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Use and significance of Anti CCP Antibodies In Rheumatoid Arthritis

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ABBREVIATIONS USED IN THE STUDY

ANTI - CCP	ANTI CYCLIC CITRULLINATED PEPTIDE
PAD	PEPTIDYL ARGININE DEIMINASE
RA	RHEUMATOID ARTHRITIS
RF	RHEUMATOID FACTOR
ACR	AMERICAN COLLEGE OF RHEUMATOLOGY
ACPA	ANTI CITRULLINATED PEPTIDE ANTIBODY
ES	EARLY SYNOVITIS
CTD	CONNECTIVE TISSUE DISORDER
SLE	SYSTEMIC LUPUS EYTHEMATOSUS
O A	OSTEO ARTHRITIS.
HBD	HEALTHY BLOOD DONORS
ELISA	ENZYME LINKED IMMUNOSORBENT ASSAY
HLA – DR	HUMAN LEUCOCYTE ANTIGEN (CLASS –DR)
Fc–PORTION	CRYSTALLIZATION (CONSTANT) FRAGMENT
Ig G	IMMUNOGLOBULIN G
ASO	ANTI STREPTOLYSIN 'O'
CRP	C – REACTIVE PROTEIN
DC	DIFFERENTIAL COUNT OF WHITE BLOOD CELLS
ESR	ERYTHROCYTE SEDIMENTATION RATE
HB	HAEMOGLOBIN
APR	ACUTE PHASE REACTION
CDC	CETRE FOR DISEASE PREVENTION AND CONTROL
PPV	POSITIVE PREDICTIVE VALUE
NPV	NEGATIVE PREDICTIVE VALUE

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CERTIFICATE

This is to certify that the dissertation entitled **"USE AND SIGNIFICANCE OF ANTI CCP ANTIBODIES IN RHEUMATOID ARTHRITIS"** is a bonafide work done by **Dr.D.Ayisha,** post graduate student in the Department of Microbiology, under the supervision of DR. **ANBU.N.ARAVAZHI, M.D.,** Professor and Head, Department of Microbiology, Coimbatore Medical College, and under the guidance of **DR.K.RAJENDRAN, B.Sc, M.D.,** Professor, Department of Microbiology, Coimbatore Medical College, Coimbatore, in fulfillment of the regulations of the Tamil Nadu Dr. M.G.R Medical University, towards the award of M.D. Degree (Branch - IV) in Microbiology.

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INTRODUCTION

Rheumatoid Arthritis (RA) is the most common systemic inflammatory, auto immune Rheumatic disease of unknown etiology[1,2,3,4] affecting nearly 1% [1,3,5,6,7,8.9,10] of the adult population worldwide. It is characterized by chronic and erosive polyarthritis,[10,11] (usually involving small, peripheral joints in a symmetric distribution) caused by abnormal growth of synovial tissue or pannus, and causes irreversible joint deformity[9] that can lead to severe disability[1,3,8,12,13] with considerable morbidity[6]. Although the precise aetiology of RA remains unknown[1], there is strong evidence for autoimmunity, since several auto antibodies are associated with the disease[6].

The potential of the synovial inflammation to cause cartilage damage and bone erosions and subsequent changes in joint integrity is the hallmark of the disease. Despite its destructive potential, the course of RA can be quite variable. Some patients may experience only a mild oligo articular illness of brief duration with minimal joint damage, but most will have a relentless progressive polyarthritis with marked functional impairment.

The disease occurs frequently in women than in men (2.5 – 3**:**1). It afflects the people of all races equally. The disease can

begin at any age, peak onset typically occurs in the fourth and fifth decades of life.[7]

In some families, multiple members can be affected, suggesting a genetic basis for the disorder. Genetic studies have demonstrated that a genetic predisposition resides in the HLA-DR locus[7,12.] There is also evidence that environmental factors, such as infectious agents, oral contraceptives and smoking, may play a role[7].

For decades, RA is diagnosed primarily according to clinical manifestations based upon ACR criteria [3,5,7,14,15], in which the only serological marker is RF test.

Rheumatoid factor (RF) is an antibody directed against the Fc region of IgG that has been used as a diagnostic marker for Rheumatoid Arthritis.[2,3,6,7,16.] and is recommended as a screening test[3.] and can be detected in up to 80% of RA patients [6,17.]However it is nonspecific[16] and may be present in 5-10 % of healthy elderly persons or in patients with other autoimmune and infectious diseases[2,6,11].The test is performed on a routine basis in most clinical laboratories[1,7] as per ACR criteria.

During the first few months of the disease, the (1987) revised criteria of the American College of Rheumatology (ACR) is rarely met. About one- third of the patients with persistent arthritis

do not fulfill the classification criteria, so it is often difficult to diagnose RA, in the very early stages of the disease[6]. On the other hand, numerous studies have shown that substantial irreversible joint damage occurs within the first 2 years [6,18,19] of the disease. In many cases, irreversible damage of the joint cartilage has already occurred by the time laboratory and radiological parameters have confirmed the clinical diagnosis of RA.

So it is therefore crucial to have a reliable and specific test to identify the RA patients prior to the occurrence of joint damage.[17]The other most specific auto antibody system for RA is the family of auto antibodies directed to Citrulline – containing proteins, including antiperinuclear factor (APF) in 1964[7], antikeratin antibodies (AKA) in 1979,[7] antifilaggrin antibodies (AFA) and anti-Sa[3,7,11]. Because of rigorous technical requirements for their detection, antiperinuclear factor and antikeratin antibodies have never been widely used as markers for Rheumatoid Arthritis, despite their high specificity[3,6.]

Recently, a new serological test (biological marker) [20] the anti Cyclic Citrullinated Peptide (anti-CCP) [2,20] was developed[6.] Citrulline is formed by deamination of arginine residues in several proteins by the action of enzyme peptidyl arginine deiminase (PAD) [6,7,16,21,20] which is present abundantly in inflammatory synovium & cause

local citrullination of proteins such as fibrin[6,7].Citrullinated extracellular fibrin in the RA synovium may be one of the major autoantigens driving local immune response suggested by the discovery of local production of anti – CCP antibodies in the joint.

The test could also be helpful in the diagnosis of RA in those cases that do not completely fulfil the 1987 ACR criteria.[7] Anti –CCP was reported to have a higher specificity for the diagnosis of RA,especially in patients with early disease .[3,10] Anti CCP antibodies are diagnostic & prognostic marker of early onset of RA,and predate arthritis by several years.[6,,21,22]

It was also found that there is an association between anti - CCP and the disease severity in early RA[6,11,23]. A-CCP is the predictor of bone damage.[3,7,23]

The high specificity (98%) of anti-CCP in patients with RA can exclude other rheumatic or immune diseases,[6,7,9] (like SLE & OA) in patients with positive anti- CCP.

20% of new patients with RA are seronegative in the firstyear,when early diagnosis is essential to prevent erosive joint disease[7,18,24]. It have been helpful to see the anti-CCP data during the first, vitally important year of disease.

Around 40 % of RF seronegative patient appear to be anti–CCP positive,which substantiates additional diagnostic potential of anti-CCP. Specificity of anti -CCP antibodies is more than 90 %.

It has been recognised in the last decade, RA needs to be diagnosed early & treated promptly with Disease Modifying Anti Rheumatic Drugs (DMARD) in order to successfully interfere with disease process[18,19].The ultimate challenge for future is to initiate therapy in early phase that the actual development of RA is prevented, for which early diagnosis is more important to limit the radiological progression of the disease (bony deformity) by initiating treatment earlier and also important for providing patients with best outcome & quality of life[16]. It is useful in diagnosis and exclusion of RA.[24.]

Another potential marker for increased risk of RA may be C-reactive protein(CRP).[11,18,21,22] may also be elevated in patients with RA.[17,22,23,25]Additionally ESR is also determined in patients with RA[11,22,23,25]. Hence the study.

AIMS & OBJECTIVES

Aim:

The aim of the present study is to evaluate the use and significance of AntiCCP antibodies in Rheumatoid Arthritis, and to compare it with other Arthritis - Early Synovitis (ES), Connective Tissue Disorders (CTD) including SLE, and Osteo Arthritis (OA), and in Healthy Blood Donors as control.

Objectives:

- To evaluate the diagnostic utility of Anti- CCP (cyclic citrullinated peptide) antibody in Rheumatoid arthritis.
- To study & compare the presence of Anti- CCP antibody in Rheumatoid arthritis (RA) with other arthritis. – Early Synovitis (ES),Connective Tissue Disorders (CTD) including SLE, and Osteo Arthritis (OA).
- To evaluate the significance of Anti- CCP antibody in Sero Negative Rheumatoid Arthritis.
- To assess the sensitivity and specificity of the Anti CCP antibody test with RF test in RA and other arthritis.

REVIEW OF LITERATURE

History [26]

The 1st known traces of arthritis date back at least as far as **4500 BC.**

- A text dated **123 AD** first describes **symptoms** very similar to Rheumatoid arthritis.
- The **art of Peter Paul Rubens** may possibly depict the effects of Rheumatoid arthritis.
- The first recognized description of rheumatoid arthritis was in **1800** by the French physician **Dr Augustin Jacob Landré - Beauvais (1772-1840).**
- The name "Rheumatoid Arthritis" itself was coined in **1859** by British Rheumatologist **Dr Alfred Baring Garrod**.
- **1937** – RF-"Rheumatoid Factor" **Erik waaler** [2,13]
- **1964** – APF-anti perinuclear antibody [7,13,27] - **Neinhuis and Mandema**[9]
- **1979** – AKA-anti keratin antibody.[7,13,27]
- **Walther van venrooij** and colleagues –A CCP- anti cyclic citrullinated peptide.[2]

Affected famous personalities[26]

- Auguste Renoir, impressionist painter, whose later 'softer' style

might have reflected in some way his severe disability.

- Christiaan Barnard, the first surgeon to perform a human-to-human heart transplant had to retire owing to the condition. He also wrote a book on living with arthritis.
- James Coburn claimed to have healed the condition using pills containing a sulfur-containing compound on his return to acting.
- Erik Lindbergh, aviator and member of the X-Prize administration. Erik has been a spokesman for the arthritis drug Enbrel, as a result of his success with the treatment.
- Kathleen Turner and Aida Turturro have worked to raise public awareness of the condition.
- Billy Bowden, international cricket umpire who had to retire from active playing due to RA.
- Melvin Franklin, bass singer of the Temptations. He was treated for RA with cortisone shots so he could perform.
- Christopher Lee, British actor, used special, ergonomically designed props when he works on set.

RHEUMATOID ARTHRITIS

The name is based on the term "**Rheumatic Fever**" an illness which includes joint pain and is derived from the Greek word ***Rheumatos*** **("flowing").** The suffix - ***oid*** **("resembling")** - joint *inflammation that resembles rheumatic fever*. [26]

Rheumatoid Arthritis (RA) is the commonest inflammatory joint disease.[1,2,3] It is characterized by chronic polyarthritis in symmetrical distribution with multiple deformities and systemic involvement.[11,25,28,29] This leads to irreversible joint disability.[25] It is associated with considerable morbidity[6].

RA is characterized by inflammation of the synovial membrane of diarthrodial joints[7]. Early indications of RA are swelling and pain of the proximal interphalangeal and metacarpophalangeal joints. Later, the larger joints become affected, especially those of the knee, elbow and ankle[7]. Many studies show that the synovial membrane inflammation, in most cases lead to progressive destruction of cartilage and bone leading to irreversible deformity and disability[1,3,8,12,13]. In a study by A.J.W.Zendman, et al ,says that RA severely affects the quality of life[16] of a patient and also has major economic consequences for society.

Since RA is a systemic autoimmune disease, other parts or

organs of the body may become affected at a later stage, example - Rheumatoid Nodule[7,30,31].

Etiology

Although the precise etiology of RA remains unknown[1,2,6] there is strong evidence for autoimmunity since several auto antibodies are associated with the disease.[6,30,31]

Family studies indicate a genetic predisposition. Severe RA is found at approximately 4 times the expected rate in first-degree relatives of individuals with disease associated with the presence of the autoantibody[6,] Rheumatoid factor. Moreover, monozygotic twins are at least four times more likely to be concordant for RA than dizygotic twins. [23]

Genetic studies have demonstrated that a genetic predisposition resides in the HLA-DR locus as reported in many studies and texts[7,12,30,31.] HLA-DR 1 is more important in Indians, and HLA-DR 15 in Japanese. As reported by CDC review, it is strongly associated with the inherited tissue type Major Histocompatibility Complex (MHC) antigen HLA-DR4 in more erosive disease [27,31,32] (most specifically DR0401 and 0404)[4, 40]. Hence family history is an important risk factor.

Risk factors: [7,22,32]

A range of environmental and genetic variables have been evaluated as potential risk factors for RA

- Hormonal exposures
- Tobacco use[33]
- Dietary components
- HLA genotype, and
- Microbial exposures

But to date no definitive risk factors for RA have been identified.

Epidemiology[26,31,32]

RA is seen throughout the world and affects all races.

Disease prevalence is similar to that of developed countries, but higher than reported from China, Indonesia, Phillippines, and Rural Africa. North Indian population is genetically closer to Caucasians (1-1.5%) [31,34] than to other ethnic groups.[35] Higher prevalence rates in pima group of Indians of Arizona[31] & in some Native American groups have (5-6%). And people from the Caribbean region & black Africans, have lower prevalence rates .[31,34]

Mahajan et al,in his study around Kashmir, in 2003, reported 23.9 % had Rheumatological problems, 24.9% had OA.[36]

The prevalence rate is 1%.[1,3,5,6,7,8,9,28.] Before the age of 45years the female, male ratio is 6:1. Women are affected three to five times as often as men. (3 - 5**:**1ratio)[7,25] suggesting a role for sex hormones. (0.5 -3.8% in women ,1.37 % in men.) [24.] The incidence of RA is six times greater in old women compared to young women.

The prevalence increases with age in 5 % of women, and 2%of men over 55 years,[31] and sex differences diminish in the older age.Onset is most frequent during the fourth and fifth decades of life[7]. With 80% of all patients developing the disease between the ages of 35 and 50 years, as shown in study by Athena Linos et al [33]. Mean ages of RA patients ranged from 50.0 to 52.2 yrs and % of female patients from 80.9% to 89.6%.It is 4 times more common in smokers than non-smokers.[37]

As per CDC [40] an estimated 1.293 million adults aged 18 and older (0.6%) had RA in 2005, down from the previous 1990 estimate of 2.1 million. The prevalence among women in 1995 was approximately double that in men (1.06% versus 0.61%).[32] This study observed almost a 2:1 ratio in prevalence for women to men.Prevalence is decreasing now.[32]

Genetic concordance in monozygotic twins is approximately 12-15%[31.] First-degree relative's prevalence rate is 2-3%[31].

Pathogenesis [26,31]

It is characterised by

- Persistent cellular activation
- Auto immunity
- Presence of immune complexes at articular and extra articular sites.

This leads to

- Chronic inflammation
- Granuloma
- Joint destruction.

Microvascular injury, thrombosis, and neovascularization with edema and infiltration by lymphocytes (CD4 Tcells), plasma cells and macrophages often collected into aggregates around small blood vessels.[26] Locally produced antibodies to tissue components and immune complexes can activate complement and generate anaphylatoxins and chemotactic factors.T cells produce cytokines such as IFN. It remains unclear whether the persistent T cell activity represents a response to a persistent exogenous antigen or to altered autoantigens such as collagen, immunoglobulin, one of the heat shock proteins, or CCP.

Effusion of synovial fluid into joint space, hypertrophy and congestion of synovial membrane and underlying connective tissue.Inflammatory granulation tissue-pannus, spreads over and under the articular cartilage with formation of lymphoid follicles resembling lymph node (granuloma), progressively erodes and destroys the bone. Adjacent muscles get inflammed due to infiltration. Immunofluorescence confirms the Rheumatoid Factor auto antibody synthesis by plasma cells in synovium and lymph node.[31]

Recent evidence suggests that antibodies may be produced against other self-antigens, such as CCP, which are generated within the synovium, and this may contribute to RA synovitis.

RA-associated autoantibodies (RF):

Throughout the last decades several autoantibody systems have been described that are associated with RA.

RF - the oldest and most widely known of these autoantibodies, is directed to the Fc part of IgG molecules.[3,6,7,16] RF can be detected in up to 80% of RA patients,[8,17] but the studies by Swedler et al, and Nehir samanchi et al shows, these antibodies are found also in several other auto immune diseases as well as in healthy individuals - 5-10 % [6,11] (especially elderly) lowering its specificity for RA.[6,11,8]

The frequency of Rheumatoid Factor in the general population

increases with age, and 10–20% of individuals > 65 years have a positive test. In addition, a number of conditions besides RA are associated with the presence of RF. These include Systemic Lupus Erythematosus, Sjogren's syndrome, chronic liver disease, Sarcoidosis, Interstitial Pulmonary Fibrosis, Infectious Mononucleosis, Hepatitis B, Tuberculosis, Leprosy, Syphilis, Subacute Bacterial Endocarditis, Visceral Leishmaniasis, Schistosomiasis, and Malaria.Due to the above factors RF's diagnostic specificity has lowered.

So the presence of Rheumatoid Factor does not establish the diagnosis of RA. Therefore the RFactor test alone is not useful as a screening procedure. Both anti-CCP antibody and RF are recommended screening tests for Rheumatoid Arthritis.[3]

A negative RF does not rule out RA, rather the arthritis is known as ***seronegative***. This is the case in about 15% of patients. Up to 20% of new patients with RA are seronegative in the first year, when early diagnosis is essential to prevent erosive joint disease[38]. Previous studies show, around 40% of RF-seronegative patients appear to be anti-CCP-positive. This substantiates the additional diagnostic potential of CCP.[8,4,16,39]

RA-Specific Autoantibodies

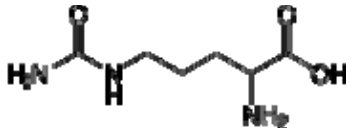
The autoantibody systems with the greatest clinical potential for

RA are the antibodies directed to citrulline containing epitopes[6,7]

The presence of autoantibodies against citrullinated proteins in RA patients was first described in the mid-seventies when the biochemical basis of antibody reactivity against **keratin** and **filaggrin** was investigated, APF (antiperinuclearantibodies) - 1964, AKA (antikeratin antibodies) -1979, though specific because of technical difficulties not widely used. Recently Anti Cyclic citrullinated peptides (**Anti CCP**) detected with high sensitivity and specificity for RA.[6]

Citrulline:

During inflammation, the enzyme **peptidylarginine deiminase** incorporates citrulline into proteins, the enzymatic conversion of peptidyl-arginine to peptidyl-citrulline.



Citrulline has been named after the Latin word for watermelon,*Citrullus vulgaris*, whichcontains large amounts of this amino acid.

Citrulline is a non standard amino acid, as it is not incorporated into proteins during protein synthesis. “Cyclic citrullinated peptide" is also known as "CCP". It is a cyclic peptide, can incorporate the amino acid citrulline and can be generated via post

translational modification of arginine residues by peptidyl arginine deiminase (PAD) enzymes.[5,6,7,10,16,21]

PAD enzymes are present in the inflamed synovium and that their activity is regulated at the transcriptional and translational levels In addition, these enzymes require relatively high Ca^2+ concentrations, about 100 times higher than normally present in the cytosol of a living cell.[6,7]

Conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom in the side chain of this amino acid, and is associated with the loss of a positive charge (at neutral pH). The neutral oxygen group of the citrulline residue is the part that is recognized by the autoantibodies.

Interestingly, citrullination occurs primarily in dying cells. Indeed, during inflammation, when many cells die by apoptosis or necrosis, in the inflamed synovia of RA and non-RA patients. Paradoxically, the presence of citrullinated proteins in most cases does not lead to the generation of anticitrullinated protein antibodies. This phenomenon might be related to the genetic background of the patient. It has been known for some time that there is a rather strong correlation between RA and certain HLA-DR alleles, particularly HLADRB1- 0401 and HLA-DRB1- 0404)[6.]

Citrulline antibody: [40,41]

It is an antibody (an immune protein) directed against a circular peptide (a ring of amino acids) containing an unusual ("non-standard") amino acid called citrulline that is not normally present in peptides or proteins.

It is frequently detected in RA is patients. Recently, these antibodies have turned up as powerful biomarkers, which are accepted as a major diagnostic tool in diagnosing RA in a very early stage of disease.

Clinical Features [26,30,31]

Complaints of pain over the affected joints, symmetrical joint involvement and morning stiffness, with characteristic changes of the **hand** including

- Radial deviation at the wrist with ulnar deviation of the digits, often with palmar subluxation of the proximal phalanges **("Z" deformity).**
- Hyper extension of the proximal interphalangeal joints, with compensatory flexion of the distal interphalangeal joints **(swan-neck deformity).**
- Flexion contracture of the proximal interphalangeal joints and extension of the distal interphalangeal joints

(boutonnière deformity), and

- Hyperextension of the first interphalangeal joint and flexion of the first metacarpophalangeal joint with a consequent loss of thumb mobility and pinch.

Typical **joint** changes may also develop in the feet, including

- Eversion at the hind foot (subtalar joint)
- Plantar subluxation of the metatarsal heads
- Widening of the forefoot
- Hallux valgus, and lateral deviation and dorsal subluxation of the toes.

Later, disability is more related to structural damage to articular structures.

Disability

- Daily living activities are impaired in most individuals.
- After 5 years of disease, approximately 33% of sufferers will not be working.
- After 10 years, approximately half will have substantial functional disability.

Prognostic factors [4,32,31]

Poor prognostic factors include[4]

- Persistent synovitis
- Early erosive disease
- Extra-articular findings (including subcutaneous rheumatoid nodules)
- Positive serum RF findings
- Positive serum anti-CCP autoantibodies
- Carriership of HLA-DR4 "Shared Epitope" alleles
- Family history of RA
- Poor functional status
- Socioeconomic factors
- Elevated acute phase response ESR
- Elevated C-reactive protein [CRP]
- And increased clinical severity

Mortality[32]

Estimates of the life-shortening effect of RA vary. Most sources cite a lifespan reduction of 5 to 10 years.

Laboratory diagnosis:

Currently, the classification of RA relies mainly on the criteria

described by the American College of Rheumatology (ACR). [1,5,7,14,15] These criteria, originally formulated 50 years ago and last adjusted in 1987[25] are based mainly on clinical parameters and a single serological test RF. Since these parameters are often only sufficiently fulfilled when the damaging effects of the inflammatory process are already in progress, this set of criteria is not very suitable for the early diagnosis of RA .In the ACR criteria for RA serologic support is restricted to the determination of Rheumatoid Factor (RF).[42] RF(1937) has been widely used as a screening test for patients with arthritis[3]. However, its diagnostic specificity for RA is poor, since RF is also found in many other rheumatic and non rheumatic diseases, infectious conditions and even in a noticeable proportion of normal healthy subjects, particularly in ageing individuals[6,11,25] .The resulting lack of specificity for RA can lead to wrong diagnosis and unwanted treatment.

However, especially during the first few months of the disease, the 1987 revised criteria of the ACR are rarely met.About one-third of the patients with persistent arthritis do not fulfill the classification criteria,so it is often difficult to diagnose RA in the very early stages of the disease [25].

On the other hand, numerous studies have shown that substantial irreversible joint damage occurs within the first 2 years .In

many cases, irreversible damage of the joint cartilage has already occurred by the time laboratory and radiological parameters have confirmed the clinical diagnosis RA.So it is important to start treatment earlier. Current therapeutic strategies in RA are with increasingly aggressive regimens.Therefore, diagnostic tests with high-specificity are desirable for deciding on the optimal treatment[25.]

Missed diagnosis of Rheumatoid Arthritis (RA) has major medical and cost implications, since this set of ACR criteria is not very suitable for the early diagnosis of RA.A specific and sensitive (serological) marker, which is present very early in the disease, is needed which should ideally be able to predict the erosive or nonerosive progression of the disease[25.]

The shortcomings of the RF test have kept the search for more specific RA markers alive. Most autoantibody systems described during recent decades, Anti Perinuclear Factor (APF) – 1964, and Anti Keratin Antibody (AKA) tests – 1979 [3,7,11] have failed to mature into mainstream tests for RA because of low sensitivity, and technical inconvenience [6,3].

Another group of autoantibodies have recently been detected in serum of patients with RA, in which patients develop antibodies to modified (citrullinated) arginine residues, and this has

resulted in the development of the anti-cyclic citrullinated peptide antibodies (anti CCP). The only antibody system that combines good sensitivity with superior specificity for RA is that targeting citrullinated epitopes.

Antibodies to CCP (anti-CCP) can also be used to evaluate patients with RA. Although these antibodies are most commonly found in Rheumatoid Factor–positive patients, on occasion they can be detected in the absence of Rheumatoid Factor. In addition, the anti-CCP test has a similar sensitivity and a better specificity for RA than R F[34] When the citrulline antibody is detected in a patient's blood, there is 90-95% likelihood that the patient has Rheumatoid Arthritis[40.]

The presence of anti-CCP is associated with the aggressive nature of the disease, with a tendency for developing bone erosions. It is most useful to confirm the diagnosis and establish a likely prognosis. A role in differential diagnosis comes from patients with erosive systemic lupus erythematosus (SLE) other arthritis and excludes them.The combined presence of RF positivity and anti-CCP positivity has 99.5% specificity for RA [43.]

The value of anti-CCP antibodies and RF for predicting the outcome of RA, clinical signs of disease activity, and the

severity of radiologic joint damage has been investigated recently. The studies by van Jaarsveld et al, Kroot et al, and Meyer et al, all support the thesis that RA patients, positive for CCP, develop significanly more radiological damage than CCP-negative patients.Lately, Visser et al, assessed a clinical prediction model in early RA patients for the three forms of arthritis out come: self-limiting, persistent nonerosive and persistent erosive arthritis in which CCP was strongly associated with erosive arthritis, more than RF.

"**RA passport"** should contain

- **Serological data** (RF, OR and anti-CCP2)
- **Genetic data** (e.g. HLA-DR4) and
- **Several clinical parameters,**

as suggested by Visser and coworkers [17].

Investigations [30, 31]

1.Neutrophils

Increase in systemic vasculitis, sepsis & decrease in rheumatoid arthritis, lupus & drugs.

2.Lymphocytes

Increase in infections, decrease in lupus, and in those with corticosteroid therapy.

3.Eosinophils

Increase in systemic vasculitis, decrease in those with corticosteroid therapy.

4.Platelets

Increase in inflammation, decrease in lupus & drugs.

5. CRP[41]

CRP is also known as **C - reactive protein**. It is an acute phase protein present in hepatocytes and plays an important role to the body's response to inflammation. [18,11,21,22.]

For the below mentioned reason the protein is called as 'C'reactive protein - Gram positive, somatic portion of pneumococci contains species specific carbohydrate known as 'C' substance (antigen), which forms precitipitate with a protein (β globulin) in the blood in acute inflammatory conditions. Its production is stimulated by

1. Bacterial infection
2. Inflammation
3. Malignancy
4. Tissue destruction.

Rapid Serum assays are useful in early detection of acute inflammation[17,22,23,25.] Increase of CRP denotes - onset of inflammation. Rapid decrease occurs when infection subsides. It is a close mirror of degree of inflammation.

CRP detection was done by method of latex agglutination test.It must be noted that even in known cases of inflammatory disease, such as RA and lupus, a low CRP level is possible, and is not indicative of no inflammation. CRP appears and disappears more quickly. Therefore, CRP level may drop to normal following successful treatment. CRP test is also used to assess the effectiveness of a specific arthritis treatment and monitor periods of disease flareup. Its value is as a general indicator of response to treatment and not specific to rheumatoid arthritis.

Another blood test often ordered in conjunction with CRP is known as ESR.Both CRP and ESR give similar information about non-specific inflammation.

6. <u>ESR</u> [23,25,22,6] Erythrocyte Sedimentation Rate:

It is an indirect measure of the **APR –** Acute Phase Reaction.It chan ges from very low to very high levels, mirrors the degree of inflammation, rise rapidly at onset, & fall as inflammation subsides.So it is a direct measure of the APR. Erythrocytes donot clump normally

due to their repellent electrostatic negative charge or zeta potential greater than the attractant electrical charge of the plasma constituents. In APR altered plasma protein concentrations- fibrinogen, increase in dielectric constant overwhelm the zeta potential & allow erythrocytes to clump (rouleaux).So it sediment fastly, which is measured as ESR.

ESR doesn't appear and disappear more quickly and may remain elevated for a longer period. ESR increases in APR,Increased level of immunoglobulin,Myeloproliferative disorders & Autoimmune isorder.

7. RF

RF is an autoantibody directed to the Fc part of IgG molecules.[6,3,7,16]. These antibodies are found in RA, in several other auto immune diseases and as well as in healthy individuals - 5-10 % [6,11] (in elderly people). This lowers its specificity.

RF is detected by the method of latex agglutination test.

8.Anti CCP Test

Antibody to cyclic citrullinated peptide,detected by ELISA technique.

Treatment: [26,30,31]

Chemically synthesised **DMARDs -** (disease modifying anti rheumatic drugs)

- Azathioprine
- Ciclosporin(cyclosporin A)
- D- penicillamine
- Gold salts
- Hydroxychloroquine
- Leflunomide
- Methotrexate (MTX)
- Minocycline

- Sulphasalazine(ssz)

Low dosages of daily cortisone (e.g., prednisone)are added to a proper specific anti-rheumatic treatment.

2. Cytotoxic drug

- Cyclophosphamide

3. Biological agent

- Tumor necrosis factor alpha (tnfα) blockers etanercept, infliximab, adalimumab.
- Interleukin 1 (IL-1) blockers
- Monoclonal antibodies against B cells - rituximab
- T cell costimulation blocker
- Interleukin 6 (IL-6) blockers

4. Anti inflammatory drugs

- Glucocorticoids
- Non-steroidal anti-inflammatory drug

5. Analgesics

Paracetamol, Opiates, Diproqualone and Lidoquine

6. Also includes **rest and physical activity.**

Osteoarthritis [30,31]

Osteoarthritis (OA) is the most common type of arthritis.

Definition

OA is joint failure, in which all structures of the joint have undergone pathologic change, often in concert. Pathologically it is defined as a condition of synovial joints characterised by focal loss of hyaline articular cartilage with proliferation of new bone and remodelling of joint contour. It involves both small and large joints.

Prevalence

OA is uncommon in adults under age 40 and highly prevalent in those over age 60. But steady rise from age 30, such that by 65, 80% will develop symptoms.Prevalence is high especially in the elderly. The Prevalence is increasing nowadays.

Risk factors:

- **Constitutional susceptibility**

  Ageing, Heredity, Gender, Hormonal status &Obesity.

- **Mechanical factors**

Trauma & Joint shape alignment usage - Occupational or recreational.

Commonly affected joints

- Hip & Knee joints are commonly affected.
- Also cervical and lumbo sacral spine.
- In the hands, the distal and proximal interphalangeal joints and the base of the thumb are often affected.
- First metatarsal phalangeal joint (MTP).
- Usually spared are the wrist, elbow, and ankle.

Pathology & Etiology

- Mechanical
- Metabolic
- Genetic or
- Constitutional insults damage the synovial joint

Panarticular involvement is present. Cartilage initially shows surface fibrillation irregularity and focal erosions develop outgrowths

of new cartilage and with neurovascular invasion from the bone, this cartilage ossifies. **Osteophytes** are an important radiographic hallmark of OA. The capsule which stretches, becomes edematous, and can become fibrotic.

Clinical Features

Joint pain from OA is activity-related. Pain comes on either during or just after joint use and then gradually resolves and relieved by rest. In knees, buckling may occur. Heberden's node are present.

Diagnosis

Diagnosis based upon Structural abnormalities or on the symptoms they evoke.

- Symptoms - usually joint pain, determine disability.
- Structural abnormalities - Cartilage loss and osteophytes.

Examination of the synovial fluid is often more helpful diagnostically than an x-ray.

Treatment

Nonpharmacotherapy

Since OA is a mechanically driven disease, ways of lessening focal load across the joint include

(1) Avoiding activities that overload the joint,

(2) Improving the strength and conditioning of muscles

that bridge the joint.

Pharmacotherapy

- Acetaminophen,
- Non steroidal Anti-Inflammatory Drugs (NSAIDs), and
- COX-2 Inhibitors.
- Intra articular Injections:
    - Glucocorticoids and Hyaluronic Acid, and
- Surgery.

Systemic lupus erythematosus (SLE) [44,30,31]

Definition

SLE is an autoimmune disease in which organs and cells undergo damage mediated by tissue-binding autoantibodies and immune complexes.

Prevalence

90% of patients are women of child-bearing age group. Both sexes, all ages, and all ethnic groups are susceptible. Prevalence in the United States is 15–50 per 100,000; the highest prevalence among ethnic groups studied is in African Americans.

Etiology and Pathogenesis

Interactions between susceptibility genes and environmental factors result in abnormal immune responses. Cell antigens,

autoantibodies, and immune complexes persist for prolonged periods of time, allowing inflammation and disease to develop Anti nuclear antibodies, and Anti ds DNA. SLE is a multigenic disease.

Diagnostic Criteria

- Malar rash
- Discoid rash
- Photosensitivity
- Oral ulcers
- Arthritis
- Renal disorder
- Haematological disorder and Anti nuclear antibodies.

Treatment

- NSAID
- Topical sunscreen
- Methotrexate
- Glucocorticoids
- Methyl prednisolone
- Cyclophosphamide
- Azathioprine
- Hydroxychloroquine.

MATERIALS & METHODS

Study Design:

This is a combined Cross Sectional, Case Control, and Prospective study.

Study Place and Study Period:

The present study was conducted at the Microbiology Diagnostic Laboratory, Coimbatore Medical College Hospital, Coimbatore. This study period extended from June 2009 to May 2010.

Study Subjects:

The total number of subjects in this study for evaluation were 250, which included both males and females.The study subjects were selected according to the inclusion criteria's mentioned below, from the patients who attended the Out Patient Clinics at the Rheumatology and Orthopaedics Department and Healthy Blood Donors who attended the Blood bank, Coimbatore Medical College Hospital, Coimbatore.

The study subjects, in both genders were divided into five groups. Each group include 50 patients. Four groups based upon the clinical conditions and fifth group, healthy individuals (blood donors) as control, as follows.

Group I:

Rheumatoid Arthritis (RA) - 50 patients.

Group II:

Early Synovitis (ES) - 50 patients.

Group III:

Connective Tissue Disorders including Systemic Lupus Erythematosus (SLE) - 50 patients.

Group IV:

Osteo Arthritis (OA) - 50 patients, and

Group V:

Healthy Blood Donors (HBD) - 50 patients.

INCLUSION CRITERIA:

Early Synovitis:

- Patients with complaints of joint pain. (Synovitis - joint pain, blotted feeling, redness, fever for > 6wks & < 12 months duration.)[5,18,19]
- Joint pain with no h/o injury or sepsis[1]
- Without any bony deformity.
- Not already on treatment for RA.

(Inclusion[19]of patients fulfiling ≥ 2 clinical and ≥1 laboratory criterion and duration of symptoms ≤ 12 weeks.

Clinical:

Absence of trauma ,Joint swelling in at least 1 joint, Joint pain in at

least 1 joint, Morning stiffness > 60 minutes.

Laboratory:

Positive Rheumatoid Factor, ESR > 20 mm/h & CRP > 5 mg/L

CTD (SLE):

- Arthritis along with vasculitis
- Photosensitivity &
- Malar rash

OA:

- Joint pain with no h/o injury or sepsis.
- Brief morning stiffness (< 30mts)
- Localised pain. Aggravated by use &relieved by rest.
- No symmetrical involvement of joints.

Healthy Blood Donors:

Healthy persons without any infection, or any communicable diseases.

RA:

RA patients were selected according to the ACR (American College of Rheumatology) criteria[14.]Criteria a–d must be present for at least 6weeks.To diagnose as RA any 4 of the below should be present.

a.Morning stiffness	Stiffness in and around the joints lasting 1 hr before maximal improvement
b.Arthritis of three or > joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (proximal interphalangeal, metacarpophalangeal, wrist, elbow, knee, ankle, and metatarso phalangeal joints)
c.Arthritis of hand joints	Arthritis of wrist, metacarpo phalangeal joint, or proximal interphalangeal joint.
d.Symmetric arthritis	Simultaneous involvement of the same joint areas on both sides of the body.
e.Rheumatoid nodules	Subcutaneous nodules over bony prominences, extensor surfaces, or juxtaarticular regions observed by a physician
f.Serum RF	Positive serum Rheumatoid Factor
g.Radiographic changes	Typical changes of RA on postero anterior hand and wrist radiographs that must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints.

EXCLUSION CRITERIA:

RA: If does not fit in to the ACR criteria.

Early Synovitis:

- Complaints of joint pain (synovitis< 6wks & > 12months)
- Joint pain with h/o injury or sepsis.[5]
- With bone and joint deformity.
- Already on treatment for RA.

CTD: Bony deformity without vasculitis.

OA : Joint pain with h/o injury or sepsis & Joint pain at rest.

HBD: Persons with any infection, or communicable diseases.

Sample for study:

Blood was taken after getting oral consent from Rheumatoid arthritis (RA), Early Synovitis (ES) & Connective Tissue Disorders including Systemic Lupus Erythematosus (SLE) patients from Rheumatology out patient clinics, and from Osteo Arthritis (OA) patients who attended the Orthopaedics out patient clinics,and also from the Healthy Blood Donors (HBD) who attended the Blood Bank. Serum was separated from the blood and used as the sample.

Collection of the Blood:

5 ml of Venous Blood was collected aseptically, divided into two portions.Three ml in a test tube with EDTA – for testing TC,DC, and ESR. And two ml in plain test tube (without EDTA) .Serum was separated and stored at – 20 ^{0}C for testing CRP,RF and Anti CCP.

ESR [22,23 25]

Method : Westergrens method:

In a 200 mm capillary tube, patient's blood was taken and kept stand still in erect posture. Results were read after one hour from the top column of the tube.Normal value < 5-10 mm

CRP [21,22,23,25]

Type of the Test:

Latex Agglutination test [14] (rapid slide test - qualitative)

Principle of the test:

The test is based upon the immunologic reaction between CRP Antigen, and latex particles coated with mono specific goat antihuman CRP Antibodies, if positive indicated by a distinctly visible agglutination of latex particles.if negative a smooth suspension is formed.

Contents of the kit:

1. Latex reagent
2. Positive control
3. Negative control
4. Disposable slides
5. Disposable applicator sticks
6. Disposable plastic droppers& teats

Storage: 2 - 8^0 c.

Specimen; Serum (separated from blood)

Do's & Don't's:

- All the reagents were brought to room temperature before testing.
- Icteric, lipaemic or haemolysed samples were discarded.

Procedure :

- Pipetted one drop of Positive control, Negative control, and serum on the slide.
- Added one drop of reagent on all sample and controls.
- Mixed, and tilted the slide to and fro for 2 minutes, and read the results.

Interpretation of Results:

Interpretation	**Observation**
Distinct coarse agglutination	Within 0.5 mts(30 sec) - strong positive
Fine agglutination	After full 2 mts - weak positive
Smooth suspension	Negative

Distinct agglutination indicates CRP content > than 6 mg /litre in undiluted serum specimen.

RHEUMATOID FACTOR [14]

Method of Detection: **Latex Agglutination test:** [15]

Principle:

Human globulin IgG is coated with latex particles.

Serum was mixed with latex reagent. Agglutination appears if the sample is positive for Rheumatoid factor. No agglutination appears and remains as smooth suspension, if the sample is negative forRF.(Latex coated with IgG + serum = IgG antibody binds with IgG & cause latex particles to flocculate.) It is a rapid qualitative slide test.

Contents of the kit:

1. Latex reagent
2. Positive control
3. Negative control
4. Disposable slides
5. Disposable applicator sticks
6. Disposable plastic droppers& teats

Storage: 2 - 8^0c

Specimen: Serum, separated from blood.

Do's & Don'ts:

- All the reagents were brought to room temperature before testing.
- Icteric, lipaemic or haemolysed samples were discarded.

Procedure :

- Pipetted one drop of Positive control, Negative control, and serum on the slide.
- Added one drop of reagent on all samples and controls.
- Mixed, tilted slide to and fro for 2 minutes, and read the results.

Interpretation of results:

Distinct agglutination indicates RF content > than 20 IU RF/ml in undiluted serum.

Interpretation	Observation
Distinct coarse agglutination	Within 0.5 mts - strong positive
Fine agglutination	After full 2 mts - weak positive
Smooth suspension	Negative

ANTI-CCP ANTIBODY

Name of the Kit Used:

Anti-CCP antibody was studied with ELISA method[11] by using GENESIS CPA (citrullinated protein antibodies) ELISA kit for detection of Rheumatoid arthritis specific Ig G antibodies to citrullinated protein.

Principle of the Test :

- Diluted serum samples are incubated with Recombinant Citrullinated rat flaggrin immobilised on micro titre wells.
- After washing away unbound components, Rabbit antihuman IgG conjugated to horseradish peroxidase is added to the wells and this binds to surface bound antibodies in the second incubation.
- Unbound conjugate is removed by washing and a solution containing 3,3', 5, 5"- tetramethylbenzidine and enzyme substrates added to trace specific antibody binding.
- Addition of the stop solution terminates the reaction and provides the appropriate pH for colour development.
- The optical densities of the standards control and samples are measured using a microplate reader at 450 nm. Optical density is directly propotional to the concentration of citrullinated protein antibodies in the sample.

Materials used for the test:

1. Microplate – 96 wells
2. Sample diluent
3. Wash buffer
4. Conjugate

5. TMB (tetramethylbenzidine) substrate

6. Stop solution

7. Standards

8. Positive control

9. Negative control

Storage of kit: 2-8^{0}C

Preparation of Reagent:

1. Diluted the sample diluent to 1:14 ratios in distilled water

2. Diluted the wash buffer to 1:9 ratios in distilled water. (50 ml wash buffer to 450ml of Distilled water)

Procedure:

- Diluted patients sample to 1:100 ratios in diluted sample diluent (10 µl serum +1ml Diluent).
- Assembled the number of strips required for the assay.
- Dispensed 100 µl of standard, the negative control, positive control and diluted patient's sample into the appropriate wells.
- Incubated for 30 minutes at room temperature.
- Decanted the contents and washed the wells 3 times with wash buffer using automated washer

and didn't allowed the wells to dry.

- Dispensed 100 µl of conjugate into each well.
- Incubated for 30 minutes at room temperature.
- Decanted the contents and washed the wells 4 times with wash buffer using automated washer and didn't allowed the wells to dry.
- Dispensed 100 µl of TMB substrate into each well.
- Incubated the plates for 10 minutes.
- Added 100 µl of Stop solution to each well.
- Took readings of the optical density of each well by using Microplate Reader within 10 minutes. (620nm reference filter is used.)

Results:

- Samples with OD ≥ OD (optical density) of 6.25 U/ml Standard are positive.
- Samples with OD ≤ OD (optical density) of 6.25 U/ml Standard are Negative.

The results obtained were tabulated and analyzed.

RESULTS

The present study was conducted with the patients who attended the Out Patient Clinics at the Rheumatology and Orthopaedics Department of Coimbatore Medical College Hospital, Coimbatore. 200 subjects (including both males and females) were recruited for the study. The subjects were categorized into four groups – RA, ES, CTD, and OA (each group 50) based upon the clinical conditions, and 50 Healthy Blood Donors who attended the Blood bank, Coimbatore Medical College Hospital, Coimbatore, were selected as control and categorized into fifth group.

In the present study which was carried out on patients having various arthritic diseases,

Age wise distribution of them were listed in **Table and chart – 1.**

Sex wise distribution of various arthritic diseases - M/F ratio – RA - 1:2.33, ES –1:2.57, CTD – 1:2.12, OA – 1:2.84 was tabulated in **Table and chart – 2.**

Age / sex distribution of various arthritic diseases were listed in **Table and chart – 3.**

Anti-CCP & RF test results in various arthritic diseases & HBD-

Anti-CCP was positive in 39 of 50 RA patients (78%),

14 of 50 (28%) Early Synovitis cases, 2 of 50 (4%) CTD(SLE) cases, ,and none were positive among (50) OA and (50) HBD, and lists of **RF** test results were 38 of 50 RA patients (76%) , 21 of 50 (42%) - ES patients, 11 of 50 (22%) - CTD including SLE patients , 6 of 50 (12%) - OA patients and 4 of 50 (8%) - of HBD were positive for RF were tabulated as shown in **Table and Chart – 4.**

Sensitivity & Specificity of RF test in RA – (RF Positive in RA -38, NON RA 17) was 76% & 86% respectively was tabulated in **Table and Chart – 5.**

The sensitivity & Specificity for Anti CCP test for RA was 78% & 98.6 % respectively was calculated in **Table and Chart - 6**.

Early synovitis was not included for the calculation of specificity in both RF and Anti CCP tests because it is an undifferentiated arthritis, can later convert either into RA or non RA and cannot be categorized into a single group.

The distribution of positivity's of **Anti CCP and / or RF** on the groups were –

In **RA** - RF +ve and Anti-CCP +ve (both +ve) were - 33 (66%), RF -ve and Anti-CCP +ve were 6 (12%) , RF+ve and Anti-CCP -ve were 5 (10%), RF -ve and Anti-CCP –ve (both -ve) were 6 (12%) were listed in the **Table and Chart - 7.**

In CTD (**SLE)** RF +ve and Anti-CCP +ve (both +ve) were - 2 (4%), RF -ve and Anti-CCP +ve were nil , RF+ve and Anti-CCP -ve were 9 (18%), RF -ve and Anti-CCP -ve (both -ve) were 39 (78%) were listed in the **Table and Chart - 7.**

In **OA -** RF +ve and Anti-CCP +ve (both +ve) were - nil, RF -ve and Anti-CCP +ve were nil, RF+ ve and Anti-CCP -ve were 6 (12%), RF -ve and Anti-CCP -ve (both -ve) were 44 (88%) were listed in the **Table and Chart - 7.**

Also in **HBD** - RF +ve and Anti-CCP +ve(both +ve) were - nil, RF -ve and Anti-CCP +ve were nil, RF+ve and Anti-CCP -ve were 4 (8%), RF -ve and Anti-CCP -ve (both -ve) were 46 (92%) were listed in the **Table and Chart - 7.**

In 50% of Sero Negative RA patients Anti CCP Test was positive (among 12 RF Negative cases 6 were positive) as listed in **Table and Chart – 8.**

The Anti CCP & RF test results for **ES** (Anti CCP was positive in 14 & RF+ve in 21) were tabulated in **Table and Chart – 9.**

Sensitivity, Specificity, PPV (positive Predictive value) & NPV (Negative Predictive value) of

Anti CCP (78%, 98.6%, 95.1% & 93%)

RF (76%, 86%, 64.4% & 91.4%)

Anti CCP and RF (66%, 98.6%, 94.2% & 89.7%)

Anti CCP / RF (88%,86%,67.7% & 95.5%) Tests were listed in **Table and Chart – 10.**

From the above results, of all the above different arthritic diseases, *Anti CCP is more sensitive and more specific for RA and RF is almost equally sensitive but less specific for RA.*

TABLE – 1

Age wise Distribution of various Arthritic Diseases

Category	Age 20-30yrs	Age31-40yrs	Age41-50yrs	Age 51-60yrs
RA 50)	7	12	14	17
ES (50)	10	16	16	8
CTD (50)	8	18	18	6
OA (50)	2	13	24	11

TABLE – 2

Sex wise Distribution of various Arthritic Diseases

CATEGORY	M	%	F	%	M/F Ratio
RA (50)	15	30	35	70	1: 2.33
ES (50)	14	28	36	72	1: 2.57
CTD (50)	16	32	34	68	1: 2.12
OA (50)	13	26	37	74	1: 2.84

TABLE –3

Age/Sex Distribution of various arthritic groups

CATEGORY	Age 20-30yrs		Age 31-40yrs		Age 41-50yrs		Age 51-60yrs	
	M	F	M	F	M	F	M	F
RA (50)	2	5	5	7	4	10	4	13
ES (50)	2	8	4	12	5	11	3	5
CTD (50)	2	6	6	12	6	12	2	4
OA (50)	0	2	3	10	6	18	4	7

TABLE – 4

Anti-CCP & RF Results in various ArthriticDiseases & HBD

CATEGORY	Anti CCP Positivity	RF Positivity
RA (50)	39 (78%)	38 (76%)
ES (50)	14 (28%)	21 (42%)
CTD (50)	2 (4%)	11 (22%)
OA (50)	0	6 (12%)
HBD (50)	0	4 (8%)

TABLE - 5

Sensitivity & Specificity of RF Test in RA

RF	Positive	Negative	Total
RA(50)	38	12	50
Non RA (150) SLE+ OA + HBD	21	129	150
Total	59	141	200

Sensitivity 76% Specificity 86 %

ES not included because of undifferentiated state.

TABLE- 6

Sensitivity & Specificity of Anti CCP Test in RA

TEST	Anti ccp Positive	Anti ccp Negative	Total
RA(50)	39	11	50
Non RA(150) SLE+ OA + HBD	2	148	150
Total	41	159	200

Sensitivity: 78% Specificity: 98.6 %

ES not included because of undifferentiated state.

TABLE-7

Distribution of Positivities of Anti CCP and /or RF on the groups

	In RA patients n = (50)	SLE n = 50	OA n = 50	Control groups (HBD) n = 50
Anti CCP positive	39	2	0	0
RF positive	33 (66%)	2 (4%)	0	0
RF negative	6 (12%)	0	0	0
Anti CCP negative	11	48	50	50
RF positive	5 (10%)	9 (18%)	6 (12%)	4 (8%)
RF negative	6 (12%)	39(78%)	44(88%)	46 (92%)

TABLE – 8

Use of Anti CCP Test in Sero Negative RA patients

Anti CCP	Sero Negative (RF-ve) RA(12)
Positive	6
Negative	6

50 % of seronegative arthritis cases are positive for anti CCP.

TABLE – 9

Anti CCP& RF Test Results in ES Patients

TEST	Positive in ES (50)
Anti CCP	14
RF	21

To be followed up further to confirm the convertability to established RA.

TABLE-10

Sensitivity, Specificity, PPV & NPV of Anti CCP & RF tests

Tests	Sensitivity %	Specificity %	PPVTest %	NPV test %
Anti CCP	78	98.6	95.1	93
RF	76	86	64.4	91.4
ANTI CCP & RF	66	98.6	94.2	89.7
ANTI CCP /RF	88	86	67.7	95.5

DISCUSSION

RA is associated with only a few specific auto antibodies, including APF, AKA and anti-CCP and several less specific auto antibodies including RF[4]. Despite a well-documented lack of specificity, RF continues to be a serological test for RA because of its inclusion in ACR criteria. However to date no single autoantibody has demonstrated adequate positive diagnostic value to form the basis of clinical decisions. So auto antibodies present in RA, Early Synovitis, connective tissue disorders including SLE and OA have been evaluated in the present study, mainly for their diagnostic value.

The modern trend in management of RA is to start the treatment as early as possible, based on the concept that early control of inflammation results in reduced joint damage. If undiagnosed the patients will not get treatment at an early date or if wrongly diagnosed as RA , will be unnecessarily treated with Anti Rheumatic drugs. It is therefore important to differentiate between RA and other forms of arthritis early, before the onset of symptoms.

In recent research by Schellekens GA,[17] he has observed the diagnostic significance of a novel RA specific autoantibody, determined by ELISA using synthetic peptides containing citrulline. The present study was done to determine the sensitivity and specificity

of the RF and anti CCP antibodies, alone or in combination in relation to RA & other arthritis.

In the present study which was carried out on patients having different Arthritic diseases, Anti CCP test results suggest the significance of anti CCP positivity in diagnosis of RA and differentiating RA from other arthritis. To minimize the errors patients were selected according to the inclusion criteria's.

For specificity calculation of RA, other arthritis and control groups were included, but ES was not included because it is in an undifferentiated form (not confined to any single entity, which may later convert into RA or other form of arthritis).

Of all the above different Rheumatological diseases, Anti CCP was more sensitive and more specific for RA, whereas RF was almost equally sensitive but less specific than Anti CCP for RA, because it is positive in other arthritic diseases and healthy persons also.

The results were in agreement with other studies in which the Anti CCP was positive in RA, ES and CTD including SLE, but the RF was non specific and present in all groups including control.

Age/Sex distribution of various Arthritic diseases:

Age :

In RA age group 51-60 years were more affected than other groups.

In age group31-40yrs and 41-50yrs ES & CTD were more common. OA was very common in age group 41-50yrs .The incidence of all types of arthritis is very low in age group of 20-30 years.

Sex

M/F ratio - in **RA** - 1: 2.33, **ES** –1**:** 2**.**57, **Connective tissue Disorder** (SLE) –1**:** 2**.**12, and in **OA** was -1**:** 2**.**84. Males are less affected than the females in all arthritic diseases.

Sensitivity of RF in RA:

On analyzing the sensitivity of RF, the present study shows 76% sensitivity in RA patients. This goes in parallel with the study by **Lee and Schur et al** [24] who found a sensitivity of 71.6% for RF.

The findings of the present study is comparable with the findings of the previous studies by **Ulrich Sauerland et al** [24] with 69.7% sensitivity, **Raphaela Goldbach-Mansky** et al [45] with 66% sensitivity, But in the studies by **Munevver Serdaroflu et al** [25,] he observed 65% sensitivity ,**Sibel Altun et al**[5] observed 60% sensitivity, **K.P. Machold et al**[19] 55% sensitivity,**Nehir Samanci et al** [11] 44.8% sensitivity, J**. van Aken et al** [12] 41% sensitivity . Sensitivity for RF was higher in the present study than the previous studies**.** This discrepancy may be due to method of selection of cases, and type of kits used for testing.

In contrast a study by **Dubucquoi *et al.*[24]** showed 94% sensitivity for RFwhich is higher than the present study.The controversy may be due to low sample size, and short period of study.

Specificity of RF

In the present study specificity of RF was 86%, which is in consistent with the study by **Sibel Altun et al[5]** which showed the specificity of RF as 86.4%. But a study by **Lee and Schur[24]** observed specificity of 80.3% which is lower than the present study.

In **Dubucquoi *et al* [24]** study the specificity of RF was 53% only. The indifference may be due to small sample size because the study was carried out as a cross sectional study which included different groups, and also may be due to short duration of study.

Sensitivity of Anti CCP

The sensitivity of **Anti CCP** is 78% in the present study. This result was similar to the previous studies by **Dubucquoi and colleagues[16]** which showed 77% sensitivity for ACCP, **Ulrich Sauerland et al** [24] study showed 74% sensitivity and **Sibel Altun et al** [5] observed 70% sensitivity . **Dubucquoi *et al.*[24]** observed a sensitivity of 85% which was **higher** than the present study. This difference may be due to small sample size of the present study because the study was carried out as a cross sectional study including

different groups, and may also be due to short duration of study.

In the present study **Anti-CCP positivity is** 78% in RA patients which is **higher than** the studies by **K.P.Machold et al**[19] and **Bizzaro et al,** which showed only 41%positivity. **Nehir Samanci et al**[11] observed 49.0% sensitivity, and **J. van Aken et al** [12] 50% sensitivity, **Lee and Schur**[24] found 66% sensitivity, **Münevver Serdaroflu et al** [25], and **Raphaela Goldbach-Mansky et al** [45] found 50% sensitivity,and **Jensen et al** [5,23] also showed 55.4% sensitivity. The disparity may be due to the usage of different types of kits for testing (advanced kits) and small sample size.

Specificity for Anti-CCP

In the present study specificity for anti-CCP is 98.6%, which is similar to the study by **Sibel Altun et al** [5.] with 98.6% specificity. The present study goes in parallel with the previous studies by **Bizzaro et al**.[5]&**Gerard A. Schellekens et al** [8] both showing 98% specificity, and **Dmitry Karayev et al** [39] 97% specificity. **Lee and Schur**[24] & **Raphaela Goldbach-Manskyet al**[45] observed 90% specificity and **Jensen et al** [5,23] 96.7% specificity.

But the studies by **Nehir Samanci et al**[11] found 99% specificity, and **Münevver Serdaroflu et al** [25] showed 100% specificity for anti-CCP which was higher than the present study.This

discrepancy may be due to sample selection criteria's and type of kits used for testing.

Sensitivity of both RF & Anti CCP antibody

In the present study both **RF and Anti-CCP** were positive in (66%) 33 of 50 RA patients. The results are comparable with the study by **Sibel Altun et al**[5] who showed 59.3% positivity. This value is higher than the study by **Schellekens et al** [5] who found both anti CCP and RF positivity as (39%) 58of 149 RA cases. In contrast a study by **Dmitry Karayev et al** [39] showed 99.6% sensitivity.

This may be due to prompt selection of cases in the present study and using advanced type of kit.

Anti-CCP or RF Positive (any one +ve) in the present study is (88%) which is higher than the study by **Bizzaro et al** [5] who reported 31.6% positivity in (31 of the 98) RA patients.

Specificity of both Anti CCP & RF:

The present study results were similar with the study by **Sibel Altun et al**[5] who showed 98.6% specificity for both tests positive in combination.

CTD (**SLE)**

Anti CCP in CTD (SLE)

In CTD including SLE only 2 of 50 (4%) were anti-CCP

positive which is similar to the study by **Sibel Altun et al**[5] showing 1 of 25 (4%) as positive in SLE patients.In an another study by **Medivake et al**[5]**.** 3 of 231(1.2%) SLE patients were anti CCP positive. **Hoffman IE**[46]and **Ulrich sauer land** [24] study and a recent article by **Gottenberg et al**.[24] also confirmed the lesser prevalance of anti CCP in SLE.

In a study by **RDL** [10] anti CCP positivity in SLE was 9.7%. which is higher and not in agreement with the present study. In contrast **O Kasapcopur et al** [28] study showed absence of anti-CCP antibody which is also **not in agreement** with the present study. The indifference might have over come if the study was carried with large sample size, and for a period of long duration.

RF in SLE

Likewise in the present study with SLE - **RF** was **positive** in 11 of 50 (22%) which goes in parallel with the study by **Sibel Altun et al**[5] with 20% positivity.These results indicate that anti-CCP antibody assay can be a useful indicator in differentiating SLE from RA than RF test.

Other Arthritis (OA) and HBD:

The present study results showed none of the OA and Healthy Blood Donors had positive Anti-CCP values which is in

positive correlation with the study by **Sibel Altun et al**[5] who also observed that none of the OA and Healthy Blood Donors had positive anti-CCP values.

Another study by **Nehir Samanci et al** [11] showed only one as (1.2%) anti-CCP positive in the control group which is not in consistent with the present study. Absence of anti CCP antibodies in OA and HBD signifies its specificity in RA and will help in excluding them from RA.

In the present study **RF** was positive in 6 of 50 (12%) OA patients (all the 6 were anti CCP –ve) and in 4of 50 (8%) HBD, which goes in agreement with the study by **Sibel Altun et al** [5] showing (10%) RF positivity in OA patients and 10% in HBD. The results denotes the non specificity of RF because of its presence in healthy persons and other arthritic cases.

Another study by **Nehir Samanci et al** [11] shows 44.8% RF positivity in OA cases and 4.8% in controls which is higher than the present study. This disparity of the results could be due to age mis-matched controls and small sample size (due to categorization of subjects into many groups). An ideal control population would have been an age-matched normal control population.

RF –ve (sero negative):

In the present study 6 of 12 (50%) sero negative patients were positive for anti CCP, this goes in parallel with **Eric-Jan J. A. et al** [14] study which showed 43%anti CCP positivity. Similarly in a study by **Kroot EJ, and a Vallbracht et aI,**[16] 40% of seronegative patients were anti-CCP-positive which substantiates the additional diagnostic potential of Anti CCP in seronegative patients also.In another study **by Quinn et al** [18] sensitivity was 60%, which is higher rate than the present study.

Gerard A. Schellekens et al [8] study shows 35% specificity and in a **multicentre** study [18] &a study by **Munevver Serdaroflu et al** [25] the specificity was 20%.Even though the % is less, it also showed the anti CCP positivity in seronegative patients, signifies the diagnostic potential of anti CCP which will be helpful in treating the undiagnosed or missed diagnosis (seronegative) cases of RA and can prevent the post sequlae.

If positive, the anti-CCP test is an important surrogate marker especially for RF-negative RA.

ES :

In the present study 14 of the 50 ES patients were positive for Anti CCP and 21 of 50 were +ve for RF. A study by **Sibel Altun et al**[5]

showed that a strong correlation was observed between Anti-CCP and RA and in ES patients. (5 of the 18 ES patients who were positive for Anti-CCP were subsequently diagnosed to have RA. None of the remaining Anti-CCP-negative 13 patients developed RA.) Similarly Bizzaro **et al. and Visser et al**.[5] also found a correlation between Anti-CCP and RA in ES patients. **Jansen et al** [5] study revealed a sensitivity of 55.4% and a specificity of 96.7%. In **dubucquoi and colleagues**[24] study the sensitivity was 65% (at 96% specificity).

Annemarie et al, also concluded in their study that the positivity of Anti-CCP is an important indicator in the discrimination of early diagnosis of RA from undifferentiated polyarthritis. In a study by **Vangalen**[17] 83% of anti CCP positive patients,and in a study by **viteuco et al** 90% of anti CCP positive patients, at base line fulfilled the ACR criteria after 1 year follow up.in **Vangalen study** 93% fulfilled the ACR criteria after 3 years followup.

The above studies signifies that the Anti CCP is of more value in early diagnosis of Rheumatoid arthritis especially in ES patients even before the symptoms appear. So anti CCP positivity (biomarker) in ES helps as an early predictor in diagnosing (future) RA.

Sensitivity, Specificity, PPV & NPV of Anti CCP & RF tests in RA:

Sensitivity, Specificity, PPV & NPV of

Anti CCP - 78%, 98.6%, 95.1% & 93%

RF - 76%, 86%, 64.4% & 91.4%.

Anti CCP & RF - 66%, 98.6%, 94.2 % & 89.7%.

ANTI CCP / RF - 88%, 86%, 67.7% & 95.5.%.

The results obtained from the study shows that **Anti CCP** test alone is *more sensitive and specific* for RA, and **RF** is *equally sensitive but less specific* than anti CCP test.

Anti CCP and **RF** - **both positive** was *moderately sensitive and equally specific* for RA (similar to anti CCP), less sensitive and more specific than RF alone,and Anti CCP/ RF. But **Anti CCP/ RF** - **any one test positive** was *more sensitive and less specific* than the combination of both tests and Anti CCP test alone and more sensitive but equally specific when compared to RF test alone.

This shows that the anti- CCP positivity gives additive value as a diagnostic test for RA and the combination of tests are valuable as a diagnostic tool in RA and in differentiating or excluding it from other arthritis. Majority of the previous studies show greater significance of anti CCP in RA,and ES (undifferentiated arthritis) as a diagnostic tool and early predictor in ES, also in excluding them from non RA.

Limitation

This study is mainly a cross sectional study, with various groups of small sample size. The period of study is also short so limiting the follow up to observe the convertibility of ES into established RA and non RA and to assess the prognosis in confirmed RA.

A large sample size, and a longitudinal study with periodical follow up will definitely be of greater value in prediction of anti CCP positivity.

Future scope of the present study

- The study can be further extended to involve different types of kits to test their sensitivity and specificity, for better prediction.
- The study can be made clinically relevant in comparing with erosiveness/deformity with the anti CCP values.
- Further research should be conducted to identify risk factors for aggressive disease, using serological as well as clinical markers.
- Studies with repeated assessments of biomarkers prior to RA development may provide further insight into the timing of biomarker elevation in preclinical RA.

Hope further research will reveal the significance of protein citrullination in the immunopathology of RA.

SUMMARY

- In the present study, Anti CCP anti bodies in Rheumatoid Arthritis and other arthritis patients were assessed and also in healthy blood donors as control.
- The anti CCP test shows high positive rate in RA patients than other arthritic disease patients, and negative in OA and in Healthy Blood Donors .This reconfirmed the better specificity of anti CCP test in RA and thus it can be used as a diagnostic tool.
- Anti-CCP antibodies determination, proved to be a powerful diagnostic tool,especially in sero negative (RF negative) patients.
- Anti CCP was very sensitive in Early Synovitis also.This confims it's importantance in the discrimination of early diagnosis of RA in undifferentiated arthritis.
- Since Anti CCP positivity is very low in SLE it helps in differentiating RA from other connective tissue disorders including SLE patients.
- Anti CCP was negative in OA and HBD. So it will be useful in excluding these cases from RA.
- Even though RF is equally sensitive as Anti CCP in RA, it is less specific when compared with Anti CCP .

- Anti-CCP testing combined with RF testing has additional value over RF testing alone in patients with early undifferentiated arthritis.

CONCLUSION

The ACPA era has just begun, and is creating a revolution in Rheumatology. As per the findings of the present study Anti CCP test is more sensitive and highly specific. Anti CCP antibody is a very valuable serological indicator in diagnosis of RA.Besides being a specific marker in advanced RA, the anti CCP antibody in ES is a very important predictor (diagnostic marker) of RA.

Anti CCP has the hallmark of establishing as a diagnostic tool and provides additive sensitivity to RF. The presence of both RF and Anti CCP in serum is a strong indicator of RA.

Association of Anti-CCP with other Connective Tissue Disorders including SLE is very low when compared to its significant association with RA.Thus it helps in distinguishing RA from other erosive disorders.

Anti CCP is positive in 50% of Seronegative arthritis (RF negative) patients.Thus it proved to be a more sensitive diagnostic tool, which helps in detection (not missing the diagnosis) of RA,very early in course.

Absence of Anti CCP in OA and HBD makes it more specific for RA.But the presence of RF to a lesser extent in the above conditions makes (RF) it less specific.

In conclusion, based upon the higher sensitivity and specificity of the Anti CCP test in RA, the current study is of diagnostic, and public importance, because it suggests that Anti CCP test should be included in the investigation of undifferentiated arthritis, since a considerable amount of Rheumatic disease associated work disability starts in the first few years of the disease.

Anti-CCP antibodies have all the hallmarks of establishing themselves firmly in the diagnostic algorithm of Rheumatoid Arthritis providing additive sensitivity to Rheumatoid Factor. So it should also be included among the diagnostic criteria of RA along with RF.

It can also be used as a prognostic indicator in RA patients on treatment.

Chart -1.Age Wise Distribution of Various Arthritic Diseases

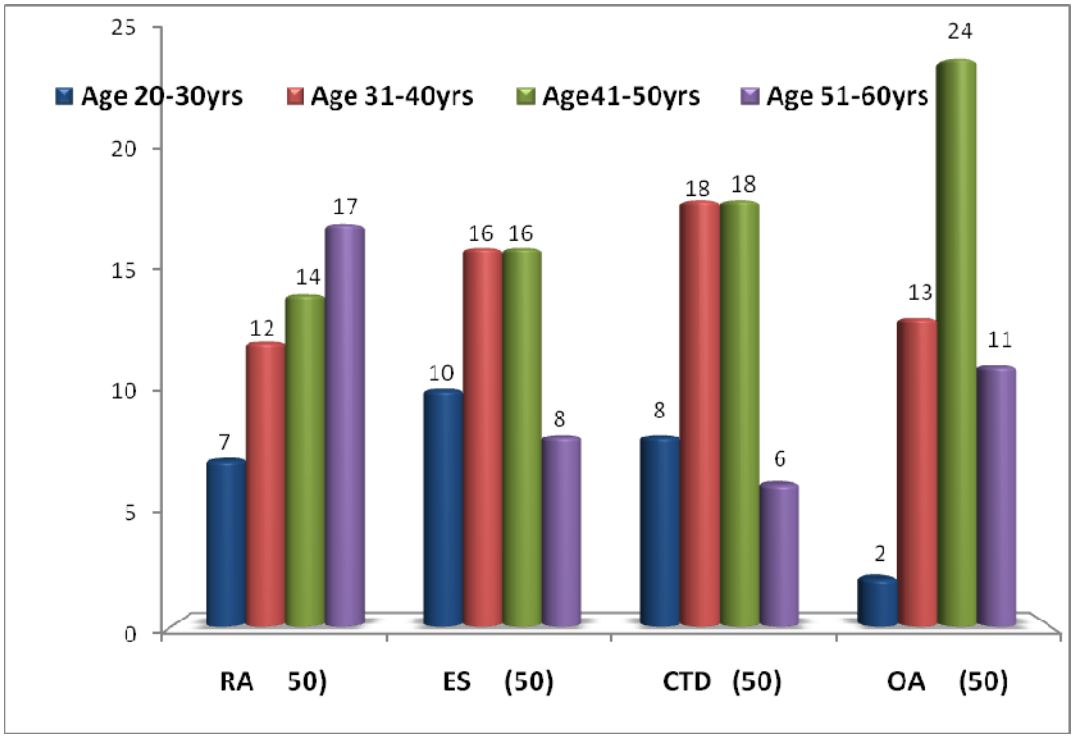


Chart-2.Sex Wise Distribution of Various Arthritic Diseases

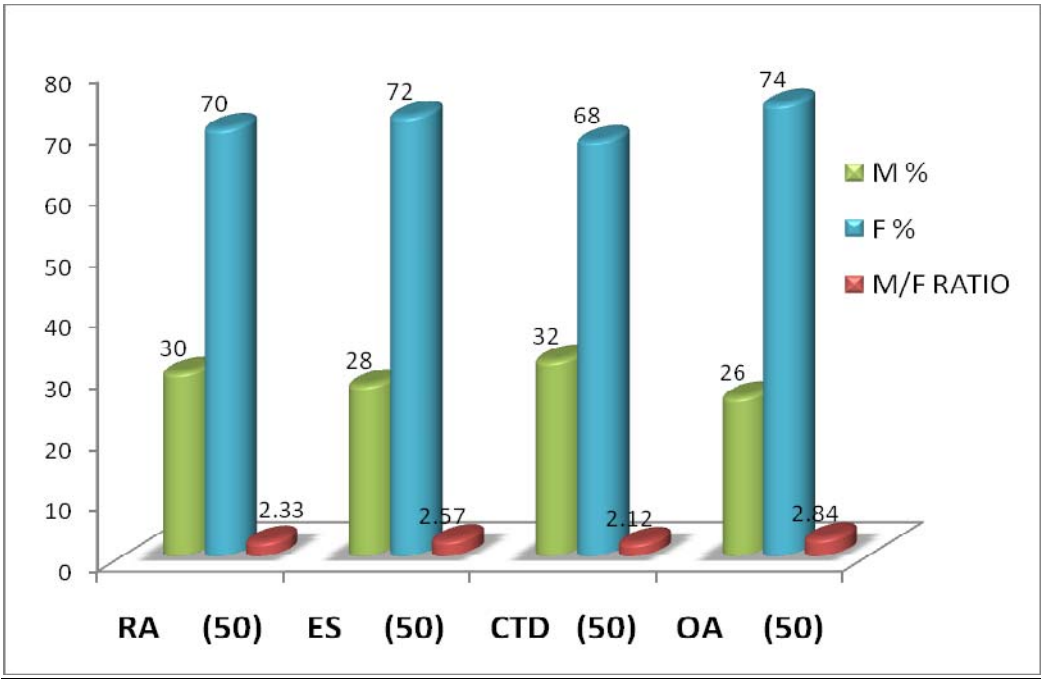


Chart -3. Age/Sex Distribution Of Various Arthritic Diseases

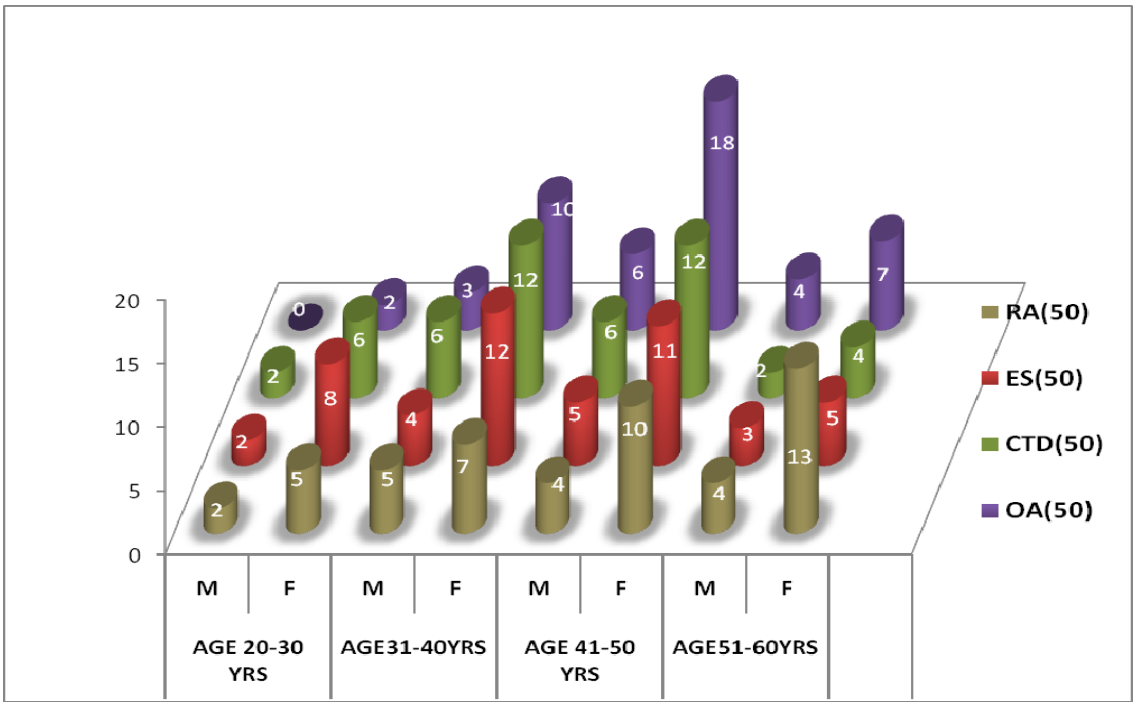


Chart - 4. Anti-CCP& RF Test Results in all Arthritic groups & HBD

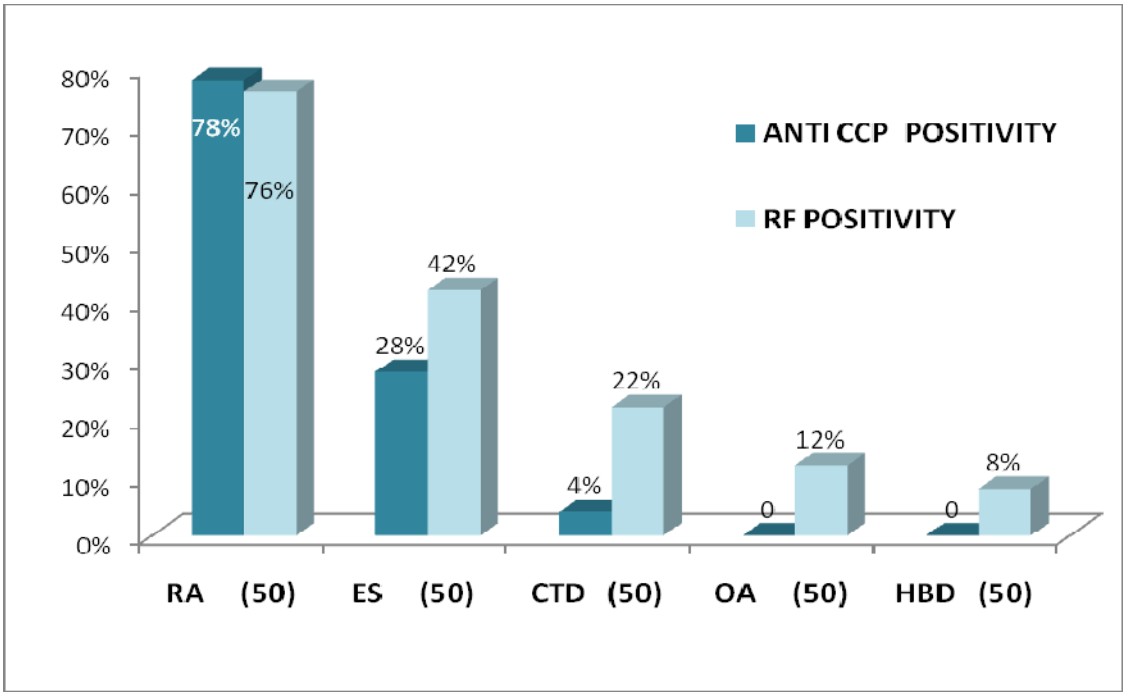


Chart - 5.Sensitivity & Specificity of RF Test in RA

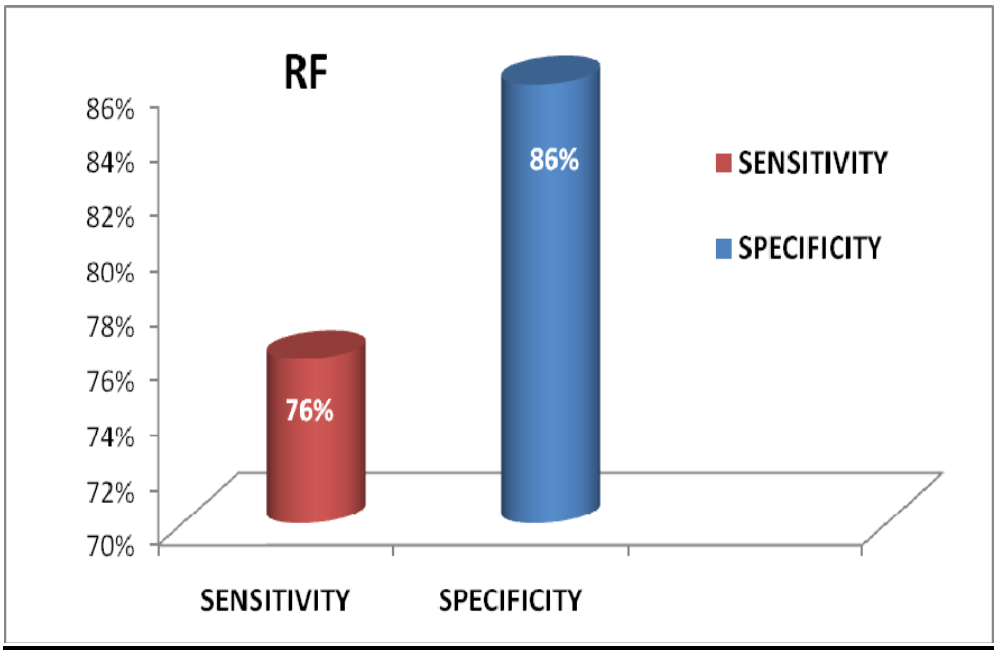


Chart – 6.Sensitivity & Specificity of Anti CCP Test in RA

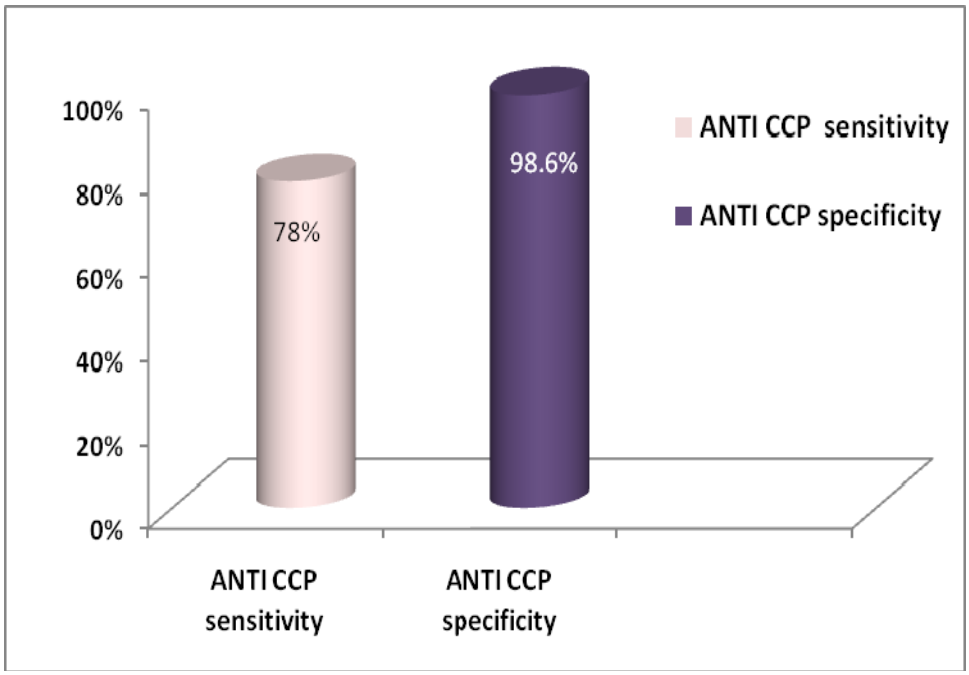


Chart- 7. Distribution of Positivities of Anti CCP and /or RF on the groups

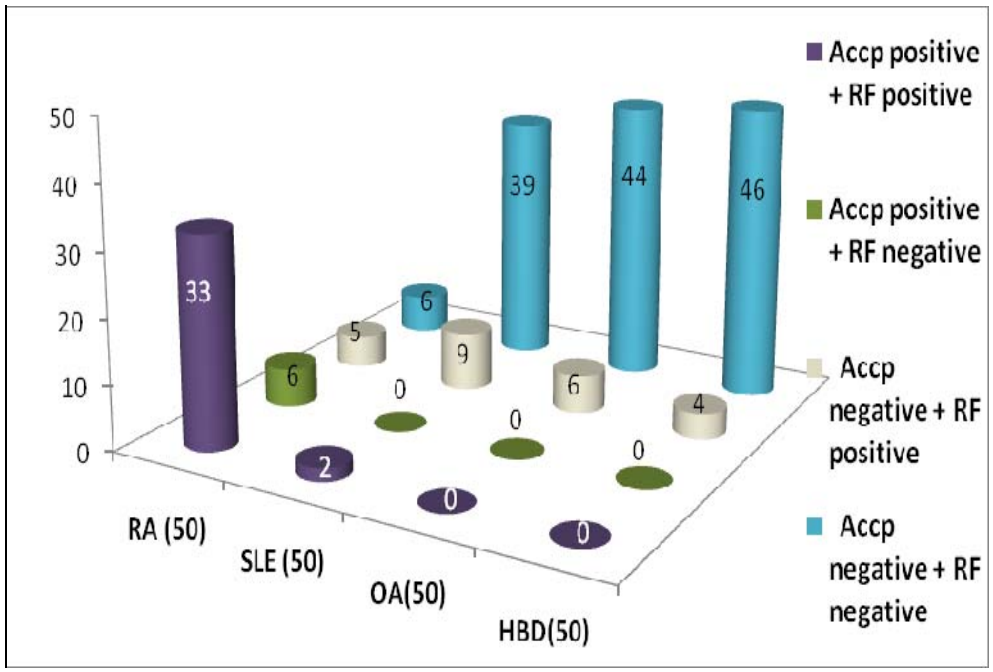


Chart - 8.Use of Anti CCP Test in Sero Negative RA patients

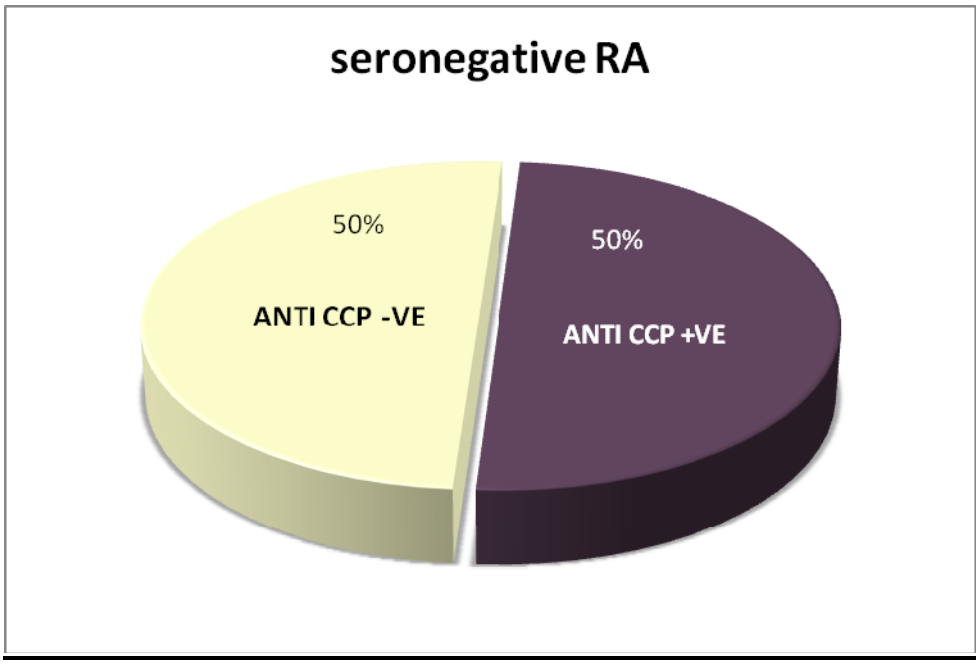


Chart-9.Anti CCP& RF Test Results in ES Patients

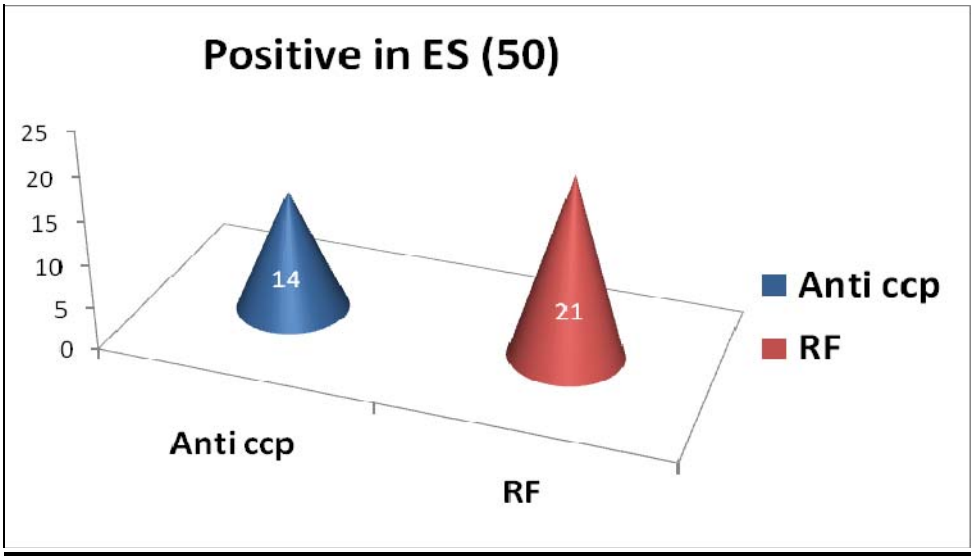
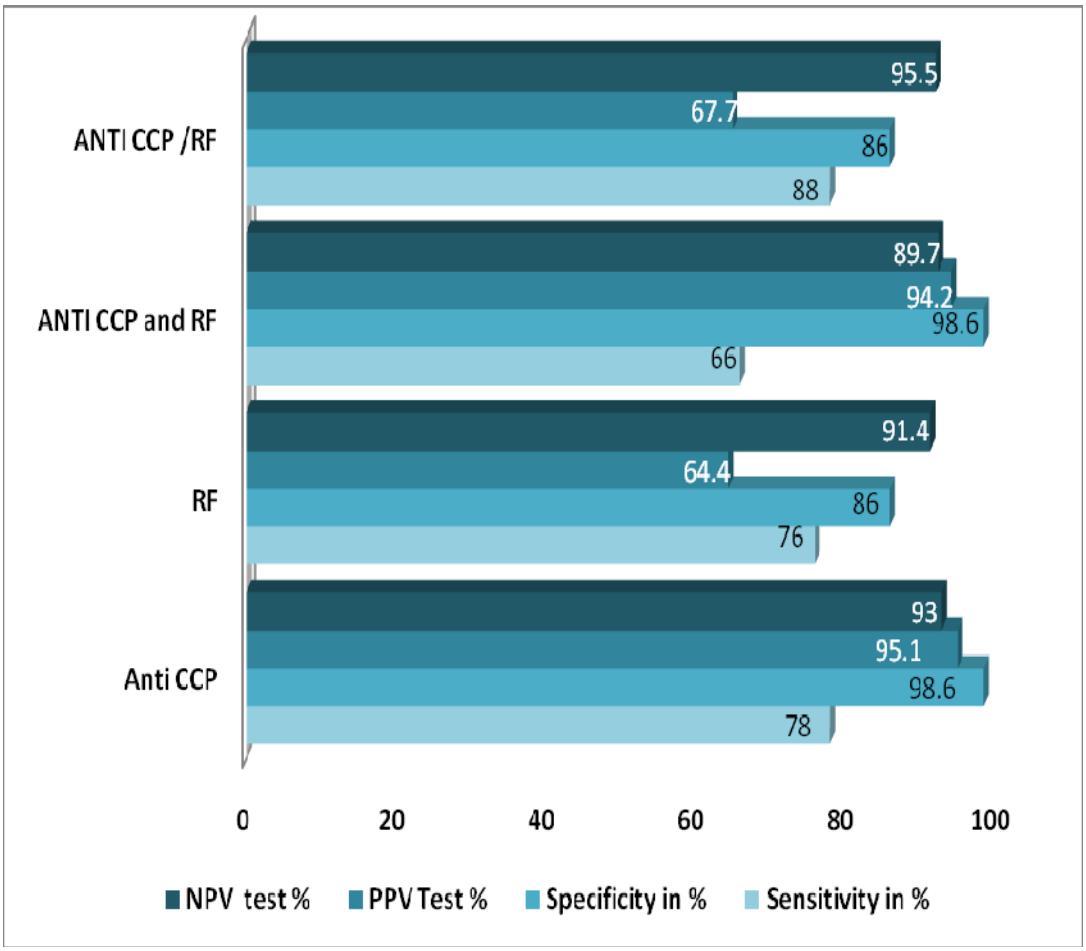
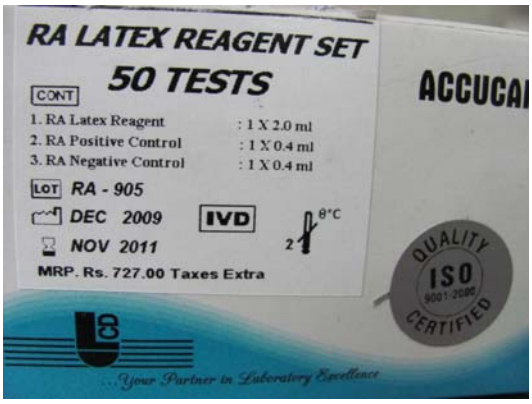


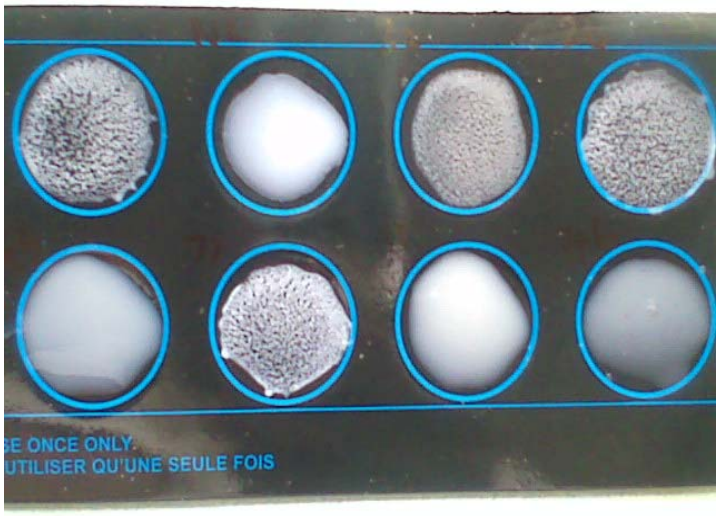
Chart-10.Sensitivity, Specificity, PPV & NPV of Anti CCP & RF tests



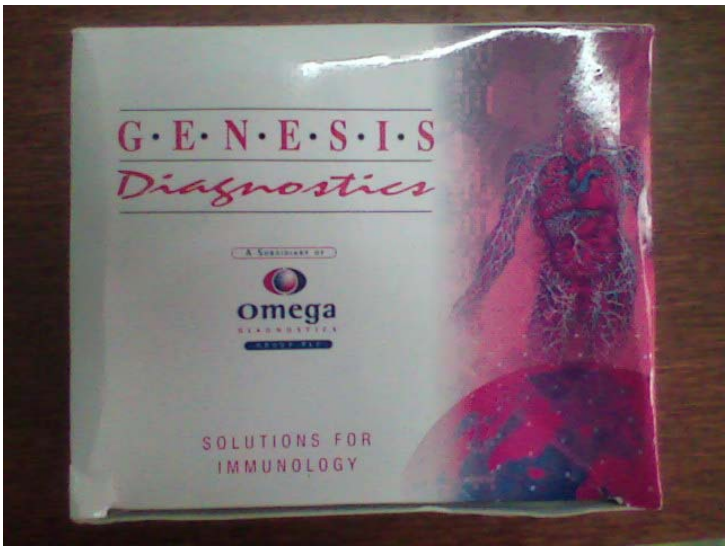
Colour Plate -1. RA Test Kit with Reagents



Colour Plate -2. RA Test Result



Colour Plate - 3. Anti CCP Test Kit

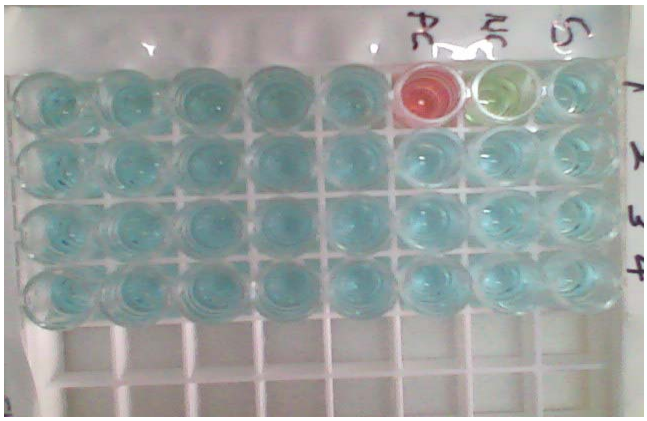


Colour Plate - 4.Anti CCP Test Reagents

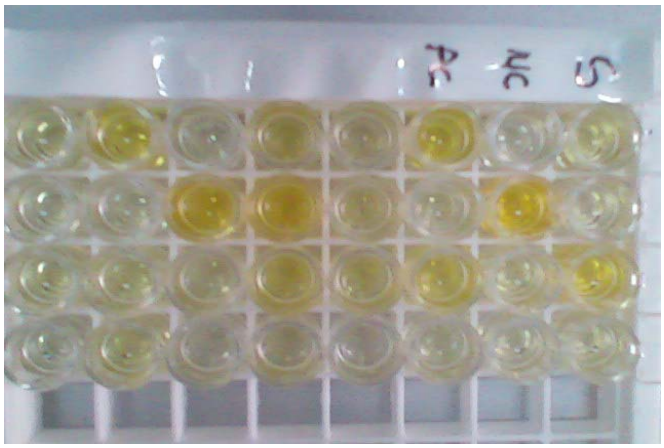
Colour Plate - 5. Anti CCP Test Procedure

5- a.Diluted Samples

5- b.Test Procedure

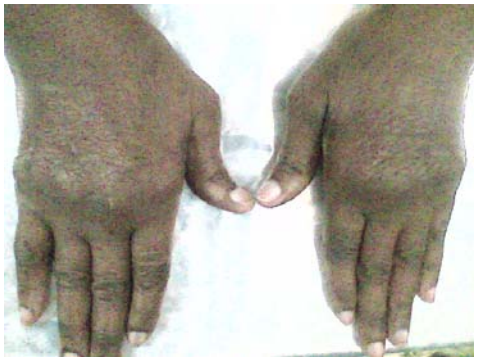


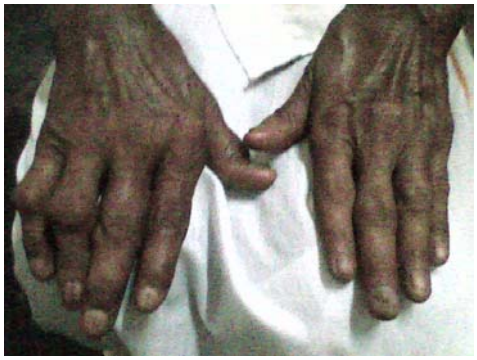
5-c. Results



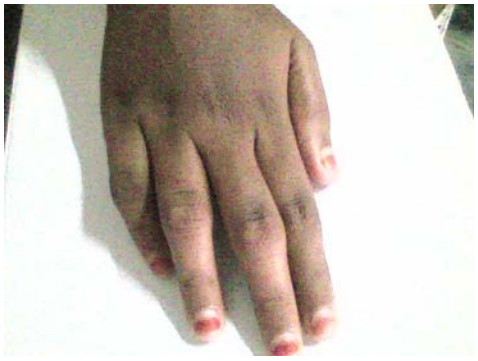
Colour Plate -6. Various Arthritic Diseases

6-a Rheumatoid Arthritis

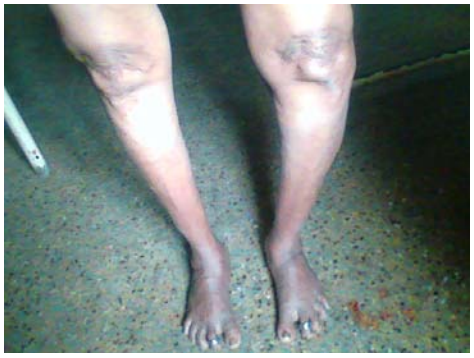




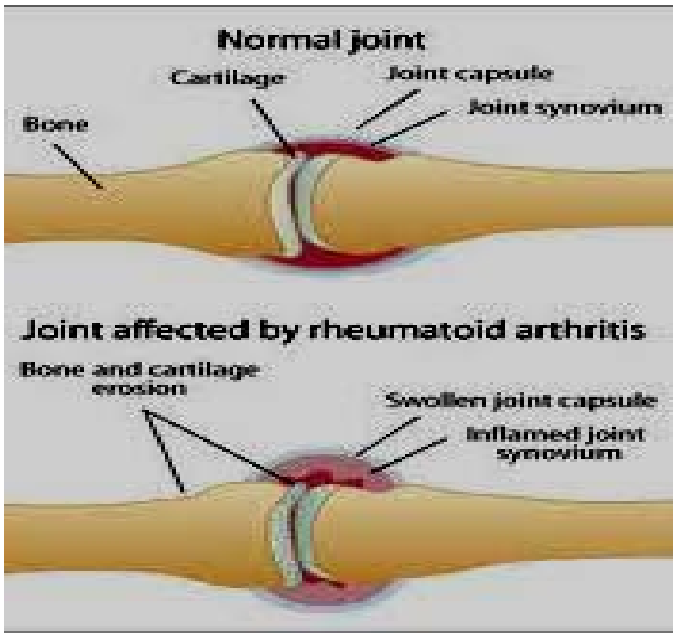
6-b.Early Synovitis



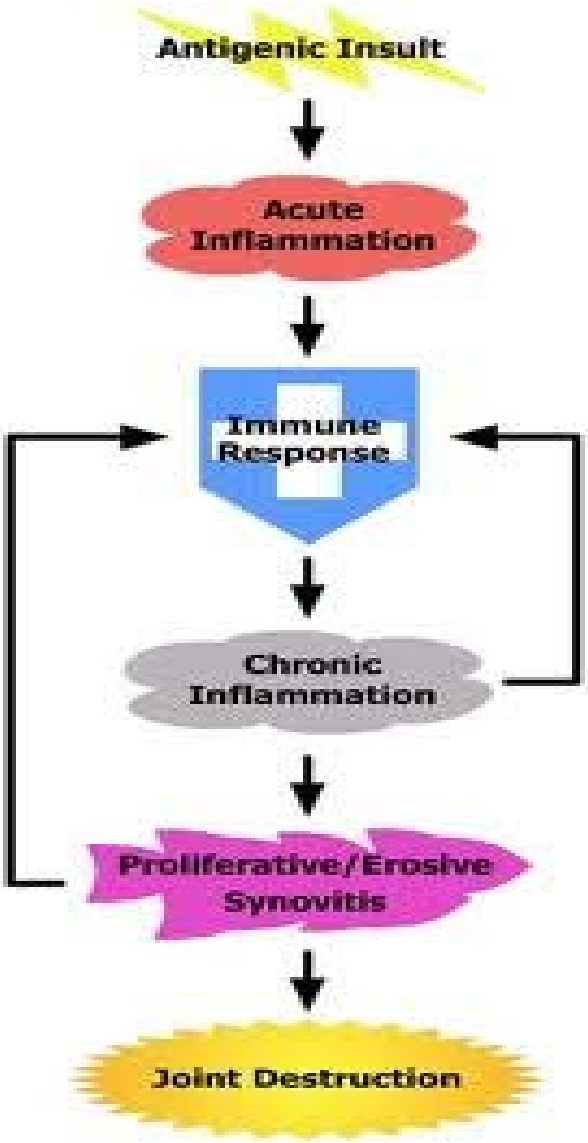
6-c.Osteo Arthritis



RHEUMATOID ARTHRITIS – NORMAL & AFFECTED JOINTS



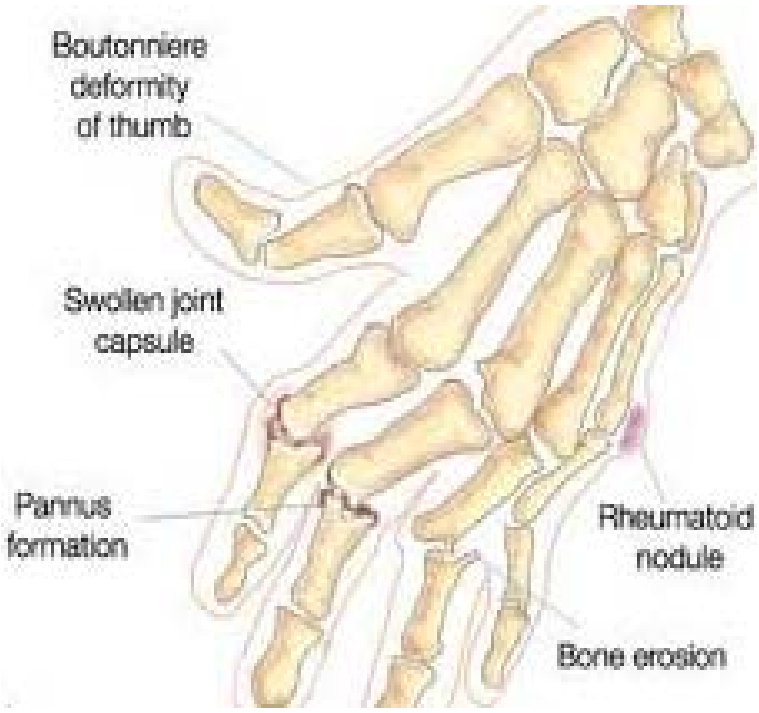
PATHOGENESIS



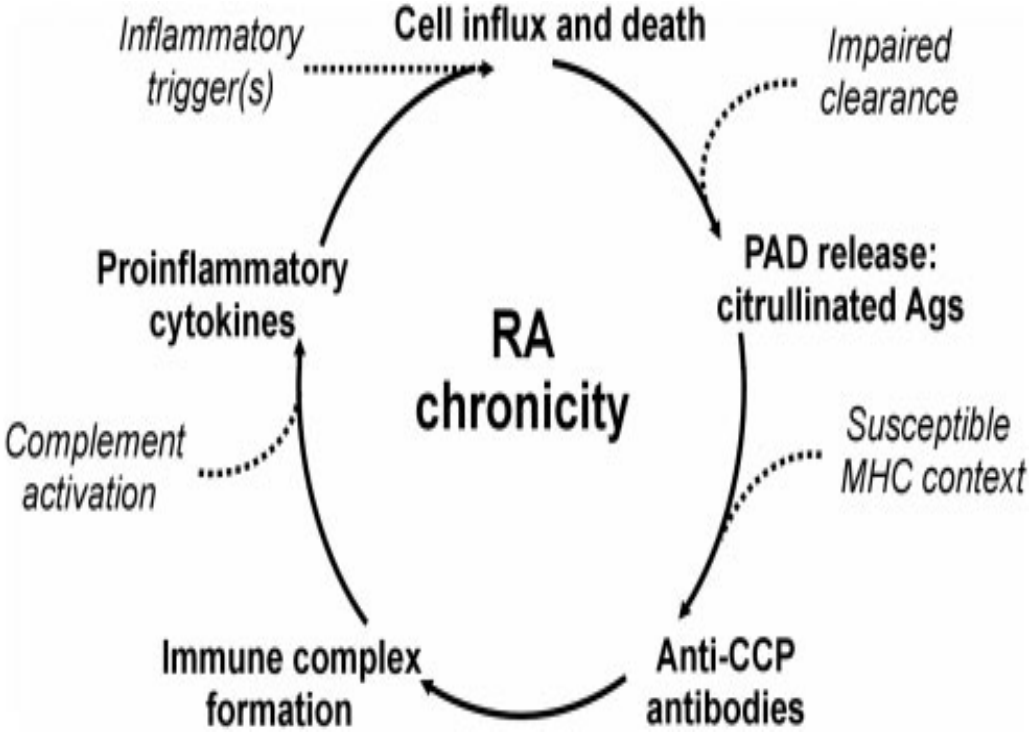
OSTEO ARTHRITIS

SLE –MALAR RASH

HAND INVOLVEMENT OF RA



RA CHRONICITY



DEAMINATION OF ARGININE