even in early RA, signifying that systemic bone loss is a distinctive of ACPA-positive RA [7]. A similar finding is reported by **Orsolini and his colleagues** [20] who demonstrated a negative association between titers of anti-CCP and BMD at many locations in RA cases. In the same line, **Guler and his colleagues** [21] found that anti-CCP had a limited negative association with lumbar BMD and a negative association with femoral BMD. Sharp scores were significantly greater among anti-CCP-positive cases compared with anti-CCP-negative cases and anti-CCP concentrations showed a significant correlation with Sharp scores ($r=0.240$, $p=0.032$). **Wysham *et al.*** [22] showed that high-positive anti-CCP ($\beta=-0.054$, $p=0.016$), and lower appendicular lean mass index (ALMI) ($\beta=-0.053$, $p<0.0001$), were significantly linked to lower BMD in an independent manner.

The present study demonstrated that significant difference was found regarding age, postmenopausal state, duration of menopause, duration of RA (years), duration of morning stiffness (minutes), TJC, SJC, PGA (mm), DAS28-ESR, DAS28-CRP, laboratory findings and frequency of drug intake between RA patients with +ve versus -ve RF. In agreement with our results, **Hwang and his colleagues**[23] revealed no change in the

mean age between RF-positive and RF-negative subjects. Also, past medical history and laboratory findings were identical between both groups.

The present study revealed that there was significant higher ratio of osteoporosis in cases with +ve RF in comparison with RA cases with -ve RF. BMD at the lumbar spine, femoral neck and distal end of radius showed significant decrease in RA cases with +ve RF and patients with -ve RF. History of fragility fracture showed significant increase in RA cases with +ve RF when compared to -ve RF cases. Also, **Bugatti *et al.*** [7] showed that RF was linked to a low BMD in lumbar and hip regions, and only at high titrations. In the presence of RF and ACPA, the latter had a negative effect on lumbar Z-score even at low RF titers.

Our study showed that BMD at the, lumbar spine, femoral neck and distal end of radius showed significant decrease in postmenopausal RA patients when compared to premenopausal RA cases. There was significant difference between premenopausal RA patients in comparison with postmenopausal RA cases. Osteoporosis demonstrated significant increase in postmenopausal RA cases in comparison with premenopausal RA cases. In line with our finding, **Coulson *et al.*** [24] showed that postmenopausal state and higher modified health assessment questionnaire score (mHAQ) had detrimental effects on lumbar spine score. BMD at the lumbar spine, femoral neck and distal end of radius showed significant decrease in RA cases with current use of corticosteroids in comparison with RA patients without current use of corticosteroids. This may be explained by long-term therapy with GCs contributes to reduced osteogenesis and OP through promoting osteoclast-mediated bone degradation and collagen and bone matrix decomposition and by suppressing osteoblasts' activity [25].

The present study revealed that T score at lumbar spine, femoral neck and distal end of radius showed significant positive correlation with age, duration of disease, DAS28-ESR and DAS28-CRP. Linear regression analysis was conducted to assess factors that can predict the T score in RA patients. Current use of Steroids, DAS28.ESR, DAS28.CRP and Anti-CCP titer were significant predictors of T score at the lumbar spine, femoral neck and the distal end of radius in RA patients. In line with our finding, **Hafez *et al.*** [26] showed that T score at lumbar, radius and femur showed significant negative correlation with ESR, disease duration, DAS score 28, SENS score and HAQ. Contrarily, **Coulson *et al.*** [24] did not report a significant association with prednisone and fracture risk or T score.

CONCLUSION

Bone loss and fragility fracture are common in RA and increased in seropositive RA, post-menopausal state and corticosteroids use. The presence of ACPA and RF are strongly predictive for the development of osteoporosis and erosions in RA cases. It would be a more appropriate approach to carefully monitor osteoporosis in seropositive RA.

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