MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY

Dissertation submitted to

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the award of the degree of

DOCTOR OF MEDICINE

IN

PHARMACOLOGY

INSTITUTE OF PHARMACOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003

APRIL 2016

CERTIFICATE

This is to certify that the dissertation entitled, **"MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY"** submitted by DR.A.GOMATHI, in partial fulfillment for the award of the degree of Doctor of Medicine in Pharmacology by The Tamilnadu Dr.M.G.R.Medical University, Chennai is a Bonafide record of the work done by her in the Institute of Pharmacology, Madras Medical College during the academic year 2013-16.

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CERTIFICATE OF THE GUIDE

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Place:

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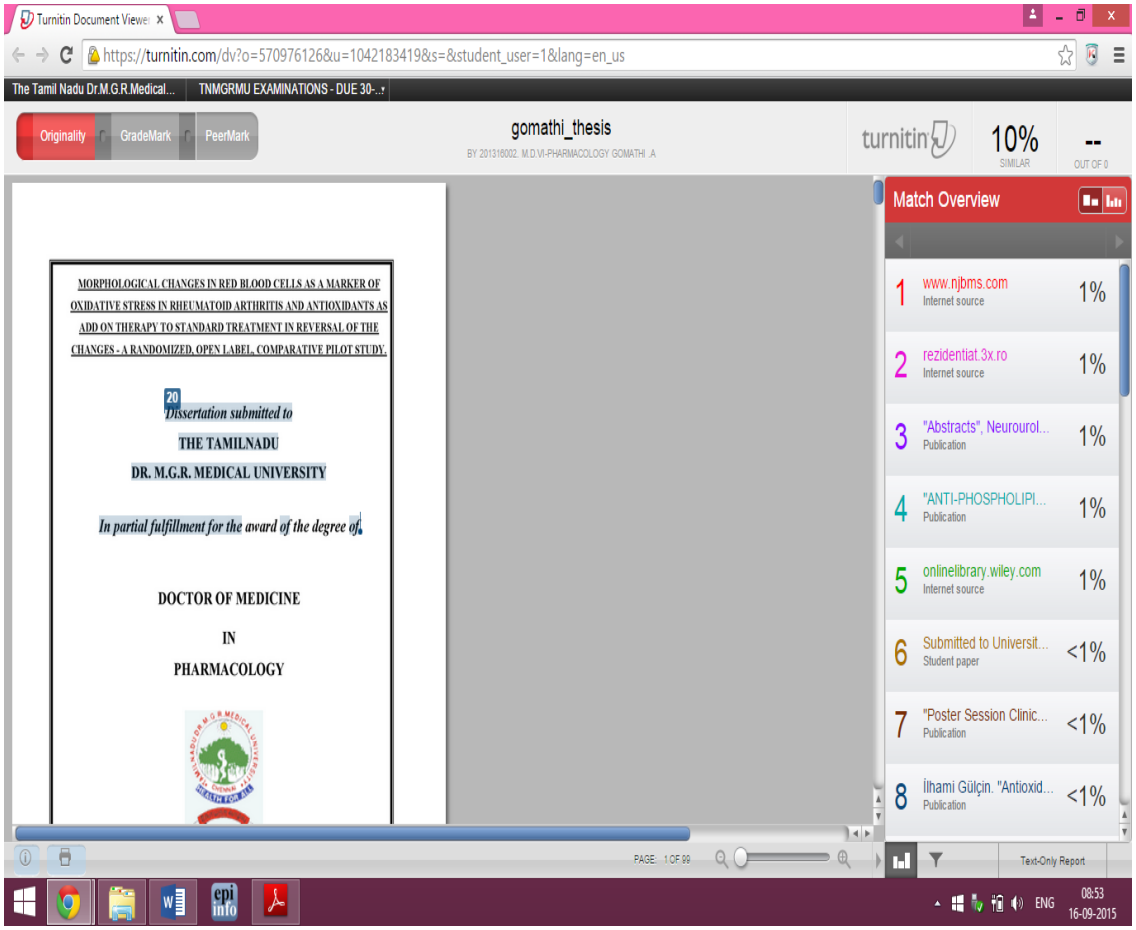
DECLARATION

I, Dr.A.GOMATHI solemnly declare that the dissertation titled "**MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY"** has been prepared by me and submitted to TN Dr.MGR Medical University, Chennai in partial fulfilment of the rules and regulations for the M.D degree examination in Pharmacology.

Date: Dr.A.GOMATHI

Place:

TURNITIN ANTI-PLAGIARISM SOFTWARE – CERTIFICATE



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ABBREVIATIONS

ACR – American College of Rheumatism

ACP – Anticyclic Citrullinated Protein Antibody

COX – Cyclooxygenase

CD4 – Cluster Differentiation

CRP – C- Reactive Protein

CYP4F2 – Cytochrome 4F2

CYP 3A – Cytochrome 3A

DNA – Deoxyribonucleic acid

DIP – Distal Interphalangeal Joint

DMARDS – Disease Modifying Anti Rheumatoid Drugs

EBV – Ebstein Barr Virus

ESR – Erythrocyte Sedimentation Rate

GLUT -1 –Glucose Transporter -1

GSH – Glutathione

HLA – Human Leucocyte Antigen

H2O2 – Hydrogen Peroxide

IL – 1 – Interleukin -1

LDL – Low Density Lipoprotein

MHC – Major Histocompatibility Complex

MRI – Magnetic Resonance Imaging

MCP –Metacarpophalangeal Joint

MTP – Metatarsophalangeal joint

NSAIDS – Nonsteroidal anti-inflammatory drugs

NO – Nitric oxide

0_2^- - Superoxide

OH – Hydroxyl radical

PIP – Proximal interphalangeal joint

PDA – Patent ductus arteriosus

PUFA – Poly Unsaturated Fatty Acid

RA – Rheumatoid Arthritis

RBC – Red blood cell

ROS -Reactive oxygen specisis

RF – Rheumatoid Factor

TNF–α – Tumour necrosis factor – α

WBC – White blood cell

CONTENTS

MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY.

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ABSTRACT

AIM:

To evaluate morphological changes in red blood cells due to oxidative stress in patients with Rheumatoid Arthritis and to study the efficacy of Antioxidants(Vitamin C and E) as an add on therapy to standard treatment in the management of these patients compared to standard treatment alone.

METHODOLOGY:

This was an open label randomised comparative pilot study. In this study 96 patients were screened, 60 patients were included. They were randomly divided into 30 each in study and control group. Control group received T. Hydroxy chloroquine 400mg OD and T. Indomethacin 25 mg BD (standard treatment), study group received standard treatment plus T. Vitamin C 500mg + C. Vitamin E 400mg for 8 weeks. They were followed for 4 weeks. Improvement of patients was monitored by RBC morphological assessment, pain by visual analogue scale, tender joint score, swollen joint score,

Disease activity score 28, Inflammatory markers (ESR, CRP), and haemoglobin level every 4 weeks till 12 weeks.

RESULTS:

All the 60 patients included in this study completed the study. After 8 weeks of treatment in the study group there is statistically significant improvement in pain score, tender joint score, swollen joint score and DAS score. Similarly, statistically significant reduction in inflammatory markers like ESR, CRP and significant improvement in Haemoglobin level were seen. In the follow up period the improvement in study group was sustained.

CONCLUSION:

RBC morphology as a biomarker of oxidative stress and adding antioxidant to regular treatment is cost effective and novel approach to the treatment of Rheumatoid arthritis.

KEY WORDS:

Rheumatoid Arthritis, Oxidative Stress, Anti-Oxidants.

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory symmetrical arthritis mostly involving smaller joints with systemic extra articular manifestations involving many organs. [1]

RA is a relatively common disease with prevalence of 1% and more in women than men. Peak incidence is between 2nd and 4th decade of life but there is no age bar.[2] Once initiated, the chronic inflammation in the synovial tissue causes progressive joint destruction and deformity in the limbs, resulting in deterioration of quality of life in the individual.[3]

The exact aetiology is not known, but autoimmunity plays a major role in the pathogenesis. Some of the environmental factors that probably triggers the immune system are cigarette smoke, emission from automobile, radiation and products of bacterial and viral infections etc.in a genetically prone person.[4]

Presence of high level of Nitric oxide (NO) in rheumatoid synovial fluid suggest that oxidative stress initiates the destruction of synoviocytes. [5]

The marker for oxidative stress like Thioredoxin, the products of lipid peroxidation in the synovial fluid are significantly high in RA patients compared to other forms of arthritis.[4],[6] The current treatment involves physical rest, anti-inflammatory agents and Disease Modifying Anti Rheumatoid Drugs (DMARDS). This treatment does not arrest the progress of disease. [7]

Since free radical injury is one of the triggering factor in RA[8] ,addition of antioxidant like Vitamin E and vitamin C can be added to the DMARDS to achieve better goal in the treatment of RA in future.

REVIEW OF

LITERATURE

LITERATURE REVIEW

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a common chronic systemic inflammatory disease mainly involving smaller joints of hands and also other organ systems. The common symptoms include stiffness of joints, pain, swelling in the joints. [2]

ETIOLOGY:

Even though the exact cause for RA remains unknown, autoimmunity plays an important role for its progression and chronicity. [9]

AGE:

RA occurs at an age group with increasing prevalence up to the 7^{th} decade of life. Even then the most common age group affected are 4^{th}-5^{th} decade of life.[2]

GENDER:

Women are commonly affected than men in the ratio of 3:1. After the menopause incidences are equal in both men and women which indicate the role of sex hormone in the aetiology.[2]

RACE:

RA is present throughout the world in all racial groups. But the prevalence is more in Pima Indians in North America (5%) and lower in Black Africans and Chinese (0.3%). [2]

GENETICS:

The risk for RA increases 9 fold in the presence of positive family history. It is more frequently associated with HLA DR4 and HLA DR1 antigen in the MHC region. HLA DR4 patients are more prone for erosive joint disease and have poor prognosis.[4]

AUTOANTIBODIES:

The commonly associated antibodies in RA are Rheumatoid factor (RF) which is an antibody against Fc portion of immunoglobulin and Anticyclic citrullinated protein antibodies (ACPA). These antibodies causes synovial inflammation through complement pathway. [10]

SMOKING:

Cigarette smoking is the strongest risk factor for ACPA positive and RF positive RA. Depending upon the intensity (no of cigarettes/day) and duration of smoking, the risk for RA increases. Smoking induces citrullination of some Self proteins creating a new epitopes that induce autoimmune reactions. [2]

SOCIECONOMIC STATUS:

People of poor socioeconomic status are more susceptible to RA.[1]

OCCUPATION:

Risk for RA is 20% more in manual labourer than others. Susceptible labourer include coalminer, granite workers, fish industry workers, workers exposed to silica and other organic solvents.[1]

INFECTIOUS AGENT:

Pathogenesis through single infectious etiological agent is not proved. However pathogens like Retro virus, EBV, Mycoplasma, Mycobacteria etc. are associated with RA as a triggering agent to autoimmune response.[2]

OXIDATIVE STRESS:

The disturbance in equilibrium between free radicals and antioxidant defence mechanism produce oxidative stress which induces the Autoimmunity and causing inflammation in the joint. [4],[11]

Various studies indicates that dietary deficiency of Vitamin E, Vitamin C selenium, and copper is associated with increased risk of RA.[6],[12]

PATHOGENESIS:

The phases of RA inflammation:

1. Initiation phase of nonspecific inflammation.

2. Amplification phase resulting from T cell activation.

3. Promotion of inflammation by activated T cell by stimulating T cell and B cell.

4. Stage of chronic inflammation with tissue injury. [2],[13]

PATHOGENESIS OF INFLAMMATORY RESPONSE:

Antigen is phagocytosed by antigen presenting cell. This complex is presented to T lymphocyte which attaches the antigen to MHC portion of cell wall causing its activation.

Activated T cell stimulates T cell and B cell production, there by promoting further inflammation.

Activated T cell produce cytokine which are toxic to tissue and also stimulate further inflammation by stimulating macrophages.

Activated B cell produce plasma cell leading to production of antibodies. The antibodies combines with complement causing accumulation of poly morphonuclear leucocytes which release cytokines, oxygen free radicals hydroxyl radical causing cellular damage to synovium and chondrocytes.[3]

The main reactive oxygen species produced by chondrocytes are nitric oxide (NO) and superoxide radical (O_2^-). The free radicals damage chondrocytes and extracellular matrix of articular cartilage and induce inflammation. [11], [14]

PATHOGENESIS OF RHEUMATOID ARTHRITIS:

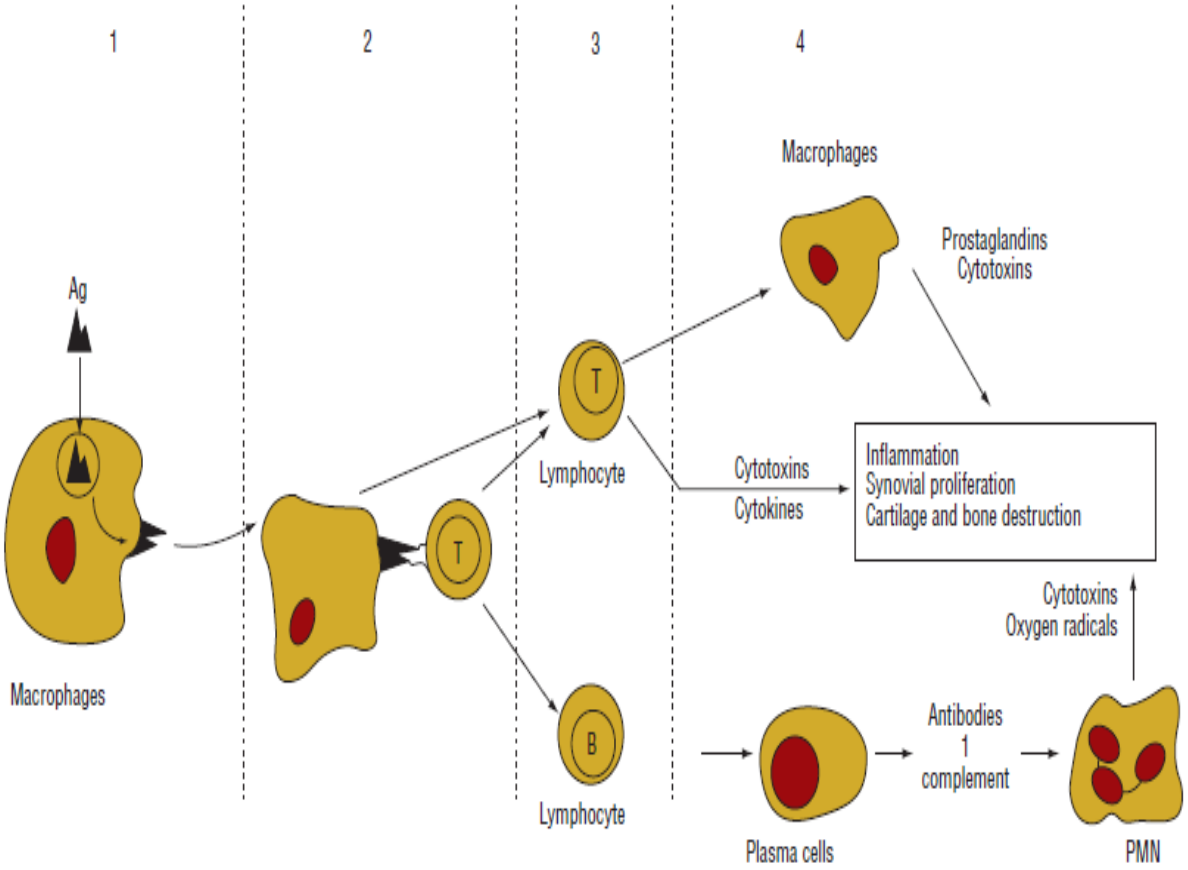


FIGURE 94-1. Pathogenesis of the inflammatory response. Phase 1: Antigen-presenting cell phagocytizes antigen. Phase 2: Antigen is presented to a T lymphocyte. The T lymphocyte attaches to antigen at the major histocompatibility complex portion of cell wall causing activation. Phase 3: An activated T cell stimulates T and B lymphocyte production, promoting inflammation. Phase 4: Activated T cells and macrophages release factors that promote tissue destruction, increase blood flow, and result in cellular invasion of synovial tissue and joint fluid. (Ag, antigen; PMN, polymorphonuclear leukocyte.)

MORPHOLOGY:

RA causes broad spectrum morphological changes. The synovium of joint is thickened oedematous and hyperplastic, transforming its smooth contour to bulbous form.

The characteristic histological findings are,

1. Synovial cell proliferation and hyperplasia

2. Increased vascularity due to angiogenesis

3. Dense perivascular cell infiltrate mainly CD4Tcell, macrophages, plasma cell

4. Neutrophil and fibrin aggregates on the surface of synovium

5. Increased osteoclastic activity

6. Pannus formation

Mass of synovium and stroma with granular tissue, fibroblast and inflammatory cell grows and erode the cartilage. After destruction of cartilage, pannus cause ankylolysis.[9]

SKIN:

Rheumatoid nodules are seen in the extensor surfaces of forearm. They present as firm, non-tender, round mass. Microscopically they have a central zone of fibrinoid necrosis surrounded by macrophages, granulation tissue and lymphocytes.[9]

BLOOD VESSEL:

Rheumatoid vasculitis is the dangerous complication of RA. It affects medium and small arteries except kidney. By obliterating the end arteries, it results in peripheral neuropathy, gangrene and ulcer. Leucocytic vasculitis produce cutaneous ulcer, purpura, and nail bed infarct.[9]

CLINICAL FEATURES:

ONSET:

The clinical course RA is variable. In 50% of cases it occurs gradually with initial prodromal symptoms of fatigue, anorexia, and musculoskeletal symptoms which last for weeks and months. Involvement of joints occur later. In 10% cases it occurs abruptly with rapid onset of polyarthritis.[13]

ARTICULAR INVOLEMENT:

Synovial inflammation causes pain, swelling, tenderness, stiffness and limitation of movement. Pain is the common symptom which increases more on movement.

Joint stiffness >1 hour is characteristic feature of RA. Accumulation of synovial fluid, thickened joint capsule, hypertrophied synovium causes joint swelling.

RA can affect any joint. The joints commonly affected are metacarpo phalangeal joint, proximal interphalangeal joint, wrist, ankle, knee metatarsophalangeal joint, shoulder, hip, elbow and upper cervical spine.

Symmetrical synovitis of PIP and MCP joints of hands and sparing of DIP is the characteristic feature of RA.

Involvement of upper cervical spine cause atlanto-axial subluxation which leads to spinal cord compression and death.

Persistent inflammation of joints leads to weakness of ligaments, tendon and joint capsule and degradation of cartilage which further leads to characteristic deformities like,

1. Radial deviation of wrist and ulnar deviation of digits with palmar subluxation of proximal phalanges. (Z deformity).

2. Flexion contracture of PIP and extension of DIP (Boutonniere deformity).

3. Hyper extension of PIP and flexion of DIP (Swan neck deformity).

4. Eversion of hind foot.

5. Widening of fore foot and hallux valgus.

6. Lateral deviation and dorsal subluxation of toes.[15]

EXTRA ARTICULAR MANIFESTATION:

1. Rheumatoid nodules
2. Rheumatoid vasculitis
3. Ocular involvement – Episcleritis, Scleritis, and keratoconjunctivitis sicca
4. Heart – Pericarditis, conduction abnormality, aortic valve incompetence
5. Pleuropulmonary manifestation – Pleural and Pulmonary fibrosis, interstitial pneumonitis, Pleuropulmanory nodules

6. CNS – proliferative synovitis and joint deformities produce neuropathy of median, ulnar, radial and anterior tibial nerve.
7. Lymph nodes – Enlarged
8. Muscles – Muscle atrophy, myositis, focal necrosis and rheumatoid nodule
9. Bone – osteoporosis
10. Others – Felty syndrome, amyloidosis, secondary Siogren syndrome[16]

COMPLICATIONS:

1. Ruptured tendon
2. Septic arthritis
3. Ruptured joint (Baker's cyst)
4. Amyloidosis
5. Spinal cord compression
6. Side effects of therapy[16]

LABORATORY INVESTIGATIONS:

There is no specific test for the diagnosis of RA.

1. RHEUMATOID FACTOR (RF):

It is an antibody specific for Ig M and present in 60-70% of patients with RA. It is not a specific test for RA because it is present in other conditions also. But it has a prognostic significance. Patient with high titre have more severe progression of disease with extra articular manifestations.[13]

2. ANTICYCLIC CITRULLINATED PROTEIN ANTIBODIES(ACCP) :

It is present in 50- 80 % of patients with RA. It is the specific test (90-95 %) and it is detectable in the very early stage of the disease.[13]

3. ACUTE PHASE REACTANT :

I) ERYTHROCTE SEDIMENTATION RATE (ESR):

It is a nonspecific test and increased in all patients with RA. Its level falls in response to therapy and has a prognostic significance

II) C-REACTIVE PROTEIN (CRP):

Increased CRP level indicates the severity of disease activity and prognosis of joint damage. [13]

4. **HEMATOLOGICAL ABNORMALITY:**

Anaemia in RA correlates with the disease activity. The cause of anaemia is not correctly understood. Some of the probable cause for anaemia in RA are

1. Anaemia of chronic disease associated with high serum ferritin concentration

2. Poor response to erythropoietin

3. Drug induced megaloblastic anaemia due to methotrexate and NSAIDS administration

Thrombocytosis is often associated with RA along with anaemia. [17]

5. SYNOVIAL FLUID ANALYSIS :

Although none of the findings is specific to confirm the presence of inflammatory arthritis. The characteristic of synovial fluids are turbid, decreased viscosity, increased protein, decreased or normal glucose, decreased C3, C4,WBC count >2000/µL.[13]

6. IMAGING TECHNIQUE :

1. RADIOGRAPHIC TECHNIQUE:

In the early disease radiographic findings are not much useful for the diagnosis, they only reveal soft tissue swelling and joint effusion.

As the disease progress, radiographic findings shows the extent of cartilage destruction and bone erosions and estimate the aggressive nature of the disease and shows the impact of therapy.

Characteristic X-ray findings are

1. Soft tissue swelling
2. Periarticular osteoporosis
3. Decreased joint space
4. Erosion of bone [13]

2. 99Tc Bisphosphonate scan and MRI:

More valuable in detecting the early inflammatory changes in joint tissue, but not routinely indicated. [13]

DIAGNOSIS:

Diagnosis of RA depends mainly on clinical features and exclusion of other inflammatory arthritis. If the initial symptoms are nonspecific, diagnosis becomes very difficult in the early stage.

Now the diagnosis is mainly based on Revised American college of Rheumatology criteria

The 1987 revised ACR criteria for RA

1. Arthritis of 3 or more joints: out of 14 joints (Rt or Lt MCP, PIP, elbow, ankle, knee, MTP joint) at least 3 joints have soft tissue swelling,
2. Morning stiffness;

   Stiffness present in and around the joint which last for at least 1hour before maximal improvement
3. Arthritis of joints in hand – at least one of PIP, MCP, Wrist
4. Symmetrical arthritis
5. Rheumatoid nodules – subcutaneous nodules over bony prominence, extensor surfaces or juxta articular regions

5. Serum rheumatoid factor

6. Radiographic changes – the postero anterior view of hand and wrist joints shows erosion or unequivocal decalcification of bone localized to or most marked in involved joint.

Out 7 criteria 4 should be there to classify the patient as having RA.[13]

DIFFERENTIAL DIAGNOSIS:

1. Sero negative spondylo arthropathy
2. Post viral arthritis
3. Polymyalgia Rheumatica
4. Acute osteoarthritis[13]

CLINICAL COURSE AND PROGNOSIS:

The course of RA is difficult to predict and variable in individual. Most patient will have persistent but fluctuating disease with variable degree of joint abnormality and functional disability.

Within 10 years - 50% have work disability

Within 20 years –80% will be severely disabled and 20% require joint replacement

In RA median life expectancy was shortened by 3- 7years and 2.5 fold increased risk of mortality was seen in patient with severe articular disease.

Prognostic factor indicating more chance of developing joint deformities are

1. Presence of >20 inflammed joints
2. Rheumatoid nodules
3. Increased ESR
4. High titre of serum Rheumatoid factor
5. X- ray evidence of bony erosion
6. Persistent inflammation
7. Advanced age at the onset

8. Presence of comorbid condition
9. Presence of HLA DR4 [15]

MANAGEMENT:

The main aim of RA management is to improve or maintain functional status thereby improving quality of life. The ultimate goal is to achieve complete remission of disease.

Management of RA involves an interdisciplinary approach.

1. **NON PHARMACOLOGICAL THERAPY**
    a. Rest
    b. Weight reduction
    c. Physical therapy
    d. Occupational therapy
    e. Use of assisted device
2. **PHARMACOLOGICAL THERAPY**
    a. NSAIDs –Diclofenc, Indomethacin etc.
    b. DMARDS-Methotrexate, Hydroxy chloroquine etc
    c. Corticosteroids -Prednisolone
    d. Biological agent –Etanercept. Anakinra etc
    e. Immunosuppressant – Cyclosporin, Azathioprine
3. **SURGICAL THERAPY** [13]

PHARMACOLOGICAL THERAPY:

NSAIDs:

They provide symptomatic relief by reducing pain and stiffness. They reduce the inflammation by inhibiting COX enzymes which is required for the synthesis of prostaglandin a major mediator of inflammation.

The NSAIDs commonly used are Indomethacin, Aspirin, Diclofenac, Ibuprofen, selective COX 2 inhibitors like Celecoxib, Etoricoxib. [18]

DMARDS:

They have main role in the management of RA. They act by inhibiting cytokine, thereby reducing the inflammation, reduction of joint swelling and joint erosion and cause fall in acute phase reactant.

They should be started singly or in combination before the appearance of erosion in bone. Early introduction of DMARDs reduce the mortality rate. The beneficial effects are not produced immediately and it takes weeks or months for their effect.

Commonly used DMARDs are Methotrexate, Hydroxy chloroquine, Sulphasalazine, other DMARD rarely used are Gold and Pencillamine.[19]

CORTICOSTEROIDS:

The use of Corticosteroids in RA was first identified by Prof. Philip Showalter Hench, for which he was awarded the Nobel Prize in 1950. Due to their immunosuppressant and anti-inflammatory property, they provide rapid control of

disease. They are given as early intensive short term regimen to induce remission or intramuscular depot preparation to control the disease.

They are also used in long term at very low doses 5mg/day or less described as physiological dose along with other therapy, as RA patients don't make normal glucocorticoid response to stress. [19]

BIOLOGICAL AGENT:

These are recombinant monoclonal antibodies which bind and inhibit cytokines mainly IL-1 and TNF-α thereby preventing further inflammation. Compared to other DMARDs they produce quicker response. Biological agents are mainly indicated for rapidly progressive disease and severe refractory cases. Major drawback of these agents are expensive and serious adverse effect.

Commonly used agents are

1. TNF-α inhibitors – Etanercept, Infliximab, Adalimumab
2. IL- 1 agents – Anakinra, Abatacept [20]

IMMUNOSUPPRESSIVE AGENTS:

They produce the therapeutic effects similar to DMARDs. Due to variety of toxic effects they are indicated in

1. Patient who have failed therapy with DMARDs and biological agents
2. Patient with extra articular disease like Rheumatoid vasculitis

Commonly used immunosuppressant are Leflunamide, Azathioprine, Cyclosporine, and Cyclophosphamide. [20]

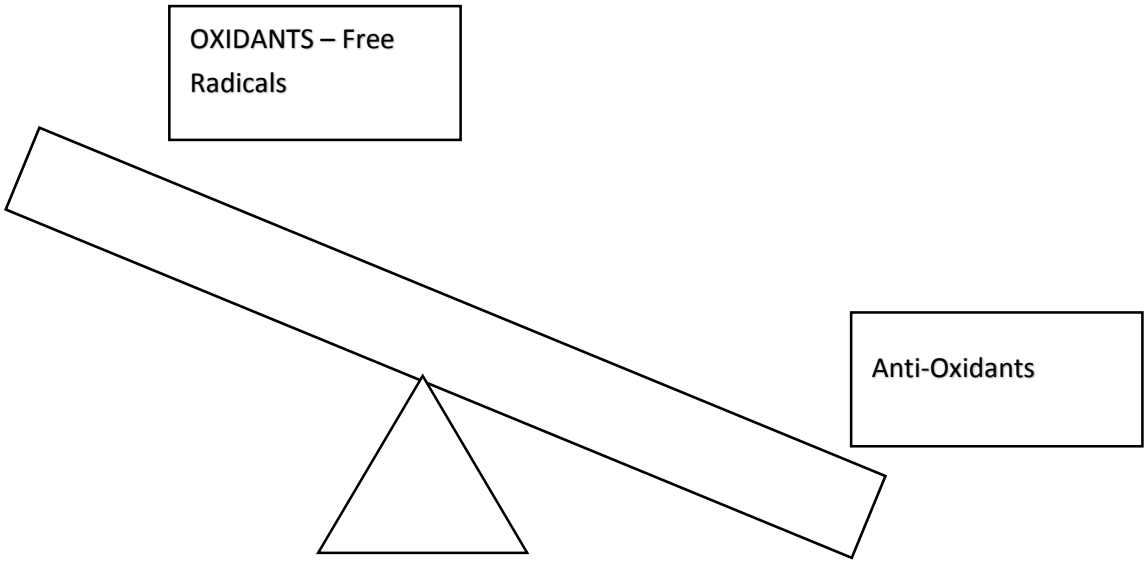
SURGICAL THERAPY:

In severely damaged joints surgical procedure are done to relieve the pain and to reduce the disability. Commonly used procedures are,

1. Reconstructive hand surgery – cosmetic improvement and functional benefit
2. Arthroplasty and total joint replacement if larger joints are involved
3. Open or Arthroscopic synovectomy—mainly for persistent mono arthritis of knee joint
4. Early Tenosynovectomy of wrist to prevent tendon rupture[16]

OXIDATIVE STRESS

Several oxidants are produced during normal course of metabolism in all cells including RBCs. In a normal cell there is a balance between pro-oxidant and anti-oxidant. This balance can be shifted to pro-oxidants when the production of oxygen species is increased greatly or when the level of antioxidants are diminished. This state is called "Oxidative Stress". The free radicals cause damage to lipids, proteins, and nucleic acids in cell membrane and plasma lipoproteins. [21]



FREE RADICAL:

Free radicals are highly reactive molecular species with an unpaired electron, which is very short lived before they collide with another molecule and abstract or donate an electron to achieve stability. This generate a new radical causing a chain reaction.

Damage caused to tissues by oxygen radicals is called **oxidative damage** and the factors that protect tissue damages are called **Anti-Oxidants**.[22]

SOURCES:

ENDOGENOUS:

1. Respiratory chain in mitochondria

2. Enzymes like xanthine oxidase, nitric oxidase synthase

3. Phagocytosis

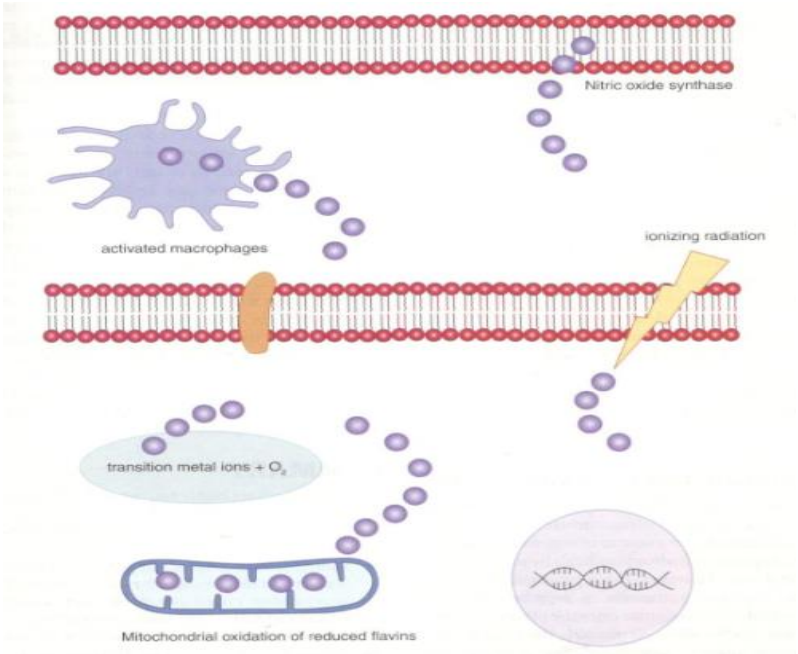
4. Transition of metal mediated oxidation

5. Exercise

6. Ischemia/reperfusion injury

EXOGENOUS:

1. Environmental pollutant
2. Smoking
3. Radiation
4. Ultraviolet light
5. Toxins including ionising radiation [22]



SOURCES OF FREE RADICALS

TYPES OF FREE RADICALS:

Oxygen derivatives - common

- Superoxide anion radical ($O2^{-}$)
- Hydrogen peroxide($H2O2^{-}$)
- Hydroxyl radical (OH^{-})

Nitrogen derivative:

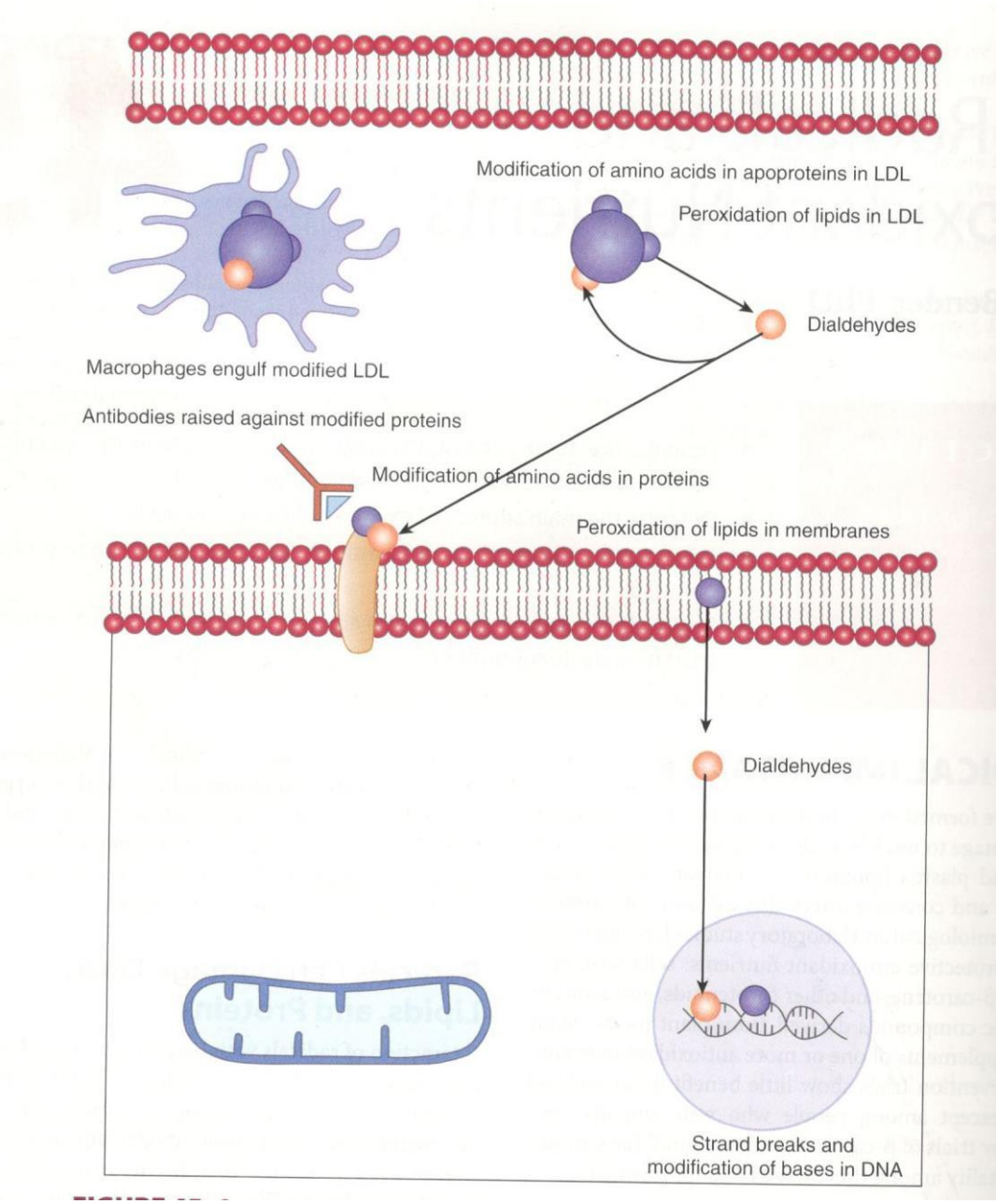
- Nitro radical ($ONOO^{-}$)
- Nitric oxide (NO)[21],[23]

OXIDATIVE DAMAGE IN CELL:

Tissue damage caused by free radical is called oxidative damage which cause injury to all important cellular components like membrane lipids, proteins and DNA.

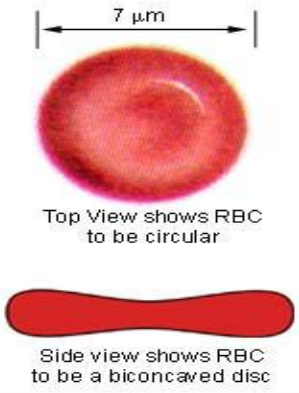
1. Cell membrane lipid → Produce Chronic inflammatory disease like Rheumatoid arthritis
2. Cell membrane and cytoplasmic proteins → Produce modified protein →Antibody formation → Autoimmune disease
3. DNA damage → Mutation → Initiation of cancer
4. Plasma LDL → Oxidized LDL →Engulfed by macrophages → Atherosclerosis [22]

OXIDATIVE DAMAGE TO CELL



RED BLOOD CELL

Red blood cell (RBC) also known as Erythrocytes are formed in the myeloid tissue or most commonly known as red bone marrow The formation of RBCs is called erythropoiesis. Red blood cells lose nuclei upon maturation, and take on a biconcave, dimpled, shape which helps to carry oxygen and to pass through capillaries. They are about 7-8 micrometers in diameter



Normal RBC count 4.5-5.5 million/cu mm in men, 4-4.5 million/cu mm in women. The main function is the transportation of oxygen throughout the body and to carry out carbon dioxide from tissue. Normal life span of RBC is 120 days. Main site of destruction is reticuloendothelial system in spleen and liver.[24]

STRUCTURE OF RBC:

The red blood cell membrane consists of three basic components, lipid bilayer, transmembrane (integral) proteins and a cytoskeletal network. The main constituent of the lipid bilayer is cholesterol and phospholipid. Cholesterol provides flexibility and stability to membrane. Integral protein embedded in lipid bilayer. Underneath the cell membrane there is a cytoskeleton network. This is

made up of contractile protein mainly spectrin and actin which maintain the shape of RBC.[24]

RBCs lack a nucleus (no DNA) and no organelles like mitochondria, ribosome and endoplasmic reticulum. Cytoplasm of RBC contains haemoglobin, enzymes for glycolytic and HMP shunt pathway, reduced glutathione and vitamin C and Vitamin E.[25], [55]

ERTHROCYTE METABOLISM:

In RBCs, ATP is produced through anaerobic glycolysis because it does not have mitochondria. This ATP is necessary for maintenance of the biconcave shape and flexibility of the cell which allows the cells to squeeze through narrow capillaries. Decreased ATP production makes RBCs rigid and fragile and more prone to hemolysis.[25], [26]

Glucose enters into RBCs through Na independent facilitated diffusion (GLUT-1). 80% of the entered glucose is utilized in glycolytic pathway for energy production and the remaining 20% glucose enters into HMP shunt pathway to produce NADPH with the help of Glucose 6 Phosphate dehydrogenase enzyme. This NADPH reduces oxidized glutathione into reduced glutathione which protects the RBCs from oxidative damage.[27], [55]

OXIDATIVE STRESS IN RBC:

The RBCs are the first cell to be affected by oxidative stress. As they pass through lungs they carry free radicals along with oxygen. The RBCs contain high level of cytoplasmic antioxidant both Enzymatic and non-enzymatic in order to

protect the RBC from deleterious effect of oxidative stress. α-tocopherol, ascorbic acid and reduced glutathione are the major endogenous antioxidants.

α–tocopherol protects the PUFA in the RBC membrane against lipid peroxidation. Ascorbic acid, a potent cellular antioxidant protects the cell membrane from oxidative damage and also regenerate oxidized α-tocopherol. Reduced glutathione in erythrocytes maintain haemoglobin in its native form. It also plays a major role in maintenance of membrane thiol group.

ROS induced cell membrane damage allows the GSH to pass through the membrane causing depletion of GSH in the cytoplasm of RBC. As the erythrocytes have no nucleus and ribosome and cannot regenerate GSH and enzyme thus becoming vulnerable to oxidative damage.[28]

Glutathione maintains the reduced state sulphhydryl group in proteins including haemoglobin. Oxidation of sulphhydryl groups leads to the formation of denatured protein that forms insoluble masses called as "**Heinz bodies"** that attaches to cell membrane. Oxidation of cell membrane proteins causes rigidity of the RBC which are removed from the surface by macrophage in the spleen and liver.[29] Higher degree of oxidation ultimately results in hemolysis of RBCs. Reticulocytes are visualised in the peripheral blood as polychromatophillic red cells which is the cause for macrocytosis.[30], [56]

Several Eicosanoid isomers are produced by non-enzymatic free radical catalysed oxidation of arachidonic acid. Out of which most characterised isoeicosanoids is 8isoPGF2α. They are produced due to lipid peroxidation of PUFA by free radicals. Unlike prostaglandin these compounds are initially formed

esterified in phospholipids, then gets hydrolysed in free form by phospholipase which then circulate, get metabolised and excreted in urine. In vivo their production is suppressed by **Antioxidant** but not by COX-1 and CO2 inhibitors.

As these Isoprostanes can activate prostanoid receptors they may contribute to the pathophysiology of various inflammatory diseases which are insensitive to COX inhibitors. Measuring the level of Isoprostanes is considered as the most accurate method to assess oxidative stress in-vivo.[31]

8 IsoPGF2 α is also a potential mediator of oxidative damage which causes injury to RBC cell membrane and produce irregularly contracted crenated RBCs and sphreocytes.[32]

The following are the structural changes in the RBC due to oxidative hemolysis

1. **Irregularly contracted and crenated cells** – Due to altered cell membrane integrity [33]

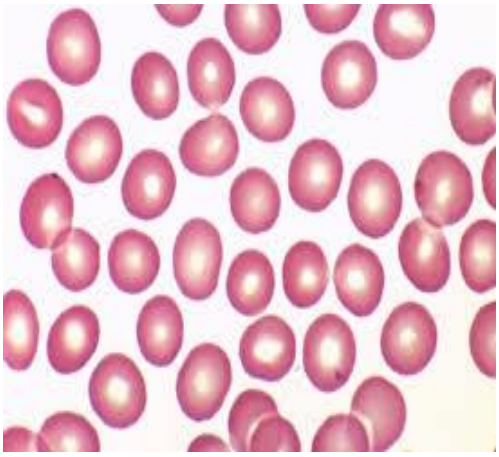
2. **Presence of Heinz bodies** – Due to Denatured haemoglobin [30]

3. **Bite cells** - Remaining cells after removal of Heinz bodies in the cells by spleen[30]

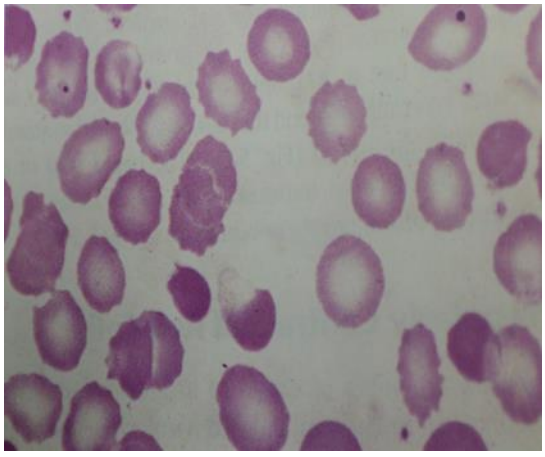
4. **Spherocytes -** spheroidal less disc cell than normal RBC [34]

5. **Macrocytosis** – Indicates presence of reticulocytes [30]

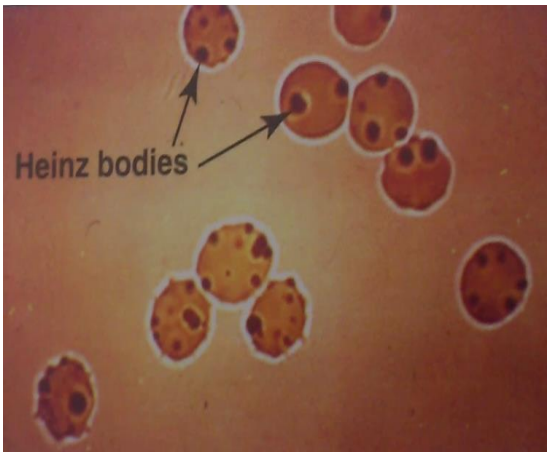
NORMAL RBC



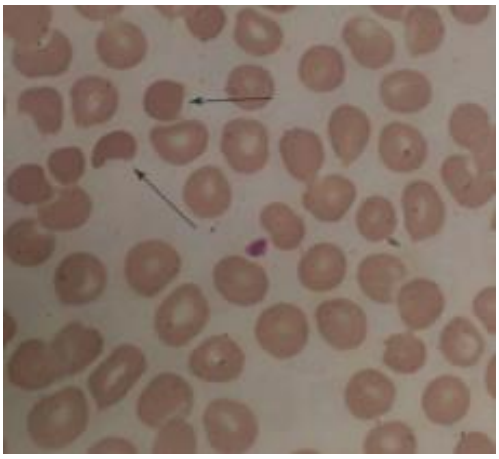
CRENATED RBC



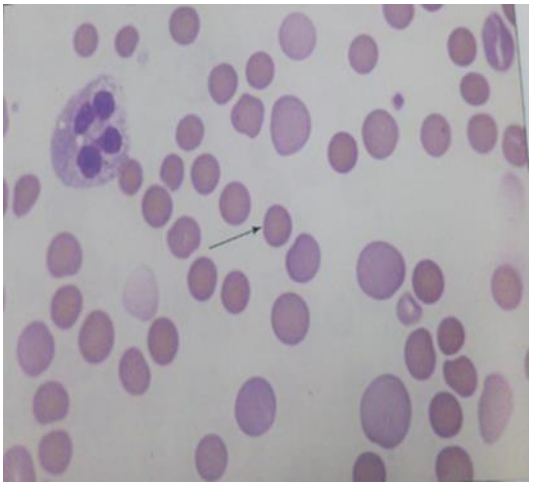
HEINZ BODIES



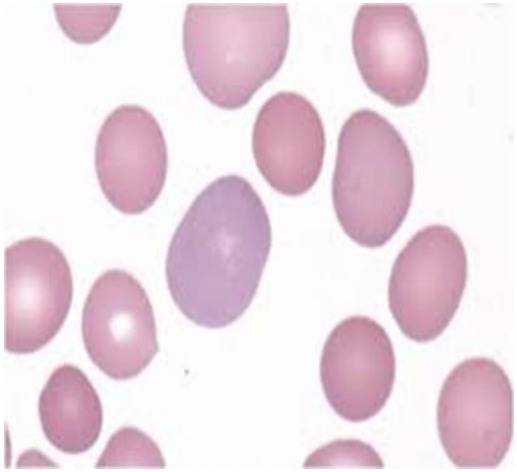
BITE CELLS



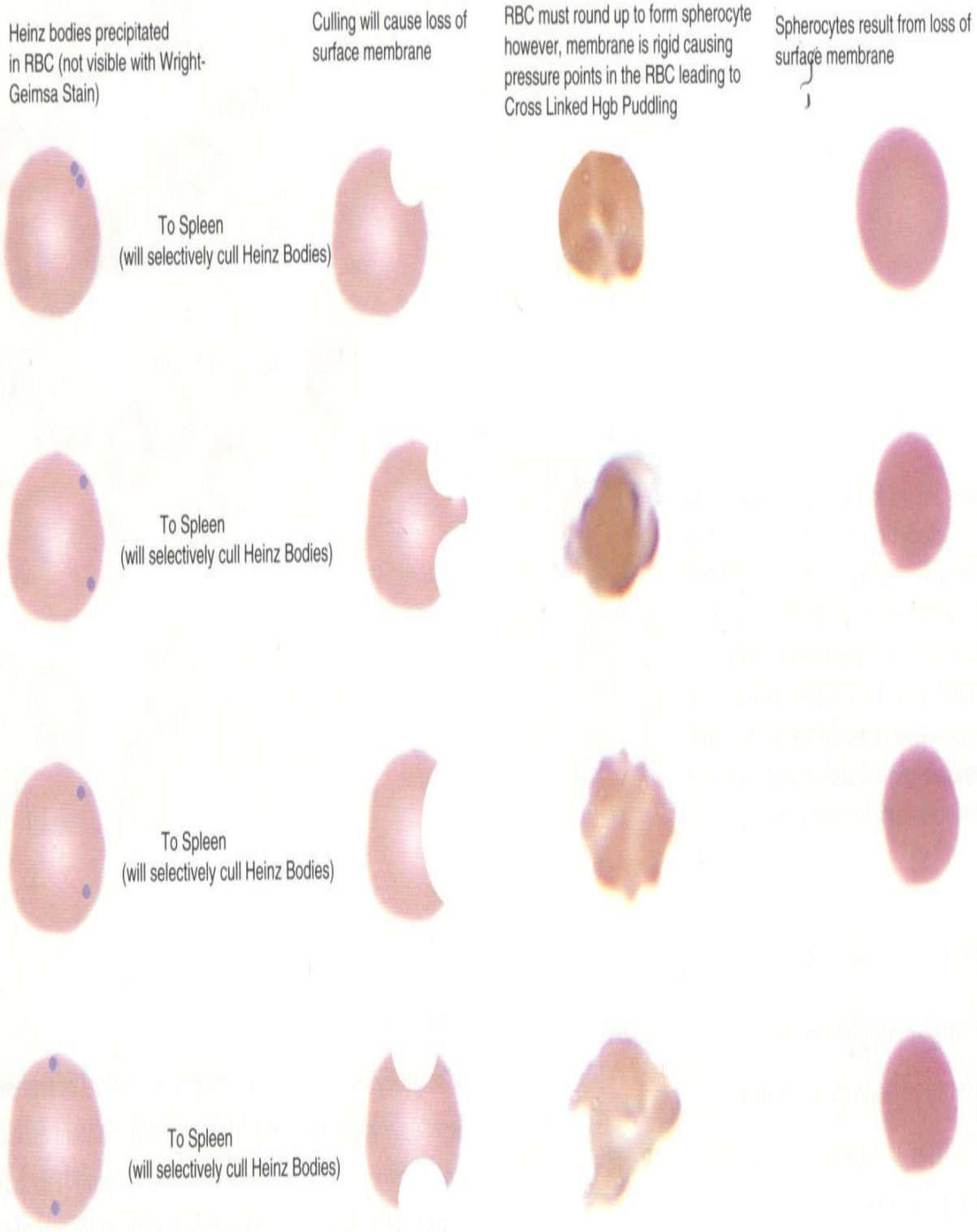
SPHEROCYTES



RETICULOCYTES



The Pathophysiology of RBC Changes in Drug Induced Oxidative Hemolysis



ANTIOXIDANTS

DEFINITION:

An antioxidant is a stable molecule that interact and neutralize free radicals preventing them from causing tissue damage.[35]

CLASSIFICATION:

- Endogenous
  - Ubiquinone
  - Uric acid
  - Superoxide dismutase
  - Catalase
  - Glutathione
- Exogenous
  - Vitamin – E
  - Vitamin – C
  - Carotenoids
  - Lycopene
  - Flavanoids
  - N – acetyl cysteine
  - Lipoicacid
  - Copper
  - Selenium
  - Zinc[35]

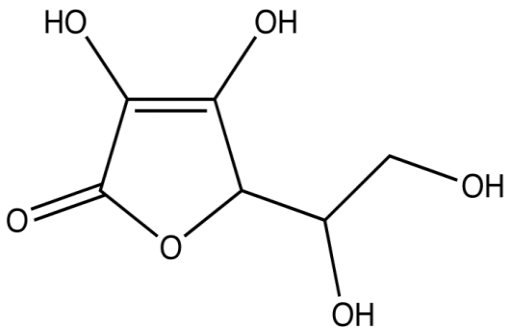
USES:

- Cerebrovascular disease
- Type II diabetes mellitus
- Alzheimer’s disease
- Autoimmune disorders
- Essential hypertension
- Cardiovascular disease
- Osteoarthritis
- Peptic ulcer [35]

ASCORBIC ACID (VITAMIN C)

- A Water soluble vitamin
- Important essential nutrient

STRUCTURE



HISTORY

Vitamin C was isolated by Joseph Svierbely, Albert Szent Gorgyi, and Charles Glenking.[36]

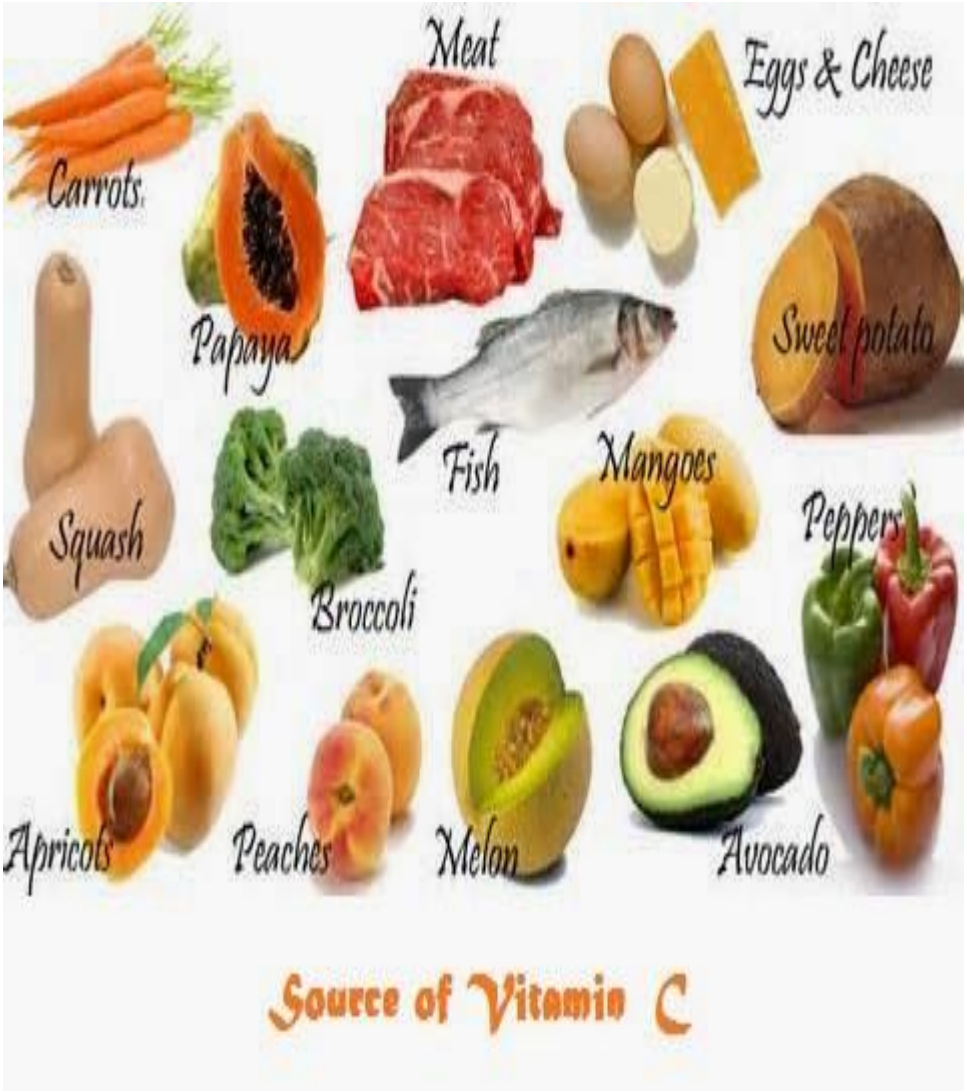
ACTIVE FORM:

- Dihydroascorbic acid (DHA)
- Ascorbic acid (AA) [36]

DIETARY SOURCES

PLANT SOURCES

- Indian goose berry
- Chilli pepper
- Guava
- Black currant
- Red pepper


Meat
Eggs & Cheese
Carrots
Papaya
Sweet potato
Fish
Mangoes
Peppers
Squash
Broccoli
Apricots
Peaches
Melon
Avocado
Source of Vitamin C

- Lemon
- Orange
- Broccoli

ANIMAL SOURCE

- Lamb brain
- Chicken liver
- Calf liver [36]

BIOSYNTHESIS

- Vitamin C is an essential nutrients for human beings
- Plants and some animals can synthesize their own vitamin C [36]

DAILY REQUIREMENTS

Adults – 75 -90 mg/day

Lactating mother – 105 mg/day

Pregnant women – 85 mg/day [36]

PHARMACOKINETICS

- Absorption takes place by simple diffusion, active transport
- Excessive intake reduces its absorption[37]

DRUG INTERACTIONS

- Improves the absorption of iron
- Due to its reducing property, interferes with the laboratory test for urine glucose

- High dose reduces effect of oral anti-coagulants
- Reduces copper , pyridoxine , cyanocobalamine metabolism [37]

FUNCTIONS:

1. ANTIOXIDANT

- Effective antioxidant - reduces oxidative stress by stabilizing the ROS by donating the electrons
- Protects oxidative damage to DNA
- Synergises with vitamin –E to reduce lipid peroxidation
- Maintains antioxidant pool by regenerating other antioxidants [37]

2. ENZYMATIC COFACTOR

- Helps in synthesis of collagen by translation hydroxylation of proline and lysine
- catalyse ferrochelatase and incorporates iron into protoporphyrin IX in formation of heme
- acts as co-factor in synthesis of carnitine from methionine and lysine
- Important cofactor in hydroxylation reaction of dopamine and serotonin
- Vital role in adrenal steroid synthesis[37]

3. METABOLISM OF CHOLESTEROL

- Cholesterol is routinely transformed into bile acids in the body. For this transformation reaction, hydroxyl group is incorporated into cholesterol nucleus with the help of vitamin C.

- By increasing the expression of LDL receptor in the liver it helps in LDL degradation[38]

4. HYPERTENSION

- It protects the Nitric Oxide from inactivation by ROS and causes vasodilatation.
- Maintains endothelial function
- Modulate the autonomic nervous system by restoring sympathovagal balance and spontaneous baroreceptor activity.[38]

4. DIABETES

- Improves insulin sensitivity by stabilizing peroxynitrate radicals
- Regenerates other antioxidants and helps in maintaining antioxidant pool[38]

5. **ANTI INFLAMMATORY ACTION**

- Vitamin C has both anti-inflammatory and analgesic action
- Supresses the production of 8isoPGF2α, the first identified isoprostane produced by non-enzymatic free radical induced lipid peroxidation.[30]

6. CATARACT

- Prevents the oxidative damage of lens protein [39]

7. CANCER

- Prevents cancer by inhibiting formation of carcinogenic N nitraso compounds[39]

8. IMMUNITY

- Found in high concentration in immune cells

  It modulates

  - Lymphocytes and cytokine production
  - Phagocytic activity
  - Cell adhesion molecules of immune cells
- It has natural anti allergic property[39]

9. RHEUMATOID ARTHRITIS

- Effective antioxidant reduces oxidative stress
- Helps in formation other antioxidant like Vitamin E
- Anti-inflammatory and analgesic action[30],[56]

8. MISCELLANEOUS

- By Improving endothelial function it decrease the atherosclerosis formation
- Promotes wound healing
- Reduces ferric to ferrous state, thereby increases iron absorption[,,39]

Deficiency causes

- Scurvy
- Bleeding tendencies
  - Gum bleeding
  - Petechiae

- Ecchymosis

- Increased keratinisation
- Weak bones
- Stunted growth
- Delayed wound healing
- Teeth malformation
- Decreased iron content of body
- Painful joints[40]

ADVERSE EFFECTS: (High dose >2g/day)

- Mild GI upset
- Sleep disturbances
- Rashes
- Facial flushing
- Headache
- Oxalate stones [41]

USES:

- Scurvy
- Common cold
- To improve iron status of the body
- Diabetes mellitus
- Essential hypertension
- Prolonged leg ulcer, Bed sores [42]

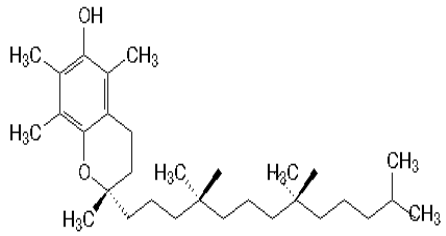
VITAMIN E

- A Fat soluble vitamin
- Vitamin E available as eight forms (Tocopherol -4 , Tochotrienols -4)
- Most common active form is alpha tocopherol[43]

HISTORY:

In 1922, a fat soluble dietary constituent was found for prevention of foetal death and sterility in rats and called as Factor S and anti-sterility factor. In 1936 it was isolated from wheat germ oil and renamed as Vitamin E.[(43),(57)]

CHEMICAL STRUCTURE:

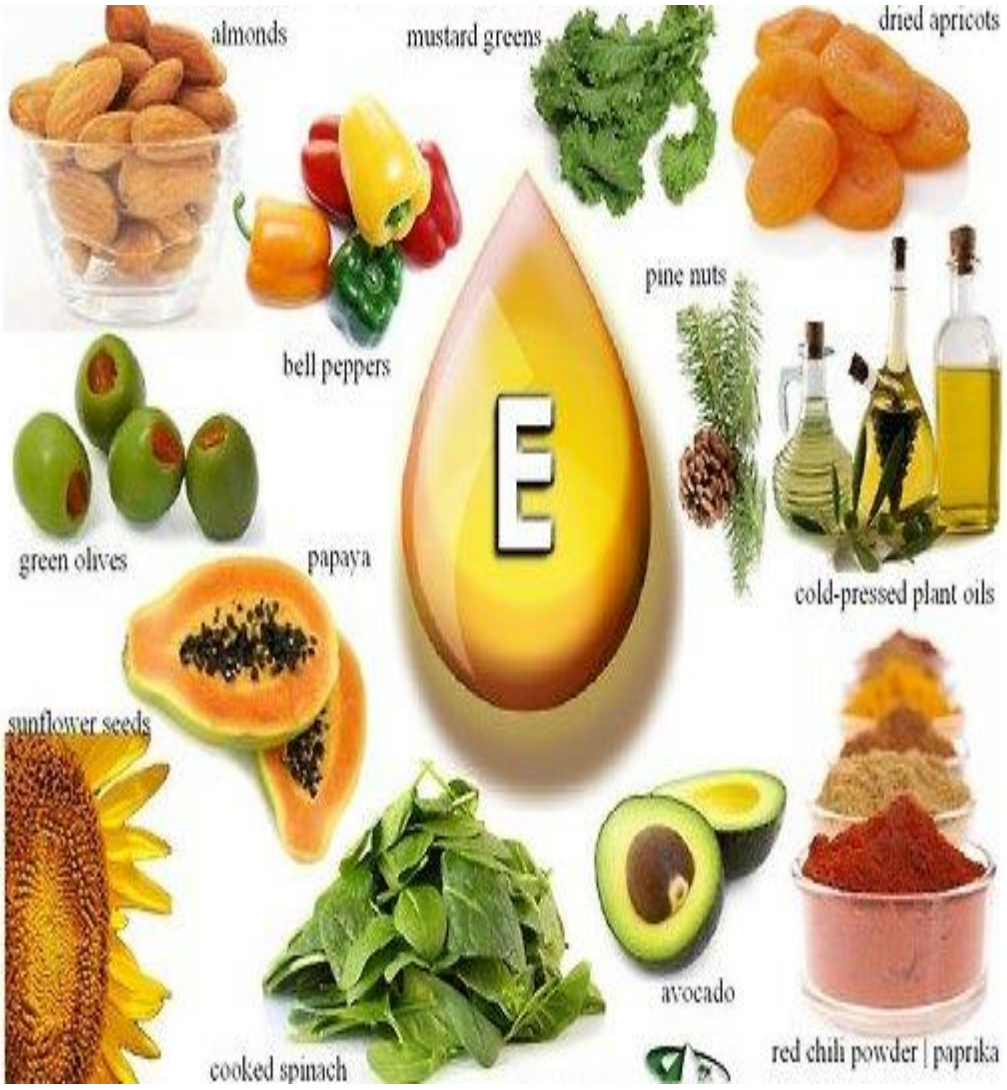


Vitamin E (α-tocopherol)

Vitamin E contains chromanyl ring and hydroxyl group (which donate a hydrogen atom to reduce free radical) and hydrophobic side chain (which allows for penetration into biological membrane). [(44), (58)]

SOURCES:

- Soya bean oil
- Green leafy vegetables
- Nuts
- Safflower oil
- Fish
- Spinach
- Sunflower oil
- Cereals
- Wheat germ oil
- Egg yolk [(35)]


almonds
mustard greens
dried apricots
bell peppers
pine nuts
E
green olives
papaya
cold-pressed plant oils
sunflower seeds
avocado
cooked spinach
red chili powder | paprika

DAILY REQUIREMENTS:

New born – 4-5 mg/ day

Children – 6-11 mg/day

Adults-15 mg/day [44]

PHARMACOKINETIC PARAMETERS:

- In intestine , vitamin E is absorbed along with chylomicrons
- Metabolised through cytochrome liver enzymes CYP4F2and CYP3 A
- Excreted in urine and bile[59]

DRUG INTERACTIONS:

- Reduces absorption of vitamins A and K
- Reduces Iron absorption
- Increases oral anticoagulant effect[58]

FUNCTIONAL ROLE:

1. **ANTI OXIDANT**
    - Acts as a peroxyl radical scavenger, preventing the propagation of free radicals by reacting with them to form a tocopherol radical, which will further reduced by a hydrogen donor (such as vitamin C) and returns to its reduced state.
    - It is present in the cell membranes and inhibits lipid peroxidation.[45]

2. **ANTI INFLAMMATORY ACTION**

- Inhibits non-enzymatic free radical catalysed oxidation of arachidonic acid, therefore production of iso-prostanes (PGF2α) is suppressed.[30]

3. **METABOLISM OF CHOLESTEROL**

- By inhibiting LDL oxidation prevents their accumulation in arterial intima thereby helps in the prevention of atherosclerosis.[46]

4. **ANTI CLOTTING**

- It inhibits vitamin –K dependent carboxylase by attaching to thiol group [46]

5. **ANALGESIC ACTION**

- It has central analgesic action by reducing central pain processing (↓ Nitric oxide) [46]

6. **DIABETES**

- Synergistic with vitamin – C
- Insulin resistance is improved by increasing tyrosine kinase activity[46]

7. **RHEUMATOID ARTHRITIS**

- Protect the cell membrane from free radical injury and provide anti-inflammatory action
- Uncouple joint inflammation and joint destruction
- Provide analgesic action by central mechanism(↓NO)[30],[47]

DEFICIENCY SYMPTOMS:

- RBC deformation
- Myopathies
- Decreased immunity
- Retinal changes
- Spinocerebellar ataxia
- Neuropathies [48]

USES

- Type II diabetes
- Essential hypertension
- Post herpetic neuralgia
- Pregnancy induced hypertension
- Osteoarthritis
- Rheumatoid arthritis
- Haemolytic disease
- Intermittent claudication
- Benign breast tumour
- Nocturnal leg cramps
- Sterility
- Recurrent abortion
- Systemic lupus Erythematosis
- Atherosclerosis [49],[50]

ADVERSE DRUG REACTIONS:

- Vomiting
- Flatulence
- Abdominal pain
- Loose stool [51]

INDOMETHACIN

- It is an NSAID drug
- Indole acetic acid derivative

MECHANISM OF ACTION:

- Non selective inhibitor of cyclooxygenase
- inhibits both COX1 and COX2[52]

PHARMACOKINETICS:

- Absorbed orally
- Metabolised in liver and excreted in urine
- 90% bound to plasma proteins
- T1/2 2-5 hr[(52)]

DOSE: 20-50mg BD-QID

ADVERSE EFFECT:

- Gastric irritation, nausea, anorexia, bleeding, diarrhoea
- Frontal headache ,dizziness, ataxia, hallucination, depression, confusion
- Hypersensitivity reaction, Rash, Leucopenia [(52)]

CONTRAINDICATIONS:

- Machinery operators
- Driver
- Epileptics
- Psychiatric disorder[52]

USES:

- Rheumatoid arthritis, Gout, Ankylosing spondylitis
- Bartter syndrome
- Malignancy associated fever
- Medical closure of PDA [52]

HYDROXY CHLOROQUINE

- 4 amino quinoline derivative of antimalarial drug
- Induce remission in 50%of patients with RA

MECHANISM OF ACTION:

- Exact mechanism not known
- Suppress the T Lymphocyte response to mitogen
- Decrease the leucocyte chemotaxis
- Stabilize the lysosomal enzyme
- Inhibition of DNA and RNA synthesis [52]

PHARMACOKINETICS:

- Orally absorbed
- High affinity for nuclear chromatin and melanin and is concentrated in spleen , lung liver, skin, kidney
- 50% plasma protein bound
- T1/2 3-10 days [53]

DOSE:

400mg/day for 4-6weeks followed by 200mg/day for maintenance

ADVERSE EFFECT:

Common:

Anorexia, nausea, vomiting, itching, difficulty in accommodation

Long term:

Corneal deposit, loss of hearing, retinal damage, Photo allergy

Parenteral;

Convulsion, hypotension, arrhythmia [53]

USES:

- Malaria
- Rheumatoid arthritis
- Lepra reaction
- Infectious mononucleosis
- Extra intestinal amoebiasis [53]

OBJECTIVES

OBJECTIVE

AIM:

To study the efficacy of Vitamin C and Vitamin E in Rheumatoid Arthritis.

PRIMARY OBJECTIVE:

To evaluate morphological changes in red blood cells due to oxidative stress in patients with Rheumatoid Arthritis and to study the efficacy of Antioxidants (Vitamin C and E) as an Add on therapy to standard treatment in the management of these patients compared to standard treatment alone.

SECONDARY OBJECTIVE:

Clinical improvement of patient by measuring painful joint score, swollen joint score, tender joint score.

METHODOLOGY

METHODOLOGY

The study was conducted in patients with Rheumatoid arthritis, diagnosed within 1 year and now attending outpatient department of Rheumatology, Rajiv Gandhi Government General Hospital, Chennai.

Study design:

A randomized, open label, prospective, interventional, comparative, parallel group pilot study.

Study population:

Adult patients with Rheumatoid Arthritis diagnosed based on American college of Rheumatology criteria.

Study center:

Institute of Pharmacology, Madras Medical College & Institute of Rheumatology, Rajiv Gandhi Government General Hospital, Chennai.

Study period:

August 2014-March 2015

Study duration:

Treatment period of 8 weeks and

Post treatment follow up period of 4 weeks per patient.

Sample size:

60 patients (Control group - 30, study group - 30).

Inclusion criteria:

- ❖ Age: 40-70 years
- ❖ Sex-both genders
- ❖ Patients diagnosed as Rheumatoid Arthritis based on American college of association criteria.
- ❖ Patients willing to give written informed consent

Exclusion criteria:

- Arthritis incompatible with RA by ACR criteria
- Patient on oral and parenteral corticosteroids
- Pregnant and lactating women
- Patients with co-existing liver disease, heart disease, dyslipidaemia or malignancy
- Patients with Haematological disorders

Study procedure:

The study was conducted after obtaining the approval from Institutional Ethics Committee, Madras Medical College and it was done in accordance with declaration of Helsinki and Good Clinical practice (GCP) guidelines.

Patients diagnosed with Rheumatoid arthritis attending the Outpatient department, Institute of Rheumatology Madras Medical College and Rajiv Gandhi

Government General Hospital, were explained about the study purpose, procedure and benefits of the study.

Written informed consent was obtained from those subjects who are willing to participate in the study in the prescribed format in regional language prior to performance of any study related procedures. If the patients were illiterate, left thumb Impression was obtained. This was done in the presence of an impartial witness.

The demographic details of the patients were obtained and recorded. Patients were screened by History, General and Systemic examinations and lab investigations. Patient who fulfil the inclusion and exclusion criteria were enrolled and Randomized into either study group or standard group.

The following lab investigation were performed during screening.

1. Hb, ESR
2. Liver function test(LFT)
3. Renal function test (RFT)
4. C-Reactive protein
5. Rheumatoid factor
6. Chest X ray
7. X ray both hands

RECRUITMENT:

- 96 patients were screened and 30 patients in each group (control and study groups) who fulfilled the inclusion and exclusion criteria were recruited into the study.
- No drop-outs of patients in both groups.

Randomization:

The enrolled patients were randomized by simple randomization into either control group or study group and received the respective therapy.

- ❖ Control group (n=30) –Standard therapy
- ❖ Study group (n=30) – Standard therapy + study drug

TREATMENT PLAN:

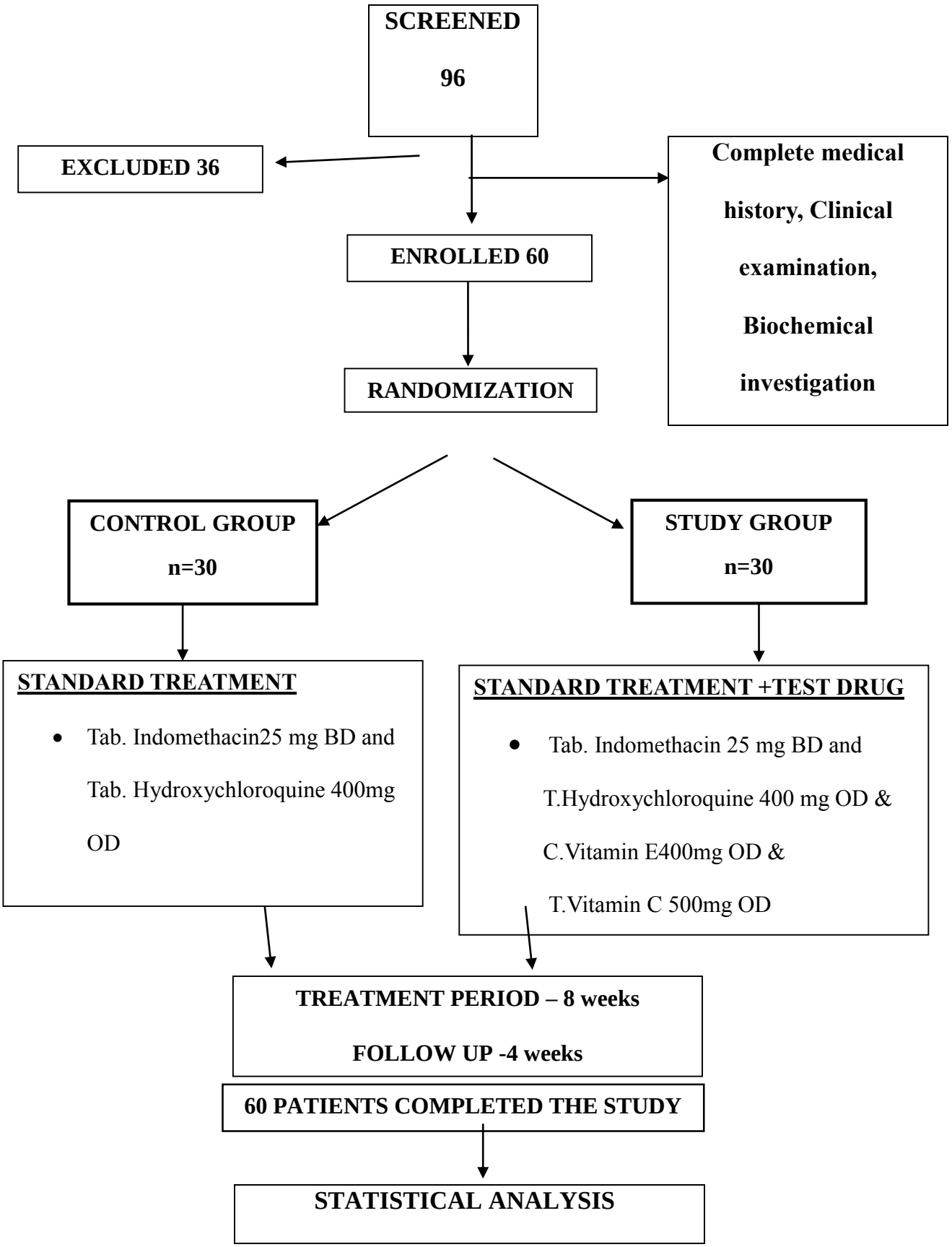
Control group

- **Standard treatment**
- Tab. Indomethacin 25 mg twice daily and Tab. Hydroxychloroquine 400mg once daily for 8 weeks

Study group

Patients received standard treatment plus capsule Vitamin E 400 mg and Tab. Vitamin C 500 mg once daily for 8 weeks

STUDY FLOW CHART



STUDY VISIT

SCREENING:

- Written informed consent obtained.
- Demographic details obtained.
- Medical history taken and recorded.
- Vital signs recorded, General, systemic & local examination done.
- Laboratory investigations done.
- Enrollment done.

VISIT 1

- Randomization done.
- Vital signs recorded.
- Clinical examination of joints was done
- Pain assessment done by Visual analogue scale.
- DAS scoring done
- RBC morphology assessment done.
- Study drugs were issued for 4 weeks to respective groups.
- Instructed to return the empty strips during subsequent visit.
- Patients were instructed to report if any adverse events occur.

VISIT 2 (end of 4 weeks)

- Vital signs recorded.
- Patients were asked to return empty strips to check compliance.
- Clinical examination of joints was done.
- Adverse events monitored.
- Pain assessment done by Visual analogue scale.
- DAS scoring done.
- RBC morphology assessment done.
- Study medication issued for subsequent 4 weeks.

VISIT 3 (end of 8weeks)

- Vital signs recorded.
- Clinical examination of joints was done.
- Adverse events monitored.
- Pain assessment done by Visual analogue scale.
- DAS scoring done.
- RBC morphology assessment done.

VISIT 4 (end of 12weeks)

- Vital signs recorded.
- Clinical examination of joints was done.
- Adverse events monitored.
- Pain assessment done by Visual analogue scale.
- DAS scoring done
- RBC morphology assessment

ASSESSMENT PARAMETERS

RBC morphology assessment:

1ml of blood was collected from the patients and centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and the packed cells were diluted with equal volume of 0.9% normal saline and centrifuged again. The supernatant was again discarded and the packed cells were reconstituted as 10% v/v suspension with 0.9% normal saline and a drop of this suspension was put on a glass slide under a cover slip and studied under High Power Microscope for assessment of morphological changes in the red blood cells.

Under high power microscope 100 cells are seen in the centre of field and the number of abnormal cells were counted, then number of percentage of abnormal cell was noted in both the groups.

Differences in the percentage of abnormal cells before and after treatment is recorded in both the groups.

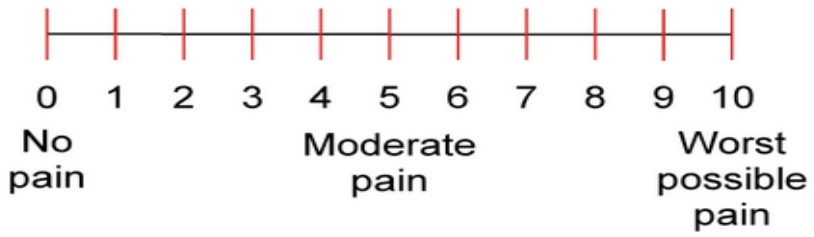
Clinical assessment – based on patient's symptoms improvement

Pain – visual analogue scale

Joint Swelling - based on DAS score

Joint Tenderness – based on DAS score

VISUAL ANALOGUE SCALE (VAS):



The Visual Analogue Scale consists of a 10 cm line with 0 at one end representing no pain, and 10 at the other end representing the worst pain ever experienced.

DAS SCORE:

DAS 28 is the quantitative measure of Rheumatoid Arthritis disease activity and used to monitor the treatment of RA. DAS stands for "Disease Activity Scoring" and 28 refers to number of joints that are examined for the assessment. DAS is calculated using a formula that includes the number of tender joints, swollen joints (28 joints maximum), ESR, and pain by Visual analogue scale.

Score 1 was given to swelling of single joint and 0 to absence of swelling

Score 1 was given to tenderness over the joint and 0 to absence of tenderness

Total number of swollen joint and tender joint was calculated.

ESR was measured in mm hour

Pain is measured by VAS by 0-100mm

DAS28 = 0.56*√(tenderjoints) + 0.28*√(swollenjoints) + 0.70*Ln(ESR)+ 0.014* VAS

DAS28 score >5.1 →high disease activity,

DAS28 < 3.2 →low disease activity.

DAS28 lower than 2.6.→patient in remission

By comparing a patient's DAS28 score over multiple time points

We can see the improvement of response.

The EULAR response criteria are defined as follows

PRESENT DAS28	DAS IMPROVEMENT OVER TIME POINTS		
	> 1.2	0.6-1.2	< 0.6
<3.2	GOOD RESPONSE	MODERATE RESPONSE	NO RESPONSE
3.2-5.1	MODERATE RESPONSE	MODERATE RESPONSE	NO RESPONSE
>5.1	MODERATE RESPONSE	NO RESPONSE	NO RESPONSE

Lab investigations:

The following laboratory investigations and assessment of symptoms were performed in the patients on screening and at the end of 8 weeks.

- Haematology - Haemoglobin, ESR
- Blood glucose
- Blood urea
- Serum creatinine
- Liver function test (SGOT, SGPT)
- X ray – chest PA view
- X ray – both hands
- ECG all leads

INSTRUCTION TO PATIENTS

The patients were instructed clearly regarding the regular intake of the medicines. They were given proper advice to report for assessment and drug receiving. They were counselled to report if any acute complaints, reactions occur

Follow up:

The patients were followed up for a post treatment period of 4 weeks, without the study drug for the assessment of symptoms of Rheumatoid arthritis.

After the completion of 12 weeks of study period, the patients were provided appropriate medical care at Institute of Rheumatology Rajiv Gandhi Government General Hospital, Chennai.

Adverse events:

Any adverse event reported by the patient or observed by the physician during the study was recorded. The onset of adverse event, causal relationship to the study drug and action taken will be recorded. Appropriate medical care was provided.

Withdrawal:

During the study period the subject was allowed to withdraw his/her voluntary consent and opt out of study. Similarly at the discretion of the investigator, the subjects were withdrawn from the study if any serious adverse event reported by the patient or observed by the physician.

RESULTS

STATISTICAL ANALYSIS

The obtained data was analysed statistically.

Distribution of age was analysed using one way ANOVA and Sex distribution was analysed by Pearson chi- square test.

The difference within the groups in pain assessment score, swollen joint score, tender joint score and DAS score, RBC morphology was analysed using students paired t-test. Similarly the difference between the control and test groups was analysed using independent t-test.

The biochemical investigations were performed at baseline and at the end of 4,8,12 weeks. The differences within the groups before and after treatment were analysed using student's paired t- test.

Statistical analysis was done by using Epi Info software.

P value <0.05 was considered to be statistically significant.

TABLE-1: AGE DISTRIBUTION

AGE(YEARS)	CONTROL	STUDY
21-30	5	4
31-40	13	13
41-50	8	9
51-60	4	4
TOTAL	30	30

Table – 1 shows the age distribution across the two groups.

Most of the patients are in the age group of 31-50 years

Figure -1: Age Distribution – Graphical representation of Table – 1

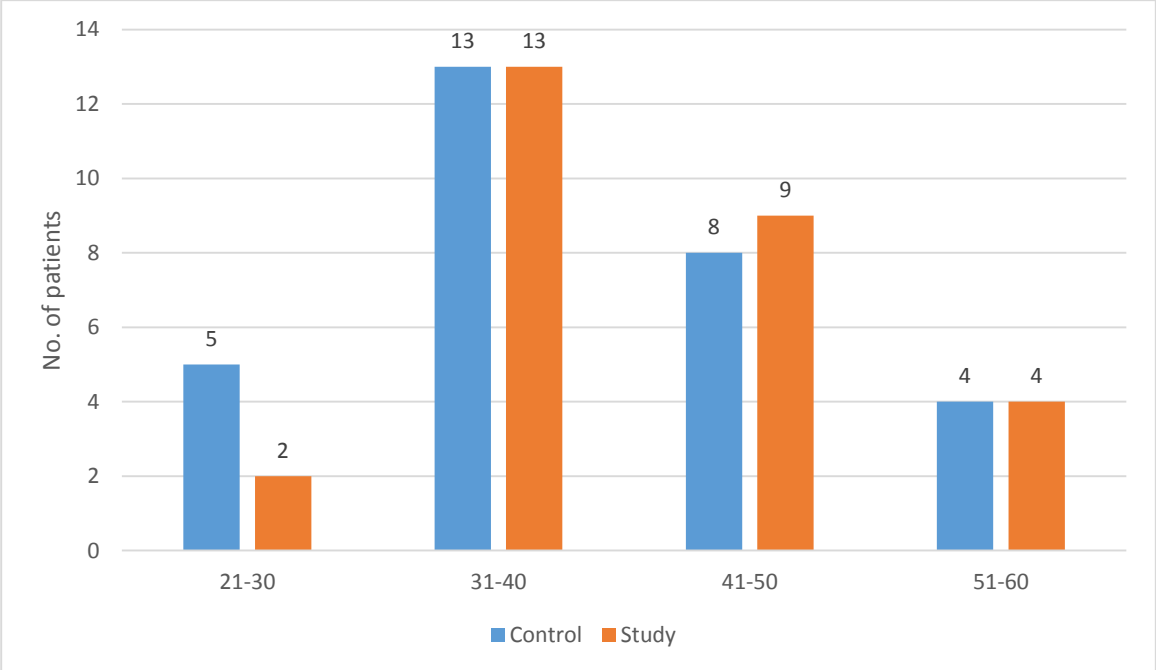


TABLE-2: MEAN AGE DISTRIBUTION

GROUP	NO. OF PATIENTS (n)	MEAN AGE IN YEARS	S.D
CONTROL	30	39.86	9.43
STUDY	30	40.43	8.75
p-VALUE	0.8091		

Table 2 shows the mean age distribution between the groups.

The mean age in the control group is 39.86 and the mean age in the study group is 40.43. The difference in mean age between the groups is statistically not significant.

Figure – 2: Mean Age Distribution

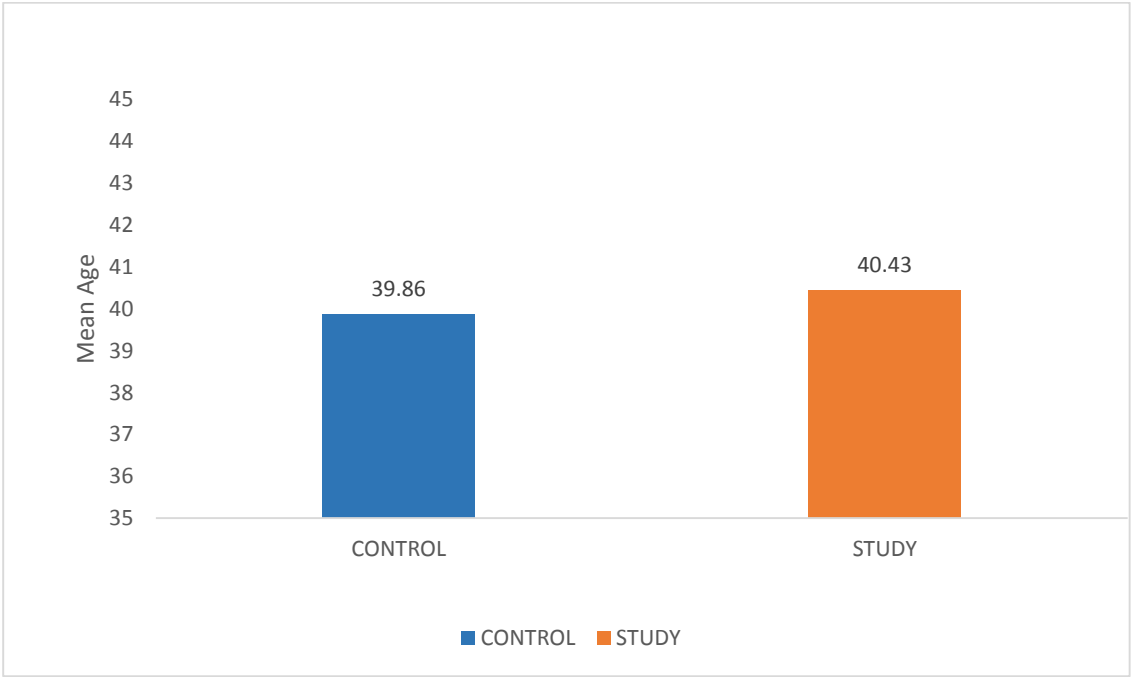


Table –2 Graphical representation of mean age distribution

TABLE-3: GENDER DISTRIBUTION

GROUP	MALE		FEMALE		TOTAL	
	N	%	n	%	N	%
CONTROL	7	23	23	76	30	100
STUDY	6	20	24	80	30	100
p-VALUE	1.0					

Table – 3 shows the gender distribution between the groups.

Females outnumber males in both the groups. There is no statistical significance in gender distribution between the two groups.

Figure – 3: GENDER DISTRIBUTION

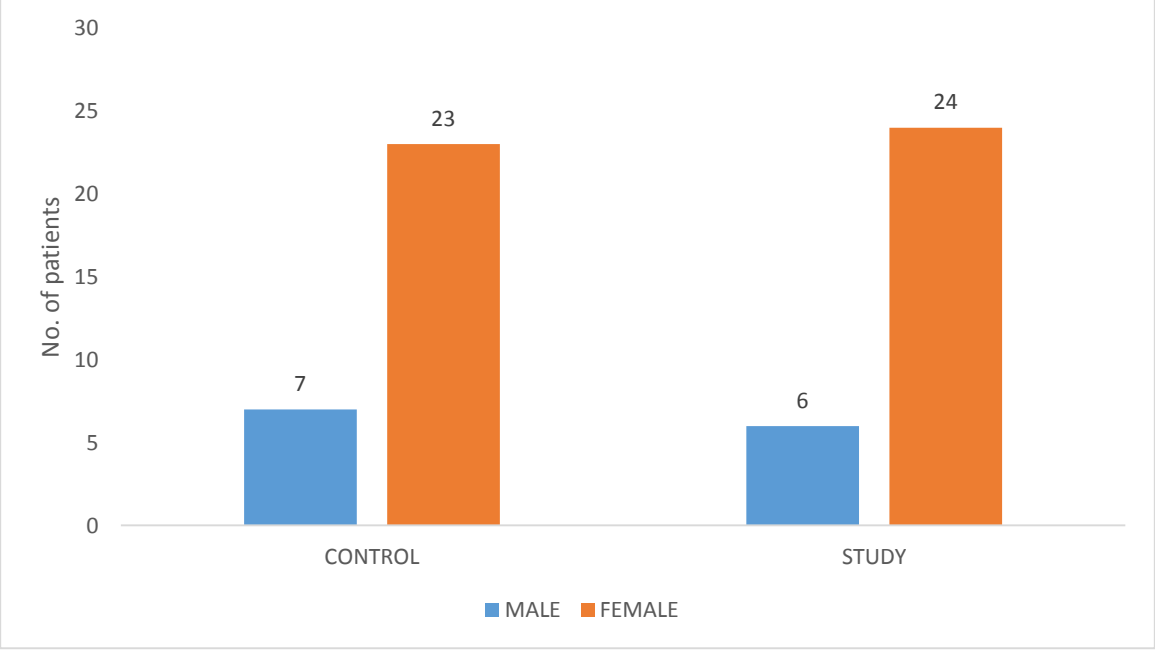


Table – 3 Graphical representation of gender distribution

TABLE-4: VAS PAIN SCORE:

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	7.06	1.26	6.30	0.915	0.14
STUDY	6.90	0.923	3.06	0.97	0.001
p value	1.0		0.042		

Table 4 shows VAS score,

On comparing the two groups,

There is statistically no significant change in the control group (p = 0.14). In the study group the reduction of VAS score at the end of 8 weeks is significant (p=0.001).

The difference between the control and study groups on day 0 (p = 1.0) is statistically insignificant. At the end of 8weeks statistically significant (p=0.042) reduction in pain score is noted in the study group.

Figure 4: VAS PAIN SCORE

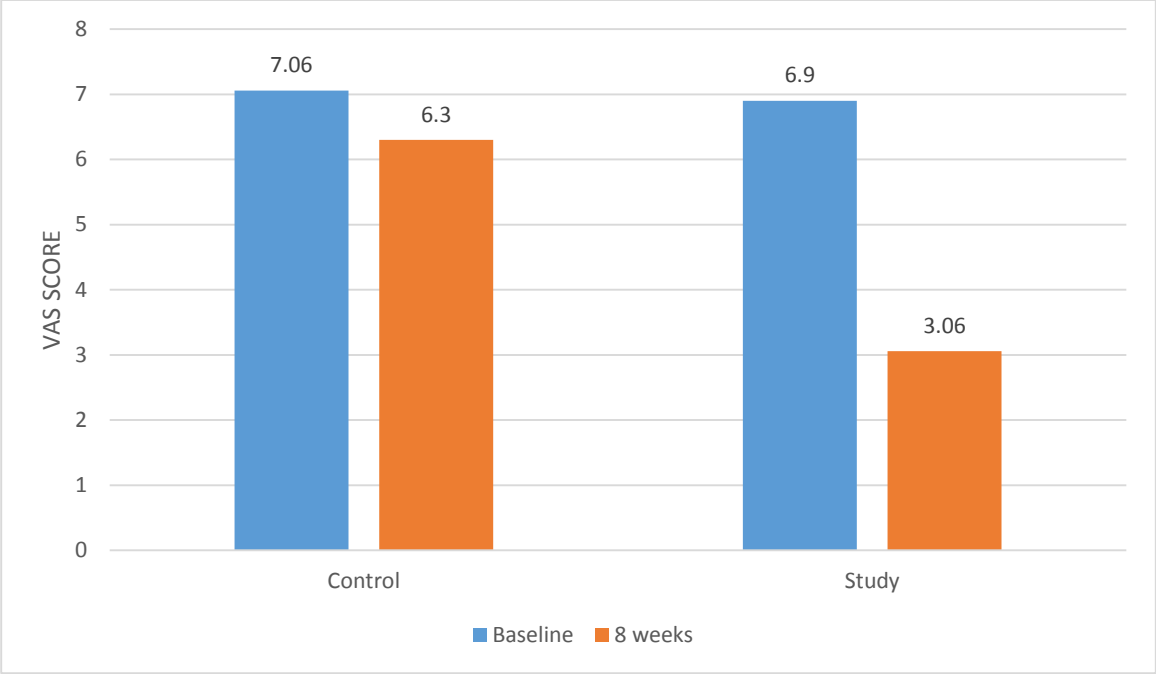


Figure 4 is the graphical representation of Table 4.

TABLE-5: TENDER JOINT SCORE:

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	14.2	3.82	12.06	3.32	0.06
STUDY	14.6	4.04	3.06	0.97	0.0001
p value	0.07		0.0001		

Table 4 shows Tender joint score.

On comparing the two groups,

There is statistically insignificant change in the control group (p = 0.06). At the end 8 weeks the study group showed significant reduction of Tender joint score (p=0.0001).

The difference between the control and study groups on day 0 is statistically insignificant (p = 0.07). There is statistically significant reduction (p=0.0001) in tender joint score is noted in study group at the end of 8 weeks.

FIGURE 5: TENDER JOINT SCORE

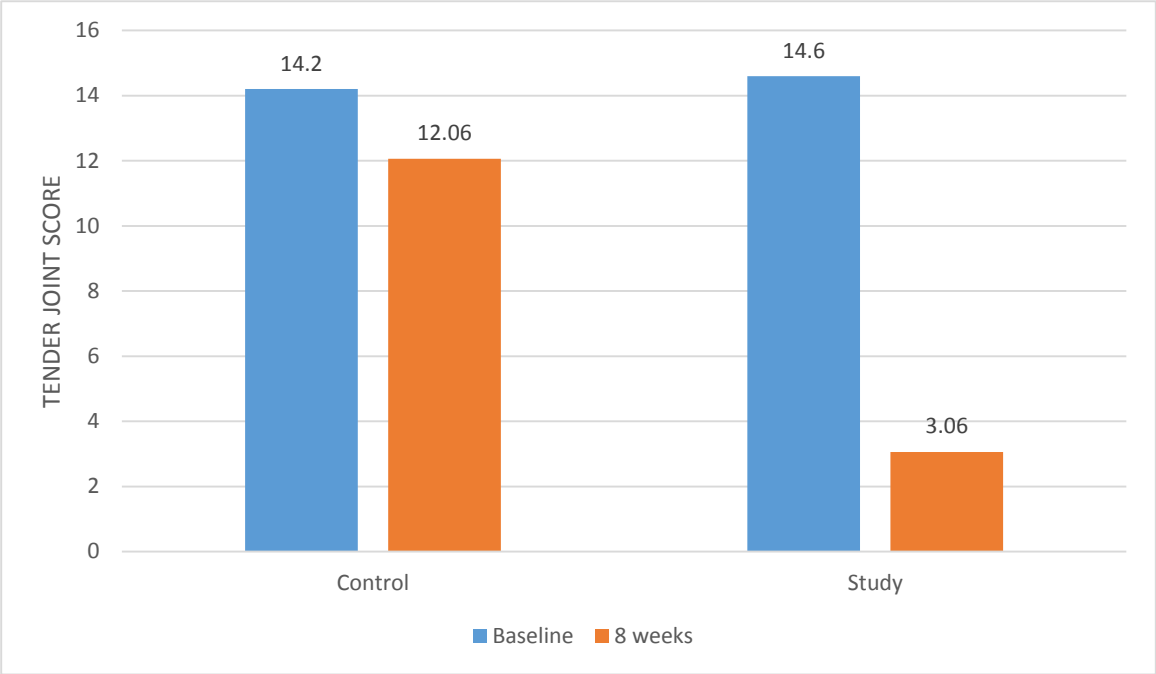


Figure 5 is the graphical representation of Table 5.

TABLE-6: SWOLLEN JOINT SCORE:

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	7.73	2.46	6.03	1.73	0.121
STUDY	7.66	2.76	2.56	1.59	0.001
p value	0.927		0.0001		

Table 4 shows swollen joint score,

On comparing the two groups,

There is statistically no significant change in the control group (p = 0.121), but in the study group there is significant reduction of swollen joint score (p=0.001) at the end of 8weeks is noted.

The difference between the control and study groups on day 0 (p = 0.927) is insignificant. After 8 weeks statistically significant reduction (p=0.0001) in swollen joint score is noted in the study group.

FIGURE 6: SWOLLEN JOINT SCORE

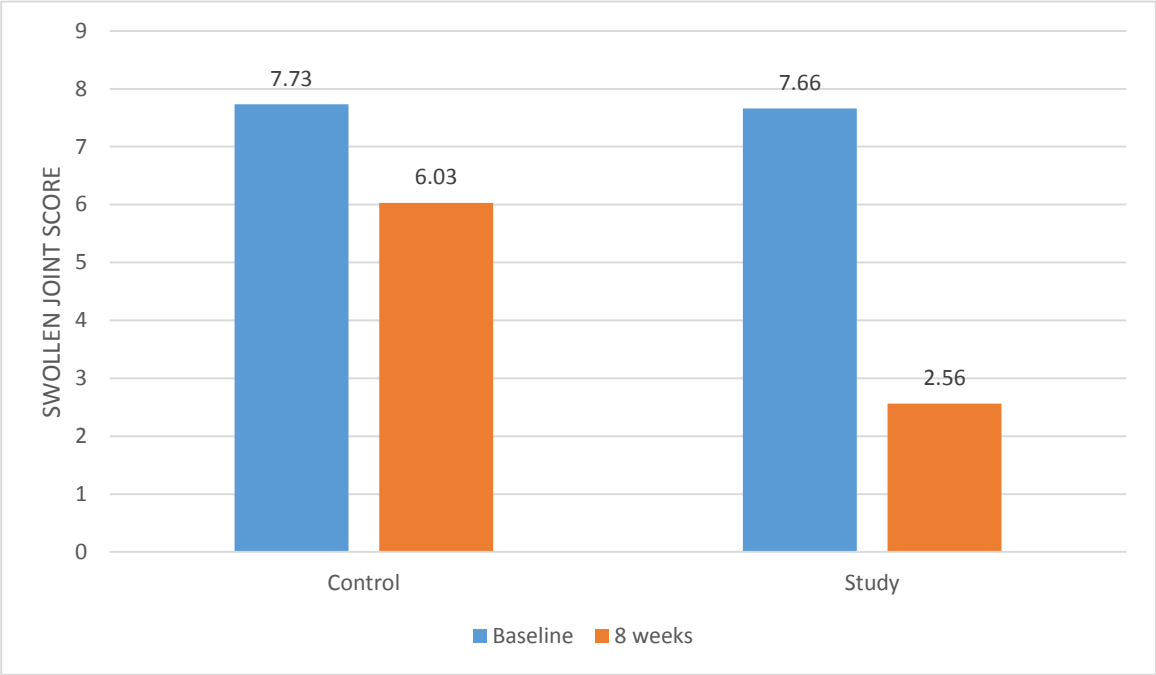
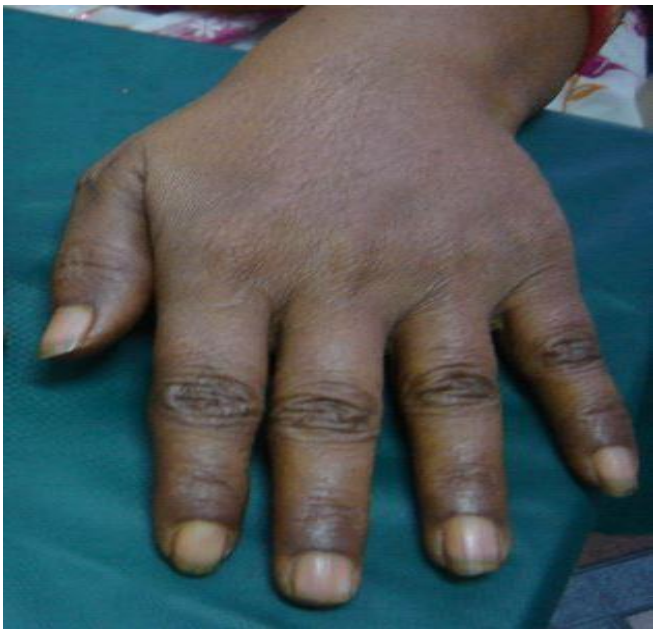
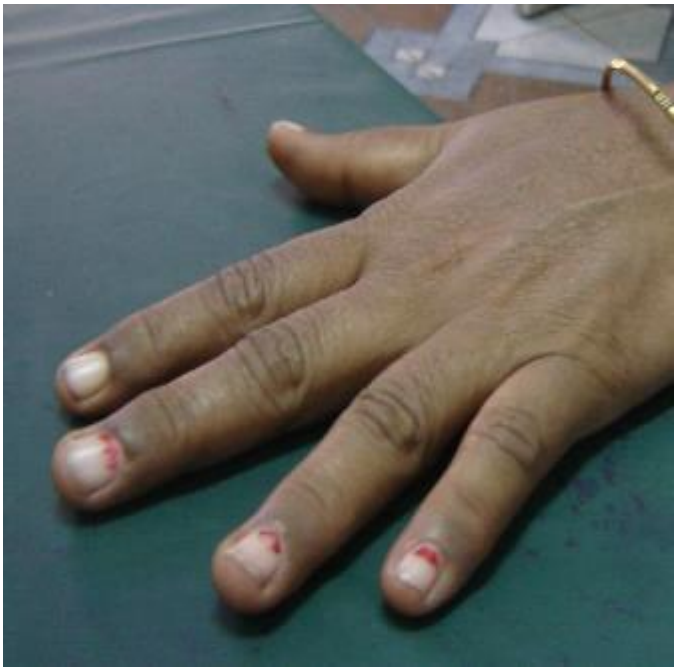


Figure 6 is the graphical representation of Table 6.

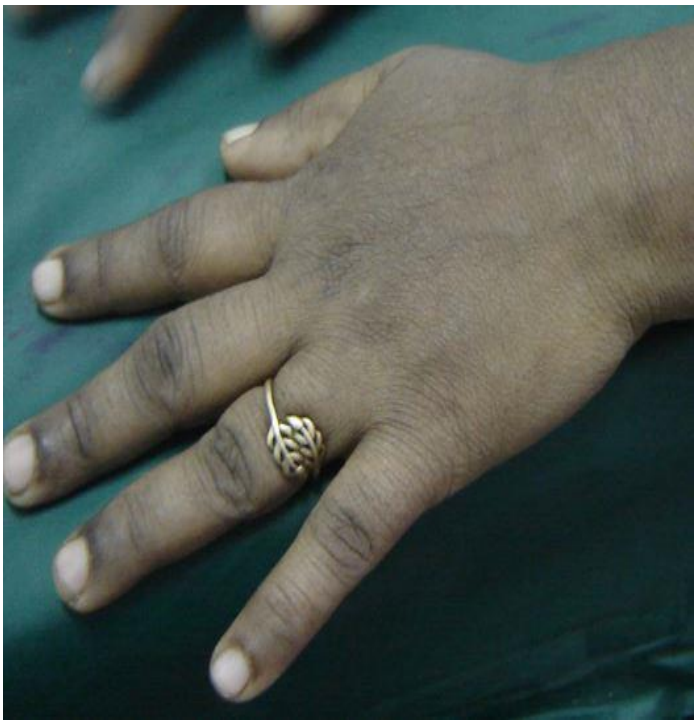
SWOLLEN FINGER JOINTS BEFORE THE TREATMENT



AFTER TREATMENT



SWOLLEN FINGER JOINT BEFORE TREATMENT



AFTER TREATMENT

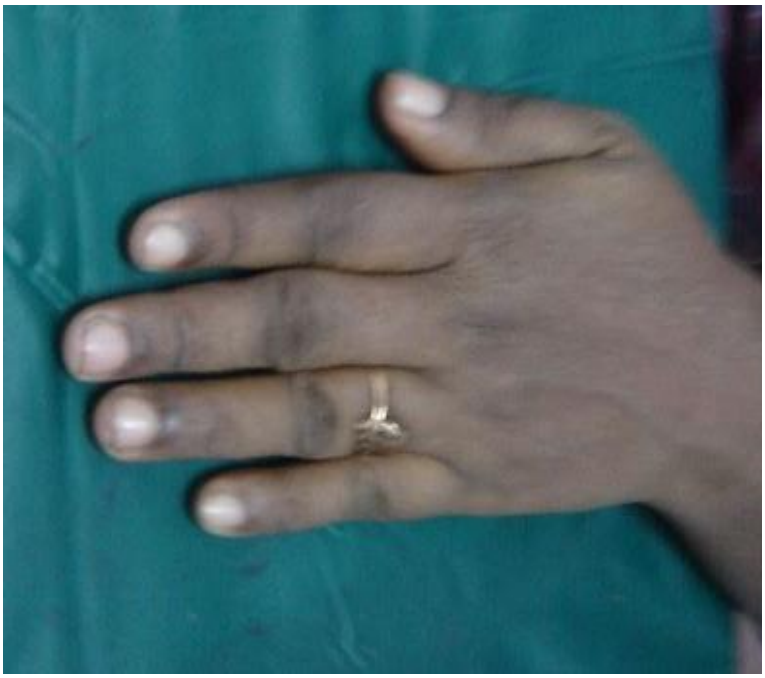


TABLE-7: DAS SCORE:

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	5.39	0.18	4.80	0.15	0.42
STUDY	5.26	0.2	3.10	0.39	0.001
p value	0.558		0.032		

Table 7 shows DAS score,

On comparing the two groups, there is statistically no significant change in the control group (p = 0.42), but in the study group reduction of DAS score at the end of 8 weeks is significant (p=0.001).

The difference between the control and study groups on day 0 is insignificant (p=0.558). After 8 weeks statistically significant (p=0.032) reduction in DAS score is noted in the study group.

After 8 weeks DAS score in study group is 3.1 and the reduction from base line is 2.16 which indicates good response to antioxidant treatment. In the control group the DAS score reduction is only 0.59 which indicates poor response to standard treatment.

FIGURE 7: DAS SCORE

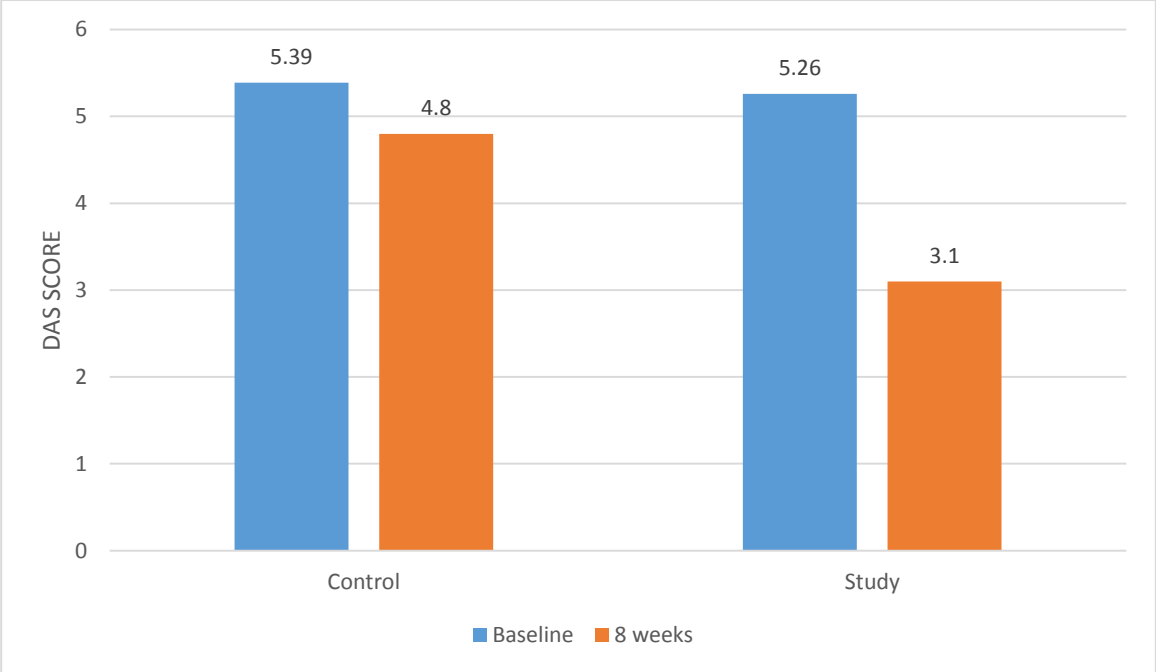


Figure 7 is the graphical representation of Table 7.

TABLE-8: MEAN ESR

GROUPS	DAY 0 (mm/hr)		AT THE END OF 8 WEEKS (mm/hr)		% REDUCTION FROM BASELINE	p value
	MEAN	SD	MEAN	SD		
CONTROL	27.27	2.75	25.3	0.915	7%	0.06
STUDY	28.20	4.51	12.06	0.97	57%	0.0001
p value	0.337		0.001			

Table 8 shows mean ESR

On comparing the two groups, there is statistically no significant change in the control group (p = 0.06) but in the study group the reduction in the ESR at the end of 8 weeks is statistically significant (p=0.0001)

The difference between the control and study groups on day 0 is insignificant (p =0.337). After 8 weeks statistically significant reduction in ESR (p=0.0001) is noted in the study group.

TABLE -8A : PERCENTAGE REDUCTION OF ESR

ESR REDUCTION (mm/hr)	STUDY GROUP	PERCENTAGE	CONTROL GROUP	PERCENTAGE
<5	2	13%	21	70%
5 to 10	4	23%	2	7%
10-15	14	47%	0	0
15-20	9	30%	0	0
OTHERS(same / increase)	1	3%	7	23%

Figure 8: MEAN ESR

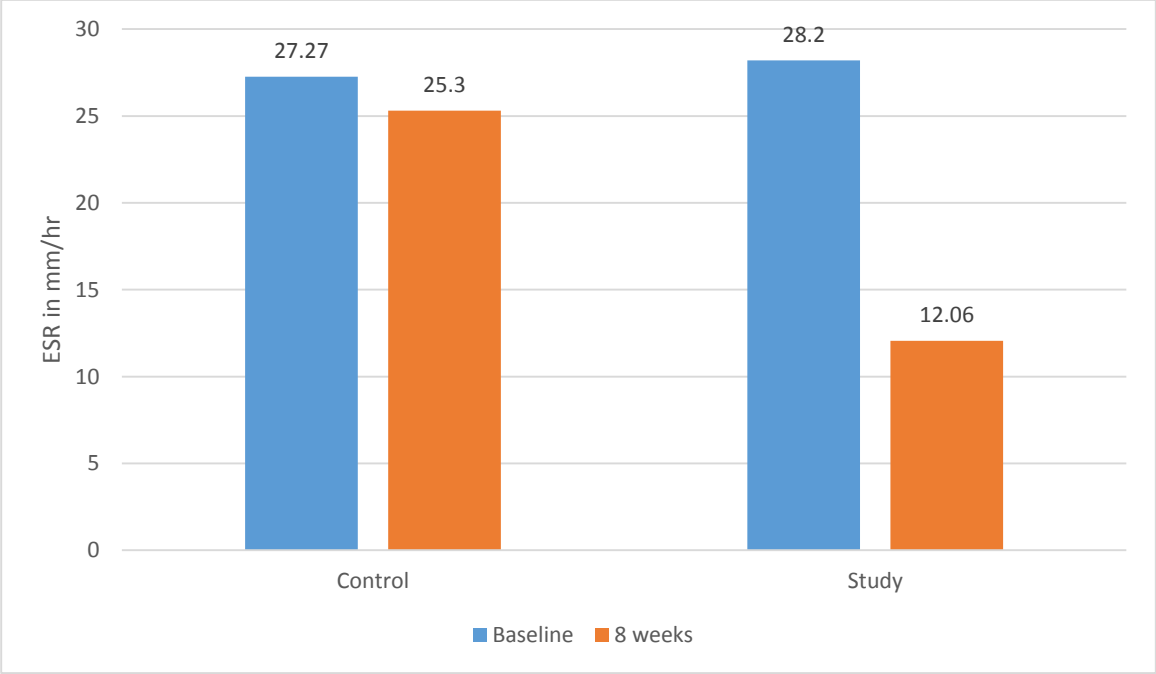


Figure 8 is the graphical representation of Table 8.

TABLE-9: C-REACTIVE PROTEIN

MEAN CRP		BASE LINE		AT THE END OF 8 WEEKS	
		NO OF PATIENTS	%	NO OF PATIENTS	%
CONTROL	>6	30	100	25	83
	<6	0	0	5	17
STUDY	>6	30	100	3	10
	<6	0	0	27	90

Table 4 shows CRP, between study and control groups

On comparing the two groups, at the end of 8 weeks 90% of patients in the study groups shows <6 value of CRP. But in the control groups only 17% of patients shows reduction of CRP to <6 value.

FIGURE-9: C-REACTIVE PROTEIN

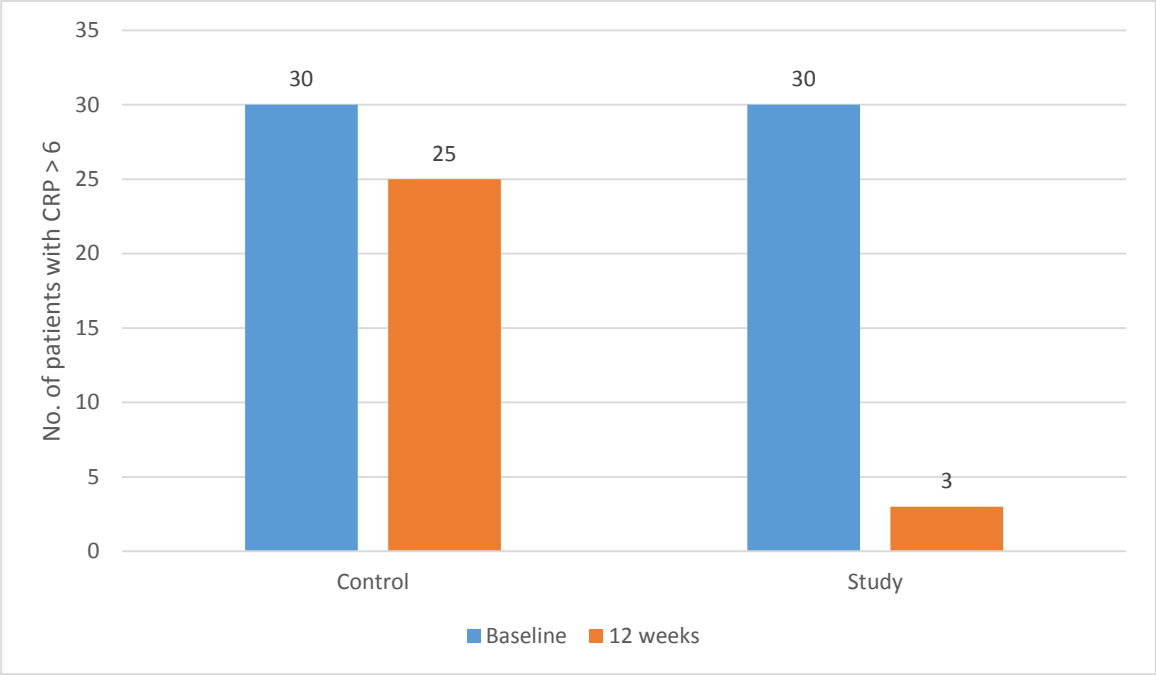


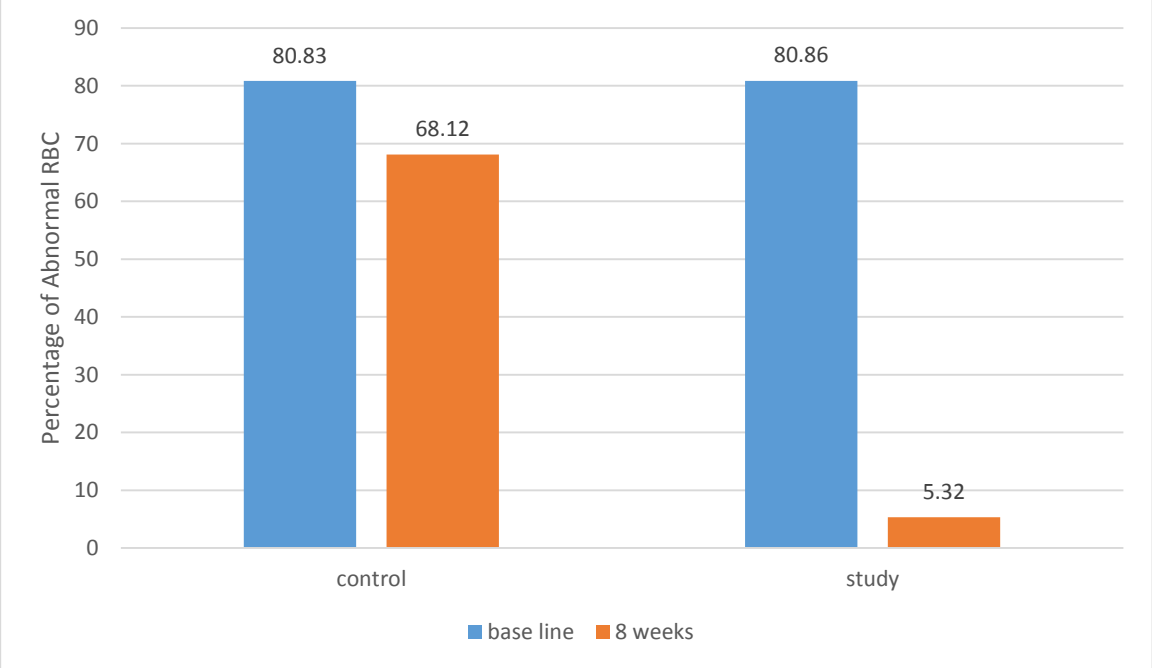
Figure 9 is the graphical representation of Table 9.

TABLE-10: % OF ABNORMAL RBC

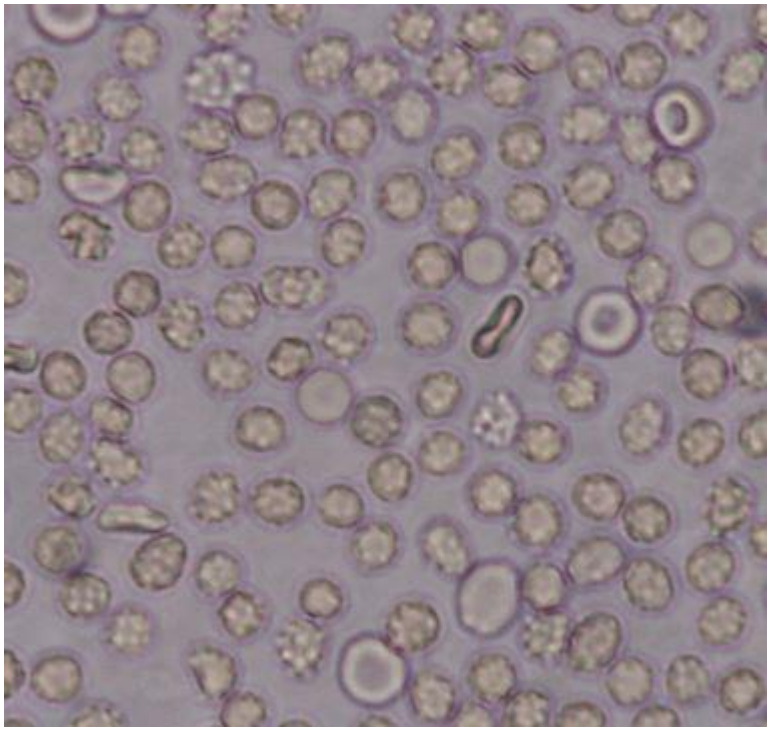
GROUPS	PERCENTAGE AT BASE LINE		PERCENTAGE AT THE END OF 8WEEKS		P VALUE
	MEAN	SD	MEAN	SD	
CONTROL	80.83%	15.2	68.12%	12.32	0.43
STUDY	80.86%	16.7	5.32%	0.42	0.0001

Percentage of abnormal RBCs in both groups at the base line was approximately 80%. After 8 weeks it was reduced to 68.12% in the control group but in the study group it was greatly reduced to 5.32%.

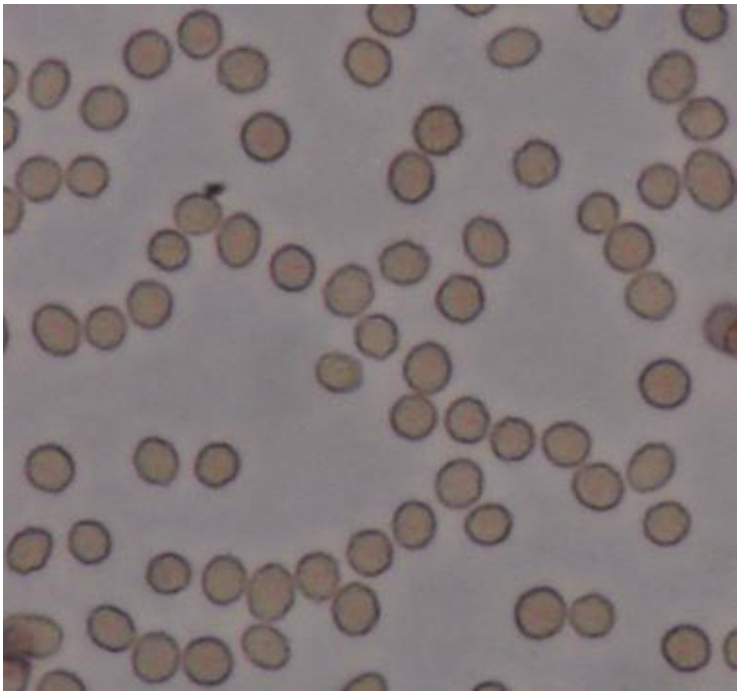
FIGURE – 10: PERCENTAGE OF ABNORMAL RBCs



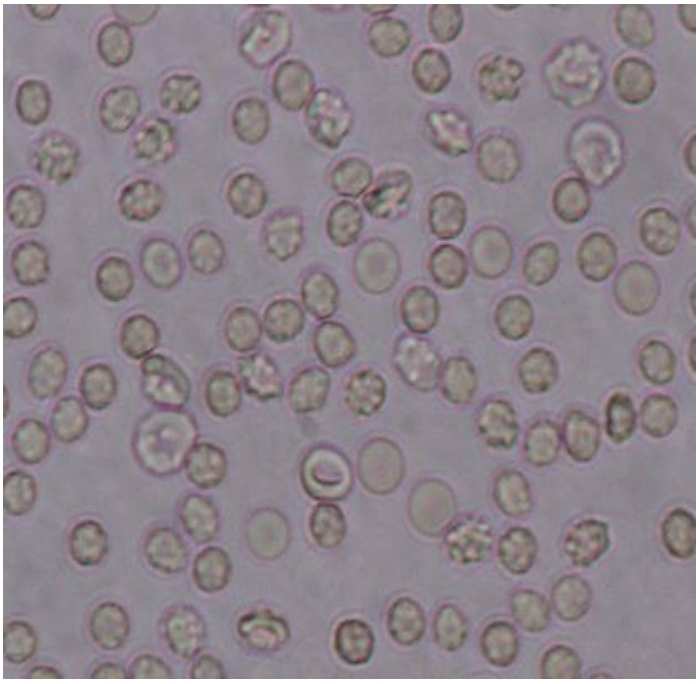
MORPHOLOGY OF RBCS BEFORE TEATMENT



AFTER TREATMENT



MORPHOLOGY OF RBCS BEFORE TRATMENT



AFTER TREATMENT

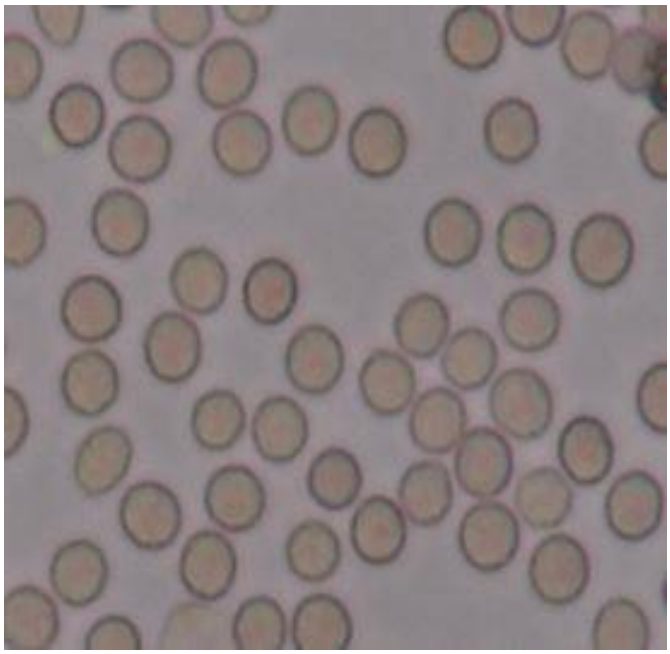


TABLE-11 : MEAN HAEMOGLOBIN

GROUPS	DAY 0(gm/dl)		AT THE END OF 8 WEEKS(gm/dl)		p value
	MEAN	SD	MEAN	SD	
CONTROL	9.32	0.39	9.38	0.23	0.22
STUDY	9.36	0.69	10.86	0.35	0.042
p value	0.162		0.043		

Table 11 shows mean haemoglobin

On comparing the two groups the increase in haemoglobin is statistically not significant in the control group (p = 0.22). In the study group increase of haemoglobin at the end of 8 weeks (p=0.042) is statistically significant.

The difference between the control and study groups on day 0 (p = 0.162) is statistically insignificant .At the end of 8 weeks statistically significant (p<0.043) increase in haemoglobin is noted in the study group.

TABLE 11A: PERCENTAGE INCREASE OF HAEMOGLOBIN

HB INCREASE FROM BASELINE (gm/dl)	STUDY GROUP	% INCREASE	CONTROL GROUP	% INCREASE
	No.of patients		No.of patients	
<0.5	1	3%	16	53%
>0.5	3	10%	4	13%
>1	9	30%	1	3%
>1.5	16	53%	0	0%
OTHERS(same/decrease)	1	3%	9	30%

FIGURE-11: MEAN HAEMOGLOBIN

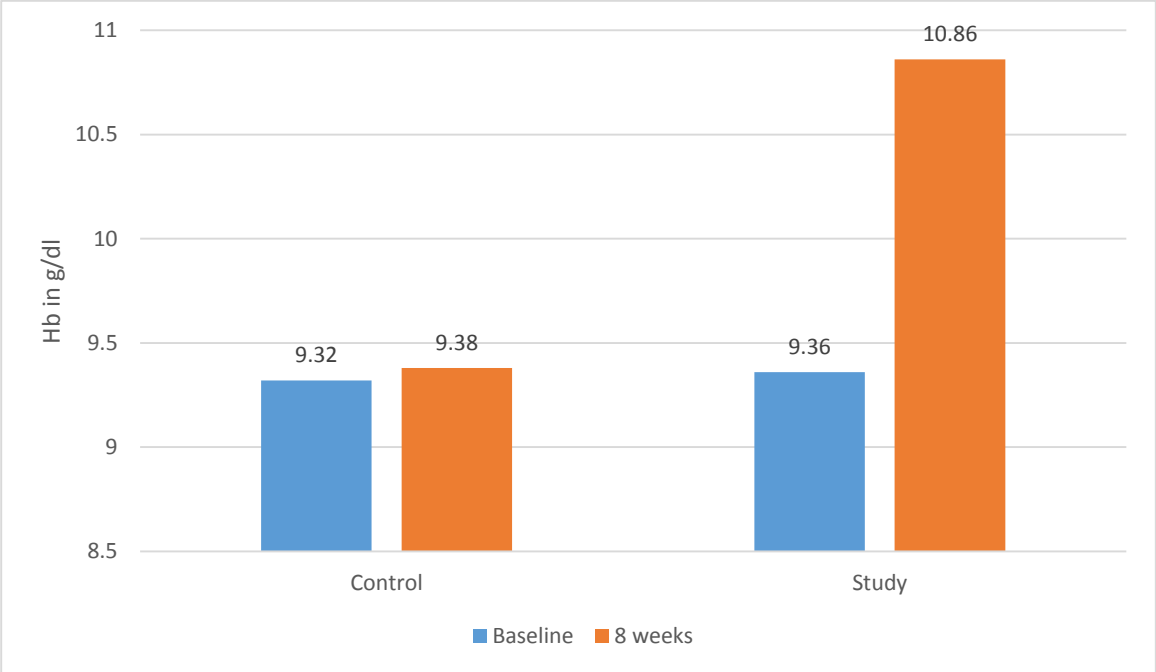


Figure 11 is the graphical representation of Table 11.

TABLE-12: MEAN SYSTOLIC BLOOD PRESSURE

GROUPS	DAY 0(mmHg)		AT THE END OF 8 WEEKS(mmHg)		p value
	MEAN	SD	MEAN	SD	
CONTROL	129.33	2.59	128.2	4.28	0.159
STUDY	128.13	2.46	120.17	3.34	0.0001
p value	0.55		0.0001		

Table 12 shows mean systolic blood pressure

On comparing the two groups,

There is statistically no significant change in the control group (p = 0.159), but in the study group there is reduction of mean systolic BP at the end of 8weeks (p=0.001)

The difference between the control and study groups on day 0 (p = 0.55) is insignificant. After 12 weeks statistically significant reduction (p=0.001) in mean systolic BP is noted in the study group.

FIGURE-12: MEAN SYSTOLIC BLOOD PRESSURE

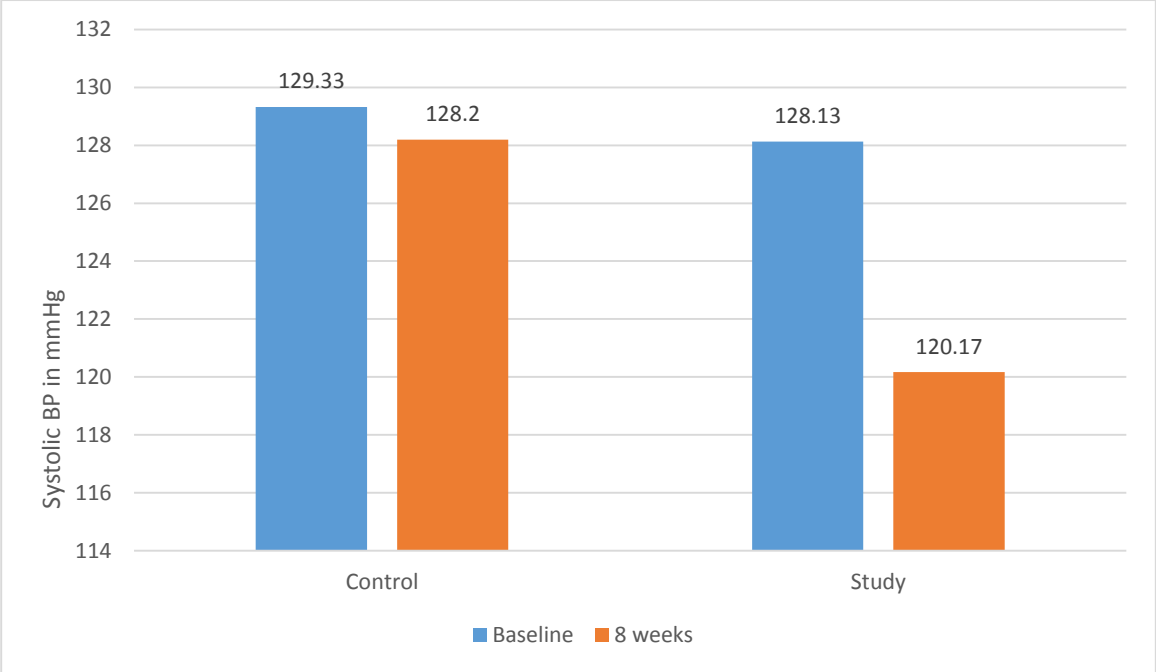


Figure 12 is the graphical representation of Table 12.

TABLE-13: MEAN DIASTOLIC BLOOD PRESSURE:

GROUPS	DAY 0(mmHg)		AT THE END OF 8 WEEKS(mmHg)		p value
	MEAN	SD	MEAN	SD	
CONTROL	85.13	4.27	83.8	5.19	0.324
STUDY	84.4	2.9	80.27	2.08	0.001
p value	0.28		0.001		

Table 13 shows mean diastolic BP

On comparing the two groups,

There is statistically no significant change in the control group (p = 0.324). At the end of 8 weeks the reduction of diastolic BP (p=0.001) is statistically significant in the study group.

The difference between the control and study groups on day 0 (p = 0.28) is insignificant. After8 weeks statistically significant reduction in diastolic BP (p=0.001) is noted in the study group.

FIGURE-13: MEAN DIASTOLIC BLOOD PRESSURE:

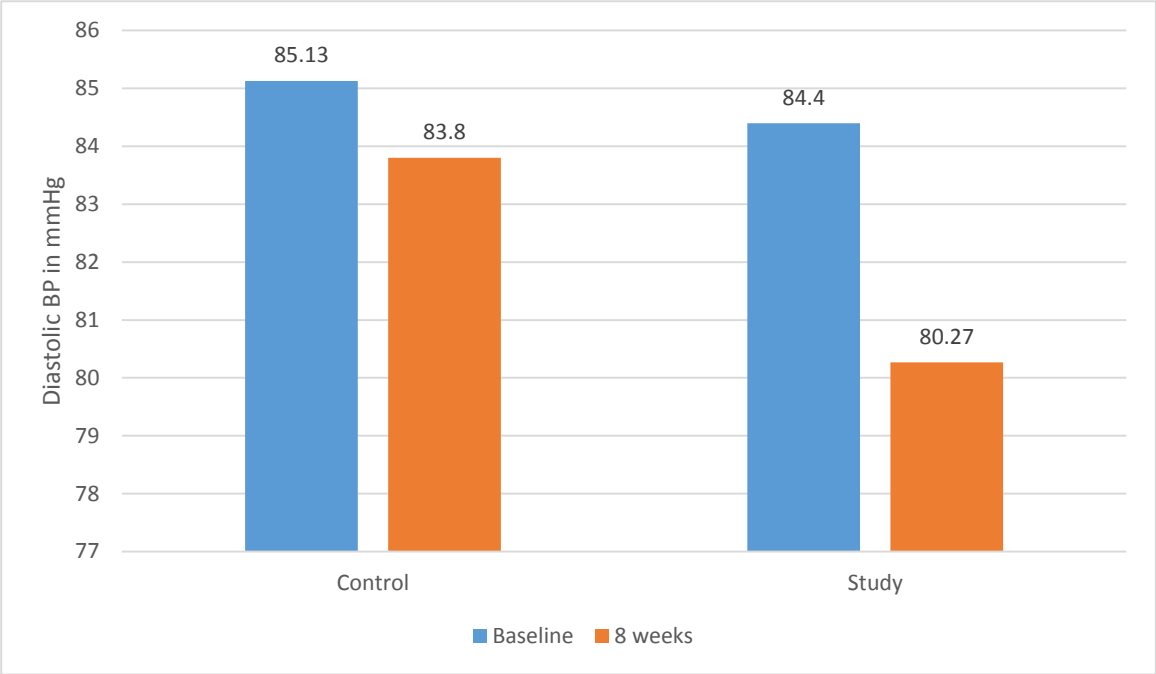


Figure 13 is the graphical representation of Table 13.

TABLE14: PERCENTAGE DECREASE IN THE VALUE OF PARAMETERS DURING THE STUDY PERIOD

INVESTIGATIONS	% decrease from Baseline Value	
	Control Group	Study Group
MEAN VAS SCORE	10.76	51.42
MEAN TENDER JOINT SCORE	15.07	79.04
MEAN SWOLLEN JOINT SCORE	21.91	66.57
MEAN DAS SCORE	9.09	30.97
MEAN ESR	7	57
CRP (No. of persons with > 6)	16.66	90
RBC MORPHOLOGY SCORE	12.71	75.56
MEAN SYSTOLIC BP	0.8	6
MEAN DIASTOLIC BP	1.7	4.8
MEAN HAEMOGLOBIN	-1.16	-16

TABLE-14A: BIOCHEMICAL INVESTIGATIONS- (STUDY GROUP)

INVESTIGATIONS	STUDY GROUP		p-VALUE
	BASELINE MEAN	8 WEEKS MEAN	
HEMOGLOBIN (g/dl)	9.36	10.86	0.043
BLOOD UREA (mg/dl)	25.33	24.14	0.182
SERUM CREATININE (mg/dl)	0.77	0.77	0.839
BILI RUBIN(mg/dl)	0.93	0.67	0.142
SGOT (IU/L)	30.37	30.80	0.636
SGPT (IU/L)	27.67	26.07	0.108

Table 14Ashows the biochemical investigations mean values done in the study group at baseline and 12 weeks.

Comparison showed that there was no statistically significant difference in the biochemical investigation mean value except haemoglobin ($p<0.001$)

TABLE-14B BIOCHEMICAL INVESTIGATIONS

(CONTROL GROUP)

INVESTIGATIONS	STUDY GROUP		p-VALUE
	BASELINE MEAN	8 WEEKS MEAN	
HEMOGLOBIN (g/dl))	9.32	9.38	0.220
BLOOD UREA (mg/dl)	27.4	24.87	0.080
SERUM CREATININE (mg/dl)	0.82	0.75	0.960
BILI RUBIN(mg/dl)	0.59	0.67	0.142
SGOT (IU/L)	29.03	28.40	0.433
SGPT (IU/L)	30.73	30.67	0.946

Table 14B shows the biochemical investigations mean values done in the study group at baseline and 12 weeks.

Comparison showed that there was no statistically significant difference in the biochemical investigation mean values.

TABLE-15: ADVERSE EVENT PROFILE

ADVERSE EVENT	CONTROL GROUP (30) No. of patients	STUDY GROUP (30) No. of patients
NAUSEA	**5(17%)**	**4(13%)**
VOMITING	**1(3%)**	**1(3%)**
ABDOMINAL PAIN	**4(13%)**	**2(7%)**
HEAD ACHE	**2(7%)**	**1(3%)**
METALLIC TASTE	**1(3%)**	**0**

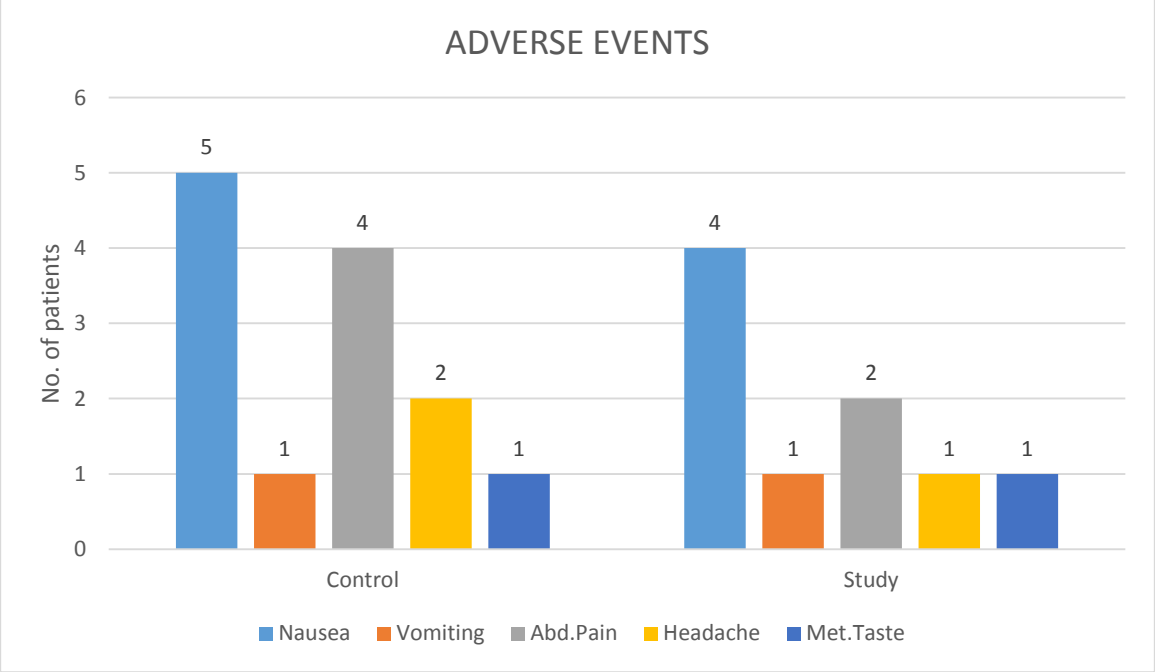


TABLE 15A: INCIDENCE OF ADRs

	CONTROL GROUP (30) No. of patients (%)	STUDY GROUP (30) No. of patients (%)
NUMBER OF ADRs	13 (43%)	8(27%)

FIGURE 15A: ADVERSE EVENT

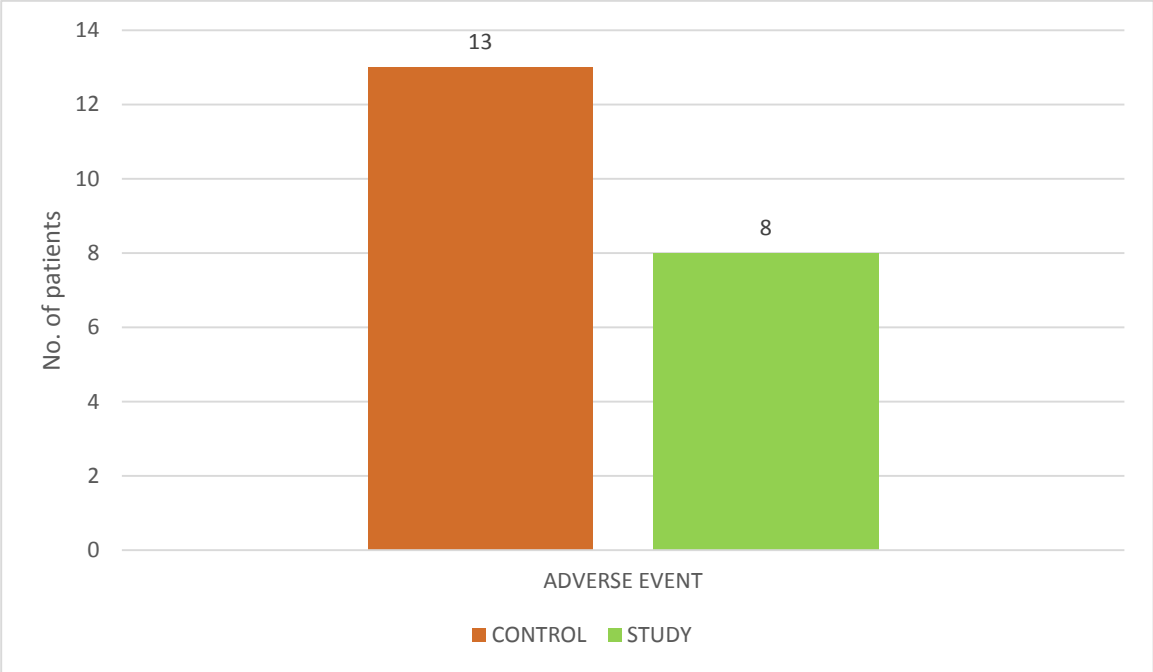


Figure 16: MEAN VAS PAIN SCORE DURING STUDY AND FOLLOW UP PERIOD

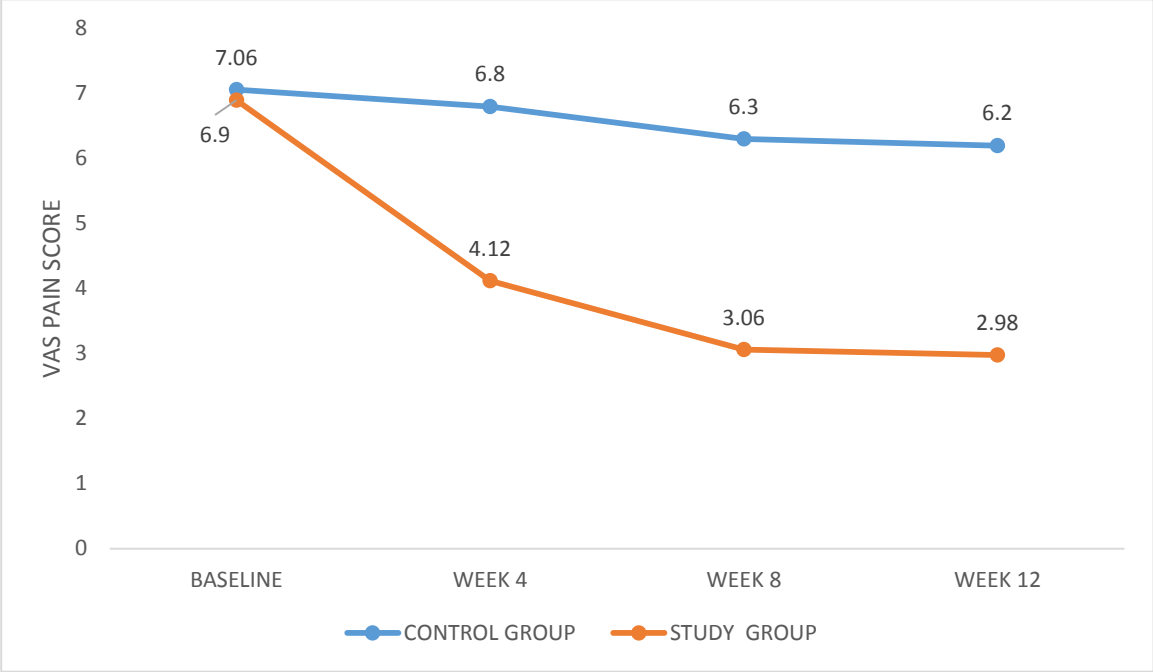


FIGURE 17: MEAN TENDER JOINT SCORE DURING STUDY AND FOLLOW UP PERIOD

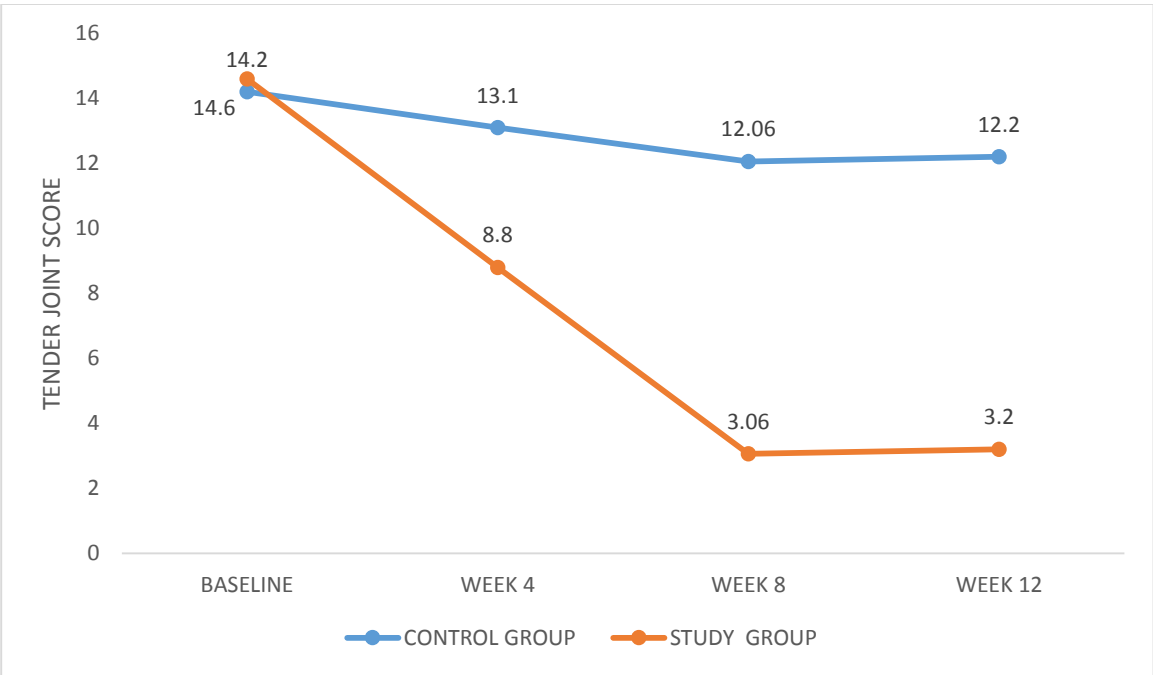


FIGURE 18: MEAN SWOLLEN JOINT SCORE DURING STUDY AND FOLLOW UP PERIOD

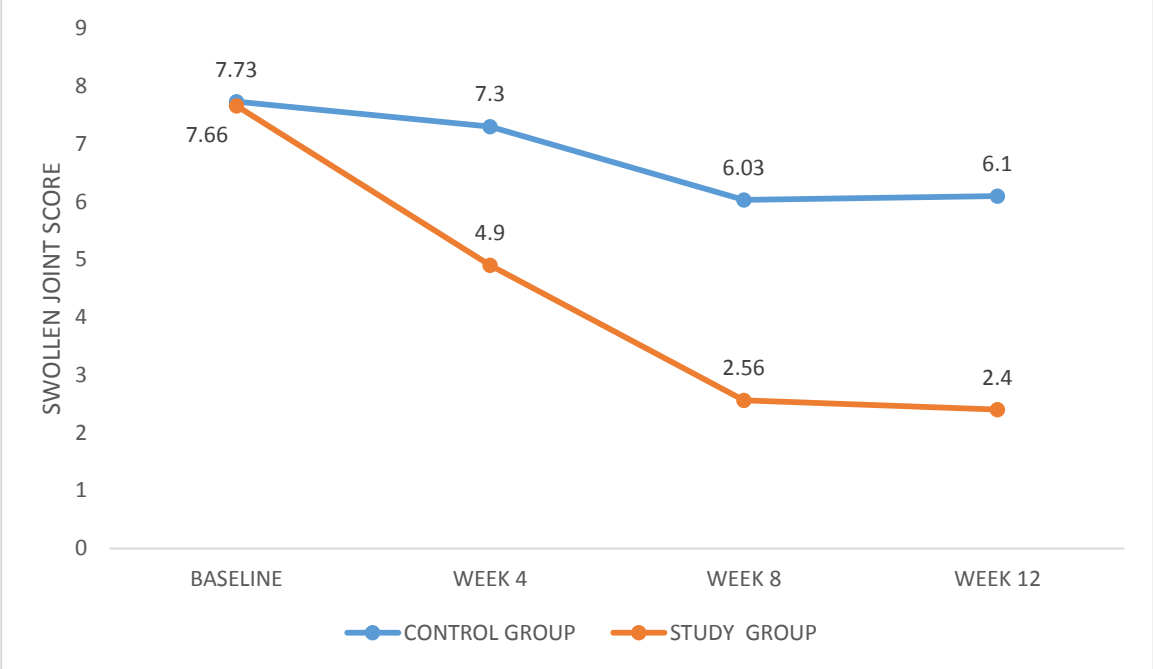


FIGURE 19: MEAN DAS SCORE DURING STUDY AND FOLLOW UP PERIOD

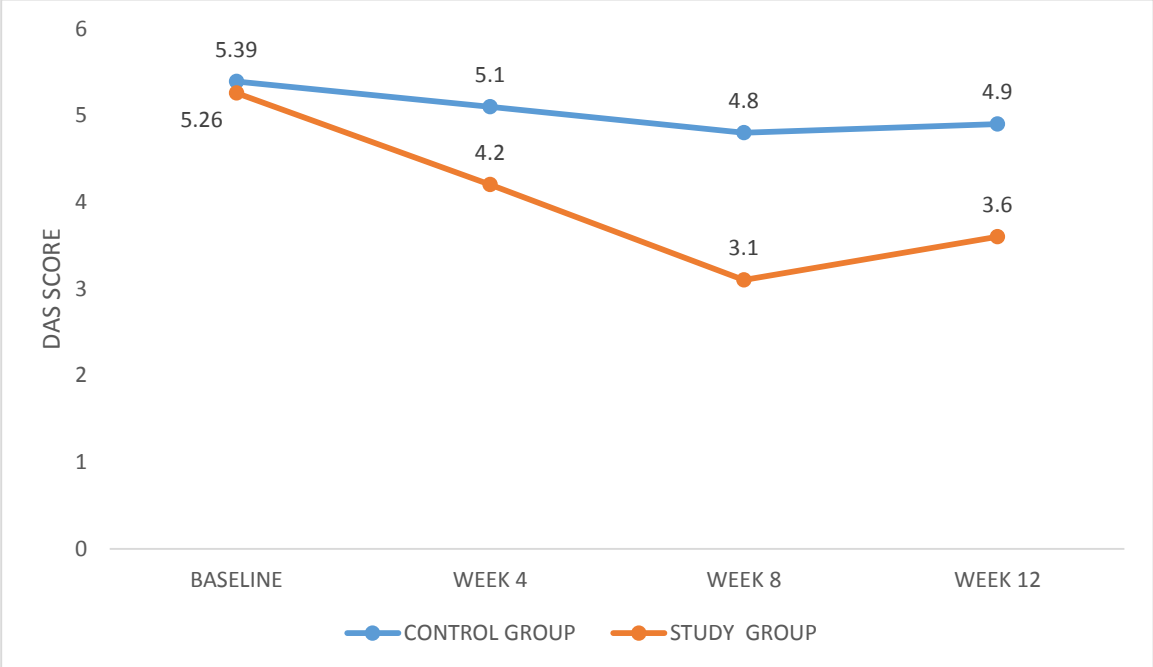


Figure 20: MEAN ESR DURING STUDY& FOLLOWUP PEROIOD

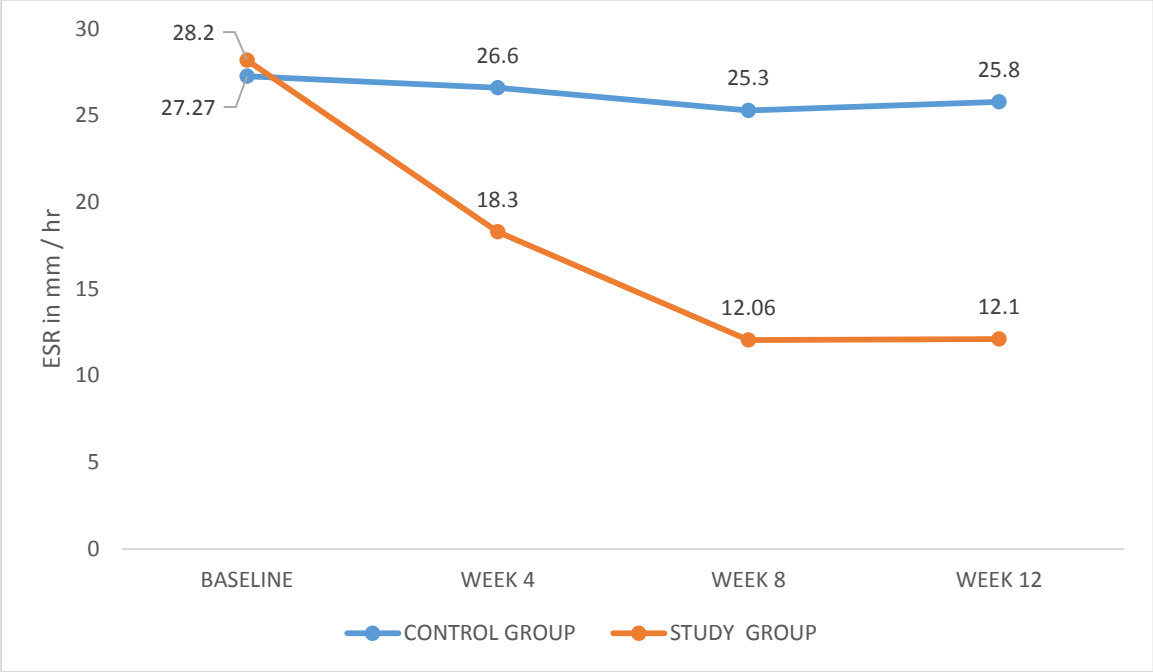


FIGURE 21: MEAN HAEMOGLOBIN DURING STUDY & FOLLOWUP PERIOD

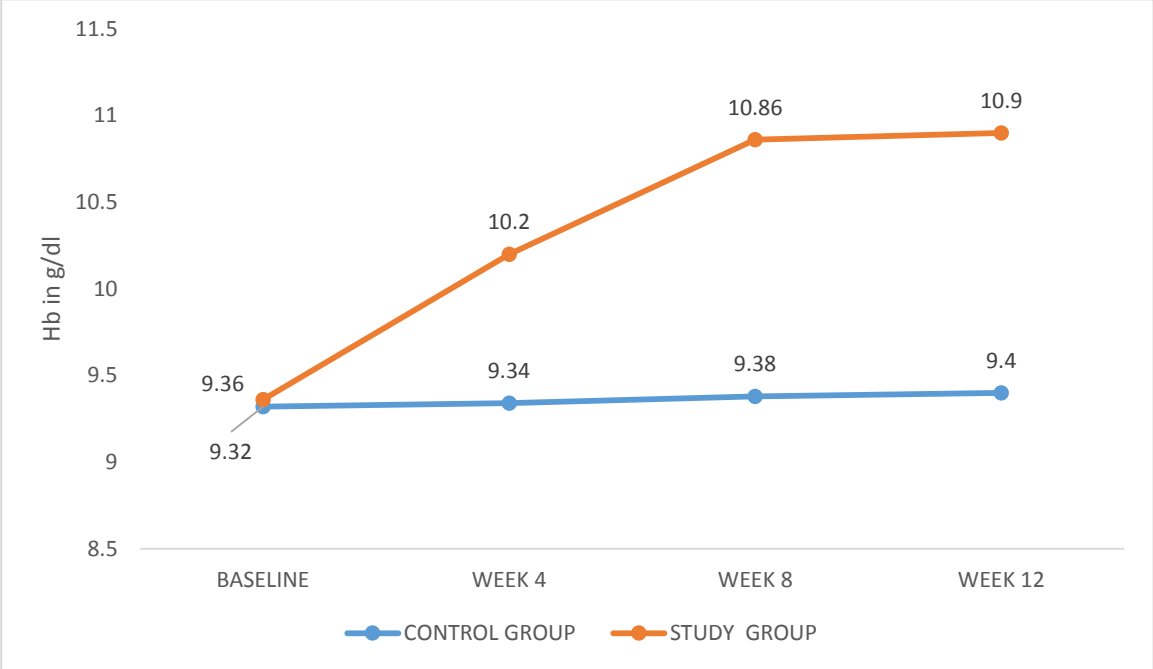
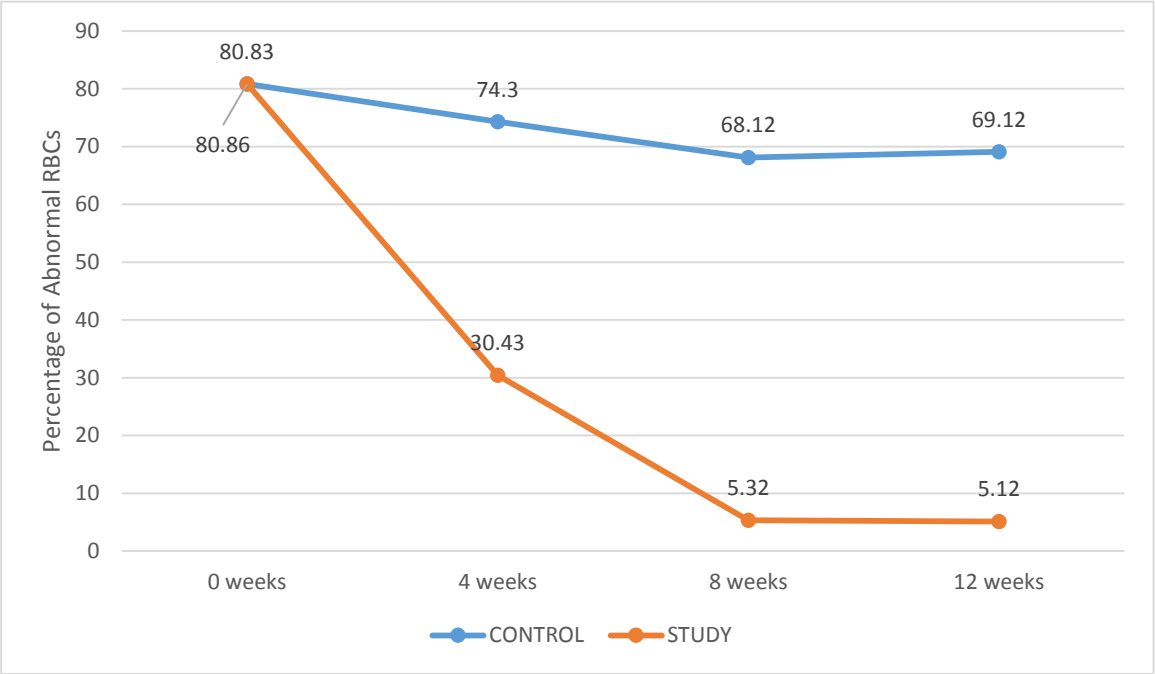


FIGURE 22: MEAN % of ABNORMAL RBCS DURING STUDY AND FOLLOW UP PERIOD



DISCUSSION

DISCUSSION

RA is an autoimmune induced chronic inflammatory disease affecting mainly joint spaces and various organs. Inspite of the treatment, the disease progresses and destroys the joints spaces and various tissues leading to deformities.[3]

Oxidative stress is one of the important etiological factor in the pathogenesis of RA. Therefore adding antioxidant to the regular treatment can arrest the disease progress. In this study α-tocopherol and ascorbic acid are added to the conventional treatment in RA.

Most of the patients with RA have anaemia along with elevate ESR level. The cause of anaemia and the type of anaemia was not clearly understood and there is no reference regarding the type of anaemia and the cause in RA. [17]

RBCs are the first target for oxidative damage caused by free radicals.[54] Therefore in this study the RBC morphology was used as a biomarker for oxidative stress. ROS causes haemolysis in RBCs which alters the shape and size of the RBCs. It also denatures the haemoglobin causing" Heinz bodies". [32]

Crenated edges, Heinz bodies, Spherocytes are some of the morphological alteration seen due to haemolysis. The lifespan of RBCs and oxygen carrying capacity is diminished as a consequences which leads to anaemia and tissue destruction. In this study Vitamin E 400mg and Vitamin C 500mg once daily after breakfast was given along with conventional treatment for a period of 2 months. The follow up period was 4 weeks.

The level of oxidative stress was measured by the level of haemoglobin and percentage of RBCs haemolysis in these patients, as a biomarker for oxidative stress.

Clinically the patients were assessed for painful joint, tender joint, and swollen joints score and DAS score. The laboratory markers like ESR and CRP were also used as a marker for inflammation.

Out of 96 patients screened 36 were excluded due to various reasons and 60 patients were divided into study group 30 patients and control group 30 patients by simple randomisation.

The majority of patients in this study group were between 31-40 years, and out of which women were 76% and men were 24%. The improvement in the patients was assessed by pain score, tender joint score, swollen joint score, and DAS score.

In the control group pain score was slightly reduced ($p=0.14$) at the end of 8weeks. In the study group pain score was reduced significantly ($p=0.001$) at the end of study.

The tender joint score was reduced in the control ($P=0.06$) which is not significant. In the study group at the end of 8 weeks, the tender joint score was reduced to ($p=0.0001$) which is highly significant.

Similarly the swollen joint score was significantly reduced in the study group ($p=0.001$) than the control group ($p=0.121$)

The disease activity score at the end 8 weeks in the study group showed significant reduction of ($p=0.001$) than the control groups ($p=0.42$).

The laboratory markers like ESR was approximately 28mm/hr in both the groups initially. At the end of 8weeks the ESR level was reduced to12mm/hr in the study group, where as in the control group there was only a slight fall to 25mm/hr only.

The CRP was decreased in nearly 90 % of the patients in the study group to <6, where as in the control group only 17% of patients showed reduction.

Nearly 80 % of the RBCs in both the group of patients were altered due to ROS induced haemolysis having crenated edges, Heinz bodies, spherocytes and macrocytosis. Presence of macrocytosis (25-30%) in the blood smear (Normal being between 0.5% -1%) indicates reactive erythropoiesis induced by haemolysis in RA.[29]

After treatment with antioxidants the number of abnormal RBCs in the study group was reduced to 5%, and in the control group it was reduced to 68% only. The reduction in haemolysis of RBCs is correlated to clinical improvement like pain score, joint score, proves the role of ROS in the pathogenesis of Rheumatoid arthritis.

The haemoglobin level was very much reduced in both the groups with mean 9.3g/dl initially. At the end of 8 weeks the haemoglobin level was increased to 10.8gm/dl in the study group and, in the control group it was only 9.38gm/dl only.

This proves that anaemia in RA is due to haemolysis caused by oxidative damage only not due to decreased erythropoiesis or iron deficiency as mentioned in the standard text book.[17]

All the patients included in the study group showed slight increase in systolic and diastolic blood pressure of 130/85mmHg before the study. At the end of 8 weeks there was a significant reduction in systolic and diastolic blood pressure of 120/80mmHg in the study group compared to control groups.

There was slight decrease in adverse events like abdominal pain, nausea, and vomiting in the study group compared to control group.

All the patients in both the groups were followed for the period of 4 weeks. During the follow up period all the patients received T. Indomethacin 25mg BD, T. Hydroxy chloroquine 400mg OD. At the end of 4 weeks all the tests were repeated along with clinical examination.

In the control group only mild improvement in the joint score was seen, to the same extent as during the study period.

In the study group the improvement in the joint score and RBC biomarker and inflammatory markers was sustained during the follow up period. There was no exacerbation of the disease after 4 weeks.

The improvement in the RBC morphology was well correlated with the clinical improvement in the patient who received antioxidants, which confirms the role of ROS in the pathogenesis of Rheumatoid arthritis.

CONCLUSION

CONCLUSION

In this study, it has been proved that anaemia in RA is due to haemolysis caused by free radical injury and not due to defective erythropoiesis.

Hence RBC morphology was used as a bio-marker of oxidative stress in RA. Anti-oxidants like vitamin C and E were added to the regular treatment for 8 weeks.

The haemolysis was almost completely arrested along with significant clinical improvement which was monitored by various scores. This once again proves that role of free radical in the pathogenesis of RA.

Treatment with antioxidants like Vitamin C and Vitamin E arrested the disease progress and has a disease modifying action. It also improved the quality of life in patients with RA.

Therefore, using RBC morphology as a bio-marker for oxidative stress and adding antioxidant to the regular treatment is cost effective and novel approach to the management of RA.

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APPENDICES

MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY.

CASE REPORT FORM

NAME: AGE/SEX : PLACE:

OP No: DIAGNOSIS:

Inclusion criteria: **YES/NO**

- Age - 40 years to 70 years
- Sex - both genders
- Patients willing to give written informed consent.
- Patients diagnosed as Rheumatoid Arthritis based on American college association criteria.

Exclusion criteria: **YES/NO**

- Arthritis incompatible with RA by ACR criteria
- Patient on oral and parenteral corticosteroids
- Pregnant and lactating women

- ❖ Patients with co-existing liver disease, heart disease, dyslipidaemia or malignancy
- ❖ Patients with Haematological disorders

Subject initials: Subject number:

Subject: Included/Excluded Reason if excluded:

Informed Consent Obtained: Yes/No

CONTROL/ TEST :

Signature of principal investigator

VISIT 1

1. Vitals:
2. Medical History:
3. General /systemic examination:
4. Investigations:

   Haematology:

   Hb:

   Blood Urea: Blood sugar: Serum creatinine:

   SGPT: SGOT:

   ECG:

   X-ray Hand AP

X-ray chest PA view:

5. Visual analogue pain scale:
6. DAS score
7. RBC morphological assessment

VISIT 2

1. Vitals:
2. Visual analogue pain scale:
3. DAS score
4. RBC morphological assessment
5. Adverse Events:

VISIT 3

1. Vitals:
2. Visual analogue pain scale:
3. DAS score
4. RBC morphological assessment
5. Adverse Events:

VISIT 4

1. Vitals
2. Visual analogue pain scale:
3. DAS score
4. RBC morphological assessment

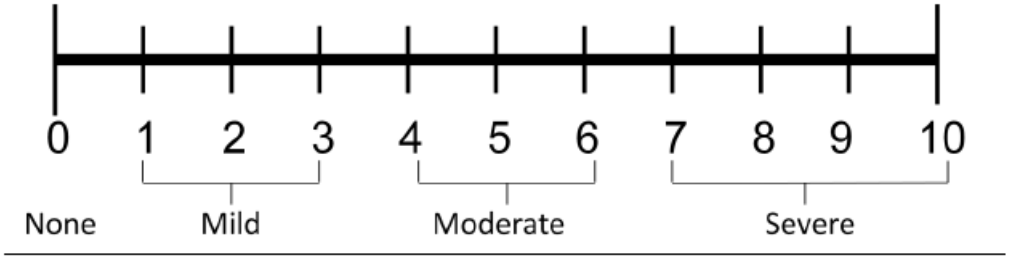
The Numeric Pain Rating Scale Instructions

General Information:

- The patient is asked to make three pain ratings, corresponding to current, best and worst pain experienced over the past 24 hours.
- The average of the 3 ratings was used to represent the patient's level of pain over the previous 24 hours.

Patient Instructions (adopted from (McCaffery, Beebe et al. 1989):

"Please indicate the intensity of current, best, and worst pain levels over the past 24 hours on a scale of 0 (no pain) to 10 (worst pain imaginable)"

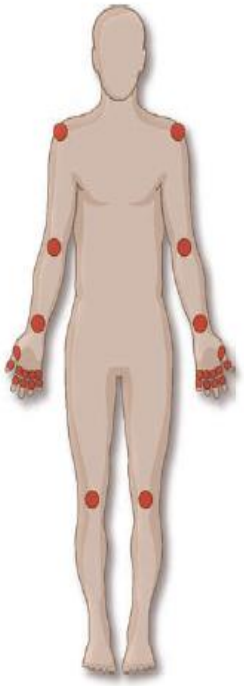


DAS28

DISEASE ACTIVITY SCORE IN 28 JOINTS (DAS28)

The DAS28 is a frequent outcome measure used in therapeutic trials and is also used to guide treatment decisions and describe disease activity across populations. It is the basis for several other RA measurement tools, including the EULAR response criteria.

FORM A		LEFT		RIGHT	
		SWOLLEN	TENDER	SWOLLEN	TENDER
Shoulder					
Elbow					
Wrist					
Metacarpophalangeal (MCP)	1				
	2				
	3				
	4				
	5				
Proximal Interphalangeal (PIP)	1				
	2				
	3				
	4				
	5				
Knee					
Subtotal					
TOTAL		SWOLLEN		TENDER	



FORM B	
Swollen (0–28)	
Tender (0–28)	
ESR (or CRP)	
VAS disease activity (0–100mm)	
DAS28=0.56*√(TENDER JOINTS) + 0.28* √(SWOLLEN JOINTS) + 0.70*LN(ESR/CRP) + 0.014*VAS	

Source: DAS-Score.nl. Available at http://www.das-score.nl/www.das-score.nl/index.html. Accessed February 5, 2009.

By comparing a patient's DAS28 score over multiple time points, you can substantiate his/her improvement or response. The EULAR response criteria are defined as follows:

PRESENT DAS28	DAS28 IMPROVEMENT OVER TIME POINTS		
	>1.2	0.6–1.2	<0.6
<3.2	good response	moderate response	no response
3.2–5.1	moderate response	moderate response	no response
>5.1	moderate response	no response	no response

HOW TO CALCULATE A DAS28 SCORE

1. Perform a swollen and tender joint examination of your patient, noting each affected joint on Form A. When complete, add all of the swollen and tender joints and record the totals in the appropriate boxes on Form B.
2. Obtain and record the patient's erythrocyte sedimentation rate (ESR) in mm/h in the appropriate box on Form B. Note: C-reactive protein (CRP) levels may be used as a substitute for an ESR.
3. Obtain and record the patient's general health on a Visual Analog Scale (VAS) of 100 mm in the appropriate box on Form B. Note: DAS28 calculations may be performed without a VAS measurement.
4. Plug the appropriate values into the formula at the bottom of Form B (many online calculators are available to compute this value including **http://www.das-score.nl/www.das-score.nl/dasculators.html**).
5. A DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity. A patient is considered to be in remission if they have a DAS28 lower than 2.6.

Information to Participants

Title: **MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY.**

Investigator:

Name of Participant:

This study is conducted in Rajiv Gandhi Govt. General Hospital, Chennai. You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concern

THE PURPOSE OF THE STUDY

Rheumatoid arthritis (RA) is a chronic multisystem disease of unknown cause causing persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution. A growing body of evidence shows that oxidative stress plays a key role in the pathogenesis of RA. In this study we want to test the morphological changes in red blood cells in oxidative stress of RA and the effect of Antioxidants in reversing these changes.

We have obtained permission from the Institutional Ethics Committee.

The study design

All patients in the study will be divided into 2 groups. You will be assigned to either of the groups. One group will receive the standard treatment & the other group will receive standard treatment + Antioxidants

Study Procedures

The study involves evaluation of red blood cell morphology at your initial visit and you will be expected to review at the end of 4,8,12 weeks. You will be required to visit the hospital thrice during the course of the study. At each visit, the study physician will collect about 2 ml of blood for studying red blood cell morphology and document any changes from your baseline evaluation.

In addition, if you notice any adverse events, you have to report it. You will be required to return unused study medicines when you report for your scheduled visits. This will enable correct assessment of the study results.

Possible benefits to the participant – Antioxidants with your standard medications will reduce oxidative stress and your future risk of developing complications due to RA

Possible benefits to other people - The results of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

Confidentiality of the information of the participant

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, Institutional Ethics Committee and any person or agency required by law like the Drug Controller General of India to view your data, if required. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

Decision to not participate in the study

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. You will be taken care of and you will not lose any benefits to which you are entitled.

Decision to withdraw from the study once started

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. However, it is advisable that you talk to the research team prior to stopping the treatment/discontinuing of procedures etc.

The results of this study will be informed to you at the end of the study.

Signature of Investigator Signature of Participant

Date Date

ஆய்வு தகவல் தாள்

ஆய்வு தலைப்பு :

இரத்த சிவப்பணுவின் அமைப்பில் ஏற்படும் மாற்றத்தின் மூலம் முடக்குவாத நோயின் போது ஏற்படக்கூடியஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை (ஆக்ஸிஜனேற்ற அழுத்தம்) கண்டறிதல் மற்றும் ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை குறைப்பதில் விட்டமின் E மற்றும் விட்டமின் C யின் பங்கு வழக்கமான சிகிச்சை முறையுடன் ஓர் ஒப்பிடுதல் ஆய்வு.

ஆய்வாளர் :

பங்கேற்பாளர் :

இந்த ஆய்வு இராஜீவ் காந்தி அரசு பொது மருத்துவமனையில் நடைபெற உள்ளது. நீங்களும் இந்த ஆய்வில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதிலுள்ள தகவலின் அடிப்படையில் இந்த ஆய்வில் பங்கேற்பதா அல்லது வேண்டாமா என்று நீங்கள் முடிவு செய்து கொள்ளலாம். உங்களது சந்தேகங்களை எங்களிடம் கேட்டு நிவர்த்தி செய்து கொள்ளலாம்.

இந்த ஆய்வின் நோக்கம்:

முடக்குவாத நோயானது மூட்டுகளில் ஏற்படும் மூட்டு அழற்சியினால் ஏற்படுகிறது. இதற்கான சரியான காரணம் இதுவரை கண்டுபிடிக்கவில்லை. பல்வேறு ஆய்வு முடிவுகளின்படி ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸானது (ஆக்ஸிஜனேற்ற அழுத்தம்) முடக்குவாதத்தினால் ஏற்படும் மூட்டு அழற்சிக்கு முக்கிய காரணமாக உள்ளது. இந்த ஆய்வில் ஆக்சிடேட்டிவ் ஸ்டிரஸ்ஸை இரத்த சிவப்பணுவின் அமைப்பில் ஏற்படும் மாற்றத்தின் மூலம் கண்டறிதல், மேலும் விட்டமின்கள் **C** மற்றும் **E** எவ்வாறு இதனை சரிசெய்ய பயன்படுகின்றன என்பதை கண்டறிதல்.

இந்த ஆய்விற்கு இன்ஸ்டிட்யூசனல் எத்திக்கல் கமிட்டி சம்மதம் பெற்றிருக்கிறோம்.

ஆய்வின் செயல்முறை:

இந்த ஆய்வில் கலந்து கொள்பவர்கள் **A** மற்றும் **B** என்று இரு குழுக்களாக பிரிக்கப்படுவீர். **A** குழுவில் இருப்பவர்கள் வழக்கமான சிகிச்சையும் **B** குழுவில் இருப்பவர்கள் வழக்கமான சிகிச்சையுடன் விட்டமின் **C** மற்றும் விட்டமின் **E** மருந்தும் பெறுவர்.

இந்த ஆய்வில் முதல் மற்றும் 4 வார முடிவில் இரத்த பரிசோதனை செய்யப்படும். அதற்காக எடுக்கப்படும் இரத்தத்தின் அளவு அதிகபட்சம் 2 மி.லி. இந்த ஆய்வின் பாது ஏதேனும் பக்கவிளைவுகள் ஏற்பட்டால் உடனடியாக எங்களிடம் தெரிவிக்க வேண்டும். மேலும் நீங்கள் உபயோகப்படுத்தாத மாத்திரைகளை எங்களிடம் திரும்ப தருமாறு கேட்டுக் கொள்கிறோம்.

ஆய்வினால் ஏற்படும் நன்மைகள்:

இந்த ஆய்வில் கலந்துக் கொள்வதன் மூலம் நீங்கள் நோயின் தன்மையில் முன்னேற்றம் பெறலாம். மேலும் வருங்காலத்தில் பிறநோயாளிகளும் பயன்பெற இந்த ஆய்வு உதவியாக அமையும்.

மருத்துவ சிகிச்சையின் தகவல்கள் குறித்த விவரங்கள்:

உங்கள் மருத்துவ சிகிச்சை குறித்த தகவல்கள் ரகசியமாக பாதுகாக்கப்படும் (பெயர், மருத்துவ பரிசோதனை முடிவு, மருத்துவ ஆய்வு முடிவு) இந்த தகவல் தாளில் கையெழுத்திடுவதின் மூலம் உங்களை பற்றிய குறிப்புகளோ, எடுத்து கொண்ட சிகிச்சை முறையை பற்றியோ ஆய்வாளரோ இன்ஸ்டிட்யூசன் எத்திக்கல் கமிட்டியை சார்ந்தவர்களோ தேவைப்பட்டால் அறிந்து கொள்ளலாம் என்று சம்மதிக்கிறீர்கள். முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆய்வின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வில் பங்கேற்காவிட்டாலும் நீங்கள் வழக்கமான சிகிச்சையை தொடர்ந்து பெறலாம்.

இந்த ஆய்வில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆய்விலிருந்து பின் வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்பு சிகிச்சையின் முடிவுகளை ஆய்வின் போதோ அல்லது ஆய்வின் முடிவின் போதோ தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

ஆய்வாளர் கையொப்பம் பங்கேற்பாளர்/பாதுகாவலர் கையொப்பம்

தேதி :

INFORMED CONSENT FORM

MORPHOLOGICAL CHANGES IN RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN RHEUMATOID ARTHRITIS AND ANTIOXIDANTS AS ADD ON THERAPY TO STANDARD TREATMENT IN REVERSAL OF THE CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY.

Name of the Participant:

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in this study.

1. I have read and understood this consent form and the information provided to me.

2. I have had the consent document explained to me.

3. I have been explained about the nature of the study.

4. I have been explained about my rights and responsibilities by the investigator.

5. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC.I understand that they are publicly presented.

7. I have understand that my identity will be kept confidential if my data are publicly presented

8. I have had my questions answered to my satisfaction.

9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

1. Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____

Signature/Guardian_____ Date_____

2. Name and Signature of impartial witness (required for illiterate patients):

Name _____

Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

சுய ஒப்புதல் படிவம்

ஆய்வு தலைப்பு :

இரத்த சிவப்பணுவின் அமைப்பில் ஏற்படும் மாற்றத்தின் மூலம் முடக்குவாத நோயின் போது ஏற்படக்கூடியஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை (ஆக்ஸிஜனேற்ற அழுத்தம்) கண்டறிதல் மற்றும் ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை குறைப்பதில் விட்டமின் E மற்றும் விட்டமின் C யின் பங்கு வழக்கமான சிகிச்சை முறையுடன் ஓர் ஒப்பிடுதல் ஆய்வு.

பெயர் : வயது : தேதி : வெளிநோயாளி எண்:

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக அறிந்து கொண்டேன். எனது சந்தேங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச் சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என் சம்மதிக்கிறேன்.

இந்த ஆய்வின் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்படாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன். இந்த ஆய்விற்காக இரத்தப் பரிசோதனை செய்துக் கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்று தெரிவிக்கிறேன் என்று புரிந்து கொண்டேன். இச்சுய ஒப்புதல் படிவத்தின் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்று தெரிந்து கொண்டேன்.

பங்கேற்பாளர் /பாதுகாவலர் கையொப்பம் தேதி :

ஆய்வாளர் கையொப்பம் தேதி :

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. A.Gomathi,
Postgraduate MD (Pharmacology),
Madras Medical College,
Chennai – 600 003.

Dear Dr. A.Gomathi,

The Institutional Ethics Committee has considered your request and approved your study titled **Morphological changes in red blood cells as a marker of oxidative stress in Rheumatoid Arthritis and antioxidants as add on therapy to standard treatment in reversal of the changes – A Randomized open label, comparative pilot study No.40082014.**

The following members of Ethics Committee were present in the meeting held on 05.08.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Dr.G.Muralidharan, Director Incharge, Inst.of Surgery	: Member
6. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
7. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
8. Prof.Tito, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
9. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
10. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
11. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003