ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

Internship Training Report

Submitted to

Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Biochemistry

Submitted by:
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(Reg No 11410884)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
May, 2016

ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

CERTIFICATE

This is to certify that **Mr. Ganesh Sudhakar Kote.** Bearing **Registration Number** 11410884.has completed his Master of Science in Clinical Biochemistry internship under our guidance and supervision. This report is record of the candidate own work carried out by him under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

Internal Supervisor	*External Supervisor*
Dr. Pranav kumar Prabhakar	**Dr.Hemant Shinde**
Associate professor	**Director of Labworldplus**
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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of ***DrPranav Kumar Prabhakar (Internal supervisor),*** Lovely Professional University andDr.HemantShinde(***External supervisor***), Lab World plus Laboratory BorivaliThis work has not been submitted in part or in full in any other university for any degree or diploma.

ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

Abstract

AIM **–** Adenosine Deaminase(ADA) is theBiochemical Marker of Rheumatoid Arthritis

Introduction

Adenosine deaminase is the ectoenzyme found in almost all human tissue cell like epithelial cell, endothelial cell and lymphoid cell. Adenosine deaminase (ADA) play an important role in purine metabolism to convert the adenosine, the product of AMP, into inosine, precursor of uric acid, which is further excreted through kidney. Adenosinedeaminase also play important role in T cell proliferation and stimulation as costimulatorysignal to help the antigen presenting cell in T cell binding, CD26 is the ligand of ADA present on the surface of antigen presenting cell. Level of ADA is increased in rheumatoid factor positive test rheumatoid arthritis patient.

Materials and Methods

The study was piloted at ***Lab Worldplus Laboratory Borivali***. This study was conducted on 51 patients of rheumatoid arthritis,of age group of 30-60 years, who were diagnosed by clinical analysis, rheumatoid factor (Group 1). Fifty-one healthy individual with no known history of any disease matched by age and sex with group 1 were taken as controls (Group 2). After taking informed consent and noting the name, age and sex, venous blood samples were drawn from both the groups. Serum ADA level was estimated by enzyme kinetic method with the pathozyme diagnostics reagent kitandErbaChem 7 semi auto analyzer.

Result

Mean serum ADA level was found 31.18±6.3IU/L in the patient with rheumatoid arthritis positive who is under medication and 18.94 ±5.37IU/L in healthy control group.

Conclusion

Serum ADA level were found to be higher in the case of the patients with rheumatoid arthritis who were under treatment but within biological reference range similar toordinary healthy control group.

Keyword: Rheumatoid arthritis; Adenosine deaminase; Dipeptides (CD26); Adenosine monophosphate

Acknowledgement

First and foremost I would like to thank **Dr. HemantShinde**, Biochemist, and Director of the laboratory, for giving me permission to work in the lab. Without it, my work would not have been possible.

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Next, I would like to thank **Dr.HemantShinde**, (counselting.Biochemist and Director of LABWORLDPLUS LABORATORY) my mentor who has enlightened me with the working of a clinical laboratory. He has helped me understand the role of a Clinical Biochemist and encouragedto better it. It is his support and guidance that made it possible to work on this particular topic. He has helped me run the tests and interpret them relating it to the quality assessment and maintenance of biochemistryanalyzers.

I would like to extend my thanksto the entire family of LABWORLDPLUS LABORATORY and Dr. JARIVALA LABORATORYfor supporting me in every step. They have provided me with an ambience that has made it possible to conduct my study smoothly. Most importantly, I would like to thank my parents, especially my brother to whom I will be forever indebted. They have been the backbone of my journey till this stage. It is their vision that has made me able to excel in my education.

Finally, I thank the **ALMIGHTY** for giving me hope and strength to complete my work.

Ganesh Kote

Reg No: 140884

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Introduction

Rheumatoid arthritis is long-lasting inflammatory illness, in which inflammation and induration in joint of hands and feet, hyperplasia of synovial cells, damage of bone and cartilages are the chief complication happened in rheumatoid arthritis (1,2). Although the exact etiology or causative agent of rheumatoid arthritis is unidentified (3,4), several time is entitled as autoantibody synthesis disorder and autoimmune disease. Atherosclerosis, insulin resistance, depression. are the tributary complication also seen in chronic rheumatoid arthritis patient(5) .Identification of rheumatoid arthritis is in acute condition is must because curability percentage of rheumatoid arthritis is more in acute condition as compare to in the chronic condition, which diminish the patient economical with physical stress burden also. In existing clinical practice rheumatoid antibody (RA) test is used as prime diagnosis test of RA, along with RA, CRP, ANTI CCP, ANA are the second generation test also using in chronic rheumatoid arthritis diagnosis. ADA (Adenosine deaminase) is an enzyme also called as adenosine hydrolase,ADA is involved in purine metabolism. ADA Catalyzed the adenosine which is originated from the food and damaged tissue or cells in to inosine (6).ADA has major role in evolution and functions of lymphocyte and development of macrophages from monocyte (7). ADA has two isoform ADA1 &ADA2,ADA1 is existing in human body cell like lymphocyte and macrophage ,ADA not only present in cytoplasm or nucleus of but it's also contemporary on the cell membrane attached to dipeptide peptidase4.ADA 2 was first identified in human spleen but then after it was subsequently founded in other tissue also like macrophages with ADA (7).ADA2 is the predominated enzyme found in human plasma and is augmented in many disease like Rheumatoid arthritis, psoriasis and sarcoidosis,liquid chromatography .

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Arthritis

Is a long-lasting inflammatory disorder which customarily occurs in a joint of body, the mutual sing and symptom are joint pain and stiffness, which normally worsen with age.Rheumatoid arthritis and osteoarthritis are the conjoint arthritis occur in worldwideinhabitants.

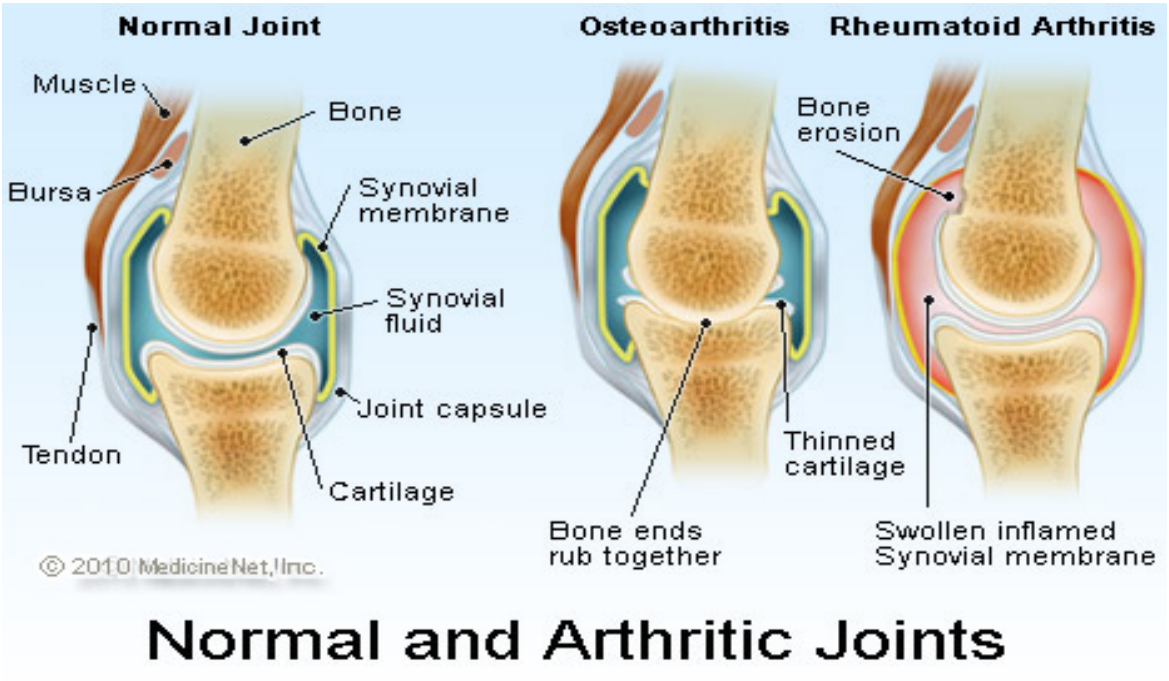

Figure no 1-Normal and Arthritis joint anatomy and physiology (1)

Types of arthritis

- Osteoarthritis
- Rheumatoid arthritis
- Juvenile Idiopathic arthritis
- Infectious arthritis
- Crystal induce arthritis

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Osteoarthritis

It's joint degenerative and deputize type of arthritis. Ordinarilybefallen in senior citizen those has spanned their sixties age. Osteoarthritis (OA) its naturally and mechanically occurring irretrievable disorder, in which not only cartilage devastation but also subchondral bone, muscle, fats and synovial tissue play an important role in pathogenesis of osteoarthritis. Elderly people having greater prevalence's to development of osteoarthritis as compare to younger people, excluding trauma in any aged. Knee, hip and shoulder joint are the prime site of osteoarthritis causes due to aging, inflammation and trauma. To apprehend why aging predisposes to the development of OA, a link between aging progressions and the pathological changes in the OA joint desires to be established. On a molecular level, aging research has revealed intrinsic changes in the structure of extracellular matrix proteins such as collagen or proteoglycans. Sti□ening of the collagen network or increased glaciation provoke a functional impairment of cartilage and joint function. Aging also has profound e□ects on cellular processes notably leading to enhanced apoptosis and reduced cellular regeneration (9). Non enzymatic collagen cross-linking leads abnormalities in bone toughness and sti□ness. Bone plasticity is further suppressed by an increase of osteon density, which leads to a lower potency of crack-bridging mechanisms(10). Infiltration of inflammatory cells in synovial cavity frontrunners to causes inflammation in the synovial cavity accumulated with inflammatory cells and their immunological product like interleukin, cytokine other are accountable for activation of cell mediated immunity and destruction of cartilage lead to causes deteriorating osteoarthritis.

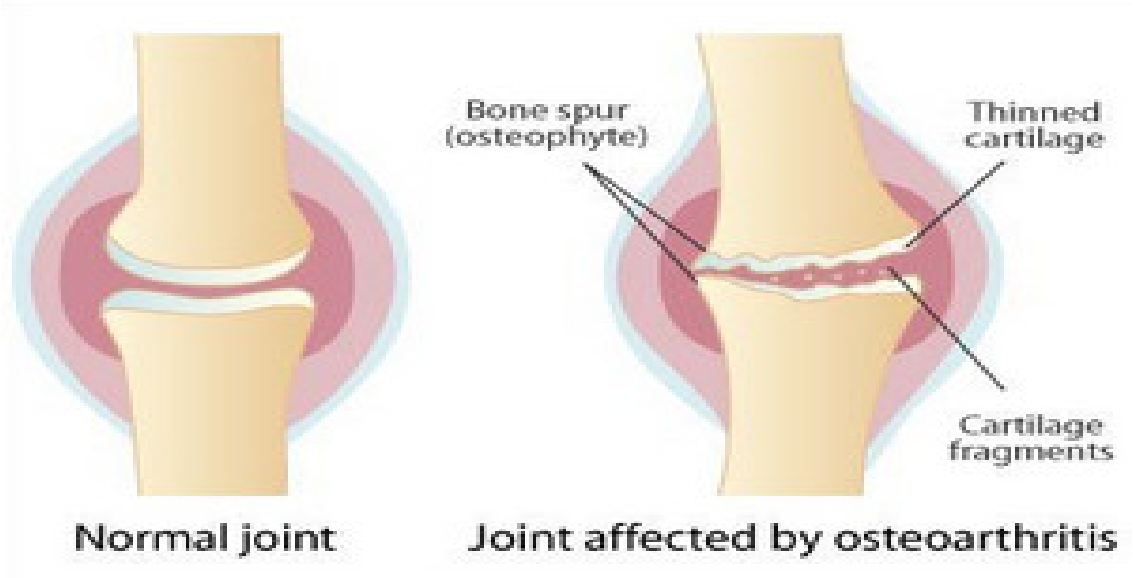

Figure no 2 - Anatomy of normal joint and osteoarthritis joint (3)

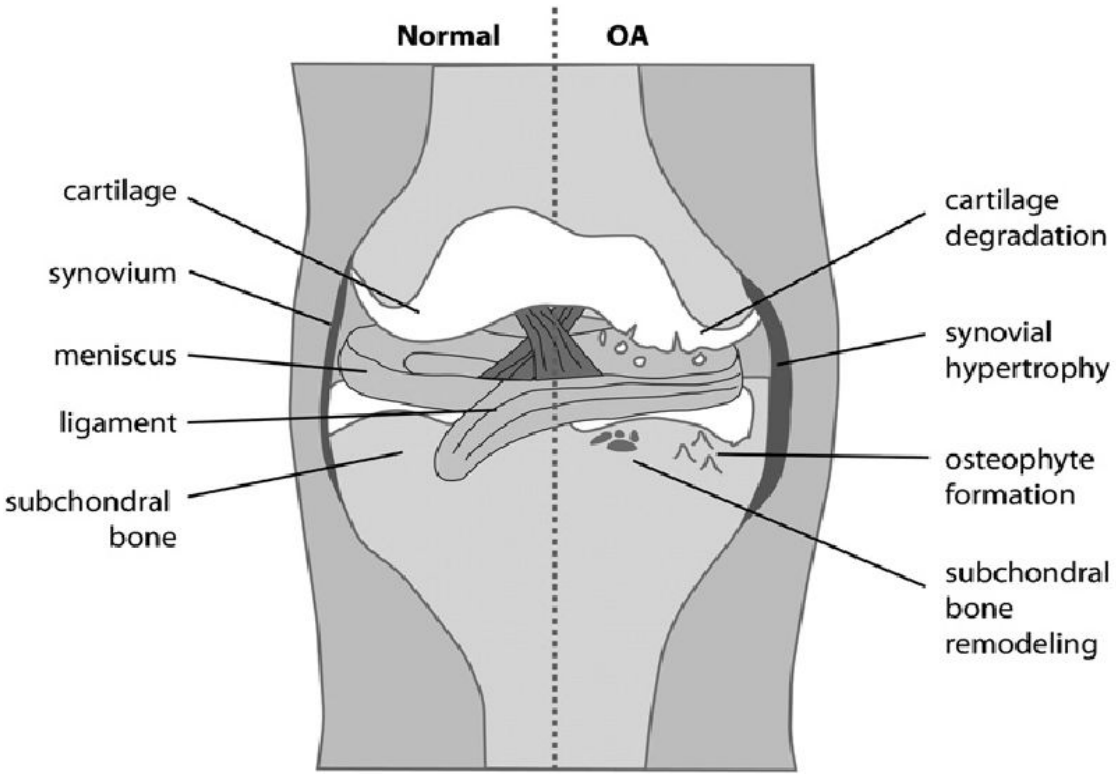

Figure no 3 - Anatomy of normal joint and osteoarthritis joint (3)

Symptom of osteoarthritis

Succeeding symptom ordinarily occur in rheumatoid particularly in osteoarthritis

A. Pain in joints

B. Joint swelling

C. Stiffness

D. Joint deformities

Singe of osteoarthritis

A. Coarse crepitus

B. Bony enlargement

C. Deformities

D. Instability

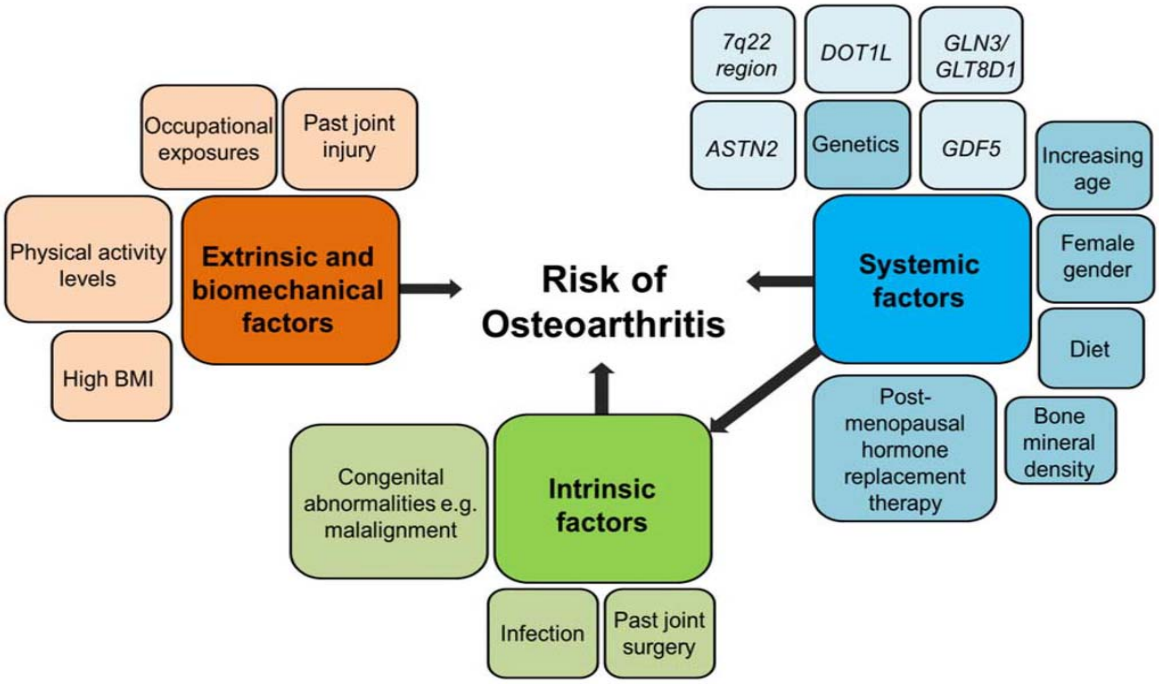

Figure no. 4 - Risk of osteoarthritis. (9)

PATHOGENESIS OF OSTEOARTHRITIS

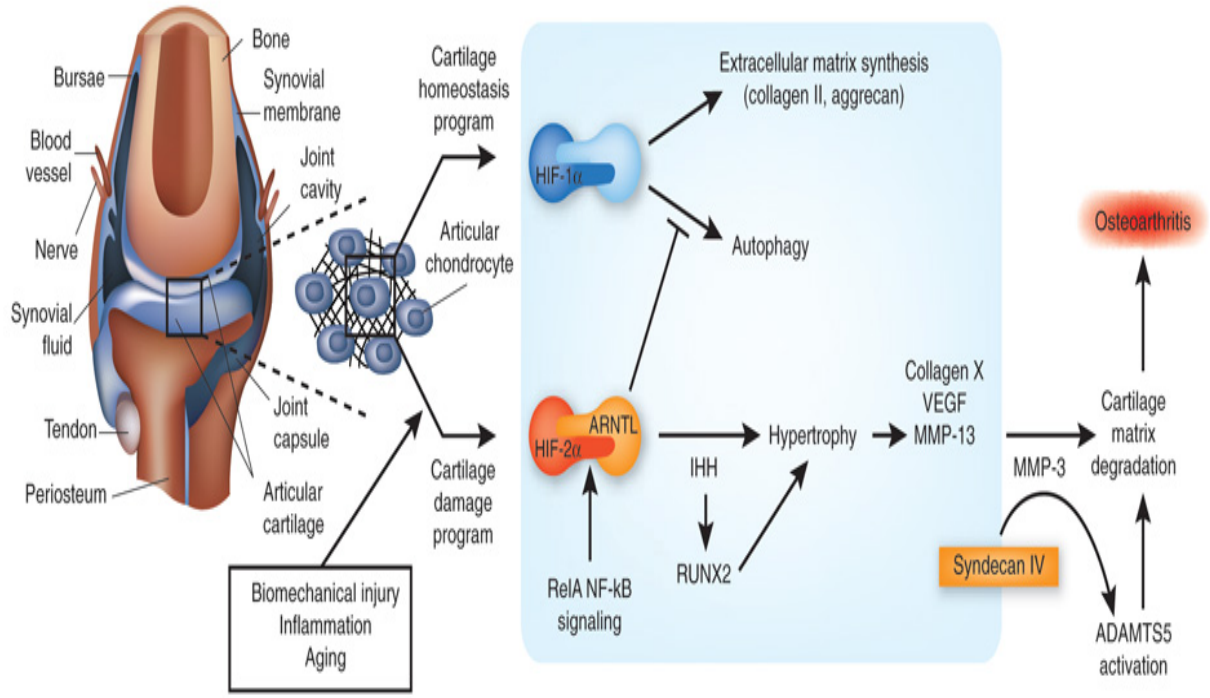

Figure no 5 - Pathogenesis of osteoarthritis (11)

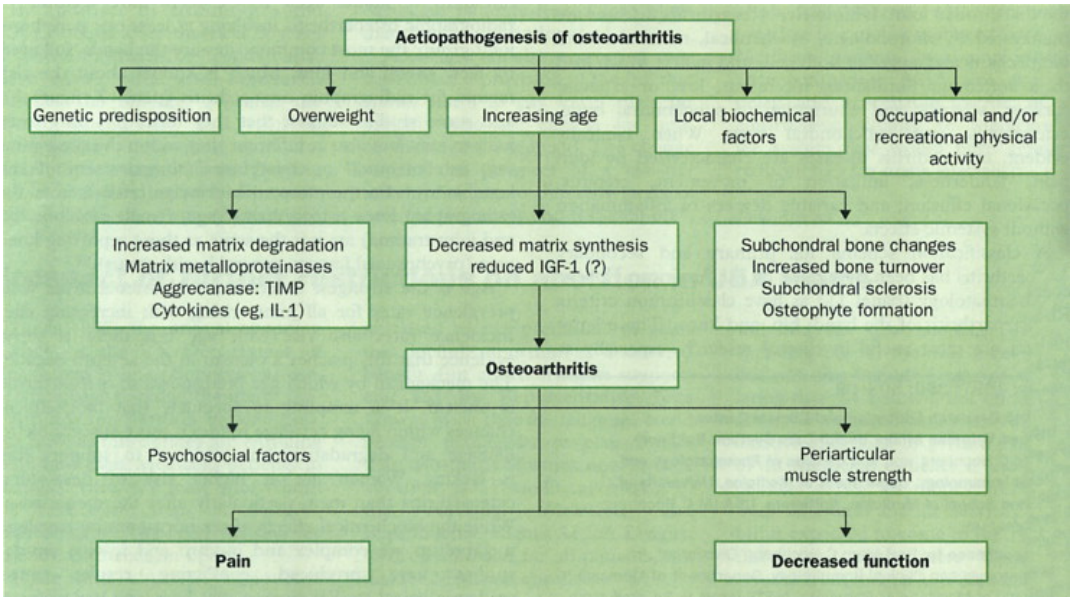

Figure no 6 - Aetiopathogenesis of osteoarthritis (11)

Infectious arthritis

Bacterial infections that causes acute suppurative arthritis usually pass in the joints from distant sites by hematogenous extent.in neonates havesophisticated incidence of contiguous spread from underlying epiphyseal osteomyelitis.*H.influenza* arthritis are dominantly ensue in neonates or children those younger than 2 year of age.*S.aurues* is the main causative agent in older children and adult and gonococcus. Individual with sickle cell has higher chances to get salmonella infection in joint at any age.Inadequate immunity with complement deficiency (C5,C6,C7) are more susceptible to gonococcus infection and hence arthritis. The classical example is the sudden development an acute painful and swollen joint that has a restricted range of motion. Systemic finding of fever, leukocytosis and elevated sedimentation rate are the common finding in bacterial arthritis .in speeded gonococcus infection the symptoms are more subacute.In 90% of nongonococcal case, the infection involves only a single joint, most commonly the knee followed frequency by the hip, shoulder,elbow,wrist and sternoclavicular joints. The axial joints are more often involved in drug user. Joint aspiration is diagnostic if it yields purulent fluid in which causal agent can be found (11).

Pathogenesis of infectious arthritis

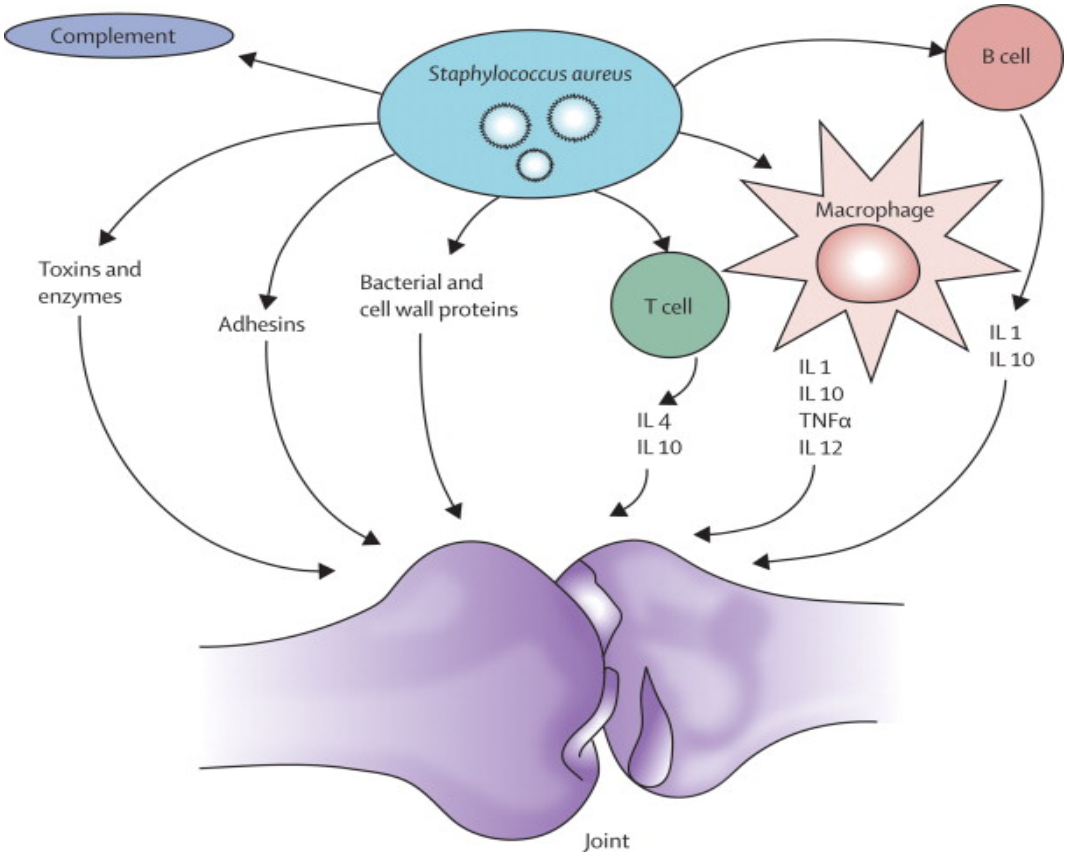

Figure no 7 - Pathogenesis of infectious arthritis (22)

Crystal induce arthritis

Bestowing to the renowned Unani physician, Buqrat (Hippocrates), Niqris is a joint disease which is caused due to surplus of one of the four humors, which under certain circumstances, drop or flow into a joint causing pain and inflammation (12). Gout is the term used to describe a group of disorders which results from tissue deposition of crystals of monosodium urate monohydrate from hyperuricemic body fluids. It is usually a monoarticular arthritis, although uncommonly presents as a polyarticular disease. It is characterized by intra-articular deposition of uric acid crystals. It is associated with hyperuricemia, which may be produced by thiazide diuretics associated with hyperuricemia: alcoholism, obesity, hypertension, dyslipidemia, hyperglycemia, diabetes mellitus, lithiasis, renal failure, and medication use such as diuretics, cyclosporine, and low-dose aspirin (13).

Classification of gout

Acute Gout:Acute monoarthritis results from an acute attack. Severe pain, erythema, and swelling are the distinctive features of the disease. The most commonly affected joint is the first metatarsophalangeal joint (podagra), followed by knee, ankle/metatarsus, wrist, and fingers. Polyarticular gout is less common but can occur, in those individuals who had repeated disease flares. The risk for gout is directly proportional with the degree of hyperuricemia. Acute gout is self-limited and symptoms typically resolve over the course of days to weeks (14).

IntercriticalGout: Patients of gout are generally asymptomatic in between sporadic episodes of acute arthritis. The management of patients with intercritical gout focuses on the prevention.

Chronic TophaceousGout:Large deposits of uric acid occur within joints or in the soft tissues, particularly around the pinna of ear, in chronic tophaceous gout. In these patients, there is substantial X-ray changes, calcification of urate deposits with soft tissue swelling and even erosions of phalangeal bone(15).

The clinic pathological features of gout are as follows:

- Males usually affected;
- Onset 40- 60 years, familial tendency;
- Acute inflammatory monoarthritis- more than one joint involved in 10%;
- Raised plasma uric acid (0.5mmol/l);
- Untreated patients may progress to chronic gouty arthritis and renal failure
- Deposition of monosodium urate crystals in joints;
- Variable incidence of uric acid renal calculi;
- Mild intermittent proteinuria with focal interstitial nephritis;(16)

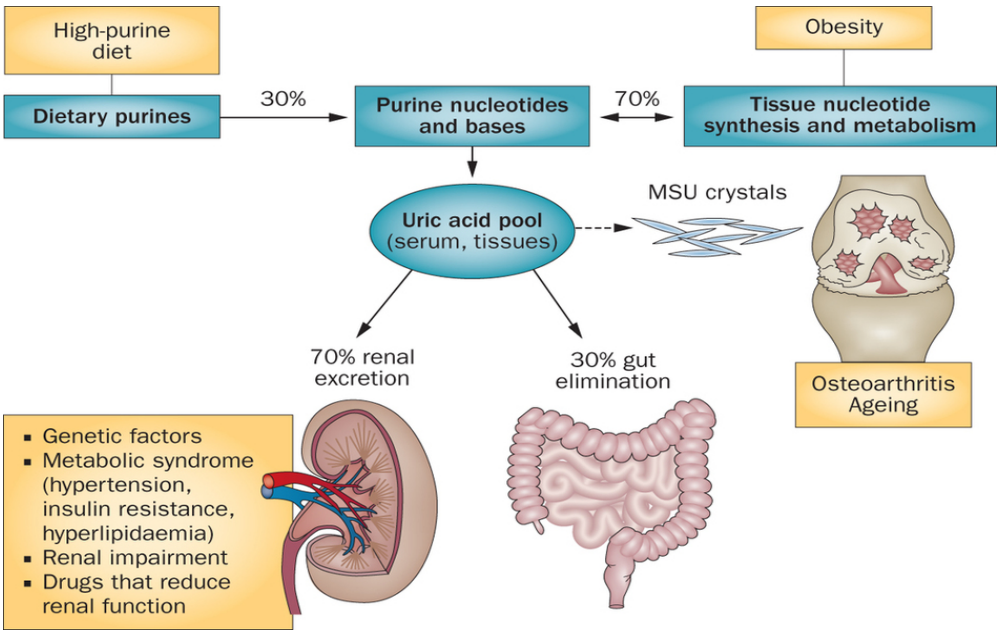

Figure no 8 - Pathogenesis of crystal induce arthritis

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Rheumatoid arthritis (RA)

Rheumatoid arthritis is an autoimmune disease. Inordinary condition immune system of human body work as security protector for their body that is to defend the human from foreign harmful agents, bacteria, virus, fungus, except in rheumatoid arthritis. In this disease immune system of body work in contrast to their own system in which immune system recognized their own protein specially in joints as foreign substance or antigen and resume antibody synthesis, such antibody is called as rheumatoid antibody (RA). Activation of cell mediated immune system against their own joint protein causes inflammation around the joint leads to causes swelling and pain around the joint get difficulties in walking. If inflammation goes unchecked, it can damage cartilage, the elastic tissue that covers the ends of bones in a joint, as well as the bones themselves. Over time, there is loss of cartilage, and the joint spacing between bones can become smaller. Joints can become loose, unstable, painful and lose their mobility. Joint deformity also can occur. Joint damage cannot be reversed, and because it can occur early, doctors recommend early diagnosis and aggressive treatment to control RA. Rheumatoid arthritis most commonly affects the joints of the hands, feet, wrists, elbows, knees and ankles. The joint effect is usually symmetrical. That means if one knee or hand if affected, usually the other one is, too. Because RA also can affect body systems, such as the cardiovascular or respiratory systems, it is called a systemic disease. Systemic means “entire body”(17).

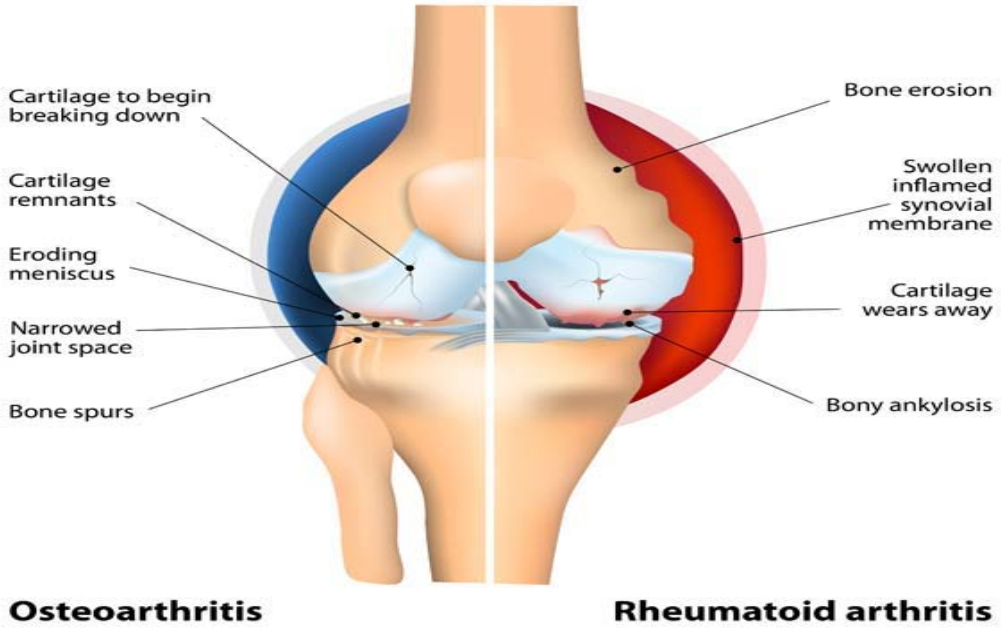

Figure no. 9 Image of Osteoarthritis & Rheumatoid Arthritis

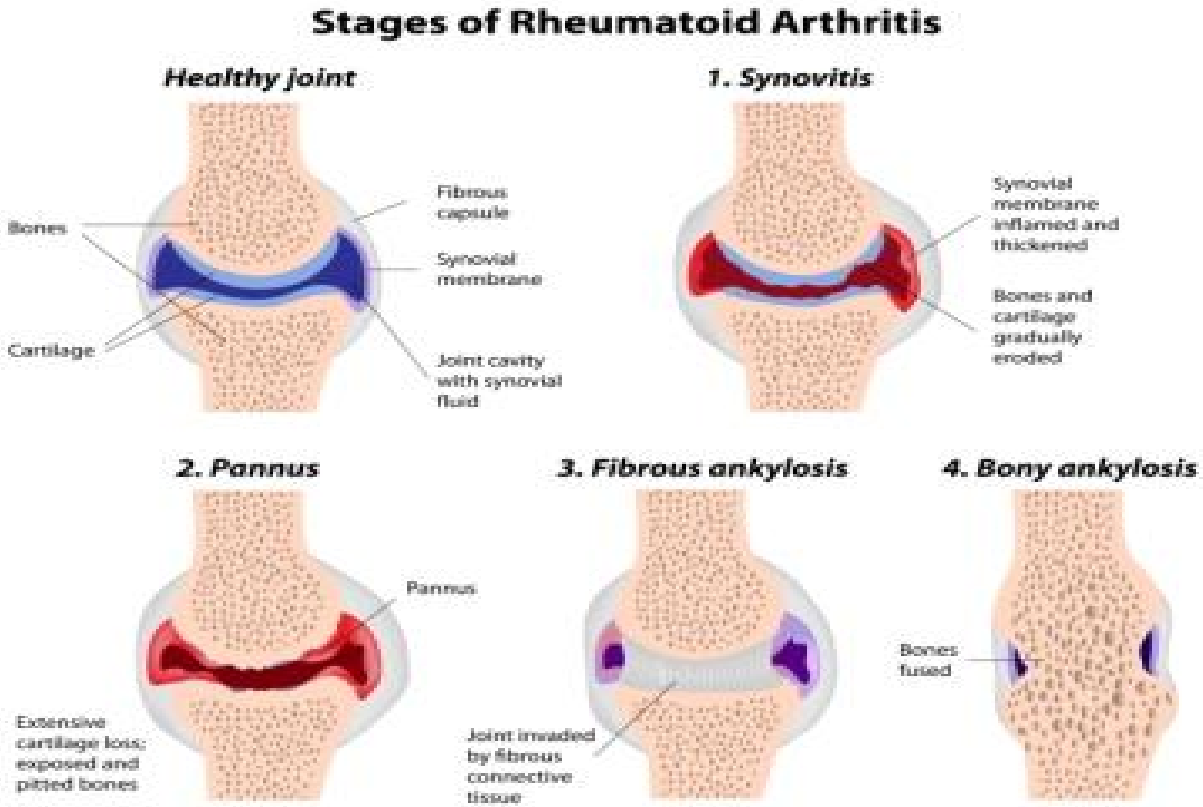

Figure no. 10 Stages of rheumatoid arthritis (17)

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Criteria for the Classification of Rheumatoid Arthritis

For classification purposes, a patient has rheumatoid arthritis if he or she has satisfied at least four of these seven criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with two clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is not to be made.

Morning stiffness

- Morning stiffness in and around the joints
- Lasting at least 1 hour before maximal improvement

Arthritis of three or more joint area

At least three joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician

- The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.

Arthritis of hand joints

At least one area swollen (as defined in 2) in a wrist or in an MCP or PIP joint

Symmetrical arthritis

Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body

- Bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry

Rheumatoid nodules

Subcutaneous nodules over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician

Serum rheumatoid factor-

Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects

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Radiographic changes

Radiographic changes typical of rheumatoid arthritis on poster anterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in, or most marked adjacent to, the involved joints

- Osteoarthritis changes alone do not qualify

Symptom of Rheumatoid Arthritis

In the early stages, people with RA may not initially see redness or swelling in the joints, but they may experience tenderness and pain.

These following joint symptoms are clues to RA:

- Joint pain, tenderness, swelling or stiffness for six weeks or longer
- Morning stiffness for 30 minutes or longer
- More than one joint is affected
- Small joints (wrists, certain joints of the hands and feet) are affected
- The same joints on both sides of the body are affected

Along with pain, many people experience fatigue, loss of appetite and a low-grade fever.The symptoms and effects of RA may come and go. A period of high disease activity (increases in inflammation and other symptoms) is called a flare. A flare can last for days or months.

Ongoing high levels of inflammation can cause problems throughout the body. Here of some ways RA can affect organs and body systems:

- **Eyes.** Dryness, pain, redness, sensitivity to light and impaired vision
- **Mouth.** Dryness and gum irritation or infection
- **Skin.** Rheumatoid nodules – small lumps under the skin over bony areas
- **Lungs.** Inflammation and scarring that can lead to shortness of breath
- **Blood Vessels.** Inflammation of blood vessels that can lead to damage in the nerves, skin and other organs
- **Blood.** Anemia, a lower than normal number of red blood cells

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Pathogenesis of rheumatoid arthritis

Over view of pathogenesis

Rheumatoid arthritis is prolonged and broadminded auto immunological disorder, which is Couse not only contribution of hereditarily defect but other aspect like environmental and life style related factored also join in development of pathogenesis of rheumatoid arthritis. although actual etiology of rheumatoid arthritis unidentified until now(18).HLA-DRB1*04 epitopes are express in 80 % population in arthritis patent, over expression of such allele increase the risk of other complication like nodular arthritis(19). Other RA-associated loci are PTPN22, PADI4, STAT4, TRAF1-C5 and TNFAIP3, although non-MHC risk alleles may represent only 35% of the genetic burden of RA.Chronic infection of bacterial , fungal and smoking also promote the arthritis pathogenesis, bacterial toxin and smoking citrullinet the college ii protein which next become a foreign or antigen of macrophages to resume immunological reaction in body(20 Activated macrophages and dendrical cell ,B cell initiated cytokine and chemokine synthesis which is increase immunological degranulation process from activated cell to release the lysosome enzyme. This enzyme then degrades the articular cartilage of joint Couse joint pain and swelling around. Infiltration of inflammatory cell in synovial membrane of joint causes inflammation and angiogenesis which further differentiate into synovitis of joint, which then increases the lining of synovial membrane and forms villi,osteoclastic rich portion or pannus break down the bones and enzyme synthesis from neutrophils degrade the cartilage (22).

Role of inflammatory cells in RA pathogenesis

Macrophages, dendrical cell, B lymphocyte are the professional antigen presenting cell. All are performancenoteworthy role in innate immunity to present the antigen toward T cell

receptor for indorse immunological reaction to eliminate the antigen from human body .In Rheumatoid arthritis activation of inflammatory cell stimulate cd4 T cell which produce interleukin -2 and IFN-Alfa. Infiltrated INF-Alfa increases monocyte activation, cytokine, prostaglandin synthesis from activated cell with increase matrix's metalloprotein concentration in synovial membrane Couse bone destruction with cartilage degradation also. B lymphocyte has twin role in rheumatoid pathogenesis to first work as APC and then synthesis of plasma cell to produce antibody ,autoantibody and cytokine which help in inflammatory reaction and activation of T-cell and RF production,RF (rheumatoid factor) is auto IgG antibody produce from activated plasma cell in contrast to citrullinated peptide (22).

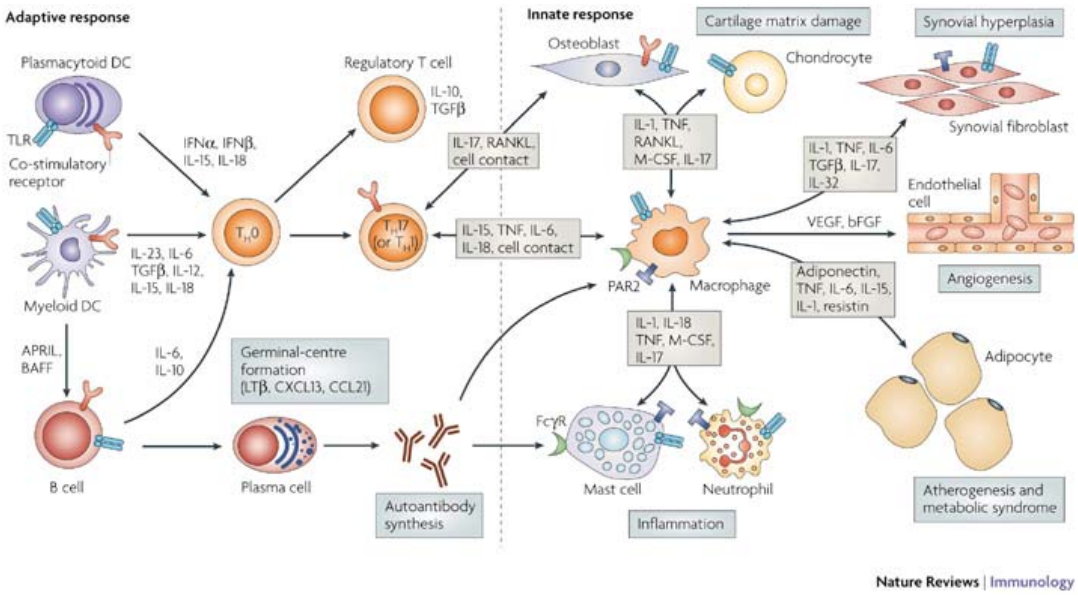

Figure no 11 - Pathogenesis of Rheumatoid arthritis (23,27)

Table No-1: Role of Cytokine in rheumatoid arthritis

Cytokine	**Role in RA pathogenesis**
TNF-Alfa	Local effects Increased monocyte activation, cytokine release, PG release Increased polymorphonuclear leucocyte priming, apoptosis and oxidative burst T-cell apoptosis, clonal regulation, TCR dysfunction ,Increased endothelial cell adhesion molecule expression, cytokine release , Decreased synovial fibroblast proliferation, collagen synthesis Increased MMP and cytokine release
IL-6	Local effects- Osteoclast activation ,Neutrophil recruitment ,Pannus formation via promotion of VEGF production ,B-cell proliferation and antibody production T-cell proliferation and differentiation
	Systemic effects - Acute-phase protein production Anemia (via hepcidin production) CVD promotion ,Osteoporosis ,HPA axis dysregulation (fatigue and depression)
IL-1	Local effects- Increased synovial fibroblast cytokine, chemokine, MMP and PG release Increased monocyte cytokine, reactive oxygen intermediate and PG release , Osteoclast activation Endothelial cell adhesion molecule expression .
	Systemic effects- Acute-phase protein production ,CVD promotion , HPA axis dysregulation (fatigue and depression
IL-17	Recruitment of monocytes and neutrophils by increasing local chemokine production , Facilitation of T-cell infiltration and activation Amplification of

	immune response (e.g. by induction of IL-6 production) Increased synovial fibroblast cytokine and MMP release , Osteoclast genesis and cartilage damage ,Synergistic activity with IL-1b, TNF-a and IFN-g
VEGF	Angiogenesis, contributing to pannus formation

Inflammation

Inflammation of rheumatoid arthritis, TNF-a, IL-6 and IL-1 are key mediators of cell migration and inflammation (24). IL-6, in particular, acts directly on neutrophils through membrane-bound IL-6R, which in turn contributes to inflammation and joint destruction by secreting proteolytic enzymes and reactive oxygen intermediates.

Bone and Cartilage destruction

Bone and cartilage destruction are thebroad-spectrum pathological condition happened in rheumatoid arthritis. Osteoclast is the principle cell of bone originated from mononuclear cell of monocyte from blood, the process of osteoclast formation called oclastognesis.MCSF (macrophage colony stimulating factor) factor essential for osteoclast genesis and RANK and RANKL ligand interaction. Expression of RANKL requires proinflamatory cytokine such as TNF-alfa, IL-1, IL-6 and IL-17 (25).MCSF,IL-11,IL-6 is show significant role in osteoclast formation from peripheral blood monocyte in RANK independent pathway (26).In normal synovial joint synovial membrane has two type of cell which play significant role synthesis of hyaluronic acid and do nourishment to avascular cartilage present in the synovial joint. In rheumatoid arthritis inflammation and infiltration of inflammatory cell into synovial cavity due to which increase proliferation of synovial cell in cavity causes pannus formation. Pannus is the inflammatory process usually observed in rheumatoid arthritis, proliferation of synovial

membrane cell which then enter into the avascular cartilage of joint cartilage. Reduce the synovial cavity space with cell proliferation process stimulate the VEGF, which then initiate the angiogenesis in proliferative cell for nourishment and oxygen transportation in avascular cartilage with increased IL-1 and TNF-Alfa ,IL-6 stimulate proliferative synovial membrane cell to synthesis MMP (matrix metal protein) which then start the degradation of cartilage with new vessel damage leads to swelling and induration with increase infiltration of macrophages in to cavity again increase cartilage degradation.(24,27)

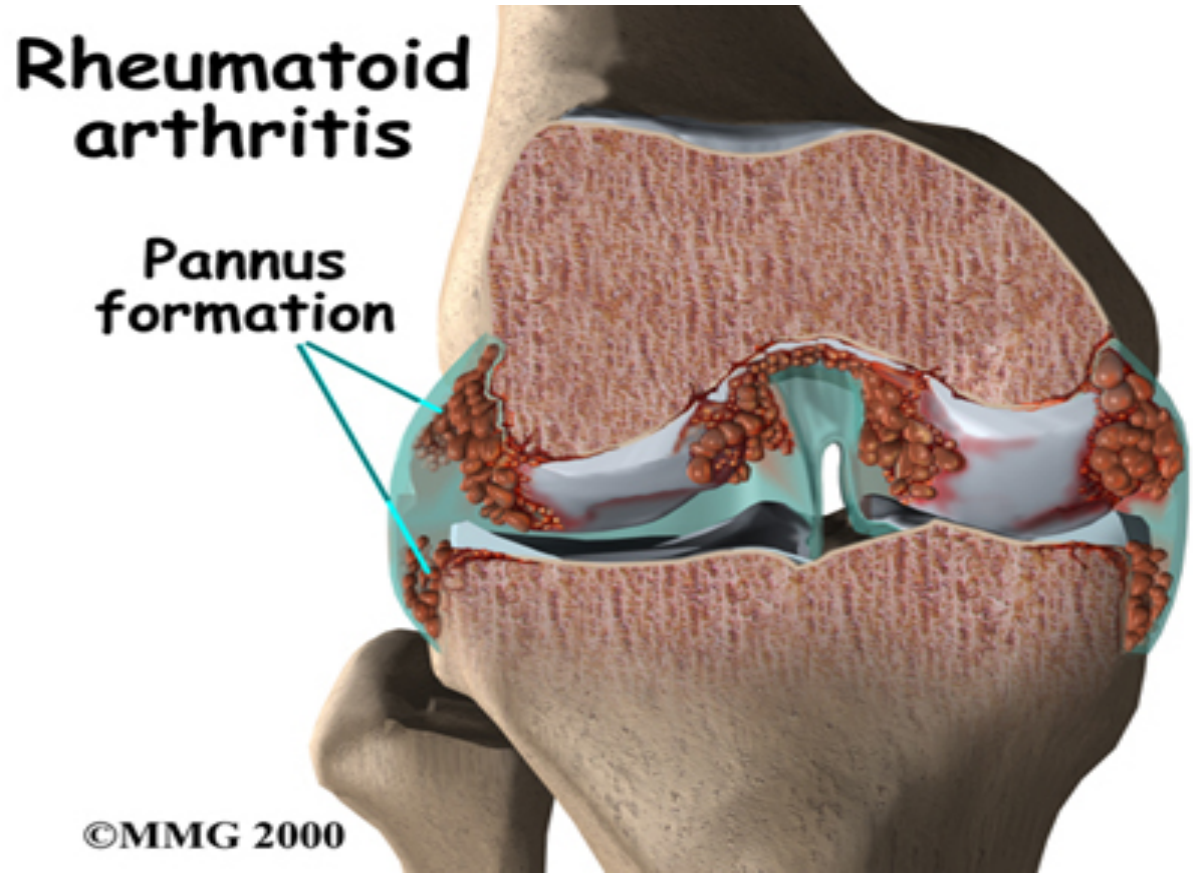

Figure no. 12 - Pannus formation in rheumatoid arthritis(24)

Role of cytokine in secondary complication in rheumatoid arthritis

Chronic inflammatory reaction in rheumatoid arthritis increases the level of cytokine or interleukin IL-6, IL-1, IL-11, TNF-Alfa in circulation of RA patient. All these interleukin and TNF-Alfa have a significant role in development of secondary complication in chronic RA

patient. Anemia, chronic vascular disease (CVD), osteoporosis, depression and fatigue are secondary complication mostly observed in rheumatoid arthritis.(28)

Anemia

Rheumatoid arthritis is the long-lasting inflammatory disease in which IL-6 high concentration of this interleukin has important role in anemia development in rheumatoid arthritis patient(29). Hepcidin its glycoprotein hormone synthesis from hepatocyte its inhibited the iron from macrophages in spleen and iron uptake in the duodenum to decrease the level of iron in circulation. In rheumatoid arthritis high level of IL-6 stimulate the Hepcidin synthesis which further decrease the level of heamoglobulin and iron to develop iron deficiency anemia in rheumatoid arthritis patient (30). High level of IL-6 increase the level of Hepcidin in RA patient with decrease the level of iron.

CVD(Chronic vascular disease)

Vascular disease is the prime secondary complication occurs in rheumatoid arthritis.IL-6 is an interleukin increase the dyslipidemia in rheumatoid arthritis patent increase the atherosclerosis risk. Chronic inflammatory reaction increases the reactive oxygen species in circulation which increase dyslipidemia in which low level of total cholesterol and high density lipoprotein and increase the triglycerides LDL concentration circulation. IL-6

increase the ROS production which increase the oxidized LDL in circulation lead to cause atherosclerosis in rheumatoid patient(31).

Osteoporosis is a mutual systemic manifestation of RA. The increased prevalence observed in this patient population consequently results in an elevated risk of bone fracture(32).

Fatigue and depression

Persistent fatigue and high rates of depression are commonly reported in patients with RA(33). Corticotrophins-releasing hormone, a key regulator of the hypothalamicpituitaryadrenal (HPA) axis and the overall stress system, is associated with fatigue, dysthymia, irritability and depression(34). HPA axis dysregulation has been reported to be caused in part by the release of various cytokines, including TNF-a, IL-1 and IL-6 (24). Thus the fatigue and depression frequently observed in persons with RA are primarily mediated by the up-regulation of cytokines known to be associated with its pathology.

ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

Adenosine deaminase enzyme (ADA)

Adenosine deaminase its ecto enzyme. Play a significant role in purine metabolism. In purine metabolism its convert adenosine or 2-deoxyadenosin into inosine and ammonia.ADA its globular enzyme with a TIM barrel fold consisting of eight parallel beta strand forming a barrel decorated by Alfa helix (35) in past yearit's called as cytoplasmic enzyme but in now scientist has discovered that it's not a cytoplasmic protein it presents on the surface of many cell such as dendrical cell, lymphocyte because of this is called as ecto enzyme(36). ADA has another function in which its help in development of immune cell like t and b lymphocyte activation to produce cytokine and immunoglobulin. Congenital defect in ADA synthesis causes severe combined immunodeficiency in patient with neurological defect, liver problem and skeletal defect also occur in congenital defect in ADA (37).ADA is not a membrane protein so that its require anchoring protein. Three ADA anchoring protein have been discovered. CD26, and adenosine anchoring protein A1 and A28. In T-cell activation in cell mediated immunity ADA has costimulatory role. Its highly present in lymphoid and its concentration increased in chronic immunological disorder like rheumatoid arthritis, pulmonary and non-pulmonary tuberculosis. In rheumatoid arthritis infiltration of inflammatory in to synovial cavity causes cytokine synthesis and t cell activation increases the level of adenosine deaminase in peripheral blood of patient .

Structure of ADA

Adenosine deaminase (ADA) it's a enzyme play a significant role in purine metabolism. It catalyze the hydrolytic deaminase of adenosine or 2-deoxyadenosine to inosine or 2-deoxyinosine and ammonia .human ADA gene consist of 363 amino acid there is in high

degree of amino acid sequence conservation amongst species(38).ADA is globular, soluble and omnipresent enzyme ,with TIM barrel fold consisting of eight parallel beta strand forming a barrel decorated by Alfa helices(39). ADA is ecto enzymes found on the surface of many cell type .it's mostly express on the surface of T cell and dendrical as antigen presenting cell. Ecto ADA is not integral protein because of this they require anchoring protein .there are three anchoring protein has been discovered such as CD26, adenosine receptor A1 and A28 binding with these protein ecto ADA mediate as co stimulatory signaling (40).The congenital defect in ADA enzyme synthesis leads to causes severe combined immunodeficiency (SCID), which is characterized by the absence of functional T and B lymphocyte affected individual, skeletal, neurological and liver abnormalities that occur in some patients may be due to the metabolic disorder, but these are of less clinical relevance than the immunodeficiency

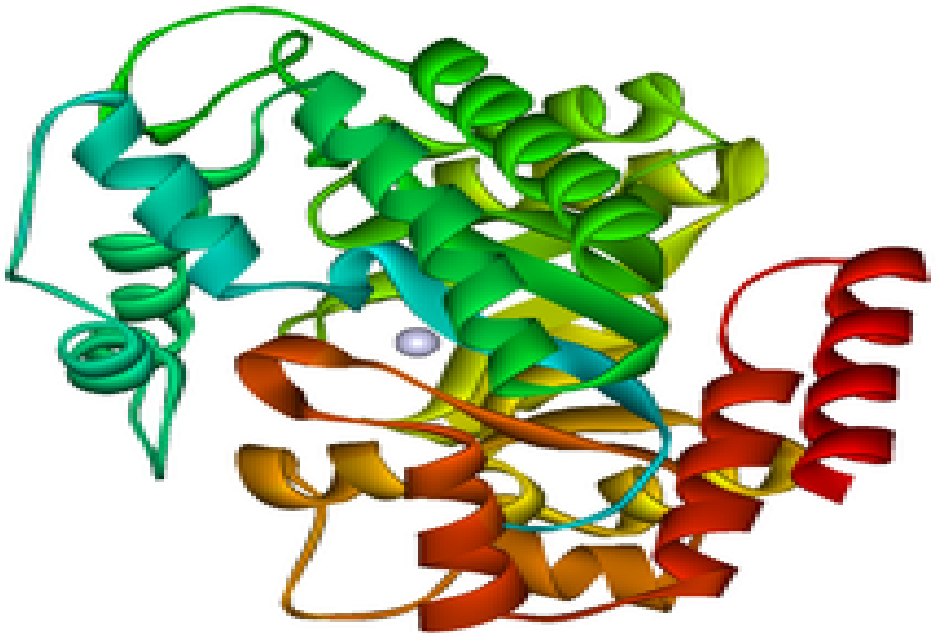

Figure no 13- Structure of adenosine deaminase (39)

ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

ISOFORM

There are 2 isoforms of ADA: ADA1 and ADA2.

- ADA1 is bring into being in most body cells, mainly lymphocytes and macrophages, where it is existing not only in the cytosol and nucleus but also as the ecto- form on the cell membrane attached to dipeptides peptidase-4 (aka, CD26). ADA1 is involved generally in intracellular activity, and be existent both in small form (monomer) and large form (dimer) The interconversion of small to large forms is regulated by a 'conversion factor' in the lung.
- ADA2 was first identified in human spleen (41).It was subsequently found in other tissues including the macrophage where it co-exists with ADA1. The two isoforms regulate the ratio of adenosine to deoxyadenosine potentiating the killing of parasites. ADA2 is found predominantly in the human plasma and serum, and exists solely as a homodimer

Clinical important of ADA

ADA has two isoforms that is ADA1 and ADA2, both are clinically important for the diagnosis of disease. ADA2 is highly present in human blood and its rate increased in different inflammatory disease like rheumatoid arthritis, psoriasis and sarcoidosis. Deficiency of ADA level in blood leads to causes SCID (severe combined immunodeficiency),defective T-cell receptor signaling. Elevated levels of ADA has also been associated with AIDS

Role of ADA in T cell stimulation

ADA has dual role in human body, first its catalyze the adenosine into inosine to preserve the cell of body from cell injure then second its work in the cell mediate immunity as co-

stimulatory in T cell activation.ADA is not a intracellular enzyme its present on the surface of various endothelial cell,epithelial cell then lymphoid cell of human. That's reason is called as ecto enzyme. In T cell stimulation ADA bind with their CD 26 receptor to help in stimulation of T cell for further reaction processes(42). CD 26 is a 110 kDa surface bound ectopeptidase, which bind ADA on the surface of T cell.

Structure of CD26 receptor

Dipeptide peptidase or CD26 its type of trans membrane glycoprotein express s homodimer on the surface of verity of epithelial, endothelial and lymphoid cell. As an exopeptidase it cleaves N terminal dipeptides from polypeptides with proline or alanine in the second last position, thereby regulating at the activity of a variety of biologically important peptides(43). The human CD26 gene encode a protein of 766 amino acid (110kDa) which is anchor to the lipid bilayer by single hydrophilic helix of 23 amino acid located at the n terminals and has short cytosolic tail of 6 amino acid .A flexible stack of 20 amino acid link the membrane anchor to beta –propeller domain which contain eight blade with four antiparallel stand.(44).Seven out of nine glycosylation site are located in the βpropeller domain. The catalytic domain in the C-terminus, which contains two glycosylation sites, spans residues Gln508-Pro766. CD26 belongs to the s9b family of serine proteases. Other members are, fibroblast activation protein (FAP)(15), DPP6(16), DPP8(17), DPP9(18)and DPP10(19). The best studied are CD26 and FAP, sharing a sequence identity of 54%(45). Both are integral membrane proteins and require dimerization for catalytic activity(46). Nucleophile-acid-base (Ser-Asp-His) is the linear order of the catalytic triad in this family of peptidases, an arrangement not common for typical serine type proteases (trypsin or chymotrypsin-like enzymes) but characteristic of the α/β-hydrolase fold(47). The catalytic triad of CD26 is Ser630-Asp708-His740, but other amino acids are also essential for enzymatic activity, such

as Glu205, Glu206, Tyr547 and His750. The highly conserved Glu205Glu206 motif interacts with the free amino terminus of the P2-residue, thus determining the dipeptidyl «amino»peptidase activity of the enzyme, and the point mutations Glu205Lys and/or Glu206Leu abolish enzyme activity(48). The Tyr547, which may stabilize the oxyanion formed in the tetrahedral intermediates by a strong hydrogen bond, is also essential for catalytic activity(44,49). In addition, His750 of CD26 is essential to the dimerization, and its replacement by a negatively charged Glu results in nearly exclusive monomer expression with a 300-fold decrease in catalytic activity(45). An interesting feature of CD26 traffic is its ability to enter into an endocytosis/exocytosis cycle, which involves re-entry into the Golgi apparatus and results in glycosylation changes. This might explain the different forms of CD26 during T cell activation. However, it has been recently demonstrated that none of the nine N-linked glycosylation sites of CD26 contributes significantly to its dimerization and peptidase activity.

Binding of dipeptidyl peptides to adenosine deaminase (ADA)

Dipeptidyl peptidase its type II plasma membrane protein expressed on all mammalian tissue cells as non-covalently linked 210 kDahomodimer. It's also called as CD 26 expressed on the most antigen presenting cells like dendrical cell. As exopeptidase its Cleves the dipeptide coming from polypeptide those having proline or alanine amino acid on their second last position of N terminal peptide chain. Apart from its enzymatic activity CD26play an important role in the cell adhesion and T cell proliferation. Binding of CD26 on their adenosine deaminase receptor which is present on the T cell plasma membrane to avoid binding of adenosine which is generated from adenosine monophosphate and resume the T cell stimulation In the deficiency of adenosine deaminase ,adenosine which produce from AMP bind the adenosine deaminase receptor to inhibit the binding of CD26to its receptor.

ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

Accumulation of adenosine and its derivative become toxins, this toxins then inhibit the proliferation and activation of lymphoid cell. Causes immune disorder .

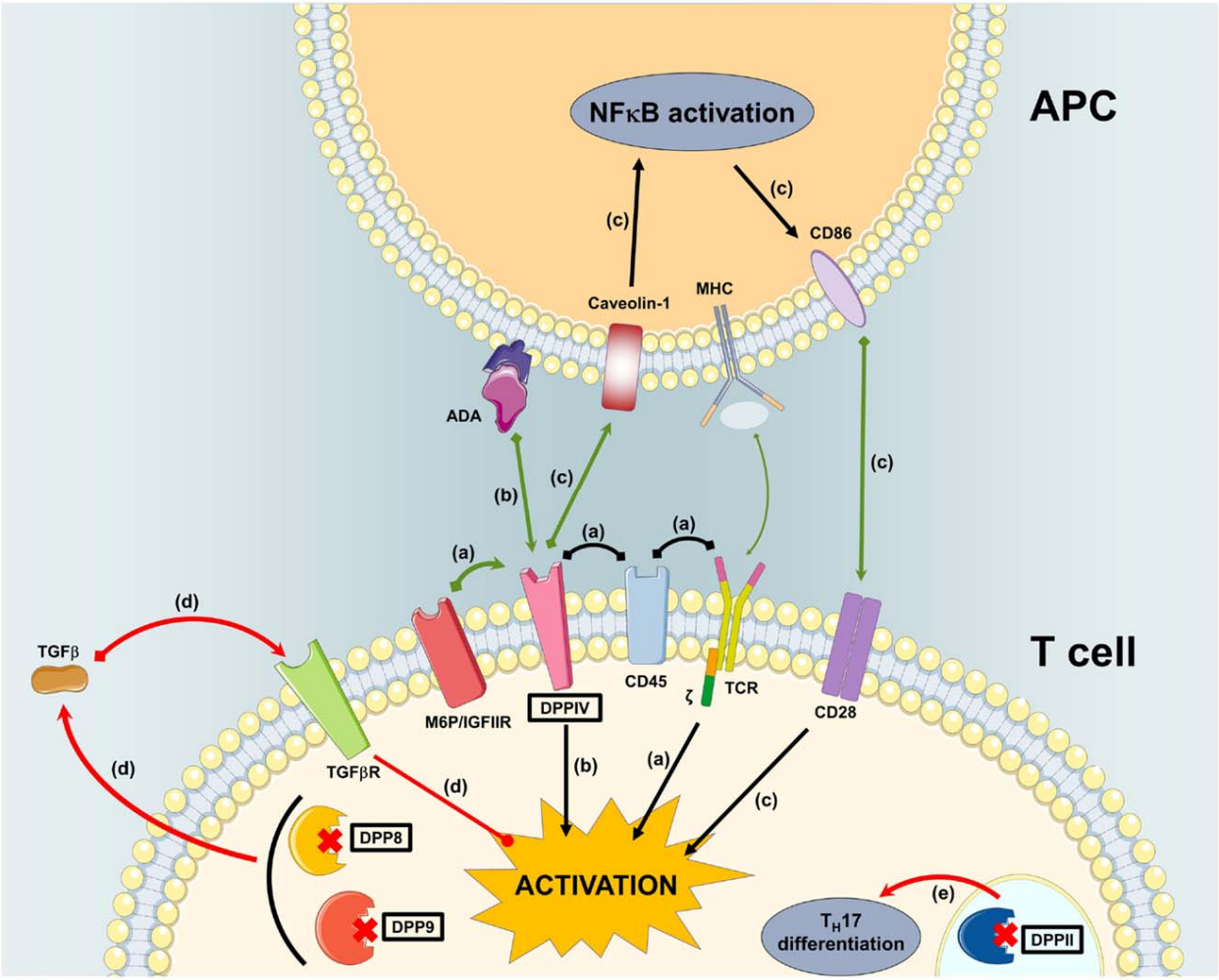

Figure no 14- Binding of ADA and CD26 on T cell to APC (34)

Materials and Methods

The study was conducted at LAB WORLDPLUS LABORATORY BORIVALI. The study comprised of 51patients of rheumatoid arthritis in the age group of 30-60 years who were diagnosed by clinical analysis, rheumatoid factor (Group 1). 51 healthy individual with no known history of any disease matched by age and sex with group 1 were taken as controls (Group 2). After taking informed consent and noting the name, age and sex, venous samples were drawn from both the groups. Serum ADA was estimated by Gusti and Galantimethod(8) of enzymatic analysis with the Pathozyme diagnostics reagent kit. The concentration of ADA and was measured using ErbaChem 7 semi auto analyzer.

Estimation of ADA (Adenosine deaminase)

Aim –estimation of adenosine deaminase in rheumatoid arthritis reactive and non-reactive patient serum sample.

Method- Enzymatic Kinetic Method

Principle -The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP).Hypoxanthine then converted in to uric acid and hydrogen peroxide by xanthine oxidase .Hydrogen peroxidase is further reacted with N-Ethyl-N-(-2hydroxy-3-sulfopropyl)-3-methylaniline and 4-aminoantipyrine in the presence of peroxidase to generate quinine dye which is monitored in a kinetic manner

Reagent composition

Reagent 1: Enzyme Reagent

Reagent2: Substrate Reagent

ADENOSINE DEAMINASE IS THE BIO CHEMICAL MARKER OF RHEUMATOID ARTHRITIS

Material required

-Clean and dry glassware

-Micropipettes and tips

-Bio chemistry Analyser

Sample- Serum sample

Assay Procedure

Reagent 1	360 micro/l
Serum	10 micro/l

Mix and incubate for 3 min at 37 degrees Celsius

Reagent 2	180 micro/l

Mix and aspirate immediately, measure the increase in the absorbance every minute during three minute

Calculation

Delta O.D /min x 1743

Linearity – up to 2000 IU/l

[Test book of clinical chemistry, Ed. By N. W. Tietz, W.B. Saunders Co., Philadelphia (1999][Kobayashi, Ikeda T, Marumo F, Sato C: Adenosine deaminaseisoenzymes in liver disease. Am. J. Gastroenterol, 88; 266-271 (1983)

Results

The results obtained were subjected to descriptive statistical analysis to find the mean, standard deviation, and P value. The statistical analysis was done by SSPS software. The difference between the two groups was done by means of 'T' test for independent mean value.

The statistical analysis is tabulated below:

Table no 2: Statically analysis of ADA level in Rheumatoid Factor reactive and Non-reactive patient

	ADENOSINE DEAMINASE	
	Rheumatoid factor reactive patient	Rheumatoid factor nonreactive patient
Mean	**31.18**	**18.94**
Standard deviation	**6.36**	**5.05**
Student t value	**10.44**	
P value	**<0.00001**	

In this study, significant difference was found between the mean value of serum ADA among RA patients (Table 2) when compared with the control group and this may be due to increase activity of ADA releasing from the damaged cells.

Table No. 3-Concentration ADA and Rheumatoid Factor in Rheumatoid

Arthritis Patients.

Following table contain readings of Adenosine Deaminase and Rheumatoid Factor in Rheumatoid arthritis and Non Rheumatoid Arthritis Patients 'were estimated in "Labworldplus Laboratory". The normal range of ADA was 08-43 IU/L in Serum and normal range of Rheumatoid Factor was 0-20 IU/ml .Figure I shows that concentration of ADA in fifty one Rheumatoid factor non-reactive patient in which 10 IU/L is the lowest concentration and 31IU/L is the highest concentration of ADA founded .Figure II contain concentration of ADA in Rheumatoid Factor reactive patient in which 23 IU/L is the lowest concentration of ADA founded and 42 IU/L is the highest concentration. Figure III contain concentration of ADA comparison in RF reactive and RF non-reactive patents. In which level of ADA concentration in RF reactive patient shows in blue color and ADA concentration in RF non-reactive patient shows in red in color

ADA in RF reactive patient vale in IU/L	ADA in RF non-reactive patient value in IU/L	RF VALUE IN RA PATIENT IN IU/ml	RF VALE IN CONTROL PEOPLE IN IU/ml
23	10	24	12
26	20	30	6
34	15	23	12
39	22	28	8
19	17	29	3
27	13	34	10
25	19	25	13
22	29	20	4
20	25	33	1
30	21	45	11
25	19	37	19
23	14	39	12
32	26	45	17
34	23	26	14
39	31	31	6
27	12	26	4
34	15	38	5
23	19	24	12
34	24	34	18
23	28	30	16
27	22	33	5

33	15	33	3
39	19	39	14
42	24	35	16
35	21	36	17
29	19	28	17
36	17	24	13
33	15	27	4
27	22	32	3
28	16	39	13
25	19	36	1
30	23	42	10
27	20	44	8
39	16	36	11
42	13	26	15
40	23	34	13
34	16	20	18
38	9	29	19
26	11	23	17
30	18	25	15
36	18	30	9
24	15	36	4
33	22	30	10
36	27	28	3
39	23	28	17
40	20	22	13
38	15	28	16
23	13	33	19
29	24	39	15
42	10	35	12

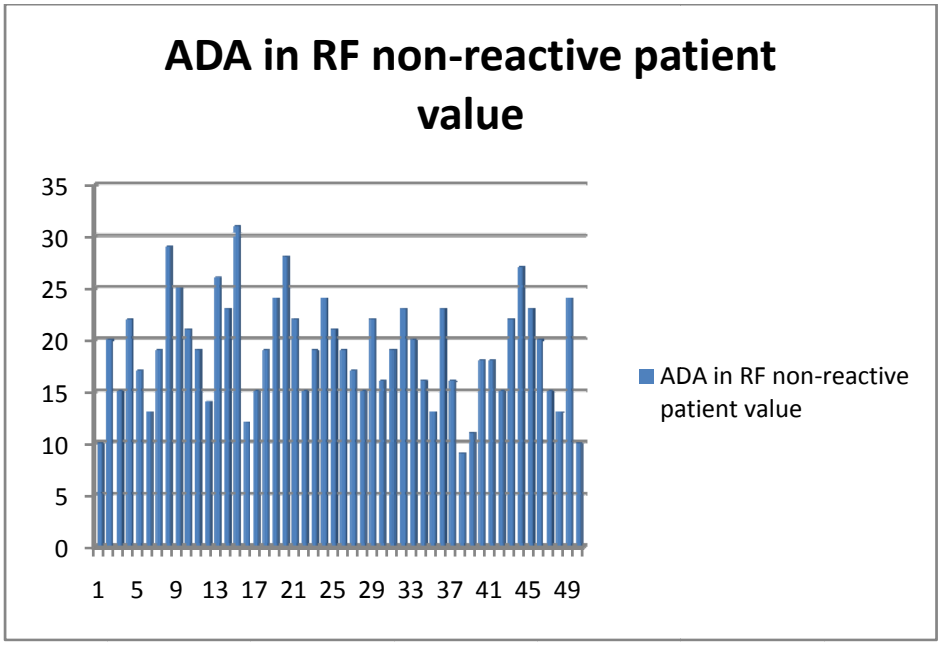

Figure I – ADA in RF non-reactive patient value in bar chart (Table no. 3)

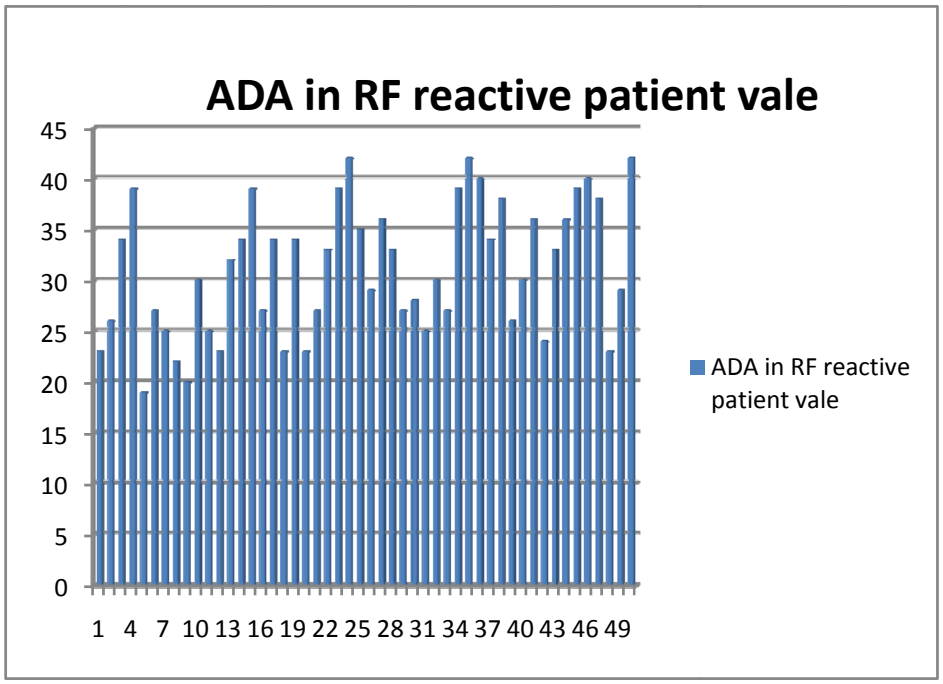

Figure II – ADA in RF reactive patient vale in bar chart (Table no. 3)

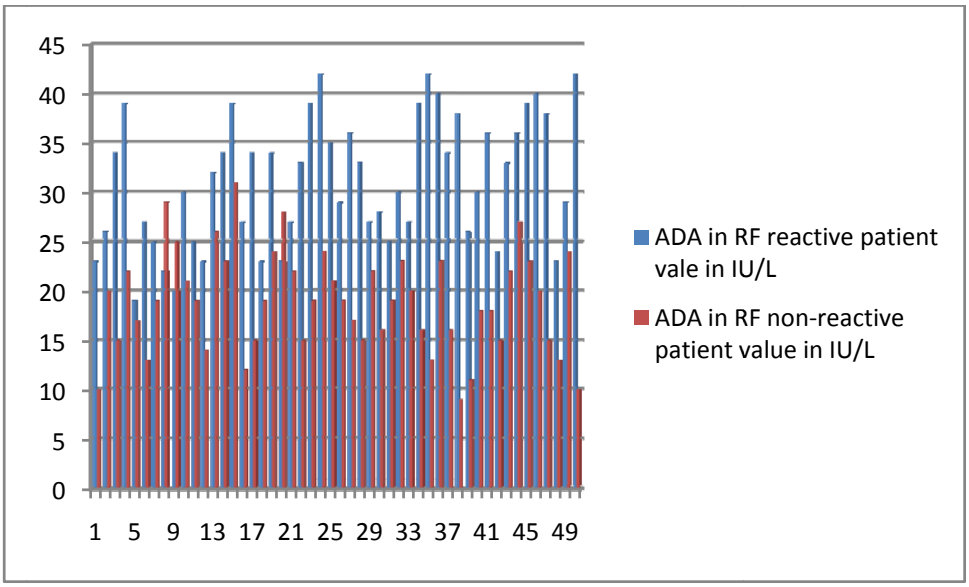

Figure III – ADA value in RF reactive and non reactive patient (Table no. 3)

Discussion and Conclusion

Rheumatoid arthritis is a prolonged inflammatory progressive disease. Inrheumatoid arthritis innate immunity and cell mediated immunity show a joint venture role to initiate the inflammation in rheumatoid arthritis. Intruded inflammatory cell in to the synovial cavity yield proinflamatory cytokine such as Interleukin 6 and tissue necroting factor which are then encourage the T cell activation process.ADA is the ecto enzyme present on the peripheral of lymphoid cell of human. ADA playsa dynamic role in T and B cell development and also defends them from toxin which is generated from adenosine when its pass in T cell through adenosine deaminase receptor. Diagnoses of rheumatoid arthritis adenosine deaminase enzyme play an important starring role. Level of adenosine deaminase is surge in rheumatoid arthritis because of ADA is ecto enzyme present on T cell and assistance in T cell stimulation, in T cell stimulation ADA performance an important role as co-stimulatory in binding of antigen presenting cell expressed MHC molecule holding antigenic peptide to T cell for proliferation of them and elimination antigen from blood circulation. High activated T cell from inflammatory cell in rheumatoid arthritis rise the level of Adenosine deaminase in body.In our initial level study ,which was held in labworlplus laboratory we were analyzed ADA level in already diagnosed rheumatoid arthritis patient shows that higher value of ADA but within biological reference range were observed as compare to other same number of healthy control patient without any disease history.ADA level increased in rheumatoid arthritis due to activation cell mediated cell immunity and cellular death increases the level of ADA in arthritis patient

Conclusion

During recent years, a huge number of potential biomarkers for the diagnosis RA have been investigated but only ADA is reliable, cost-effective and currently tested routinely in clinical practice. During inflammations of RA, ADA is released in extra cellular location, resulting in the considerable increase of its activity by activating CMI, which ultimately help to better understanding of some pathophysiological aspects of a disease and may help to relieve the triggering factors of inflammation and promote new therapeutic approaches.

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