AIM OF THE WORK

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

SUBJECTS

Patients

Forty-three premenopausal rheumatoid arthritis (RA) female patients attending the Outpatient Clinic of Physical Medicine, Rheumatology and Rehabilitation Department, Main University Hospital were enrolled in this study.

They were further categorized into 2 groups according to anti-cyclic-citrullinated peptide antibodies (ACPA) into 31 ACPA positive patients, and 12 ACPA negative patients. The categorization of the patients into 2 groups according to ACPA levels was done at the end of the study, after data collection and before statistical analysis was done. (Figure 9)

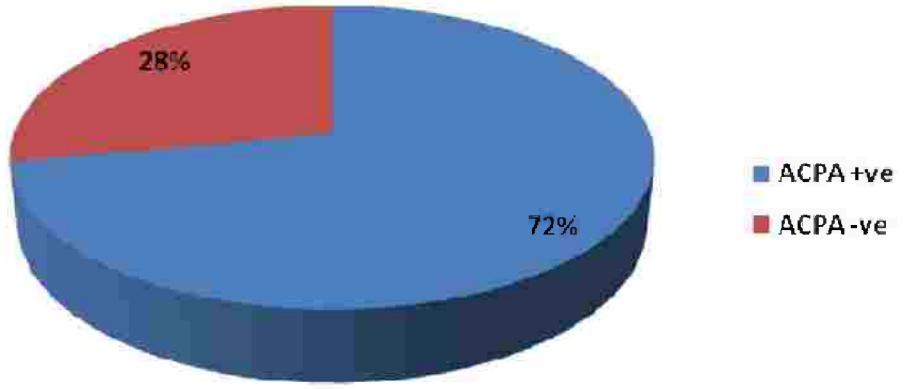


Figure (9): Percentage of patient in the ACPA positive and negative groups.

Inclusion criteria

1. Fulfillment of the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA.[35] (Annex I)
2. Rheumatoid arthritis patients in the pre-menopausal period to exclude postmenopausal and age related osteoporosis (OP).

Exclusion criteria

1. Patients with a history of taking treatment for OP.
2. Patients with concurrent medical conditions known to affect bone metabolism (including renal or liver disease, malignancy or endocrine disorders).[195]

Control subjects

Thirty age matched healthy premenopausal females were included in the study as the control group.

The study was explained to the participants and an informed consent was given by each, after approval from the local ethical committee of Faculty of Medicine, Alexandria University.

METHODS

The following data were obtained from each participant:

I. Demographic characteristics

1. Name.
2. Age.
3. Marital status.
4. Parity (number of children).
5. Occupation: participants were categorized according to type of job into:
   a. House wives.
   b. Jobs that required prolonged standing example; nurses and teachers.
   c. Desk jobs example; secretaries, accountants and tailors.

II. Personal history

1. Menstrual history and age of menarche.
2. History of smoking: each participant was asked about their smoking history in detail (number of cigarettes/day and duration).
3. Coffee intake: each participant was asked about the average number of cups of coffee they drank/ day.
4. Dietary habits: each participant was asked about the amount and frequency of dairy products intake since childhood and were then categorized into 2 categories:
   a. Participant that received adequate amounts of dairy products/day (those who received daily requirements of dairy products according to age).
   b. Participant that received inadequate amounts of dairy products/day (those who didn't receive daily requirements of dairy products according to age).
5. Calcium and vitamin D supplements: previous intake and amount of calcium and vitamin D supplements during the course of the disease was recorded for each participant and accordingly were categorized into 3 groups:
   a. Never received calcium and vitamin D supplements,
   b. Infrequently received calcium and vitamin D supplements,
   c. Daily received calcium and vitamin D supplements.
6. Personal history of fragility fracture was recorded.

III. Family history:

1. Family history of an autoimmune disease (RA) in a first degree relative was recorded.
2. Family history of fragility fracture was recorded.

IV. Drug history was obtained from the patients:

1. Non-steroidal anti-inflammatory drugs (NSAIDs) intake: number of patients that received NSAIDs during the course of their disease was recorded in the patients' groups.
2. Cumulative intake of corticosteroids (Cs): It was estimated by detailed history taking and by reviewing each prescription, where Cs received by each patient during the course of their disease were converted to its equivalent dose of prednisolone and the total amount of prednisolone was summed up in grams (gms).[196, 197]

3. Disease modifying anti-rheumatic drugs (DMARDs) intake: detailed history of the type and duration of DMARDs as well as the combination therapy was recorded.

V. Historical data for the present condition was obtained from the patients:

1. Onset of the disease.
2. Disease duration.
3. Duration of morning stiffness.
4. History of fatigue.

VI. Disability assessment by Health Assessment Questionnaire (HAQ). (Annex II)

The HAQ is a comprehensive measure of outcome in patients with a wide variety of rheumatic diseases, including rheumatoid arthritis, osteoarthritis, and other rheumatic conditions.[198]

Physical function refers to the ability to move one's body purposefully to accomplish a task. These tasks include self-care, such as dressing, eating and hygiene, transferring, ambulation, moving objects, and household chores. Accomplishing these tasks requires some measure of strength, flexibility, and endurance; and functional limitations can result from any process that compromises any one of these factors.[199]

Rheumatoid arthritis can have a profound impact on physical functioning, due to the widespread polyarthritis that is often present and in particular the frequent involvement of hand joints. Joint pain, swelling, and stiffness due to active synovitis can cause functional limitations by decreasing strength and flexibility. Pain and fatigue can also decrease endurance. Joint deformities and instability, muscle wasting, and deconditioning may occur as a consequence of longstanding synovitis can also result in functional limitations. Psychologic status, mood, and comorbid conditions may also affect physical function.[200] From the patient's perspective, functional capacity is the most relevant outcome.[42]

The HAQ includes items that assess fine movements of the upper extremity, locomotor activities of the lower extremity, and activities that involve both the upper and lower extremities during the past week. Standard scoring takes into account the use of aids and devices or assistance from another person. There are 20 items in eight categories, each category contains at least two specific sub-category questions. For each item, there is a four-level response set that is scored from 0 to 3, with higher scores indicating more disability. To calculate the HAQ the score of each question is divided by the number of questions answered. There must be responses in at least 6 of the 8 categories or else a HAQ cannot be computed. The use of aids or devices or physical assistance increases the score by two. The overall HAQ score ranges from zero to three. Scores of 0 to 0.3 represents no disability (normal), more than 0.3 to 1 generally represent mild to moderate disability, 1 to 2 represent moderate to severe disability, and 2 to 3 indicate severe to very severe disability. [201]

VII. Body Mass Index (BMI)

The BMI was calculated for all participants.[202]

$$\text{BMI}=\frac{Weight^{2} \text{ in kilograms}}{Height \text{ in meters}}$$

VIII. Disease severity assessment by Disease Activity Score 28 (DAS 28) (Annex III)

Disease activity was assessed using the disease activity score based on 28 joints-erythrocyte sedimentation rate (DAS 28-ESR) and C-reactive protein (DAS 28-CRP). It is a central measurement in the follow-up of patients with RA. It takes center stage because it comprises signs and symptoms of the disease (such as inflammatory pain, swelling, and stiffness) which is responsible for progression of joint damage. The nature of RA makes disease activity assessment more complicated than in many other diseases, such as diabetes or hypertension.[42]

The DAS 28 is a simple, easy, widely used and validated measure to assess disease activity in clinical practice as well as clinical trials. It is a statistically driven index combining tender joint count, swollen joint count, acute phase reactants level and patient global assessment score.[36]

IX. Thorough clinical evaluation including:

Musculoskeletal examination[203] with stress on joint examination as a key element in the assessment of the clinical status of RA. It is regarded as the most important measure for assessment in both clinical trials and daily practice. Clinical evaluation of joints involved:

a. Assessment of inflammatory activity including tenderness, pain, and synovial effusion or swelling.
b. Assessment of joint damage including collateral ligament laxity, subluxation, mal-alignment, bone-on-bone crepitations, and reduced range of motion (ROM).
c. Neck, back and sacroiliac joint (SIJ) involvement was considered when patient complained of pain and/or tenderness was elicited on examination.
d. Tempro-mandibular joint (TMJ) involvement was considered when patient complained of pain on opening of the mouth and/or mastication, difficulty in opening the mouth and/or tenderness was elicited on examination.
e. Ankle and feet involvement was considered when patient complained of pain or swelling and/or tenderness was elicited on examination.
f. Assessment of extra-articular features including assessment for secondary fibromyalgia (FM),[25,204] rheumatoid nodules (RN), rheumatoid vasculitis,[23,26,31] and pleuropulmonary,[25,204] neurologic, digestive, cardiovascular, cutaneous, hematologic (eg; anaemia, thrombocytosis), and ocular complications.[23,26,31]
g. Associated conditions as hypertension (HTM), diabetes mellitus (DM), etc. were assessed in all patients.

X. Laboratory Investigations:

I. Routine laboratory investigations were done including:[205]
   a. Complete blood picture (CBC),
   b. ESR first hour in mm/Hr,
   c. CRP in mg/L.

II. Rheumatoid factor (RF) in IU/ml was measured for all patients by fluoroimmunoassay.[206]

III. Anti-cyclic-citrullinated peptide antibodies (ACPA) in U/ml was measured for all patients by ELISA.[207]

XI. Bone turnover markers were measured including:

A. Total procollagen type 1 amino-terminal propeptide (TP1NP). [208]

Electro chemiluminescence immunoassay of TP1NP was done using Elecsys 2010 immunoassay analyzer. (Figure 10)

Figure (10): Elecsys 2010 immunoassay analyzer.

This test is intended for use in monitoring therapy following the diagnosis of OP in postmenopausal women, and in patients diagnosed with Paget's disease of the bone.

More than 90 % of organic bone matrix consists of type 1 collagen. Type 1 collagen is derived from type 1 procollagen which is synthesized by fibroblasts and osteoblasts. Type 1 procollagen contains both amino- (N) and carboxy- (C) terminal extensions. These extensions are removed by specific proteases during the conversion of procollagen to collagen and its subsequent incorporation into bone matrix. The extension measured by this assay is the amino terminal, hence P1NP.

This marker P1NP is therefore a specific indicator of type 1 collagen deposition and thus may be defined as a true bone formation marker.

P1NP is released into the blood during type 1 collagen formation as a trimeric structure (derived from the trimeric collagen structure) and a degraded monomeric structure. The P1NP assay detects both fractions present in the blood and therefore termed total P1NP.

Test principle for TP1NP

- Serum sample (20 µl) was incubated with biotinylated monoclonal P1NP specific antibody followed by addition of streptavidin labeled micro-particles and a monoclonal P1NP specific antibody labeled with a ruthenium complex.
- Streptavidin coated micro-particles were added to the mixture. After incubation the complex became bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture was aspirated into the measuring cell where the micro-particles are magnetically captured on to the surface of the electrode. Unbound substances are removed with ProCell. (Figure 11)

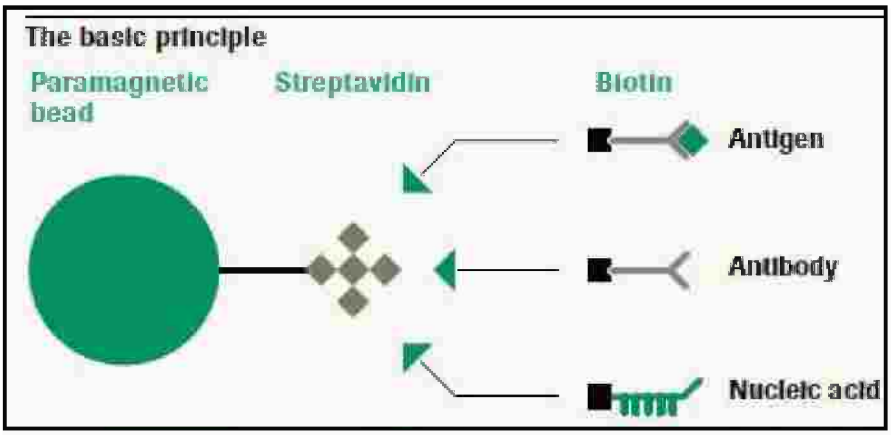


Figure (11): The basic principle for bone turnover markers measurement using the elecsys 2010 immunoassay analyzer.[209]

- Application of voltage to the electrode induced chemiluminescent emission which was measured by a photomultiplier. (Figure 12, 13)

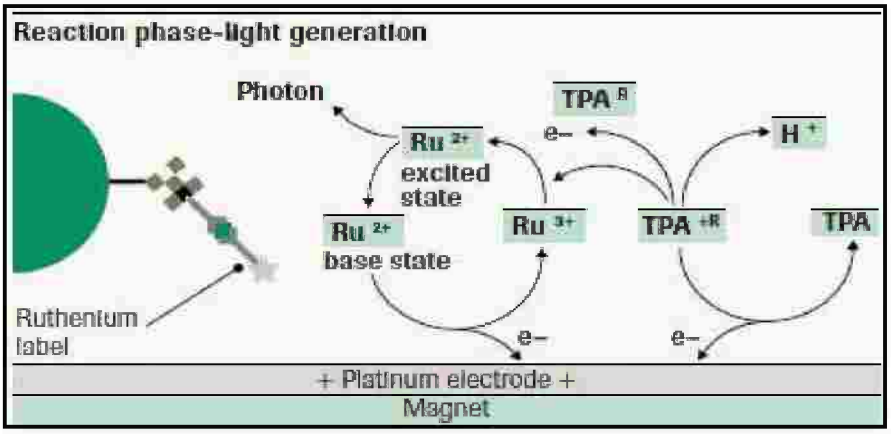


Figure (12): Reaction phase-light generation for bone turnover markers measurement using the elecsys 2010 immunoassay analyzer.[209]

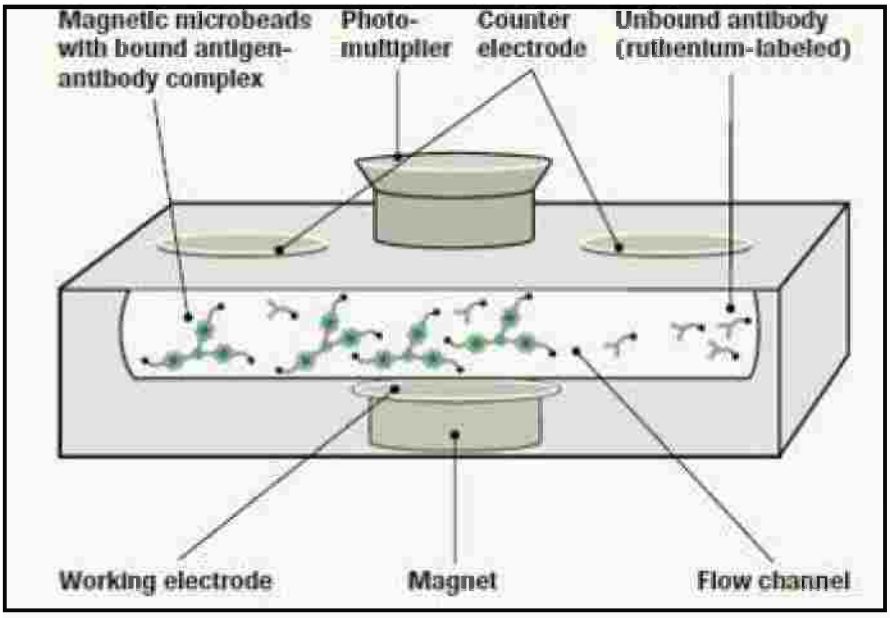


Figure (13): Over view of a reaction cell for bone turnover markers measurement using the elecsys 2010 immunoassay analyzer.[209]

- Results were determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

B. β-CrossLaps (β-CTx)[210]

Electro chemiluminescence immunoassay of β-CTx was done using Elecsys 2010 immunoassay analyzer.(Figure 10)

This test is used as an aid in assessing bone resorption. It is used in monitoring antiresorptive therapies in postmenopausal women, and individuals diagnosed with OP.

More than 90 % of organic bone matrix consists of type 1 collagen. During normal bone metabolism, mature type 1 collagen is degraded and small fragments pass into the blood stream and are excreted by the kidneys. In physiologically or pathologically elevated bone resorption, type 1 collagen is degraded to an increased extent, and accordingly a rise in the level of collagen fragments in the blood.

By determining these bone resorption markers (especially relevant are β-CTx), the activity of osteoclasts can be detected.

The immuno assay is specific for crosslinked isomerized type 1 collagen fragments. The assay specificity is guaranteed through the use of two monoclonal antibodies each recognizing linear β-8AA octapeptides.

Test principle for B-CTx

- Serum sample (50 µl) was incubated with biotinylated monoclonal anti-β-CTx specific antibody followed by addition of streptavidin labeled micro-particles and a monoclonal anti-β-CTx specific antibody labeled with a ruthenium complex.
- Streptavidin coated micro-particles were added to the mixture. After incubation the complex became bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture was aspirated into the measuring cell where the micro-particles are magnetically captured on to the surface of the electrode. Unbound substances are removed with ProCell. (Figure 11)
- Application of voltage to the electrode induced chemiluminescent emission which was measured by a photomultiplier. (Figure 12, 13)
- Results were determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

C. N-MID Osteocalcin (OCN)[211]

Electro chemiluminescence immunoassay of N-MID OCN was done using Elecsys 2010 immunoassay analyzer. (Figure 10)

Osteocalcin is the most important non collagen protein in bone matrix. It is a bone specific calcium binding protein and is vitamin K dependent. During bone synthesis, OCN is produced by osteoblasts.

Serum OCN is related to the rate of bone turnover in various disorders of bone metabolism and therefore is termed a bone turnover marker. The determination of serum OCN is used for monitoring antiresorptive therapeutic efficiency.

In blood, OCN is present as an intact molecule (amino acids 1-49) as well as the large N-MID fragment (aminoacids 1-43). Intact OC is unstable due to protease cleavage between amino acids 43 and 44. The N-MID fragment resulting from cleavage is considerably more stable. The assay thus detects the stable N-MID fragment as well as the (still) intact OCN. The test is independent on the unstable C terminal fragment (amino acids 43-49) of the OCN molecule and thus ensures constant measurement results.

The immunoassay uses two monoclonal antibodies directed against epitopes on the N MID fragment and the N terminal fragment.

Test principle for N-MID OCN

- Serum sample (20 µl) was incubated with biotinylated monoclonal N-MID osteocalcin-specific antibody and a monoclonal N-MID osteocalcin- specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- Streptavidin coated micro-particles were added to the mixture. After incubation the complex became bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture was aspirated into the measuring cell where the micro-particles are magnetically captured on to the surface of the electrode. Unbound substances are removed with ProCell. (Figure 11)
- Application of voltage to the electrode induced chemiluminescent emission which was measured by a photomultiplier. (Figure 12, 13)
- Results were determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

XII. Radiological Investigations

1. Plain X-ray (PXR) of both hands, wrist and feet were done

The radiological damage of the joints of the hands and feet was assessed by a trained rheumatologist according to the modified total sharp score (mTSS).[103,104] (Table 2, Figure 14)

The modification proposed in 1985 is now considered the standard for the Sharp method. Erosion (E) scores range from 0 to 280 per patient (hands 160, feet 120). The score for joint space narrowing (JSN) ranges from 0 to 168 per patient (hands 120, feet 48). Subluxation is not scored.[104]

Table (2): Detailed scoring system for erosions and joint space narrowing in mTSS.[104]

	E	JSN
For each hand:	• 1st IP, • 4 PIPs (2nd to 5th), • 5 MCPs.	• 4 PIPs, (2nd to 5th), • 5 MCPs.
For each wrist:	• 1st proximal MC bone, • Multiangular as one unit, (trapezium and trapezoid) • Scaphoid bone • Lunate bone • Distal radius, • Ulnar bone.	• 3 CMC (3rd to 5th), • Multiangular-navicular joints, • Capitate- Scaphoid joints. • Scaphoid -lunate joints.
For each foot:	• 1st IP, • 5 MTPs.	• 1st IP, • 5 MTPs.
Score for each area:	0-5 points in the hands & 0-10 points in the feet. 0= NO erosions 1=discrete interruption of cortical surface 2-5/2-10= more or larger defects of cortical surface.	1-4 points. 0=NO narrowing 1=focal joint narrowing, 2=diffuse narrowing (<50% of the original space), 3= diffuse narrowing (>50% of the original space), 4=Ankylosis.
Total score:	0-280 Hands: 160 Feet: 120	0-168 Hands: 120 Feet: 48

E: erosions, JSN: joint space narrowing, PIP: proximal interphalangeal joint, MCP: metacarpophalangeal joint, CMC: carpometacarpal, IP: interphalangeal joint, MTP: metatarsopharengeal joint.

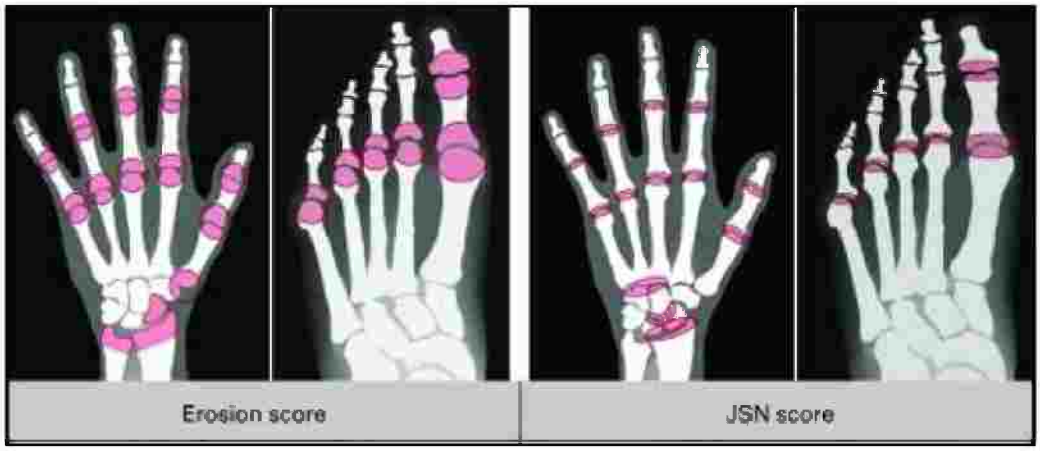


Figure (14): Modified sharp score calculation.[104]

2. Dual-energy X-ray absorptiometry (DXA) was done

Dual-energy X-ray absorptiometry (DXA) is recognized as the reference method to measure bone mineral density (BMD) with acceptable accuracy and good precision and reproducibility.[212]

Areal BMD in g/cm^2 was measured by a Lunar prodigy advance DXA scanner. (Figure 15) BMD was performed in the anteroposterior view for the lumbar spine, left (Lt) hip and Lt distal forearm by trained technicians; they were represented by the average of the 1st and 4th lumbar vertebrae (L1–L4), Lt total femur and Lt total radius BMD score respectively. When orthopaedic material was present in the left side, the right side was measured.[212]

Figure (15): Lunar prodigy advance DXA scanner.

T-score was calculated as a relationship between 2 norms by comparing the patient's BMD to that of young normal adults of the same sex. The BMD diagnosis of normal, osteopenia, OP and established OP is based on the WHO diagnostic classification.[112] (Table 3)

Table (3): The WHO definitions based on BMD measurement at the spine, hip or forearm by DXA devices.[112]

Normal: BMD is within 1 SD of a "young normal" adult (T-score at -1.0 and above).
Low bone mass ("osteopenia"): BMD is between 1.0 and 2.5 SD below that of a "young normal" adult (T-score between -1.0 and -2.5).
Osteoporosis: BMD is 2.5 SD or more below that of a "young normal" adult (T-score at or below -2.5).
Established osteoporosis: Patients in this group who have already experienced one or more fractures are deemed to have severe or "established" osteoporosis.

SD: Standard deviation

In premenopausal women, men less than 50 years of age and children, the WHO BMD diagnostic classification should not be applied. In these groups, the diagnosis of OP should not be made on the basis of densitometric criteria alone. The International Society for Clinical Densitometry (ISCD) recommends that instead of T-scores, ethnic or race adjusted Z-scores should be used.[128]

Z-score was calculated as a relationship between 2 norms by comparing the patient's BMD to the expected BMD for the patient's age and sex. The difference between the patient's score and the norm is expressed in standard deviations (SD) above or below the mean. Usually, 1 SD equals 10 to 15 percent of the BMD value in g/cm2. Depending upon the skeletal site, a decline in BMD is expressed in absolute terms (g/cm2) and in standard deviations (T-scores and Z-scores).[112] (Table 4)

Table (4): The International Society for Clinical Densitometry definitions based on BMD measurement at the spine, hip or forearm by DXA devices.[128]

Normal: BMD is within 2 SD of an age & sex matched normal adult (Z-score above -2.0).
Reduced bone mineral density for chronological age/Below the expected range for age: BMD is 2.0 SD or more below that of an age & sex matched normal adult (Z-scores at -2.0 or lower)

SD: Standard deviation

Statistical analysis

Statistical analysis was done using IBM SPSS statistics program version 21

- Independent sample t test was used to study statistical significant difference in mean quantitative variables (e.g HAQ, mTSS, DAS 28-ESR) among two groups of ACPA positive and ACPA negative. The use of parametric tests was due to normally distributed quantitative variables by KS test.
- Mann-Whitney test was used to study the statistical significant difference in the median Quantitative variables between ACPA positive and ACPA negative groups. The use of non-parametric tests was due to abnormally distributed quantitative variables by K.S test.
- Chi square test was used to study significant association between two categorical variables. Fisher exact and Montecarlo tests were used if more than 20% of total expected cell counts <5 at .05 level of significance.
- One way ANOVA and Kruskal Wallis tests were done to compare the mean, median quantitative variables respectively between the several categories of ACPA positive, ACPA negative and control groups. For significant results, pair wise comparison was done using adjusted p value.
- Pearson & spearman correlation tests were used to test statistical significant linear relationship between two quantitative variables. The choice of either of them depends on data distribution.
- Two way ANOVA test was done to detect statistical significant effect of two categorical variables on different quantitative variables each independently and the presence of significant interaction between these categorical variables. The quantitative variables must be normally distributed among different groups or transformation was done using MedCalc program.

All statistical tests were done at 0.05 significance level.

RESULTS

Forty three premenopausal female patients fulfilling the ACR/EULAR 2010 classification criteria for diagnosed of rheumatoid arthritis (RA) were enrolled in this study. They 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. The control group consisted of 30 age matched healthy premenopausal females.

Demographic characteristics of the studied groups

Age distribution

In the **ACPA positive patients**, the mean age was 39.41±6.81 years and in the **ACPA negative patients**, the mean age was 43.1±6.07 years. In the control group the mean age was 40.32±6.93 years. There was no statistically significant difference in the mean age among the studied groups (F = 1.68, p = 0.192). (Figure 16, table 5)

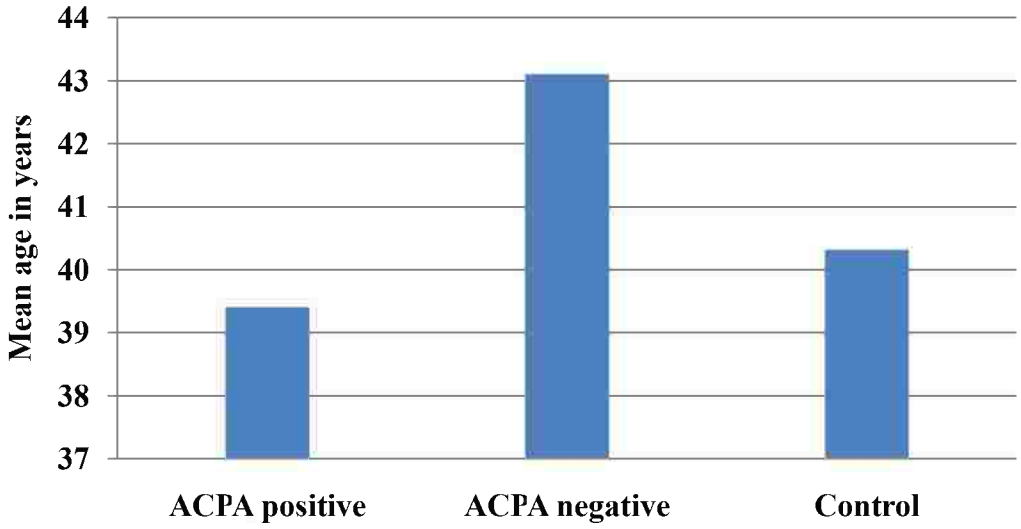


Figure (16): Mean age among studied groups.

Marital status

In the ACPA positive group: the majority were married; 27 patients (87.1%), while 2 patients (6.5%) were divorced, 1 patient (3.2%) was a widower and 1 patient (3.2%) was single.

In the ACPA negative group: the majority were married; 10 patients (83.3%) and 2 patients (16.7%) were divorced.

In the control group: the majority were married; 25 females (83.3%), while 1 female (3.3%) was divorced, 2 females (6.7%) were widowers and 2 females (6.7%) were single.

There was no statistical significant difference in the marital status among the studied groups (p=0.671). (Figure 17, table 5)

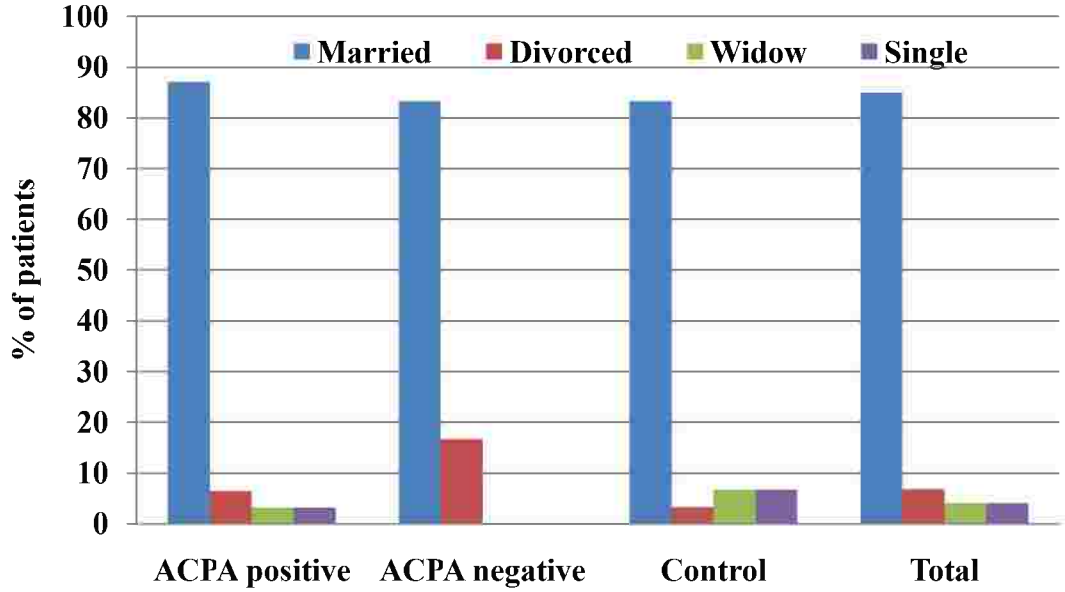


Figure (17): Marital status distribution among the studied groups.

Parity distribution

In the ACPA positive group the median number of children was 3 children (ranging from 0-9), in the ACPA negative group the median number of children was 3 children (ranging from 0-5) and in the control group the median number of children was 2 children (ranging from 0-8).

There was no statistical significant difference in the median number of children among the studied groups (p=0.213). (Table 5)

Occupation

In the ACPA positive group the majority 27 patients (87.1%) were house wives, while 2 patients (6.45%) had jobs that required standing for long durations (nurse/ teacher) and 2 patients (6.45%) had desk jobs (secretary/ tailor).

Only a single patient (who worked as a tailor) stopped work because of her medical condition (RA).

In the ACPA negative group the majority 7 patients (58.3%) were house wives, 3 patients (25%) had jobs that required standing for long durations (nurse/ nanny) and 2 patients (16.7%) had desk jobs (secretary/ accountant).

In the control group the majority 17 females (56.6%) were house wives, while 9 females (30%) had jobs that required standing for long durations (nurse/ teacher) and 4 patients (13.4%) had desk jobs (secretary/ accountant).

There was no statistical significant difference in the work type among the studied groups (p=0.065). (Figure 18, table 5).

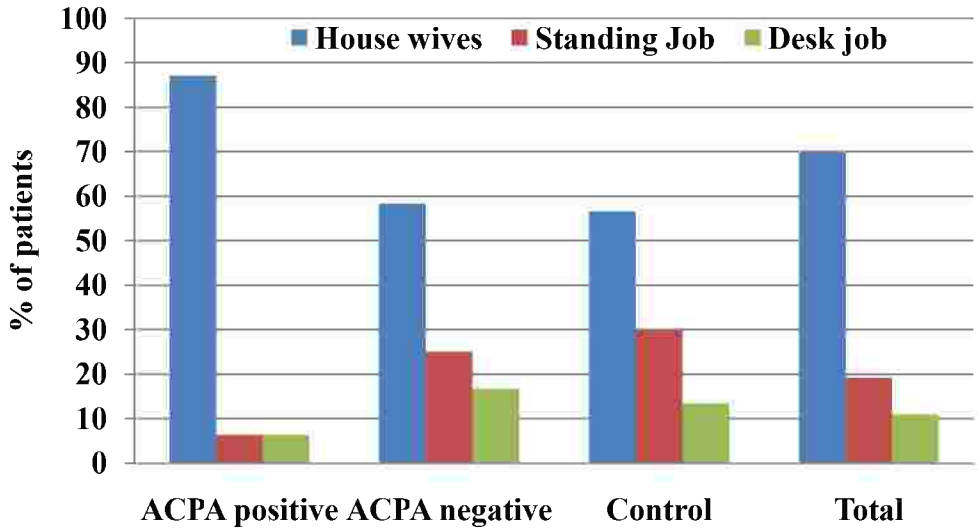


Figure (18): Occupation distribution among the studied groups.

Table (5): Summary of demographic characteristics of the studied groups.

	ACPA positive	ACPA negative	Control	p value
	Mean ±SD			
Age	39.41±6.81	43.1±6.07	40.32±6.93	F= 1.68 p=0.192
Marital status	No (%)			
Married	27(87.1%)	10(83.3%)	25(83.3%)	X^2 p=0.67
Divorced	2(6.5%)	2(16.7%)	1(3.3%)	
Widower	1(3.2%)	-	2(6.7%)	
Single	1(3.2%)	-	2(6.7%)	
Parity	Median (range)			K
No. of children	3(0-9)	3(0-5)	2(0-8)	p=0.213
Occupation	No. (%)			
House wives	27(87.1%)	10(83.3%)	25(83.3%)	X^2 p=0.065
Standing jobs	2(6.5%)	2(16.7%)	1(3.3%)	
Desk jobs	2(6.5%)	-	2(6.7%)	

ACPA: anti-cyclic citrullinated-peptide, SD: standard deviation, No.: number, %: percentage, F: one way ANOVA, X2: Pearson Chi square, K: Kruskal-Wallis test, significance level <0.05.

Personal history of the studied groups

Age of menarche

In the ACPA positive group the median age of menarche was 13 (ranging from 10-16), in the ACPA negative group the median age of menarche was 12 years (ranging from 10-13) and in the control group the median age of menarche was 13 years (ranging from 10-15).

There was no statistical significant difference in the median age of menarche among the studied groups (p=0.213). (Table 6)

History of smoking

In the ACPA positive group all 31 patients (100%) were non smokers. Similarly in the ACPA negative group all 12 patients (100%) were non smokers. While in the control group the majority 28 females (93.3%) were non smokers and only 2 females (6.7%) were ex smokers (very short duration 3 month).

There was no statistical significant difference in the smoking history among the studied groups (p=0.505). (Table 6)

Coffee intake

The median number of cups of coffee per day was 0 cups/day (ranging from 0-4) *in the ACPA positive group*, 0 cups/day (ranging from 0-2) *in the ACPA negative group* and 0 cups/day (ranging from 0-2) *in the control group*.

There was no statistical significant difference in the median number of cups of coffee/day among the studied groups (p=0.778). (Table 6)

Dietary habits

Detailed history of dairy product intake since childhood was taken from each participant. They were categorized into 2 groups; those who took daily adequate amounts of dairy products and those who had infrequent intake of dairy products.

In the ACPA positive group 18 patients (58.1%) had adequate daily intake of dairy products, while 13 patients (41.9%) had infrequent intake of dairy products.

In the ACPA negative group 10 patients (83.3%) had adequate daily intake of dairy products and only 2 patients (16.7%) had infrequent intake of dairy products.

In the control group 22 females (73.3%) had daily intake of dairy products and 8 females (26.7%) had infrequent intake of dairy products.

There was no statistical significant difference among the studied groups as regards to the daily intake of dairy products (X^2=2.443, p=0.295). (Table 6)

Calcium and vitamin D supplements

Detailed history of calcium and vitamin D supplements intake was taken from each participant. They were categorized into 3 groups; those who never received calcium and vitamin D supplements, those who infrequently received calcium and vitamin D supplements and those who daily received calcium and vitamin D supplements.

In the ACPA positive group 16 patients (51.6%) had never received calcium and vitamin D supplements, 10 patients (32.3%) had infrequently received calcium and vitamin D supplements and only 6 patients (16.1%) had regularly received calcium and vitamin D supplements.

In the ACPA negative group 8 patients (66.7%) had never received calcium and vitamin D supplements, while 3 patients (25%) had infrequently received calcium and vitamin D supplements and only 1 patient (8.3%) had regularly received calcium and vitamin D supplements daily.

In the control group 18 patients (60%) had never received calcium and vitamin D supplements, 11 patients (36.67%) had infrequently received calcium and vitamin D supplements and only 1 patient (3.33%) had regularly received calcium and vitamin D supplements.

There was no statistical significant difference among the studied groups as regards calcium or vitamin D supplement intake (p=0.713). (Table 6)

Personal history of fragility fractures

There was no personal history of fragility fractures in any of the studied groups.

Table (6): Summary of personal history of the studied groups.

	ACPA positive	ACPA negative	Control	p value
Age of menarche	**Median (range)**			K
	13(10-16)	12(0-13)	13(10-15)	p=0.213
Smoking	**No. (%)**			X^2
Non-smokers	31(100%)	12(100%)	28(93.3%)	p=0.505
Ex-smokers	-	-	2(6.7%)	
Coffee intake	**Median (range)**			K
Cups/day	0(0-4)	0(0-2)	0(0-2)	p=0.213
Dietary habits	**No. (%)**			
Adequate	18(58.1%)	10(83.3%)	22(73.3%)	X^2=2.443
Inadequate	13(41.9%)	2(16.7%)	8(26.7%)	p=0.065
Calcium Supp	**No. (%)**			
Never	16(51.6%)	8(66.7%)	18(60%)	X^2
Infrequently	10(32.3%)	3(25%)	11(36.67%)	p=0.713
Regularly	5(16.1%)	1(8.3%)	1(3.33%)	

ACPA: anti-cyclic citrullinated-peptide, Supp: supplements, No.: number, %: percentage, X2: Pearson Chi square, K: Kruskal-Wallis test, significance level <0.05.

Family history in the studied groups

Family history of an autoimmune disease in first degree relatives

Five patients (16.1%) in the ACPA positive group and 3 patients (25%) in the ACPA negative group had history of autoimmune disease (RA) in a first degree relative, while none of the control group had history of autoimmune disease (RA) in a first degree relative.

Family history of fragility fractures

There was no family history of fragility fractures in any of the studied groups.

Drug history of the patients groups

Non-steroidal anti-inflammatory drugs (NSAIDs)

Twenty five (80.7%) patients in the ACPA positive group and 7 (58.3%) patients in the ACPA negative group received NSAIDs during the course of their disease.

There was no statistical significant difference regarding the number of patients that received NSAIDs among the patients' groups (p= 0.14). (Table 7)

Corticosteroids (Cs)

Twenty nine (93.5%) patients in the ACPA positive group and 9 (75%) patients in the ACPA negative group received Cs in the course of their disease.

The cumulative Cs intake was estimated by summation of the total amount of prednisolone or its equivalent received by each patient over the course of the disease in grams (gms), through detailed history taking and reviewing of each prescription. The median cumulative Cs intake was 2.15 gm (0-12.26) in the ACPA positive group and 0.86 gm (0-5.33) in the ACPA negative group.

There was no statistical significant difference among the patients' groups as regards cumulative Cs intake (p=0.052). (Table 7)

Methotrexate (MTX)

Twenty eight (90.3%) patients in the ACPA positive group and 8 (66.67%) patients in the ACPA negative group received MTX therapy during the course of their disease. The median MTX dose/week in the ACPA positive group was 12.5 mg (ranging from 0-25) and in the ACPA negative group was 12.5 mg (ranging from 0-25).

While the median MTX intake duration in the ACPA positive group was 1.67 years (ranging from 0-10) and in the ACPA negative group was 0.5 years (ranging from 0-6).

There was no statistical significant difference regarding the MTX dose/week and intake duration among the patients' groups (p= 0.529, p=0.592) respectively. (Table 7)

Hydroxychloroquine (Hcq)

Eleven (35.5%) patients in the ACPA positive group and 2 (16.67%) patients in the ACPA negative group received Hcq. The median Hcq intake duration in the ACPA positive group was 0 years (ranging from 0-5) and in the ACPA negative group was 0 years (ranging from 0-0.3).

There was no statistical significant difference regarding the duration of Hcq intake among the patients' groups (p=0.243). (Table 7)

Sulfasalazine (SSZ)

Eight (25.8%) patients in the ACPA positive group and 4 (33.3%) patients in the ACPA negative group received SSZ. The median SSZ intake duration in the ACPA positive group was 0 years (ranging from 0-6) and in the ACPA negative group was 0 years (ranging from 0-3). There was no statistical significant difference regarding the duration of SSZ intake among the patients' groups (p=0.82). (Table 7)

Leflunomide (LEF)

Fifteen (48.4%) patients in the ACPA positive group and 5 (41.67%) patients in the ACPA negative group received LEF. The median LEF intake duration in the ACPA positive group was 0 years (ranging from 0-2) and in the ACPA negative group was 0 years (ranging from 0-0.3). There was no statistical significant difference regarding the duration of LEF intake in the patients' groups (p=1.0). (Table 7)

Azathioprine (AZA)

Only one patient (3.22%) ***in the ACPA positive group*** received AZA for 6 months. There was no statistical significant difference regarding the number of patients that received AZA among the patients' groups (p= 0.8). (Table 7)

Tocilizumab

One patient (3.22%) in the ***ACPA positive group*** and another patient (8.33%) in the ***ACPA negative group*** received Tocilizumab. They received 6 injections each with 4 weeks interval, with no complications. There was no statistical significant difference regarding the number of patients that received Tocilizumab among the patients' groups (p=1.0). (Table 7)

Table (7): Drug history in the patients groups.

	ACPA positive	ACPA negative	Mann Whitney U test
	No. (%)		
NSAIDs	25(80.7%)	7(58.3%)	p=0.14
Cumulative Cs (gms)	29(93.5%)	9(75%)	p=0.052
	Median (range)		
	2.15(0-12.26)	0.86(0-5.33)	
MTX	28(90.3%)	8(66.67%)	p=0.529
	Median (range)		
Dose/week	12.5(0-25)	12.5(0-25)	
Duration in yrs	**Median(range)**		p=0.592
	1.67(0-10)	0.5(0-6)	
Hcq	11(35.5%)	2(16.67%)	p=0.243
Duration in yrs	**Median (range)**		
	0(0-5)	0(0-0.3)	
SSZ	8(25.8%)	84(33.3%)	p=0.82
Duration in yrs	**Median (range)**		
	0(0-6)	0(0-0.3)	
LEF	15(48.4%)	5(41.67%)	p=1.0
Duration in yrs	**Median (range)**		
	0(0-2)	0(0-0.3)	
AZA	1(3.22%)	-	p=0.8
Tocilizumab	1(3.22%)	1(8.33%)	p=1.0

ACPA: anti-cyclic citrullinated-peptide, No.: number, %: percentage, NSAIDs: Non-steroidal anti-inflammatory drugs, Cs:corticosteroids, MTX: methotrexate, Hcq: hydroquine, SSZ: sulfasalazine, LEF:leflunomide, AZA: azathioprine, gms: grams, yrs: years, significance level <0.05.

Summary for drug history

In the ACPA positive group 3 patients (9.7%) were MTX naïve, 1 patient (3.2%) received MTX as a monotherapy, 8 patients (25.8%) received MTX in combination with 1 other DMARD, 10 patients (32.3%) received MTX in combination with 2 other DMARDs and 9 patients (29%) received MTX in combination with 3 other DMARD.

In the ACPA negative group 4 patients (33.3%) were MTX naïve, 1 patient (8.3%) received MTX as a monotherapy, 2 patients (16.7%) received MTX in combination with 1 other DMARD, 2 patients (16.7%) received MTX in combination with 2 other DMARDs and 3 patients (25%) received MTX in combination with 3 other DMARD. (Figure 19)

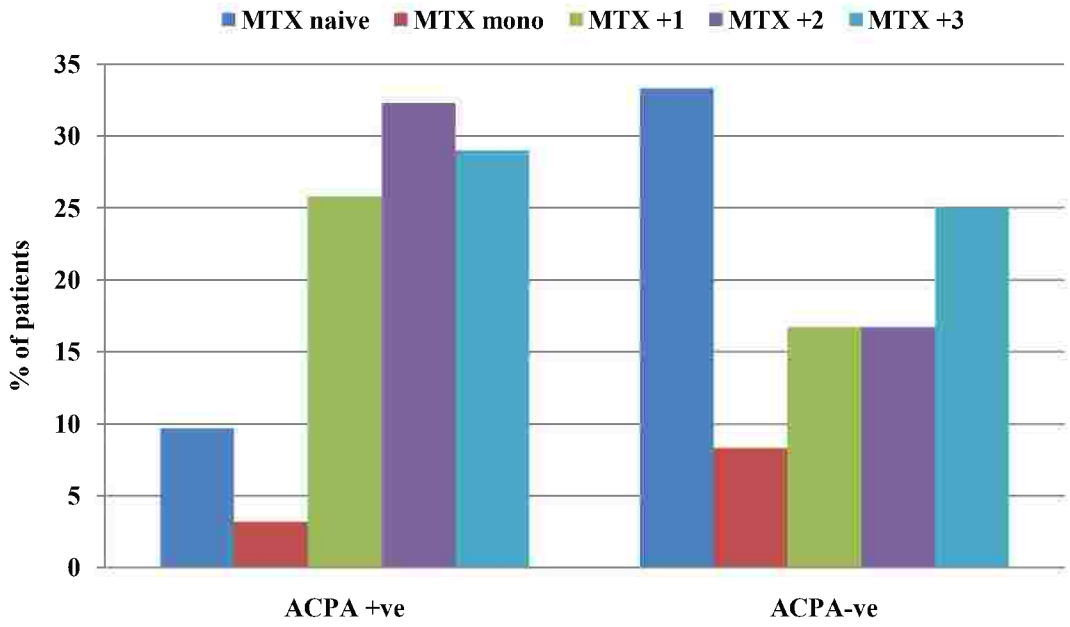


Figure (19): Summary of drug therapy in the patients groups.

Historical data for the present condition

Disease onset

In the ACPA positive group 17 patients (54.8%) had gradual onset of disease symptoms while 14 patients (45.2%) had a rapid onset of disease symptoms and in the ACPA negative group 6 patients (50%) had gradual onset of disease symptoms and 6 patients (50%) had a rapid onset of disease symptoms. There was no statistical significant difference in the disease onset among the patients' groups (X^2= 0.081, p=0.775). (Table 8)

Disease duration

The median disease duration was 4 years ranging from 5 months to 20 years in the ACPA positive group and 4.5 years ranging from 4 months to 15 years in the ACPA negative group. There was no statistical significant difference in the median disease duration among the patients' groups (U= 337.5, p=0.477). (Figure 20, Table 8)

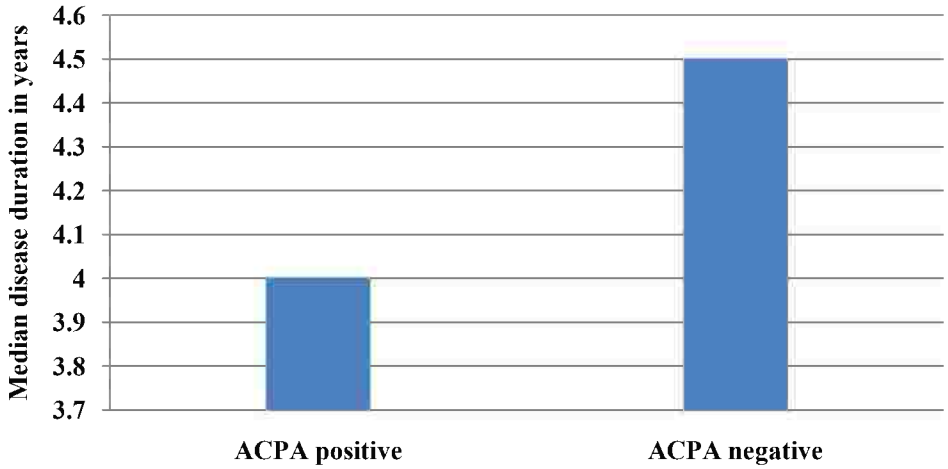


Figure (20): Median disease duration in years.

Duration of morning stiffness

The median duration of morning stiffness was 30 minutes ranging from 0 to 120 minutes in the ACPA positive group and 15 minutes ranging from 0 to 30 minutes in the ACPA negative group.

There was a statistical significant difference in the median duration of morning stiffness among the patients' groups (U= 270, p=0.022), where the ACPA positive patients had a higher median duration of morning stiffness. (Figure 21, Table 8)

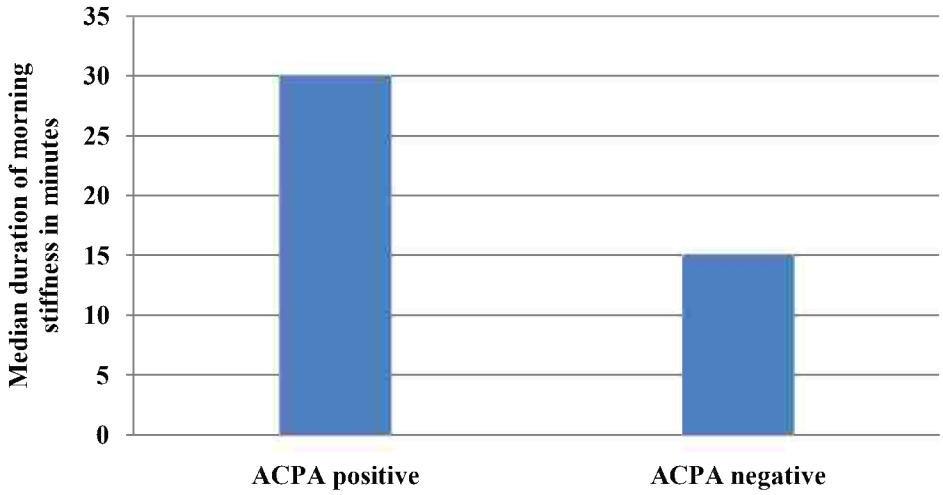


Figure (21): Median duration of morning stiffness in minutes among the patients' groups.

Frequency of fatigue in patients

Twenty eight patients (90.3%) complained of easy fatigability in the ACPA positive group and 10 patients (83.3%) complained of easy fatigability in the ACPA negative group. There was no statistical significant difference in the frequency of fatigue among the patients' groups (p=0.608). (Figure 22, Table 8)

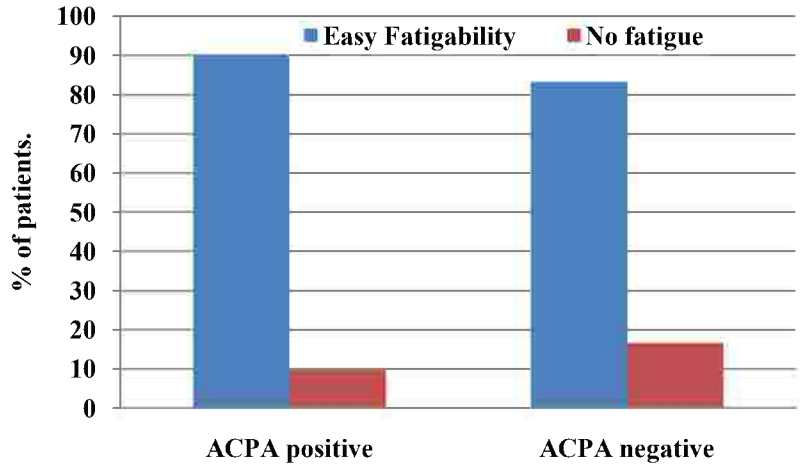


Figure (22): Percentage of patients complaining of easy fatigability.

Table (8): Summary of historical data of the present condition.

	ACPA positive	ACPA negative	p value
	No. (%)		
Disease Onset			
Gradual	17(54.8%)	6(50%)	X^2=0.081 p=0.505
Rapid	14(45.2%)	6(50%)	
	Median (range)		
Disease duration in yrs	4(0.4-20)	4.5(0.3-15)	U=337.5 p=0.213
Duration of morning stiffness in min	30(0-120)	15(0-30)	**U=270 p=0.022***
	No. (%)		
Frequency of Fatigue	28(90.3%)	10(83.3%)	X^2 p=0.608

ACPA: anti-cyclic citrullinated-peptide, No.: number, %: percentage, F: one way ANOVA, X2: Pearson Chi square, U: Mann Whitney U test, yrs: years, min: minutes, significance level <0.05.

Disability in patients

Disability was assessed by the health assessment questionnaire (HAQ)

In the ACPA positive patients: most of the patients 24 (77.4%) had moderate to severe disability, 6 patients (19.4 %) had mild to moderate disability and only 1 patient (3.2 %) had severe to very severe disability, with a mean score of 1.48 ± 0.459.

In the ACPA negative patients: 6 patients (50 %) had moderate to severe disability, 5 patients (41.7 %) had mild to moderate disability and only 1 patient (8.3 %) had no disability, with a mean score of 1.03±0.479.

There was a statistical significant difference in the disability score among the ACPA positive and negative group (t= -2.82, p= 0.007), where the ACPA positive group showed a higher disability (HAQ) score. (Figure 23, table 9)

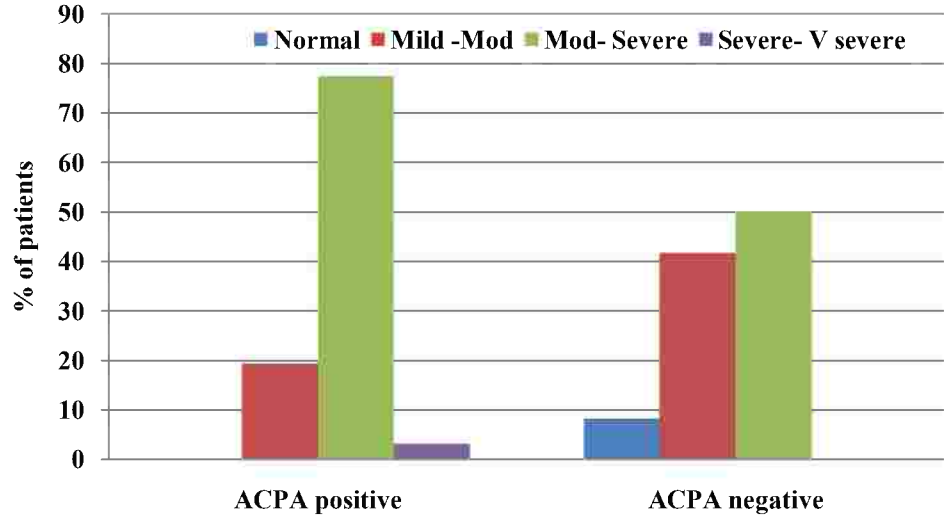


Figure (23): Distribution of patients according to degree of disability by HAQ.

Table (9): Disability in the patients' groups.

	ACPA positive	ACPA negative	Independent sample t test
	Mean ± SD		
HAQ	1.48±0.459	1.03±0.479	**t=-2.82 p=0.007***

ACPA: anti-cyclic citrullinated-peptide, HAQ: health assessment questionnaire, SD: standard deviation, significance level <0.05.

Body mass index (BMI) among patients and control

The mean BMI was 29.72±5.87 in the ACPA positive patients, 32.98±6.59 in the ACPA negative patients and 31.53±6.72 in the control group. There was no statistical significant difference in the mean BMI among the studied groups (F = 1.53, p = 0.222). (Table 10)

Table (10): Body mass index in the studied groups.

	ACPA positive	ACPA negative	Control	One way ANOVA
	Mean±SD			
BMI	29.72±5.87	32.98±6.59	31.53±6.72	F=1.53 p=0.222

ACPA: anti-cyclic citrullinated-peptide, BMI: body mass index, SD: standard deviation, significance level <0.05.

Musculoskeletal Examination

Disease activity in patients

Disease activity was assessed using the disease activity score based on 28 joints-erythrocyte sedimentation rate (DAS 28-ESR) and C-reactive protein (DAS 28- CRP).

DAS 28-ESR

In the ACPA positive patients: most of the patients 28 (90.3%) had high disease activity, while only 3 patients (9.7%) had moderate disease activity, with a mean score of 6.58± 1.33.

In the ACPA negative patients: 4 patients (33.3%) had high disease activity, 5 patients (41.7%) had moderate disease activity and 3 patients (25%) had low disease activity, with a mean score of 4.74± 1.28.

There was a statistical significant difference in the DAS 28-ESR score between the patients' groups (t= -4.131, p< 0.001) where the ACPA positive group showed a higher DAS 28-ESR score.

DAS 28-CRP

In the ACPA positive patients: most of the patients 19 (61.3%) had high disease activity, 10 patients (32.2 %) had moderate disease activity and only 2 patients (6.5 %) had low disease activity, with a mean score of 5.66± 1.4.

In the ACPA negative patients: 3 patients (25 %) had high disease activity, 6 patients (50 %) had moderate disease activity and 3 patients (25%) had low disease activity, with a mean score of 4.53± 1.32.

There was a statistical significant difference in the DAS 28-CRP score between the studied groups (t= -2.41, p= 0.02) where the ACPA positive group showed a higher DAS 28-CRP score. (Figure 24, Table 11)

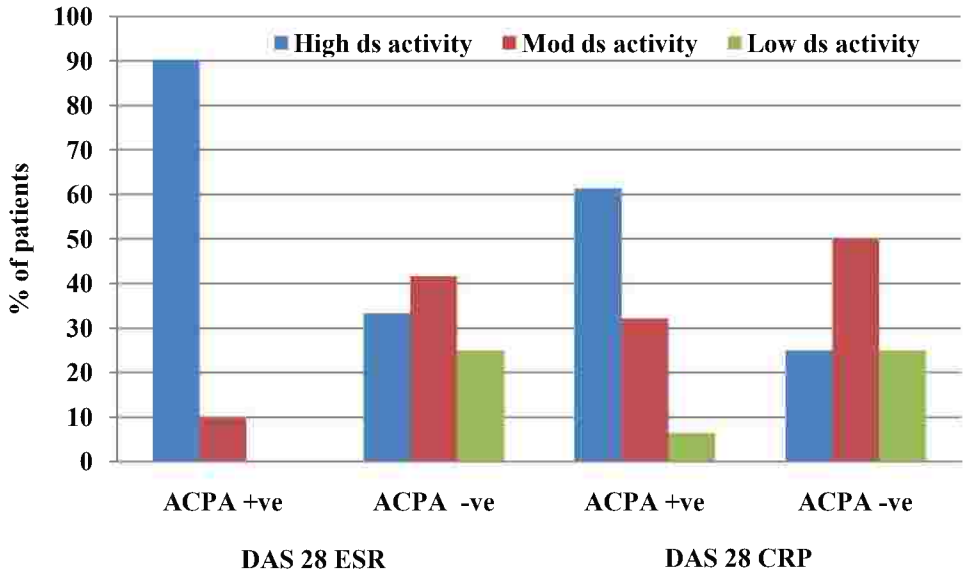


Figure (24): Percentage distribution of patients according to severity of disease activity by DAS 28.

Table (11): Disease activity in the patients groups.

	ACPA positive	ACPA negative	Independent sample t test
	Mean ± SD		
DAS 28-ESR	6.58±1.33	4.74±1.28	**t= -4.131 p<0.001***
DAS 28-CRP	5.66±1.4	4.53±1.32	**t= -2.41 p=0.02***

ACPA: anti-cyclic citrullinated-peptide, DAS 28: disease activity score based on 28 joints, ESR: erythrocyte sedimentation rate, CRP:C-reactive protein, SD: standard deviation, significance level <0.05.

Neck involvement

Eight patients (25.8 %) in the ACPA positive group and 3 patients (25%) in the ACPA negative group had neck pain and tenderness. There was no statistical significant difference among the patients' groups (p=1.0). (Figure 25, Table 12)

Back involvement

Six patients (19.4%) in the ACPA positive group and 1 patient (8.3%) in the ACPA negative group had back pain and tenderness. There was no statistical significant difference among the patients' groups (p=0.652). (Figure 25, Table 12)

Sacroiliac joint (SIJ) involvement

Two patients (6.5%) in the ACPA positive group had bilateral SIJ pain and tenderness. None of the ACPA negative group had SIJ pain and tenderness. There was no statistical significant difference among the patients' groups (p=0.588). (Figure 25, Table 12)

Tempro-mandibular joint (TMJ) involvement

Nine patients (29%) in the ACPA positive group and 3 patients (25%) in the ACPA negative group had bilateral TMJ involvement. There was no statistical significant difference among the patients' groups (p=1.0). (Figure 25, Table 12)

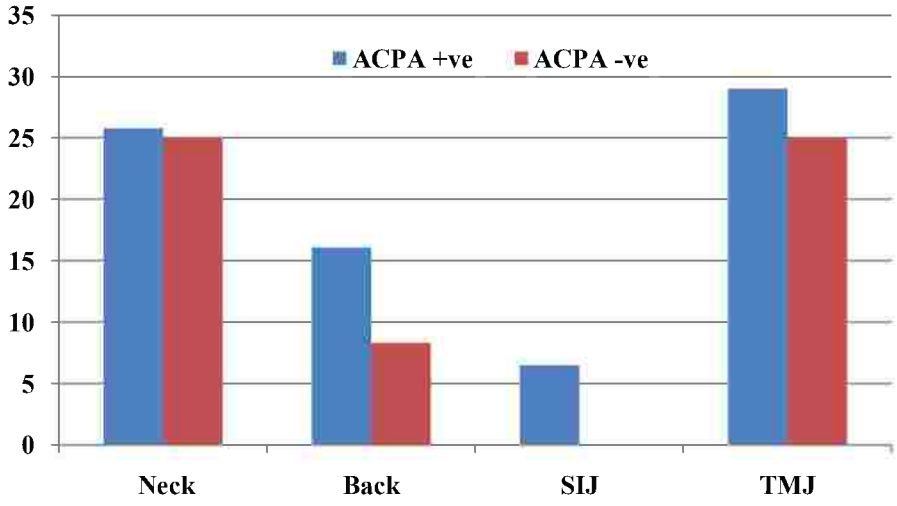


Figure (25): Percentage of patients with axial and TMJ involvement.

Feet involvement

Feet involvement was considered present when there was tenderness or swelling in one or more of the small joints of the feet.

Only 2 patients (6.5%) had unilateral foot involvement while 13 patients (41.9 %) had bilateral feet involvement in the ACPA positive group. (Figure 26) Four patients (33.3 %) had unilateral foot involvement and only 1 patient (8.3%) had bilateral feet involvement in the ACPA negative group.

There was a statistical significant difference in the number of patients with feet involvement among the patients' groups (p=0.019), where the ACPA positive group had a higher number of patients with feet involvement. (Figure 26, 28, Table 12)

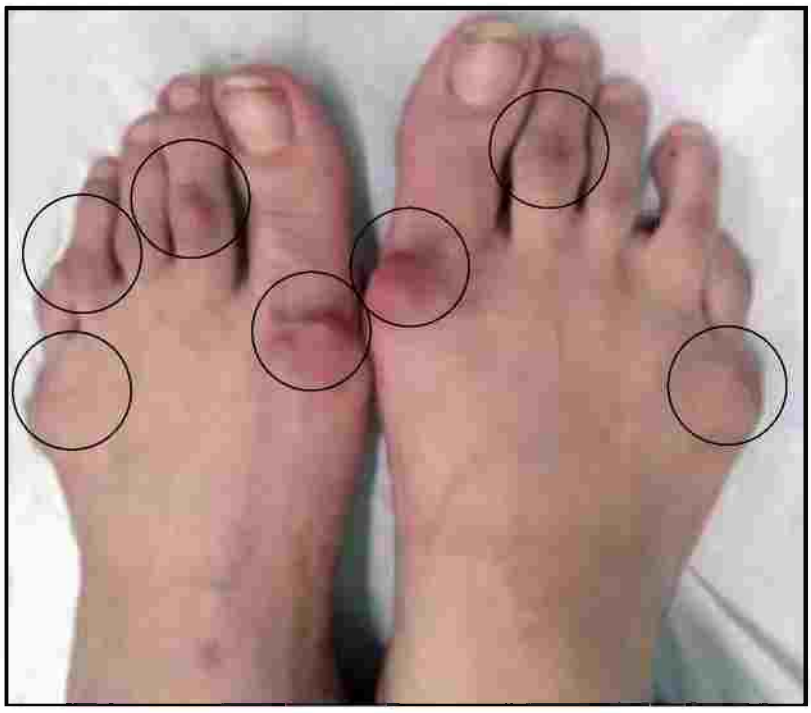


Figure (26): Feet involvement in a 41 year old ACPA positive patient showing multiple swollen joints with deformities and rheumatoid nodules.

Ankle involvement

Ankle involvement was considered present when there was tenderness or swelling in the ankle joint.

Only 2 patients (6.5%) had unilateral ankle involvement while 17 patients (54.8%) had bilateral ankles involvement in the ACPA positive patients. (Figure 27) Three patients (25 %) had unilateral ankle involvement and 3 patients (25 %) had bilateral ankles involvement in the ACPA negative patients.

There was no statistical significant difference among the patients' groups (p=0.089). (Figure 27, 28, Table 12)

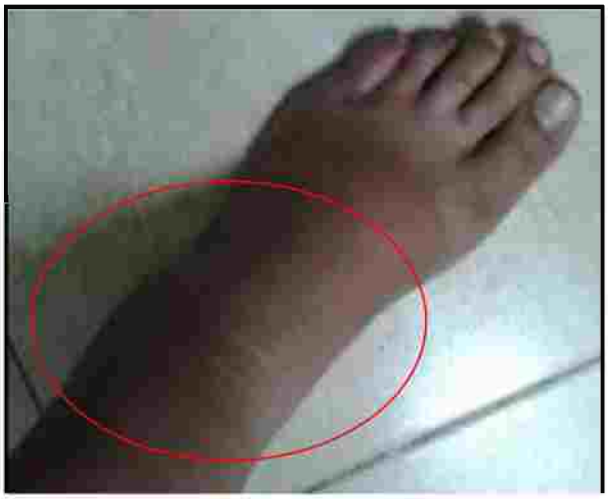


Figure (27): A 45 year old ACPA positive RA patient with right ankle involvement in the form of swelling and tenderness.

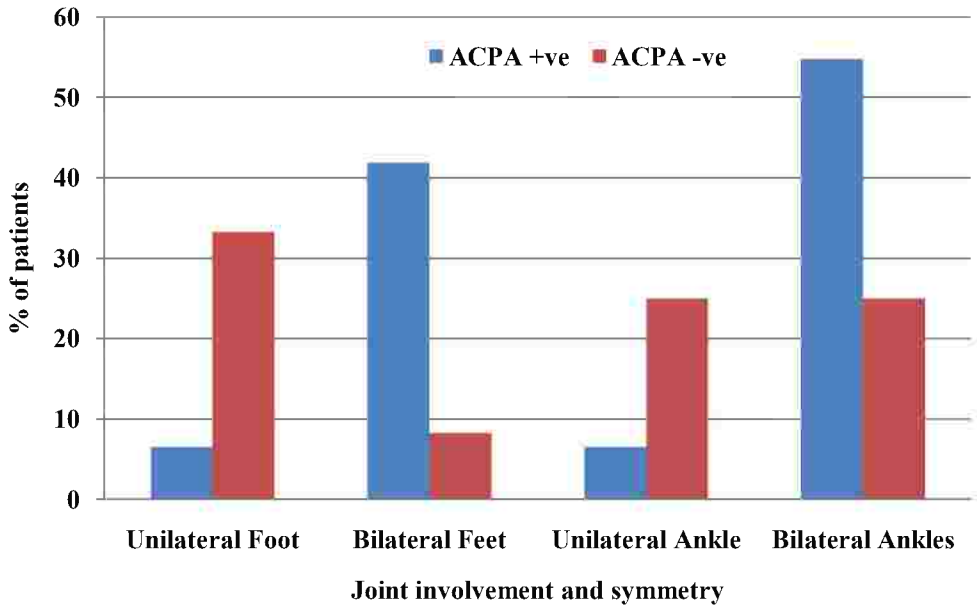


Figure (28): Percentage of patients with ankle or feet involvement.

Table (12): Musculoskeletal examination in the patients groups.

Area of involvement	ACPA positive	ACPA negative	Chi square test (X^2)
	No. (%)		
Neck	8(25.8%)	3(25%)	p=1.0
Back	6(19.4%)	1(8.3%)	p=0.652
SIJ	2(6.5%)	-	p=0.588
TMJ	9(29%)	3(25%)	p=1.0
Feet	15(48.4%)	5(41.6%)	**p=0.019***
Unilateral	2(6.5%)	4(33.3%)	
Bilateral	13(41.9%)	1(8.3%)	
Ankle	19(61.3%)	6(50%)	p=0.089
Unilateral	2(6.5%)	3(25%)	
Bilateral	17(54.8%)	3(25%)	

ACPA: anti-cyclic citrullinated-peptide, No.: number, %: percentage, SIJ: sacroiliac joint, TMJ: tempro-mandibular joint, significance level <0.05.

Tenosynovitis

Only 2 patients (6.5 %) in the ACPA positive group had tenosynovitis; in the form of extensor tenosynovitis of both upper limbs (UL) in one patient and unilateral flexor tenosynovitis in another patient. None of the ACPA negative patients had tenosynovitis. There was no statistical significant difference regarding the number of patients developed tenosynovitis among the patients' groups (p= 0.588).

Rheumatoid nodules (RN) in patients

Five patients (16.1%) had RNs in the ACPA positive group (2 patients had 1 RN, 1 patients had 2, another patient had 3 and 1 patient had 14 RNs), (Figure 30) with a mean number of RNs of 0.677±2.56, while only 1 patient (8.3%) had 2 RNs in the ACPA negative group, with a mean number of RNs of 0.167 (±0.577).

There was no statistical difference in the number of RNs in the patients among the patients' groups (t= -0.679, p= 0.501). (Figure 29)

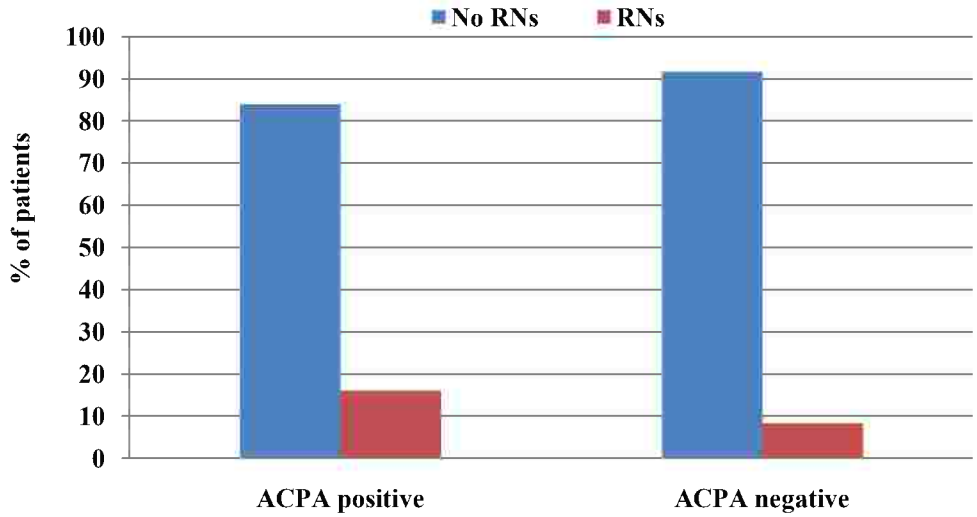


Figure (29): Percentage of patients with RNs.

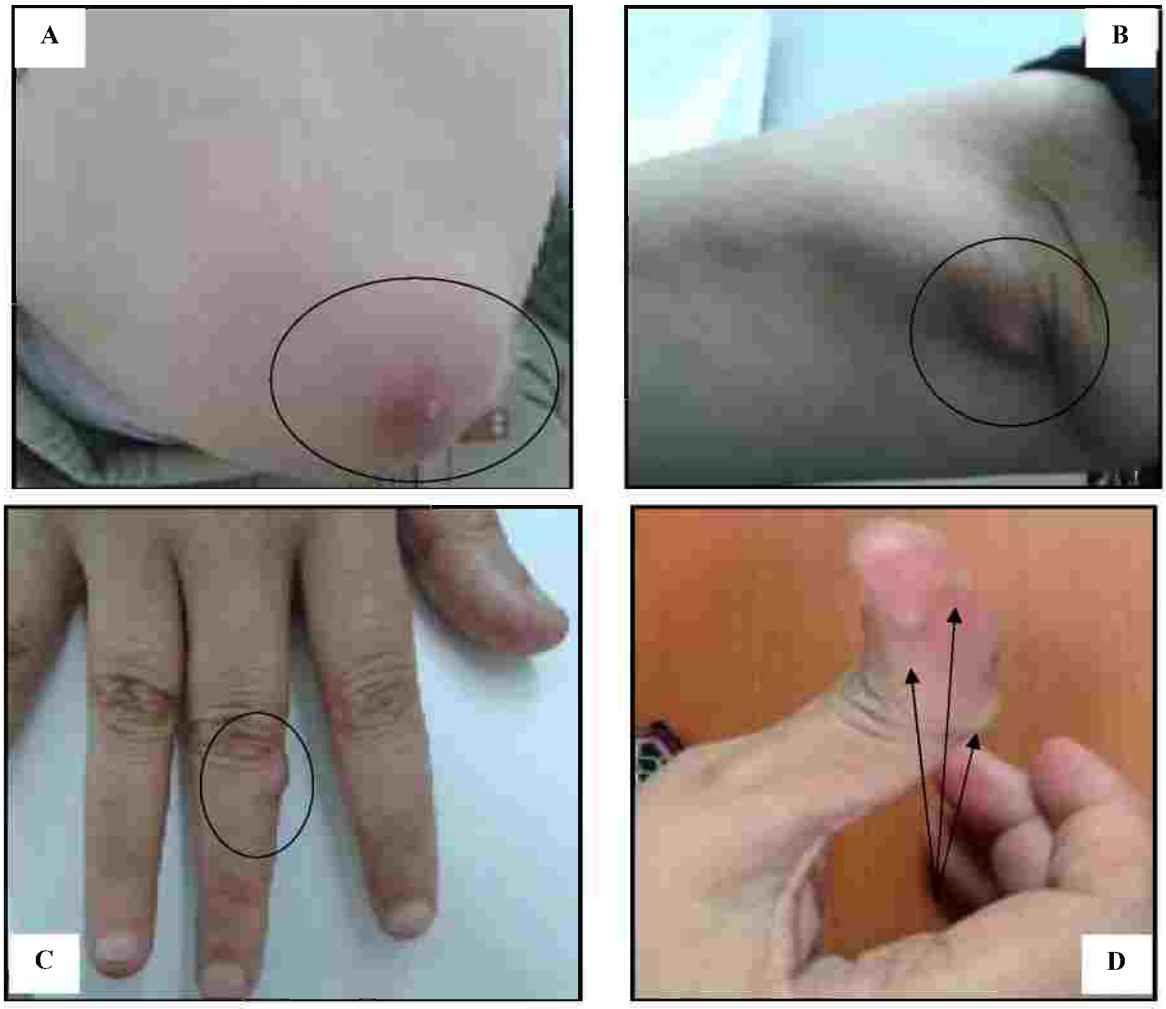


Figure (30): A 41 year old ACPA positive patient with multiple RNs in the (A) Right elbow (B) Left Elbow (C) Right middle finger and (D) thumb.

Deformities in patients

Deformities occur from persistent synovitis which is either neglected or resistant to treatment.

Upper limb deformities included

Shoulder deformities: in the form of bilateral limitation in the range of motion (ROM) in 1 patient (3.2%) in the ACPA positive group, while another patient had periarthritis of the left shoulder which improved by physiotherapy and home exercise program. (Table 13)

Elbow deformities: in the form of unilateral limitation in the ROM in 1 patient (3.2%) and bilaterally in another patient (3.2%) in the ACPA positive group. (Figure 31, Table 13)

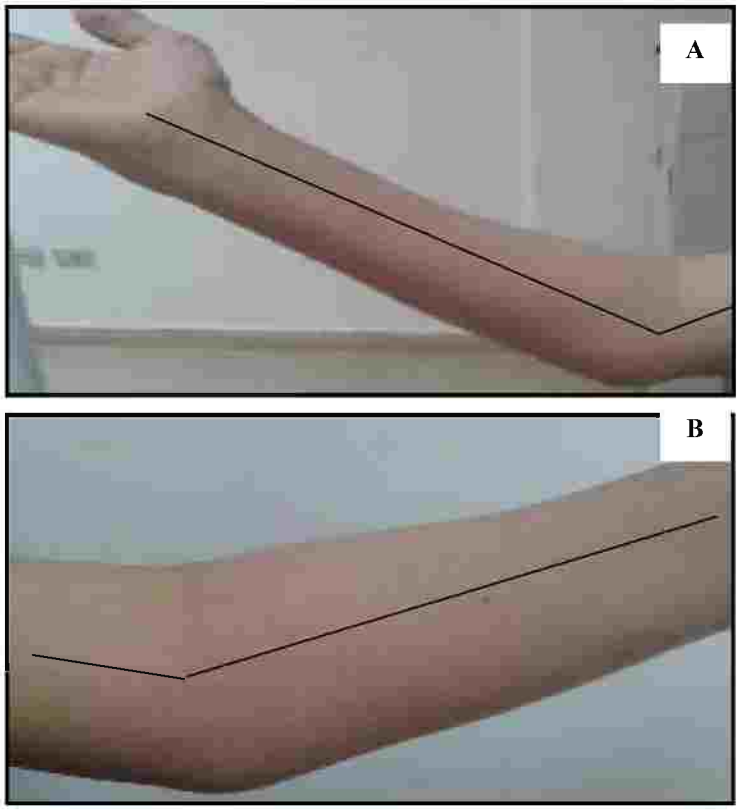


Figure (31): A 40 year old ACPA positive RA patient with bilateral elbow deformities (A) right and (B) left in the form of limited extension and increase in the carrying angle of the right elbow.

Wrist deformities: Ulnar deviation was present in 7 patients (22.6%) (4 unilaterally and 3 bilaterally) and limitation in the ROM in a single wrist in 2 patients (6.4%) in the ACPA positive group. (Figure 32) While in the ACPA negative group; ulnar deviation was present in 2 patients (16.7%) (1 unilaterally and 1 bilaterally) and limitation in the ROM in a single wrist in 1 patient (8.3%).

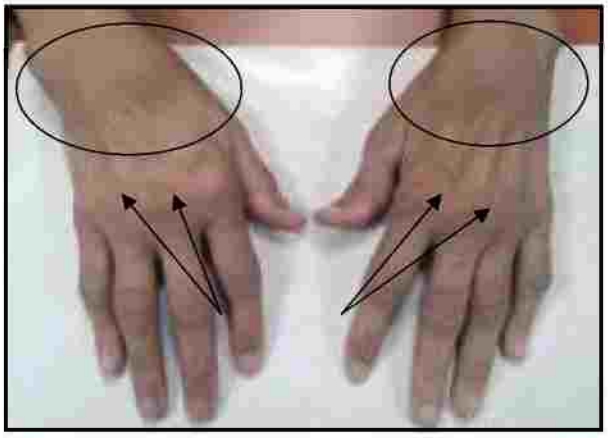


Figure (32): A 43 year old ACPA positive RA patient with chronic synovitis, effusion, (circles) tenderness and ulnar deviation of both wrist joints, severe wasting of the small muscles of the hands (arrows) and multiple finger deformities.

One patient in the ACPA positive group had undergone synovectomy with fixation and fusion of the right wrist due to persistent chronic synovitis. (Figure 33)

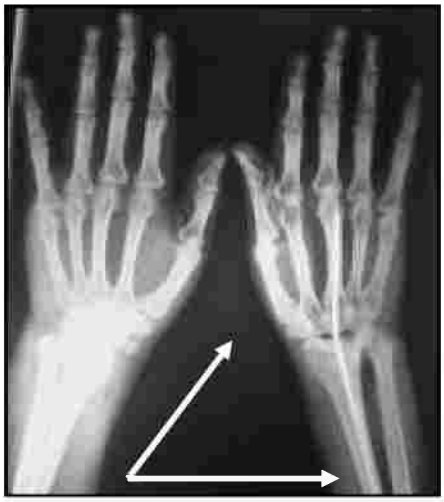


Figure (33): Plain X-ray of both hands and wrist of a 35 year old ACPA positive patient showing extensive erosive and destructive changes in the right wrist joint where synovectomy with K-wire fixation was done.

Finger deformities

Z-shaped deformities of the thumb was present in 4 patients (12.9%) (1 unilaterally and 3 bilaterally) in the ACPA positive group.

Boutonnieres deformity was present in 4 patients (12.9%) in the ACPA positive group and in 1 patient (8.3%) in the ACPA negative group.

Swan neck deformity was present in 4 patients (12.9%) (3 unilaterally and 1 bilaterally) in the ACPA positive group and unilaterally in 1 patient (8.3%) in the ACPA negative group. (Figure 34, 35, Table 13)

Other Deformities of the fingers included: medial deviation of the little finger and lateral deviation of the ring finger was present in 1 patient (3.2%) in the ACPA positive group, while limitation in ROM of the medial 4 fingers was present in one hand in 1 patient (8.3%) in the ACPA negative group.

There was no statistical significant difference in upper limb deformities among the patients' groups (p=0.43). (Table 13)

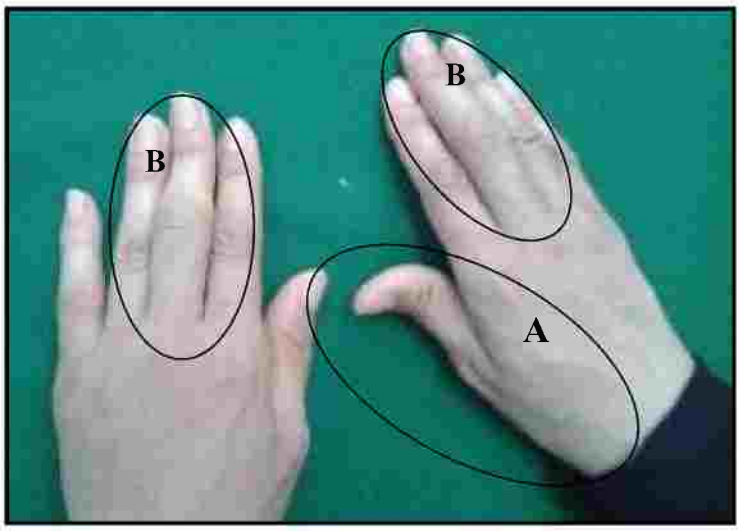


Figure (34): A 41 years old ACPA positive RA patient showing finger deformities (A) Right Z-thumb, (B) Bilateral middle finger boutonnieres deformity.

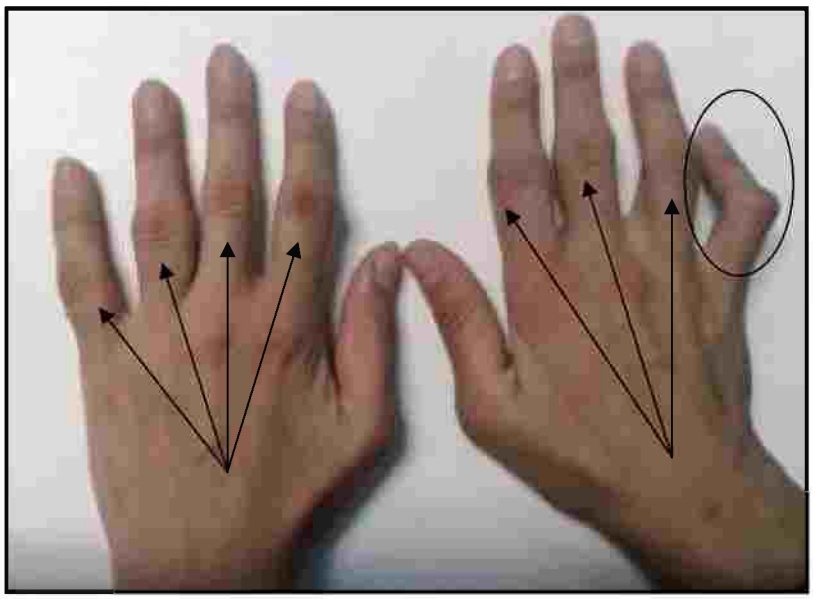


Figure (35): A 32 year old ACPA positive RA patient with swelling in several PIP joints of both hands (arrows) and boutonniere deformity in the right little finger (circle).

Table (13): Deformities in the upper limbs in the patients' groups.

		ACPA +ve		ACPA -ve	
		Unilateral	Bilateral	Unilateral	Bilateral
		No (%)			
Shoulder	Limited ROM	-	1(3.2%)	-	-
	Periarthritis	1(3.2%)	-	-	-
Elbow	Limited ROM	1(3.2%)	1(3.2%)	-	-
Wrist	Limited ROM	2(6.4%)	-	1(8.3%)	-
	Ulnar deviation	4(12.9%)	3(9.6%)	1(8.3%)	1(8.3%)
	Synovectomy	1(3.2%)	-	-	-
Fingers	Z-thumb	1(3.2%)	3(9.6%)	-	-
	Boutonniere	4(12.9%)	-	1(8.3%)	-
	Other deformities	1 (3.2%)	-	1 (8.3%)	-
Total deformities in UL		15(48.4%)	8 (25.6%)	4 (33.2%)	1 (8.3%)
		23 (74%)		5 (41.5%)	
Median (range):		0 (0-6)		0 (0-6)	
Mann Whitney U test		p= 0.43			

ACPA: anti-cyclic citrullinated-peptide, +ve: positive, -ve: negative, No: number, %: percentage, ROM: range of motion, UL: upper limb, significance level<0.05.

Lower limb deformities included

Knee deformities: limitation in the ROM in one knee joint was present in 1 patient (3.2%) in the ACPA positive group. (Figure 36)

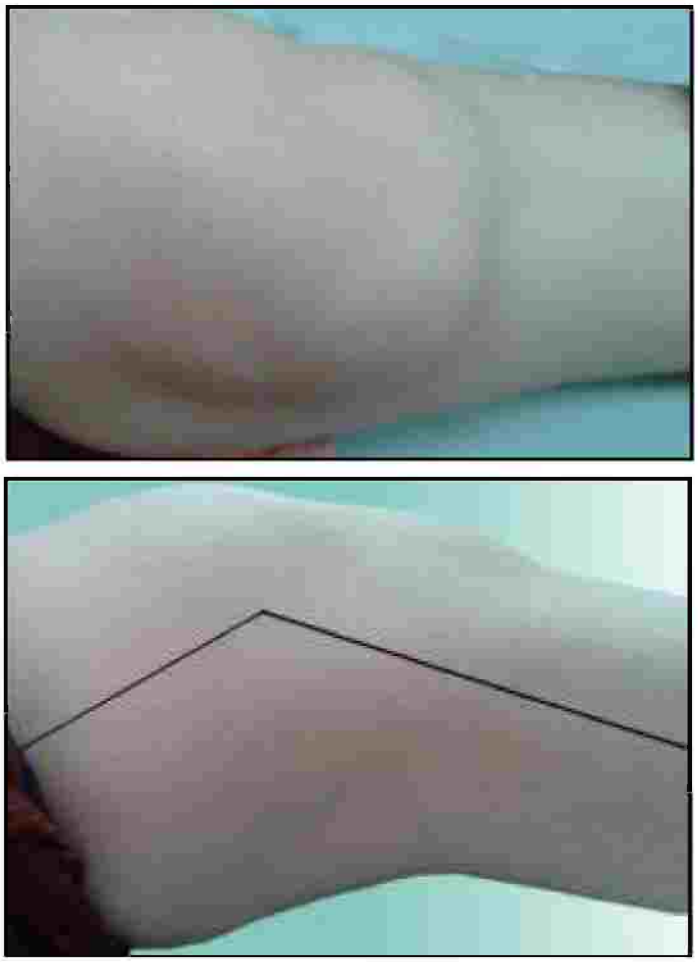


Figure (36): A 40 year old ACPA positive RA patient with severe swelling and effusion of the right knee with limitation in flexion and extension.

Deformities in the big toe: was present unilaterally in 1 patient (3.2%) in the ACPA positive group.

One patient in the ACPA positive group uses walking aids (right elbow crutch) due to the severe pain in the lower limb joints.

There was no statistical significant difference in lower limb deformities among the studied groups (p=0.64). (Table 14)

Table (14): Deformities in the lower limbs in the patients' groups.

		ACPA +ve		ACPA –ve	
		Unilateral	Bilateral	Unilateral	Bilateral
		No (%)			
Knee	Limited ROM	1(3.2%)	-	-	-
Toes	Limited ROM	1 (3.2%)	-	-	-
Total deformities in LL		2 (6.4%)		-	
Median, range:		0 (0-1)		0	
Mann Whitney U test		p= 0.64			

ACPA: anti-cyclic citrullinated-peptide, +ve: positive, -ve: negative, No: number, % percentage, ROM: range of motion, LL: lower limbs, significance level <0.05.

Eye complications

One patient in the ACPA positive group developed RA corneal ulcer which was resistant to treatment with high dose oral Cs and MTX, but it was responsive to topical cyclosporine eye drops. (Figure 37)

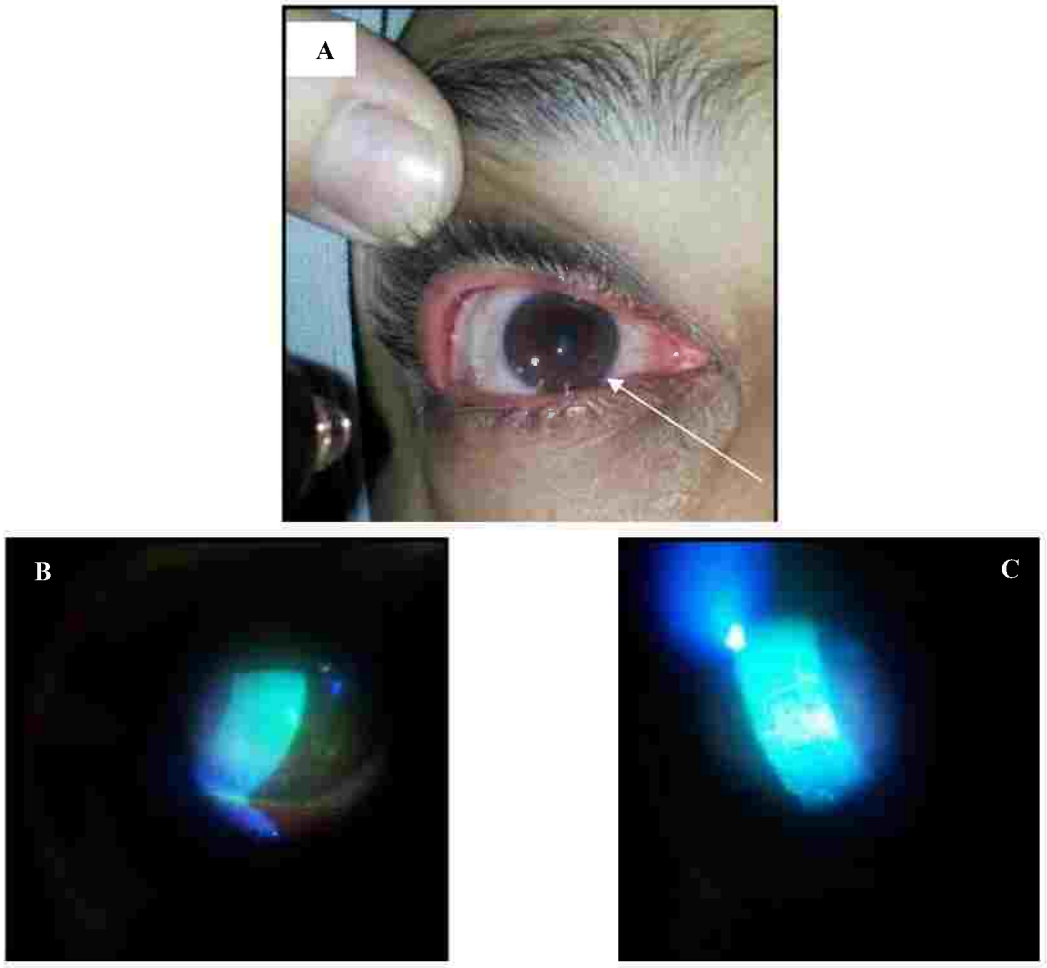


Figure (37): RA patient severe sicca syndrome complicated by (A) corneal ulcer with surrounding infiltration, (B,C) staining of corneal erosions and corneal infiltration with fluorescin stain & cobalt blue light slit lamp examination.

Associated conditions

Several conditions can be found in RA patient which can affect the treatment strategy and can also affect the patient's quality of life.

Hypertension (HTN)

Two patients (6.4%) in the ACPA positive group, 1 patient (8.3%) in the ACPA negative group and 2 individuals (6.7%) in the control group had HTN. There was no statistical significant difference among the studied groups (p=1.0). (Table 15)

Diabetes Mellitus (DM)

Three patients (9.7%) in the ACPA positive group, 4 patients (33.3%) in the ACPA negative group and 2 individuals (6.7%) in the control group had DM. There was no statistical significant difference among the studied groups. (p=0.081). (Table 15)

Depression

One patient (3.2%) in the ACPA positive group had depression, while none in the ACPA negative group or in the control group had depression. There was no statistical significant difference among the studied groups (p=1.0). (Table 15)

Vasculitis

Three patients (9.7%) in the ACPA group and 1 patient (8.3%) in the ACPA negative group developed vasculitis, while none in the control group developed vasculitis. There was no statistical significant difference among the studied groups (p=1.0). (Table 15)

Sjogren's syndrome (Secondary)

Three patients (9.7%) developed Sjogren's syndrome in the ACPA positive group, while none in the ACPA negative group or in the control group developed Sjogren's syndrome. There was no statistical significant difference among the studied groups (p=0.548). (Table 15)

Secondary Fibromyalgia (FM)

Seven patients (22.6%) in the ACPA positive group and 2 patients (16.7%) in the ACPA negative group developed Secondary FM, while none in the control group developed FM. There was no statistical significant difference among the studied groups (p=1.0). (Table 15)

Secondary Knee osteoarthritis (OA)

Two patients (6.5%) in the ACPA positive group, 1 patient (8.3%) in the ACPA negative group developed secondary knee OA proven by X-ray, while 2 individuals (6.7%) in the control group had knee OA. There was no statistical significant difference regarding the number of patients developed secondary OA among the studied groups (p= 1.0). (Table 15)

Entrapment neuropathies (Secondary)

Carpel tunnel syndrome (CTS)

Three patients (9.7%) in the ACPA positive group and 1 patient (8.3%) in the ACPA negative group developed CTS, while none in the control group had CTS. There was no statistical significant difference regarding the number of patients that developed CTS among the studied groups (p= 1.0). (Table 15)

Ulnar neuritis at the elbow

One patient (3.2%) in the ACPA positive group developed ulnar neuritis at the elbow, while none in the ACPA negative group or in the control group developed ulnar neuritis at the elbow. There was no statistical significant difference regarding the number of patients developed ulnar neuritis at the elbow among the studied groups (p= 1.0). (Table 15)

Table (15): Associated conditions in the studied groups.

Associated condition	ACPA+ve	ACPA-ve	Control	Chi square test (X^2)
	No (%)			
Hypertension	2(6.4%)	1(8.3%)	2(6.7%)	p=1.0
Diabetes Mellitus	3(9.7%)	4(33.3%)	2(6.7%)	p=0.081
Depression	1(3.2%)	0	0	p=1.0
Vasculitis	3(9.7%)	1(8.3%)	0	p=1.0
Sjogren's Syndrome	3(9.7%)	0	0	p=0.548
Fibromyalgia	7(22.6%)	2(16.7%)	0	p=1.0
Osteoarthritis Knee	2(6.5%)	1(8.3%)	2(6.7%)	p=1.0
Entrapment neuropathies				
CTS	3 (9.7%)	1(8.3%)	1(3.33%)	p=1.0
Ulnar neuritis at the elbow	1(3.2%)	0	0	p=1.0

ACPA: anti-cyclic citrullinated-peptide, +ve: positive, -ve: negative, CTS: carpel tunnel syndrome, significance level<0.05.

Laboratory Investigations

Hemoglobin (Hb)

The mean Hb level was 11.529 gm/dl (±1.49) in the ACPA positive group and 12.355 gm/dl (±1.34) in the ACPA negative group. Nineteen (61%) patients had anemia in the ACPA positive group and only 2 (16.7) patients in the ACPA negative group

There was no statistical significant difference in the mean Hb level in the patients' groups (t=1.617, p=0.114). (Table 16)

Red blood cells (RBC):

The mean RBC count was $4.57x10^6$ cell/mm^3 (±0.449) in the ACPA positive group and $4.62x10^6$ cell/mm^3 (±0.398) in the ACPA negative group.

There was no statistical significant difference in the mean RBC count in the patients' groups (t=0.35, p=0.728). (Table 16)

White blood cells (WBC)

The median WBC count was $7.44x10^3$ cell/mm^3 (ranging from 3.65-11.41) in the ACPA positive group, and $6.59x10^3$ cell/mm^3 (ranging from 4.3-9.24) in the ACPA negative group.

There was no statistical significant difference in the mean WBC count in the patients' groups (p=0.693). (Table 16)

Platelets (PLT)

The mean PLT count was $309x10^3$ cell/mm^3 (±69.1) in the ACPA positive group and $286x10^3$ cell/mm^3 (±66.9) in the ACPA negative group.

There was no statistical significant difference in the mean PLT count in the patients' groups (t=-0.948, p=0.349). (Table 16)

Erythrocyte sedimentation rate (ESR)

The mean level of ESR in the first hour was 60.097 mm/Hr (± 32.55) in the ACPA positive group and 47.25 mm/Hr (± 29.29) in the ACPA negative group.

There was no statistical significant difference in the mean ESR level in the patients' groups (t=-1.192, p=0.24). (Table 16)

C-reactive protein (CRP)

The median level of CRP was 9.6 mg/L (ranging from 1.4-52) in the ACPA positive group, and 5.88 mg/L (ranging from 2.97-49.2) in the ACPA negative group.

There was no statistical significant difference in the median CRP level in the patients' groups (p=0.446). (Table 16)

Rheumatoid factor (RF)

The median level of RF was 120 IU/ml (ranging from 10-896) in the ACPA positive group, and 10.1 IU/ml (ranging from 6-15) in the ACPA negative group.

There was a statistical significant difference in the median RF level in the patients' groups (p<0.001) where the ACPA positive group had higher RF levels. (Table 16)

Anti-cyclic-citrullinated peptide antibodies (ACPA)

The median level of ACPA was 90 U/ml (ranging from 20.3-1000) in the ACPA positive group, and 12.75 U/ml (ranging from 2-18) in the ACPA negative group.

There was a statistical significant difference in the median ACPA level in the patients' groups (U=642, p<0.001) where the ACPA positive group had higher ACPA levels. (Table 16)

Table (16): Laboratory investigations in the patients groups.

	ACPA positive	ACPA negative	Independent sample t test
	Mean ± SD		
Hb gm/dl	11.529±1.49	12.355±1.34	t=1.617, p=1.0
RBC ($x10^6$ c/mm^3)	4.57±0.449	4.62±0.398	t=0.35, p=0.728
	Mean (range)		Mann Whitney U test
WBC ($x10^3$ c/mm^3)	7.44 (3.65-11.41)	4.62 (4.3-9.24)	p=0.693
	Mean ± SD		Independent sample t test
PLT ($x10^3$ c/mm^3)	309±69.1	286±66.9	t= -0.948, p=0.349
ESR (mm/Hr)	60.097±29.29	47.25±29.29	t= -1.192, p=0.24
	Mean (range)		Mann Whitney U test
CRP (mg/L)	9.6 (1.4-52)	5.88 (2.97-49.2)	p=0.446
RF (IU/ml)	120 (10-896)	10.1 (6-15)	**p<0.001***
ACPA (U/ml)	90 (20.3-1000)	12.75 (2-18)	**U= 642, p<0.001***

Hb: hemoglobin, RBC: red blood cells, WBC: white blood cells, PLT: platelets, ESR:erythrocyte sedimentation rate, CRP: c-reactive protein, RF: rheumatoid factor, ACPA: anti-cyclic-citrullinated peptide antibodies, sig: significance, SD: standard deviation, No.: number, %: percentage, SIJ: sacroiliac joint, c: cell, Hr: hour, mg:milligram, L: liter, IU: international unit, U: unit, ml: milliliter, significance level<0.05.

Bone Turnover Markers

Total procollagen type 1 amino-terminal propeptide (TP1NP)

The mean level of serum TP1NP was 36.01±19.266 ng/ml in the ACPA positive group, 28.615±15.91 ng/ml in the ACPA negative group and 41.81±15.51 ng/ml in the control group. There was no statistical significant difference between the 3 groups as regards the mean levels of serum TP1NP (F=2.19, p=0.121). (Table 17)

β-CrossLaps (β-CTx)

The mean level of serum β-CTx was 0.364±0.221 in the ***ACPA positive group***, 0.337±0.231 in the ***ACPA negative group*** and 0.233±0.0779 in the ***control group***. There was a statistical significant difference in the mean level of β-CTx between ***the control group*** and the ***ACPA positive group*** (p=0.011), where the ACPA positive group had a higher level of serum β-CTx. (Figure 38, Table 17)

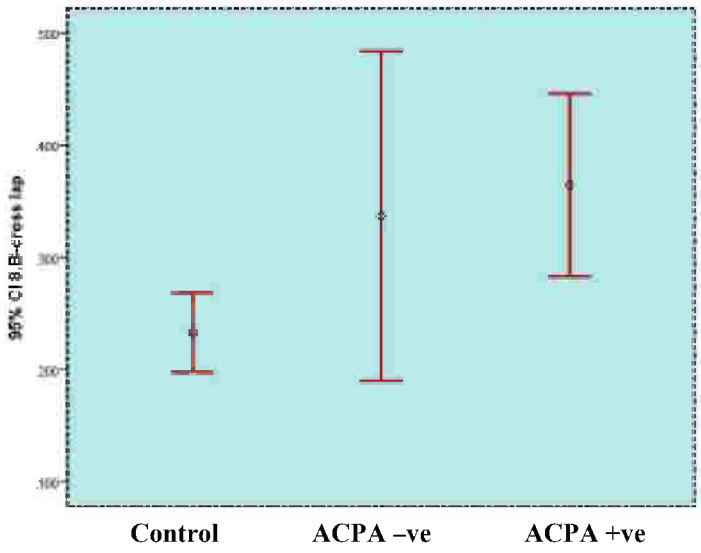


Figure (38): Mean level of serum β-CrossLap in the studied groups.

N-MID Osteocalcin (OCN)

The median level of serum OCN was 20 (ranging from 6.59 to 41.15) in the ACPA positive group, 13.84 (ranging from 3.02 to 49.63) in the ACPA negative group and 20.42 (ranging from 13.75 to 38.14) in the control group. There was a statistical significant difference in the median level of serum OCN between the control group and the ACPA negative group (p=0.032), where the ACPA negative group had a lower level of serum OCN. (Figure 39, Table 17)

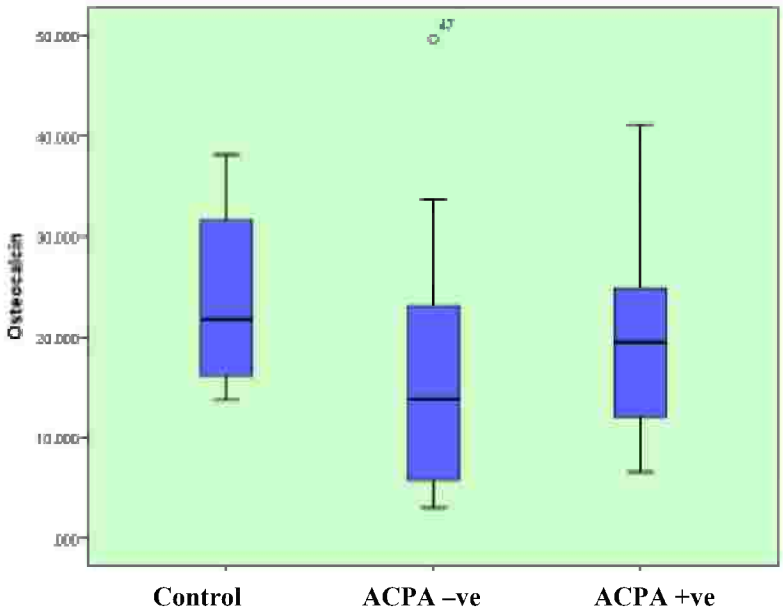


Figure (39): Median level of serum NMD-Osteocalcin in the studied groups.

Table (17): Correlation between the studied groups as regards serum bone turnover markers.

Item	ACPA +ve	ACPA -ve	Control	Statistical significance
	Mean(SD)			OnewayANOVA
TP1NP ng/ml	36.01(±19.266)	28.615(±15.91)	41.81(±15.51)	F=2.19 p=0.121
β -CTx ng/ml	0.364(±0.221)	0.337(±0.231)	0.233(±0.0779)	**F=24.7 p=0.013***
Control	**p=0.011****			
ACPA +ve				
ACPA -ve				
	Median(min-max)			KruskalWallis
OCN ng/ml	20 (6.59-41.15)	13.84 (3.02-49.63)	20.42 (13.75-38.14)	**K=6.79 p=0.034***
Control		**p=0.032****		
ACPA +ve				
ACPA -ve				

ACPA: anti-cyclic-citrullinated peptide antibodies, F: One way ANOVA test, K: Kruskal Wallis, SD: standard deviation +ve: positive, -ve: negative, TP1NP: Total procollagen type 1 amino-terminal propeptide, β-CTx: β-CrossLaps, OCN: osteocalcin, significance level<0.05.

Modified Total Sharp Score (mTSS)

The mean mTSS was 77.69(±48.23) in the ACPA positive group, 41.42(±16.88) in the ACPA negative group.

There was a statistical significant difference in the mean mTSS among the ACPA positive and the ACPA negative group (t= -3.41, p=0.002) where the ACPA positive group showed a higher mTSS and therefore high joint damage and destruction. (Table 18, Figures 40-43)

Table (18): Modified total sharp score in the patients groups.

	ACPA positive	ACPA negative	Independent sample t test
	Mean ± SD		
mTSS	77.69±48.23	41.42±16.88	**t=-3.41 p=0.002***

ACPA: anti-cyclic-citrullinated peptide antibodies, mTSS: modified total sharp score, SD: standard deviation, significance level<0.05.

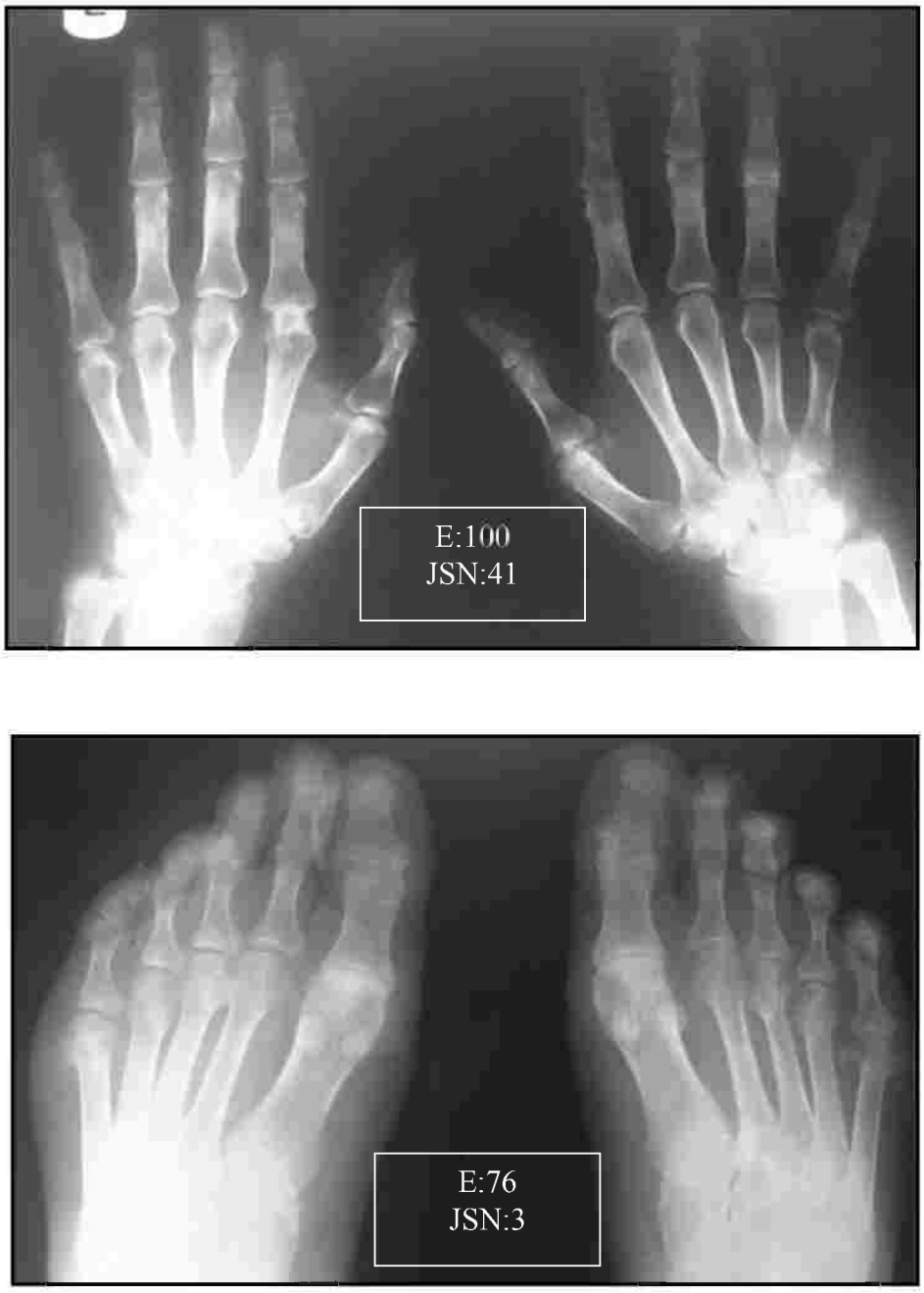


Figure (40): Plain X-ray of both hands and feet of a 45 years old ACPA positive RA patient with mTSS: 220. (E:erosions, JSN: joint space narrowing).

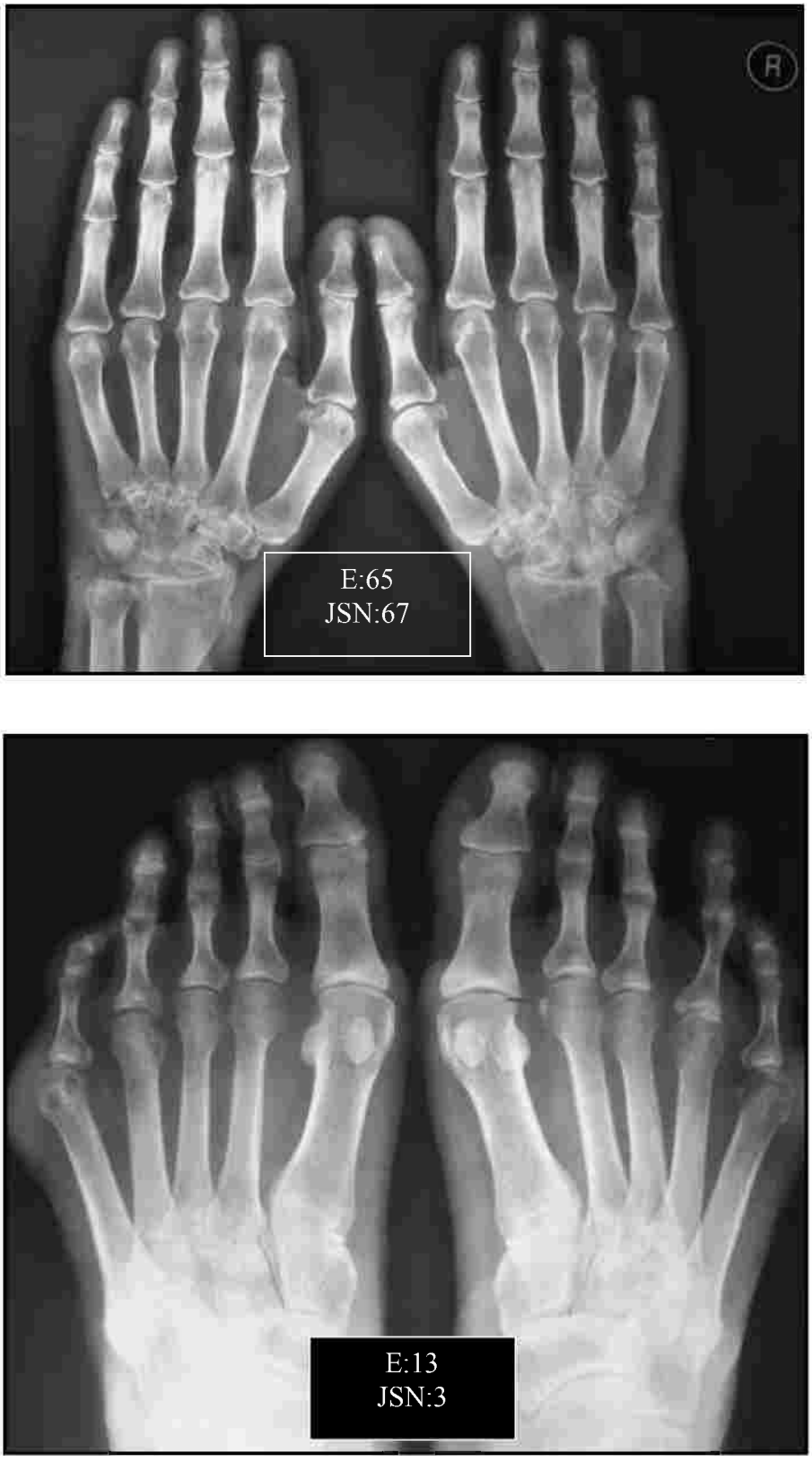


Figure (41): Plain X-ray of both hands and feet of a 42 years old ACPA positive RA patient with mTSS: 148. (E:erosions, JSN: joint space narrowing).

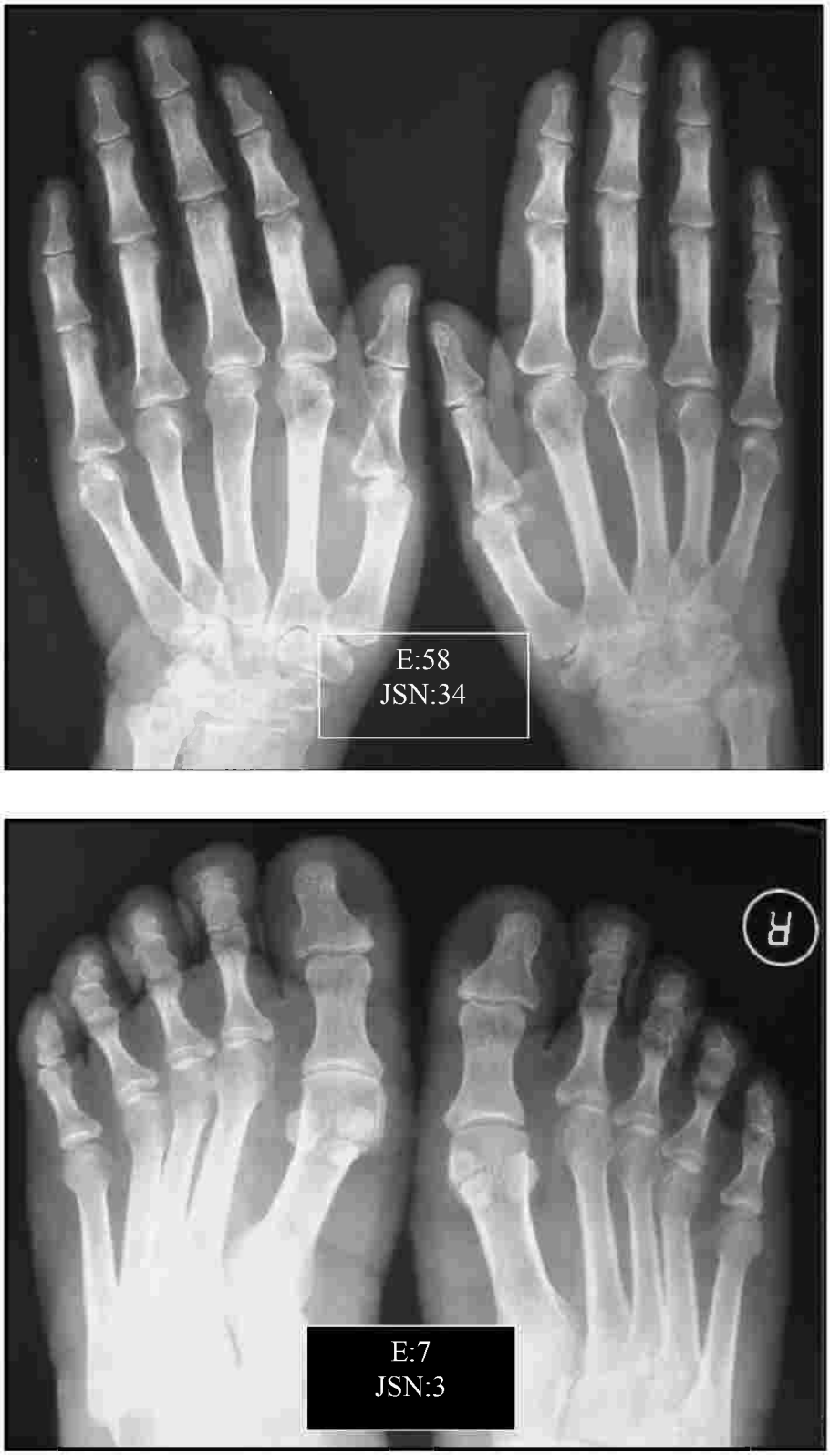


Figure (42): Plain X-ray of both hands and feet of a 32 years old ACPA positive RA patient with mTSS: 102. (E:erosions, JSN: joint space narrowing).

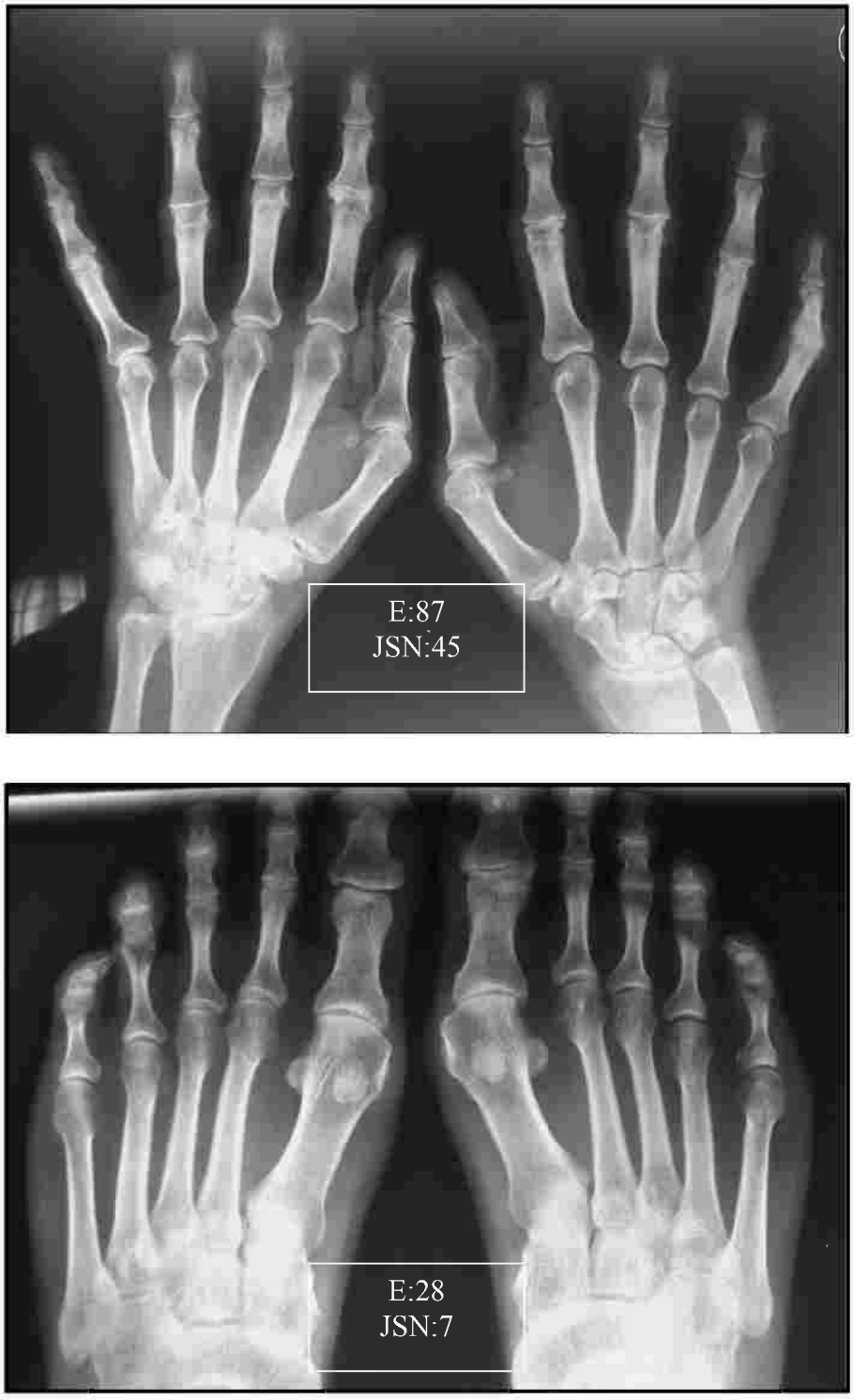


Figure (43): Plain X-ray of both hands and feet of a 32 years old ACPA positive RA patient with mTSS: 171. (E:erosions, JSN: joint space narrowing)

Dual Energy X-ray absorptiometry (DXA)

Lumbar spine DXA

The lumbar spine bone mineral density (BMD) was represented by the 1st and 4th lumbar vertebrae (L1–L4) BMD average score measurement. The mean L1-L4 BMD was 1.074(±0.143) gm/cm^2 in the ACPA positive group, 1.176(±0.110) gm/cm^2 in the ACPA negative group and 1.26(±0.116) gm/cm^2 in the control group.

There was a statistical significant difference in the mean level of L1-L4 spine BMD between the control group and the ACPA positive group ($p< 0.001$) as well as between the ACPA negative and the ACPA positive group ($p= 0.035$), where the ACPA positive group showed a lower L1-L4 spine BMD than the control as well as the ACPA negative group. (Table 19)

Hip DXA

The left (Lt) hip BMD was represented by the Lt total femur BMD score. The mean Lt total femur BMD was 0.978(±0.104) gm/cm^2 in the ACPA positive group, 1.035(±0.097) gm/cm^2 in the ACPA negative group and 1.129(±0.111) gm/cm^2 in the control group.

There was a statistical significant difference in the mean level of Lt total femur BMD between the control group and the ACPA positive ($p< 0.001$) and between the control group and the ACPA negative group ($p= 0.019$) where the ACPA positive group as well as the ACPA negative group showed a lower Lt total femur BMD than the control group. (Table 19)

Forearm DXA

The Lt forearm BMD was represented by the Lt total radius BMD score. The mean Lt total radius BMD was 0.644(±0.075) gm/cm^2 in the ACPA positive group, 0.677(±0.073) gm/cm^2 in the ACPA negative group and 0.709(±0.065) gm/cm^2 in the control group.

There was a statistical significant difference in the mean level of Lt total radius BMD between the control group and the ACPA positive group ($p< 0.001$), where the ACPA positive group showed a lower Lt total radius BMD. (Table 19)

Table (19): Correlation between the studied groups as regards DXA score in the different regions.

Item	Control	ACPA +ve	ACPA -ve	Statistical Significance	
	Mean(SD)			Oneway ANOVA	Linear trend
L1-L4 Spine BMD	1.26 (±0.116)	1.074 (±0.143)	1.176 (±0.110)	**F=17.317 p<.001***	**F=34.50 p<.001***
Control		**<0.001****			
ACPA +ve					
ACPA -ve		**0.035****			
Lt hip BMD	1.129 (±0.111)	0.978 (±0.104)	1.035 (±0.097)	**F=16.1 p<.001***	**F=31.93 p<.001***
Control		**<0.001****	**0.019****		
ACPA +ve					
ACPA –ve					
Lt forearm BMD	0.709 (±0.065)	0.644 (±0.075)	0.677 (±0.073)	**F=6.67 p<.002***	**F=13.32 p<.001***
Control		**<0.001****			
ACPA +ve					
ACPA –ve					

ACPA: anti-cyclic-citrullinated peptide antibodies, F: One Way ANOVA test, SD: standerd deviation, +ve: positive, -ve: negative, L1-L4: 1st and 4th lumbar vertebrae, Lt: left, BMD: bone mineral density, significance level<0.05.

T-score

The T-score was calculated for all patients to classify patients according to the World Health Organization (WHO) classification for bone loss by DXA score as normal: T-score at -1.0 and above, osteopenia: T-score between -1.0 and -2.5 and osteoporosis: T-score at -2.5 and below.

There was a statistical significant difference in the L1-L4 spine T- score and the overall T-score among the studied groups ($p<0.001$) and ($X^2=29.217$, $p<0.001$) respectively, where the ACPA positive group included more patients with osteopenia and osteoporosis especially at the spine. (Table 20, Figures 44-48)

Table (20): Correlation between the studied groups as regards T-score.

		ACPA +ve	ACPA-ve	Control	Total	Chi square test (X^2)
		Number(%)				
L1-L4 spine T-score	Normal	13(42%)	8(66.7%)	27(90%)	48(65.8%)	**p<0.001***
	Osteopenia	13(42%)	4(33.3%)	3(10%)	20(27.4%)	
	Osteoporosis	5(16%)	0	0	5(6.8%)	
Lt hip T-score	Normal	25(80.6%)	11(91.7%)	30(100%)	66(90.4%)	p=0.063
	Osteopenia	5(16.1%)	1(8.3%)	0	6(8.2%)	
	Osteoporosis	1(3.2%)	0	0	1(1.4%)	
Lt forearm T-score	Normal	21(67.7%)	9(75%)	24(80%)	69(94.5%)	p=0.483
	Osteopenia	7(22.6%)	2(16.7%)	6(20%)	4(5.5%)	
	Osteoporosis	3(9.7%)	1(8.3%)	0		
Overall T-score	Normal	9(29%)	9(75%)	27(90%)	45(±61.6)	**X^2=29.217 p<0.001***
	Osteopenia	11(35.5%)	1(8.3%)	3(10%)	15(±20.6)	
	Osteoporosis	11(35.5%)	2(16.7%)	0	13(±17.8)	

ACPA: anti-cyclic-citrullinated peptide antibodies, %: percentage, +ve: positive, -ve: negative, L1-L4: 1st and 4th lumbar vertebrae, Lt: left, normal: T-score >-1.0, osteopenia: T-score < -1.0 and >-2.5, osteoporosis: T-score < -2.5, *Results ≤.05 are significant.

Z-score

The Z-score was calculated for all patients according to the International Society for Clinical Densitometry (ISCD) recommendations in premenopausal females to assess bone loss by DXA score as normal: Z-score at -2.0 and above, and below normal for age: Z-score at -2 and below.

There was a statistical significant difference in the L1-L4 spine Z- score and the overall Z-score among the studied groups (X^2=30.482, p<0.001) and (X^2=29.15, p<0.001) respectively, where the ACPA positive group included more patients with below than normal score for age especially at the spine. (Table 21, Figures 44-48)

Table (21): Correlation between the studied groups as regards Z-score.

		ACPA+ve	ACPA -ve	Control	Total	Chi square test (X^2)
		Number (%)				
L1-L4 spine Z-score	N >-2	12(38.7%)	11(91.7%)	30(100%)	53(72.6%)	**X2=30.482 p<0.001***
	BNA≤-2	19(61.3%)	1(8.3%)	0	20(27.4%)	
Lt hip Z-score	N >-2	27(87.1%)	12(100%)	30(100%)	69(94.5%)	p=0.129
	BNA≤-2	4(12.9%)	0	0	4(5.5%)	
Lt forearm Z-score	N >-2	25(80.6%)	10(83.3%)	29(96.7%)	64(87.7%)	p=0.305
	BNA≤-2	6(19.4%)	2(16.7%)	1(3.33%)	9(12.3%)	
Overall Z-score	N >-2	14(45.2%)	10(83.3%)	29(96.7%)	53(72.6%)	**X2=29.15 p<0.001***
	BNA≤-2	17(54.8%)	2(16.7%)	1(3.3%)	20(27.4%)	

ACPA: anti-cyclic-citrullinated peptide antibodies, N=normal, BNA= below normal for age, sig=significance, %: percentage, +ve: positive, -ve: negative, L1-L4: 1st and 4th lumbar vertebrae, Lt: left, *Results ≤.05 are significant.

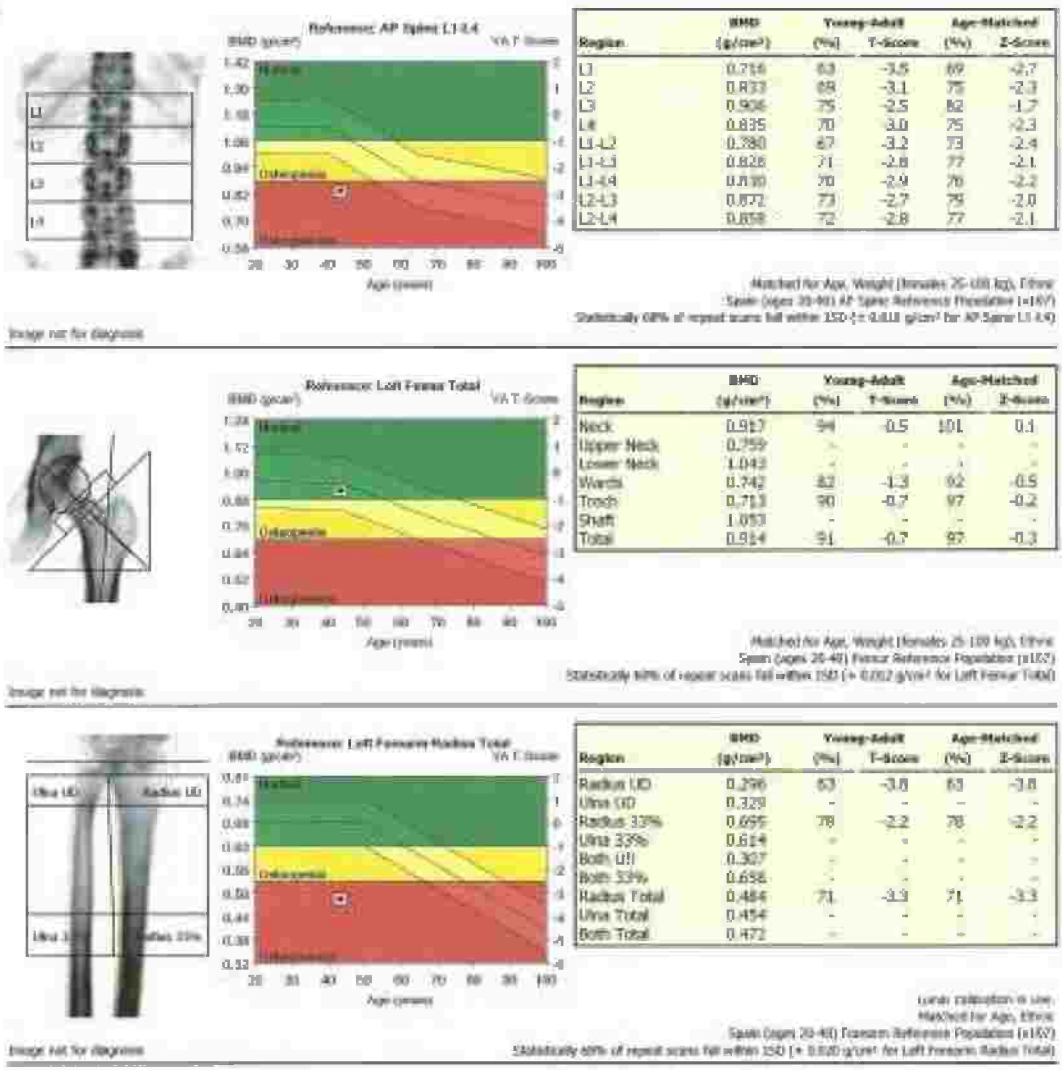


Figure (44): The DXA of a 40 year old ACPA positive RA patient of 5 years disease duration with high disease activity showing osteoporosis by T- score and reduced bone mass by Z- score at the lumbar spine and left forearm.

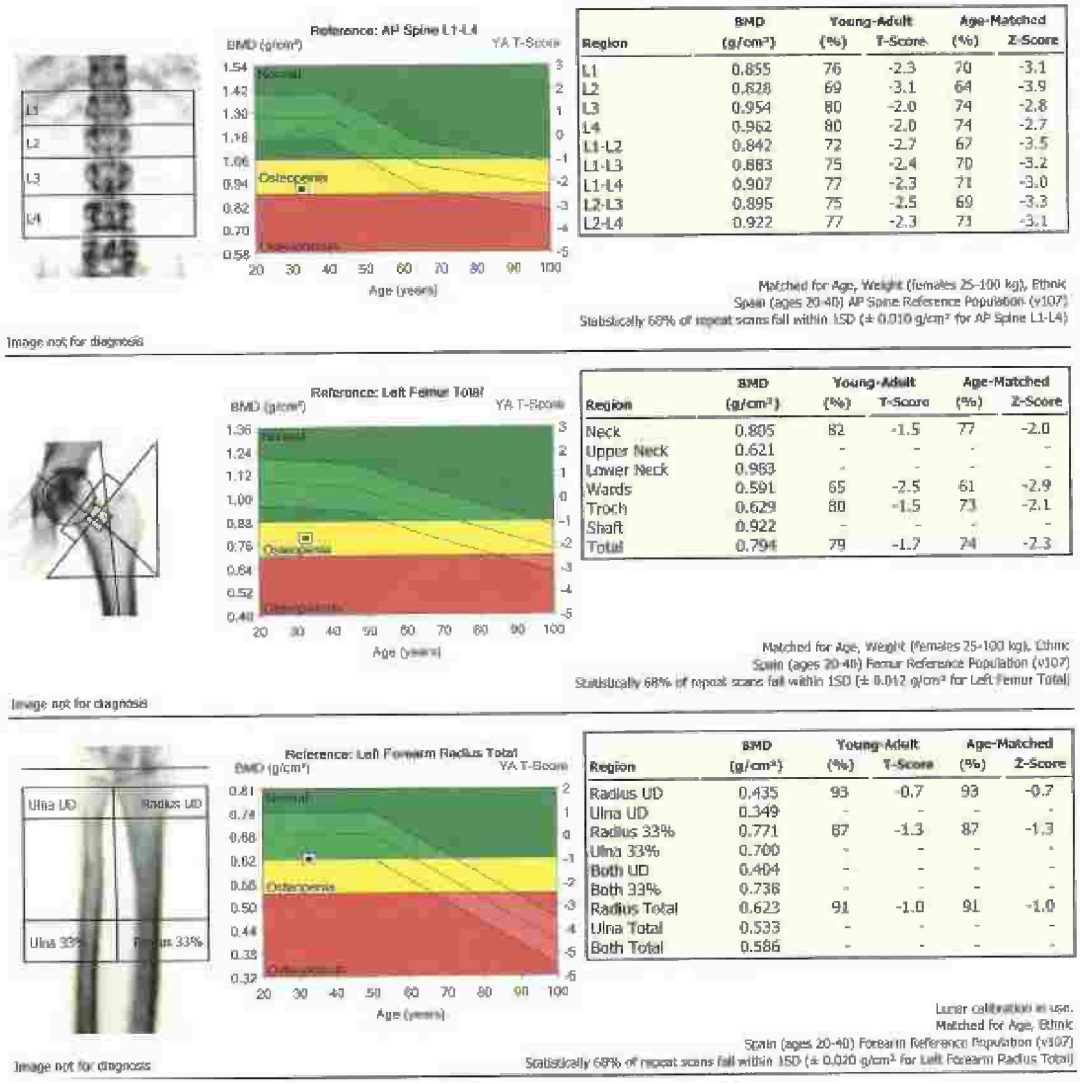


Figure (45): The DXA of a 33 year old ACPA positive RA patient of 7 years disease duration with high disease activity showing osteoporosis at the lumbar spine, and osteopenia at the left hip and forearm by T- score and reduced bone mass at the left hip and lumbar spine by Z- score.

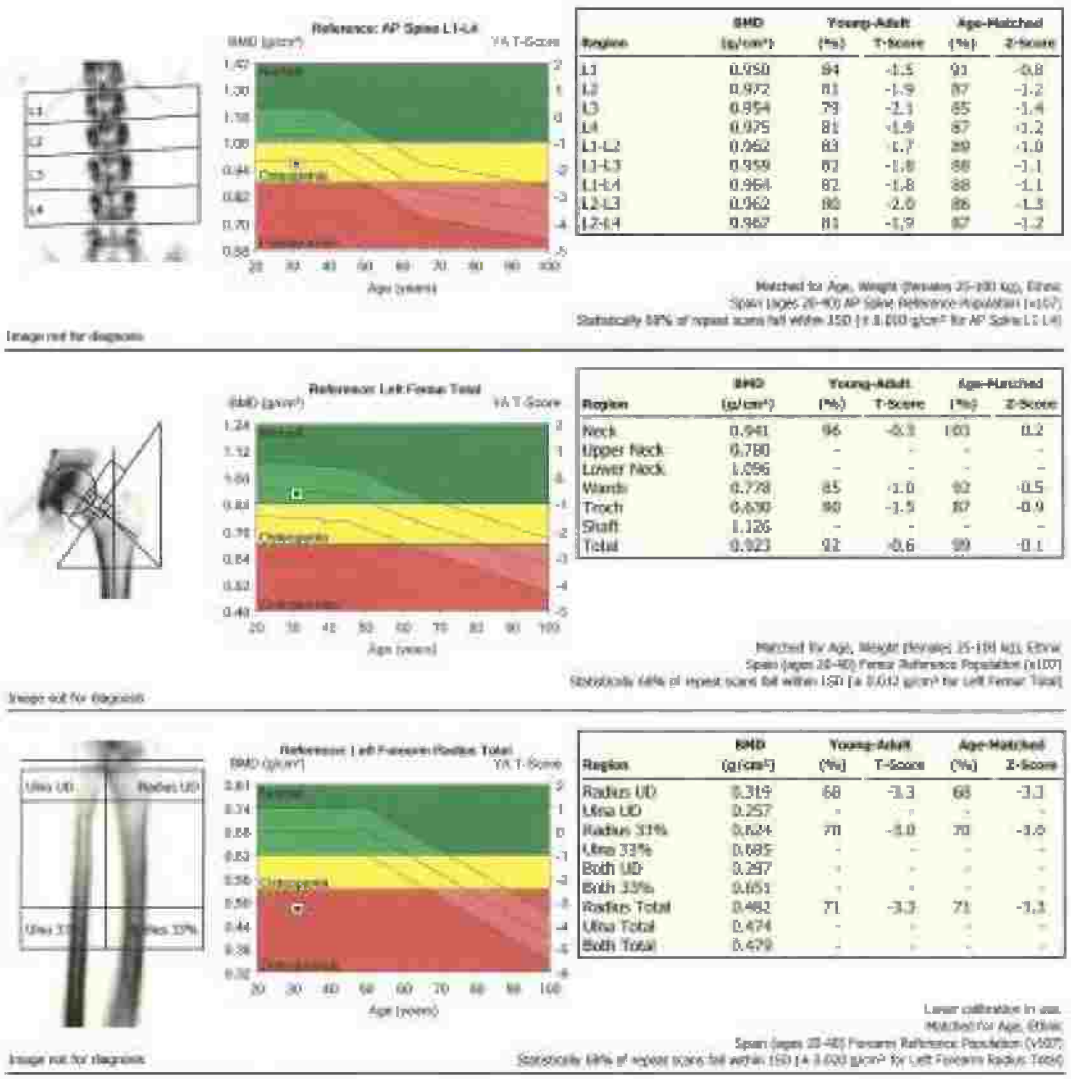
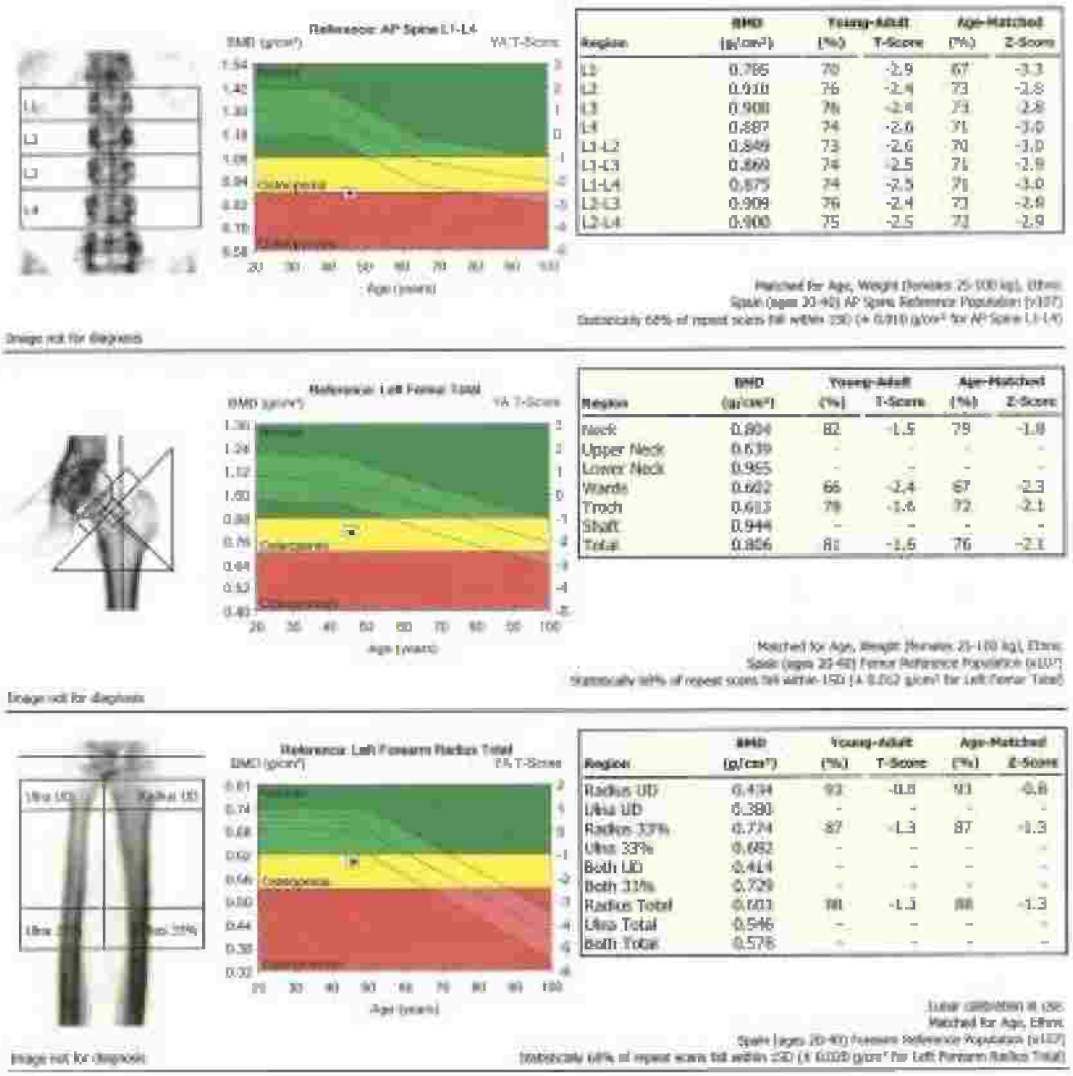


Figure (46): The DXA of a 32 year old ACPA positive RA patient of 5 years disease duration with high disease activity showing osteoporosis at the left forearm and osteopenia at the lumbar spine by T- score and reduced bone mass at the left forearm by Z- score.



Region	BMD (g/cm²)	Young-Adult (%)	T-Score	Age-Matched (%)	Z-Score
L1	0.785	70	-2.9	67	-3.3
L2	0.910	76	-2.4	73	-2.8
L3	0.908	76	-2.4	73	-2.8
L4	0.887	74	-2.6	71	-3.0
L1-L2	0.849	73	-2.6	70	-3.0
L1-L3	0.869	74	-2.5	71	-2.9
L1-L4	0.875	74	-2.5	71	-3.0
L2-L3	0.909	76	-2.4	73	-2.9
L2-L4	0.900	75	-2.5	73	-2.9

Region	BMD (g/cm²)	Young-Adult (%)	T-Score	Age-Matched (%)	Z-Score
Neck	0.804	82	-1.5	79	-1.8
Upper Neck	0.639	-	-	-	-
Lower Neck	0.965	-	-	-	-
Wards	0.602	66	-2.4	67	-2.3
Troch	0.613	78	-1.6	72	-2.1
Shaft	0.944	-	-	-	-
Total	0.806	81	-1.6	76	-2.1

Region	BMD (g/cm²)	Young-Adult (%)	T-Score	Age-Matched (%)	Z-Score
Radius UD	0.434	93	-0.8	93	-0.8
Ultra UD	0.380	-	-	-	-
Radius 33%	0.774	87	-1.3	87	-1.3
Ultra 33%	0.682	-	-	-	-
Both UD	0.414	-	-	-	-
Both 33%	0.729	-	-	-	-
Radius Total	0.603	88	-1.3	88	-1.3
Ultra Total	0.546	-	-	-	-
Both Total	0.578	-	-	-	-

Figure (47): The DXA of a 46 year old ACPA positive RA patient of 19 years disease duration with high disease activity showing osteoporosis at the lumbar spine and osteopenia at the left hip and forearm by T- score and reduced bone mass at the lumbar spine and left hip by Z- score.

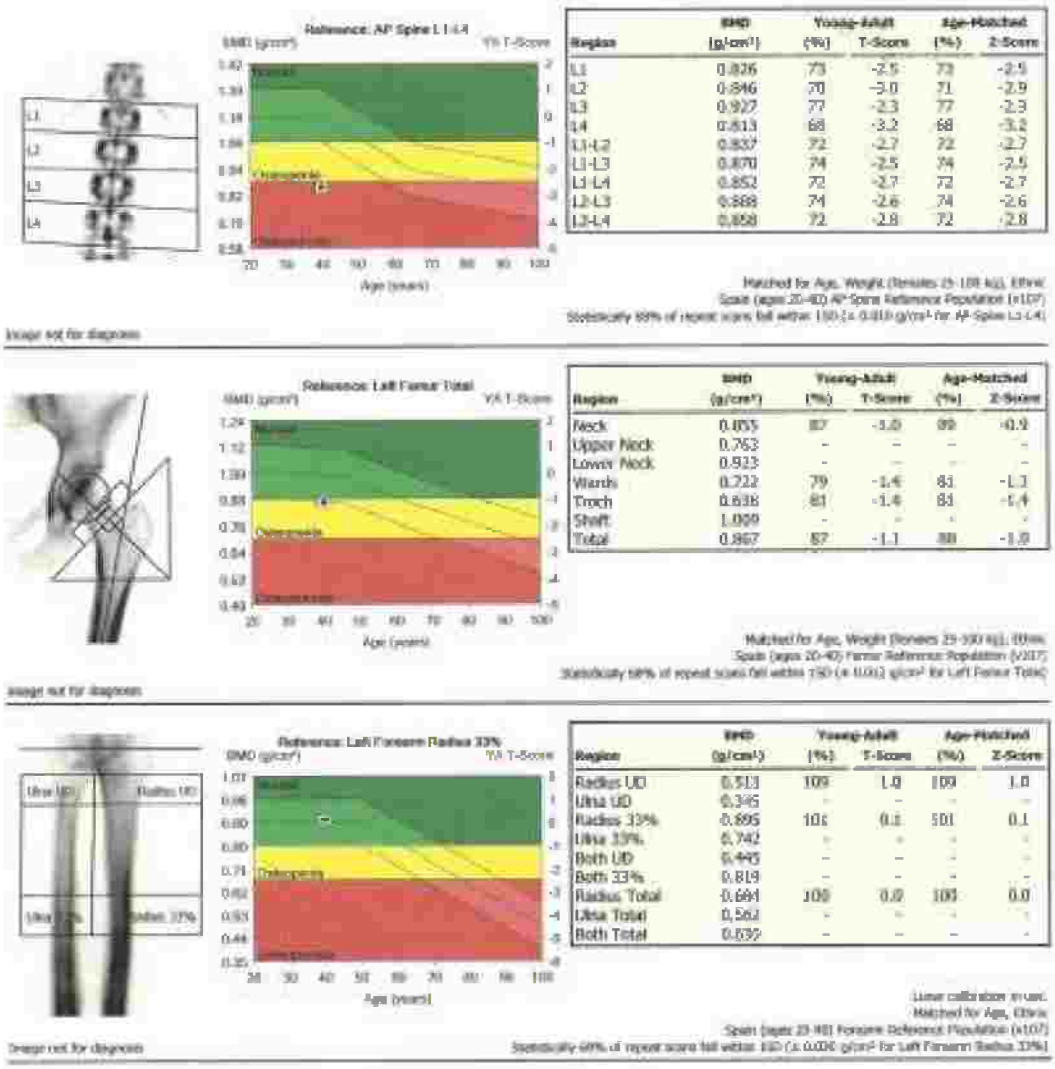


Figure (48): The DXA of a 39 year old ACPA positive RA patient of 6 years disease duration with high disease activity showing osteoporosis at the lumbar spine and osteopenia at the left hip and reduced bone mass at the lumbar spine by Z- score.

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.

Correlation between bone turnover markers and other patient & disease parameters: (Table 22)

In the ACPA positive group

There is a statistical significant moderate positive linear relation between the age of the patient and serum β-CTx level (r=0.37, p=0.041), where the older the patient, the higher the serum β-CTx level.

There is a statistical significant moderate negative linear relation between DAS 28-ESR & DAS 28-CRP and serum TP1NP level (r=-0.429, p=0.016) & (r=-0.356, p=0.049) respectively, where the higher the DAS 28-ESR & DAS 28-CRP score, the lower the serum TP1NP level.

There is a statistical significant moderate positive linear relation between DAS 28-CRP and serum β-CTx level (r=0.376, p=0.037), where the higher DAS 28-CRP score is, the higher the serum β-CTx level.

There is a statistical significant large positive linear relation between serum TP1NP and serum OCN level (r=0.612, p<0.001), where the higher serum TP1NP level, the higher the serum OCN level.

There is a statistical significant moderate negative linear relation between serum β-CTx and serum OCN level (r=-0.375, p=0.041), where the higher serum β-CTx level is, the lower the serum OCN level.

In the ACPA negative group

There is a statistical significant large positive linear relation between disease duration and serum OCN level (rs=0.665, p=0.021), where the longer the disease duration, the higher the serum OCN level.

There is a statistical significant large positive linear relation between serum TP1NP and serum OCN (r=0.905, p<0.001), where the higher serum TP1NP level is, the higher the serum OCN level.

Table (22): Correlation between bone turnover markers and other patient & disease parameters.

	TP1NP		OCN		β-CTx	
	ACPA+ve	**ACPA-ve**	**ACPA+ve**	**ACPA-ve**	**ACPA+ve**	**ACPA-ve**
Age	rs=-0.276, p=0.133	rs=-0.322, p=0.308	rs=-0.206, p=0.274	rs=-0.133, p=0.681	**rs=0.37, p=0.041***	rs=0.308, p=0.331
Disease duration	rs=-0.02, p=0.915	rs=0.417, p=0.178	rs=0.197, p=0.297	**rs=0.665, p=0.021***	rs=0.103, p=0.582	rs=-0.098, p=0.762
Duration of MS	rs=0.064, p=0.731	rs=-0.467, p=0.126	rs=0.143, p=0.45	rs=-0.333, p=0.29	rs=0.249, p=0.109	rs=0.033, p=0.918
BMI	r=-0.232, p=0.21	r=-0.427, p=0.166	rs=-0.368, p=0.054	rs=-0.235, p=0.463	rs=0.095, p=0.613	rs=0.319, p=0.313
DAS ESR	**r=-0.429, p=0.016***	r=-0.07, p=0.983	r=-0.217, p=0.25	r=0.084, p=0.794	r=0.29 , p=0.114	r=0.062, p=0.848
DAS CRP	**r=-0.356, p=0.049***	r=0.004, p=0.99	r=-0.201, p=0.287	r=0.048, p=0.882	**r=0.376, p=0.037***	r=0.084, p=0.795
HAQ	r=-0.249, p= 0.177	r=-0.233, p=0.466	r=-0.288, p= 0.122	r=-0.365, p=0.243	r=0.337, p= 0.064	r=-001, p=0.997
Cumulative Cs	rs= 0.08, p=0.67	rs=0.322, p=0.308	rs= 0.226, p=0.23	rs=0.244, p=0.445	rs= -0.117, p=0.531	rs=-0.283, p=0.373
ACPA	r=-0.078, p=0.677	r=0.179, p=0.578	rs=0.356, p=0.053	rs=0.063, p=0.846	rs=-0.342, p=0.06	rs=0.462, p=0.131
OCN	**r= 0.612, p<0.001***	**r=0.905, p<0.001***				
β-CTx	r=-0.123, p= 0.509	r=-0.293, p= 0.355	**r=-0.375, p=0.041***	r=-0.21, p= 0.512		

TP1NP: Total procollagen type 1 amino-terminal propeptide, OCN: NMD-Osteocalcin, β-CTx: β-CrossLaps, +ve: positive, -ve: negative, MS: morning stiffness, BMI: body mass index, DAS: disease activity score, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, HAQ: health assessment questionnaire, Cs: corticosteroids, ACPA: Anti-cyclic-citrullinated peptide antibodies, r: Pearson's correlation, rs: Spearmen's correlation, significance level<0.05.

Correlation between mTSS and other patient & disease parameters: (Table 23)

In the ACPA positive group

There is a statistical significant large positive linear relation between both disease duration & DAS 28-CRP and mTSS (rs=0.761, p<0.001 & r=0.837, p=0.042 respectively), and a statistical significant moderate positive linear relation between cumulative Cs dose and mTSS (r=0.432, p=0.027), where the longer the disease duration or the higher the DAS 28-CRP score or cumulative Cs dose, the higher the mTSS.

There is a statistical significant moderate negative linear relation between BMI and mTSS (r= -0.398, p=0.044), where the lower the BMI, the higher the mTSS.

In the ACPA negative group

There is a statistical significant large negative linear relation between serum OCN level and mTSS (r=-0.624, p=0.03), where the higher the serum OCN level, the lower the mTSS.

Table (23): Correlation between mTSS and other patient & disease parameters.

	mTSS	
	ACPA +ve	**ACPA -ve**
Age	r=0.114, p=0.579	r=0.01, p=0.976
Disease duration	**rs= 0.761, p<0.001***	rs= -0.056, p=0.862
Duration of MS	r=0.089, p=0.667	r=0.28, p=0.378
BMI	**r=-0.398, p=0.044***	r=0.016, p=0.961
DAS ESR	r=-0.097 , p=0.639	r=-0.487, p=0.108
DAS CRP	**r=0.837, p=0.042***	r= -0.436, p=0.157
HAQ	r= -0.2, p= 0.326	r= 0.348, p=0.268
Cumulative Cs	**rs= 0.432, p= 0.027***	rs= -0.411, p=0.148
ACPA	r=0.011, p=0.958	r=0.463, p=0.129
TP1NP	r= -0.006, p= 0.975	r= -0.59, p= 0.071
OCN	r= 0.145, p= 0.052	**r= -0.624, p= 0.03***
β-CTx	r= 0.064, p= 0.755	r= -0.049, p= 0.881

mTSS: modified total sharp score, +ve: positive, -ve: negative, MS: morning stiffness, DAS: disease activity score, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, HAQ: health assessment questionnaire, Cs: corticosteroids, ACPA: Anti-cyclic-citrullinated peptide antibodies, TP1NP: Total procollagen type 1 amino-terminal propeptide, OCN: NMD-Osteocalcin, β-CTx: β-CrossLaps r: Pearson's correlation, rs: Spearmen's correlation.

Correlation between BMD in the different regions and other patient & disease parameters: (Table 24)

In the ACPA positive group

There is a statistical significant moderate negative linear relation between DAS 28-CRP and both forearm & lumbar spine BMD (r=-0.417, p=0.025) & (r=-0.355, p=0.042) respectively, as well as between HAQ and both forearm & lumbar spine BMD (r=-0.359, p=0.05) & (r=-0.426, p=0.012) respectively; where the higher the DAS 28-CRP or the HAQ score, the lower the forearm & lumbar spine BMD.

Similarly there is a statistical significant moderate negative linear relation between cumulative Cs dose and both hip & lumbar spine BMD (r=-0.456, p=0.013) & (r=-0.44, p=0.015) respectively, as well as between mTSS and both forearm & hip BMD (r=-0.521, p=0.008) & (r=-0.402, p=0.046) respectively; where the higher the cumulative Cs dose or mTSS, the lower the forearm, hip & lumbar spine BMD.

On the other hand there is a statistical significant moderate positive linear relation between forearm BMD and both hip & lumbar spine BMD (r=0.432, p=0.022), (r=0.447, p=0.015) respectively, as well as between hip BMD and lumbar spine BMD (r=0.55, p=0.002); where the lower the forearm BMD, the lower both the hip & lumbar spine BMD and the lower the hip BMD, the lower the lumbar spine BMD.

Table (24): Correlation between BMD in the different regions and other disease parameters.

	Forearm BMD		Hip BMD		Lumbar Spine BMD	
	ACPA+ve	**ACPA-ve**	**ACPA+ve**	**ACPA-ve**	**ACPA+ve**	**ACPA-ve**
Age	rs=-0.074, p=0.702	rs=-0.191, p=0.574	rs=-0.025, p=0.897	rs=0.318, p=0.34	rs=-0.138, p=0.467	rs=0.245, p=0.467
Disease duration	r=-0.045, p=0.815	r=-0.308, p=0.356	r=-0.241 p=0.175	r=-0.377 p=0.203	r=0.145, p=0.415	r=0.233, p=0.444
Duration of MS	rs=0.06, p=0.756	rs=-0.227, p=0.502	rs=-0.036, p=0.852	rs=-0.227, p=0.502	rs=0.05, p=0.794	rs=0.076, p=0.823
BMI	rs=0.358, p=0.056	rs=-0.35, p=0.291	rs=0.284, p=0.135	rs=0.528, p=0.095	rs=-0.007, p=0.969	rs=0.65, p=0.3
DAS ESR	rs=-0.233, p=0.225	rs=-0.315, p=0.345	rs=-0.159, p=0.409	rs=0.082, p=0.81	rs=-0.22, p=0.243	rs=0.538, p=0.088
DAS CRP	**r=-0.417, p=0.025***	r=-0.574, p=0.065	r=-.136 p=0.458	r=0.028 p=0.923	**r=-0.355, p=0.042***	r=0.147 p=0.615
HAQ	**r=-0.359, p= 0.05***	r=-0.06, p=0.861	r=-0.033 p=0.852	r=-0.104 p=0.721	**r=-0.426 p=0.012***	r=0.08 p=0.784
Cumulative Cs	r=- 0.18, p=0.351	r=-0.493, p=0.123	**r= -0.456, p=0.013***	r=-0.232, p=0.492	**r= -0.44, p=0.015***	r=0.313, p=0.349
ACPA	rs=-0.116, p=0.55	rs=-0.372, p=0.261	rs=-0.015, p=0.937	rs=-0.322, p=0.335	rs=-0.182, p=0.336	rs=-0.179, p=0.599
MTX duration	r=- 0.098, p=0.613	r=- 0.034, p=0.926	r=0.181, p=0.347	r=0.248, p=0.426	r=- 0.31, p=0.872	r=0.215, p=0.551
MTX amount	r=0.205, p=0.348	r=- 0.482, p=0.198	r=0.084, p=0.702	r=- 0.386, p=0.304	r=0.186, p=0.385	r=0.371, p=0.326
TP1NP	r=-0.015, p=0.94	r=0.137, p=0.687	r=0.156, p=0.418	r=-0.434, p=0.183	r=0.049, p=0.798	r=-0.083, p=0.808
OCN	rs=-0.109, p= 0.579	rs=0.136, p=0.689	rs=-0.212, p= 0.28	rs=-0.409, p=0.212	rs=-0.223, p= 0.245	rs=0.336, p=0.312
β-CTx	rs=- 0.19, p=0.922	rs=-0.2, p=0.555	rs=-0.022, p=0.911	rs=-0.109, p=0.75	rs= -0.103, p=0.589	rs=0.236, p=0.484
mTSS	**r=-0.521, p= 0.008***	r=0.008, p= 0.982	**r=-0.402, p= 0.046***	r=0.029, p= 0.932	r=-0.185, p= 0.365	r=-0.282, p= 0.401
Forearm BMD			**r=0.432, p= 0.022***	r=0.52 , p= 0.101	**r=0.447, p= 0.015***	r=0.082, p= 0.822
Hip BMD					**r=0.55, p= 0.002***	r=-0.004, p= 0.991

BMD: bone mineral density, +ve: positive, -ve: negative, MS: morning stiffness, DAS: disease activity score, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, HAQ: health assessment questionnaire, Cs: corticosteroids, ACPA: Anti-cyclic-citrullinated peptide antibodies, r: Pearson's correlation, rs: Spearmen's correlation, mTSS: modified total sharp score, MTX: methotrexate. TP1NP: Total procollagen type 1 amino-terminal propeptide, OCN: NMD-Osteocalcin, β-CTx: β-CrossLaps

Correlation between Bone turnover markers and other disease parameters

There is no statistical significant relation between serum bone markers and the marital status, amount of dairy intake or disease onset in the studies groups. (Table 25)

Table (25): Correlation between bone turnover markers and other factors.

	TP1NP		OCN		β-CTx	
	F	**p**	**F**	**p**	**F**	**p**
Marital status	0.412	0.745	0.238	0.869	1.648	0.189
Onset	0.342	0.562	0.001	0.981	1.835	0.183

F: two way ANOVA, TP1NP: Total procollagen type 1 amino-terminal propeptide OCN: NMD-Osteocalcin, β-CTx: β-CrossLaps

Correlation between BMD and other disease parameters

There is no statistical significant relation between BMD (forearm, hip or lumbar spine) and the marital status, amount of dairy intake or disease onset in the studies groups. (Table 26)

Table (26): Correlation between BMD and other factors.

	Forearm BMD		Hip BMD		Lumbar Spine BMD	
	F	p	F	p	F	p
Marital status	1.226	0.309	0.466	0.760	0.837	0.507
Diary intake	1.386	0.257	1.766	0.179	1.491	0.233
Onset	1.664	0.205	1.872	0.180	0.072	0.790

F: two way ANOVA, BMD: bone mineral density