EFFECT OF RHEUMATOID ARTHRITIS ON BONE MINERAL DENSITY AND ITS CORRELATION WITH INFLAMMATORY MILIEU IN RHEUMATOID ARTHRITIS PATIENTS

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(GENERAL MEDICINE)

JULY, 2020 **DR. RAMANAND S**

AIIMS, JODHPUR

EFFECT OF RHEUMATOID ARTHRITIS ON BONE MINERAL DENSITY AND ITS CORRELATION WITH INFLAMMATORY MILIEU IN RHEUMATOID ARTHRITIS PATIENTS

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(GENERAL MEDICINE)

JULY, 2020 **DR. RAMANAND S**

AIIMS, JODHPUR

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

DECLARATION

I hereby declare that the thesis titled **"EFFECT OF RHEUMATOID ARTHRITIS ON BONE MINERAL DENSITY AND ITS CORRELATION WITH INFLAMMATORY MILEU IN RHEUMATOID ARTHRITIS PATIENTS"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

Dr Ramanand S
Department of General Medicine
All India Institute of Medical Sciences
Jodhpur

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

CERTIFICATE

This is to certify that the thesis titled "**EFFECT OF RHEUMATOID ARTHRITIS ON BONE MINERAL DENSITY AND ITS CORRELATION WITH INFLAMMATORY MILEU IN RHEUMATOID ARTHRITIS PATIENTS**" is the bonafide work of Dr Ramanand S carried out under our guidance and supervision, in the Department of General Medicine, All India Institute of Medical Sciences, Jodhpur.

Guide

Dr Mahendra Kumar Garg
Professor and Head
Department of General Medicine
AIIMS, Jodhpur

Co-Guide

Dr Maya Gopalakrishnan Associate Professor Department of General Medicine AIIMS, Jodhpur	
Dr Satyendra Khichar Associate Professor Department of General medicine AIIMS, Jodhpur	
Dr Ravindra Kumar Gayaprasad Shukla Additional Professor Department of Endocrinology AIIMS Jodhpur	
Dr Kamla Kant Shukla Associate Professor Department of Biochemistry AIIMS Jodhpur	

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

CERTIFICATE

This is to certify that the thesis titled "**EFFECT OF RHEUMATOID ARTHRITIS ON BONE MINERAL DENSITY AND ITS CORRELATION WITH INFLAMMATORY MILEU IN RHEUMATOID ARTHRITIS PATIENTS**" is the bonafide work of Dr Ramanand S carried out under our guidance and supervision, in the Department of General Medicine, All India Institute of Medical Sciences, Jodhpur.

Dr Mahendra Kumar Garg
Professor & Head
Department of General Medicine
AIIMS, Jodhpur

Acknowledgement

"*Alone we can do so little; together we can do so much*"

-Helen Keller

Nothing worth achieving has ever been achieved without the guidance of our teachers and the support of family and friends. The immortal words of Helen Keller could not be more undeniable as I stand at the cusp of one of the most prestigious achievements of my life.

I would not be at this landmark moment without the trust and patient patronage of Dr Mahendra Kumar Garg, my MD thesis Guide and Head of Department of General Medicine, All India institute of Medical Sciences, Jodhpur. I am truly honoured and grateful for the opportunity to learn under his tutelage.

I am also indebted to my Co-guides Dr Maya Gopalakrishnan, Associate Professor, Department of General Medicine, Dr. Satyendra Khichar, Associate Professor, Department of General Medicine, Dr. Ravindra Kumar Gayaprasad Shukla, Associate Professor, Department of Endocrinology and Dr Kamla Kant Shukla, Associate Professor, Department of Biochemistry, All India institute of Medical Sciences, Jodhpur. Their step by step support and guidance has been instrumental in creating the environment of success and excellence needed for a dissertation from our esteemed institute.

I extend my sincere gratitude to all faculty members of our beloved department, Dr. Gopal Krishna Bohra (Additional Professor), Dr. Bharat Kumar, Dr. Naresh Kumar Midha, Dr. Deepak Sharma, Dr. Satyendra Khichar, Dr. Amit Rohila, Dr. Durga Shankar Meena, for their guidance.

I am also thankful to Dr Parag Vijayvergia, Dr. Swapnil Tripathi, Dr. Neeraja Vijayan, Dr. Pranav kumar, Dr. Sachin, Dr. Manika (fellow Junior Residents) who worked with me in rheumatology clinic which made it easy for me to include patients for my research.

Family is the backbone which has prevented me from ever faltering in the quest for excellence and knowledge. I cannot express in words my appreciation and love for my parents, Mr

Sivaprasad S and Mrs Rajasree A and brother, Rajanand S for their immense support and understanding even when I was not worthy of it.

I acknowledge the nurturance I have received from my seniors -Dr. Parag Vijayvergia, Dr. Neeraja Vijayan, Dr. Sonu Pandit, Dr. Saurabh Kumar and Dr. Akhilesh Kumar PH, Dr. Swapnil Tripathi, Dr. Prakrati Yadav, Dr.Veena Surendran, Dr. Kartikey Saini, Dr. Tejaswee Banavathu, Dr Vishwanath Jha, Dr. Nakka Vihari, Dr. Naveen, Dr. Arjun of the Department of General Medicine. Without their help and reprimands, I would have been clueless as to how to proceed with the work involved in this dissertation. I also thank my esteemed colleagues – Dr. Naja K, Dr. Isha Stutee, Dr. Shilpi Goyal, Dr. Pranav S Kumar, Dr. Pankaj Sukhadiya, Dr Srividhya for keeping me motivated. I would extend my gratitude to my beloved juniors, Dr. Sachin, Dr. Venkata Teja, Dr. Mayur, Dr. Sanjay and Dr. Ninad, Dr. Lochan, Dr. Naveen, Dr. Shubham, Dr. Manika, Dr. Divya, Dr. Akash, Dr. Pankaj, Dr. Naman, Dr. Shawon, Dr. Samarth, Dr Ayush Garg who have stepped up in times of need to help balance the duties of a junior resident of our beloved department and the commitments to my thesis.

All the faculties of the Department of General Medicine, All India institute of Medical Sciences, Jodhpur have been pillars of support and understanding and this would not have been possible without them.

Last but by far not the least, I express thanks to all my patients without whom this milestone was not possible.

INDEX

	SOCIO-DEMOGRAPHIC AND CLINICAL PROFORMA	
17.	Annexure 2: ACR-EULAR CRITERIA FOR DIAGNOSIS OF RHEUMATOID ARTHRITIS	
18.	Annexure 3: DAS 28 score form	
19.	BMD DXA REPORT	

LIST OF ABBREVIATIONS

ACR	AMERICAN COLLEGE OF RHEUMATOLOGY
ACPA	ANTI CYCLIC CITRULLINATED PEPTIDE ANTIBODIES
AIIMS	ALL INDIA INSTITUTE OF MEDICAL SCIENCES
ANA	ANTI NUCLEAR ANTIGEN
RA	RHEUMATOID ARTHRITIS
BMD	BONE MINERAL DENSITY
ESR	ERYTHROCYTE SEDIMENTATION RATE
RF	RHEUMATOID FACTOR
MHC	MAJOR HISTOCOMPATIBILITY COMPLEX
HLA	HUMAN LEUKOCYTE ANTIGEN
BMI	BODY MASS INDEX
GC	GLUCOCORTICOID
FRAX	FRACTURE RISK ASSESMENT TOOL
RANK	RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KB
RANK-L	RANK LIGAND
OPG	OSTEOPROTEGERIN
DXA	DUAL XRAY ABSORPTIOMETRY
WHO	WORLD HEALTH ORGANISATION
SD	STANDARD DEVIATION
TMB	TETRAMETHYL BENZIDINE
HRP	HORSERADISH PEROXIDASE
OD	OPTICAL DENSITY
SE	SUSCEPTIBILITY EPITOPE
ACPA	ANTI CITRULLINATED PEPTIDE ANTIBODIES
GWA	GENOME WIDE ASSOCIATION
SNP	SINGLE NUCLEOTIDE POLYMORPHISM
PADI	PEPTIDYL ARGINASE DEIMINASE

TLR	TOLL LIKE RECEPTOR
FLS	FIBROBLAST LIKE SYNOVIOCYTES
TNF-α	TUMOUR NECROSIS FACTOR- α
MCP	METACARPOPHALANGEAL
PIP	PROXIMAL INTER PHALANGEAL
MTP	META TARSO PHALANGEAL
TMJ	TEMPORO MANDIBULAR JOINT
DIP	DISTAL INTER PHALANGEAL
DMARD	DISEASE MODIFYING ANTI RHEUMATIC DRUG
GERD	GASRTO ESOPHAGEAL REFLUX DISEASE
DLBCL	DIFFUSE LARGE B CELL LYMPHOMA
CVD	CARDIOVASCULAR DISEASES
SCORE	SYSTEMIC CORONARY RISK EVALUATION
ILD	INTERSTITIAL LUNG DISEASE
UIP	USUAL INTERSTITIAL PNEUMONIA
NSIP	NON SPECIFIC INTERSTITIAL PNEUMONIA
CDAI	CLINICAL DISEASE ACTIVITY INDEX
SDAI	SIMPLE DISEASE ACTIVITY INDEX
RADAI	RHEUMATOID ARTHRITIS DISEASE ACTIVITY INDEX
OP	OSTEOPOROSIS
HAQ	HEALTH ASSESMENT QUESTIONNAIRE
PINP	PROCOLLAGEN TYPE 1 N-TERMINAL PRO PEPTIDE
IFN γ	INTERFERON γ
DKK-1	DICKKOPF-1
IQR	INTER QUARTILE RANGE
LBM	LEAN BODY MASS
SPRA	SEROPOSITIVE RHEUMATOID ARTHRITIS
SNRA	SERONEGATIVE RA

LIST OF TABLES

24	Lumbar spine BMD distribution among patients with DAS 28 ESR </>3.2	
25	Left forearm BMD distribution among patients with DAS 28 ESR</>3.2	
26	BMD in RF negative and RF positive patients (N=75)	
27	BMD distribution in Early and Established RA (N=102)	
28	Left Hip BMD distribution in Vitamin D deficiency	
29	Lumbar spine BMD distribution in Vitamin D deficiency	
30	Left Forearm BMD distribution in Vitamin D deficiency	
31	DAS28 ESR distribution among Left Hip T score categories	
32	DAS28 ESR distribution among Lumbar spine T score categories	
33	DAS28 ESR distribution among Left forearm T score categories	
34	DAS28 ESR distribution among Left Hip T score categories	
35	DAS28 ESR distribution among Lumbar spine T score categories	
36	DAS28 ESR distribution among Left forearm T score categories	
37	Univariable linear regression analysis of Vitamin D deficiency with T score category (osteopenia/osteoporosis)	
38	Serum calcium distribution among Left hip T score categories	
39	Serum calcium distribution among Lumbar spine T score categories	
40	Serum calcium distribution among Left forearm T score categories	
41	DAS28 ESR distribution among Left Hip Z score categories	
42	DAS28 ESR distribution among Lumbar spine Z score categories	
43	DAS28 ESR distribution among Left forearm Z score categories	
44	Univariable linear regression analysis of Vitamin D deficiency with Z score category	
45	Serum calcium distribution among Left Hip Z score categories	
46	Serum calcium distribution among Lumbar spine Z score categories	
47	Serum calcium distribution among Left forearm Z score categories	
48	Association of IL-1 with osteopenia/osteoporosis	
49	Association of IL-6 with osteopenia/osteoporosis	

LIST OF FIGURES

SUMMARY OF THE PROJECT

Background: Rheumatoid arthritis is an inflammatory disorder, associated with significant systemic and local bone loss. Bone mineral density loss in rheumatoid arthritis is known but its association to disease severity is not clearly studied.

Objectives:

1. To estimate Bone Mineral Density in Rheumatoid arthritis patients
2. To determine the frequency and risk factors of osteopenia/osteoporosis in Rheumatoid arthritis patients

Methods: In this study we prospectively enrolled rheumatoid arthritis patients from January 1st 2021 to July 31st 2022 at All India Institute of Medical Sciences (AIIMS), Jodhpur. Patients were assessed clinically for disease activity and bone mineral density. Patients were tested for routine biochemical investigations, BMD-DXA scan, IL-1 and IL-6.

Results: One hundred and two patients were enrolled in the study. In the study population, prevalence of osteopenia and osteoporosis was calculated using T score. 43.1% patients had osteopenia and 15.7% had osteoporosis at left hip, 42.2% had osteopenia and 16.7% had osteoporosis at lumbar spine, 18.6% had osteopenia and 17.6% had osteoporosis at left forearm. There was significant variation in BMD across the age groups at lumbar spine (P value-0.031) and left forearm (P value < 0.0001). Lower values of BMD were observed in female patients but was not statistically significant. There was statistically significant reduction in BMD with disease activity at Left Hip, however significance was not observed at Lumbar spine and Left forearm. There was statistical significance in the Lumbar spine BMD across the DAS 28 ESR <3.2 and DAS 28 ESR >3.2 subgroup. Lower values of BMD were observed in RF positive patients but was not statistically significant. Lower values of BMD were observed in Established RA patients but was not statistically significant. There was no statistically significant association between vitamin D deficiency, steroid cumulative dose, IL-1levels, IL-6 levels and BMD.

Conclusion: Our study population had more prevalence of osteopenia and osteoporosis at left hip, lumbar spine and left forearm compared to cases and controls in similar studies. BMD reduction at left hip can be used as a predictor of disease severity.

INTRODUCTION

Rheumatoid arthritis is the most common inflammatory arthritis. It is chronic, symmetrical, polyarticular inflammatory erosive arthritis. The history of RA is very long and complex one and evidence says that it is present for very long times while some argue that it is a disease of the modern world.

The evidence of RA like illness has been documented in 1500 BC by Ebers papyrus and in 400 BC by Hippocrates. In modern times evidence is from 1800 by Augustin Jacob and then in 1890 when rheumatoid arthritis term was coined by Alfred Garrod (1). Studies on RA in 2001 showed a global prevalence of 0.5 -1 % (2) , recent studies have shown that global prevalence is 0.24% with 2-3:1 female to male ratio (3).

RA involves a complex interplay among environmental triggers, genotype, and chance. Genetic factors play a clear role in RA risk, progression and severity. Monozygotic twins shares RA on about 12%–15% of occasions compared to 1% for the general population and around 2%–5% for fraternal twins or other first-degree relatives (4). Relatively low concordance shows that many other factors are also included in the pathogenesis. The most important genetic risk allele for RA resides in the MHC class II locus, and accounts for around 40% of the genetic influence. Individuals with MHC class II HLA-DR4 alleles has an odds ratio of about 5:1 for developing RA. The link between HLA-DR and RA was described in the 1970s on the basis of the observation that HLA-DR4 is present in 70% of RA patients, compared to about 30% in controls (4).

Chronic inflammation of RA can cause deleterious effects like bone loss. Bone loss is quite often seen in chronic inflammatory diseases. During chronic inflammation, large amount of body's energy is diverted to the immune system activation, and this can lead to signs and symptoms that enhances bone loss. Reduced functional capacity and lack of physical exertion associated with joint pain and deformities can affect healthy life and lead to progressive bone loss.

Osteoporosis (OP) is characterized by low bone mass and microarchitectural deterioration of bone tissue, which results in bone fragility and increased susceptibility to fracture. Fragility fracture can be defined as spontaneous fracture that can result from minimal or no identifiable trauma and can be regarded as a sign of OP (5).

The prevalence of OP in general population usually ranges from 9 to 38% for women and 1 to 8% for men depending on various countries (5). A study calculated prevalence of global OP at lumbar spine or femoral neck in Spanish female population to be 12.7% (6). In women older than 50 years, prevalence was found to be 22.8% at lumbar spine and 9.1% at femoral neck (6). Whereas, the prevalence of OP in RA was found to be around 30% (up to 50% in post-menopausal women), which is a twofold increase above the general population (7,8). More than that, RA patients can have fractures with higher bone mineral density (BMD) compared to patients without RA (9). The spine is the most often affected site and the incidence of vertebral fractures in RA patients might be about 5 times the rate in healthy controls (10,11).

OP and RA shares many common risk factors such as female gender (female: male ratio in RA: 3–4:1) and smoking. Other general OP risk factors including age, low BMI, menopause, thyroid disorders or diabetes (7,12,13) are equally applicable to patients with RA and to the general population. Other risk factors for OP in RA includes systemic inflammation related to disease activity, bone erosions due to local effect of immune cells, glucocorticoid (GC) treatment and physical activity impairment (13).

The fracture risk assessment tool (FRAX), is frequently used worldwide to determine fracture risk, and has RA as one among the seven most important risk factors for fragility fractures.

There are three different forms of skeletal involvement in patients with RA, and they all are having a common pathophysiologic mechanism: alteration in bone remodeling. The first one is juxta-articular osteoporosis or peri-articular bone loss related to modification in bone remodeling favoring bone resorption. Here there is a loss of peri-articular cortical and trabecular bone, which appears at the beginning of the disease and can be seen in hand radiographs. The second type of bone loss in RA is marginal bone erosion. Here, immediate peri-articular cortical bone is lost due to synovial membrane inflammation. The third pattern

is a generalized osteoporosis involving the whole skeleton, including distant sites of joint inflammation.

The close association of inflammation and bone loss is linked to the interactions between cells of the immune system and those of bone. Osteoclasts or the bone-resorbing cells are stimulated by various inflammatory cytokines in different phases of their lifespan leading to bone loss at various parts of the skeleton. The production of local and systemic cytokines, can stimulate recruitment of osteoclast precursors and can regulate formation and function of osteoclast. The various inflammatory cytokines are stimulators for RANKL synthesis, and their increased production during the inflammatory process can exceed the production of its physiologic inhibitor and decoy receptor osteoprotegerin (OPG). The RANKL/OPG ratio imbalance is responsible for bone loss in various inflammatory diseases.

Bone mineral density is measured by using dual X-ray absorptiometry (DXA); it is the actual expression of the bone in absolute terms of grams of mineral (primarily, as g/cm2 of calcium) per square centimeter of the scanned bone. The difference between the patient's BMD and mean BMD of young females aged in the range of 20-29 years (divided by the standard deviation (SD) of the reference population) yields the T-score; comparing the BMD of a particular age, sex, and ethnicity-matched adult reference population is called the Z-score. As defined by the World Health Organization (WHO), osteoporosis is present when BMD is 2.5 SD or more below the average value for young healthy women (a T-score of <-2.5 SD). A second, higher threshold describes "low bone mass" or osteopenia as a T-score that lies between -1 and -2.5 SD. "Severe" or "established" osteoporosis denotes osteoporosis that has been defined in the presence of one or more documented fragility fractures (14). DEXA scan is a high-precision X-ray that measures your bone mineral density and bone loss. If your bone density is lower than normal for your age, it indicates a risk for osteoporosis and bone fractures. DEXA stands for dual energy X-ray absorptiometry. Rheumatoid arthritis (RA) is an established risk factor for osteoporosis with all main guidelines recommending dual-energy X-ray absorptiometry (DEXA) for bone mineral density (BMD) assessment in RA. However, factors which determine this bone mineral (BM) loss in RA have not been well determined.

Furthermore, impact of Vitamin-D deficiency on BMD in RA has not been evaluated. This study is being done in a population where Vitamin-D deficiency/insufficiency is common. Glucocorticoid use has conventionally been associated with adverse impact on bone health. There are conflicting data available on the impact of glucocorticoid use on bone health in RA. Hence, the aim of this study is to quantify the occurrence of osteopenia and osteoporosis in Rheumatoid arthritis patients in AIIMS Jodhpur, to determine the clinical, biochemical, and radiological predictors of BM loss; and to assess the impact of treatment on bone health in RA.

REVIEW OF LITERATURE

History of RA

The first description of RA was found in the dissertation of Augustin Jacob Landré-Beauvais from the year 1800. He hypothesized that patients with "rheumatism" were suffering from a condition which is previously uncharacterized, and he named it as "Primary Asthenic Gout or *Goutte Asthénique Primitive*".

Alfred Garrod first distinguished gout from all other arthritic conditions. He found that there is excess of uric acid in the blood of gout patients, which was not found in blood of other arthritis patients. He considered RA as a distinct condition, and called it "Rheumatic Gout." The fourth son of Alfred Garrod, Archibald Garrod, also conducted research on arthritis and RA. He was the author of '*Treatise on Rheumatism and Rheumatoid Arthritis'*. He coined the term "Rheumatoid Arthritis". He also proposed that RA was present during past and was problematic for our ancestors and was not a disease of the modern era. His book is main source for the *Ancient Origin* school of thought regarding the RA etiology.

In 20th century, American physician Charles Short challenged paleopathological claims of Archibald Garrod and tried to discredit the *Ancient Origin* hypothesis. He hypothesized RA was actually a disease of modern origins, due to the lack of evidence demonstrating otherwise.

Apart from the historical medical writings, postmortem examination of human remains was also a clue for gathering information regarding the historical background of this disease. Two preliminary paleopathological studies carried out independently by Professor Flinders Petrie and Sir Armand Ruffer in the 19th and 20th centuries respectively discuss human remains from Egypt that shows skeletal damage similar to RA. Their work showed that evidence forrheumatic diseases could be seen in ancient human remains.

There is a third school of thought regarding RA etiology: *New World to Old World* transfer concept. People supporting this view argue that since some of the oldest paleopathological specimens displaying RA were found in the Americas, RA must have been transmitted to Old

World through some unspecified vector after Columbus' discovery of the Americas. But, studies have found the presence of RA in the Old World before Columbus' voyage in 1492, which discredits this theory.

ETIOPATHOGENESIS

RA is the most common inflammatory arthropathy. The majority of evidences points to an immune-mediated etiology associated with stromal tissue dysregulation which together propogate chronic inflammation and articular destruction. A pre-RA phase which lasts months to years exists, which is characterized by the presence of circulating autoantibodies, increasing concentration of inflammatory cytokines and altered metabolism. There are five phases, genetic or predetermined risk, asymptomatic inflammation, undifferentiated synovitis, classical RA, and evolution of chronic inflammation and autoimmunity.

Dysregulated immune function was first implicated in the pathogenesis of RA by the discovery of anti-immunoglobulin G (IgG) antibodies known as rheumatoid factors, first by Erik Waaler and then more widely by H.M. Rose in the 1940s. RA starts with a high-risk genetic background that, in combination with epigenomic marks launches a cascade of events inducing synovitis and ultimately chronic destructive arthritis.

Genetics of Rheumatoid Arthritis

Genetic factors plays a role in RA risk, progression and severity. Monozygotic twins share RA on about 12%–15% of occasions compared to 1% for the general population and around 2%–5% for fraternal twins or other first-degree relatives. This relatively low concordance implicates many other factors, including those in the environment and the microbiome in pathogenesis. Gene sequences are not the sole determinants of heritability, epigenetic marks also contribute, especially for monozygotic twins (15). The most important genetic risk allele for RA resides in the class II MHC locus, which accounts for about 40% of the genetic influence. The odds ratio of developing RA in individuals with MHC class II HLA-DR4 alleles is about 5:1. This link between HLA-DR and RA was initially described in the 1970s with the observation that HLA-DR4 is present in 70% of RA patients, compared with about 30% of controls.

RA-associated alleles present citrullinated peptides to T cells more efficiently, which leads to production of higher amounts of cytokines IL-17 and IFN-g than to native peptide. Adaptive immune responses to citrullinated peptides are also characterized by the presence of ‘‘anti-citrullinated peptide antibodies’’ (ACPAs), which can be observed in 80%–90% of RA patients. Genome-wide association studies (GWASs) and meta-genomic analyses have identified around 100 single nucleotide polymorphisms (SNPs) and genes associated with ACPA+ RA other than the HLA. Non-MHC linkages are associated with peptidyl arginase deiminase (PADI) and PTPN22. PADI gene products are enzymes that convert arginine to citrulline, which can create new potential antigens that bind to RA-associated. The PTPN22 allele with an amino acid substitution (R620W) doubles the risk of developing ACPA+ but not ACPA Negative RA.

Interactions between Genes and Environment: RA as a ‘‘Mucosal Disease’’?

RA is considered an immune-mediated disease with a lot of genetic influence. Origin of the disease may involve the interface between external influences and the immune system, especially at the mucosal surfaces. Three locations have been related with RA, namely (1) the lungs, (2) the oral mucosa, and (3) the gastrointestinal tract. It is thought that local tissue stress leads to post-translational modification of peptides and subsequent antibody formation. Studies have showed the role of cigarette smoking as a environmental risk factor for RA, suggesting a role for pulmonary mucosal biology in disease etiology. Other pulmonary exposures can also increase risk, e.g., silica or textile dust. Exposure to inhaled toxic chemicals in cigarette smoke can increase PADI expression in the airway and increase protein citrullination (16).

The relationship between RA and microbiome has been known for many years. Periodontitis can lead to increased susceptibility to RA. P. gingivalis is the most common bacteria implicated in periodontitis. *P. gingivalis* could express PADI and potentially citrullinate peptides in the oral mucosa that could promote ACPA generation in the context of inflammation (17). A recent study identified that A. actinomycetemcomitans can cause hypercitrullination due to a toxin, leukotoxin A (LtxA), mediated on neutrophils and was detected in RA patients oral microbiome (18). Prevotella copri species were enriched in the analysis of the gastrointestinal microbiome in early RA patients and Bacteroides species were decreased in the same patients (19). However, the overrepresentation of Prevotella was not observed in chronic RA or in other forms of arthritis.

Epigenetics: Linking Environmental Stress and the Genome

Epigenetic modifications contributes to regulation of gene expression. A variety of epigenetic mechanisms are known in RA, including DNA methylation, microRNA expression and histone modification. Stable epigenetic marks have been identified in RA that alter cell function and permanently imprint some lineages, most notably synovial fibroblast-like synoviocytes (FLS)

CLINICAL FEATURES

A) ARTICULAR MANIFESTATION

The presenting symptoms of RA typically result from inflammation of the joints, tendons, and bursae. Patients often complain of early morning joint stiffness lasting more than 1 h that eases with physical activity. The earliest involved joints are typically the small joints of the hands and feet. The initial pattern of joint involvement may be monoarticular, oligoarticular (≤4 joints), or polyarticular (>5 joints), usually in a symmetric distribution.

The classic joint distribution in early disease includes the small joints of the hands and feet (MCP, PIP, and MTPs). With time, intermediate (wrists, elbows, and ankles) and large (hips, shoulders, and cervical spine) joints may become involved. With more advanced and longer duration of disease, RA may also involve atypical joints, including the temporomandibular joint (TMJ), cricoarytenoid, and sternoclavicular joint. RA rarely affects the DIP joints and almost never targets the thoracic or lumbosacral spine (20).

B) EXTRAARTICULAR MANIFESTATIONS

Around 50% of RA patients exhibit extra articular manifestations of the disease (21) . Generally, those RA patients having high titers of RF, ANA, disease-associated HLA genes (especially homozygous DRB1*04 subtype), history of smoking are most likely to have EAM which includes rheumatoid nodules, vasculitis, pulmonary, neurologic, cardiac, hematological, and cutaneous complications. The presence of ACPAs is also associated with a more progressive joint damage and severe EAM (22).

Skin manifestations: Rheumatoid nodules are the most common skin manifestation, affecting upto 20% of RA patients (23) and is mainly due to small vessel vasculitis. They commonly appear on extensor surface which is attributable to pressure, like elbows and forearms. Nodules are seen in patients with high titres of Rheumatoid factor. Other manifestations of rheumatoid small vessel

vasculitis affecting the skin are splinter haemorrhages, periungual infarcts, leg ulcers, digital gangrene and sharply demarcated painful ulcerations (24). Pyoderma gangrenosum is characterized by recurrent, non infective ulceration secondary to necrotizing vasculitis and is also associated with RA.

Ocular manifestations: Most common ocular EAM is dry eyes due to secondary Sjogren's syndrome, occurrs in upto 25% of RA patients (25). Episcleritis and scleritis are described in 0.2-3% of RA patients, with the necrotizing form of scleritis being associated with severe pain and increased mortality (25).

Gastrointestinal manifestations: They can vary from mild GI disturbance to life threatening vasculitic manifestations. Intestinal rheumatic vasculitis is the most severe form, reported in 10-38% of RA patients (26). Amyloidosis is another rare GI manifestation of RA with prevalence reported upto 7-13% (27). It can manifest as intractable diarrhoea, malabsorption and esophageal dysmotility causing GERD. Asymptomatic elevation of alkaline phosphatase and gamma glutamyl transferase have been reported and they correlate with disease activity (28). Felty syndrome manifests with hepatosplenomegaly and neutropenia, and is commonly seen in long standing and severe RA. It can present with features of portal hypertension and collateral formation (29).

Neuropsychiatric manifestations: A wide variety of central and peripheral nervous system abnormalities have been described in RA. Peripheral nerve system abnormalities have been found in around 20% of RA patients and include entrapment neuropathies, mononeuritis multiplex, distal sensory neuropathy and sensorimotor neuropathy (30). They form as a result of vasculitis of vasa nervosa causing nervous demyelination. Central nervous system involvement in RA patients includes a wide variety of manifestations, such as cervical myelopathy, cerebral vasculitis, meningitis, optical atrophy and formation of rheumatoid nodules (21). The most common manifestation of CNS involvement is cervical myelopathy due to atlanto-axial subluxation, reported in up to 40% of RA patients. Upto 40% of RA patients have been diagnosed with anxiety and major depressive disorders, which has been attributed to chronic pain (31).

Renal manifestations : Renal adverse effects of therapies and secondary amyloidosis represent the most common causes of kidney involvement .On the other hand, renal involvement as direct

result of the disease itself is less common and usually manifests as mesangioproliferative glomerulonephritis (GN) or membranoproliferative GN (32).

Rheumatoid arthritis and malignancies: An increased risk of Hodgkin's and Non-Hodgkin's lymphoma, particularly Diffuse Large B cell Lymphoma (DLBCL) has been described in RA patients (33). The risk of developing lymphoma is particularly increased in patients with high and longstanding disease activity, thus reflecting the role of chronic activation of B cells in the pathogenesis of lymphoproliferative disorders in RA as in other autoimmune conditions..

Cardiovascular manifestations: The leading cause of death among RA patients is cardiovascular diseases (CVD), with a risk 50% higher than that observed in the general population. In order to identify patients at higher CV risk, the EULAR update on CVD risk management recently proposed CVD risk assessment in all RA patients by Systematic Coronary Risk Evaluation (SCORE) algorithm at least every 5 years in case of low CVD risk (SCORE<5%) and sooner in case of intermediate or high risk (SCORE ≥5%, <10% and ≥10%,respectively) (34).

Pulmonary manifestations of RA

Pulmonary involvement can even precede articular manifestation, but it usually occurs within 5 years of disease onset (35). Airway disease can present as bronchiectasis, bronchiolitis, airway hyperreactivity, cricoarytenoid arthritis in large airways and constrictive and obstructive bronchiolitis in smaller airways. Clinically significant ILD is seen in around 10% of RA patients (36). Usual interstitial pneumonitis (UIP) followed by non-specific interstitial pneumonitis (NSIP) is the most common subtype but others like organizing pneumonia, desquamative interstitial pneumonia, lymphocytic interstitial pneumonia, diffuse alveolar haemorrhage can also occur (37). Pleural effusions, pleuritis, pleural thickening, empyema, pneumothorax, and trapped lung syndrome are all examples of pleural involvement with pleural effusion being the commonest. The vascular component of pulmonary involvement includes pulmonary hypertension and vasculitis.

Rheumatoid vasculitis: It is characterized by severe injury to involved blood vessels most frequently small vessels. It is more common in males, seropositive patients with long duration and severity of the disease.

DIAGNOSIS OF RHEUMATOID ARTHRITIS

The first attempt to define RA was done by a committee on American Rheumatism Association in 1956 (38) whereby they classified patients into 3 categories – definite, probable and possible RA. In the definite group there should be almost no question that every patient has rheumatoid arthritis and in the probable group the likelihood should be great that every patient has rheumatoid arthritis. Eleven criteria with 19 exclusions were proposed. "Definite" RA required at least 5 criteria and 6 weeks of joint symptoms while "Probable" RA required at least 3 criteria and 4 weeks of duration.

In an attempt to improve the specificity of diagnosis, the same committee of ARA revised the criteria in 1958 and 1987 (38). A new category of "Classic" RA was added where patients qualified 7 out of the 11 original criteria. The duration required for probable RA was increased from 4 to 6 weeks. This revised criteria was then used for nearly 30 years. The 1987 criteria failed to detect patients with early RA who would benefit from early initiation of aggressive immunotherapy. Its main purpose was to distinguish established RA from other forms of arthritis, rather than detecting early RA who would benefit from intervention. These classification criteria were developed before the diagnostic and prognostic importance of ACPAs were recognized. Thus, only serum RF was included as a serological marker. In 2007, a joint committee was formed by ACR/European league Against Rheumatism (EULAR) in Zurich to develop new criteria for diagnosis of RA. The categories of the 2010 ACR/EULAR criteria (39) are grouped into four classifications, with point scores for each: joint symptoms; serology (including RF and/or ACPA); symptom duration, whether <6 weeks or >6 weeks; and acute-phase reactants (CRP and/or ESR). Prospective validation of the 2010 criteria was carried out in several cohorts prior to its implementation, with reported sensitivities ranging from 0.50 to 0.60 and specificities from 0.88 to 0.97 (40). In patients with RA lasting for less than 3months (referred to as Early RA), the sensitivity of this criteria is only 62 to 74 (41). There have been recent studies evaluating the role of ultrasound with power Doppler to diagnose early RA and to correlate the disease severity with DAS 28 and SDAI scores (42). 2010 ACR guidelines still continues to be used for RA diagnosis and enrollment in clinical trials.

TREATMENT

Treatment goals are as follows (43):

(1) Early, aggressive therapy to prevent joint damage and disability

(2) Frequent modification of therapy with utilization of combination therapy where appropriate.
(3) Individualization of therapy in an attempt to maximize response and minimize side effects.
(4) Achieving, whenever possible, remission of clinical disease activity

Disease-modifying antirheumatic drugs (DMARDs) are a class of drugs indicated for the treatment of inflammatory arthritis including rheumatoid arthritis (RA),

DMARDs are further categorised as

- Conventional synthetic
- Targeted synthetic
- Biological
- Biosimilars

Conventional DMARDs (CsDMARD) include methotrexate (oral and subcutaneous), anti-malarials (hydroxychloroquine/chloroquine), sulfasalazine and leflunomide. Other drugs which are not used nowadays are gold, d-penicillamine, cyclosporine and azathioprine.Targeted synthetic DMARDs (TsDMARDs) includes tofacitinib, baricitinib and upadacitinitib Biological DMARDs is further divided into subclasses depending on the drug mechanism (44).

- TNF-alpha inhibitor: Infliximab, adalimumab, golimumab, certolizumab pegol, etanercept
- B cell targeted therapy:
    - B cell depleting agent- Rituximab
    - B cell function inhibitor- Ofatumumab, belimumab, atacicept, tabalumab
- T cell targeted therapy
    - CD28/CTLA4- Abatacept
    - CD80/CD86- Belatacept
- Interleukin inhibitors:
    - IL-1 – Anakinra, canakinumab, rilonacept
    - IL-6 – Tocilizumab
    - IL-17- Secukinumab
- Growth and differentiation factors
    - RANKL inhibitor- Denosumab
    - GM-CSF inhibitor- Mavrilimumab

NSAIDs and glucocorticoids are used in acute flare for symptomatic management.

Non pharmacological interventions are physical exercise and cognitive behavioural therapy.

Every patient's treatment should be focused towards achieving a goal of sustained remission or low disease activity. Various scales for assessment of disease activity are available, and they are frequently used in deciding the line of treatment. The most commonly used are DAS 28 ESR and DAS 28 CRP. Others are clinical disease activity index (CDAI), simple disease activity index (SDAI), Rheumatoid arthritis disease activity index (RADAI). These scores 25 also include patient's global health assessment in addition to joint involvement and lab parameters.

INFLAMMATION AND BONE LOSS IN RA

Osteoporosis (OP) is characterized by low bone mass and microarchitectural deterioration of bone tissue, which results in bone fragility and increased susceptibility to fracture. Fragility fracture can be defined as spontaneous fracture that can result from minimal or no identifiable trauma and can be regarded as a sign of OP (5). The prevalence of OP in general population usually ranges from 9 to 38% for women and 1 to 8% for men depending on various countries (5). A study calculated prevalence of global OP at lumbar spine or femoral neck in Spanish female population to be 12.7% (6). In women older than 50 years, prevalence was found to be 22.8% at lumbar spine and 9.1% at femoral neck (6). Whereas, the prevalence of OP in RA was found to be around 30% (up to 50% in post-menopausal women), which is a twofold increase above the general population (7,8). More than that, RA patients can have fractures with higher bone mineral density (BMD) compared to patients without RA (9). The spine is the most often affected site and the incidence of vertebral fractures in RA patients might be about 5 times the rate in healthy controls (10,11).

OSTEOPOROSIS RISK FACTORS IN RA

OP and RA share many common risk factors such as female gender (female: male ratio in RA: 3–4:1) and smoking. Other OP risk factors such as age, low BMI, menopause, diabetes or thyroid disorders (7,12,13,45) are equally applicable to patients with RA and to the general population. Other risk factors that can account for OP in RA include systemic inflammation associated with disease activity, local effect of immune cells leading to bone erosions,

glucocorticoid (GC) therapy and impairment of physical activity (13). OP and fractures are more frequent in patients with high disease activity (according to DAS28), RA disease duration ≥ 10 years, high HAQ score or high titers of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) positivity (7,10,11,13,45). Whereas, recent publications show that patients achieving early RA remission can have a similar OP risk profile to that of the general population (45).

Regarding treatment options in RA, GC needs mention. GCs suppress osteoblast bone formation, which is associated with a rapid suppression of procollagen type 1 N-terminal pro-peptide (PINP, a biomarker of bone formation), leading to an early reduction in trabecular bone (46). But, GCs also suppress osteoclast activity, which is increased in active arthritis patients, which can have a protective effect in some cases (47). Some studies even shows that GC use in RA could be beneficial, with a low impact on BMD due to their anti-inflammatory and suppressive effect on arthritis activity (13,48–50). Other pharmacological agents increasing fracture risk are opioids, SSRI, anti-psychotics, benzodiazepines and PPI (51).

BONE HOMEOSTASIS AND BONE REMODELING AND THE IMMUNE SYSTEM

The entire skeleton is renewed in around every 10 years. This dynamic process of bone formation and resorption is known as bone remodeling. RA is a prototype osteoimmunologic disease where one of the most characteristic findings is bone loss. There are three kinds of bone loss in RA: local, juxta-articular and systemic causing periarticular osteopenia, bone erosions and generalized osteopenia and/or osteoporosis far from inflamed joints, respectively (52–54).

Increased Bone Resorption in RA

Osteoclasts are the main cells responsible for bone loss in RA patients. They originate from hematopoietic stem cells of the macrophage/monocyte lineage. Numerous molecules and signaling pathways are involved in the processes of osteoclast differentiation and activation. The receptor activator of nuclear factor (NF)-kB (RANK) and its ligand (RANKL) are the most important among them. They are proteins belonging to the TNF superfamily. Osteoprotegerin (OPG) is another protein of the TNF superfamily, which has a regulatory role in bone remodeling (55), it works as a RANKL decoy receptor, blocking its effect and

therefore inhibiting osteoclastogenesis. The RANK/RANKL/OPG pathway is essential in regulating bone remodeling.

Different types of T cells (Th1, Th2, Th17, and Treg) also play a crucial role in bone metabolism in RA. Th1 and Th2 play a negative regulatory role on osteoclastogenesis, secreting inhibitory cytokines like interferon gamma (IFN-γ) and IL4 (56). Regulatory T cells (Treg) also work as negative regulators of osteoclastogenesis, whereas Th17 cells are critical stimulators of osteoclastogenesis in RA. Th17 cells produce RANKL and IL-17, a cytokine that in turn stimulates RANKL production by fibroblasts and osteoblasts. Although proinflammatory and osteoclastogenic role of IL17 (57) has been described in RA, treatment with IL-17 inhibitors has not demonstrated any clear efficacy in RA patients (58).

B cells are able to produce RANKL under stimulation. In RA, activated B cells of synovial fluid and peripheral blood have been found to secrete high RANKL levels, thus taking part in osteoclastogenesis and bone resorption (59). Numerous other cytokines involved in the pathogenesis of RA have also been described, among which TNFα and IL-6 are found to have a direct effect on bone remodeling in RA (52,53,60). They are two of the main therapeutic targets of the novel RA therapies.

TNF-α stimulates bone resorption by promoting osteoclast differentiation by increasing RANKL expression in T and B lymphocytes and osteoclasts. It also promotes RANK expression in osteoclast precursors (61). TNF-α also causes inhibition of bone formation through stimulation of Dickkopf-1 (DKK-1) production. TNF-α has a net osteoclastogenic effect. Therapy with TNF-α inhibitors (TNFi) has shown efficacy in prevention of radiographic progression (62).

IL-6 is another key cytokine in the pathogenesis of RA (63). IL-6 promotes bone resorption by enhancing the expression of RANKL by osteoblasts, fibroblasts and T cells (64) and is involved in the differentiation of Th17 cells (65). Therapy with IL-6 inhibitors is found effective in controlling inflammation and also the radiological progression of RA (66).

Association of denosumab, a RANKL inhibitor human antibody, with methotrexate and other therapies for controlling RA reduces bone erosions, increases BMD and decreases biomarkers

of bone resorption, so it can be considered a potential treatment option for erosive RA (67). However, it has not been approved for RA treatment.

One of the most important signaling routes in the bone formation by osteoblasts is the Wnt pathway. There are different endogenous inhibitors of this pathway, among which DKK-1 and sclerostin are the most important (68). In RA, there is an increase in the expression of these inhibitory factors of the Wnt pathway and therefore a reduction in bone formation. DKK-1 elevation is associated with an increased risk of erosions in RA patients (69). Its levels seem to depend on the pro-inflammatory state, while inhibiting TNF-α reduces them. Blocking DKK-1 by monoclonal antibodies reduces the occurrence of bone erosions regardless of the inflammatory state in arthritis animal models (70). Therefore, DKK-1 plays an important role in the development of erosions in a pro-inflammatory environment Sclerostin is another inhibitor of the Wnt pathway, mainly secreted by osteocytes.

ROLE OF AUTOANTIBODIES IN OSTEOPOROSIS ASSOCIATED TO RA

RA is a systemic inflammatory disease in which the development of different autoantibodies is an early pathogenic event that is associated with structural joint damage, the appearance of erosions and juxta-articular osteopenia (71,72). The most frequent autoantibodies associated with RA are RF and ACPA. ACPA are more specific of RA and very rare in general population, having demonstrated evidence of their prognostic role on radiological progression and the appearance of erosions.

RA-Related Autoantibodies as Drivers of Bone Resorption

Systemic osteoporosis in RA is a complex process including sustained inflammation, glucocorticoid use, decrease of physical activity and as a consequence of some disease modifying anti-rheumatic drugs (DMARDs). At present, there is enough evidence to support that autoantibodies play also a role in the pathogenesis of bone loss, either systemic or local, in RA. Different animal models have demonstrated that ACPA can induce osteoclasts differentiation and activation even before arthritis onset (73,74). Furthermore, Kleyer et al. have demonstrated a decrease in systemic cortical bone mass in a limited population of healthy ACPA-positive subjects without arthritis (75). In a study, ACPA positive subjects showed a significantly lower systemic bone mass at hip and lumbar spine, but not at

periarticular level in metacarpophalangeal joints. This effect was independent of the effect of classical risk factors for low bone mass, such as female gender, menopause or BMI (76,77). Anti-carbamylated proteins antibodies (anti-CarPA) have evidence regarding their role in the pathogenesis of RA compared to anti-acetylated proteins or other modifications. Anti-CarPA have shown a clear overlap with ACPA, but some studies have identified them as an independent prognostic biomarker of erosions (78). Regueiro et al. described that high titers of anti-CarPA were associated with lower systemic BMD, either at lumbar spine or hip, in these patients, but not at local level in metacarpophalageal joints, and this association was independent of ACPA titers (79).

BONE MINERAL DENSITY AS POSSIBLE SEVERITY MARKER IN RA

Currently, the diagnostic and therapeutic strategies aim at the early detection and treatment of the disease (80). Indeed, in the PEARL study the implementation of early DMARD treatment in tight control and treat to target strategies have led to prevention of erosive disease and arrest of radiological progression (81), both due to a better control of the disease and a reduced use of long-term osteopenizing drugs. The association of RA-related autoantibodies with worse BMD suggests that measurement of bone mass could help to predict prognosis of patients with early arthritis. There is evidence that measurement of BMD by dual X-ray radiogrammetry (DXR) at metacarpal diaphysis in the non-dominant hand of RA patients is associated with disease progression, appearance of bone erosions and even, in some studies, with increased mortality (82,83). In addition, DXR is a very sensitive procedure to detect loss of BMD in the hand, which in long-standing RA has been associated with high titers of autoantibodies, mainly ACPA, radiographic progression and the appearance of erosions (82).

Meha Sharma et al, 2018 (85) did a study to determine the occurrence and predictors of BM loss in the young premenopausal women with RA. In the young premenopausal females with RA having median symptom and treatment duration of 30 months, with moderate disease activity (DAS-28,4.88±1.17), occurrence of osteoporosis and osteopenia was 7.29% and 25% at spine, 6.25% and 32.29% at hip, and 17.7% and 56.25% at wrist, respectively(significantly higher than controls). RA patients had lower BMD at total femur, lumbar spine (LS), radius total, and radius ultra distal. Total lean mass(LM) and BM content were significantly lower in RA ($P=0.022$ and <0.001, respectively). In RA, BMD at majority of sites(LS, neck of

femur, greater trochanter, radius total, and radius 33%) had the strongest positive correlation with LM followed by body fat percent. RA patients with most severe disease had lowest BMD at different sites and lowest LM. The study concluded that LM and disease severity

Harris A. Ahmad et al, 2018 (84) studied the relationship between low bone mineral density (BMD), anti-cyclic citrullinated peptide-2 (anti-CCP2) antibodies, and disease activity in patients with established rheumatoid arthritis (RA). A total of 149 patients (all women) were included (47 anti-CCP2 antibody negative [−], 102 anti-CCP2+). Mean disease duration was greater in the three anti-CCP2+ groups vs. the anti-CCP2− group. BMD was lower in the anti-CCP2+ vs. the anti-CCP2− groups BMD decreased with increasing anti-CCP2 titer ($P < 0.001$ for left and right hands Among patients with established RA, data suggest that anti-CCP2+ patients, particularly those with high anti-CCP2 antibody titers, have lower hand BMD, and patients with lower hand BMD are less likely to have low disease activity.

C. A. F. Zerbini et al, 2016 (86) studied Biologic therapies and bone loss in rheumatoid arthritis, study concluded that treatment with biologic drugs is associated with the decrease in bone loss. Studies with anti-TNF blocking agents show preservation or increase in spine and hip BMD and also a better profile of bone markers.

Asadullah Makhdoom et al, 2017 (87) studied the Bone mineral density level by dual energy X-ray absorptiometry in rheumatoid arthritis. In the studied 229 rheumatoid arthritis patients, 33(14.4%) were males. Five (15.1%) males had normal bone density, 14(42.4%) had osteopenia and 14(42.4%) had osteoporosis. Of the 196(85.5%) females, 45(29.9%) had normal bone density, 72 (37.7%) had osteopenia and 79(40.30%) had osteoporosis. Of the 123(53.7%) patients aged 30-50 years, 38(30.9%) had normal bone density, 59(48.0%) had osteopenia, and 26(21.1%) had osteoporosis. Of the 106(46.3%) patients over 50 years, 12(11.3%) had normal bone density, 27 (25.5%) had osteopenia and 67(63.2%) had osteoporosis. The study concluded that Osteoporosis and osteopenia were common among rheumatoid arthritis patients.

Krishnamurthy et al, 2016 (74) studied the role of ACPAs in osteoclast (OC) activation and to identify key cellular mediators in this process. Study result showed protein citrullination by PADs is essential for OC differentiation. Polyclonal ACPAs enhance OC differentiation

through a PAD-dependent IL-8-mediated autocrine loop that is completely abolished by IL-8 neutralisation. Some, but not all, human monoclonal ACPAs derived from single SF B-cells of patients with RA and exhibiting distinct epitope specificities promote OC differentiation in cell cultures. Transfer of the monoclonal ACPAs into mice induced bone loss that was completely reversed by the IL-8 antagonist reparixin.

Peter Pietschmann et al, 2022 (88) studied mechanisms of systemic osteoporosis in rheumatoid arthritis. Based on animal and human data, the pathophysiology of osteoporosis, a frequent comorbidity in conjunction with RA, was delineated. Autoimmune inflammatory processes, which lead to a systemic upregulation of inflammatory and osteoclastogenic cytokines, the production of autoantibodies, and Th cell senescence with a presumed disability to control the systemic immune system's and osteoclastogenic status, may play important roles in the pathophysiology of osteoporosis in RA. Consequently, osteoclast activity increases, osteoblast function decreases and bone metabolic and mechanical properties deteriorate.

Piero ruscitti et al, 2015 (89) studied the role of IL1 β in the bone loss during rheumatic diseasesThe main factor required for osteoclast activation is the stimulation by receptor activator of nuclear factor kappa-B ligand (RANKL) expressed on osteoblasts. In this context, interleukin- (IL-) 1*β*, one of the most powerful proinflammatory cytokines, is a strong stimulator of in vitro and in vivo bone resorption via upregulation of RANKL that stimulates the osteoclastogenesis. The resulting effects lead to an imbalance in bone metabolism favoring bone resorption and osteoporosis.

METHODOLOGY

OBJECTIVES:

1. To estimate Bone Mineral Density in Rheumatoid arthritis patients

2. To determine the frequency and risk factors of osteopenia/osteoporosis in Rheumatoid arthritis patients

MATERIALS AND METHODS

STUDY SETTING:

Patients attending to the out-patient and in-patient services of Department of Internal Medicine of All India Institute of Medical Sciences, Jodhpur, Rajasthan.

STUDY DURATION: From January 1st 2021 to July 31st 2022

STUDY DESIGN:

The study was conducted as Cross sectional study after seeking informed written consent from the study participants and approval by Institutional Ethics Committee. Baseline assessment of various variables were collected which includes:

1. Socio-demographic: Name, age and gender.
2. Clinical: duration of rheumatoid arthritis, details and duration of treatment, general physical examination including DAS 28 score and deformities present.
3. Investigations: All patients underwent the following investigations as per clinical indication.
    a. Baseline hematological and biochemical assessment as per routine clinical care including Complete hemogram, serum electrolytes, blood glucose, Erythrocyte sedimentation rate, High sensitivity C reactive protein, RA factor, anti-Cyclic citrullinated peptide, Renal function test, and Liver function test, Serum calcium, Serum vitamin-D3, Serum phosphate, Serum Alkaline phosphatase

4. All patients underwent DEXA (Dual energy Xray absorptiometry) scan to determine Bone Mineral Density.
5. Serum Interleukin-1 and Serum Interleukin-6

Each patient's BMD was measured at enrollment using a dual-energy X-ray absorptiometry scanner (Hologic Corp, model no-Horizon A S/N 303237M) for the femoral neck (FN), Lumbar spine and Left forearm. For postmenopausal women and men aged 50 years and older, osteoporosis was defined as a T-score of –2.5 or less at the FN based on the normal reference database for young white females. The instrument was calibrated on a daily basis using the phantom provided by the manufacturer, and the coefficient of variation (CV) at different sites was found to be <0.5% over the duration of the study. The manufacturer's appointed service engineer reviewed the calibration data and did scanner maintenance check to ensure the system's performance before at the beginning and at the end of the study to confirm that no instrumentation drift occurred during the study.

IL-1:

For the detection of IL-1, the IL-1 ELISA kit was used (Catalogue no.- 850.006.096)

Principal of test- A capture antibody highly specific for IL-1β has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of IL-1β samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti IL-1β secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of IL-1β present in the samples and standards. The absorbance of the colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can be used to accurately determine the concentration of IL-1β in any sample tested.

Specimen collection- Blood samples were collected and allowed to clot for 10-20 minutes at room temperature and then serum was centrifuged at 2000-3000 RPM for 20 minutes and then stored at -20 centigrade.

Reagent preparation-

All reagents were brought to room temperature before use.

Standard: Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 500 pg/ml of IL-1β. Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 500 to 15.6 pg/ml. A fresh standard curve should be produced for each new assay.

Wash buffer: Dilute the (200x) concentrate wash buffer 200 fold with distilled water to give a 1X working solution. Pour entire contents (10ml) of the concentrate wash buffer into a clean 2,000 ml graduated cylinder. Bring final volume to 2000 ml with glass distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2º-25ºc.

Assay Procedure-

1. All the serum specimens, and kit reagents were brought to room temperature (20-25 º C).
2. Total 87 coated strips were placed into the holder.
3. Blank, 6 standard diluents and 80 samples were used .
4. Add 100µl of samples, control and diluted standards and 50µl diluted biotinylated antibody. Covered the plate with a sealer. Incubate 3 hours at 37°C.
5. Wash three times then add 100µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Incubate 30 minutes at 37°C.
6. Removed the sealer and washed the plate 3 times with wash buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.

7. Added 100µl of TMB substrate. Protect from light. Let the colour develop for 10-15 min
8. Added 100µl Stop Solution to each well, the blue colour changed into yellow immediately.
9. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

Calculation of results-

1. A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and drawn a best fit curve through the points on the graph.
2. Using the standard curve and OD of the samples concentration of IL-1 was calculated.

IL-6:

For the detection of IL-6, the IL-6 ELISA kit was used (Catalogue no.- EH0201).

Principal of test – Capture antibody was pre coated onto 96 well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP- streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the OD absorbance at 450 nm in a microplate reader, and then the concentration of target can be calculated.

Specimen collection- Blood samples were collected and allowed to clot for 10-20 minutes at room temperature and then serum was centrifuged at 2000-3000 RPM for 20 minutes and then stored at -20 centigrade.

Reagent preparation-

All reagents were brought to room temperature before use.

Standard: Add 1ml sample dilution buffer into one standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

Label 7 EP tubes with ½, ¼, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the sample dilution buffer into each tube. Add 0.3 ml of the above standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample dilution buffer was used for thr blank control.

Wash buffer: Dilute 30 ml(15ml for 48T) concentrated wash buffer into 750 ml (375ml for 48T) wash buffer with deionized or distilled water.

Assay Procedure-

1. All the serum specimens, and kit reagents were brought to room temperature (20-25 ^{o} C).
2. Total 87 coated strips were placed into the holder.
3. Blank, 6 standard diluents and 80 samples were used.
4. Add 100µl standard or sample to each well and incubate for 90 minutes at room temperature.
5. Aspirate and wash plates 2 times. Add 100µl Biotin labeled antibody working solution to each well and incubate for 60 minutes at 37^{o}c.
6. Aspirate and wash plates 3 times. Add 100µl SABC working solution into each well and incubate for 30 minutes at 37^{o}c.
7. Aspirate and wash plates 5 times. Add 90µl TMB substrate solution. Incubate 10-20 minutes at 37^{o}c.
8. Added 50µl Stop Solution to each well, the blue colour changed into yellow immediately.
9. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

Calculation of results-

1. A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal.
2. Using the standard curve and OD of the samples concentration of IL-6 was calculated.

STUDY PARTICIPANTS:

INCLUSION CRITERIA: -

1. Age >18 Years
2. Patient having rheumatoid arthritis according to EULAR ACR criteria (2010)

EXCLUSION CRITERIA:

1. Patients meeting criteria for other connective tissue disorders like SLE, polymyositis or scleroderma.
2. Pregnant women will be excluded

METHODOLOGY:

Patients will be diagnosed with RA according to ACR EULAR criteria (2010)

⇩

All patients will undergo baseline investigations as per routine clinical care and DEXA scan to determine BMD

⇩

CALCULATION OF Z SCORE AND T SCORE

⇩

Correlating BMD with clinical, biochemical, and radiological parameters to look for predictors of Bone Mineral loss in Rheumatoid arthritis patients

⇩

All patients who have been diagnosed to have osteopenia/osteoporosis will be managed appropriately

SAMPLING AND SAMPLING SIZE:

Due to the ongoing COVID 19 pandemic the sample size was kept time bound and all patients who presented to department of medicine from the time of approval of thesis by Institute Ethics Committee to July 31st 2022 was enrolled.

STUDY DURATION:

From January 1st 2021 to July 31st 2022 at the outpatient services of Department of Internal Medicine, All India Institute of Medical Sciences, Jodhpur, Rajasthan.

DATA COLLECTION:

Data were collected on the first visit for the baseline assessment using following questionnaires and scales (Annexure 1,2,3,4):

1. Socio-demographic proforma
2. DAS-28 ESR score.

Data were collected regarding symptoms and investigations which were maintained in excel sheet.

Statistical analysis

Kolmogorov–Smirnov test was used to check for normality of variable distribution. Descriptive statistics will be presented as mean with standard deviation in case of continuous variables and median with interquartile range in case of categorical variables. Normally distributed data will be analyzed using Unpaired t-test. Non-normally distributed data will be analyzed using non-parametric test such as Mann-Whitney test. Chi-Square test will be used to assess categorical variables. Adjusted odds ratios will be calculated for respective risk factors for osteoporosis using multivariable logistic regression. For determining risk factors, we will perform univariable logistic regression analysis followed by multivariable logistic regression with osteoporosis as the dependent variable and other risk factors as independent variables. The results of the multivariable analysis will be reported as adjusted Odds Ratio with 95% Confidence Intervals. A two tailed p value less than 0.05 will be considered significant. SPSS version 20 (Chicago, IL, USA) was used for data analysis.

RESULTS

Population characteristics

Total 160 patients of RA were screened during the study duration. Out of this, 102 patients met the inclusion criteria and were enrolled in the study while the rest of the patients were not having complete data.

Age and gender distribution

The mean age of the study population was 46.7±12.3 years (range 19-76 years) with age distribution as given below (Table 1 and Fig 2). Out of 102 patients, 85 (83.3%) were females and 17 (16.7%) patients were males (Fig 1).

Body mass index distribution

The mean BMI of the study population was 22.8±3 kg/cm^2 (range 14.2-34.6) with BMI distribution according to Asian criteria as given below (Table 1 and Fig 3).

Table 1: Demographical profile of study population (N=102)

Demographic detail	**Number (%)**
Gender	
Female	85 (83.3%)
Male	17 (16.7%)
Age distribution (Mean ± SD)	46.7 ± 12.3 years
<25 years	2 (1.96%)
26-35 years	19 (18.63%)
36-45 years	28 (27.45%)
46-55 years	26 (25.49%)
56-65 years	23 (22.55%)
>65 years	4 (3.92%)

BMI distribution (Mean ± SD)	22.8±3 kg/cm^2
Underweight	7 (6.9%)
Normal	46 (45.1%)
Overweight	21 (20.6%)
Pre-obese	17 (16.7%)
Obese	11 (10.8%)
Disease Activity (DAS28-ESR)	
Remission	8 (7.8%)
Low disease activity	9 (8.8%)
Moderate disease activity	51 (50.0%)
High disease activity	3 (33.3%)

Fig.1: Gender distribution among RA patients (N=102)

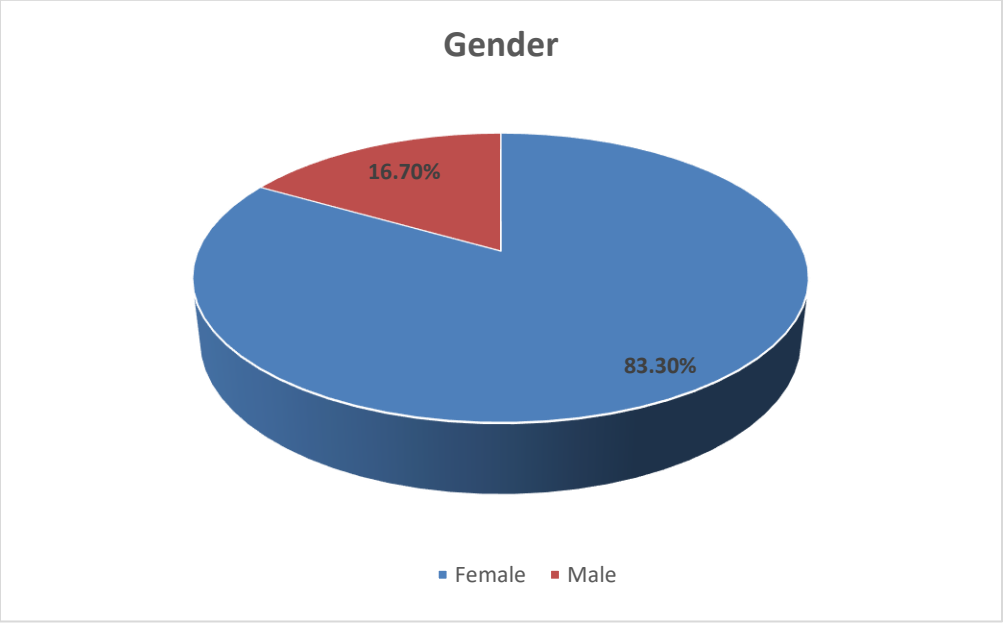


Fig 2: Age distribution of RA patients (N=102)

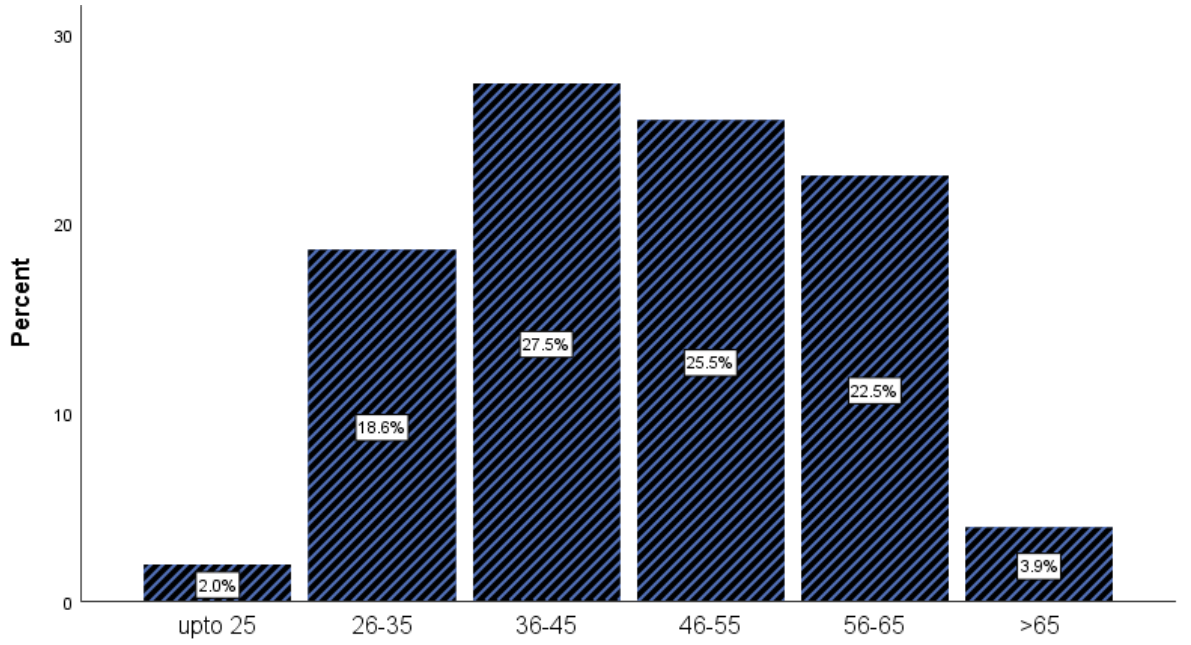
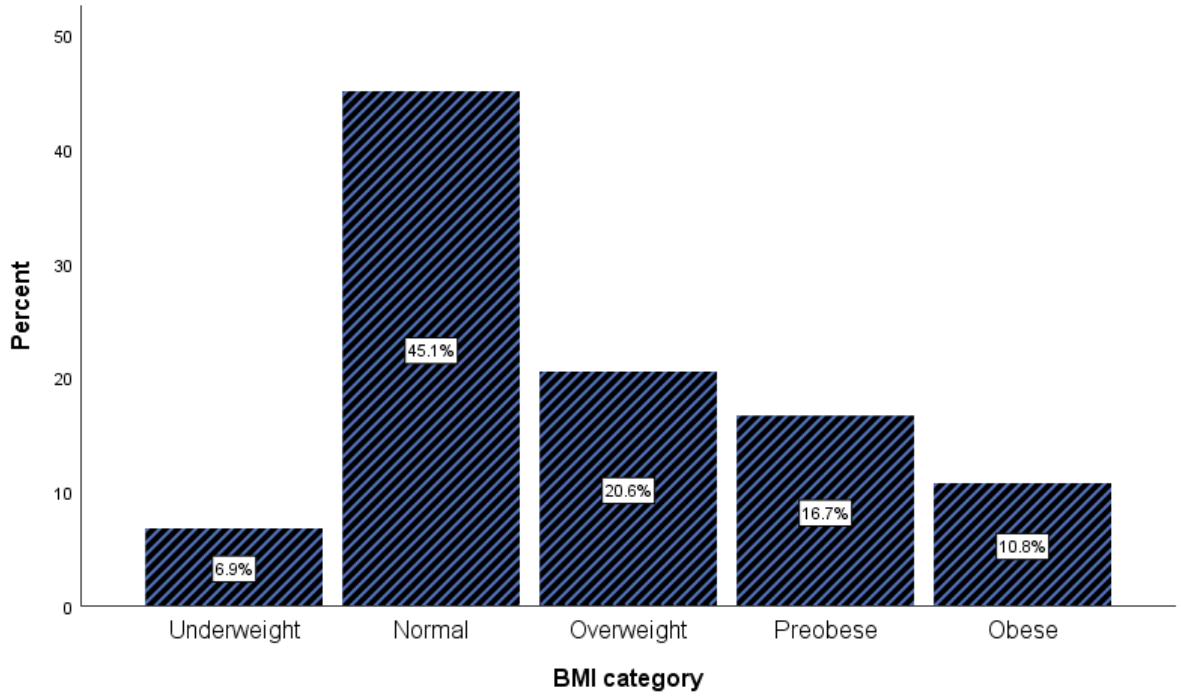


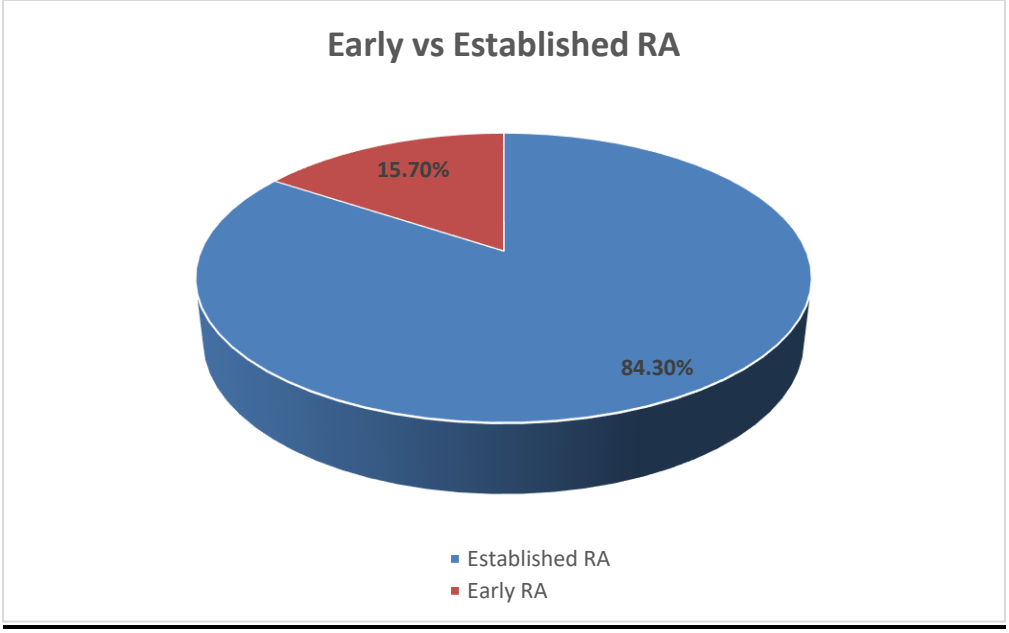
Fig 3: Body Mass Index (BMI) distribution of RA patients (N=102)



Disease characteristics

At the time of recruitment into the study, median disease duration was 48±36 months. Out of these, 16 patients (15.7%) belonged to early RA while 86 (84.3%) patients had established RA (Fig 4).

Fig 4: Disease duration of RA patients (N=102)



The median number of tender and swollen joints was 4 and 3 respectively. Serum level of rheumatoid factor was available for 75 patients, out of which 85.3% (n=64) were rheumatoid factor positive while 14.7% (n=11) were RF negative (Fig 5). Mean DAS 28 ESR score was 4.5±1.2. Eight of our study patients (7.8%) were in remission while 34 (33.3%) patients had high disease activity. Around 50.0% of patients in the study population had moderate disease activity (Fig 6).

Fig 5: Prevalence of Rheumatoid factor (N=75)

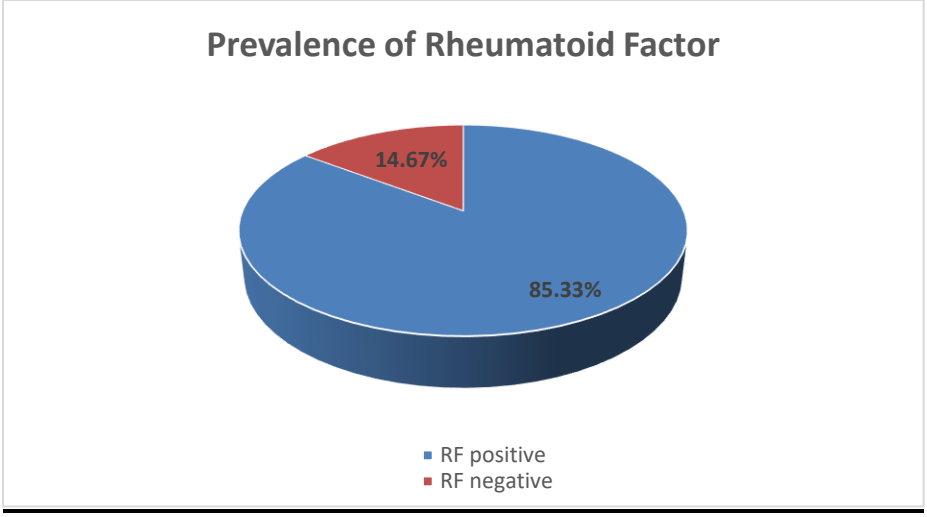
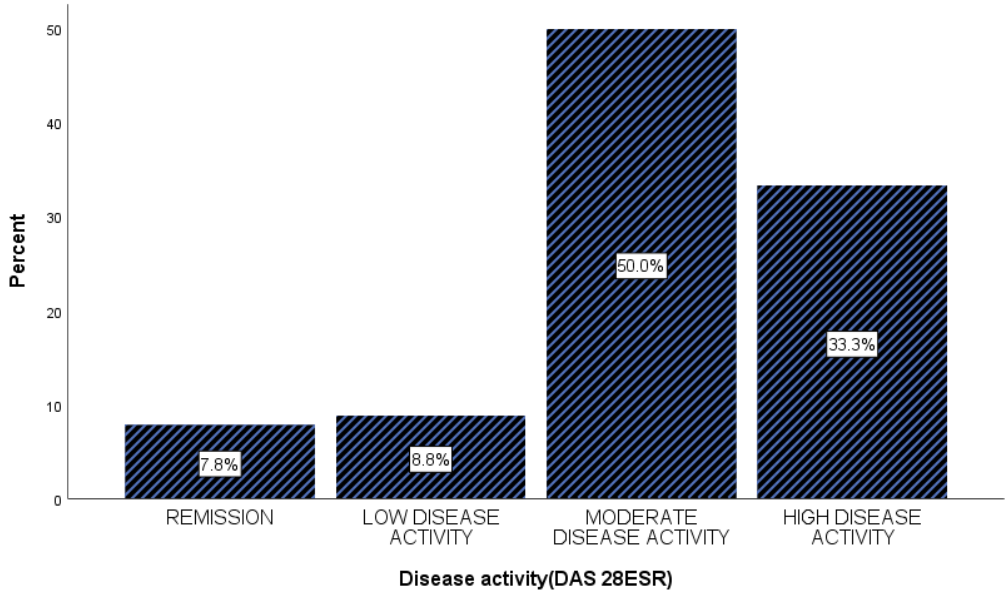


Fig 6: Disease activity by DAS28-ESR (N=86)



Treatment characteristics

On review of previous treatment details, we found that 68 patients (66.7%) had previous history of treatment for RA with a median duration of treatment before enrollment of 15±30 months. 39.2% percentage of patients had a previous history of steroid intake (n=40). Mean cumulative steroid dosage used was 251.23±68 mg of prednisolone.

Laboratory features:

Laboratory parameters are being depicted as mean±standard deviation in table 2.

TABLE 2: Laboratory parameters of RA patients (N=102)

Parameter	Values (Mean±SD) / (Median±IQR)
Hemoglobin (gm/dl) (N=97)	11.4±1.8
Platelet count(10^3/uL) (N=97)	330±136
ESR (mm**) (**N=95)	48.5±28.5
Hs CRP (mg/L**)** (N=96)	7.5±17.5
Total protein (mg/dl) (N=94)	7.4±0.6
Albumin (mg/dl) (N=94)	3.9±0.4
Globulin (mg/dl) (N=94)	3.3±0.8
Calcium (mg/dl) (N=79)	9.5±0.4
Phosphorus (mg/dl) (N=72)	3.9±0.6
Vitamin D (mg/dl) (N=80)	20.5±20.9
IL-1 (ng/L) (N=37)	30.7±9.5
IL-6 (ng/L) (N=27)	4.9±68.8

* All nonnormally distributed variable expressed as median±IQR

Bone mineral density in RA patients

BMD was measured at Left hip, Lumbar spine and left forearm using DEXA scan and mean BMD was 0.69, 0.90 and 0.54 gm/cm^2 at Left hip, Lumbar spine and Left forearm respectively (Table 3). Mean and median T score and Z score is depicted in Table 4 and Table 5.

Table 3: BMD in RA patients

SITE	BMD (gm/cm^2) (Mean±SD)	95% CI
Left Hip (N=100)	0.69±0.21	0.64-0.73
Lumbar spine (N=98)	0.90±0.15	0.87-0.93
Left forearm (N=99)	0.54±0.10	0.52-0.56

Table 4: T score in RA patients

SITE	T SCORE (Mean±SD) / Median±IQR	95% CI
Left Hip (N=100)	-1.23±1.33	-1.49 to -0.96
	-1.30±1.50	
Lumbar spine (N=99)	-1.34±1.39	-1.62 to -1.07
	-1.20±1.70	
Left forearm (N=98)	-0.74±1.88	-1.11 to -0.36
	-0.45±2.40	

Table 5: Z score in RA patients

SITE	Z SCORE (Mean±SD) / Median±IQR	95% CI
Left Hip (N=100)	-0.40±1.07	-0.61 to -0.18
	-0.40±1.40	
Lumbar spine (N=99)	-0.71±1.22	-0.96 to -0.47
	-0.70±1.7	
Left forearm (N=98)	-0.02±1.66	-0.35 to -0.31
	0.05±2.1	

Prevalence of osteopenia and osteoporosis in RA patients

In the study population, prevalence of osteopenia and osteoporosis was calculated using T score. 43.1% patients had osteopenia and 15.7% had osteoporosis at left hip, 42.2% had osteopenia and 16.7% had osteoporosis at lumbar spine, 18.6% had osteopenia and 17.6% had osteoporosis at left forearm. Prevalence of osteopenia/osteoporosis at different sites is being depicted in Fig (7,8 and 9), Table 6

Table 6: Prevalence of osteopenia/osteoporosis at different sites in RA patients

BMD category (Based on T score)	Left Hip (N=100)	Lumbar spine (N=99)	Left forearm (N=98)
Normal	40 (40%)	39 (39.4%)	61 (62.2%)
Osteopenia	44 (44%)	43 (43.4%)	19 (19.4%)
Osteoporosis	16 (16%)	17 (17.2%)	18 (18.4%)

Fig 7: Prevalence of Osteopenia/osteoporosis at left Hip (N=100)

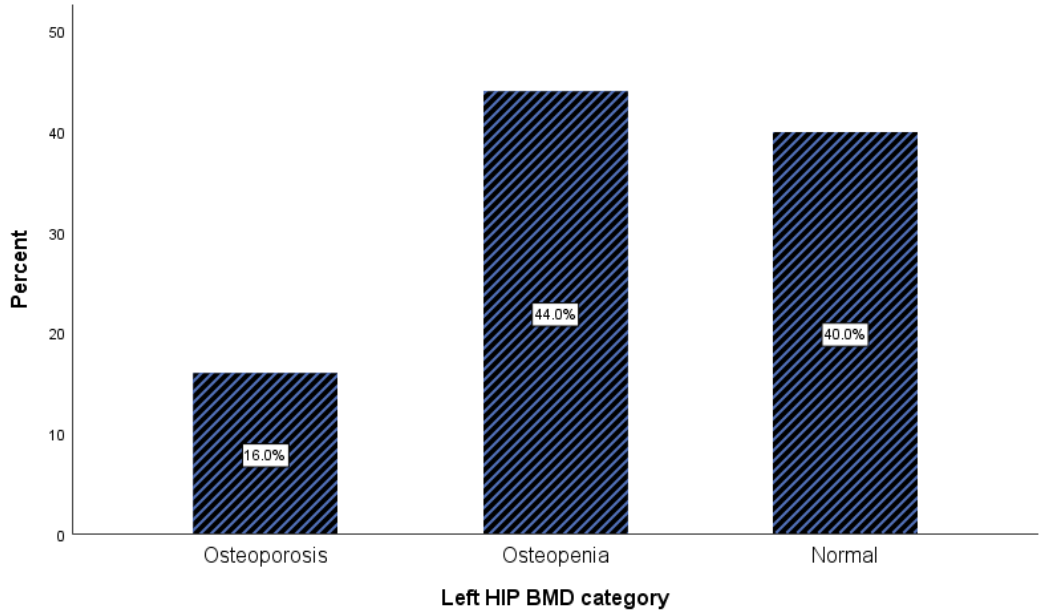


Fig 8: Prevalence of Osteopenia/osteoporosis at lumbar spine (N=99)

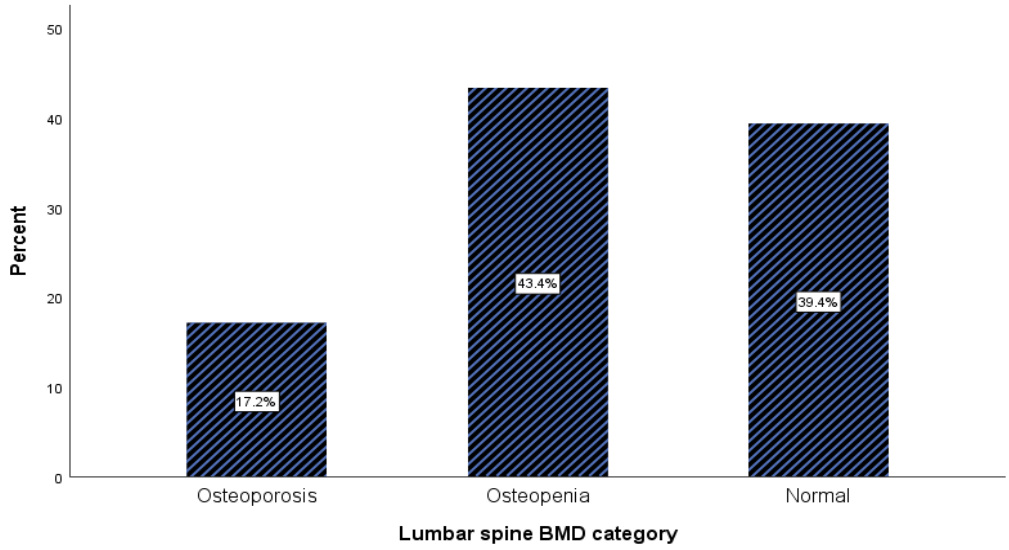
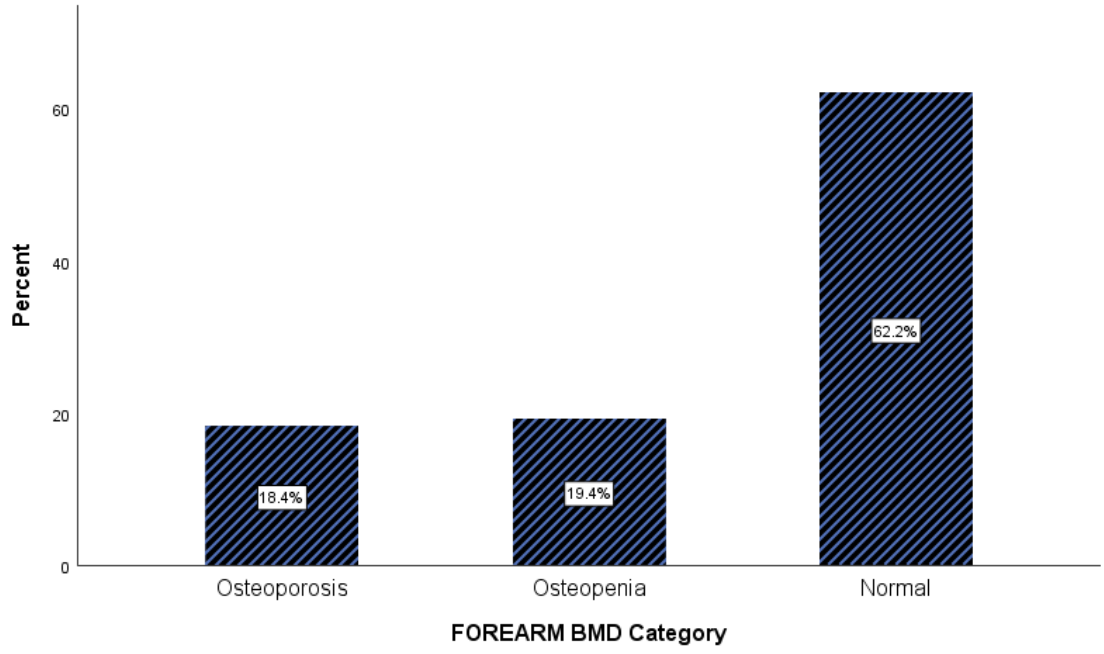


Fig 9: Prevalence of Osteopenia/osteoporosis at left forearm (N=98)



Prevalence of low BMD for age using Z score in RA patients

In the study population, BMD was below the expected range for age in 6%, 15.2% and 9.2% patients at left hip, lumbar spine and left forearm respectively. Table 7, Fig 10,11,12.

Table 7: Prevalence of low BMD for age using Z score in RA patients

BMD for age (Based on Z score)	Left Hip (N=100)	Lumbar spine (N=99)	Left forearm (N=98)
Below the expected range for age	6 (6%)	15 (15.2%)	9 (9.2%)
Within the expected range for age	94 (94%)	84 (84.8%)	89 (90.8%)

Fig 10: Prevalence of low BMD for age using Z score at Left hip

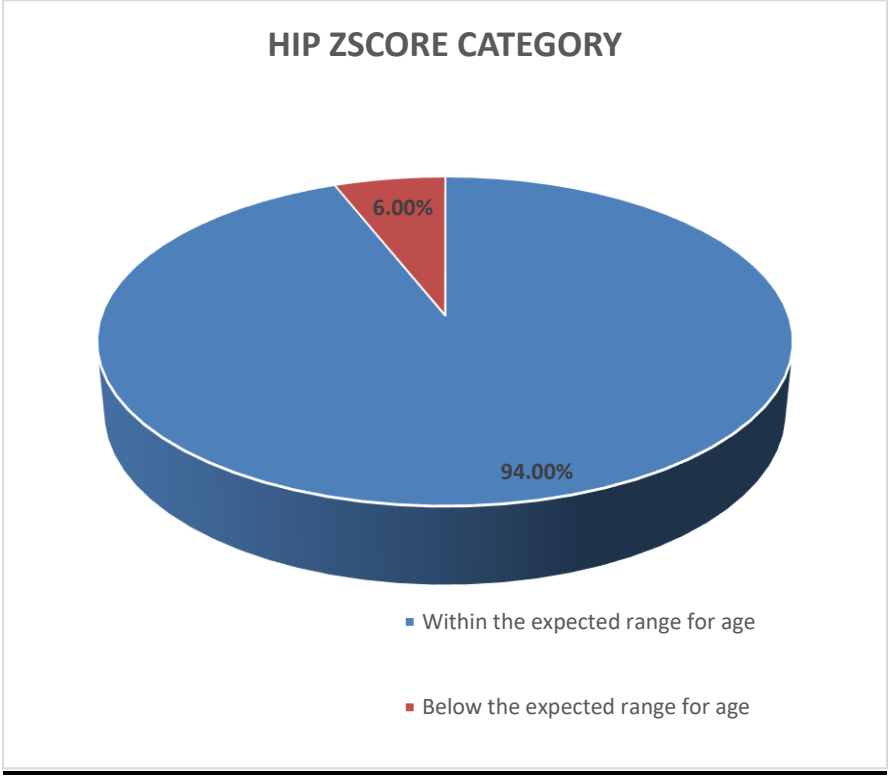


Fig 11: Prevalence of low BMD for age using Z score at L spine

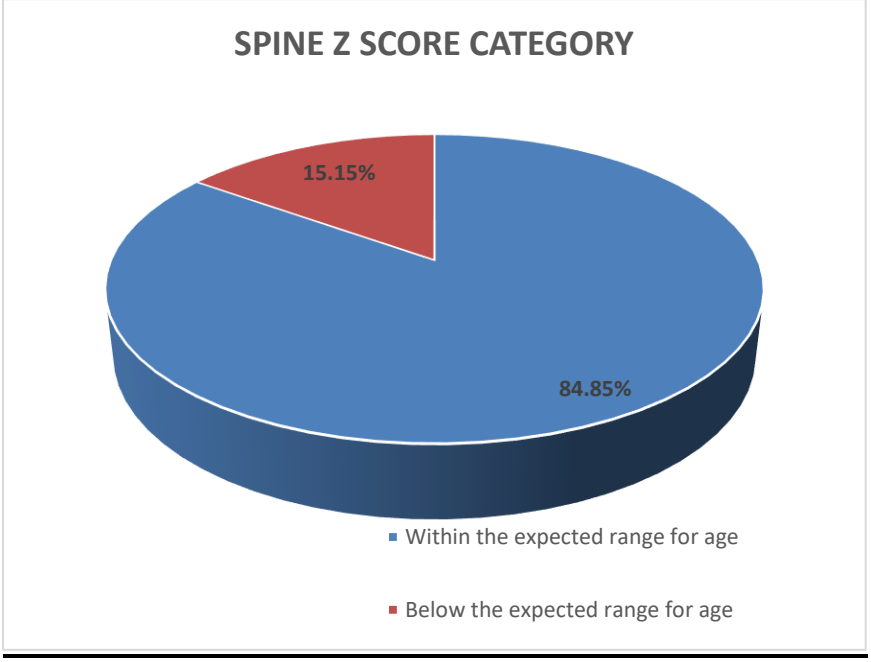
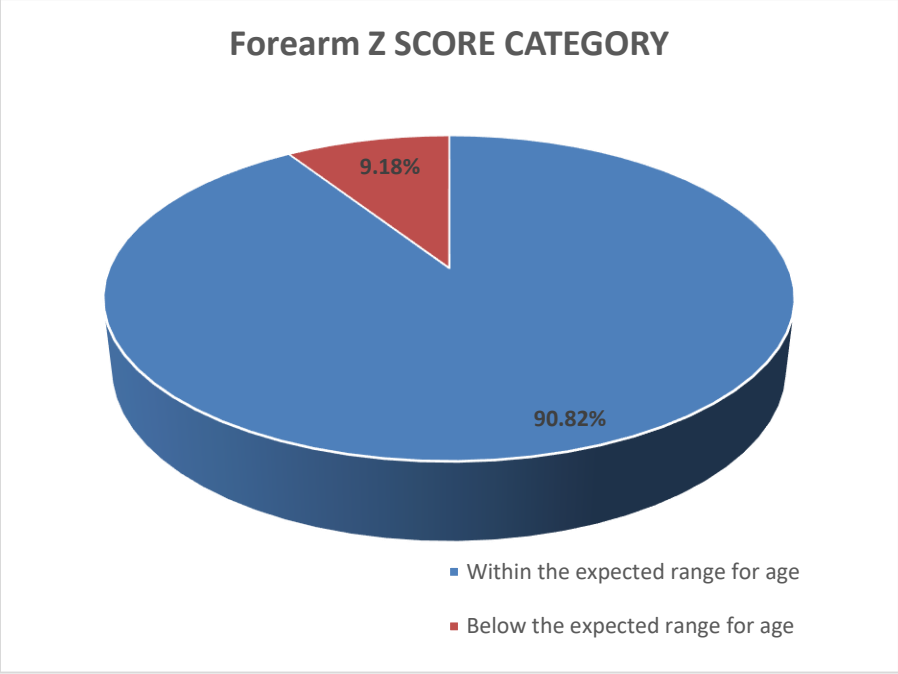


Fig 12: Prevalence of low BMD for age using Z score at Left forearm



BMD distribution among age categories in RA patients

BMD distribution among different age categories in the study population is depicted in Table 8,9,10

Table 8: Left HIP BMD distribution among different age categories

AGE CATEGORY	L HIP BMD (gm/cm^2)(Mean±SD)	P value*
<25	0.72±0.02	0.187
26-35	0.75±0.17	
36-45	0.74±0.18	
46-55	0.67±0.24	
56-65	0.65±0.21	
>65	0.49±0.38	

*one way ANOVA

Table 9: Lumbar spine BMD distribution among different age categories

AGE CATEGORY	L SPINE BMD (gm/cm^2) (Mean±SD)	P value*
<25	0.84±0.13	**0.031**
26-35	0.93±0.13	
36-45	0.97±0.14	
46-55	0.89±0.15	
56-65	0.86±0.15	
>65	0.74±0.25	

*one way ANOVA

Table 10: Left forearm BMD distribution among different age categories

AGE CATEGORY	L FOREARM BMD (gm/cm^2) (Mean±SD)	P value*
<25	0.636	< **0.0001**
26-35	0.58±0.06	
36-45	0.60±0.08	
46-55	0.51±0.10	
56-65	0.49±0.11	
>65	0.42±0.10	

*one way ANOVA

Association of Gender with BMD in RA patients

BMD distribution among males and females are depicted in Table11,12,13. Lower values of BMD were observed in female patients but was not statistically significant.

Table 11: Left Hip BMD distribution among sex categories

SEX CATEGORY	L HIP BMD (gm/cm^2)	P value*
Female (N=83)	0.69±0.21	0.335
Male (N=17)	0.70±0.18	

*unpaired students T test

Table 12: Lumbar spine BMD distribution among sex categories

SEX CATEGORY	L SPINE BMD (gm/cm^2)	P value*
Female (N=81)	0.89±0.16	0.055
Male (N=17)	0.96±0.10	

*unpaired students T test

Table 13: Left forearm BMD distribution among sex categories

SEX CATEGORY	L FOREARM BMD (gm/cm^2)	P value*
Female (N=81)	0.53±0.09	0.971
Male (N=17)	0.63±0.10	

*unpaired students T test

BMD distribution among DAS28 ESR subgroups in RA patients

BMD comparison was done among DAS 28 ESR subgroups based on disease activity, which showed statistically significant reduction in BMD with disease activity at Left Hip, however significance was not observed in Lumbar spine and Left forearm (Table 14,15,16).

Table 14: Left Hip BMD distribution among DAS28 ESR subgroups

DAS 28 ESR SCORE CATEGORY	L HIP BMD (gm/cm^2)	P value*
REMISSION (N=8)	0.75±0.16	**0.019**
LOW DISEASE ACTIVITY (N=9)	0.78±0.21	
MODERATE DISEASE ACTIVITY (N=50)	0.73±0.20	
HIGH DISEASE ACTIVITY (N=33)	0.60±0.20	

*one way ANOVA

Table 15: Lumbar spine BMD distribution among DAS28 ESR subgroups

DAS 28 ESR SCORE CATEGORY	L SPINE BMD (gm/cm^2)	P value*
REMISSION (N=8)	0.88±0.12	0.864
LOW DISEASE ACTIVITY (N=8)	0.94±0.06	
MODERATE DISEASE ACTIVITY (N=50)	0.91±0.15	
HIGH DISEASE ACTIVITY (N=32)	0.89±0.18	

*one way ANOVA

Table 16: Left forearm BMD distribution among DAS28 ESR subgroups

DAS 28 ESR SCORE CATEGORY	L FOREARM BMD (gm/cm^2)	P VALUE*
REMISSION (N=8)	0.55±0.07	0.905
LOW DISEASE ACTIVITY (N=9)	0.56±0.11	
MODERATE DISEASE ACTIVITY (N=49)	0.55±0.55	
HIGH DISEASE ACTIVITY (N=32)	0.53±0.12	

*one way ANOVA

BMD distribution among patients in Remission

BMD comparison was done between patients in remission and not in remission and found no statistical significance (Table 17,18,19).

Table 17: Left Hip BMD distribution among patients in Remission

DAS28 ESR SCORE	L HIP BMD (gm/cm^2)	P value*
DAS28<2.6 (N=8)	0.75±0.16	0.332
DAS28>2.6 (N=92)	0.68±0.21	

*unpaired students T test

Table 18: Lumbar spine BMD distribution among patients in Remission

DAS28 ESR SCORE	L SPINE BMD (gm/cm^2)	P value*
DAS28<2.6 (N=8)	0.88±0.12	0.371
DAS28>2.6 (N=90)	0.90±0.16	

*unpaired students T test

Table 19: Left forearm BMD distribution among patients in Remission

DAS28 ESR SCORE	L FOREARM BMD (gm/cm^2)	P value*
DAS28<2.6 (N=8)	0.55±0.07	0.117
DAS28>2.6 (N=90)	0.54±0.10	

*unpaired students T test

BMD distribution among patients with High disease activity

BMD comparison was done between patients with high disease activity and no high disease activity and found no statistical significance (Table 20,21,22).

Table 20: Left Hip BMD distribution among patients with High disease activity

DAS28 ESR SCORE	L HIP BMD (gm/cm^2)	P value*
DAS 28<5.1 (N=67)	0.74±0.20	0.868
DAS 28>5.1 (N=33)	0.60±0.20	

Table 21: Lumbar spine BMD distribution among patients with High disease activity

DAS28 ESR SCORE	L SPINE BMD (gm/cm^2)	P value*
DAS 28<5.1 (N=66)	0.91±0.13	0.079
DAS 28>5.1 (N=32)	0.89±0.19	

Table 22: Left forearm BMD distribution among patients with High disease activity

DAS28 ESR SCORE	L FOREARM BMD (gm/cm^2)	P value*
DAS 28<5.1 (N=66)	0.55±0.09	0.088
DAS 28>5.1 (N=32)	0.53±0.12	

Table 23: Left Hip BMD distribution among patients with DAS 28 ESR</>3.2

DAS28 ESR SCORE	L HIP BMD (gm/cm^2)	P value*
DAS28<3.2 (N=17)	0.76±0.19	0.317
DAS28>3.2 (N=83)	0.67±0.21	

*unpaired students T test

Table 24: Lumbar spine BMD distribution among patients with DAS 28 ESR </>3.2

DAS28 ESR SCORE	L SPINE BMD (gm/cm^2)	P value*
DAS28<3.2 (N=16)	0.91±0.10	0.018
DAS28>3.2 (N=82)	0.90±0.16	

*unpaired students T test

Table 25: Left forearm BMD distribution among patients with DAS 28 ESR</>3.2

DAS28 ESR SCORE	L FOREARM BMD (gm/cm^2)	P value*
DAS28<3.2 (N=17)	0.55±0.09	0.245
DAS28>3.2 (N=81)	0.54±0.10	

*unpaired students T test

Association of RF positivity with BMD in RA patients

Serum level of rheumatoid factor was available for 75 patients, out of which 85.3% (n=64) were rheumatoid factor positive while 14.7% (n=11) were RF negative. Anti CCP antibodies data was available for 14 patients (13.7%), of those 11 tested positive (78%) and 3 tested negative (22%). BMD was calculated in both subgroups as depicted in the table 26. Lower values of BMD were observed in RF positive patients but was not statistically significant.

TABLE 26: BMD in RF negative and RF positive patients (N=75)

Parameter	RF negative (11)	RF positive (64)	P value*
L HIP BMD	0.79±0.17	0.69±0.21	0.498
L SPINE BMD	0.94±0.11	0.89±0.15	0.128
L FOREARM BMD	0.55±0.10	0.54±0.10	0.209

*Unpaired T test

Association of BMD with disease duration in RA

Patients were divided into early and established RA on the basis of disease duration. BMD was calculated in both subgroups and are being depicted in the table 27. Lower values of BMD were observed in Established RA patients but was not statistically significant.

TABLE 27: BMD distribution in Early and Established RA (N=102)

Parameter	Early RA (16)	Established RA (84)	P value*
L HIP BMD	0.72 ± 0.20	0.68 ± 0.21	0.511
L SPINE BMD	0.92 ± 0.15	0.90 ± 0.16	0.988
L FOREARM BMD	0.58 ± 0.10	0.54 ± 0.10	0.767

*Unpaired T test

Association of BMD with Steroid cumulative dose

Correlation was assessed between steroid cumulative dose and BMD. There was no correlation between steroid cumulative dose and BMD.

Association of Vitamin D deficiency with BMD in RA patients

Patients were divided into Vitamin D deficiency and No vitamin D deficiency on the basis of Vitamin D levels. BMD was calculated in both subgroups and are being depicted in the table 28,29,30. There was no statistically significant association between vitamin D deficiency and BMD.

TABLE 28: Left Hip BMD distribution in Vitamin D deficiency

Vitamin D levels	**L HIP BMD (gm/cm^2)**	**P value***
Vitamin D deficiency (N=38)	0.76±0.21	0.567
No vitamin D deficiency (N=62)	0.64±0.20	

*unpaired students T test

TABLE 29: Lumbar spine BMD distribution in Vitamin D deficiency

Vitamin D levels	L SPINE BMD (gm/cm^2)	P value*
Vitamin D deficiency (N=37)	0.91±0.14	0.128
No Vitamin D deficiency (N=61)	0.90±0.17	

*unpaired students T test

TABLE 30: Left Forearm BMD distribution in Vitamin D deficiency

Vitamin D levels	L FOREARM BMD (gm/cm^2)	P value*
Vitamin D deficiency (N=38)	0.56±0.08	0.070
No Vitamin D deficiency (N=60)	0.54±0.11	

*unpaired students T test

Association of BMD with Serum calcium levels

Correlation was assessed between Serum calcium levels and DAS28 ESR. There was no correlation between S. calcium and DAS28 ESR.

Association of Disease activity (DAS 28 ESR) with osteopenia/osteoporosis

Patients were divided into normal BMD and abnormal BMD (osteopenia/osteoporosis). Mean DAS 28 ESR were calculated across the groups and are being depicted in Table 31,32,33

TABLE 31: DAS28 ESR distribution among Left Hip T score categories

LHIP T SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
Normal BMD (N=40)	4.43±1.25	0.780
Osteopenia+osteoporosis (N=60)	4.62±1.29	

*unpaired students T test

TABLE 32: DAS28 ESR distribution among Lumbar spine T score categories

LSPINE T SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
Normal BMD (N=39)	4.58±1.20	0.699
Osteopenia+osteoporosis (N=60)	4.50±1.32	

*unpaired students T test

TABLE 33: DAS28 ESR distribution among Left forearm T score categories

LFOREARM T SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
Normal BMD (N=61)	4.43±1.24	0.850
Osteopenia+osteoporosis (N=37)	4.69±1.34	

*unpaired students T test

Association of Disease activity (DAS 28 ESR) with osteoporosis

Patients were divided into osteoporosis and no osteoporosis. Mean DAS 28 ESR were calculated across the groups and are being depicted in Table 34,35,36

TABLE 34: DAS28 ESR distribution among Left Hip T score categories

LHIP T SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
No osteoporosis (N=84)	4.40±1.26	0.352
Osteoporosis (N=16)	5.31±1.05	

*unpaired students T test

TABLE 35: DAS28 ESR distribution among Lumbar spine T score categories

LSPINE T SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
No osteoporosis (N=82)	4.44±1.27	0.504
Osteoporosis (N=17)	5.00±1.23	

*unpaired students T test

TABLE 36: DAS28 ESR distribution among Left forearm T score categories

L FOREARM T SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
No osteoporosis (N=80)	4.42±1.26	0.732
Osteoporosis (N=18)	5.00±1.28	

*unpaired students T test

Association of Vitamin D deficiency with Osteopenia/osteoporosis

Analysis was done to assess association of Vitamin D deficiency with Osteopenia/osteoporosis, considering Vitamin D deficiency as independent variable, T score category at each site was used for analysis. There was no significant association of Vitamin D deficiency with Osteopenia/osteoporosis (Table 37).

Table 37: Univariable linear regression analysis of Vitamin D deficiency with T score category (osteopenia/osteoporosis)

Dependent Variable	Standard coefficient(beta)OR	P value	Confidence interval (95%)	
			Lower	Higher
LHIP T SCORE CATEGORY (N=78)	0.734	0.536	0.275	1.956
LSPINE T SCORE CATEGORY (N=77)	0.580	0.303	0.206	1.635
LFOREARM T SCORE CATEGORY (N=76)	0.784	0.639	0.284	2.168

Association of serum calcium with Osteopenia/osteoporosis

Patients were divided into normal BMD and abnormal BMD (osteopenia/osteoporosis). Mean serum calcium were calculated across the groups and are being depicted in Table 38,39,40. There was no significant difference in mean serum calcium value across the groups.

TABLE 38: Serum calcium distribution among Left hip T score categories

L HIP T SCORE CATEGORY	S. CALCIUM (Mean ± SD)	P value*
No osteopenia/osteoporosis (N=30)	9.59±0.48	0.155
Osteopenia/Osteoporosis (N=47)	9.47±0.38	

*unpaired students T test

TABLE 39: Serum calcium distribution among Lumbar spine T score categories

L SPINE T SCORE CATEGORY	S. CALCIUM (Mean ± SD)	P value*
No osteoporosis (N=31)	9.54±0.46	0.510
Osteoporosis (N=45)	9.50±0.40	

*unpaired students T test

TABLE 40: Serum calcium distribution among Left forearm T score categories

L FOREARM T SCORE CATEGORY	S. CALCIUM (Mean ± SD)	P value*
No osteoporosis (N=49)	9.54±0.43	0.513
Osteoporosis (N=26)	9.48±0.40	

*unpaired students T test

Association of Disease activity (DAS 28 ESR) with Z score category

Patients were divided based on Z score categories and Mean DAS 28 ESR were calculated across the groups and are being depicted in Table 41,42,43. There was significant association between disease activity and lumbar spine Z score category.

TABLE 41: DAS28 ESR distribution among Left Hip Z score categories

L HIP Z SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
Within the expected range for age (N=94)	4.48±1.25	0.663
Below the expected range for age (N=6)	5.70±1.07	

*unpaired students T test

TABLE 42: DAS28 ESR distribution among Lumbar spine Z score categories

L SPINE Z SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
Within the expected range for age (N=84)	4.45±1.33	**0.033**
Below the expected range for age (N=15)	5.01±0.79	

*unpaired students T test

TABLE 43: DAS28 ESR distribution among Left forearm Z score categories

L FOREARM Z SCORE CATEGORY	DAS 28 ESR (Mean ± SD)	P value*
Within the expected range for age (N=89)	4.51±1.28	0.697
Below the expected range for age (N=9)	4.67±1.39	

*unpaired students T test

Association of Vitamin D deficiency with Z score category

Analysis was done to assess association of Vitamin D deficiency with low BMD for age, considering Vitamin D deficiency as independent variable, Z score category at each site was used for analysis. There was no significant association of Vitamin D deficiency with low BMD for age (Table 44).

Table 44: Univariable linear regression analysis of Vitamin D deficiency with Z score category

Dependent Variable	Standard coefficient(beta)	P value	Confidence interval(95%)	
			Lower	Higher
LHIP Z SCORE CATEGORY (N=78)	0.141	0.098	0.014	1.431
LSPINE Z SCORE CATEGORY (N=77)	0.310	0.061	0.091	1.054
LFOREARM Z SCORE CATEGORY (N=76)	0.272	0.073	0.065	1.131

Association of serum calcium with Z score category

Patients were divided based on Z score categories. Mean serum calcium were calculated across the groups and are being depicted in Table 45,46,47. There was no significant difference in mean serum calcium value across the groups.

TABLE 45: Serum calcium distribution among Left Hip Z score categories

L HIP Z SCORE CATEGORY	**S. CALCIUM (Mean ± SD)**	**P value***
Within the expected range for age (N=72)	9.53±0.42	0.879
Below the expected range for age (N=5)	9.32±0.52	

*unpaired students T test

TABLE 46: Serum calcium distribution among Lumbar spine Z score categories

L SPINE Z SCORE CATEGORY	S. CALCIUM (Mean ± SD)	P value*
Within the expected range for age (N=64)	9.52±0.44	0.066
Below the expected range for age (N=12)	9.47±0.31	

*unpaired students T test

TABLE 47: Serum calcium distribution among Left forearm Z score categories

L FOREARM Z SCORE CATEGORY	S. CALCIUM (Mean ± SD)	P value*
Within the expected range for age (N=68)	9.55±0.40	0.631
Below the expected range for age (N=7)	9.20±0.48	

*unpaired students T test

Association of RF positivity with IL-1, IL-6 in RA patients

Correlation of RF positivity with IL-1 and IL-6 was assessed using independent samples Mann-Whitney U test. There was no significant difference in interleukin levels with RF positivity.

Association of disease duration with IL-1, IL-6 in RA patients

Correlation of disease duration with IL-1 and IL-6 was assessed using independent samples Mann-Whitney U test. There was no significant difference in interleukin levels with disease duration.

Association of IL-1, IL-6 with DAS28 ESR

Association of DAS28ESR with IL-1 and IL-6 was assessed using independent samples Mann-Whitney U test. There was no significant difference in interleukin levels between various DAS28 ESR categories.

Parameter	Remission (N=4)	Not in Remission (N=33)	P value*
IL-1 (ng/L)	27.73±4.92	31.50±11.00	0.140

*Independent samples Mann Whitney U test

Parameter	Remission (N=3)	Not in Remission (N=24)	P value*
IL-6(ng/L)	4.62	5.20±74.73	0.914

*Independent samples Mann Whitney U test

Parameter	No High disease activity (N=25)	High disease activity (N=12)	P value*
IL-1 (ng/L)	28.07±9.31	31.90±15.21	0.181

*Independent samples Mann Whitney U test

Parameter	No High disease activity (N=22)	High disease activity (N=5)	P value*
IL-6(ng/L)	5.08±47.88	4.88±232.60	0.739

*Independent samples Mann Whitney U test

Parameter	DAS 28 ESR<3.2 (N=8)	DAS 28 ESR>3.2 (N=29)	P value*
IL-1 (ng/L)	27.73±3.66	31.67±10.54	0.137

*Independent samples Mann Whitney U test

Parameter	DAS 28 ESR<3.2 (N=6)	DAS 28 ESR>3.2 (N=21)	P value*
IL-6(ng/L)	4.42±13.54	5.53±82.14	0.512

*Independent samples Mann Whitney U test

Association between IL-1, IL-6 with osteopenia/osteoporosis

Analysis was done to assess association of IL-1, IL-6 with osteopenia/osteoporosis considering IL-1, IL-6 as independent variable, T score category at each site was used for analysis. There was no significant association of IL-1, IL-6 with osteopenia/osteoporosis (Table 48,49).

TABLE 48: Association of IL-1 with osteopenia/osteoporosis

Dependent Variable	Standard coefficient(beta)	P value	Confidence interval(95%)	
			Lower	Higher
LHIP T score category	0.938	0.135	0.863	1.020
LSPINE T score category	0.963	0.229	0.906	1.024
LForearm T score category	1.011	0.586	0.971	1.053

TABLE 49: Association of IL-6 with osteopenia/osteoporosis

Dependent Variable	Standard coefficient(beta)	P value	Confidence interval(95%)	
			Lower	Higher
LHIP T score category	1.003	0.563	0.994	1.012
LSPINE T score category	1.006	0.314	0.994	1.018
LForearm T score category	0.992	0.252	0.979	1.006

Association between IL-1, IL-6 with low BMD for age

Analysis was done to assess association of IL-1,IL-6 with low BMD for age, considering IL-1,IL-6 as independent variable, Z score category at each site was used for analysis. There was no significant association of Vitamin D deficiency with low BMD for age (Table 50,51).

TABLE 50: Association of IL-1 with low BMD for age

Dependent Variable	Standard coefficient(beta)	P value	Confidence interval(95%)	
			Lower	Higher
LHIP Z score category (N=35)	0.991	0.923	0.827	1.188
LSPINE Z score category(N=26)	0.928	0.466	0.759	1.135
LForearm Z score category (N=26)	0.957	0.417	0.861	1.064

TABLE 51: Association of IL-6 with low BMD for age

Dependent Variable	Standard coefficient(beta)	P value	Confidence interval(95%)	
			Lower	Higher
LSPINE Z score category(N=26)	1.007	0.163	0.997	1.017
LForearm Z score category (N=26)	0.848	0.467	0.543	1.323

DISCUSSION

One hundred and two patients were enrolled in the study. The mean age of the patients was 46.7 ± 12.3 years which was comparable to previously available data of 30 to 50 year (90). The mean age of presentation was similar to a study by Yadav et al (47 ± 12 years) (91). There were 85 females (83.3%) and 17 males (16.7%) in the study population. Worldwide RA is twice more common in females (3) while previous Indian studies like Diggikar et al (92), Premkumar et al (93) and Yadav et al (91) have shown female proportion of 84%, 77.3% and 86% respectively. This can be explained by the increased prevalence of autoimmune and rheumatological diseases in the female patients.

Mean BMI of the study population was found to be 22.8±3 kg/cm^2 which is comparable to the mean BMI of 23.3±3.3 in study by Sharma et al (85).

In previous study by Shin et al they have showed that disease activity is more severe in the seropositive than the seronegative group, and more aggressive treatments are needed in the seropositive group (94). Serum level of rheumatoid factor was available for 75 patients, out of which sixty four (85.3%) were rheumatoid factor positive while eleven (14.7%) were RF negative which is comparable to 79.3% RF positivity in study by Kumar et al (95).

At the time of recruitment into the study, median disease duration was 48±36 months. which was comparable to 3.97 years ± 3.93 years as mentioned in study by Yadav et al (91). Out of these, sixteen patients (15.7%) belonged to early RA while eighty-six (84.3%) patients had established RA.

At presentation, patients were classified into four groups as per DAS28 ESR criteria, eight patients (7.8%) were in remission, nine patients (8.8%) had low disease activity, 51(50.0%) patients had moderate disease activity and thirty-four patients (33.3%) had high disease activity. Previous study by Kumar et al showed 39 % patient had high disease activity (95).

BMD was measured at Left hip, Lumbar spine and left forearm using DEXA scan and mean BMD was 0.69, 0.90 and 0.54 gm/cm^2 at Left hip, Lumbar spine and Left forearm respectively which was much less compared to the cases and controls in the previous study by Sharma et al (85). Mean BMD was comparable to the cases in study done by Shan fu yu et al (96) and Yu mori et al (97).

Table 52: Comparison of Mean BMD at various sites in different studies

	Our study	Sharma et al		Shan fu yu et al	Yu mori et al
SITE	BMD (gm/cm^2) (Mean±SD)	BMD (gm/cm^2) (Mean±SD)		BMD (gm/cm^2) (Mean±SD)	BMD (gm/cm^2) (Mean±SD)
		Case	Control		
Left Hip (N=100)	0.69±0.21	0.89±0.10	0.96±0.92	0.633 ± 0.120	0.69±0.14
Lumbar spine (N=98)	0.90±0.15	1.05±0.1	1.08±0.15	0.871 ± 0.170	0.82±0.18
Left forearm (N=99)	0.54±0.10	0.60±0.07	0.64±0.06	-	-

Average T score was -1.23, -1.34 and -0.45 at Left hip, Lumbar spine and Left forearm respectively. Average T score was much less at hip and forearm compared to cases and controls in previous study (85) but T score was better at left forearm compared to that of cases and controls in previous study by Sharma et al (85). Average T score in our study was better at left hip and lumbar spine compared to previous study by Joo-Hyun lee (98).

Table 53: Comparison of Mean T score at various sites in our study

	Our study	Sharma et al		Joo-Hyun Lee et al
SITE	T SCORE (Mean±SD) / Median±IQR	T SCORE (Mean±SD) / Median±IQR		
		Case	Control	
Left Hip (N=100)	-1.23±1.33	−0.9±0.8	−0.4±0.9	−1.4 ± 1.2
Lumbar spine (N=99)	-1.34±1.39	−1.1±0.9	−0.97±0.95	−1.8 ± 1.3
Left forearm (N=98)	-0.45±2.40	−1.3±1.1	−0.8±0.8	-

Average Z score was -0.40, -0.71 and -0.02 at Left hip, Lumbar spine and Left forearm respectively. Average Z score at hip and lumbar spine is comparable to the cases and better than the controls in previous study (85) but average Z score was better at left forearm compared to that of cases and controls in previous study by Sharma et al (85).

Table 54: Comparison of Mean Z score at various sites in our study

	Our study	Sharma et al	
SITE	Z SCORE (Mean±SD) / Median±IQR	Z SCORE (Mean±SD) / Median±IQR	
		Case	Control
Left Hip (N=100)	-0.40±1.40	-0.40±1.07	−0.1±0.6
Lumbar spine (N=99)	-0.71±1.22	−0.6±0.9	−0.52±0.96
Left forearm (N=98)	-0.02±1.66	−1.2±1.0	−0.8±0.8

Prevalence of osteopenia and osteoporosis at left hip and lumbar spine was higher in our study population compared to the previous study by Sharma et al. But prevalence of osteopenia at forearm was found to be lower and prevalence of osteoporosis at forearm was found to be higher compared to the cases in study by Sharma et al (85). Prevalence of osteopenia and osteoporosis at left hip was higher in our study compared to the previous study by Hafez et al (99), but prevalence of osteopenia and osteoporosis at lumbar spine and osteopenia at left forearm was comparable to the study by Hafez et al (99).

BMD distribution among different age categories in the study population showed statistically significant variation at lumbar spine and left forearm (P value=0.031 and<0.0001). After age 50, bone breakdown (resorption) outpaces bone formation and bone loss often accelerates, particularly at the time of menopause. This BMD variation among the age groups may also be attributed to the longer disease duration of RA leading to more bone loss.

BMD distribution among males and females was compared, Lower values of BMD were observed in female patients but was not statistically significant. In general, males tend to have higher bone density and content and they achieve it at later age compared with females (100).
BMD comparison was done among DAS 28 ESR subgroups based on disease activity, which showed statistically significant reduction in BMD with disease activity at Left Hip (P value-0.019),

however significance was not observed in Lumbar spine and Left forearm. Previous study by Sharma et al (85) showed statistically significant reduction in BMD with disease activity at Left Hip and left forearm (P value-<0.01 and 0.02 respectively). Previous study by Joo-Hyun Lee et al (98) showed no statistically significant correlation with DAS28 ESR. Previous study by Xun gong et al (49) showed statistically significant increase in osteoporosis with DAS28 ESR (p value 0.016). This can be explained by systemic inflammation associated with disease activity, local effect of immune cells leading to bone erosions, more use of glucocorticoid (GC) therapy and impairment of physical activity in patients with high disease activity. All of these factors are likely to affect hip joint compared to spine and forearm.

BMD was compared in RF positive and RF negative subgroups. Lower values of BMD were observed in RF positive patients but was not statistically significant. Previous study by Bugatti et al (101) also showed no statistically significant reduction in BMD with RF positivity at spine or hip. But previous study by Amkreutz et al (102) showed statistically significant reduction in BMD at Lumbar spine with RF positivity (p value-0.04). Autoantibodies such as Rheumatoid factor, anti–citrullinated protein antibodies (ACPAs) have been described as inducing bone loss in rheumatoid arthritis (RA), which can also be reflected by bone mineral density (BMD). However, in our study there was no statistically significant reduction in BMD with RF positivity. BMD was compared in Early and Established RA subgroups. Lower values of BMD were observed in Established RA patients but was not statistically significant

BMD was compared with vitamin D levels. There was no statistically significant association between vitamin D deficiency and BMD in our study population. Previous study by Labronici et al (103) also showed no significant variation in BMD with vitamin D levels.

Comparison of mean DAS28 ESR was done across Z score categories, which showed significant low BMD for age with higher disease activity at lumbar spine. There was a paucity of studies comparing Z scores in the literature review.

The correlation between IL-6 levels and different variables in patients with RA was studied, no correlation was found between IL-6 level and RF positivity, disease duration, DAS28 ESR, osteopenia/osteoporosis, low BMD for age. Previous study done by Abdel meguid et al (104) showed inverse significant correlation between IL-6 and T-score (p-0.0001).

The correlation between IL-1 levels and different variables in patients with RA was studied, no correlation was found between IL-1 level and RF positivity, disease duration, DAS28

ESR, osteopenia/osteoporosis, low BMD for age. There are numerous cytokines involved in the pathogenesis of RA, including TNFα, IL-17, IL-1, IL-6. They have a direct effect on bone remodeling in RA. Our study has only assessed the correlation of IL-1 and IL-6 with different variables in patients with RA.

CONCLUSION

Among the 102 patients included in the study, prevalence of osteopenia and osteoporosis was calculated using T score. 43.1% patients had osteopenia and 15.7% had osteoporosis at left hip, 42.2% had osteopenia and 16.7% had osteoporosis at lumbar spine, 18.6% had osteopenia and 17.6% had osteoporosis at left forearm. There was significant variation in BMD across the age groups at lumbar spine and left forearm. Lower values of BMD were observed in female patients but was not statistically significant. There was statistically significant reduction in BMD with disease activity at Left Hip, however significance was not observed at Lumbar spine and Left forearm. There was statistical significance in the Lumbar spine BMD across the DAS 28 ESR <3.2 and DAS 28 ESR >3.2 subgroup. From our study, we recommend that Left hip BMD can be used as a predictor of disease severity categories according to DAS 28.

Parameters like IL-1, IL-6 could not reliably predict the disease severity categories according to DAS 28 or BMD studied in our patients. This can be explained by understanding that the flares in disease activity in RA is multifactorial and multiple cytokines are impacting the disease severity and bone loss. So, we would like to recommend that there is no role in using IL-1 or IL-6 for guiding the treatment of RA as per treat to target system or to predict BMD loss.

REFERENCES

1. Hyndman IJ. Rheumatoid arthritis: past, present and future approaches to treating the disease. Int J Rheum Dis. 2017 Apr;20(4):417–9.

2. Gabriel SE. The epidemiology of rheumatoid arthritis. Rheum Dis Clin North Am. 2001 May;27(2):269–81.

3. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis. 2014 Jul;73(7):1316–22.

4. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum. 2000 Jan;43(1):30–7.

5. Wade SW, Strader C, Fitzpatrick LA, Anthony MS, O'Malley CD. Estimating prevalence of osteoporosis: examples from industrialized countries. Arch Osteoporos. 2014;9:182.

6. Prevalencia de osteoporosis determinada por densitometría en la población femenina española | Med. clín (Ed. impr.);116(3): 86-88, ene. 2001. | IBECS [Internet]. [cited 2022 Nov 27]. Available from: https://pesquisa.bvsalud.org/portal/resource/pt/ibc-2913

7. Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. Arthritis Rheum. 2000 Mar;43(3):522–30.

8. Hauser B, Riches PL, Wilson JF, Horne AE, Ralston SH. Prevalence and clinical prediction of osteoporosis in a contemporary cohort of patients with rheumatoid arthritis. Rheumatol Oxf Engl. 2014 Oct;53(10):1759–66.

9. Raterman HG, Lems WF. Pharmacological Management of Osteoporosis in Rheumatoid Arthritis Patients: A Review of the Literature and Practical Guide. Drugs Aging. 2019 Dec;36(12):1061–72.

10. Jin S, Hsieh E, Peng L, Yu C, Wang Y, Wu C, et al. Incidence of fractures among patients with rheumatoid arthritis: a systematic review and meta-analysis. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA. 2018 Jun;29(6):1263–75.

11. Tong JJ, Xu SQ, Zong HX, Pan MJ, Teng YZ, Xu JH. Prevalence and risk factors associated with vertebral osteoporotic fractures in patients with rheumatoid arthritis. Clin Rheumatol. 2020 Feb;39(2):357–64.

12. Osteoporosis Pathophysiology, Epidemiology, and Screening in Rheumatoid Arthritis - PubMed [Internet]. [cited 2022 Nov 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/31123839/

13. Hand bone loss in early undifferentiated arthritis: evaluating bone mineral density loss before the development of rheumatoid arthritis | Annals of the Rheumatic Diseases [Internet]. [cited 2022 Nov 27]. Available from: https://ard.bmj.com/content/65/6/736

14. Kanis JA, McCloskey EV, Johansson H, Oden A, Melton LJ, Khaltaev N. A reference standard for the description of osteoporosis. Bone. 2008 Mar 1;42(3):467–75.

15. Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GHT, Wong AHC, et al. DNA methylation profiles in monozygotic and dizygotic twins. Nat Genet. 2009 Feb;41(2):240–5.

16. Vassallo R, Luckey D, Behrens M, Madden B, Luthra H, David C, et al. Cellular and humoral immunity in arthritis are profoundly influenced by the interaction between cigarette smoke effects and host HLA-DR and DQ genes. Clin Immunol Orlando Fla. 2014;152(0):25–35.

17. Wegner N, Lundberg K, Kinloch A, Fisher B, Malmström V, Feldmann M, et al. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev. 2010 Jan;233(1):34–54.

18. Konig MF, Abusleme L, Reinholdt J, Palmer RJ, Teles RP, Sampson K, et al. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Sci Transl Med. 2016 Dec 14;8(369):369ra176.

19. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. eLife. 2013 Nov 5;2:e01202.

20. Firestein GS, Gabriel SE, McInnes IB, O'Dell JR, editors. Kelley and Firestein's textbook of rheumatology. Tenth edition. Philadelphia, PA: Elsevier; 2017. 1 p.

21. Marcucci E, Bartoloni E, Alunno A, Leone MC, Cafaro G, Luccioli F, et al. Extra-articular rheumatoid arthritis. Reumatismo. 2018 Dec 20;70(4):212–24.

22. Das S, Padhan P. An Overview of the Extraarticular Involvement in Rheumatoid Arthritis and its Management. J Pharmacol Pharmacother. 2017;8(3):81–6.

23. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular Manifestations in Rheumatoid Arthritis. Mædica. 2010 Dec;5(4):286–91.

24. Genta MS, Genta RM, Gabay C. Systemic rheumatoid vasculitis: a review. Semin Arthritis Rheum. 2006 Oct;36(2):88–98.

25. Artifoni M, Rothschild PR, Brézin A, Guillevin L, Puéchal X. Ocular inflammatory diseases associated with rheumatoid arthritis. Nat Rev Rheumatol. 2014 Feb;10(2):108–16.

26. Rheumatoid Vasculitis: A Diminishing Yet Devastating Menace - PubMed [Internet]. [cited 2022 Nov 20]. Available from: https://pubmed.ncbi.nlm.nih.gov/28631066/

27. Extra-articular rheumatoid arthritis - PubMed [Internet]. [cited 2022 Nov 20]. Available from: https://pubmed.ncbi.nlm.nih.gov/23425964/

28. Ohira H, Abe K, Takahashi A. Involvement of the liver in rheumatic diseases. Clin J Gastroenterol. 2012 Feb;5(1):9–14.

29. Ebert EC, Hagspiel KD. Gastrointestinal and hepatic manifestations of rheumatoid arthritis. Dig Dis Sci. 2011 Feb;56(2):295–302.

30. Agarwal V, Singh R, Wiclaf null, Chauhan S, Tahlan A, Ahuja CK, et al. A clinical, electrophysiological, and pathological study of neuropathy in rheumatoid arthritis. Clin Rheumatol. 2008 Jul;27(7):841–4.

31. Joaquim AF, Appenzeller S. Neuropsychiatric manifestations in rheumatoid arthritis. Autoimmun Rev. 2015 Dec;14(12):1116–22.

32. Vinicki JP, Pellet SC, De Rosa G, Dubinsky D, Laborde HA, Marini A, et al. Analysis of 65 Renal Biopsies From Patients With Rheumatoid Arthritis (1976-2015): Change in Treatment Strategies Decreased Frequency and Modified Histopathological Findings. J Clin Rheumatol. 2015 Oct 1;21(7):335–40.

33. Simon TA, Thompson A, Gandhi KK, Hochberg MC, Suissa S. Incidence of malignancy in adult patients with rheumatoid arthritis: a meta-analysis. Arthritis Res Ther. 2015 Aug 15;17(1):212.

34. Agca R, Heslinga SC, Rollefstad S, Heslinga M, McInnes IB, Peters MJL, et al. EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. Ann Rheum Dis. 2017 Jan;76(1):17–28.

35. O'Dwyer DN, Armstrong ME, Cooke G, Dodd JD, Veale DJ, Donnelly SC. Rheumatoid Arthritis (RA) associated interstitial lung disease (ILD). Eur J Intern Med. 2013 Oct;24(7):597–603.

36. Olson AL, Swigris JJ, Sprunger DB, Fischer A, Fernandez-Perez ER, Solomon J, et al. Rheumatoid arthritis-interstitial lung disease-associated mortality. Am J Respir Crit Care Med. 2011 Feb 1;183(3):372–8.

37. Kelly C, Iqbal K, Iman-Gutierrez L, Evans P, Manchegowda K. Lung involvement in inflammatory rheumatic diseases. Best Pract Res Clin Rheumatol. 2016 Oct;30(5):870–88.

38. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA. Proposed Diagnostic Criteria for Rheumatoid Arthritis *. Ann Rheum Dis. 1957 Mar;16(1):118–25.

39. Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. Rheumatol Oxf Engl. 2012 Dec;51 Suppl 6:vi5-9.

40. Bykerk VP, Massarotti EM. The new ACR/EULAR classification criteria for RA: how are the new criteria performing in the clinic? Rheumatol Oxf Engl. 2012 Dec;51 Suppl 6:vi10-15.

41. Migliore A, Bizzi E, Petrella L, Bruzzese V, Cassol M, Integlia D. The Challenge of Treating Early-Stage Rheumatoid Arthritis: The Contribution of Mixed Treatment Comparison to Choosing Appropriate Biologic Agents. BioDrugs Clin Immunother Biopharm Gene Ther. 2016 Apr;30(2):105–15.

42. Jeka S, Dura M, Zuchowski P, Zwierko B, Waszczak-Jeka M. The role of ultrasonography in the diagnostic criteria for rheumatoid arthritis and monitoring its therapeutic efficacy. Adv Clin Exp Med Off Organ Wroclaw Med Univ. 2018 Sep;27(9):1303–7.

43. Singh JA, Saag KG, Bridges SL, Akl EA, Bannuru RR, Sullivan MC, et al. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. Arthritis Care Res. 2016 Jan;68(1):1–25.

44. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res. 2018;6:15.

45. Lindner L, Callhoff J, Alten R, Krause A, Ochs W, Zink A, et al. Osteoporosis in patients with rheumatoid arthritis: trends in the German National Database 2007-2017. Rheumatol Int. 2020 Dec;40(12):2005–12.

46. Aeberli D, Schett G. Cortical remodeling during menopause, rheumatoid arthritis, glucocorticoid and bisphosphonate therapy. Arthritis Res Ther. 2013 Mar 21;15(2):208.

47. García-Magallón B, Silva-Fernández L, Andreu-Sánchez JL. Update on the use of steroids in rheumatoid arthritis. Reumatol Clin. 2013;9(5):297–302.

48. Adami G, Saag KG. Osteoporosis Pathophysiology, Epidemiology, and Screening in Rheumatoid Arthritis. Curr Rheumatol Rep. 2019 May 23;21(7):34.

49. Gong X, Xu S qian, Tong H, Wang X rong, Zong H xiang, Pan M juan, et al. Correlation between systemic osteoporosis and local bone erosion with rheumatoid arthritis patients in Chinese population. Rheumatology. 2019 Aug 1;58(8):1443–52.

50. Fenton CG, Webster JM, Martin CS, Fareed S, Wehmeyer C, Mackie H, et al. Therapeutic glucocorticoids prevent bone loss but drive muscle wasting when administered in chronic polyarthritis. Arthritis Res Ther. 2019 Aug 1;21(1):182.

51. Kendler DL, Marin F, Geusens P, López-Romero P, Lespessailles E, Body JJ, et al. Psychotropic medications and proton pump inhibitors and the risk of fractures in the teriparatide versus risedronate VERO clinical trial. Bone. 2020 Jan 1;130:115113.

52. Arboleya L, Castañeda S. Osteoinmunología: el estudio de la relación entre el sistema inmune y el tejido óseo. Reumatol Clínica. 2013 Sep 1;9(5):303–15.

53. Immune-bone interplay in the structural damage in rheumatoid arthritis | Clinical and Experimental Immunology | Oxford Academic [Internet]. [cited 2022 Nov 29]. Available from: https://academic.oup.com/cei/article/194/1/1/6411871

54. Guder C, Gravius S, Burger C, Wirtz DC, Schildberg FA. Osteoimmunology: A Current Update of the Interplay Between Bone and the Immune System. Front Immunol [Internet]. 2020 [cited 2022 Nov 29];11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.00058

55. Osteoprotegerin: A Novel Secreted Protein Involved in the Regulation of Bone Density: Cell [Internet]. [cited 2022 Nov 29]. Available from: https://www.cell.com/cell/fulltext/S0092-8674(00)80209-3?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867400802093%3Fshowall%3Dtrue

56. Srivastava RK, Dar HY, Mishra PK. Immunoporosis: Immunology of Osteoporosis—Role of T Cells. Front Immunol [Internet]. 2018 [cited 2022 Nov 29];9. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2018.00657

57. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest. 1999 May 1;103(9):1345–52.

58. Dokoupilová E, Aelion J, Takeuchi T, Malavolta N, Sfikakis P, Wang Y, et al. Secukinumab after anti-tumour necrosis factor-α therapy: a phase III study in active rheumatoid arthritis. Scand J Rheumatol. 2018 Jul 4;47(4):276–81.

59. Production of RANKL by Memory B Cells: A Link Between B Cells and Bone Erosion in Rheumatoid Arthritis. [cited 2022 Nov 29]; Available from: https://onlinelibrary.wiley.com/doi/10.1002/art.39489

60. Fang Q, Zhou C, Nandakumar KS. Molecular and Cellular Pathways Contributing to Joint Damage in Rheumatoid Arthritis. Mediators Inflamm. 2020 Mar 17;2020:e3830212.

61. Frontiers | Classical and Paradoxical Effects of TNF-α on Bone Homeostasis [Internet]. [cited 2022 Nov 29]. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2014.00048/full

62. Marotte H, Pallot-Prades B, Grange L, Gaudin P, Alexandre C, Miossec P. A 1-year case-control study in patients with rheumatoid arthritis indicates prevention of loss of bone mineral density in both responders and nonresponders to infliximab. Arthritis Res Ther. 2007 Jun 27;9(3):R61.

63. Houssiau FA, Devogelaer JP, Damme JV, Deuxchaisnes CND, Snick JV. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arthritis Rheum. 1988;31(6):784–8.

64. IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-α and IL-17 | Rheumatology | Oxford Academic [Internet]. [cited 2022 Nov 29]. Available from: https://academic.oup.com/rheumatology/article/47/11/1635/1790556

65. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006 May;441(7090):235–8.

66. Nishimoto N, Hashimoto J, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial of tocilizumab. Ann Rheum Dis. 2007 Sep 1;66(9):1162–7.

67. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: A twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. [cited 2022 Nov 29]; Available from: https://onlinelibrary.wiley.com/doi/10.1002/art.23417

68. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol. 2001 Jul;3(7):683–6.

69. Garnero P, Tabassi NCB, Voorzanger-Rousselot N. Circulating Dickkopf-1 and Radiological Progression in Patients with Early Rheumatoid Arthritis Treated with Etanercept. J Rheumatol. 2008 Dec 1;35(12):2313–5.

70. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med. 2007 Feb;13(2):156–63.

71. Rossini M, Bagnato G, Frediani B, Iagnocco A, Montagna GL, Minisola G, et al. Relationship of Focal Erosions, Bone Mineral Density, and Parathyroid Hormone in Rheumatoid Arthritis. J Rheumatol. 2011 Jun 1;38(6):997–1002.

72. van der Heijde DMFM. Radiographic imaging: the "gold standard" for assessment of disease progression in rheumatoid arthritis. Rheumatology. 2000 Jun 1;39(suppl_1):9–16.

73. Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest. 2012 May 1;122(5):1791–802.

74. Krishnamurthy A, Joshua V, Hensvold AH, Jin T, Sun M, Vivar N, et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. Ann Rheum Dis. 2016 Apr 1;75(4):721–9.

75. Kleyer A, Finzel S, Rech J, Manger B, Krieter M, Faustini F, et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. Ann Rheum Dis. 2014 May 1;73(5):854–60.

76. Castañeda S, Llorente I, García-Vicuña R, González-Álvaro I. Anti-citrullinated protein antibodies and bone loss in patients with early arthritis: comment on the article "Anti-citrullinated protein antibodies and high levels of rheumatoid factor are associated with systemic bone loss in patients with early untreated rheumatoid arthritis" by Bugatti et al. Arthritis Res Ther. 2017 Jul 3;19(1):152.

77. Anti-citrullinated protein antibodies are associated with decreased bone mineral density: baseline data from a register of early arthritis patients | SpringerLink [Internet]. [cited 2022 Nov 30]. Available from: https://link.springer.com/article/10.1007/s00296-017-3674-9

78. Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GMC, van Veelen PA, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proc Natl Acad Sci. 2011 Oct 18;108(42):17372–7.

79. Regueiro C, Ortiz AM, Boveda MD, Castañeda S, Gonzalez-Alvaro I, Gonzalez A. Association of high titers of anti-carbamylated protein antibodies with decreased bone mineral density in early arthritis patients. PLOS ONE. 2018 Aug 17;13(8):e0202583.

80. Smolen JS, Landewé RBM, Bijlsma JWJ, Burmester GR, Dougados M, Kerschbaumer A, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis. 2020 Jun 1;79(6):685–99.

81. Toledano E, Ortiz AM, Ivorra-Cortes J, Montes N, Beltran A, Rodríguez-Rodriguez L, et al. Are rheumatologists adhering to the concepts window of opportunity and treat-to-target? Earlier and more intense disease-modifying anti-rheumatic drug treatment over time in patients with early arthritis in the PEARL study. Clin Exp Rheumatol. 2018 May 1;36(3):382–8.

82. Haugeberg G, Lodder MC, Lems WF, Uhlig T, Ørstavik RE, Dijkmans B a. C, et al. Hand cortical bone mass and its associations with radiographic joint damage and fractures in 50–70 year old female patients with rheumatoid arthritis: cross sectional Oslo-Truro-Amsterdam (OSTRA) collaborative study. Ann Rheum Dis. 2004 Oct 1;63(10):1331–4.

83. Book C, Algulin J, Nilsson JÅ, Saxne T, Jacobsson L. Bone mineral density in the hand as a predictor for mortality in patients with rheumatoid arthritis. Rheumatology. 2009 Sep 1;48(9):1088–91.

84. Ahmad HA, Alemao E, Guo Z, Iannaccone CK, Frits ML, Weinblatt M, et al. Association of Low Bone Mineral Density with Anti-Citrullinated Protein Antibody Positivity and Disease Activity in Established Rheumatoid Arthritis: Findings from a US Observational Cohort. Adv Ther. 2018 Feb 1;35(2):232–42.

85. Sharma M, Dhakad U, Wakhlu A, Bhadu D, Dutta D, Das SK. Lean Mass and Disease Activity are the Best Predictors of Bone Mineral Loss in the Premenopausal Women with Rheumatoid Arthritis. Indian J Endocrinol Metab. 2018 Apr;22(2):236–43.

86. Zerbini C a. F, Clark P, Mendez-Sanchez L, Pereira RMR, Messina OD, Uña CR, et al. Biologic therapies and bone loss in rheumatoid arthritis. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA. 2017 Feb;28(2):429–46.

87. JPMA - Journal Of Pakistan Medical Association [Internet]. [cited 2022 Nov 23]. Available from: https://jpma.org.pk/article-details/8035

88. Mechanisms of Systemic Osteoporosis in Rheumatoid Arthritis - PMC [Internet]. [cited 2022 Nov 25]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9368786/

89. Ruscitti P, Cipriani P, Carubbi F, Liakouli V, Zazzeroni F, Di Benedetto P, et al. The Role of IL-1β in the Bone Loss during Rheumatic Diseases. Mediators Inflamm. 2015 Apr 12;2015:e782382.

90. Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. Am J Med. 2007 Nov;120(11):936–9.

91. Yadav. A cross-sectional study of different rheumatic diseases and their respective comorbidities at a tertiary care hospital in India [Internet]. [cited 2022 Dec 18]. Available from: https://www.indianjrheumatol.com/article.asp?issn=0973-3698;year=2019;volume=14;issue=1;spage=42;epage=48;aulast=Yadav

92. Diggikar PM, Gokhale VS, Satpathy PK, Baldania DD, Babu TV, Jain KD. A Study on Clinical Profile of Patients Presenting with Rheumatoid Arthritis in a Tertiary Care Hospital of Pune City -. Natl J Med Res. 2016;6(2):146–50.

93. Premkumar B, Srinivasamurthy M, Rajagopal K. A Retrospective Study on Clinical Characteristics of Rheumatoid Arthritis Patients. Biomed Pharmacol J. 2015 Apr 28;6(2):471–5.

94. Shin YS, Choi JH, Nahm DH, Park HS, Cho JH, Suh CH. Rheumatoid Factor is a Marker of Disease Severity in Korean Rheumatoid Arthritis. Yonsei Med J. 2005 Aug 31;46(4):464–70.

95. Kumar AA, Gupta K, S K, Kumar SA. Clinical Profile of Rheumatoid Arthritis Patients Reporting to a Tertiary Care Center – Data From Southwestern Part of India. Int J Contemp Med Res IJCMR [Internet]. 2019 Jun [cited 2022 Dec 18];6(6). Available from: https://www.ijcmr.com/uploads/7/7/4/6/77464738/ijcmr_2577_v1.pdf

96. Yu SF, Chen JF, Chen YC, Wang YW, Hsu CY, Lai HM, et al. The Impact of Seropositivity on Systemic Bone Loss in Rheumatoid Arthritis—A 3-Year Interim Analysis of a Longitudinal Observational Cohort Study. Front Med. 2022 Jun 9;9:885801.

97. Mori Y, Kuwahara Y, Chiba S, Kogre A, Baba K, Kamimura M, et al. Bone mineral density of postmenopausal women with rheumatoid arthritis depends on disease duration regardless of treatment. J Bone Miner Metab. 2017 Jan;35(1):52–7.

98. Lee JH, Sung YK, Choi CB, Cho SK, Bang SY, Choe JY, et al. The frequency of and risk factors for osteoporosis in Korean patients with rheumatoid arthritis. BMC Musculoskelet Disord. 2016 Feb 24;17:98.

99. Hafez EA, Mansour HE, Hamza SH, Moftah SG, Younes TB, Ismail MA. Bone mineral density changes in patients with recent-onset rheumatoid arthritis. Clin Med Insights Arthritis Musculoskelet Disord. 2011;4:87–94.

100. Alswat KA. Gender Disparities in Osteoporosis. J Clin Med Res. 2017 May;9(5):382–7.

101. Bugatti S, Bogliolo L, Vitolo B, Manzo A, Montecucco C, Caporali R. Anti-citrullinated protein antibodies and high levels of rheumatoid factor are associated with systemic bone loss in patients with early untreated rheumatoid arthritis. Arthritis Res Ther. 2016 Oct 6;18(1):226.

102. Amkreutz JAMP, de Moel EC, Theander L, Willim M, Heimans L, Nilsson J, et al. Association Between Bone Mineral Density and Autoantibodies in Patients With Rheumatoid Arthritis. Arthritis Rheumatol Hoboken Nj. 2021 Jun;73(6):921–30.

103. Labronici PJ, Blunck SS, Lana FR, Esteves BB, Franco JS, Fukuyama JM, et al. Vitamin D and its Relation to Bone Mineral Density in Postmenopause Women. Rev Bras Ortop Engl Ed. 2013 May 1;48(3):228–35.

104. Abdel Meguid MH, Hamad YH, Swilam RS, Barakat MS. Relation of interleukin-6 in rheumatoid arthritis patients to systemic bone loss and structural bone damage. Rheumatol Int. 2013 Mar 1;33(3):697–703.

IEC CERTIFICATE

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3544 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3379**

Project title: **"Effect of rheumatoid arthritis on bone mineral density and it's correlation with inflammatory milieu in rheumatoid arthritis patients"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Ramanand S**
Guide:	**Dr. M.K.Garg**
Co-Guide:	**Dr. Maya Gopalakrishnan, Dr. Satyendra Khichar, Dr. Ravindra Kumar & Dr. Kamla Kant Shukla**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

APPENDIX-1

All India Institute of Medical Sciences

Jodhpur, Rajasthan

Informed Consent Form

Title of Thesis/Dissertation: BONE MINERAL DENSITY AMONG RHEUMATOID ARTHRITIS PATIENTS IN AIIIMS JODHPUR

Name of PG Student: Dr. Ramanand S 8281988012

Patient/Volunteer Identification No.: _____

I,_____S/o or D/o_____

R/o_____give my full, free, voluntary

Consent to be a part of the study **"BONE MINERAL DENSITY IN RHEUMATOID ARTHRITIS PATIENTS PRESENTING TO AIIMS JODHPUR"**, the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from _____(Company Name) or from regulatory authorities. I give permission for these individuals to have access to my records.

Date : _____ _____

Place : _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date :

Place : Signature of PG Student

Witness 1 Witness 2

Signature: Signature:

Name: Name:

Address : Address :

APPENDIX-2

अखिल भारतीय आयुर्विज्ञान संस्थान

जोधपुर, राजस्थान

सूचित सहमति प्रपत्र

थीसिस का शीर्षक: एम्स जोधपुर का संधिवात गठिया रोगियों का अस्थि खनिज घनत्व

पीजी छात्र का नाम: डॉ रामानंद दूरभाष।संख्या **: 8281988012**

रोगी / स्वयंसेवीपहचानसंख्या.: _____

मैं,_____पुत्र/पुत्री_____

निवासी_____मेरीपूर्ण, नि: शुल्क, स्वैच्छिक सहमति देता हु निम्नलिखित अध्ययन का हिस्सा बनने के लिए, जिसकी प्रक्रिया और प्रकृति मेरी पूरी संतुष्टि के लिए मेरी अपनी भाषा में मुझे समझाया गया है।मैं पुष्टि करता हूं कि मेरे पास प्रश्न पूछने का अवसर था। मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और किसी भी कारण के बिना ,किसी भी समय अध्ययन से बाहर निकलने के मेरे अधिकार से अवगत हूंI मैं समझता हूं कि मेरे और मेरे किसी भी मेडिकल रिकॉर्ड के बारे में एकत्र की गई जानकारी एम्स जोधपुर से या नियामक प्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखी जा सकती है। मैं इन व्यक्तियों के लिए अपने रिकॉर्ड तक पहुंचने की अनुमति देता हूं**I**

यदि आवश्यक हो तो भविष्य के संदर्भों और अध्ययनों के लिए, एकत्र किए गए नमूने कड़े परिस्थितियों में संग्रहित किए जाएंगे I

दिनांक: _____ _____

स्थान : _____ **हस्ताक्षर/बाएंअंगूठेकीछाप**

यह प्रमाणित करने के लिए कि उपर्युक्त सहमति मेरी उपस्थिति में प्राप्त की गई है।

तारीख :_____

स्थान: _____ **हस्ताक्षर का पीजी छात्र**

साक्षी 1	साक्षी 2
हस्ताक्षर:	हस्ताक्षर:
नाम:	नाम:
स्थान :	स्थान :

APPENDIX-3

PATIENT INFORMATION SHEET

Name of the patient: **Patient ID:**

BONE MINERAL DENSITY AMONG RHEUMATOID ARTHRITIS PATIENTS IN WESTERN RAJASTHAN

1. You are participating in a study to understand osteoporosis found in a condition called rheumatoid arthritis.
2. We will be collecting information regarding your age, gender, duration of your disease and the treatment you have received.
3. Study procedure: We will be collecting your blood sample to do your routine tests as well as 2 new tests, which are done as part of our study. You will be asked to participate in a DEXA scan.The DEXA scan will expose you to minimum radiation but the benefits of doing the scan will outweigh the risks.
4. Likely benefit: If you have an underlying osteoporosis, we can treat it early which will be of benefit to you.
5. Confidentiality: All the data collected from you will be kept highly confidential.

6. Risk: Enrollment in above study poses no substantial risk to you. You can withdraw from the study at any point of time without any consequences to yourself.

   For further information / questions, the following personnel can be contacted:

   Dr Ramanand S, Junior Resident, Department of Internal Medicine, All India Institute of Medical Sciences, Jodhpur, Rajasthan. Ph: 8281988012

ANNEXURE 1

SOCIO-DEMOGRAPHIC AND CLINICAL PROFORMA

Patient ID: **Name of patient:** **Participant No:**

Age/gender: **DATE OF VISIT:**

Diagnosis by ACR-EULAR criteria	
Duration of disease	
Drug history	
Duration of methotrexate exposure	
Cumulative methotrexate dose	
Cumulative steroid dose	
Comorbidities	
Family history	
Last menstrual period(LMP)	
Urine pregnancy test	

General Examination	

DAS 28 SCORE

Systemic examination	
Joint deformities	
Extra articular manifestations	

BONE DENSITY REPORT

REGION	BMD	T-SCORE	Z-SCORE	CLASSIFICATION

ANNEXURE 2

ACR-EULAR CRITERIA FOR DIAGNOSIS OF RHEUMATOID ARTHRITIS

Parameter	Criteria	Score*
Joint involvement	1 large joint	0
	2-10 large joints	1
	1-3 small joints	2
	4-10 small joints	3
	>10 joints (at least 1 small joint)	5
Serology	Negativity for RF and anti-CCP	0
	Low level of RF or anti-CCP	2
	High level of RF or anti-CCP	3
Acute-phase reactants	Normal CRP level and ESR	0
	Abnormal CRP level or ESR	1
Duration of symptoms	<6 wk	0
	≥6 wk	1

CCP = cyclic citrullinated peptide; **CRP** = C-reactive protein; **ESR** = erythrocyte sedimentation rate; **RF** = rheumatoid factor; **wk** = week.
*A total score of more than 6 indicates a diagnosis of rheumatoid arthritis.

ANNEXURE 3

DAS28 form

Patient name …… Date of Birth __ /__/____

Observer name …… Date __ /__/____

	LEFT		RIGHT	
	SWOLLEN	TENDER	SWOLLEN	TENDER
SHOULDER				
ELBOW				
WRIST				
MCP 1				
2				
3				
4				
5				
PIP 1				
2				
3				
4				
5				
KNEE				
SUBTOTAL				
TOTAL	SWOLLEN		TENDER	

How active was your arthritis during the past week?

(Please mark the degree of activity on the scale below by placing a vertical line)

Not active at all _____ **Extremely active**

Swollen Joint Count (0-28)	
Tender Joint Count (0-28)	
ESR	
VAS disease activity (0-100mm)	

DAS28 = 0.56*√(t28) + 0.28*√(sw28) + 0.70*Ln(ESR)+ 0.014*VAS

ANNEXURE 4

BMD DXA REPORT

ALL INDIA INSTITUTE OF MEDICAL SCIENCES

BASNI PHASE 2nd
JODHPUR, RAJASTHAN 342005

Name: SALIL BORNA
Patient ID: AIIMS/JDH/2016/06/012942
DOB: 25 July 1993

Sex: Male
Ethnicity: Asian

Height: 172.0 cm
Weight: 72.8 kg
Age: 27

Referring Physician: dr.M MITTAL

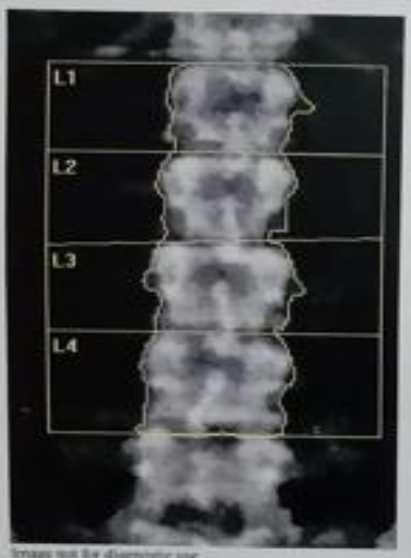


Image not for diagnostic use
k = 1.123, d0 = 48.7
168 x 140

Scan Information:

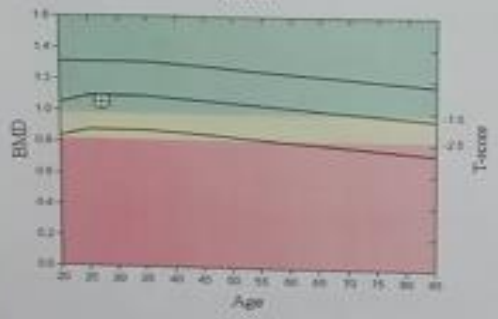
Scan Date: 24 November 2020 ID: A1124200C
Scan Type: x Lumbar Spine
Analysis: 25 November 2020 12:07 Version 13.6.0.5:3
Spine
Operator:
Model: Horizon A (S/N 303237M)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	Z-score
L1	13.04	12.34	0.947	-1.1	-1.1
L2	13.36	14.13	1.058	-0.3	-0.3
L3	14.82	16.52	1.115	0.1	0.1
L4	18.27	19.48	1.066	-0.2	-0.2
Total	**59.48**	**62.46**	**1.050**	**-0.4**	**-0.4**

Total BMD CV 1.0%, ACF = 1.030, BCF = 1.005, TH = 7.693
WHO Classification: Normal
Fracture Risk: Not Increased

Total



Comment:

T-score vs. White Male Source:2012 BMDCS/Hologic Z-score vs. White Male
Source:2013 BMDCS/Hologic

HOLOGIC