A STUDY ON 14-3-3η LEVELS IN RHEUMATOID ARTHRITIS

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"A STUDY ON 14-3-3η LEVELS IN RHEUMATOID ARTHRITIS"** is the original bonafide work done by **DR.T.POORNIMA**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.

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DECLARATION

I, **Dr. T.POORNIMA** , Post Graduate , Institute of Biochemistry, Madras Medical College, solemnly declare that the dissertation titled "**A STUDY ON 14-3-3η LEVELS IN RHEUMATOID ARTHRITIS"** is the bonafide work done by me at Institute of Biochemistry, Madras Medical College under the expert guidance and supervision of **Prof. Dr. K.PRAMILA,** M.D., Professor, Institute of Biochemistry, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch XIII) in Biochemistry.

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CONTENTS

ABBREVIATIONS

1.	RA	-	Rheumatoid Arthritis
2.	MMPs	-	Matrix Metallo-Proteinases
3.	RF	-	Rheumatoid Factor
4.	ACPA	-	Anticitrullinated Protein Antibodies
5.	CE	-	catecholestrogen
6.	8-OHdG	-	8- hydoxy -2’ deoxyguanosine
7.	SF	-	synovial fluid
8.	OHE	-	hydroxyl estrogens
9.	IL	-	interleukin
10.	MHC	-	major histocompatibility complex
11.	VEGF	-	vascular endothelial growth factor
12.	TIMP	-	tissue inhibitor of metallo proteinases
13.	OA	-	osteoarthritis
14.	RANKL	-	receptor activator of NFkB ligand
15.	PKC	-	protein kinase C
16.	ACCP	-	anticyclic citrullinated peptide antibodies
17.	SLE	-	systemic lupus erythematosus
18.	MCP	-	metacarpophalangeal joint
19.	PIP	-	proximal interphalangeal joint
20.	MTP	-	metatarsophalangeal joint

21.	C/MC	-	carpal to metacarpal ratio
22.	LGL	-	large granular lymphocytes
23.	DMARDs	-	disease modifying anti rheumatoid drugs
24.	AAP	-	4 amino antipyrine
25.	CNS	-	central nervous system
26.	ESR	-	erythrocyte sedimentation rate
27.	Hb	-	hemoglobin
28.	CKD	-	chronic kidney disease
29.	DM	-	diabetes mellitus
30.	ROC	-	receiver operating characteristic curve
31.	TNF	-	tumor necrosis factor
32.	VEGF	-	Vascular Endothelial Growth factor
33.	TGF β	-	Transforming growth factor beta.
34.	GAG	-	Glycosaminoglycan
35.	NFK	-	Nuclear factor kappa.
36.	PG	-	Prostaglandin
37.	HLA	-	Human Leucocyte Antigen.
38.	SD	-	standard deviation
39.	CV	-	coefficient of variation
40.	ACR	-	American college of rheumatology
41.	AST	-	aspartate transaminase
42.	ALT	-	alanine transaminase

43.	CNS	-	central nervous system
44.	Nsaids	-	nonsteroidal anti-inflammatory drug
45.	CSF	-	cerebrospinal fluid
46.	MAPK	-	mitogen- activated protein kinases
47.	ERK	-	extracellular signal- regulated kinases
48.	OPG	-	osteoprotegerin
49.	TIMPs	-	tissue inhibitor of metalloproteinases
50.	HADC	-	HIV associated dementia complex

Introduction

INTRODUCTION

Rheumatoid arthritis(RA) is a disease of joints. It is a type of inflammatory arthritis. Characterized by chronic symmetric erosive arthritis of the peripheral joints. The disease is not confined with joints but also presents with extra articular manifestations. Etiology includes both environmental and genetic factors.[1] Disease progresses to physical inability and joint deformity.[2]

Pathogenesis involves inflammatory mediators like cytokines, growth factors, chemokines, adhesion molecules and matrix metalloproteinases(MMPs). Diagnosis and diseases severity is assessed by evaluating the joint involvement, estimating acute phase reactants and assessing the various serological markers. Rheumatoid factor(RF) and anticitrullinated protein antibodies(ACPA) are major serological markers of RA. Lack of sensitivity of the both serological markers in diagnosis and progression of the disease leads to the necessity of search of new biomarkers for early diagnosis and halting the disease progression.

14-3-3η(eta) protein, an intracellular chaperone protein has been found to have correlation with MMPs and joint destruction.[3] The new serological marker 14-3-3η protein belong to a family of seven isoforms and are involved in the pathogenesis of joint inflammation and damage. The relationship of the new biomarker with the disease diagnosis and progression of the disease, makes the marker as an addition to existing biomarkers for more cases identification at an earlier stage and preventing the disease progression and deformity.

So this study has been done to establish the role of 14-3-3η protein in rheumatoid arthritis and establish its sensitivity and specificity and for earlier diagnosis and treatment

EPIDEMIOLOGY

- RA affects 0.3 to 1.5% of the total population worldwide.[3]
- Based on an indian epidemiologic survey

  6 to 7 million people of india are suffering with RA.[4]

    0.5 to 3.8% prevelance in women.[4]

    0.15 to 1.37% prevelance in men.[4]
- Cases are mostly clustered around 4th decade of life worldwide
- Maximum cases in indian population are found to be of age group 25-29 comparitively younger than the western diseased population.[4]
- African population shows a low prevelance.[5,6]
- China population also shows a low prevelance of the disease.[7]

Review of Literature

REVIEW OF LITERATURE

RHEUMATOID ARTHRITIS:

Rheumatoid arthritis, a disease involving the joints is a type of inflammatory arthritis. Has been classified under autoimmune diseases. The etiology of the disease is multifactorial. It includes immune cause, neuroendocrine and pychosocial variable.[8] The relationship between the etiological variables is unclear. Women compared to men are the major contribution to the disease population . 80% of the cases fall in the age group 35 to 50.[9]

ETIOLOGY AND PATHOGENESIS OF RHEUMATOID ARTHRITIS

AUTOIMMUNE ROLE IN DISEASE:

Theories on pathogenesis of rheumatoid arthritis includes autoantibodies and immune complexes, T cell mediated antigen response, T cell independent cytokine role and tumour like behavior of the synovium have also been identified.[10]

Autoantibodies identified in rheumatoid arthritis patients include

1. Rheumatoid factor (RF) is the major autoantibody found in the diseased patients
2. Autoantibodies to cartilaginous collagen have also been identified
3. Cross reactive natural autoantibodies[11] (IgM) , the role of its action is against either histone moieties or double stranded DNA

The autoantibodies have specific high binding property for Catecholestrogens (CE)modified DNA.[12] Autoantibodies indicates the oxidative lesions in DNA.

8- hydroxyl-2'deoxyguanosine(8-OHdG) levels in serum and synovial fluid also represents the oxidative status.[12]

Synovial B cells release anti collagen antibody and rheumatoid factor.

Synovial inflammation is caused by the antigen glucose-6-phosphoisomerase related to adherence to the cartilage surfaces.

The antigen antibody complexes gets adherent to the cartilage and gets immobilized which is responsible for the complement fixation.

RHEUMATOID FACTOR:

Initially rheumatoid factor along with the immune complexes was considered as the only reason for the development of the autoimmune rheumatoid arthritis. But later was determined as RF as one of the factor that leads to the development of acute inflammatory synovitis. Rheumatoid factor can be expressed even before the development of disease and vice versa even it may be seronegative in clinically evident rheumatoid arthritis. The patients with seropositive to rheumatoid factor have more severe disease when compared with the patients with seronegative results. RF factor role in the pathogenesis is through fixation and activation of classic complement pathway. IgM and IgG RF are found most abundantly in the synovial tissue of diseased joints. High affinity RF production in the rheumatoid arthritis patients will cause T cell activation. There are experimental evidences for RF role in the inflammatory process of the disease.

CARTILAGE- SPECIFIC ANTIGENS:

Synovial inflammation is the most important finding in the rheumatoid arthritis patients. The inflammatory process are produced by varied number of joint specific antigens. The autoimmunity will vary according to the stage of the disease, clinical manifestations and treatment.

ROLE OF CYTOKINES IN PATHOGENESIS OF DISEASE

Cytokines play an important role in the inflammatory process and the systemic manifestations.[13] Cytokines involve in the initiation and the progression of the inflammatory process. The over production of cytokines causes the synoviocyte proliferation and release of secondary mediators which are involved in the recruitment of inflammatory mediators. The cascade of these reactions and release of factors causes the disease progression and joint destruction .[14] Inflammatory markers are directly proportional to the disease progression and joint destruction.

The markers include

1) C-reactive protein
2) Vascular endothelial growth factor
3) Synovial matrix metalloproteinase
4) And matrix digesting enzymes[15]

CEs AND PATHOGENESIS OF RHEUMATOID ARTHRITIS:

Estrogen and the metabolites of estrogen(CE) have a major role in the disease progression and joint destruction.[12] Role of estrogen in the pathogenesis of

the disease has been the recent area of research. Estrogen is related to the development of autoimmunity and hence involves in the pathogenesis of the autoimmune diseases like rheumatoid arthritis.[16]

Estrogen and its CE metabolites are found to be risk factors for the development of RA disease. In RA patients, synovial fluid(SF) resulted to show high levels of estrogen and its hydroxylated forms like 16-OHE and 4-OHE. [15,17] 16 OHE compared to 2 OHE play an important role in rheumatoid arthritis.[11] Estrogen enhances immune response by acting as a driving force for production of IL -10 when present at physiological levels. Estradiol also enhances antibody production via interleukin 10.[18]

IMMUNOGLOBINS AND RHEUMATOID ARTHRITIS:

Class II MHC involve in the cellular immune responses in RA. Deficient galactosylation of the immunoglobulin is one of the reasons of autoimmune diseases like RA. There are evidence of defective galactosylation of immunoglobulin even before the onset of the disease. The defective galactosylation is found to be prominent on the IgG_1 , IgG_2 and IgG4 isotypes of RF[19]. The etiology involved in the role of defective galactosylation would be reduced galactosyl transferase activity in the RA B cells. Deficient galactosylation can be an early indicator or marker for the patients with early synovitis who develops into full blown rheumatoid arthritis. Autoantibodies lacking galctosylation are more pathogenic than with normal galactosylation in the pathogenesis of rheumatoid arthritis[20].

HLA-DR ASSOCIATION IN DISEASE :

The genetic basis of the rheumatoid arthritis with HLA-DR was determined in 1970. The study of MHC using complementary DNA probes against specific regions of DC loci revealed the predisposition of DR to the development of the disease[1]. The susceptibility of the disease is related to the DRβ chains- third hypervariable region(from 70 thro 74 amino acids)[21]. The epitope found in DR4 and DR14 related with the disease is the sequence-glutamine-leucine-arginine-alanine (QKRAA).

Old Nomenclature (HLA-DRB1 alleles)	New nomenclature	Association with Rheumatoid arthritis
HLA-DR1	0101	+
HLA-DR4 (Dw4)	0401	+
HLA-DR4 (DW14)	0404/0408	+
HLA-DRw14(Dw16)	1402	+
HLA-DR4(Dw10)	0402	-
HLA-DR2	1501,1502,1601,1602	-
HLA-DR3	0301,0302	-
HLA-DR5	1101-1104,1201,1202	-
HLA-DR7	0701,0702	-
HLA-DRw8	0801,0803	-
HLA-DR9	0901	-
HLA-DRw10	1001	-
HLA-DRw13	1301-1304	-
HLA-DRw14	1401	-

From Weyand CM, hicok KC,Conn DL, Goronzy JJ: the influence of HLA-DRB1 genes on disese severity in rheumatoid arthritis. Ann Intern med 117:801,1992

CYTOKINE POLYMORPHISMS AND OTHER GENETIC ASSOCIATIONS:

Tumour necrosis factor-α(TNF-α) have been identified as the major cytokine factor involved in the pathogenesis of the disease. TNF genes are located on MHC locus on chromosome 6. TNF polymorphism and disease have been reported. Polymorphorphism of TNF-α promoter region leads to altered gene transcription . For example polymorphism at positions -238 and -308. Microsatellite polymorphism of other cytokines and inflammatory mediators like interleukin-1(IL1),CCR5 and RANTES are also associated with the susceptibility of the disease[22]. The genetic basis of polymorphism other than MHC plays a minor role in the influence of the development of the arthritis but a combination of factors may lead to the course of disease.

ENVIRONMENTAL INFLUENCES ON DISEASE:

No specific individual exposure have been determined as a pivotal agent for the development of the disease. Case controlled and restrospective studies has been done to determine the influence of oral contraceptives in disease. Some data shows a temporary protective effect. The protective effect is a delaying factor not a disease preventing factor. Other endocrine factors related to disease are corticotrophin releasing hormone and estrogen synthase. Nulliparity has been determined as a risk factor for the disease. But the evidence has been controversial by other studies. Smoking is also a risk factor for rheumatoid arthritis.

SYNOVIAL INVOLVEMENT WITH THE DISEASE:

The primary target for the rheumatoid arthritis is the synovium. The disease is characterized by

1. Infiltration of the synovium with mononuclear cells
2. Synovial intimal lining hyperplasia

Synovial intimal lining is a thin interface between synovial fluid space and the synovium and is devoid of tight junctions and a definite basement membrane. There is increase in the cell number in RA. Two types of cells are present in the lining

1) Type A synoviocyte- macrophage like cell
2) Type B synoviocyte- fibroblast like cell

Both the type of cells increase in RA. Type A cells increase more in RA. Synovial macrophage do not divide in the joint and the accumulation of cells is likely to ingress new bone marrow derived precursors.

PROTEASES IN RHEUMATOID ARTHRITIS:

The progression of the disease leads to bone and cartilage destruction. The process involves increased activity of proteases like matrix metalloproteases (MMPs) and papain like cysteine proteases.

MATRIX METALLOPROTEINASES(MMPs):

Matrix metalloproteinases have been subdivided into 5 categories

1. Collagenases includes MMP-1, -8 and -13
2. Includes type I ,II and III- degrades interstitial collagens, MMP-2 and 9 which are gelatinases , type IV collagen
3. Includes MMP-3,-10 and 11- degrades non collagen matrix proteins
4. Includes MMP-14, -15, -16, -17 , -24 and -25 which are of Membrane type
5. MMP-7, -11, -12, -20 and -23.

Collagen degradation:

The expression of MMP s are very low in a normal joint tissue. Their levels are increased in arthritic joints. The collagenase activity of MMP will cleave the collagen. MMP s have specificity over collagen types like MMP -13 cleaves type II collagen, MMP-1 cleaves type I collagen and MMP-8 are effective over collagen type I.

Degradation of non collagenous components:

Mediated by stromelysins and matrilysins. *Stromelysins degrade fibronectin, elastin, laminin and aggrecan*. MMP-3 which is classified under stromelysin -1 are identified to be responsible for (i) proteoglycan loss[23] and (ii) activation of proMMP-1[24].

Matrilysin cleave proteoglycans which are induced by TNF-α and IL-1. MMP s that leaves the proteoglycans cleave the aggregan core protein at Asn341-Phe342 bond[25,26].

Membrane type MMPs role in RA:

MT1 and MT3-MMP play a role in the pathogenesis of RA disease. Among both MT1 plays a dominant role. The pathogenesis in destruction is both of direct and indirect effects. During the disease progression in rheumatoid arthritis fibroblasts and osteoclast like cells will express MT1-MMP which will mediate bone resorption[27]. MMP s destroy collagenous , non collagenous matrix molecules and diturb the joint integrity and function.

Gene expression of MMP:

Synthesis of MMP is regulated at the level of gene expression[28]. In arthritis varied from the low expression of MMP-2 and MT1-MMP in normal tissues, the expression of these metalloproteinases are increased and constitutively expressed(promoters of these genes do not contain a TATA box)[29].

The MMP- 1, -3 and -9 and -13 expression are regulated through induction by IL-1 and TNF- alpha. Induction factors regulate the MMP gene expression through signal transduction pathways such as mitogen- activated protein kinases(MAPKs)[30].

CYSTEINE PROTEASES:

Cathepsins are classified under cysteine proteases. Cathepsins act on collagenases type II, IX and XI. They are regulated by cytokines and proto-oncogenes. Cathepsin K has been found to be involved in degradation of collagen type I.

AGGRECANASES:

Aggrecan is classified under proteoglycan. It is involved in compressibility activity on the joints due to its large size and negative charge and containing large amounts of water. Two sites are susceptible for cleavage in aggrecan . one site is attacked by MMPs and the other site is attacked by aggrecanases.

BLOOD VESSELS IN ARTHRITIS:

Blood vessels are the route of red blood cells and leukocytes to the inflammatory site. Vessels involve in cell specificity to the tissue site and involved in tissue growth and nutrition.

NEW VESSELS IN RHEUMATOID ARTHRITIS:

Synovitis in rheumatoid arthritis resembles tumor and wound healing process.

Stimulus and factors involved in new vessel formation:

(i) Hypoxia which is a major contributory towards the new vessel formation in RA joint

(ii) Vascular endothelial growth factor(VEGF) – high levels are correlated with later joint manifestations such as degradation[31].

VEGF is involved in increased levels of collagenases which is involved in degradation of the extracellular matrix[32].

(iii) pro inflammatory factors like IL-8,FGF and TNF-α are angiogenic.

CARTILAGE DESTRUCTION IN RHEUMATOID ARTHRITIS:

Normal cartilage: it is a type of hyaline cartilage. It is avascular. The major components of the cartilage includes type II collagen and aggrecan. Deficiency or any gene alterations of majority of collagen leads to diseases such as premature osteoarthritis. Mutations in type II collagen leads to chondrodysplasias

Pathogenesis in Destruction of cartilage in Rheumatoid arthritis involves two process named enzymatic and mechanical.

Enzymatic process is mediated by factors such as IL-1 and TNF-α.

Proteoglycans depletion - due to catabolic effect of IL-1, MMPs and aggrecanases.

↓

Weakening and destruction of the cartilage

Depletion of proteoglycans in the rheumatoid arthritis leads to mechanical destruction of the cartilage. Streptomelysin and collagenases mRNA are increased in rheumatoid arthritis joints cartilage. Rate limiting step in the cartilage destruction is the cleavage of the collagen. MMP s released in case of rheumatoid arthritis leads to the digestion of the matrix proteins. Other enzymes such as cathepsins are involved in the non collagenous matrix proteins. Elastase, plasmin and aggrecanases also contribute a major role in the cartilage destruction.

BALANCE OF MMPs AND TIMPs IN THE DEVELOPMENT OF DISEASE:

Tissue inhibitor of metallo proteinases(TIMP) bind to the MMPs in the ratio 1:1. The binding is specific to active MMPs. There are also different forms of TIMPs as TIMP-2 which will bind to the inactive progelatinase(MMP-2). The number of TIMPs in the diseased synovial fluid is found in excess and they form complexes with active collagenases. The levels of MMP:TIMP are higher in rheumatoid arthritis when compared with the osteoarthritis(OA). The balance between the culprits MMPs and the inhibitors TIMPs decides the fate of the disease that is the destruction of the extracellular matrix. This balance has been utilized in treatment purpose.

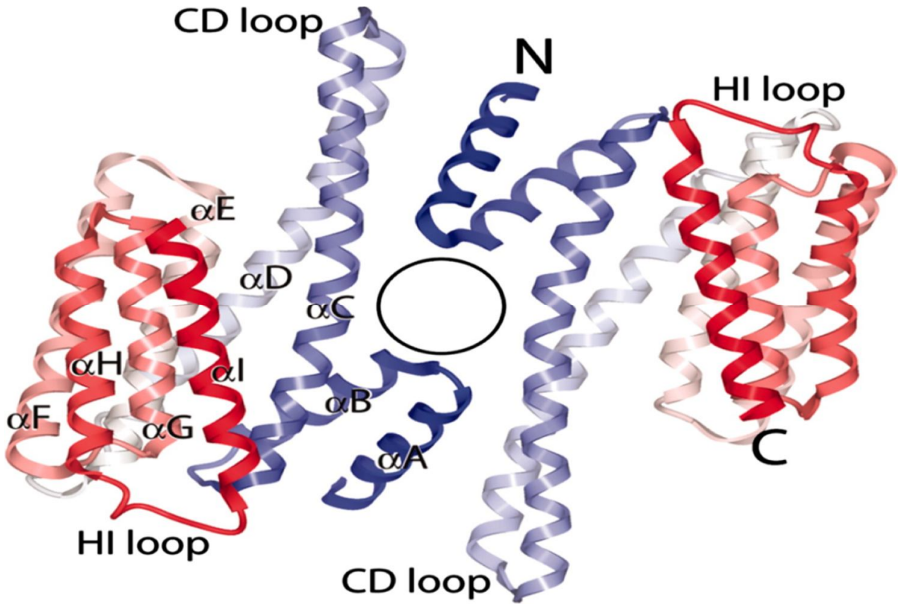
ANOTHER CHECKPOINT OF BONE DESTRUCTION- RANKL:

Osteoclasts have been identified to be the causative for the bone degradation in the diseased conditions like rheumatoid arthritis. Recent studies have proved that the relation between the receptor activator of NFkB ligand (RANKL) and bone resorption modulates the activity of the disease[33]. The osteoclast development and activation mechanisms depends on RANKL. The osteoclast precursors have expression of RANKL receptors known as RANK. When osteoclasts have expression of RANKL on their surfaces the disease progression to destruction of the joints happens through MMPs and cathepsin K. osteoprotegerin(OPG) is competes with the binding of RANKL with receptor RANK. The determination of RANK, RANKL and OPG from the diseased synovial fluid can be done to access the bone destruction. The number of RANKL

compared to OPG outnumbers in RA whereas in other diseases like OA and gout the difference in number is not that significant[34]. Osteoclasts expressing TRAP-tartrate- resistant acid phosphatase have been also expressed by the synovial fluid of rheumatoid arthritis[35].

14-3-3 ETA PROTEIN IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS:

14-3-3 ETA PROTEIN:



Courtesy : https://goo.gl/images/ttvJqZ.

14-3-3 protein is basically a chaperone protein. It belongs to a family of seven isoforms named as (i) beta (ii) gamma (iii) epsilon (iv) eta (v) tau (vi) zeta (vii) sigma. The 14-3-3 eta protein weighs 20 kDa[36]. The seven isoforms share amino acid homology partly.

Structure of the 14-3-3 family chaperones:

(i) Consists of 9 alpha helices

(ii) The amino terminal and carboxy terminal of the isoforms vary from each other Functions of these chaperone proteins:

The 14-3-3 proteins form a grove which is termed as amphipathic groove. The grove is formed by dimerization through N terminals of the proteins the dimerization can be either homo dimerization or heterodimerisation. Through the groove the chaperone protein interact with various proteins to carry out various functions like synthesis of protein, signal transduction processes, protein trafficking and cytoskeletal transport[37]. The signal transduction processes of 14-3-3 proteins are recently identified to be ones involving tryptophan, protein kinase C(PKC) and tyrosine kinase[38].

ROLE OF 14-3-3 ETA PROTEIN IN RHEUMATOID ARTHRITIS:

Among the seven isoforms it has been identified as only eta variant have been in higher number in the rheumatoid arthritis. And it has been documented that the levels of 14-3-3 eta protein in the synovial fluid is to of very much higher compared with the sera of the patients.

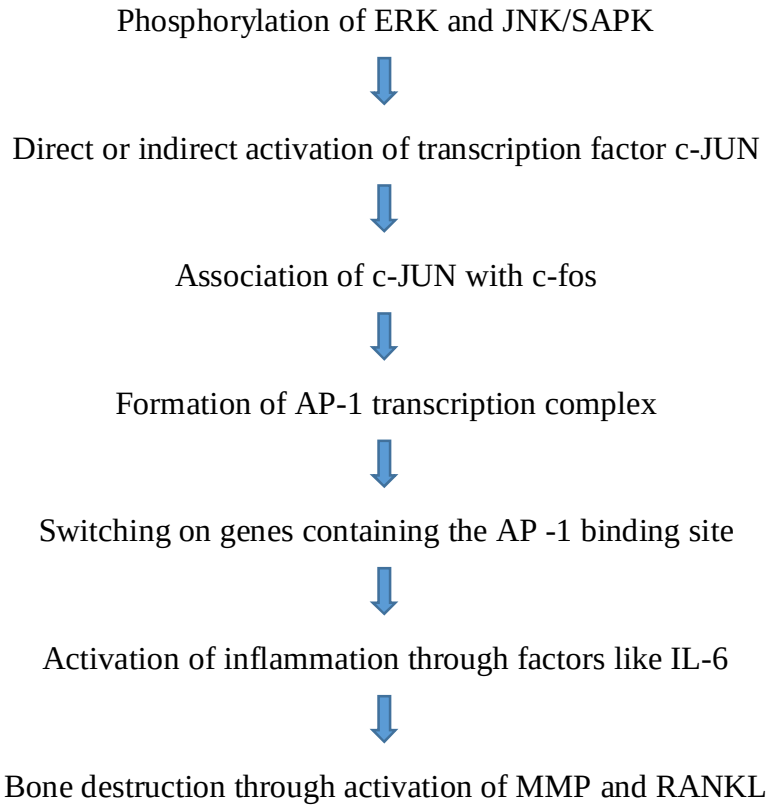
Release of the proteins in diseased condition:

Externalization of the protein in the diseased joints is by exocytosis reaction and release from the microvesicles[39]. 14-3-3 eta protein in the diseased

conditions when released to the extra cellular space acts as an inducer of various cells of the innate immunity system.

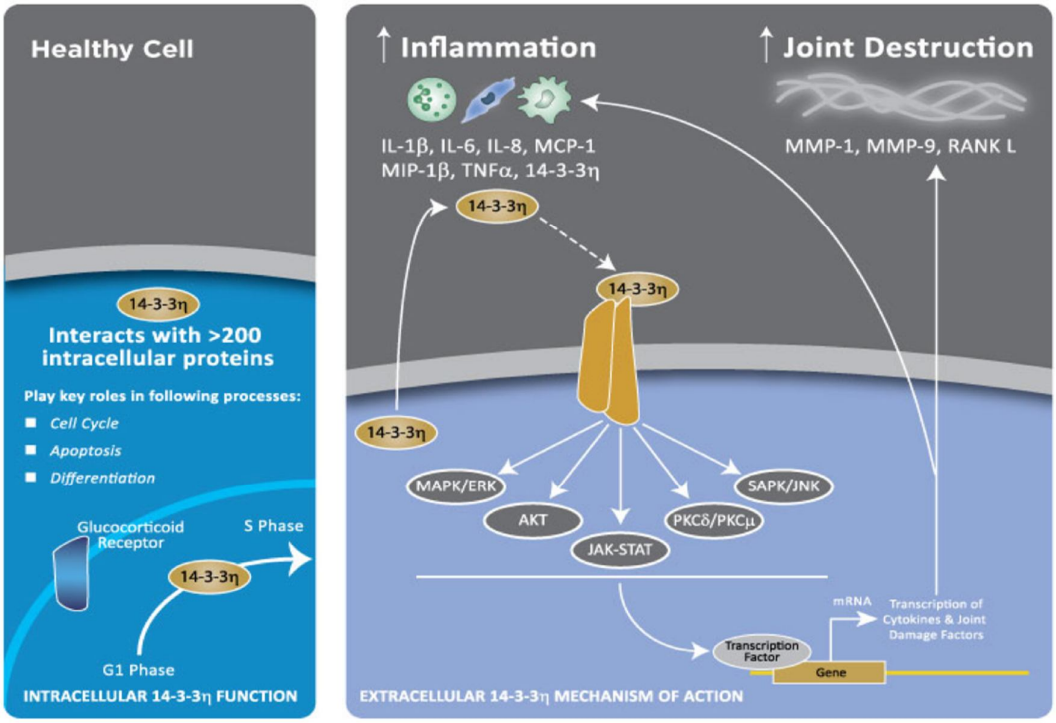
Signaling pathway of 14-3-3 eta protein:

Cell signaling studies were carried out to understand the mechanism of action of the proteins extra cellularly during inflammatory conditions. Inflammatory signals like interleukins and tumor necrosis factors activate 14-3-3 eta protein which act through various signaling pathways. Mainly acts through MAPK signaling pathway. Acts through transcription factor AP-1. Causes phosphorylation of ERK and JNK/SAPK in the cells which are stimulated by 14-3-3 proteins. The activation of cells by 14-3-3 proteins differs from activation by TNF-α as 14-3-3 does not phosphorylate p38MAPK



PATHOGENESIS OF 14-3-3 ETA PROTEIN IN DISEASE

INVOLVES IN THE FOLLOWING PATHWAY



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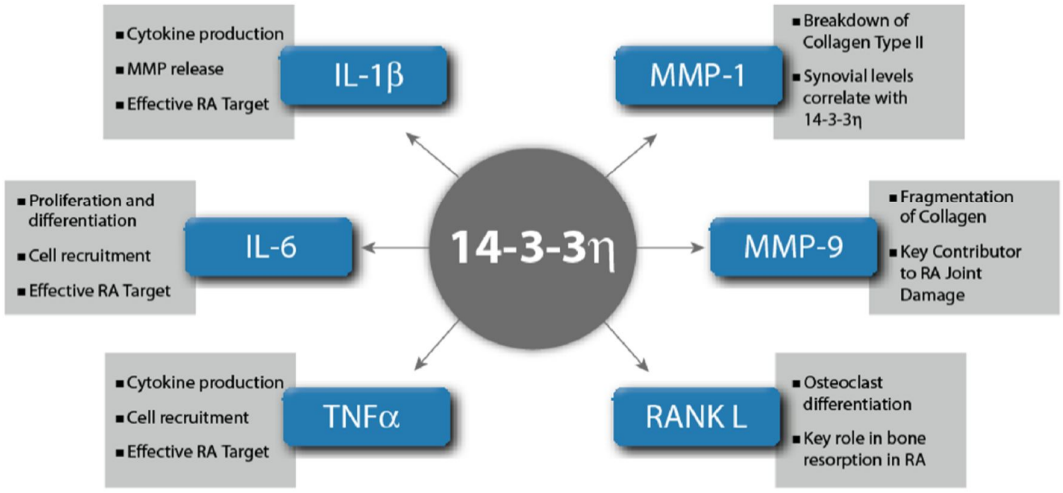
14-3-3η Induces Factors Successfully Targeted with Biological Based Therapies



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The inflammatory mediators or factors involved in the pathogenesis and the progression of the disease through 14-3-3 are

(i) MMP-1

(ii) MMP-9

(iii) RANKL

(iv) TNFα

(v) IL-6

(vi) IL-1β

(i) MMP 1: matrix metalloproteinase 1 (MMP-1) is classified under one class along with MMP-8 and 13. MMP-1 has collagenases activity. Their activity is increased in the arthritic joints. The expression of MMP-1 is regulated through IL-1 and TNFα. The levels of MMP will correlate with levels of 14-3-3 eta protein levels in the synovial fluid.

(ii) MMP-9: matrix metalloproteinases 9(MMP-9) is classified under category 2 along with MMP-2. MMP-9 is a gelatinase which causes degradation of collagen type IV. MMP-9 is determined to be major factor responsible for the joint destruction.

(iii) RANK-L: the osteoclasts cells have receptors for RANKL . osteoclast causes bone destruction. The expression of RANKL is increased in RA causing increased bone degradation. Thus the expression of RANKL is involved in the pathogenesis of the bone degradation and severity of the disease.

(iv) TNFα: it's a pleotropic cytokine. The functions of TNF-α includes cytokine production enhancement, adhesion molecule production and proliferation of MMP.

(v) IL-6: cytokine like IL-6 is involved in the initiation and the progression of the inflammatory process. Causes synoviocyte cell recruitment and release of inflammatory factors leading to progression of the disease

(vi) IL-1β: cytokines like Il-1β is also involved in the cell recruitment and inflammatory process. Causes the release of matrix metallo-proteinases which are responsible for the joint destruction.

Significance of 14-3-3 protein levels in other diseases:

Carcinoma[40]:

14-3-3 epsilon protein has been used in the prognosis and severity of hepatocellular carcinoma.

Creutzfeld-jacob disease[41]:

14-3-3 protein levels in CSF has been significant in the diagnosis of cretzfeld Jacob disease with a sensitivity of 94% and specificity of 84%.

Alzheimer's disease[42]:

14-3-3 a regulatory protein has been studied to be found in higher levels in the CSF of persons suffering from alzheimer's disease

Multiple sclerosis[43]:

14-3-3 eta protein is used in the multiple sclerosis as a marker for axonal pathology and the disease progression

Infarction[44]:

Specific isoforms of 14-3-3 proteins has been identified in the neuronal cells . these proteins have been produced as a reaction to ischemic stress. It may be a protective mechanism for the survival for the cells against death.

And HIV[45]:

It has been studied that 14-3-3 protein has been demonstrated in CSF of patients suffering from HIV associated dementia complex (HADC). The disease has features of encephalitis, leucoencephalopathy , astrogliosis and neuronal loss.

Risk factors in the development of rheumatoid arthritis:

ANTICYCLIC CITRULLINATED PEPTIDE ANTIBODIES:

RF was considered to be major contributory factor towards the diagnosis of the disease. But RF has also been positive in the sera of other diseases like systemic lupus erythematosus(SLE), Sjogren's syndrome, viral infections like mononucleosis, hepatitis, influenza, parasitc infections like trypanosomiasis etc, chronic bacterial infections and other hyperglobulinemic status.

Arginine undergoes deamination produces citrulline. The antibodies against citrulline termed as ACCP are found to present in the sera of rheumatoid arthritis patients. Recombinant fillagrin have been developed to identify these autoantibodies ACCP in sera of the patients.

TWINS- A RISK FACTOR :

There is eight fold increase in the risk of developing rheumatoid arthritis if it is a monozygotic twin[46,47]. The risk factor in the twins is due to the polymorphism of HLA-DRB chains.

SEX AND THE DISEASE:

Women are more susceptible for development of disease when compared to men. The reason for female preponderance is estrogen which has a stimulatory effect over the immune system causing the development of autoimmune

rheumatoid arthritis. There are studies proving oral contraceptives are protective[48] against severity of diseases and also studies to prove women with one child are less susceptible for the disease. It has been proved that patients improve during pregnancy. Whereas multiparity is a risk factor for the development of the disease. Smoking women are at high risk to develop rheumatoid arthritis and to be seropositive when compared to the non smoking women. The reason for increased severity in smoking individuals is due to the glutathione S transferase M1- null polymorphism.

GOUT AND RA A NEGATIVE CORRELATION:

There is an association between seronegative RA and gout. With increased levels of uric acid in patients with rheumatoid arthritis there has been a decrease in the severity or improvement in the disease activity[49]. A hypothesis has been proposed that hyperuricemia is a state of anti- inflammatory.

DIET:

No strong evidence of diet playing a role in the relation to the disease development or any progression.

ATOPY AND RA:

In some sub populations it has been demonstrated that when there is an increase in the evidence of development of atopy there is a decrease in the rheumatoid arthritis incidence[50].

EARLY RHEUMATOID ARTHRITIS:

The pattern of onset varies from individual to individual.

Insidious onset:

55%-65% cases have insidious onset[51].

Symptoms and signs includes

(i) Systemic symptoms

(ii) Articular symptoms

(iii) Nonspecific symptoms like fatique, malaise, puffy hands or diffuse musculoskeletal pain

(iv) Asymmetrical presentations leading to later symmetrical presentation

(v) Symmetrical involvement is due to bilateral release of neuropeptides

(vi) Morning stiffness due to fluid accumulation during sleep in the inflamed tissue.

(vii) Muscle atropy around the affected joint

(viii) Weakness due to pain

(ix) Low grade fever- rare presentation

(x) Weight loss due to catabolic cytokines

Acute or intermediate onset:

8-15% of patients develop symptoms acutely or intermediate. Patient will describe sudden onset of symptoms and then pain develops to other joints. Diagnosis will be difficult and may be confused with vasculitis or sepsis. Acute onset may be presented with fever.

Joint presentation in disease:

First involvement is the small joints when compared to larger joints.

The joints involved are metacarpophalangeal joints(MCP), proximal interphalangeal joints(PIP), metatarsophalangeal joints(MTP) and wrists[52]. Larger joints present later after the small joints. Present as synovitis or systemic presentations

JOINT INVOLVEMENT	MEAN % OF PATIENTS	RANGE OF % OF PATIENTS
MCP, PIP	91	74-100
WRISTS	78	54-82
KNEES	64	41-94
SHOULDERS	65	33-75
ANKLES	50	10-67
FEET	43	15-73
ELBOWS	38	13-60
HIPS	17	0-40
TEMPEROMANDIBULAR	8	0-28
SPINE	4	0-11
STERNOCLAVICULAR	2	0-6
PARA-ARTICULAR SITES	27	20-29

Modified from Guerne P-A, Weisman MH: Palindromic rheumatism: part of or apart from the spectrum of rheumatoid arthritis. Am J Med 16:451-460, 1992. Copyright 1992, with permission from Excerpta Medica, Inc.

Other patterns of disease:

Adult onset Still's disease:

Adult onset Still's disease present with an unusual presentation with fever as early and predominant symptom. The seromarkers(RF and antinuclear antibodies) will be negative in the case of Still's disease[53]. There is no sex indifference in the presentation of Still's disease.

Fever will be the only presentation before arthritis making the diagnosis difficult. At times salmon- coloured or pink macules present in the trunk or extremities. There is cervical spine involvement and neck motion will be lost. The diagnosis may be more confusing with presentation with pleural effusion, pericarditis and abnormal liver function test. There will unusual elevation of ferritin levels. Adult onset Still's disease have been a diagnosis by exclusion so far.

CRITERIA FOR DIGNOSIS OF STILL'S DISEASE:

MAJOR CRITERIA	MINOR CRITERIA
Temperature >39^{o}C for >1 week	Sore throat
Leukocytosis >10,000/mm^{3} with >80% PMNs	Lymph node enlargement
Typical rash	Splenomegaly
Arthralgias>2 week	Liver dysfunction (high AST/ALT)
	Negative ANA, RF

Diagnosis is based on if more than 2 major criteria are present. Prognosis of Still's disease is good when the systemic presentation are more when compared to articular presentation. Treatment includes aspirin or nonsteroidal inflammatory drugs (NSAIDs), oral glucocorticoids and methotrexate.

Palindromic pattern of onset:

Starts with pain in one joint and and worsens by time and later present with swelling and erythema. They resolve in reserve sequence. The palindromic disease develops to rheumatoid arthritis in most of cases who have HLA-DR4[54]. Some of the individuals develop rheumatoid arthritis from palindromic disease. Some palindromic cases resolve completely without development of disease. The development of palindromic disease to rheumatoid arthritis show seropositive rheumatic factor and CCP positive in later stage after the evident RA.

Effect of age in the onset of the disease :

RA developing in older age presents with stiffness and swelling of joints. Patients presenting at an earlier age may be presented with subcutaneous nodules or RF.

Unusual presentation along with other diseases:

RA may be presented along with other diseases like poliomyelitis, meningioma, encephalitis, cerebral palsy, strokes, neurovascular syphilitic. Joints in the paralyzed side are spared.

Arthritis robustus:

This is the unusual reaction of the patients to the disease[55]. Causes proliferative synovitis with little pain and disability. Also present with periarticular osteopenia, new bone formation and subcutaneous nodules.

JOINT INVOLVEMENT IN THE DISEASE:

CERVICAL SPINE:

Causes osteochondral destruction. Radiographic picture shows lateral narrowing of the spine. Characterized by pain. Passive motion may be positive in the diseased spine. The mechanism includes extension of inflammatory process from adjacent neurocentral joints and cervical instability.

The atlantoaxial joint may lead to subluxation. Subluxation can occur

(i) Anteriorly

(ii) Posteriorly

(iii) Vertically

Clinical symptoms include

- Spastic quadriparesis with sensory loss
- Medullary dysfunction with vertebral artery compression
- Paresthesias in shoulders or arms
- Symptoms of spinal cord compression includes
- Drop attacks
- Sphincter control is lost
- Level of consciousness is altered
- Sensation of head falling forward
- Dysphagia, dysarthria vertigo
- Hemiplegia, nystagmus

THORACIC, LUMBAR AND SACRAL SPINE:

Thoracic, lumbar and sacral spine are not involved in the rheumatoid arthritis. Apophyseal joints are not involved. Synovial cysts may cause compression over the spine causing neurological pain and deficits.

TEMPEROMANDIBULAR JOINT:

Commonly involved joint in the rheumatoid arthritis is temporomandibular joint. Radiograph will produce bone alterations such as mandibular condyle and eminentia articularis are eroded.

Physical examination: tenderness and auscultation for crepitus

Symptoms: acute pain, not able to close mouth

Radiologic finding: erosion and cysts of the mandibular cyst

CRYCOARYTENOID JOINTS:

Crycoarytenoid joint is involved in rotation of the vocal cords and plays a role in the pitch and tone of the voice.

The involvement of the joint in rheumatoid arthritis produces hoarseness of the voice in rheumatoid arthritis vocal cords are adducted to midline causing stridor ocassionally will cause aspiration of pharyngeal contents

It has been studied that about 50% of patients with rheumatoid arthritis have involvement of this joint.

EAR OSSICLES:

Rheumatoid arthritis patients complain of hard of hearing. This has been postulated to the salicylate toxicity. Causes conductive type of hearing loss. The rheumatoid ears are due to erosions and shortening of the ossicles produced by erosive synovitis

STERNOCLAVICULAR AND MANUBRIOSTERNAL JOINTS:

The synovium and the cartilaginous disc of both the joints are involved in the rheumatoid arthritis.

Symptoms: pain over the joints while lying on the sides

Radiograph: shows delineation of sternoclavicular joint

SHOULDER:

The shoulder synovium and clavicle- particularly distal third is involved in the rheumatoid arthritis. Rotator cuff and the muscles of the neck and chest are also involved. The rotaor cuff involvement is the principle reason for the symptoms

Symptoms: pain and inflammation. Decreased range of motion. obstruction to venous return from the arm.

Radiographic finding: superior subluxation, rotator cuff tears, proliferative synovitis, erosions, irregular capsular attachment, adhesive capsulitis, soft tissue swelling, subacromial bursitis and glenohumeral joint effusions[56].

ELBOW

Stable Hinge type of joint

Involvement is about 20 -65%

Symptom: may lead to loss of full extension. Shoulder and wrist movement will compensate the loss of slow motion.

HAND AND WRIST:

Both the joints involve as a functional unit.

Extensor carpi ulnaris muscle is weakened which causes radial deviation of the wrist and carpal bone respond differently.

Proximal row shows ulnar deviation

Distal row shows radial deviation

It is not mandatory that only when there is erosion of the bones it leads to deformity. Even without erosions of the bone patients present with deformity.

Wrist with Dorsal swelling many a times present as a early sign in the rheumatoid arthritis[57].

Synovial proliferation inside the wrist

Pressure increases

Tendons, ligaments and articular disk are destroyed

Communications between radius, ulnar and carpal bones are lost

Ulnar bones shows a dorsal prominence

Patients present with carpal tunnel syndrome mostly bilaterally.

Disease progression leads to (i) loss of joint space

(ii) ankylosis

carpal to metacarpal ratio(C/MC)(length of carpus/ length of third metacarpal) is used as powerful indicator about bone deformity and useful for corrective measures[58]

MRI: detects early synovial proliferation and bone erosions

Clinical tests for hand involvement: grip test

Deformities:

- SWAN NECK DEFORMITY: due to shortening of interosseous muscles and intrinsic muscles

  (i) flexion of DIP and MCP joints

  (ii) hyperextension of the PIP joint

  if accompanied with OA shows marginal erosion also

- BOUTONNIERE DEFORMITY:

  During the course of the disease chronic inflammation of the PIP joint causes flexion of the joint and DIP will remain in hypeextension to produce this deformity.

Later stages of the disease produces resorptive arthropathy

- THUMB DEFORMITIES
    1. TYPE I: MCP inflammation and stretching of joint
    2. TYPE II: CMC inflammation and volar subluxation
    3. TYPEIII: exaggerated adduction of 1st metacarpal bone, MCP- flexion and DIP- hyperextension.
- TENOSYNOVITIS
- PAINFUL LOCK OF FINGER:

Due to nodules or fibrin deposits in the tendon sheats.

HIP:

Less frequently involved joint in RA.

Symptoms: Complaints of pain the groin and lower buttock

Pain in the lateral aspect of hip

Radiographic finding: femoral head – collapse and resorption

Acetabulum remodeling and medial displacement - protrusio acetabuli

Femoral head may present with cystic lesions which will decrease joint space.

KNEES:

- Early disease: quadriceps atrophy leading to loss of full extension. In later course of the disease presents as fixed flexion contracture

- Persons who varum or valgum deformity when developing rheumatoid arthritis whose pressure on medial side will be more present with radiographic feature of erosion and thinning of cartilage

BAKER'S CYST:

Also known as popliteal cyst. Caused due to the large effusion causing increase in the pressure[59]. This may lead to the protrusion of posterior contents into the joint causing the bakers cyst.

The fluid entering the cyst will be of one way valve will not return[60] back to the posterior compartment which leads to increasing pressure in the cyst. Jayson and Dixon have demonstrated this one way communication of the cyst. The very high pressure in the popliteal space leads to the rupture of contents down to calf or rarely ruptures superiorly into the posterior thigh. Rupture can also occur posteriorly in the space between the medial head of gastrocnemius and biceps tendon insertion.

Clinical symptoms: intact cyst will cause compression on the superficial veins presented as dilatation of the veins and edema[61].

Rupture of the contents to calf will present as thrombophlebitis presenting with swelling and tenderness.

Systemic sign – leukocytosis

Local sign- crescentic hematoma beneath the ankle malleoli[62]

Diagnosis is by invasive arthrography

Invasive procedures can be avoided using ultrasonography and MRI

ANKLE AND FOOT:

Ankle and foot will not be affected in mild cases of rheumatoid arthritis. The severity of the disease causes the involvement of ankle and foot.

First sign: swelling anterior and posterior to the malleoli.

Due to inflammatory and proliferative process in the disease causes loosening of connections between the ankle, fibula and tibia. The progression of the joint involvement causes pronation deformity and eversion of the foot. Patients of RA (one third of them) develop disease of feet.

Achilles tendon: rheumatoid nodules[63] develop in this tendon which will undergo rupture spontaneously.

Rocker bottom deformity: develops due to progressive eversion of the subtalar joint and pain which leads to lateral subluxation. Subtalar joint is the one which controls the eversion and inversion of the talus. Patients will complain of walking difficulty over the uneven surface because of tis eversion deformity.

Cock-up deformity: MTP joints are involved in the RA disease significantly which in due course leads to downward subluxation of metatarsal heads leads to cock-up deformity of the PIP joints. The subluxation can cause pressure necrosis of the plantar surfaces and may develop ulceration over PIP joints.

DIP joints are rarely involved in RA.

Another cause of foot pain is tarsal tunnel syndrome.

The disease progression in the foot are as follows[64]

- Inflammation of the intermetatarsal joints
- Extend to the forefoot
- The fatty cushion of the plantar surface will extend anteriorly
- Subluxation of the toes
- Subluxation of the metatarsal heads
- Hallux valgus and stacking of toes

DIP joints are rarely involved in RA. Rigid hallux caused in RA will be very painful and may require surgical intervention[65]

Some patients with RA foot involvement may develop tarsal tunnel syndrome. Radiograph shows erosions in the foot in such patients.

EXTRA- ARTICULAR MANIFESTATIONS OF RHEUMATOID ARTHRITIS:

These manifestations depends on the duration and severity of the disease. These reason behind these extra-articular manifestations is the extra-articular foci of an immune response[66]. These extra articular manifestations are related with increased risk of mortality[67]

SKELETON:

Skeleton has a cortical and trabecular part. These parts will respond to the diseases differently. Postmenopausal women should be treated earlier and aggressively as they would be presented with overlapping osteoporosis. Bone loss may be secondary to glucocorticoid therapy.The synovial penetration cause cyst formation which will weaken the bone which is also predisposing to fracture. Adequate treatment in both RA and also osteoposrosis prevents bone loss[68]. Bone loss due to glucocorticoid therapy is of two phases. First phase involves rapid bone loss followed by chronic slower rate of bone loss[69]. And the bone loss due to the glucocorticoid therapy can be reversed[70]. The bone loss in RA leads to stress fractures common in the site of bone loss[71].

MUSCLE:

Muscle weakness is a common feature od RA . the weakness may be due to inflammation or may be a response to pain. Rarely present with muscle tenderness.

Nodular myositis: lymphocytes and plasma cells are accumulated leading to degeneration of the muscle fibers.

5 stages[72] of muscle disease in RA

1. Muscle bulk is decreased leading to atrophy
2. Peripheral neuropathy
3. Steroid myopathy
4. Myositis and muscle necrosis

5. Dystrophic process and end stage of inflammatory myositis

Biopsy: shows atrophy of type II fibers

Active myositis and focal necrosis will be seen

Also shows disproportionately high ESR

Lymphocytes in the biopsied muscle synthesize immunoglobulin M AND RF

Myositis will appear as patchy nodules. The nodules are comprised of plasma cella as well as lymphocytes.

SKIN:

- Rheumatoid nodules is the most frequent skin manifestation.
- Skin over the synovitis becomes thin and atrophic.
- Palmar erythema is another skin manifestation seen in RA
- Vasculitis features
- Livido reticularis- discoloration seen in the extremities
- Associated with antiphospholipid antibodies in the circulation[73]

EYE:

Eye manifestations are seen in the complicated RA. The rheumatoid process produces scleritis and episcleritis. Both occurs less than 1% RA patients[74]. The connective tissue of eye is highly differentiated and forms aggressive form of disease when it is involved. Will not produce discharge but cause tearing response. May cause visual loss. Scleritis may be superficial or generalized. Extension into the uveal layer causes scleromalacia perforans. Perilimbic ischemic ulcers produced by RF-IgG complexes. If these ulcers are left untreated it leads to

perforation of the anterior chamber. Can also produce chronic blepharitis in RA eye in the individuals who have keratoconjunctivitis sicca secondary to Sjogren's syndrome.

RHEUMATOID NODULES:

Nodule has a centre of necrosis surrounded by fibroblasts and inflammatory cells. Nodules have a tendency to grow and encroach the connective tissue matrix. The earliest nodules to be identified is to be of size 4mm[75]. The nodules increase in size by enchroaching the adjacent cells and form necrosis which is intiated by vasculopathy and protein destruction by connective tissue matrix. Most of rheumatoid nodules are present on the extensor surface. Eg: olecranon process of ulna.

Characteristic of nodules: they are subcutaneous, consistency vary from soft to hard and attached firmly to the periosteum.

Contents of the nodules: macrophage and nonsynoviocyte fibroblasts.

Other sites of nodules:

- Sacral nodules
- Occipital nodules
- Nodules in the larynx
- Nodules in the heart and lungs
- Nodules on the sclera
- Nodules in the vertebral bodies.
- Nodules in central nervous system(CNS)

Nodules in the sacrum are mis interpreted as bed sores.

Nodules in the sclera may go for perforation.

Nodules in the CNS involves mostly leptomeninges[76]

Development of nodules: mediated through immunological reactions. Cytokines mediate the reaction.

Initial nodule development is through the affected arterioles which leads to complement activation

Rheumatoid nodulosis[77]: positive RF with episodes of intermittent synovitis and cystic lesions of small bones of hands and feet.

The nodules enveloping cells have the cabability to c[produse collagenase and protease which lead to the disease activity[78].

DIFFERNTIAL DIAGNOSIS OF RHEUMATOID NODULES

1. Benign nodules: non tender and present in non RA individuals
2. Granuloma annulare: intracutaneous, slowly resolve, not associated with any disease
3. Xanthomatosis: no bone involvement. Occurs in patients with high lipoprotein levels
4. Tophi: occurs in gout
5. Other conditions: like acrodermatitis chronica and leprosy.

FISTULA:

Can be sterile or septic. Forms a connection to the skin and bone.

INFECTION:

Occurs as a complication of rheumatoid arthritis. Common sites of infection are pulmonary, skin infections[79,80]. Infections increase with increasing age, with extra articular manifestations, leukopenia, chronic lung disease, alcoholism, DM and glucocorticoid therapy. Pseudoseptic arthritis should be differentiated from infection[81].

The infection is worsened with the following factors

- leukopenia
- increasing age
- patients with extra articular manifestations
- associated with other diseases like chronic lung disease, diabetes mellitus, alcoholism and treatment with glucocorticoids.
- DMARD is not producing increased risk of infections[82,83], which makes DMARD safer to use than glucocorticoids.
- Infections should be identified at an earlier stage and treated.

CANCER:

Increased risk to developing cancer in rheumatoid arthritis patients. Increased risk to develop lymphoma, interstitial fibrosis leads to lung carcinoma and also increased risk to develop hodgkin's disease, non Hodgkin's lymphoma and leukemia. Lung carcinoma is of bronchoalveolar type[84]. Interestingly RA patients have a decreased incidence of gastro intestinal tumors[85].

HEMATOLOGICAL COMPLICATIONS:

Most patients have a tendency to develop normocytic hypochromic anemia. Patients may also have additional B12 or folate deficiency anemia[86].

Points to remember when diagnosing anemia in rheumatoid arthritis patients

1. High ferritin levels in anemia of chronic disease like RA when compared with iron deficiency anemia
2. Folate and B12 deficiency will mask iron deficiency anemia by increasing mean cell volume and mean cell hemoglobin levels .
3. ESR values will correlate inversely with hemoglobin levels in RA
4. Erythropoietin level[87] will not be increased to the level such with iron deficiency anemia
    - Total erythroid turnover is reduced
    - red cell aplasia is a rare finding in RA
    - eosinophilia and thrombocytosis are often associated with RA[88]
    - a subset of RA patients have large granular lymphocytes(LGLs)
    - paraproteinemia when coexists with RA have a poor prognosis

VASCULITIS:

Vasculitis in RA occurs due to the inflammatory change in the small blood vessels.

Systemic vasculitis is a dreadful complication of RA. The incidence of the systemic vasculitis have been decreased in these days because of the aggressive treatment modalities

Pathology of vasculitis in RA: panarteritis , infiltered with mono nuclear cells, fibrinoid necrosis is seen in the active lesion,obliterative endarteritis, immune complexes deposits in the vessels.

When large vessels are involved the vasculitis resembles poly arteritis nodosa[89].

Clinical presentation of vasculitis

- distal arteritis present with hemorrhage or gangrene
- ulceration
- peripheral neuropathy
- pupura which is of palpable type
- arteritis of viscera which inludes heart, kidney, liver etc
- paresthesias or burning feet
- weakness
- rheumatoid pachymengitis, a rare presentation of RA involving the brain.
- larger vessel involvement resembles polyarteritis nodosa

Risk factors for development of vasculitis in RA[90].

- High RF value
- Joint erosions
- Glucocorticoid therapy
- Cryoglobulins
- Nodules- subcutaneous
- males

RENAL COMPLICATION:

Rare involvement by the rheumatoid arthritis disease. It may be involved as a complication of treatment. Amyloidosis is a complication of RA. Renal papillary necrosis: caused by phenacetin use in the treatment.

Salicylates and other NSAIDS also produce renal changes.

Membranous neohropathy: caused by gold salts and D- pencillamine therapy
Focal necrotizing glomerulitis: seen in patients with disseminated vasculitis

PULMONARY COMPLICATION:

Pulmonary involvement in RA may present as one of the following

- Small airway disease
- Pleural disease
- Pulmonary hypertension
- Interstitial fibrosis
- Bronchiolitis
- Nodular lung disease

Small airway disease:

Diagnosed by specific pattern of pulmonary function tests. There are studies stating that small airway disease is not a direct complication but a overlapping of diseases.

Pleural disease:

Commonly an autopsy finding. Rarely present as active disease during the life time. Pleuritic pain is the complaint but a rare feature. May develop effusions

even large enough to cause dyspnea. The rheumatoid exudative effusions on examination shows low glucose levels. Another low glucose level in exudative effusion examination is tuberculosis[91]

Pulmonary hypertension:

Seen in >30 percent of RA patients[92]. Mostly they are silent without any symptoms. Diagnosed with noninvasive echocardiograms

Interstitial fibrosis:

Caused by increased activity of mesenchymal cells to RA. Present as diffuse reticular or Reticulonodular pattern. Radiograph- shows honey comb appearance. High resolution CT- shows lattice appearance. Pathological examination shows diffuse fibrosis and mononuclear cell infiltrate. The functional defect is alveolocapillary gas exchange. Mild cases shows more lymphocytes on bronchoalveolar lavage. Severe cases shows neutrophils in the bronchoalveolar lavage. Interstitial pneumonitis will be the finding in cases of RA with coexisting Sjogren's syndrome. The initial defect is diagnosed by single breath carbon monoxide diffusion capacities[93].

Bronchiolitis:

Rare presentation in RA. Pathologic examination shows fibrosis and exudates in the bronchioles and the alveoli

Nodular lung disease:

Nodules may appear single or in groups. When presented with multiple nodules they have the tendency to coalesce. Diagnosis is by needle biopsy.

CAPLAN'S SYNDROME[94]:

Pneumoconiosis with RA. It is a rare presentation with obliterative lesion seen as occupational hazard in the mining individuals.

Nodules may progress to produce cavity and complicating to fistula formation. Some of the rheumatoid nodules have been associated with coexisting bronchogenic carcinoma[95].

CARDIAC COMPLICATIONS:

May be due to granulomatous proliferation or vasculitis.

Pericarditis: there are studies showing 50% of RA patients with pericarditis. Echocardiogram shows pericardial effusion. There are also evidence of contrictive pericarditis and cardiac tamponade in rheumatoid arthritis.

Myocarditis: Myocarditis present as granulomatous or inflammatory disease. Infiltrated by mononuclear cells. Mostly would not present with any clinical symptoms.

Endocardial inflammation: Presents with mitral or atrial involvement.

Conduction defects: There are studies showing that 30% of patients with rheumatoid arthritis present with complete heart block.

Coronary arteritis: Present with vasculitis and develop myocardial infarction.

Granulomatous aortitis: granulomatous type of aortitis.

DIAGNOSIS OF RHEUMATOID ARTHRITIS:

Diagnosis is based on clinical examination, and evidences supporting the history and examination

CRITERIA FOR DIAGNOSIS- REVISED AMERICAN RHEUMATISM ASSOCIATED CRITERIA

1. EARLY MORNING JOINT STIFFNESS:

The stiffness present in morning soon after rising from bed and lasts for a minimum of 1 hour

2. ARTHRITIS OF SMALL (HAND) JOINTS:

Atleast one joint is swollen in the wrist region either MCP or PIP joint

3. ARTHRITIS INVOLVING THREE OR MORE JOINTS:

At least should present with 3 simultaneous joint involvement with soft tissue swelling. There are 14 possible joints including PIP,MTP, ankle, wrist, knee, elbow and MCP joints.

4. SYMMETRIC ARTHRITIS:

Simultaneous involvement of joints on both sides.

5. RHEUMATOID NODULES:

Subcutaneous nodules over the bony prominences

6. RHEUMATOID FACTOR:

Positivity by any standard method

7. RADIOGRAPH FINDINGS:

Erosions or bony calcifications apart from osteoarthritis.

For diagnosis: - 4 out of 7 criteria should be present

- clinical criteria should be present for a minimum of 6 weeks

IMPORTANCE OF EARLY DIAGNOSIS OF RHEUMATOID ARTHRITIS

The diagnosis of rheumatoid arthritis till date seams to be difficult because of atypical presentation of the patients. Not all the patients present with typical features and satisfies the scoring to be diagnosed as rheumatoid arthritis. It is important to diagnose earlier the disease as there are effective therapies for treating RA and a deformity and long term disease can be halted at an early stage. And also it should be considered that the non diseased patients should not treated with the drugs and chances of developing adverse effects. The difficulty in diagnosis and the necessity in the early diagnosis and treatment of RA to halt the disease progression, newer markers are needed to complement with the existing criteria for diagnosis to identify more number of cases. Thus the identification of newer marker 14-3-3η protein in the rheumatoid arthritis patients can compliment the existing diagnostic criteria.

ACR criterion for sensitivity and specificity of various diagnostic criteria

CRITERIA	**SENSITIVITY**	**SPECIFICITY**
EARLY MORNING STIFFNESS	68	65
ARTHRITIS INVOLVING MORE THAN 3 JOINTS	80	43
HAND JOINT ARTHRITIS	81	46
SYMMETRIC ARTHRITIS	77	37
RHEUMATOID NODULES	3	100
RHEUMATOID FACTOR	59	93
RADIOGRAPHIC FINDINGS	22	98

There are no single marker with good sensitivity and specificity. Therefore the diagnosis is a combination of markers and also there is need for newer markers for early diagnosis

TREATMENT OF RHEUMATOID ARTHRITIS:

Show good improvement with NSAIDs.

Glucocorticoid therapy – improve the symptoms and patients routine physical activity. Intramuscular methylprednisolone are practiced by some of the rheumatologists. But patients who are under long term glucocorticoid therapy develops osteoporosis in future.

Tetracycline derivatives inhibit MMP's

Disease modification therapy: DMARDs(disease modifying anti rheumatoid drugs) show good improvement in the patients.

Aims & Objectives

AIM AND OBJECTIVES OF THE STUDY

- The aim of the study is to estimate the levels of 14-3-3 eta protein in the serum of rheumatoid arthritis patients
- Compare the levels with rheumatoid factor positive patients and rheumatoid factor negative patients.
- Compare the levels with healthy individuals
- Establish the sensitivity and specificity of the new marker for the diagnosis of rheumatoid arthritis.
- To establish its adjuvant role in the diagnosis of the disease.

Materials & Methods

MATERIALS AND METHODS

This is a case control study and the study was conducted after getting the ethical committee approval from MADRAS MEDICAL COLLEGE.

The study population includes 60 patients and 30 healthy age and sex matched individuals.

The patients were selected from the rheumatology outpatient department from the Rajiv Gandhi Government General Hospital, Chennai. The patients were explained about the purpose of study and consent was obtained from the individuals.

INCLUSION CRITERIA:

Group 1: rheumatoid factor positive patients

Group 2: rheumatoid factor negative patients.

Group 3: age and sex matched healthy control

EXCLUSION CRITERIA:

- Patients with active infection
- SLE
- Psoaritic arthritis
- Jeuvenile arthritis
- Spondylo arthritis
- Diabetes mellitus
- Hypertension
- Other systemic diseases

SAMPLE COLLECTION:

3 ml of venous blood was collected from the patients and healthy individuals and was transferred to plain red tube and was allowed to clot. After adequate clotting, centrifuge was done to separate the serum. The serum was pipetted and stored in aliquots. The following investigations were carried out in the serum

- Serum 14-3-3□ - ELISA method
- Rheumatoid Arthritis factor - Quantitative slide agglutination method
- CRP - turbidimetric immunoassay
- Serum Uric acid - Spectrophotometric method
- ESR - Automated
- Complete blood count- Automated cell counter

ESTIMATION OF 14-3-3η PROTEIN:

METHOD: Enzyme linked immune sorbent assay – non competitive sandwich method

TEST PRINCIPLE:

The method is based on the biotin antibody sandwich technology. The ELISA plate wells will be coated with 14-3-3 eta monoclonal antibody. Then the serum containing 14-3-3 eta protein should be incubated. Then anti 14-3-3 antibodies labeled with biotin united with streptavidin- HRP is added. These form a immune complex with antigen antibody complex. Then the unbound enzymes are washed after incubation process. Then substrates A and B are added. The

solutions in the well turn to yellow which was blue initially. The concentration of the colour formed is directly proportional to the concentration of the 14-3-3 eta protein present in the serum.

REAGENTS AND MATERIALS USED:

1. Standard solution(4000ng/L)- 0.5ml
2. Dilution for standard preparation- 3 ml
3. Coated ELISA plate
4. Streptavidin-HRP
5. Washing concentrate(30X)
6. Anti YMHAH antibodies labelled with biotin
7. Chromogen solution A
8. Chromogen solution B
9. Stop solution

ASSAY PROCEDURE:

Standard preparation: The standard solution is diluted to various concentrations as follows

2000ng/L	Standard No.5	120µL Original Standard + 120µL standard diluent
1000ng/L	Standard No.4	120µL Standard No.5+120µL standard diluent
500ng/L	Standard No.3	120µL Standard No.4+120µL standard diluent
250ng/L	Standard No.2	120µL Standard No.3+120µL standard diluent
125ng/L	Standard No.1	120µL Standard No.2+120µL standard diluent

4000ng/L 2000ng/L 1000ng/L 500ng/L 250ng/L 125ng/L

Washing solution preparation: washing solution is mixed with distilled water for the washing steps in ELISA

1. The wells should be marked for blank well, standard well and sample well in the ELISA plate
2. Blank well: no sample is added to tis well, anti YMHAH antibody labelled with biotin or streptavidin-HRP is added. But the chromogen solutions A and B and the stop solution should not be added to the blank well
3. Standard wells: to the standard wells, 50μL of the standards of varying concentration are added. Then 50μL of streptomycin-HRP is added to each standard well. The labelled antibody are already added with the standard solution so no need to add the labelled antibody.
4. Sample well: 40μL of sample is added(60 patients sample and 30 healthy control individuals serum are added to the respective wells. 10μL of YMHAH antibodies are added. Then coated with 50μL of streptavidin- HRP. After addition of the sample and these antibodies. The ELISA plate should be sealed and to be shaken gently to mix the contents and then to be incubated at 37^{O}C for 60 minutes.

5. After the incubation time. ELISa plate is uncovered and dried by blotting over filter paper and then washed with washing solution using washer for five times and then dried using the filter paper blotting.
6. Then to each well except the blank well 50μL of coloring solution A is added, then 50μL of colouring solution B is added. Mixed well by shaking the plate gently and uniformly. Then the plate is incubated for ten minutes at 37^{o}C. The plate should be covered and should be stored in a dark place avoiding sunlight. Even during addition of the chromogen solutions care should be taken to avoid from light.
7. After the incubation time. Each well except the blank well is added with 50μL of stop solution is added. immediately after adding the stop solution the blue colour changes to yellow colour
8. Within ten minutes of adding the stop solution, the OD of each well should be measured at 450 nm wavelength using ELISA reader.
9. Before hand the ELISA plate should be defined and the concentrations of the standars should be fed to the reader. With known concentration of the standards, OD is measured and standard graph is plotted.
10. Using the standard graph, and the plotting the OD of the samples over the graph, the ELISA reader gives the concentration of the different samples.

OBTAINING RESULTS USING STANDARD GRAPH:

The concentration of the standards are made as the abscissa(x axis) and the OD as ordinate(y axis). Using this graph the unknown concentration of the samples are obtained by calculating the concentration using the measured OD.

Assay range	:	10ng/L to 3500ng/L
Sensitivity	:	5.22 ng/L.
Intra assay precision	:	CV<10%
Inter assay precision	:	CV<12%

ESTIMATION OF c- REACTIVE PROTEIN:

CRP is a non specific inflammatory marker. Increases during any inflammatory condition and sooner returns back to normal range after recovery.

METHOD : turbidimetric immunoassay

PRINCIPLE:

Antigen antibody reaction occurs and the signal is measured by end point method.

REAGENT CONSTITUENTS:

R1 which acts as a buffer contains

CHEMICAL	DESCRIPTION
Phosphate buffer	pH is 7.43
Sodium azide	0.09%
Polyethylene glycol	40g/L

R2 antiserum contains

CHEMICAL	DESCRIPTION
Phosphate buffer	pH is 7.43
Sodium azide	0.09%
Polyclonal goat anti- human for CRP	

Reagents are ready to use

The kit is calibrated using XSYS0053 CRP calibrator.

Results are obtained automatically not calculated.

Normal value (given in the kit insert)- 0-10 mg/L or 0-1mg/dl

Linearity of the kit – upto 22mg/dl

Lower detection limit – 0.1mg/dl

Hook effect >84mg/dl

Intra -assay precision: CV- 0.82%

Inter- assay precision: CV-2.46%

ESTIMATION OF URIC ACID:

Uric acid is the end product of purines , nucleic acids and nucleoproteins. Hyperuricemia occurs in various conditions like renal dysfunction, gout, leukemia and many other conditions.

Method: uricase, Endpoint assay.

Principle:

Uric acid is oxidised to allantoin with the help of the enzyme uricase. H202 is also produced in this reaction. The peroxide reacts with 4-AAP(4-

aminoantipyrine) and TOOS to form a coloured compound quinoneimine dye. The absorbance of the dye is measured at 546 nm which is proportional to the uric acid level

$$\text{Uric acid} + H_2O \xrightarrow{\text{uricase}} \text{allantoin} + co2 + H_2O_2$$

$$TOOS + 4AAP + 2H_2O_2 \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 4H_2O$$

Tis reaction is called as trinder reaction.

REAGENT CONSTITUENTS:

R1

CHEMICAL	CONCENTRATION
Pipes buffer whos pH is 7.0	50mmol/L
4- Aminoantipyrine	0.375mmol/L
Uricase	≥ 200 U/L

R2

CHEMICAL	CONCENTRATION
Pipes buffer whose pH is 7.0	50mmol/L
TOOS	1.92 mmol/L
Peroxidase	≥ 5000 U/L

Reagents are ready to use

CALIBRATION : calibration is done by XL MULTICAL, Cat No. Xsy0034

CONTROL : Quality control is checked with ERBA NORM Cat No. BLT0080 and ERBA PATH, Cat No. BLT00081.

REFERENCE VALUES:

Adult male : 3.5- 7.2 mg/dl

Adult female: 2.6-6.0 mg/dl

Linearity is upto 25 mg/dl

Measuring range is between 0.28-25 mg/dl

Intra assay precision: CV of 0.83%

Inter assay precision: CV of 1.93%

ESTIMATION OF RHEUMATOID FACTOR(RF):

Rheumatoid factor is an auto antibody produced against own immunoglobin

PRINCIPLE:

Latex agglutination test. Reagent will contain latex particle which will coated with human immunoglobin to produce agglutination.

PROCEDURE:

- After bringing the reagents and sample to room temperature
- a drop of positive control and patients sample are placed on a slide
- the reagent containing latex particle is added
- separate sticks are used for control and sample to mix with the reagent
- after 2 minutes look for agglutination
- this is a qualitative test.
- if agglutination is present in 2 minutes it approximately account for more than 18IU/ml

DILUTION TEST FOR POSITIVE AGGLUTINATION(SEMI-QUANTITATIVE TEST:

- serial dilutions are made with the positive sample like 1:8, 1:16, 1:32, 1:64, 1:128 with isotonic saline
- the qualitative test is repeated with the serial dilution samples and look for agglutination.

Result: the last dilution where agglutination is present is noted.

RF in IU/ml= Sensitivity of latex gammaglobulin x titre where agglutination is present.

Nomal range: less than 15 IU/ml

ESTIMATION OF HEMOGLOBIN:

METHOD: automated haemoglobin analyser

PRINCIPLE:

The blood sample is injected into the analyser through a capillary action. The measurement occurs by measuring the absobance of the blood ar a Hb/HbO2 isoelectric point. The measurement takes place at two wavelengths, 506nm and 880nm. Two wavelengths are selected to decrease the interference due to the turbidity of the sample. The wavelength is also chosen based on the interference caused by haemoglobin variants like methemoglobin and carboxyhemoglobin.

Type : quantitative measurement

Sample : whole blood.

Precision: For low value with mean of 7.3 g/dL – CV is 0.82%

For normal value with mean 13.2 g/dL- CV is 0.80%

For high value with mean 17.2 g/dL- CV is 0.78%

ESTIMATION OF ERYTHROCYTE SEDIMENTATION RATE(ESR):

PRINCIPLE:

Anti-coagulant blood on standing in a narrow glass tube for a time period without being disturbed, the RBC's settle down under the influence of gravity. The rate of settling of red cells is measured by the millimetres of clear plasma at the top of the column. It is measured after one hour and expressed in mm/hr.

METHODS:

1. Wintrobe's method
2. Westergren's method

PROCEDURE:

- The blood sample should be mixed thoroughly
- The blood is drawn into the tube upto 0 mark with the help of rubber bulb
- Excess blood should be wiped from the bottom of the tube with cotton
- then the tube is made to stand upright in a stand. Should ensure for any leakage from the tube.
- The tube should be undisturbed for 1 hour
- After one hour, the result is read

REFERENCE RANGE:

Males : 0-10 mm/hr

Females : 0-15 mm/hr

ESTIMATION OF TOTAL COUNT:

Method: automated cell counter

PRINCIPLE:

- electrical impedance system(conductivity)
- direct imaging
- or flow cytometry
- electrical impedance is by measuring the changes in the electrical impedance produced by the particle while it passes through the aperture.
- Blood cells are non conductive particle and they are mixed with a conductive diluent.
- Along with the conductive diluent while each particle moves through the aperture causes an electrical conductance between the electrodes which is measured and counted.
- direct imaging is by when the blood sample which is diluted while passing through a steady steam and a beam of light is focussed on it
- when each particle passes through the sensing zone, scatters the light
- scattered light is detected by a photodetector and which is converted to electrical impulse and measured

- flow cytometry is by when a light of single wavelength is directed over a beam of particles, the light being scattered forward or side scatters which are detected by photo detectors and measured.

Automated cell counters uses the combination of the principles

Normal range: 4000 – 110000 cells/ mm3

False Low count:

Leukocyte aggregation

False high count:

Failure to lyse RBC

Microclots

Platelet clumping

Abnormal hemoglobin

ESTIMATION OF ANTI-CITRULLINATED PEPTIDE ANTIBODY (ACPA):

METHOD: ELISA method

PRINCIPLE:

It is an Indirect solid phase enzyme immunoassay. Quantitative measurement of IgG auto antibodies against the citrullinated proteins are measured in the serum or plasma. The wells are coated with antigens. The serum containing auto antibodies are added to the wells and incubated. After incubation the plate is washed to remove the unbound particles. Then an anti-human-IgG

horseradish peroxidase conjugate solution is added to each well which will bound to the antigen antibody complex. After incubation for binding, the plate is washed to remove the unbound particles. Then a chromogenic substance is added to the wells. After the development of colour, stop solution is added. The colour turns to yellow. The concentration of the colour formed is directly proportional to the ACPA concentration present in the sample.

PRECISION:

Inter assay	:	5.53%
Intra assay	:	4.70%
Sensitivity	:	even as low as 1U/ml
Normal range	:	<15 U/ml

Statistical Analysis

STATISTICAL ANALYSIS

- The data s obtained between the cases and controls were analysed using IBM SPSS software 20.
- The significance was determined when the p value is less than 0.05.
- The RF, ESR, CRP, ACPA, 14-3-3 eta protein, total count, Hb, uric acid were compared between the cases and the control group using Students t- test.
- When more than two groups are compared like RF positive cases group, RF negative cases group and control groups. One way ANNOVA was done to compare the three groups
- Correlation of parameters between RF, ESR, CRP, ACPA, 14-3-3 eta protein are done using pearson correlation.
- Step wise linear regression was done to find the independent predictor of the disease diagnosis.
- ROC curve was done to determine the specificity and sensitivity of the marker14-3-3 eta protein.

Results

MASTER CHART

S. NO	SAMPLE NO	AGE / SEX	RF	ESR	CRP	ACPA	14-3-3ETA	UREA	CREAT	URIC ACID	SGOT	SGPT	Hb	TC	DISEASE DURATION	DEFORMITY
RF POSITVE CASES																
1	1	21/F	381	14	7	202	989.5	21	0.8	5.8	30	20	9.8	8700	10 yrs	YES
2	2	46/F	164	30	6	56	746.4	23	0.7	6.8	31	15	10.1	8700	5 yrs	no
3	3	50/F	128	80	6	55	666.6	21	0.6	2.2	21	22	10.1	6500	3 yrs	no
4	4	30/F	123	6	6	45	631.3	22	0.9	3.2	16	3.2	9	9600	3 yrs	no
5	8	31/F	478	17	6	396	1565	21	0.6	2.7	30	15	9.6	6700	14 yrs	YES
6	11	47/F	135	45	8	55	636	30	1	4.1	25	36	9.9	9600	2 yrs	no
7	13	46/F	158	50	24	59	715.8	31	0.8	2.1	26	27	9.8	8600	2 yrs	no
8	14	54/F	224	17	6	64	836	25	0.9	1.9	19	22	9.4	6800	4 yrs	no
9	32	42/F	460	122	12	321	1321	26	0.8	5.5	26	22	9	10000	3 yrs	YES
10	9	39/F	598	29	6	422	1911	23	0.8	2.8	25	35	9.8	7800	13 yrs	YES
11	44	57/F	426	15	6	389	1778	20	0.8	3.5	20	21	8.8	7400	11 yrs	YES
12	21	31/F	232	55	6	44	657.4	22	0.9	4.5	10	12	8.7	6500	2 yrs	no
13	22	38/F	145	24	7	6	501.2	20	0.9	1.3	17	15	11.2	7900	8 YRS	no
14	53	44/F	621	29	24	489	1997.3	21	1	2.6	41	31	8	12000	5 yrs	YES
15	27	58/F	132	42	5	5	488.9	28	1.2	6.5	30	13	9.2	13300	7 YRS	no
16	37	30/F	230	88	16	55	742.6	25	1	32	35	20	9.2	10200	5 yrs	no
17	29	43/F	122	45	6	4	514.1	116	0.7	3.1	23	56	12.9	9800	3YRS	YES
18	7	48/F	232	18	40	45	600.4	30	0.7	3.4	12	32	11.2	6900	3 yrs	no
19	40	38/F	116	72	6	7	485.6	32	11	3.6	36	27	10.2	10200	2 yrs	no
20	12	21/F	224	18	6	51	702.6	21	0.8	2.6	30	15	9.8	6700	2 YRS	no
21	46	63/F	502	38	12	384	1879	26	1.1	4.2	25	27	9.5	9200	6 YRS	YES
22	49	46/F	511	46	6	401	1900.3	25	0.8	1.8	41	24	9.4	6700	11 yrs	YES
23	54	35/F	142	58	24	24	667.6	30	0.9	4	33	31	11	10600	3 yrs	no
24	55	30/F	132	42	12	26	678.6	55	1	5.2	42	46	10.5	8200	3 yrs	no
25	56	51/F	320	62	58	388	1733	55	1	2.6	31	30	13.6	9800	13YRS	YES
26	57	40/F	85	62	6	6	551.5	50	1.3	2.9	36	14	11	7900	6MONTHS	no
27	25	41/F	140	15	6	56	613.5	24	0.8	1.4	32	21	12.6	9700	10YRS	no
28	59	40/F	135	15	6	5	528.2	21	0.6	2.6	35	51	12	6700	5 yrs	no
29	60	41/F	132	17	24	4	563.6	25	1	3.4	65	53	11.2	7300	4 yrs	no
30	43	39/F	326	35	48	222	964.5	17	1.2	3.7	13	14	1.07	12900	6 yrs	YES

S. NO	SAMPLE NO	AGE / SEX	RF	ESR	CRP	ACPA	14-3-3ETA	UREA	CREAT	URIC ACID	SGOT	SGPT	Hb	TC	DISEASE DURATION	DEFORMITY
RF NEGATIVE CASES																
31	5	40/F	5	25	6	4	290.6	31	1.1	4.5	39	18	13.7	7900	2 yrs	negative
32	34	29/F	4	55	12	25	402.4	56	0.8	2.3	35	25	12.4	10700	5YRS	POSITIVE
33	6	25/F	8	20	24	6	230	23	1	5.5	24	39	10.9	12400	1 year	negative
34	10	45/F	7	40	24	5	385.7	20	1	3.6	30	14	10.8	5400	4YRS	negative
35	42	45/F	6	70	6	4	220.9	25	0.9	43	30	27	10.1	8600	7 YRS	negative
36	18	21/F	3	106	6	6	462.2	19	0.9	2.7	14	17	12.4	8600	6YRS	negative
37	19	38/F	2	74	6	5	210	22	0.7	2.7	11	19	10.5	12200	7yrs	negative
38	20	45/F	4	70	13	15	544.4	25	1.7	3.2	51	26	12	11300	4 YRS	negative
39	23	45/F	7	15	5	4	467.3	26	1	3.5	38	36	10.7	7000	1 YR	negative
40	58	45/F	5	64	48	6	256.3	29	1	5.3	10	21	11.8	16100	2 yrs	negative
41	26	48/F	4	50	5	6	490.5	17	1.1	1.5	23	26	10.7	8900	1 yr	negative
42	30	31/F	8	55	5	5	537.5	35	3.5	2.6	20	30	12.4	11000	6 months	negative
43	31	51/F	8	110	6	4	323.7	24	1.2	2.7	41	44	12.4	9500	2 yrs	negative
44	15	24/F	2	30	7	6	236.6	22	0.8	3.4	30	15	10	14300	3 yrs	negative
45	33	55/F	7	99	8	15	508.7	25	1.1	3.4	13	15	10.6	6900	2 yrs	positive
46	35	30/F	4	56	10	6	391.9	26	1.1	3.4	13	15	10.6	6900	2 yrs	negative
47	36	30/F	3	16	6	5	437.6	27	1.2	4.7	12	15	12.5	6700	1 yr	negative
48	28	42/F	2	100	15	1	233.5	16	1	2.6	35	22	9.9	8400	3YRS	POSITIVE
49	38	65/F	5	46	7	4	535.6	32	1.1	3.9	22	12	9.4	12000	5 yrs	negative
50	39	45/F	5	18	6	20	410	23	1.1	2.8	25	41	12.8	10100	4 yrs	negative
51	41	32/F	4	40	8	12	445.5	24	0.9	2.7	41	21	12.6	6200	4 yrs	negative
52	16	39/F	6	35	12	4	230.5	23	0.9	2.8	33	15	10.7	6200	3 yrs	negative
53	17	30/F	4	34	12	155	382.7	20	0.7	2.9	26	29	10.2	6900	1 yr	negative
54	45	37/F	5	40	8	12	440.3	32	0.9	42	21	14	8.2	6000	2 yrs	negative
55	47	50/F	4	40	6	6	518.3	23	1.2	5.4	21	10	11	7000	4 yrs	positive
56	48	50/F	6	100	24	16	548.4	23	1	3.1	24	29	9.2	10200	3 yrs	positive
57	50	51/F	7	60	48	5	577.3	21	1.2	3.7	31	19	8.2	8600	3 yrs	negative
58	51	44/F	8	15	48	4	577.3	19	0.9	3.4	30	21	7.4	6700	3 yrs	negative
59	52	42/F	6	29	24	4	504.6	21	0.8	4.8	35	15	8.8	7200	2 yrs	negative
60	24	50/F	6	30	8	4	160.3	26	1.5	2.9	14	14	10.2	8100	1 yr	negative

S. NO	SAMPLE NO	AGE / SEX	RF	ESR	CRP	ACPA	14-3-3ETA	UREA	CREAT	URIC ACID	SGOT	SGPT	Hb	TC	DISEASE DURATION	DEFORMITY
CONTROLS																
61	1	31/F	5	13	6	3	178	37	0.8	3.1	15	16	13	5500		negative
62	2	32/F	5	14	5	2	261	25	0.7	2.1	25	22	11	6100		negative
63	3	36/F	5	7	4	1	180	24	0.6	4.2	22	23	10.9	7700		negative
64	4	36/F	4	11	4	2	221	28	0.5	3.7	17	16	9.8	5000		negative
65	5	32/F	4	12	4	4	165	27	0.6	3.2	15	16	9.7	4500		negative
66	6	43/F	4	13	4	1	154	29	0.7	3.1	26	15	906	5000		negative
67	7	32/F	2	15	4	2	56	26	0.8	2.8	25	18	9.9	5900		negative
68	8	43/F	2	20	4	3	220	33	0.9	3.5	22	15	10.1	6300		negative
69	9	28/F	2	18	5	4	250	34	1	3.6	15	18	10.2	5500		negative
70	10	26/F	2	17	5	1	54	28	0.8	3.2	25	27	9.8	6000		negative
71	11	26/F	2	18	5	2	380.3	29	0.7	3.1	22	27	11	7000		negative
72	12	29/F	1	19	5	2	180	35	0.6	3.2	28	27	10	4500		negative
73	13	19/F	1	14	5	4	225	28	0.5	2.1	22	27	9.8	5000		negative
74	14	18/F	1	12	6	1	36	25	0.4	3.5	28	29	9.7	5600		negative
75	15	31/F	6	11	6	2	174	26	0.6	2.2	26	25	8.9	6000		negative
76	16	42/F	5	13	6	3	166	24	0.7	2.4	15	14	9.7	4500		negative
77	17	27/F	4	17	6	4	280	28	0.8	2.6	17	18	9.8	5600		negative
78	18	41/F	3	12	6	1	250	29	0.5	2.5	19	18	9.5	4500		negative
79	19	41/F	2	14	4	1	223	32	0.6	2.4	16	17	8.6	6000		negative
80	20	18/F	1	16	5	1	254	34	0.7	2.2	15	18	7.4	4500		negative
81	21	18/F	7	12	4	1	261	38	0.8	2.4	18	19	9.8	4500		negative
82	22	18/F	4	17	5	2	281	26	0.5	2.6	19	17	9.7	4500		negative
83	23	18/F	5	13	4	3	296	27	0.6	2.6	19	18	9.7	5000		negative
84	24	30/F	6	12	6	5	38	28	0.7	2.4	25	27	9.8	6000		negative
85	25	28/F	4	11	6	2	44	33	0.8	3.1	18	19	9.4	7000		negative
86	26	30/F	5	8	4	2	155	27	0.9	3.2	19	19	9.4	6600		negative
87	27	32/F	4	17	4	4	168	29	0.7	3.6	22	26	10.2	5000		negative
88	28	44/f	9	18	4	3	177	36	0.7	2.5	24	29	11	4900		negative
89	29	39/f	5	19	4	5	186	35	0.4	2.8	22	23	9.7	5800		negative
90	30	38/f	4	21	4	2	220	33	0.7	2.6	28	29	9.8	6800		negative

RESULTS

TABLE 1: Shows the Age distribution of study Subjects

Age Group (in Years)	CASE		CONTROL	
	Number	**Percentage**	**Number**	**Percentage**
20 - 40 Years	27	45	24	40
41 - 60 Years	31	51.7	6	10
Above 60 Years	2	3.3	0	0
TOTAL	60	100	30	100
Mean	41.06		31.73	
Standard Deviation (sd)	10.09		7.08	

TABLE 2 : Shows the comparison between the RF value of cases and patients

RF	CASE		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**
	131.81	170.71	3.8	1.93
t-value		5.8		
P-value		0.000		
Significant		Significant		

The RF values of cases resulted a mean to be of 131.81 and controls had a mean of 3.8. The p value was 0.000(highly significant).

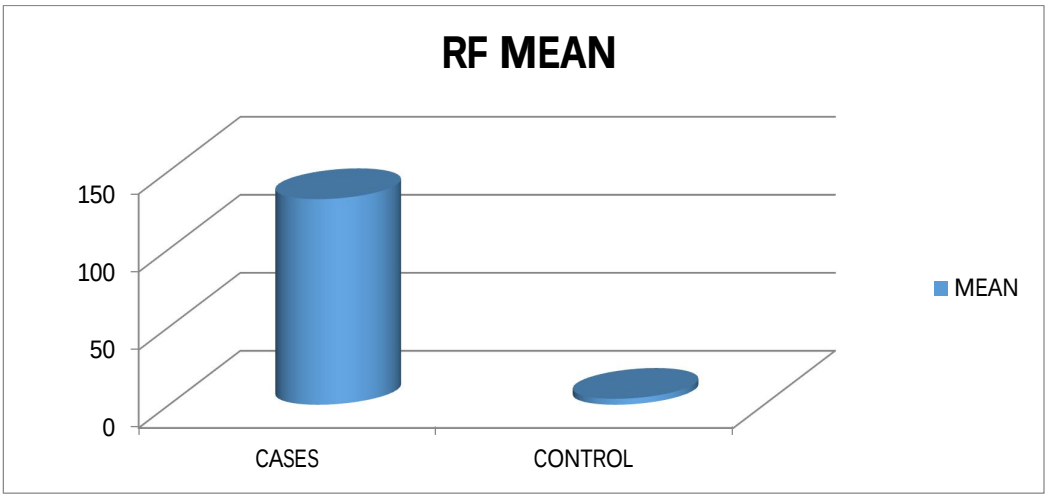


TABLE 3: Shows the comparison of ESR between cases and controls

ESR	CASES		CONTROL	
	Mean	Sd	Mean	Sd
	45.8	28	14.46	3.49
t-value			8.53	
P-value			0.000	
Significant			Significant	

The mean ESR of cases is 45.8 and controls is 14.46. and p value resulted to be highly significant

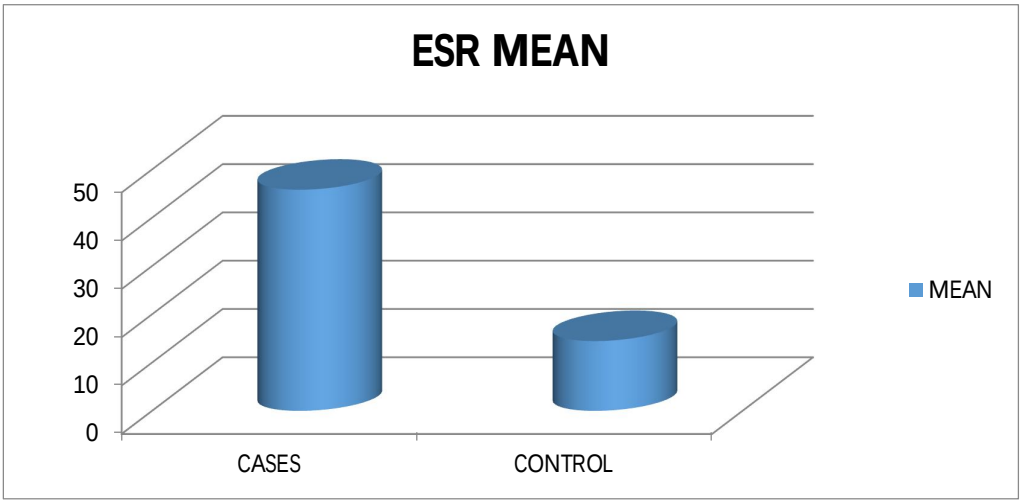


TABLE-4 : Shows the comparison of CRP between the cases and control

CRP	CASE		CONTROL	
	Mean	Sd	Mean	Sd
	13.9	13.18	4.8	0.84
t-value	5.33			
P-value	0.000			
Significant	Significant			

The mean CRP of cases showed 13.9 and mean CRP of controls resulted to be of 4.8. the p value resulted to be highly significant.

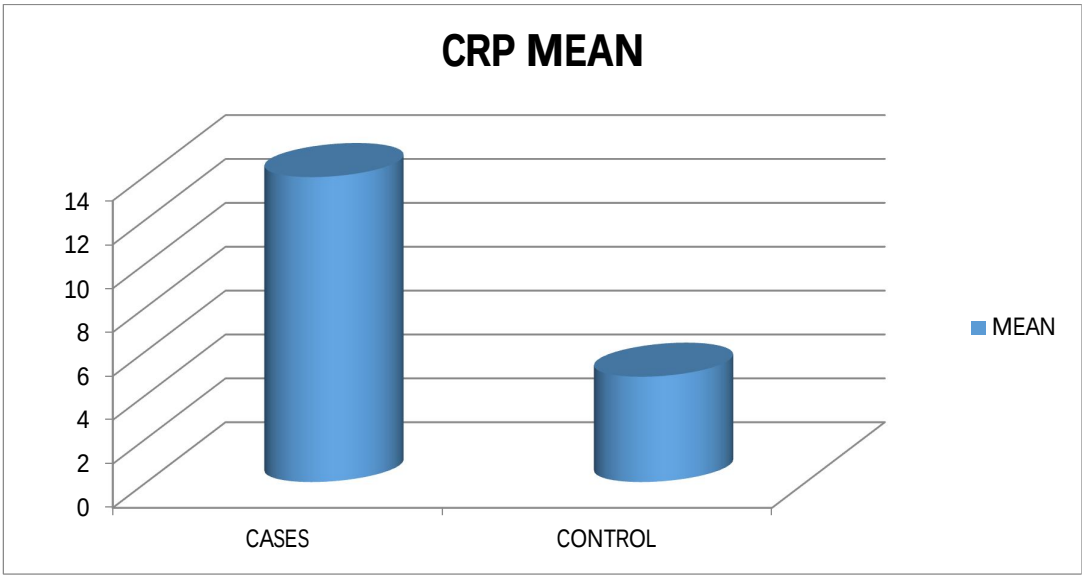


TABLE-5 : Shows the comparison of ACPA between the cases and control

ACPA	CASE		CONTROL	
	Mean	Sd	Mean	Sd
	77.66	134.97	2.43	1.25
t-value	4.32			
P-value	0			
Significant	Significant			

The mean value of ACPA of cases was 77.46 and mean of control was 2.43. The p value resulted to be of highly significant

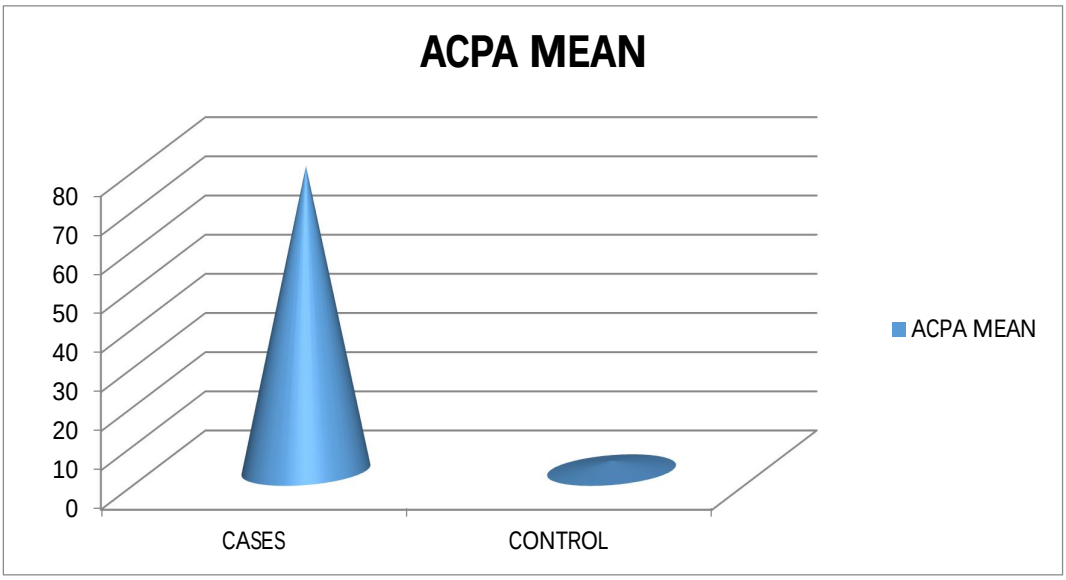


TABLE-6 : Shows the comparison of 14-3-3 eta protein between the cases and control

14-3-3 ETA	CASE		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**
	675.45	468.51	191.11	83
t-value	7.77			
P-value	0			
Significant	Significant			

The mean value of 14-3-3 eta protein of cases was 675.45 and mean of control resulted to be of 191.11. the p value resulted to be of highly significant.

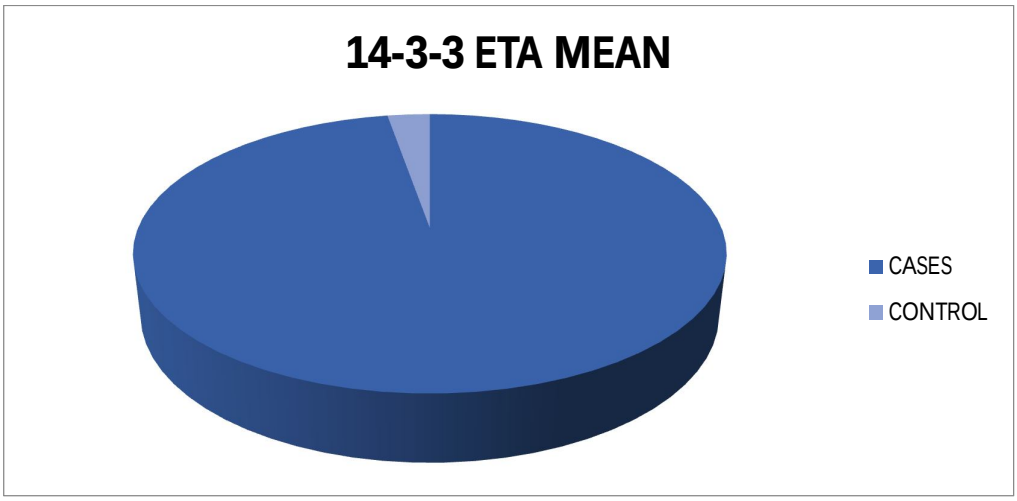


TABLE-7 : Shows the comparison of haemoglobin values between cases and control

HB	CASE		CONTROL	
	Mean	Sd	Mean	Sd
	10.34	1.88	9.18	0.91
t-value	1.23			
P-value	0.22			
Significant	Not significant			

There is no significant difference between the haemoglobin values of cases and control.

TABLE-8 : Shows the comparison of total count between cases and control

Total count	CASE		CONTROL	
	Mean	Sd	Mean	Sd
	8848.33	2267.79	5560	890.79
t-value	9.82			
P-value	0.000			
Significant	Significant			

The total count resulted to show highly significant difference between the cases and the control.

TABLE-9 : Shows the uric acid comparison between the cases and control

URIC ACID	CASE		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**
	2.82	0.57	2.89	0.52
t-value	0.54			
P-value	0.59			
Significant	Not significant			

There is no significant difference in uric acid between the cases and control.

There are significant differences in the values between cases and controls in the following parameters:

1. RF
2. ESR
3. CRP
4. ACPA
5. 14-3-3
6. TOTAL COUNT

Among these parameters total count , esr and crp are not specific markers for rheumatoid arthritis.

They are elevated in all inflammatory conditions and difficult to isolate the cause. Rheumatoid factor specific for rheumatoid arthritis. It is a marker showing autoimmune role in the disease. Rheumatoid factor leads to cytokine activation

and activation of other inflammatory factors and also leads to bone erosion. ACPA also a specific marker for rheumatoid arthritis.

14-3-3 eta protein which is a chaperone protein gets released during inflammatory signals and causes matrix metallo proteins to be increased and causes bone destruction.

TABLE 10: Shows the comparison of deformities between RF positive cases and RF negative cases

DEFRMITY HISTORY	RF positive		RF NEGATIVE	
	Number	**Percentage**	**Number**	**Percentage**
NO	19	59.4	25	78.1
YES	11	34.4	5	15.6
TOTAL	**30**	**100**	**30**	**100**
Chi-square			32.77	
p-value			0.0001	
Significant			Significant	

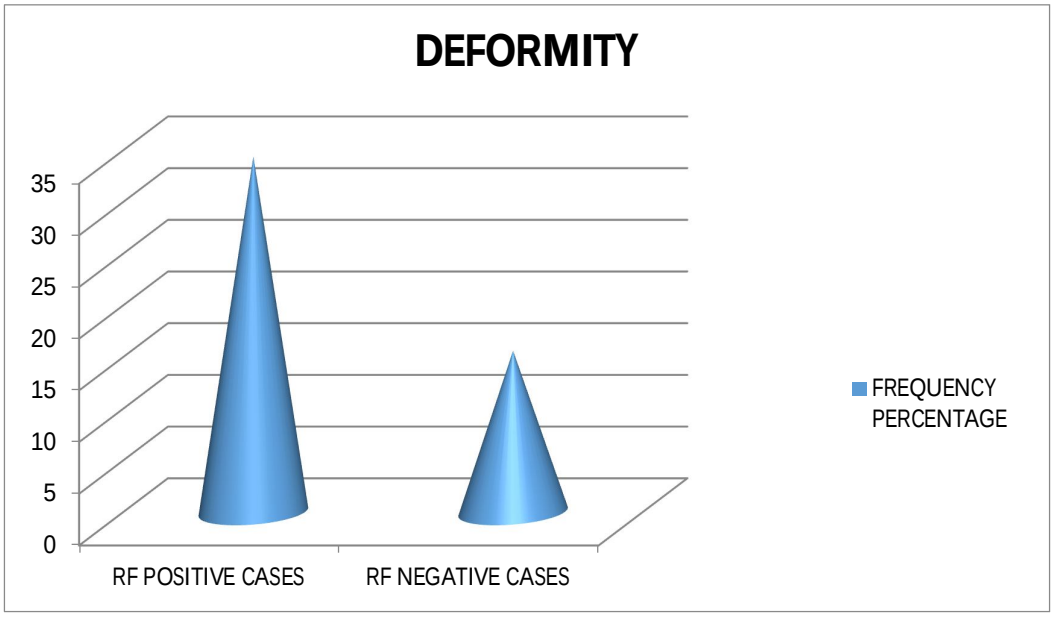
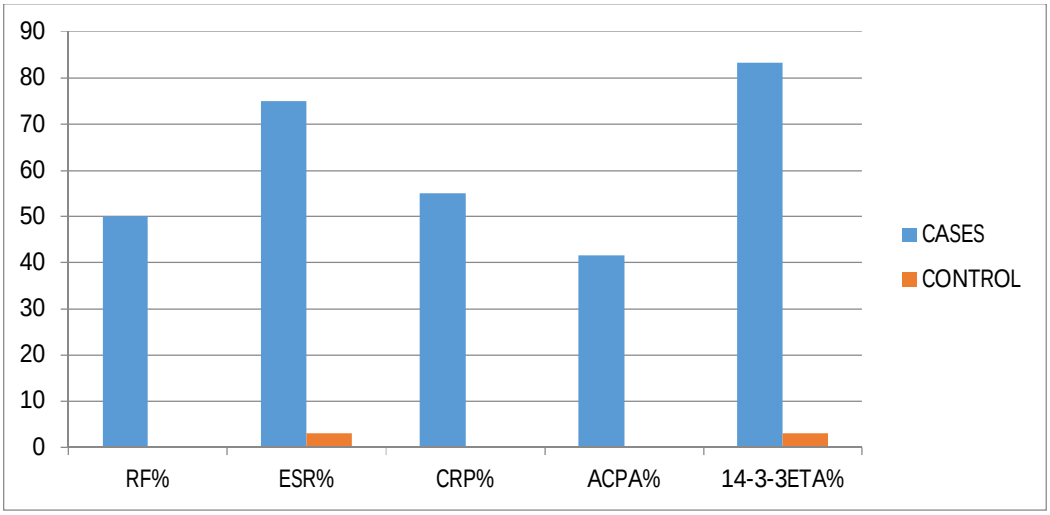


TABLE 11: Overall view of the analyte comparison between the cases and control

	CASES	CONTROL
RF%	50	0
ESR%	75	3
CRP%	55	0
ACPA%	41.6	0
14-3-3ETA%	83.3	3

The following table shows the positivity percentage of various analyte between the cases and control.

- 14-3-3 eta protein shows a higher percentage of positivity in cases and can be considered as a good marker to identify more cases
- ESR also determines more percentage of cases. But not specific to rheumatoid arthritis. ESR is marker for all inflammatory conditions.
- CRP also not a specific marker for rheumatoid arthritis. CRP increased in all inflammatory conditions
- ACPA and RF are specific for rheumatoid arthritis but not sensitive. The percentage of positivity among the cases is low



THREE GROUP COMPARISON

The study population were divided into three groups as follows:

Cases were classified into 2 groups. Patients were divided according to RF positive cases and RF negative cases.

RF positive cases - 30

RF negative cases - 30

Healthy control - 30

TABLE 12: Shows the comparison of RF value between the RF positive cases, RF negative cases and control

RF	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
	258.46	161.56	5.16	1.83	3.8	1.93
F-value			74.12			
P-value			0.0001			
Significant			SIGNIFICANT			

There is significant difference between the RF values between the three groups

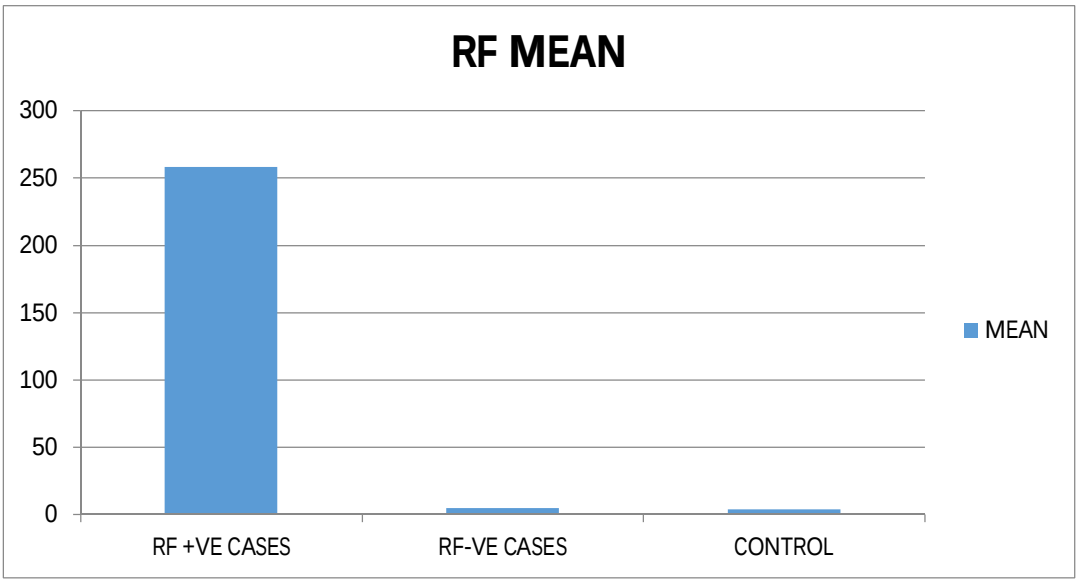


TABLE 13:

Shows the comparison between the ESR values of RF positive cases, RF negative cases and healthy control

ESR	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
	40.2	26.42	51.4	28.84	14.46	3.49
F-value			20.92			
P-value			0			
Significant			SIGNIFICANT			

There is significant difference between the ESR values of the three groups

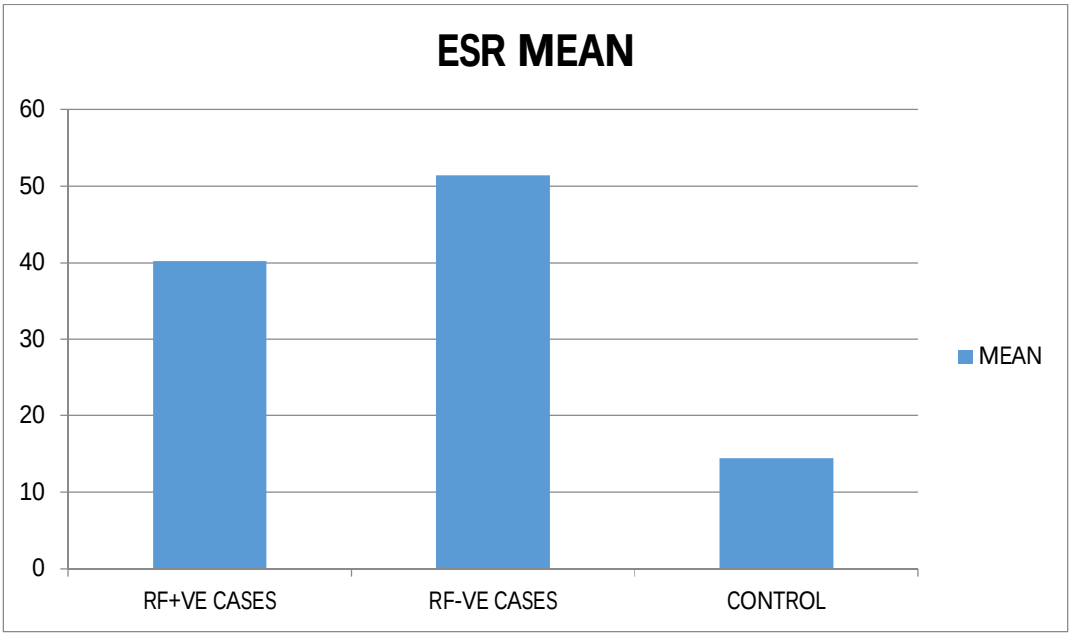


TABLE-14 : Shows the comparison of CRP values between the two groups

CRP	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	Sd	Mean	Sd	Mean	Sd
	13.7	13.59	14.1	12.99	4.8	0.84
F-value			7.02			
P-value			0.0001			
Significant			SIGNIFICANT			

There is significant difference between the CRP values of three different groups

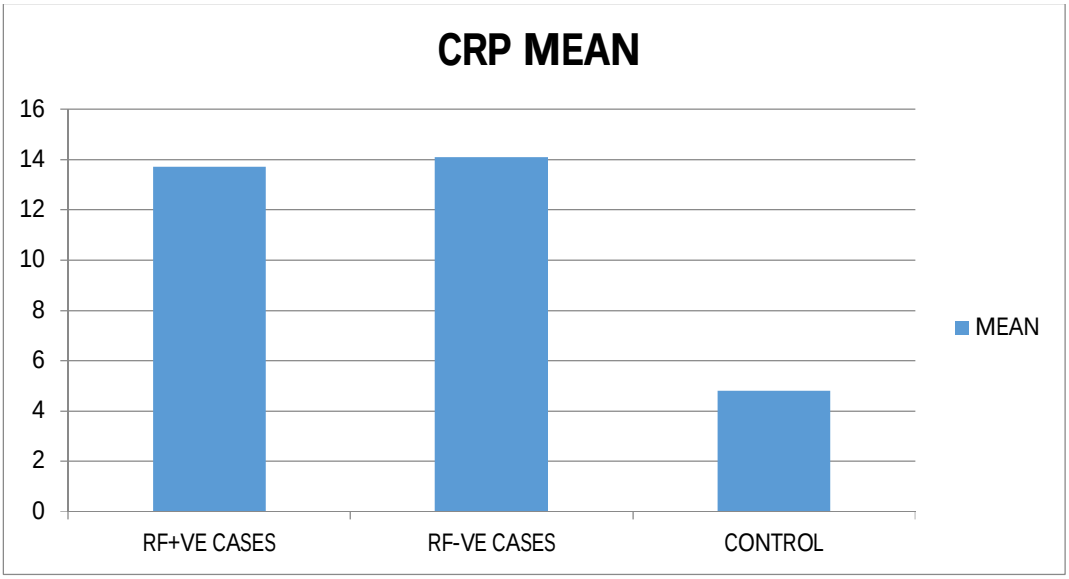


TABLE-15 : Shows the comparison of ACPA values between the three groups

ACPA	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	Sd	Mean	Sd	Mean	Sd
	142.86	165.88	12.46	27.41	2.43	1.25
F-value			19.54			
P-value			0.0001			
Significant			SIGNIFICANT			

There is significant difference between the ACPA values of the three groups

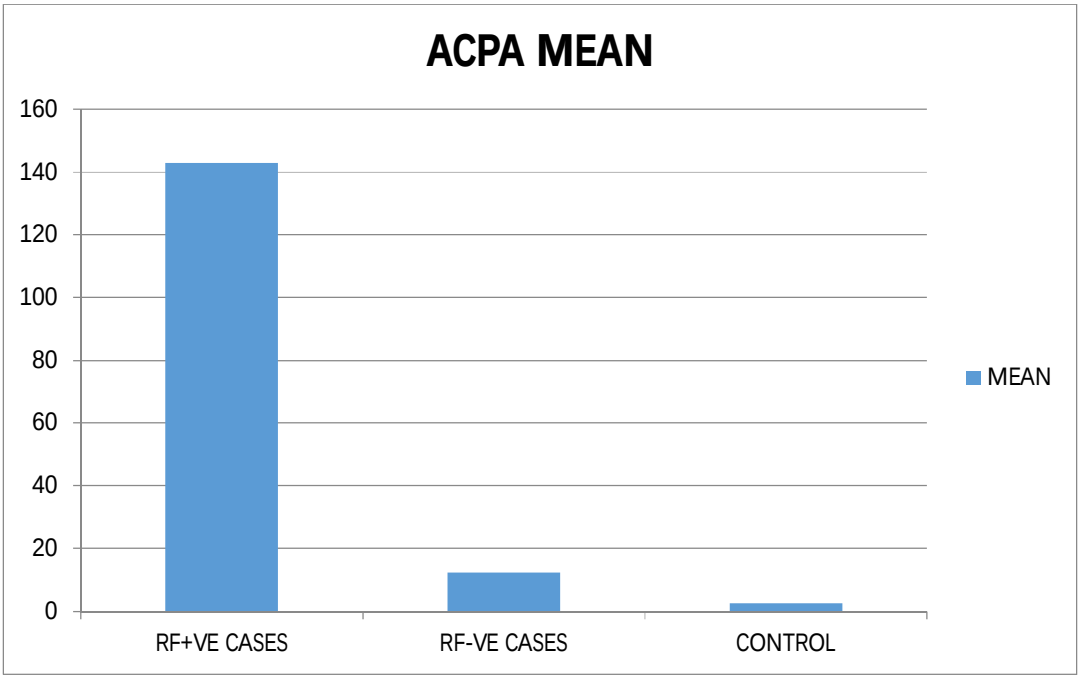


TABLE-16 :Shows the comparison of 14-3-3 eta values between the three groups

14-3-3 ETA	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
	952.21	521.16	398.68	128.41	191.11	83
F-value			47.23			
P-value			0.0001			
Significant			SIGNIFICANT			

There is significant difference between the 14-3-3 eta values between the three groups.

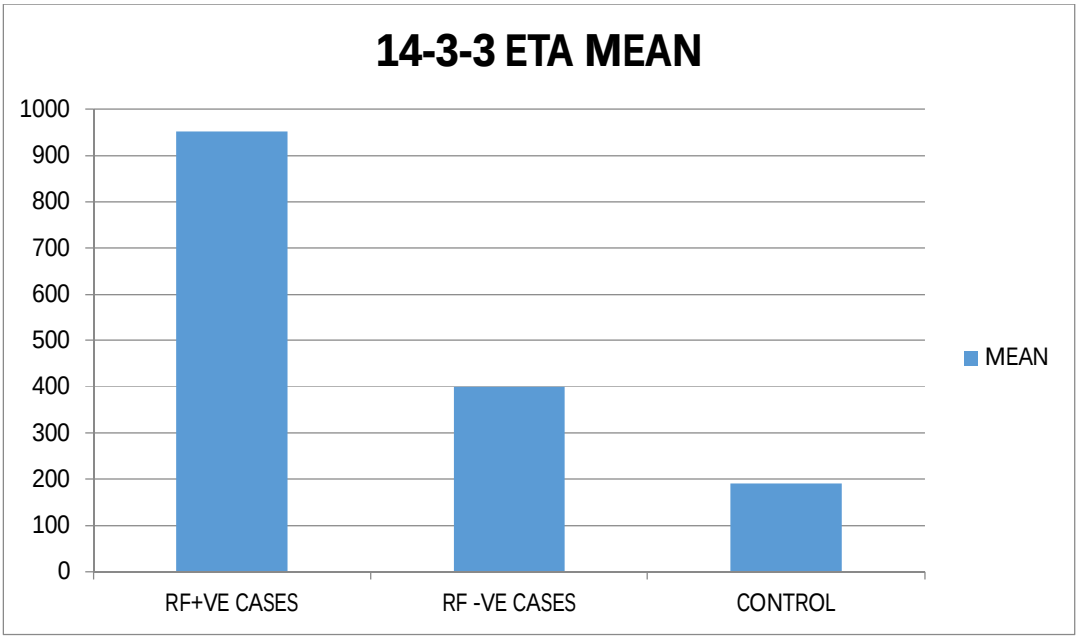


TABLE-17 : Shows the comparison of haemoglobin between the three groups

HB	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
	9.91	2.11	10.77	1.52	9.89	0.91
F-value			2.91			
P-value			0.06			
Significant			NOT SIGNIFICANT			

There is no significant difference between the haemoglobin of three groups

TABLE-18: Shows the comparison of total count between the three groups

Total count	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
	8763.3	1886.33	8933.33	2624.85	5560	890.79
F-value			28.91			
P-value			0.0001			
Significant			SIGNIFICANT			

There is significant difference between the total count values between the three groups

TABLE-19: Shows the comparison between the uric acid value of three groups

URIC ACID	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	Mean	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
	2.77	0.65	2.87	0.48	2.89	0.52
F-value			0.38			
P-value			0.68			
Significant			NOT SIGNIFICANT			

There is no significant difference between the uric acid values of the three groups.

CORRELATION STUDIES BETWEEN THE PARAMETERS

TABLE-20: Correlation with RF

	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	r	**P-value**	**r**	**P-value**	**r**	**P-value**
14-3-3 eta	0.935**	0	0.23	0.23	-0.01	0.96
ACPA	0.951**	0	-0.14	0.48	0.21	0.27
ESR	-0.03	0.89	-0.11	0.57	-0.27	0.15
TOTAL COUNT	0.02	0.93	-0.16	0.39	0	0.99
CRP	0.11	0.56	0.36	0.05	-0.11	0.57

Note : ** Correlation is Significant at 0.01 * Correlation is Significant at 0.05

There is a highly significant positive correlation between

- RF value and14-3-3 eta protein value in the RF positive cases and there is no correlation between the two in other two groups
- RF value and ACPA value in the RF positive cases and there is no correlation between the two in other two groups

TABLE-21: Correlation with 14-3-3 eta

ACPA	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	R	P-value	r	P-value	r	P-value
	0.983**	0	0.03	0.86	0	0.98
ESR	-0.01	0.95	0.02	0.9	0.28	0.13
TOTAL COUNT	0	1	-0.25	0.18	-0.17	0.37
CRP	0.17	0.37	0.17	0.36	-0.18	0.34

Note : Correlation is Significant at 0.01 * Correlation is Significant at 0.05**

There is a highly significant correlation between

- 14-3-3 eta protein and ACPA values in the RF positive cases group and there is no correlation between the two other groups
- There is no other correlation between the 14-3-3 eta protein and other parameters.

TABLE-22: Correlation with ACPA

ESR	RF POSITIVE CASES		RF NEGATIVE CASES		CONTROL	
	r	P-value	r	P-value	r	P-value
	0	0.99	-0.09	0.63	0.28	0.14
TOTAL COUNT	0.06	0.74	-0.12	0.52	-0.1	0.59
CRP	0.21	0.27	-0.05	0.79	0.05	0.79

There is no correlation between ACPA and other parameters

TABLE-23: Correlation with disease duration

14-3-3 ETA	RF POSITIVE CASES		RF NEGATIVE CASES	
	r	**P-value**	**r**	**P-value**
	0.643**	0	-0.77	0.69
RF	0.588**	0	-0.35	0.06
ACPA	0.663**	0	-0.17	0.38
ESR	-0.3	0.1	0.33	0.07
CRP	0.08	0.67	-0.09	0.64
TOTAL COUNT	-0.05	0.81	0.15	0.44

There is a highly significant positive correlation between

- Disease duration and 14-3-3 eta protein values in the RF positive cases
- Disease duration and RF value in RF positive cases
- Disease duration and ACPA values in RF positive cases

STEPWISE LINEAR REGRESSION ANALYSIS

TABLE 24:

Correlations							
		VAR00010	O1433	RF	CRP	ACPA	ESR
Pearson Correlation	VAR00010	1.000	-.513	-.400	-.373	-.309	-.544
	O1433	-.513	1.000	.931	.277	.930	.194
	RF	-.400	.931	1.000	.186	.915	.092
	CRP	-.373	.277	.186	1.000	.219	.221
	ACPA	-.309	.930	.915	.219	1.000	.084
	ESR	-.544	.194	.092	.221	.084	1.000
Sig. (1-tailed)	VAR00010	.	.000	.000	.000	.002	.000
	O1433	.000	.	.000	.004	.000	.034
	RF	.000	.000	.	.040	.000	.193
	CRP	.000	.004	.040	.	.019	.018
	ACPA	.002	.000	.000	.019	.	.217
	ESR	.000	.034	.193	.018	.217	.
N	VAR00010	90	90	90	90	90	90
	O1433	90	90	90	90	90	90
	RF	90	90	90	90	90	90
	CRP	90	90	90	90	90	90
	ACPA	90	90	90	90	90	90
	ESR	90	90	90	90	90	90

RF, CRP, ACPA, ESR and 14-3-3 eta protein shows significant predictors of the disease

Model Summary[b]										
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.781[a]	.610	.586	.30485	.610	26.243	5	84	.000	.986
a. Predictors: (Constant), ESR, ACPA, CRP, RF, O1433										
b. Dependent Variable: VAR00010										

ESR, ACPA, CRP, RF and 14-3-3 eta protein are considered to be be independent predictors of disease

ANOVA[a]						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	12.194	5	2.439	26.243	.000[b]
	Residual	7.806	84	.093		
	Total	20.000	89			
a. Dependent Variable: VAR00010						
b. Predictors: (Constant), ESR, ACPA, CRP, RF, O1433						

From the above table from spss software the individual predictors of the disease are found to be ESR, ACPA, CRP, RF and 14-3-3. Step wise regression analysis is done to predict whether the markers of interest can be used as an individual marker for diagnosing the disease or used for prognostic tool. It is based on correlation factors to determine the significance of the individual markers and then the predictor characteristics are determined by significance by ANNOVA.

ROC FOR 14-3-3 ETA PROTEIN

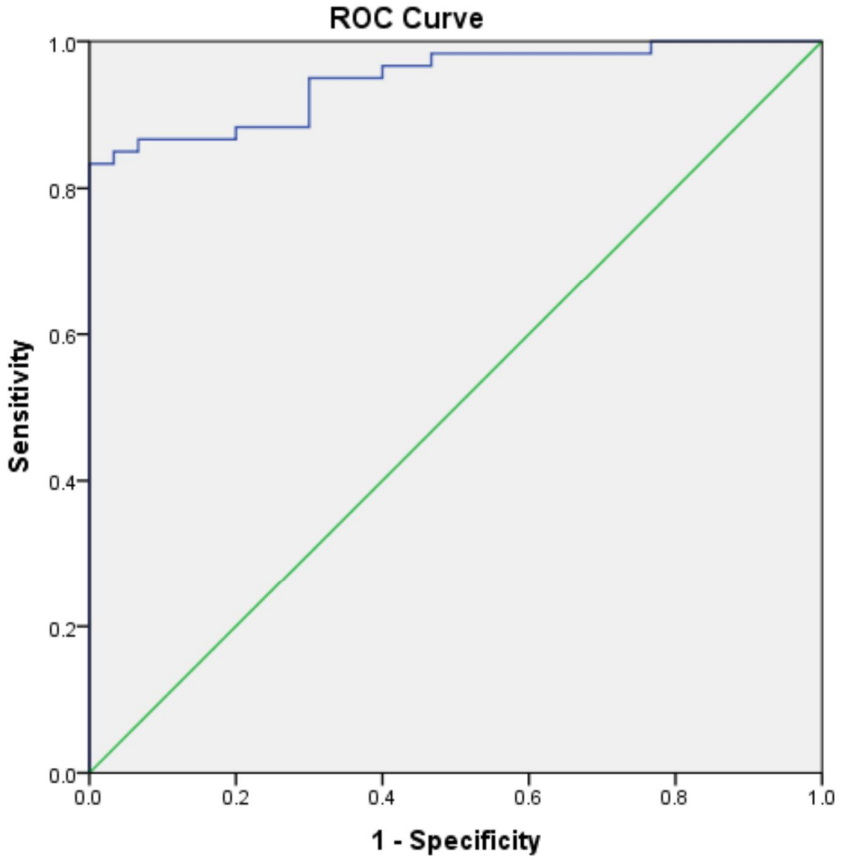
Area Under the Curve

Test Result Variable(s): O1433

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.948	.021	.000	.906	.989

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5



Coordinates of the Curve		
Test Result Variable(s): O1433		
Positive if Greater Than or Equal To[a]	**Sensitivity**	**1 - Specificity**
35.0000	1.000	1.000
37.0000	1.000	.967
41.0000	1.000	.933
49.0000	1.000	.900
55.0000	1.000	.867
105.0000	1.000	.833
154.5000	1.000	.800
157.6500	1.000	.767
162.6500	.983	.767
165.5000	.983	.733
167.0000	.983	.700
171.0000	.983	.667
175.5000	.983	.633
177.5000	.983	.600
179.0000	.983	.567
183.0000	.983	.500
198.0000	.983	.467
215.0000	.967	.467
220.4500	.967	.400
220.9500	.950	.400
222.0000	.950	.367
224.0000	.950	.333
227.5000	.950	.300
230.2500	.933	.300
232.0000	.917	.300
235.0500	.900	.300
243.3000	.883	.300
252.0000	.883	.233
255.1500	.883	.200
258.6500	.867	.200
270.5000	.867	.133
280.5000	.867	.100
285.8000	.867	.067
293.3000	.850	.067
309.8500	.850	.033
352.0000	.833	.033
381.5000	.833	.000
384.2000	.817	.000

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
388.8000	.800	.000
397.1500	.783	.000
406.2000	.767	.000
423.8000	.750	.000
438.9500	.733	.000
442.9000	.717	.000
453.8500	.700	.000
464.7500	.683	.000
476.4500	.667	.000
487.2500	.650	.000
489.7000	.633	.000
495.8500	.617	.000
502.9000	.600	.000
506.6500	.583	.000
511.4000	.567	.000
516.2000	.550	.000
523.2500	.533	.000
531.9000	.517	.000
536.5500	.500	.000
540.9500	.483	.000
546.4000	.467	.000
549.9500	.450	.000
557.5500	.433	.000
570.4500	.417	.000
588.8500	.383	.000
606.9500	.367	.000
622.4000	.350	.000
633.6500	.333	.000
646.7000	.317	.000
662.0000	.300	.000
667.1000	.283	.000
673.1000	.267	.000
690.6000	.250	.000
709.2000	.233	.000
729.2000	.217	.000
744.5000	.200	.000
791.2000	.183	.000
900.2500	.167	.000
977.0000	.150	.000
1155.2500	.133	.000

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
1443.0000	.117	.000
1649.0000	.100	.000
1755.5000	.083	.000
1828.5000	.067	.000
1889.6500	.050	.000
1905.6500	.033	.000
1954.1500	.017	.000
1998.3000	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

from the ROC analysis it has been determined that 381.5 value is the cut off to diagnose more number of true positive cases and do not include persons are not classified as diseased who are not actually diseased. Thus the cut off value determines good sensitivity and specificity.

Discussion

DISCUSSION

Rheumatoid arthritis is a chronic autoimmune inflammatory joint disorder affecting multiple joints. The disease progression leads to bone erosion and disability[96,97]. The disease pathogenesis is multifactorial. The recent advances in the treatment of rheumatoid arthritis have developed drugs which modify the disease progression such as DMARD's. Intensive treatment of the patients have shown significant improvement in the disease. With proper treatment the disease progression can be halted and the deformity can be prevented which leads to a life long disability.

Even after centuries the diagnosis of rheumatoid arthritis remain a challenging to the physicians. The specific and sensitive markers are not satisfying the identification of patients because of the prevalence of significant percentage of sero negative patients[98].

Diagnosis is based on a combination of clinical symptoms, clinical findings and laboratory investigations. Among the current investigation panel RF is a sensitive marker to identify more number of cases whereas anti CCP is a specific marker[99,100] to identify rheumatoid arthritis patients.

The lack of sensitivity and specificity of the present diagnostic panel of parameters have lead to the research of newer biomarkers to add to the present panel to identify more cases and halt the progression of the disease and prevent the disability to greater extend.

Hence this study has been undergone to determine the effectiveness of the newer biomarker 14-3-3 eta protein to diagnose more number of patients at a earlier stage and halt the progression of the disease course. 14-3-3 eta protein levels are more correlated to the matrix metallo proteinases which are markers of bone destruction process. The synovial fluid determination of 14-3-3 eta protein are more specific than the serum. As it is an invasive procedure. Serum levels of 14-3-3 eta protein have been determined in this study which has been previously determined by many studies across the world.

In this study the study population is divided into 3 groups

Group 1: RF positive patients

Group 2: RF negative patients

Group 3 : healthy controls

RF:

RF being a sensitive marker. The mean of RF was compared between the cases and controls and later with the 3 separate groups.

The mean RF of cases(131.81) and controls(3.8) and they were significantly different($p<0.01$)

The mean RF of positive cases(258.46) and negative cases(5.16) and controls (3.8) and were significantly different between the groups($p<0.05$)

There are several studies supporting RF as a sensitive marker in the diagnosis of rheumatoid arthritis[101].

ACPA:

ACPA is a specific marker in the diagnosis of rheumatoid arthritis and was compared among the groups

The mean ACPA of cases(77.6) and controls(2.43) and was significantly different($p<0.05$)

The mean ACPA of positive cases(142.86) and negative cases(12.46) and controls(2.43) and were significantly different among the groups($p<0.05$)

ACPA is considered as a specific marker and one among the diagnostic panel of rheumatoid arthritis.

ESR:

ESR is an inflammatory marker and not specific to rheumatoid arthritis but has been used as one among the markers in the diagnostic panel.

The mean value between the cases and the control showed significant difference ($p<0.05$)

There were also significant difference among the positive cases, negative cases and control groups ($p<0.05$).

ESR is a non specific inflammatory marker.

CRP:

CRP is also a non specific inflammatory marker which is increased in rheumatoid arthritis. The CRP value shows statistically significant difference among cases and controls and also among the positive cases, negative cases and controls ($p<0.05$)

14-3-3 eta protein:

The mean value of 14-3-3 eta protein of cases(675.45) and controls(191.11) resulted to significantly different($p<0.05$)

The mean value of positive cases(952.21), negative cases(398.68) and controls(191.11) also resulted to be statistically significant($p<0.05$).

14-3-3 eta protein is a chaperone whose expression[103] causes inflammatory mediators to recruit and causes release of matrix metalloproteinases and destruction of the bone joint. The levels of the new marker 14-3-3 can be equated to the bone destruction. Thus can be used as a marker for deformity. And it can also be used as an early marker of the joint damage and can be used to prevent the progression of the disease.

There are also animal studies done on dogs demonstrating the high levels of 14-3-3 eta protein in the synovial fluid in the dogs with ligament tear[104]. In the animal studies the 14-3-3 eta levels are compared with the matrix metalloproteinases and resulted to have a positive correlation between the both parameters.

CORRELATION STUDIES:

Correlation studies are done to establish relationship between the parameters diagnosing the disease

- Positive correlation is one in which both the parameters increase or decrease simulataneously and the statistical significance is calculated
- Negative correlation is one in which both parameters show difference in the opposite direction. For example for the increase in one parameter the other parameter shows significant difference

Correlation studies are done using SPSS software using pearson correlation.

In my study the various parameters are analysed to determine its relation between one another

Rheumatoid factor which is used as a diagnostic marker for rheumatoid arthritis was compared with other analytes in my study and resulted to have

- Positive correlation with 14-3-3 eta protein of RF positive patients which is highly significant correlation
- Positive correlation with ACPA of RF positive patients which is also highly significant

The new emerging marker 14-3-3 eta protein was compared with the existing markers and resulted to have

- Positive correlation with ACPA of RF positive patients and which is highly significant correlation.

To know the severity of the disease the marker should have the positive correlation with the duration of the disease so that with the level of the markers can be used as a severity marker of the disease whom are highly prone for complications like joint damage and disability.

- 14-3-3 eta protein have a positive correlation with the disease duration among the RF positive patients
- RF also has a positive correlation with the disease duration among the RF positive patients.

STEPWISE REGRESSION ANALYSIS:

Step wise regression analysis is done to determine the efficacy of individual markers as predictors of the disease.

Done using spss software using data of the study. It has been resulted that

- ESR
- CRP
- ACPA
- RF
- 14-3-3 eta

can be used as independent predictors of the disease. But ESR and CRP are not specific markers. Both are inflammatory markers which can be elevated in any inflammatory conditions cannot be used as independent markers.

RF is a sensitive marker but not specific and not all the patients present with high RF values. This leads to miss cases to be diagnosed at an earlier stage and leads to deformity development

ACPA is a specific marker of the disease and can be used as a independent marker but the sensitivity is low.

14-3-3 eta protein has diagnosed 83.3% of cases in our study and with step wise regression analysis has also been proved that can be used as an independent marker. Thus the emerging new marker 14-3-3 eta protein can be used in the routine diagnosis of the disease. The combination of 14-3-3 eta protein along with the existing markers can be used to diagnose more number of cases at an early stage and with the use of current broad spectrum of drugs rheumatoid arthritis can be halted from progression to deformity and disability.

ROC determination for 14-3-3 eta protein:

ROC is done to determine the sensitivity and specificity of the marker. And it has been determined that with the cut off value of 381.5, the newer marker has good sensitivity and specifity.

Conclusion

CONCLUSION

Rheumatoid arthritis is a chronic autoimmune disorder inflammatory disorder involving joints. It has also extra articular manifestations. This study was done to determine the role of 14-3-3 eta protein in the diagnosis of rheumatoid arthritis

- The new marker diagnosed 83.3% of patients
- There was significant difference in the values among the patients and the controls
- There was positive correlation with existing marker rheumatoid factor
- There was also positive correlation with the disease duration and hence can be used to asses the severity of the disease
- The study also proved that 14-3-3 eta protein can be used as an independent marker
- And the marker has a good sensitivity and specificity.

Thus our study has proved the role of 14-3-3 eta protein in rheumatoid arthritis and have determined its significance which can be used along with other markers to diagnose more number of cases at an early stage and halt the disease progression.

Limitation of the study

LIMITATIONS OF THE STUDY

- 14-3-3 eta protein is determined by ELISA procedure which is not a gold standard method of determination.
- Its role has not been determined whether increased in all other inflammatory diseases.
- Its role should be determined in other arthritis like osteoarthritis, psoariatic arthritis, and other inflammatory arthritis.

Scope for further studies

FUTURE PROSPECTS OF THE STUDY

- 14-3-3 eta protein determination can be used as a early marker for diagnosis.
- Even before the progression of the disease to deformity
- Its role has to be established using follow up studies to determine its use to identify patients even before the clinical arthritis.
- There are studies done to determine its role as a follow up study[105]. In this study patients with high levels of 14-3-3 eta protein with arthralgia developed arthritis in a median period of 15 years.
- Thus can be used as an early marker even before the onset of clinical arthritis

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98. Does 14-3-3 ETA Protein Offer Any Additional Diagnostic Value in Rheumatoid Arthritis? Display combined fields only if separate fields don't exist Andrew Vasconcellos1, Seema Chittalae2 and Petros Efthimiou3, 1Methodist, New York Methodist Hospital, Brooklyn, NY, 2Medicine, New York Methodist Hospital, Brooklyn, NY, 3Med/Rheumatology, New York Methodist Hospital/Weill Cornell Medical College Affiliate, Brooklyn, NY

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104. Increased levels of the 14-3-3 h and g proteins in the synovial fluid of dogs with unilateral cranial cruciate ligament rupture Kamran Sardari, Claudia Chavez-Muñoz, Ruhangiz T. Kilani, Terri Schiller, Aziz Ghahary

105. A prospective cohort study of 14-3-3η in ACPA and/ or RF- positive patients with arthralgia. Van Beers-Tas MH, Marotta A, Beers M, Maksynwych WP, van Schaardenburg D.

Annexures

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. T. Poornima
IInd year, PG Degree- M.D Biochemistry
Institute of Biochemistry
Madras Medical College, Chennai

Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY PN 14-3-3η LEVELS IN RHEUMATOID ARTHRITIS " NO.08092016 .**

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

1. Prof. C. Rajendran, MD.	Chairperson
2. Prof. Dr. M.K. Muralidharan, M.S, M.Ch., MMC ,Ch-3	Deputy Chairperson
3. Prof. Sudha Seshayyan, MD., Vice Principal, MMC.Ch- 3.	Member Secretary
4. Prof. B.Vasanthi,MD.,Prof of Pharmacology, MMC,	Member
5. Prof. P.Raghumani.MS., Professor of Surgery, Inst. of surgery	Member
6. Prof. R.Padmavathy,MD., Professor, Inst.of Pathology, MMC,Ch	Member
7. Tmt.J.Rajalakshmi, Junior Administrative Officer,MMC,Ch	Layperson
8. Thiru.S.Govindasamy., B.A.B.L., High Court, Chennai-1	Lawyer
9. Tmt.ArnoldSaulina, MA., 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

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07 SEP 2016

URKUND

Urkund Analysis Result

Analysed Document: mywork (Autosaved) (Autosaved).docx (D30403803)
Submitted: 9/6/2017 4:57:00 PM
Submitted By: poornimathangarajan@gmail.com
Significance: 0 %

Sources included in the report:

Instances where selected sources appear:

0

PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled **"A STUDY ON 14-3-3η LEVELS IN RHEUMATOID ARTHRITIS"** of the candidate **DR.T. POORNIMA** with registration Number **201523003** for the award of **M.D** in the branch of **BIOCHEMISTRY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **0 percentage** of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

PROFORMA

Date: Sample Id : _____

Name: Age : Sex :

Occupation:

Relevant History:

Duration of illness: Severity:

Hospitalisation for similar complaints:

Joint involvement: Physical activity:

Diet: Drug history:

Examination:

Joints(for crepitations ,swelling, restriction of movements):

BMI:

Diagnosis:

Investigations:

Rheumatoid Arthritis Factor: CRP:

ESR: Uric acid:

Complete blood count:

Xray findings:

14-3-3η:

INFORMATION SHEET

- Your blood sample has been accepted.
- We are conducting a study on patients with diagnosed Rheumatoid arthritisatRajiv Gandhi Government General Hospital, Chennai and for that your blood sample may be valuable to us.
- The purpose of this study is to measure the level of 14-3-3ηprotein in Rheumatoid arthritis patients and compare with healthy individuals.
- We are selecting certain cases and if your blood sample is found eligible, we may be using your blood sample to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

PATIENT CONSENT FORM

Title of the study : **"A STUDY ON 14-3-3η LEVELS IN RHEUMATOID ARTHRITIS"**

Name : Date :
Age : OP/IP No :

Sex : Project Patient No :

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree to use my personal clinical history&investigation details for the purpose of the study.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

Having understood _____s/o_____ give my consent to participate in the study conducted by DR.T.POORNIMA, Post graduate, Institute of Biochemistry, Madras Medical College, Chennai.

Signature of the investigator: Signature of the participant:

Place: Thumb impression.

Date:

நோயாளியின் ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு: **¬ hUSÁõu® ÷{õ¯õ Î P Î ß CµzuzvÀ 14&3&3 ChõGÝ® ¦µuzvß AÍÄ£ØÔ¯©¸zxÁ B´Ä.**

பெயர் : தேதி :

வயது : புறநோயாளிஎண்: பால் :

ஆராய்ச்சி சேர்க்கை எண் :

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும், அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்நான் "**¬ hUSÁõu® ÷{õ¯õ Î P Î ß CµzuzvÀ 14&3&3 ChõGÝ® ¦µuzvß AÍÄ£ØÔ¯©¸zxÁ B´Ä**" என்ற தலைப்பில் மேற்கொள்ளப்படும் இந்த ஆராய்ச்சியின் விபரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்து கொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்

தேதி கையொப்பம்

ஆராய்ச்சி தகவல் தாள்.

தங்களது இரத்தம் இங்குபெற்றுக்கொள்ளப்பட்டது.

சென்னை அரசு பொது மருத்துவமனையில் ¬ hUSÁõu® ÷{õ¯õ Î P Î ß CµzuzvÀ 14&3&3 Chõ GÝ® ¦µuzvß AÍÄ £ØÔ¯ ©¸zxÁ B´Ä" என்ற தலைப்பில் ஆராய்ச்சி நடைபெற்று வருகின்றது.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்பிகிறோம்.

இந்தஆராய்ச்சியில் உங்களுடையஇரத்தம்எடுத்து சிறப்புப்பரி சோதனைக்குஉட்படுத்திஅதன்தகவல்களைஆராய்வோம்.

அதனால் தங்களது நோயின்ஆய்வறிக்கையோஅல்லது சிகிச் சையோபாதிப்புக்குஉள்ளாகாதுஎன்பதையும்தெரிவித்துக்கொள் கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறோம்

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது.மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துகொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

தேதி: