SERUM PROLACTIN LEVELS IN PATIENTS WITH RECENTLY DIAGNOSED RHEUMATOID ARTHRITIS

Dissertation submitted for

M.D. BIOCHEMISTRY BRANCH – XIII
DEGREE EXAMINATION

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI – 600 032

TAMILNADU

APRIL 2015

BONAFIDE CERTIFICATE

This to certify that this dissertation work entitled "**SERUM PROLACTIN LEVELS IN PATIENTS WITH RECENTLY DIAGNOSE RHEUMATOID ARTHRITIS"** is the original bonafide work done by**Dr.M.KARTHIGA**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.

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SPECIAL ACKNOWLEDGEMENT

The author gratefully acknowledges and sincerely thanks Professor **Dr.R.Vimala, M.D.,** Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, for granting her permission to utilize the facilities of this Institution for the study.

ACKNOWLEDGEMENT

The author expresses her warmest respects and profound gratitude to Dr.K.Ramadevi, M.D., Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her academic enthusiasm and for facilitating her research work in the institute.

The author expresses her heartfelt gratitude to her guide and supervisor Dr.V.K. RAMADESIKAN, M.D., Professor, Institute of Biochemistry, Madras Medical College, Chennai, for his intellectual and valuable guidance, unfailing support, encouragement and continuous inspiration throughout the period of her study.

The author in particular, is extremely thankful to Dr.RAJESWARI,MD,DM, Professor and Head of the Department of Rheumatology, Government General Hospital,Chennai, for granting permission to obtain blood samples from the patients.

The author expresses her thanks to the Professors Dr.R.ChitraaM.D, Dr.K.Pramila M.D Dr.V.Amuthavalli M.D , and Dr.Periyandavar M.D., Institute of biochemistry, Madras Medical College, for their guidance, encouragement, insightful comments and suggestions.

The author expresses her warm respects and sincere thanks to her co-guide. Dr.V.G. Karpaghavalli M.D Assistant Professor, Institute of biochemistry, Madras Medical College for her guidance and support. The author expresses her warm respects and sincere thanks to other Assistant Professors, Dr.C.Shanmugapriya, Dr.Poonguzhali Gopinath, Dr.C.Mythili, Dr.V.Ananthan, Dr.S.Siva, Dr.B.SudhaPresanna, Institute of biochemistry, Madras Medical College, for their valuable suggestions regarding the practical issues of research which is something beyond the textbooks.

The author expresses warm respects to the members of the Institutional Ethical committee for approving the study.

The author expresses her special thanks to Mr.K.Suresh and Mrs.Maragatham Non-medical assistant, Institute of biochemistry, for their timely co-operation and assistance during the ELISA technique.

The author expresses her special thanks to her co-PGs Dr.P.Deepa, Dr.R.Amirtha jansi rani and Dr.P.Renuka, for their constructive criticism and unconditional support. The author expresses her thanks to all her colleagues in the institute, for their constant encouragement through out the study period.

The author is grateful to the Statistician, Mr.K. Boopathi for his help in processing the data and statistical analysis.

The author is indebted to the patients and controls from whom blood samples were collected for conducting the study.

TheauthorexpressesherspecialthankstoalltheDMLTstudents,Mrs.Eswarilabtechnician,Rheumatology lab for their timely help and co-operation during sample collection.

Finally, the author expresses her special thanks to her family members for their moral support and encouragement .

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ABBREVIATIONS

1. AA – Amino acids.
2. PRL- prolactin
3. TNF α – Tumour necrosis factor
4. TLR- Toll like receptor
5. INF – Interferon gamma
6. IL – Interleukins
7. CD- cluster differentiation.
8. TRH – Thyroid releasing hormone
9. TSH – Thyroid stimulating hormone
10. RA- Rheumatoid arthritis
11. RF- Rheumatoid factor
12. Ig- Immunoglobulin
13. ACPA- Anti citrullinated protein antibodies.
14. Anti CCP- Anti cyclic citrullinated peptide
15. PADI- Peptidyl Arginine Deiminase
16. EBV- Epstein Barr Virus
17. VEGF- Vascular Endothelial Growth factor.
18. MMP- Matrix Metalloproteinase
19. bp – base pairs
20. cys-cysteine
21. KDa- Kilo Dalton
22. RBC- Red Blood Corpuscle.
23. PBMC- Peripheral Blood Mononuclear Cells.

24. GAS – Gamma Interferon Activated sequence

25. NK-Natural Killer cells.

26. JAk- for just another kinases/ Janus kinase

27. PRLR- prolactin receptor

28. STAT-Signal Transducers And Activators of Transcription.

SERUM PROLACTIN LEVELS IN PATIENTS WITH RECENTLY DIAGNOSED RHEUMATOID ARTHRITIS.

ABSTRACT

OBJECTIVES: The role of Prolactin in autoimmune diseases & use of anti Prolactin drugs in disease remission has been described & investigated in several studies. The role of anti-prolactin drugs in remission of disease activity have been investigated. The present study was carried out to correlate serum Prolactin levels with disease severity in recently diagnosed Rheumatoid Arthritis patients.

MATERIALS and METHODS: A case-control study was carried out in 55 recently diagnosed untreated Rheumatoid factor positive & RF negative Rheumatoid Arthritis patients and 27 age & sex matched apparently healthy individuals. The diagnosis of RA was made using ACR criteria 2010. Serum Prolactin(ELISA) ,serum Anti CCP(ELISA), RF (Latex agglutination method) , TSH(ELISA),ESR(conventional Westergrens method),Serum urea & creatinine were assayed. Disease severity was assessed by DAS(28) formula. Statistical evaluation was done by independent t-test,ANOVA, Pearson correlation co-efficient

RESULTS: Serum Prolactin levels in RA patients was significantly higher (33.53±17.9 ng/mL) compoared to controls (14.4±5.9) with p value of ▪ 0.001 . A fair correlation was found between disease activity and serum Prolactin levels (r = 0.345 ; p = 0.01).

CONCLUSION : Elevated levels of serum prolactin indicates the immunomodulatory role of PRL and its relationship to diseases activity. Use of Anti Prolactin drugs may be of use in patients with hyperprolactinemia.

Key words: Prolactin , autoimmune disease , rheumatoid arthritis

INTRODUCTION

Rheumatoid Arthritis is an autoimmune disease. Affects 1 to 2% of general population. As any other autoimmune disease it is commom in women .The male to female sex ratio is 2:1[1]. This female gender bias suggest ; female hormones play a role in pathogenesis. The hormones Estrogen and Prolactin are related. The pituitary expression of Prolactin is under the control of estradiol[2].

Role of Pituitary hormones in immune system modulation has been suggested right from 1930s.Prolactin one of the anterior pituitary hormones plays a major role in immune regulation .Many clinical ,invitro, invivo animal and clinical studies suggests Prolactin exhibits immunoregulatory properties[2].At times of stress Prolactin is needed to balance the effects of glucocorticoids and other inflammatory mediators[3]. Prolactin upregulates the expression of Th1 cytokines. Th1 cytokines are involved in autoimmune diseases [4,5].

Clinical datas suggest that altered Prolactin concentration in serum exacerbates certain Autoimmune diseases but a clear causal relationship is still lacking. Certain case reports show that administration of Bromocryptine , cabergoline in Rheumatoid Arthritis patients with elevated serum Prolactin cause disease remission[6]. But these evidences were not consistent . Hence the present study was carried out to determine the status of serum Prolactin in patients with recently diagnosed Rheumatoid arthritis.

REVIEW OF LITERATURE

RHEUMATOID ARTHRITIS

Rheumatoid arthritis , the most common chronic inflammatory disease affecting synovial lining , bursae & tendon sheath is a prototype disease entity for defining the molecular & pathological basis of chronic inflammatory syndrome. The term rheumatoid arthritis was coined by Garrod.[7]

EPIDEMIOLOGY:

0.5 to 1 % of the general population is affected. The prevalence is constant across the globe irrespective of race and geographical location[1].Onset is most frequent between 4^{th} and 5^{th} decades of life. 80% of individuals develop the disease between 35 and 50 years of age. Incidence in 60 year old women is 6 times greater when compared with 18 to 29 year old women[7]. The female to male ratio is 2:1 to 3:1.

RISK FACTORS:

1. AGE: Immune senescence occurs in old age. Immune senescence is also considered as a possibility of Autoimmune diseases . Immunesenesence is characterized at molecular and cellular level by massive expansions of lymphocyte clone, telomere erosion of leukocytes , corresponding contraction of naïve T & B cell repertoires. All these represents an extensive proliferative history.

Derangements in pathways integral to antigen responsiveness and immune regulation occurs in old age. These factors combine to

- Increase susceptibility to foreign pathogens.
- Auguments reactivity to self antigens.
- Generate a repertoire of lymphocytes defective in tumor surveillance.

Hence immune senescence in old age could be considered a risk factor in development of Autoimmune diseases.[7]

2. GENES IMPLICATED IN RHEUMATOID ARTHRITIS: [1]

About 35 genes are implicated in the pathogenesis of RA identified by genome wide screen .The most common genes are

- Genes of MHC Class II
- PTPN 22- Protein tyrosine phosphatase 22(a phosphatase that regulates phosphorylation status of many kinases responsible for T - cell activation)
- Cytokine promoter polymorphism
- Signal transduction gene polymorphism
- Population specific genes eg: PADI 4 in Japanese people .

3. HLA –DR:[1]

- HLA –DR is present in 70% of Rheumatoid arthritis patients.
- The 3rd hypervariable region of DR-β chain from aminoacid 70- 74 (QKRAA,Glutamine-leucine-arginine-alanine - alanine) comprises the disease susceptibile region.

- This susceptible epitope is associated with HLA –DR4β subtypes like DRB*0401,DRB*0404,DRB*0101,DRB*1402.

- These susceptible epitopes has a role in shaping the T- cell repertoire in thymus.

- They alter the intracellular HLA trafficking and antigen loading.

4. **MICROCHIMERISM** : [1]

Maternal cells expressing the susceptibile epitope (QKRAA) can persist in a child , these non inherited maternal antigens confers increased risk of disease in these children.

5. **SEX**: [1]

Autoantibody producing B cells exposed to estradiol are more resistant to apoptosis. It might escape peripheral tolerance. Hence autoimmune diseases are more common in women.

6. **HORMONAL FACTORS**: [1]

The first 3 months of Postpartum period, the disease activity is more in a Rheumatoid Arthritis patient .This is attributed to the hormonal changes during pregnancy especially Prolactin. Decreased androgen and increased estrogen status in males is associated with Rheumatoid arthritis. Extended periods of breast feeding is associated with increased risk of Rheumatoid Arthritis. This again is associated with elevated serum Prolactin.

7. **SMOKING**:[1]

Smoking 25 cigarettes per day for >20 years confers a 15 fold increased risk of Rheumatoid arthritis in subjects who carry disease associated HLA –DRB1 allelles.

8**.** **URBAN DWELLING**: Urban dwelling provokes Rheumatoid arthritis in a genetically prone individual.[1]

9. **PATHOGENS**:[7]

- Exposure of mucosal surfaces such as lungs, periodontium, gut to infectious antigens may induce RA in susceptible individuals.
- Antibodies to nuclear antigens of Epstein Barr virus is seen in Rheumatoid arthritis patients.
- Porphyromonas gingivalis – causative organism in periodonitis is capable of generating citrullinated proteins.
- Filamentous bacteria in gut induce T cells of lamina propria to generate IL 17 that induce inflammatory arthritis.

AUTO ANTIGENS IN RHEUMATOID ARTHRITIS : [7]

Rheumatoid arthritis is an autoimmune disease, antibodies are produced against self antigens. Some of the self antigens implicated in RA are as follows

Table I Proven Antigens in RA & the available assay: -

Antigen	Molecular specificity	Assay to identify
IgG	Human Fc fragment of IgG	Rheumatoid factor
Cyclic Peptides	Citrullinated peptides	Anti – CCP Ab
Vimentin	Citrullinated Vimentin	MCV assay Mutant citrullinated Vimentin

Table II Unproven Antigens in RA (under research) :

Antigen	Cell type	Molecular specificity
Glucose 6 phosphate isomerase	Both B& T	Multiple epitope
Enolase	B	CEP-1
Fibrin	B	Alpha & β chain epitopes
Fibrinogen	B &T	Multiple epitopes
Collagen II	B& T	Multiple epitopes
hn RNPA 2	B&T	Multiple epitopes
Aggrecan	B&T	Multiple epitopes
Hcgp-39	B&T	Multiple epitopes

PATHOGENESIS:

Although it is a well known fact that Autoimmunity plays a pivotal role in pathogenesis of RA ,pathogens do have a role in Pathogenesis of RA. Predominance of CD4+ T- cells in circulation and presence of IL2 in synovial fluid and blood of these patients indicate that Rheumatoid arthritis is a immunologically mediated event[7].No single specific pathogen for RA is unlikely .Some of the infectious agents found to be associated with RA are Mycoplasma , Parvo virus B19,Retrovirus ,enteric bacteria,mycobacteria,EBV, Bacterial cell wall.The mechanisms through which these agents cause RA are direct injury , molecular mimicry and activation of TLR.

TOLL LIKE RECEPTORS(TLR) and RHEUMATOID ARTHRITIS: [1]

- TLR are a part of innate immunity .These receptors are expressed by sentinel cells and they provide the first line of defence.
- These receptors recognize constitutively preserved structures; molecular patterns like peptidoglycans , dsRNA , lipopolysaccharide ,DNA in infectious agents like bacteria and virus. Permits rapid release of inflammatory mediators and activates Antigen Presenting Cells and thereby adaptive immunity is activated.
- TLR-3 recognizes double stranded RNA
- TLR-7 recognizes single stranded RNA
- TLR-9 recognizes double stranded DNA

- TLR-2 recognizes peptidoglycan
- B lymphocytes bear TLR-7 and 9
- Dendritic cells bear either TLR-3 or both TLR-7and TLR-9.
- Fibroblast carry TLR -3
- TLR are expressed by Rheumatoid synovial tissue & cultured FLS(Fibroblast like synoviocytes)
- Repeated engagement of these TLRs would initiate RA in a genetically susceptible individual .Repeated activation of TLR could break immune tolerance and permits autoimmune response to occur in a genetically susceptible individual

NORMAL IMMUNE BALANCE & SELF TOLERANCE

RA is an autoimmune disease. Understanding of normal immune balance and self tolerance mechanisms would help us to understand the pathogenesis of autoimmune diseases.

IMMUNE BALANCE

Cytokines released from T helper lymphocytes balances cell mediated and humoral immunity.Cell mediated immunity is regulated by cytokines –IL-2 ,IFN-γ which are released by Th1 cells. The cytokines IL-4,IL-5,IL-10 released by Th2 regulates humoral immunity [8,9].IL-12,INFγ inhibits Th2 response,IL-10 and IL-4 inhibits Th1 response .Thus both Th1 and Th2 responses are mutually inhibitory[10].

Human leukocyte antigen complex [11]

The HLA complex helps the immune system to distinguish between self antigens and from proteins made by foreign invaders such as viruses and bacteria. More than 200 genes located closely on chromosome 6 comprises the MHC complex. It is categorized as class I, II,III.HLA –A, HLA-B,HLA-C are the 3 main classes of MHC class I; whose products are present on the surface of almost all nucleated cells .The 6 main MHC class II genes in humans: HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1,HLA-DRA, and HLA-DRB1. Each person's immune system react to a wide range of foreign invaders because of possible variations in HLA genes. HLA is inherited as a set of 3 haplotypes.We have a 25% chance of inheriting same HLA as any one of our siblings. 25% chance of not inheriting the same haplotype. 50% chance of sharing one haplotype with our siblings. There is 1 in 4 chance of being an identical match withour siblings

IMMUNE TOLERANCE [11]

Deletion, anergy , suppression are the three common mechanisms taking place in our body which protects us from autoimmunity.[20]The immune tolerance is classified as central and peripheral based on the lymphoid organ where it takes place.

Deletion is the process occurring in central tolerance. Anergy and suppression are the processes occurring in peripheral tolerance.

Central tolerance is the process of destruction of self reactive T- Cells in Thymus. Progenitor T cells migrate to Thymus for maturation during 8^{th} to 9^{th} week

of gestation in humans .A T- Lymphocyte express 10^5 receptors exhibiting 10^10 different antigen specificities.In thymus positive and negative selection of T cells occur. Positive selection is the process of allowing the maturation of T cells with low affinity receptors for self antigens presented by MHC molecules. Negative selection is the process where elimination of T cells which reacts strongly with self MHC molecules occur.

In thymic cortex destruction of T-cells that reacts with self antigens presented by MHC II in the presence of co-stimulatory molecules occurs. In thymic medulla destruction of T- cells that reacts with Antigens presented by MHC I in the absence of co-stimulatory molecules and MHC II occurs .The apoptosis takes place by a controlled interaction between Fas and Fas ligand.[20]

Anergy is induced by insufficient co-stimulation thereby causing unresponsiveness in an autoreactive cell clone. It is a process of peripheral tolerance.

Clonal suppression is another peripheral tolerance mechanism where cytokines released by CD4+, CD25+ -Tregulatory cells ,CD8+ suppressor cells quenches the anti- self responses.

B Lymphocytes matures in bone marrow. The specificity of B lymphocyte towards an antigen is created by random rearrangement of a series of gene segments that encodes the antibody molecule. A single naïve B cell is estimated to exhibit 10^10 different antigen specificities. This diversity is restricted by the process of negative selection i.e. B cells that reacts with self antigens are eliminated

These are the mechanisms of immune tolerance that prevent or subsides the autoimmune response.

AUTOIMMUNITY[11]

The inappropriate response of immune system towards self components is Autoimmunity. The reason is

All self reactive lymphocytes are not deleted during T-cell and B- cell maturation .

Normal healthy individuals posses self reactive cells in circulation and it is suppressed by clonal suppression; defect in clonal suppression leads to activation of these self reactive clones.

RISK FACTORS FOR AUTOIMMUNE DISEASE :

Autoimmunity is a natural phenomenon. Self reactive antibodies and autoimmune cells are present in all normal individuals. The combination of genetic predisposition and environmental factors contributes to development of disease, where the participation of genetic predisposition is 1/3rd and the remaining 2/3rd is by environmental factors.HLA haplotype, is the best available predictor of developing an autoimmune disease. The likelihood of developing similar autoantibodies is related directly to sharing HLA haplotypes with family members and the probability is even greater if two haplotypes rather than one are shared. Genes outside the MHC also contribute to the risk for developing autoimmune disease .

Environmental agents like diet , hormones , toxins , infectious substance and drugs amplify autoimmunity in genetically susceptible individuals. These agents break tolerance in genetically susceptible individuals, and increases the risk of developing autoimmune diseases.

HORMONES

Steroid hormones , estrogens and androgens are known to influence antibody production and immune cell proliferation .Thus hormones can amplify or inhibit the immune response. Women produce elevated antibody response compared to men, while men often develop more severe inflammation.

DIET & AUTOIMMUNE DISEASES

Food additives & pesticides may contribute to autoimmunity . Certain evidences are Iodine inducing antibody formation against thyroglobulin in autoimmune thyroiditis. Hypersensitivity to gluten in diet induces antibody formation against transglutaminase , actin and calreticulin.

DRUGS & TOXINS

Drugs induce autoimmune diseases. Example- Procainamide, Penicillamine result in Lupus like disorders. These symptoms ameliorate with drug withdrawal.

INFECTION

There are evidences to show that infections are associated with autoimmune diseases.

- Coxsackievirus and CMV in TypeII Diabetes mellitus and myocarditis.

- Measles and EBV in Multiple Sclerosis .
- Mycobacteria and EBV with Rheumatoid arthritis.

Association of multiple diverse microorganisms with a single autoimmune disease suggest that a common mechanism induce autoimmune disease in genetically prone individuals, which can be

- Direct viral damage
- Release of Cryptic self peptides
- Antigenic spread
- Molecular mimicry (Microbial Ag closely resembles self antigens)
- Adjuvant effect (specific activation of innate immune response by microbes eg- vaccine administration)
- Bystander activation (non specific immune response stimulated by infection results in activation of autoimmune response)

A number of animal models of autoimmune diseases had been studied using the above said mechanisms. Examples for adjuvant effect are

  - Rheumatoid arthritis in mice with collagen
  - Multiple sclerosis with myelin basic protein
  - Myocarditis with cardiac myosin

CYTOKINE IMBALANCE

A inflamed tissue is subjected to cytokines released by B cells, activated cells like endothelial cells, fibroblast , synoviocytes . To downregulate the immune response in that tissue , it utilizes the cytokines released by T cells and macrophages A balance exists between these pro-inflammatory and anti-inflammatory cytokines. Autoimmune disease of a organ occurs when this balance gets disrupted.

DYSREGULATED INTRACELLULAR SIGNAL TRANSDUCTION

Inappropriate activation of naive T cells and retention of T cells occurs in deficiency of cytotoxic T–Lymphocyte antigen CTLA-4.[21] Altered amounts of STAT-3 and deregulated signaling by NFKB are seen in certain autoimmune disases.[11].

NEO ANTIGEN FORMATION

Epitope conformation of a endogenous molecule is altered when a hapten is conjugated with it. The haptens are usually metals , toxic substance or chemical conjugates .This altered epitope in that endogenous molecule may provoke autoimmunity.

AUTOIMMUNE DISEASE

Normal minimal amount of autoantibodies present in circulation helps us in clearing cellular debris associated with inflammation. Any alteration in mechanisms that regulate inflammation or immune responses by environment or genes makes this autoimmune response exaggerate to autoimmune disease. Autoimmune diseases are classified as organ specific and systemic, based on whether autoimmune response is

directed against a particular tissue or widespread . Autoimmune diseases are characterized by presence of autoantibodies , inflammatory cells and cytokines.

AUTO ANTIBODIES IN AUTOIMMUNE DISEASE

1. Activates the complement system and cause cell lysis eg: hemolytic anemia
2. Binds with cell surface receptors and alters the function of that receptor eg:antibodies against thyrotropin receptors in Graves disease, antibodies against Ach in Myasthenia gravis
3. Deposition of antibody – complement complex over joints and vessels causing tissue damage eg: Lupus, Rheumatoid arthritis , inflammatory heart disease.

IMMUNE CELLS IN AUTOIMMUNITY & DISEASE

1. The cells involved are macrophages, neutrophils, CD4+ T Helper cells , CD8+ cytotoxic T cells, Natural killer cells , mast cells , dendritic cells.
2. Tissue macrophages and monocytes acts as antigen presenting cells to initiate an autoimmune response.
3. Macrophages destroys cells through antibody dependent cell mediated cytotoxicity or by secreting cytokines- TNF , IL-1,which in turn recruits other inflammatory cells like neutrophils and T-cells.
4. Macrophage and neutrophils damage tissues by releasing nitric oxide or hydrogen per oxide.
5. IL-4 released by Th1 acts on B cell to produce immunoglobulins.[12]

ROLE OF CD4+ T CELL: [7] CD4+ T cells on exposure to a self Ag

A. CD4+ T Cell differentiates into TH1 like effector cells

↓

release of INF-γ from TH1 cells

↓

INF-γ stimulates macrophages to release proinflamatory cytokines IL-1 & TNF

↓

INFLAMMATION

B.T-Cells release cytokines that promote B Cell proliferation and differentiation

↓

Production of immunoglobulins(polyclonal activation)

occur against self antigens – RF, ACPA

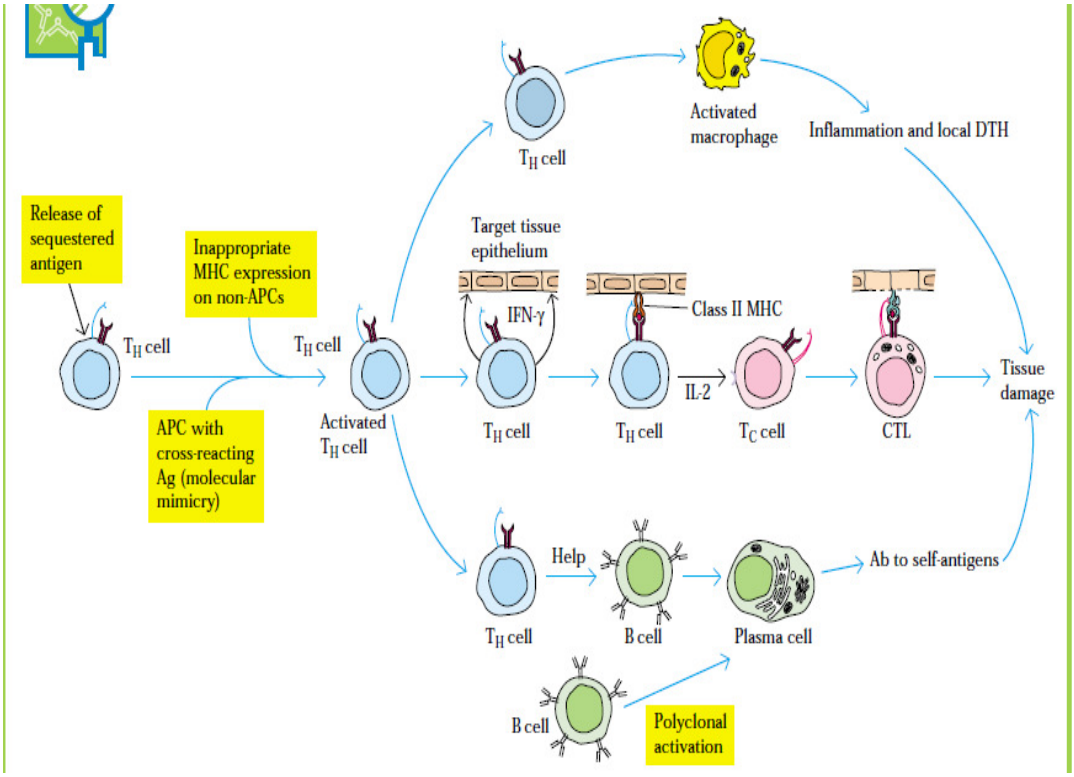
↓

Formation of immune complex

↓

Complement activation cause Tissue damage by type III hypersensitivity reaction

Figure 1- immune cells and autoimmune mechanisms



Courtesy: V.Kumar et al .1989 Annu Rev. Immunol 7:657

This figure explains various cell involvement in an autoimmune response

PROGRESSIVE NATURE OF AUTOIMMUNE DISEASE

The chronic cell and tissue injury leads to continuous release of self antigens .When the condition is favourable for antigen presentation and co-stimulation ; epitope spread occurs. Further increased activation of lymphocytes that recognizes self antigens and their expansion leads to chronicity of Autoimmune disease. [13]

AUTOIMMUNITY IN RA:[1]

Autoimmunity plays a major role in RA and autoimmune response precedes the onset of disease by many years. Autoimmune response can be directed against articular antigens and Non – articular systemic antigens .The articular antigens are TypeII collagen in cartilage and glycoprotein 39 in cartilage. The non-articular antigens are Glucose-6-phosphate isomerase, heat shock proteins , heavy chain binding proteins,hnRNPA2 .

Type II collagen

Antibodies against type II collagen in RA is capable of generating C5a when these antibodies bind with type II collagen in cartilage , this can amplify the inflammatory response and it is not an initiating event.

Glycoprotein 39

Peptides of Glycoprotein 39 from the cartilage binds with HLA DR*0401 and stimulates proliferation of T-lymphocytes. Gp39 has been detected in a small

group of patients and are found to be highly specific for RA. Presence of Gp39 is associated with less aggressive disease.

Heterogenous nuclear Ribonucleoprotein-A2(hn RNPA2)

hn RNPA2 or RA 33 occurs in one third of RA patients and other autoimmune diseases.RA-33 positive patients tend to have a less severe disease.

An algorithm including anti RA33, RF, Anti CCP had been used in patients with early synovitis to predict the progression to erosive RA.[1]

The major autoantibodies involved in RA are Rheumatoid Factor(RF) and Anti Citrullinated Protein Antibody(ACPA) and they are explained in detail.

Modification in structure of a self antigen and generation of neoepitope are the two proved mechanisms of initiation and provocation of Autoimmune response in Rheumatoid Arthritis.

CHANGE IN STRUCTURE OF THE ANTIGEN:[1]

Non antigenic soluble monomeric IgG form immune complex with Fc fragment of IgG thus the structure of Ig gets altered leading to generation of Rheumatoid factor

RHEUMATOID FACTOR :[7]

- Presence of Rheumatoid Factor is a cardinal feature of RA.The antibody that binds with Fc fragment of heavy chain of IgG is the Rheumatoid factor .Presence of RF is an evidence for autoimmunity in Rheumatoid arthritis.

- Presence of Rheumatoid factor precedes disease onset by many years. seroconversion occurs during the 1st year of disease activity.

- These antibodies activate the classic complement pathway. Large quantities of IgG RF produced by synovial tissues form complexes with one and another and gets deposited over the synovial tissues, facilitating complement fixation and release of chemokines.

- The proof of involvement of RF in RA is that RF levels increases with clinical relapse and decreases with remission of disease activity. Some patients initially though seronegative to RF subsequently convert to seropositive typically during the first year of disease activity.

- Rf directed against IgG and IgM are abundant in patients with RA .Other than IgG RF; IgM RF , IgERF & IgARF are also present in RA patients.

- 75% of patients with RA are seropositive with standard agglutination assays whereas 90% are seropositive positive when assayed for IgM RF ELISA

- The Standard agglutination tests primarily detect IgM RF , but the seronegative patients usually have IgA RF.

- Immune complex formed by IgE RF cause degranulation of mast cells.

- Rheumatoid factor present in 5% of normal individuals are considered to be germline derived and have low affinity for Fc fragment of heavychain of IgG.

- Rheumatoid factor seen in RA patients are formed due to gene rearrangement and somatic mutations of germline genes.

- Predictive value of Rheumatoid factor in diagnosis of rheumatoid arthritis is poor , only 1/3 rd of RF positive individuals had Rheumatoid arthritis.

- It is also positive in other autoimmune diseases like SLE, Sjogrens syndrome , Sarcoidosis , Interstitial pulmonary fibrosis and other diseases like chronic liver disease, tuberculosis, malaria, syphilis, hepatitis B etc.

- IgA RF is a better indicator of T-Cell dependent affinity matured antibodies directed to particular Fc- gamma epitopes relevant to RA

- Combined detection of IgM&IgA RF is a strong indicator of RA [14]

GENERATION OF NEO- ANTIGENS:

Citrullination of proteins is a post translational modification. Citrullination occurs by Peptidyl Arginine Deiminase enzyme that converts arginine to citrulline by removal of the imine group.

ANTI CITRULLINATED PROTEIN ANTIBODIES:

ACPA are immunoglobulins that bind with citrullinated proteins and are produced by patients with RA. Citrulline is a derived AA formed from posttranslational modification of Arginine by PADI

It is an important surrogate marker for diagnosis & prognosis of RA because they are

- As sensitive & more specific than IgM RF in both early & fully established RA.

- Predicts the eventual development into RA when found in undifferentiated arthritis

- Marker of erosive disease in RA

- Detected in healthy individuals years before disease onset.

Anti perinuclear factor was described in 1964 as a marker for RA . Anti keratin antibodies was described as a marker in 1974. Post translationaly modified vimentin – Sa antibody in 1994. It was discovered in 1998 that all these antibodies target citrullinated proteins . Citrullinated peptides fit better in the HLA DR4 Ag binding groove than the corresponding non- citrulline containing peptides . Different linear citrullinated peptides are seen in serum of different patients , suggesting a polyclonal response. The flanking regions around the citrulline are also important , hence all sera will not react with every peptide [14].

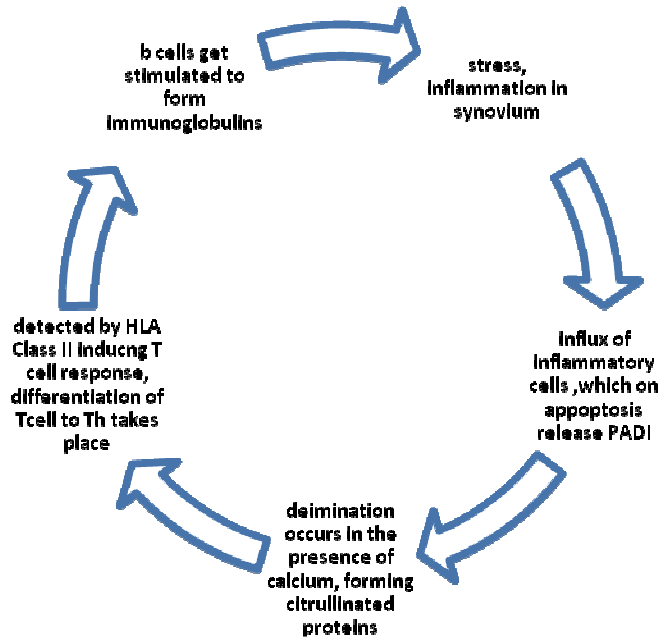
- The proteins that undergo citrullination are usually vimentin, fibrinogen, fibronectin, EBV derived peptides.

- Citrullination of proteins is mediated by the enzyme PADI- Peptidyl Arginine Deiminase Ec 3.5.3.15 [15]; there are 4 isoforms and the common ones are PADI2 and PADI4.

- The usual changes are conversion of Glycine to Arginine in the primary sequence and citrullination of these newly formed arginine or anyother arginine residues.

- Citrullination as such increases T Cell responses to arthritogenic antigens
- Antibodies against citrullinated proteins activates both the classical and alternative pathway.
- IgE ACPA sensitizes basophils and cause mast cell degranulation.
- Inheritance of specific HLA – DRB1 allelle favour T and B cell immune response to host derivatized non epitope peptide antigen.
- The induction of PADI expression and citrullination of peptides are not specific for RA, this can also occur in other inflammatory settings.[16]
- Detection of ACPA is 90% specific . Positive in 80 to 90% of patients.
- These antibodies seen in circulation are synthesized by synovial B cells. They appear long before the clinical disease.

11. **Mechanism of ACPA formation** is shown in figure 2.



- INTERPRETATION of Anti CCP:

< 20U/mL	NEGATIVE
20 to 39.9U/mL	WEAK POSITIVE
40 to 59.9 U/mL	POSITIVE
≥60U/mL	STRONG POSITIVE [17].

- Anti CCP is not useful to predict the future development of disease since 1.5% of normal individuals possessing this antibody did not develop the disease.

- Anti CCP positive individuals are more prone for aggressive and erosive disease.[18]

- Anti CCP positivity is also a independent risk factor for ischemic heart disease incidence in RA patients.[7]

ENVIRONMENTAL FACTORS ASSOCIATED WITH RHEUMATOID ARTHRITIS:

Smoking Tobacco and silica exposure are the two agents associated with Rheumatoid Arthritis.

TISSUE DAMAGE IN RHEUMATOID ARTHRITIS:

The first phase is production of activated T and B lymphocytes by neoantigen ie citrullinated protein . Peptidyl Arginine Deiminase enzyme is released

by apoptotic granulocytes and monocytes. The activated T cell in turn stimulates macrophages, synovial fibroblast , endothelial cell, mast cell , osteoclast. These activated cells release cytokines like TNF, IL-1 , IFN-γ, MMP , Osteopontin leading to synovitis , pannus formation , bone erosion , cartilage destruction.

Direct cell injury also occurs by rheumatoid factor activation of Classical complement pathway.

Less effective clearance of antigen – antibody complex occurs in RA patients causing further damage.

Spread of arthritis occurs by migration of fibroblast like synovial cells.

RF vs Anti CCP

RF is more sensitive than Anti CCP but not specific.1st generation ELISA for anti CCP used several fillagrin epitopes, had a sensitivity of only 65-70%. Cyclisation improved the sensitivity, hence 2nd generation ELISA used cyclic epitopes that mimicked true conformational epitope. 3rd generation ELISA and Chemiluminescence have increased sensitivity with similar specificity [14,15]

BIOMARKERS IN RHEUMATOID ARTHRITIS

Autoantibodies: IgM RF, IgA RF , ACPA

Acute Phase Response markers : ESR,CRP, IL-6

Synovial Vascularity: VEGF

Cartilage Metabolites: Matrix metalloproteinase MMP1&3, COMP- Cartilage Oligomeric Protein, Aggrecan cleavage fragments , c- terminal collagen II

Bone metabolites: Pyridinoline cross links , Carboxy terminal collagen I telopeptides.

MARKERS THAT ANTEDATE DISEASE : These auto –antibodies are present in serum long before onset of disease activity

1. IgM RF
2. ACPA- Anti citrullinated vimentin

   Anti citrullinated α enolase

   Anti citrullinated fibrin

PREDICTORS OF RADIOGRAPHIC PROGRESSION: Concentration of these antibodies correlate with joint damage

1. IgM RF
2. ACPA
3. VEGF
4. MMP 1&3
5. C terminal collagen II in urine

PREDICTORS OF JOINT DAMAGE IN EARLY RHEUMATOID ARTHRITIS:

1. COMP- Cartilage Oligomeric Protein.
2. C-terminal collagen I telopeptides.

SEEN IN EXTRA-ARTICULAR DISEASE:

1. IgM RF – severe extra articular disease.

2. IgA RF

MARKERS OF DISEASE ACTIVITY

1. Acute phase reactants.

2. Pyridinoline cross links from bone.

C Reactive Protein

It is an acute phase reactant indicating disease activity in many inflammatory disorders.It is used in Rheumatoid arthritis also. CRP was discovered in 1930- by Tillet and Francis, in acutely ill patients as a protein that binds with cellwall c-polysaccharide of Streptococcus pneumonia.

It is made up of 5 identical polypeptide subunits.The molecular weight is 23,028 Da. It is non-covalently associated to form a disc shaped structure with radial symmetry. It is related to Serum Amyloid P and pentraxin-3. It has a half life of 18to 20 hours.

It provides nonspecific host defence and activates classical complement pathway.

Serum Concentration of 5-10mg/L suggest inflammation and its rise is proportional to tissue damage [19].

PRL & IMMUNE SYSTEM

Role of PRL in immune system was described as early as 1930s.Prolactin known as a lactogenic hormone is a cytokine .In addition to secretion from acidophills of anterior pituitary it is also secreted by lymphocytes, deciduas of uterus, amnion, chorion of placenta, mammary gland and many regions of brain. The role of Prolactin in immune regulation and autoimmune diseases had been explained by many invitro and invivo animal and human studies . Prolactin regulates the maturation of $CD4^-$ and $CD8^-$ to CD4+ and CD8+. Prolactin decreases or prevent the apoptosis of lymphocytes. Interferes with peripheral tolerance. Prolactin enhances the proliferative response to certain antigens and mitogens. Prolactin upregulates the expression of Th1 cytokines. Th1 cytokines are involved in autoimmune diseases.

Let us go through Prolactin hormone in a new aspect.

PROLACTIN

Prolactin (PRL) the lactogenic hormone belongs to Group – 1 □ helix bundle protein hormones". This group also includes Growth hormone synthesized by anterior pituitary and placental prolactin like peptides synthesised by placenta [22,23]

STRUCTURE

PRL is made of a single polypeptide chain with 199 AA . Its molecular weight is approximately 23 KDa .It has 3 intramoleclar disulphide bridges cys4- cys 11, cys58-cys 174, cys 191-cys199 [24].Prolactin is arranged as 4 antiparallel alpha helix. [25,26]

SYNTHESIS

Prolactin is a polypeptide of 199 amino acids synthesized by acidophilic cells (‘lactotrophs’) of anterior pituitary gland. It is synthesized as a prohormone with 227 AA. The cleavage of signal peptide- 28 AA gives mature human prolactin [27,28]

PROLACTIN VARIANTS

The normal circulatory serum PRL is monomeric 23KDa form. In addition to 23KDa form, many other prolactin variants are also seen in circulation .These variants are formed by proteolytic cleavage, post translational modifications, alternative splicing of the primary transcript. [27]. Alternative splicing is the minor contributor to Prolactin pool,it forms a 137 AA long Prolactin [29]

By means of proteolytic Cleavage 14KDa,16KDa and,22KDa fragments are produced. They are found in pituitary extracts and human serum. Their biological activities are not well defined. These forms may be preparative artifacts [27].

The post translational modifications occurring are dimerization , polymerization, phosphorylation, glycosylation, sulfation and deamidation [30].Dimerization and polymerization of monomeric prolactin or its aggregation with immunoglobulins form macroprolactin whose biological activity is low[27]. Phosphorylation of Prolactin occurs within secretory vesicle of lactotrophs just before exocytosis[31.] Non phosphorylated Prolactin is the active form. Phosphorylated Prolactin is the autocrine regulator(inhibitor) of non phosphorylated Prolactin secretion. Phosphorylated Prolactin also inhibits the signal transduction pathways activated by Prolactin [32].Glycosylation lowers the biological activity and clearance rate of Prolactin [33]

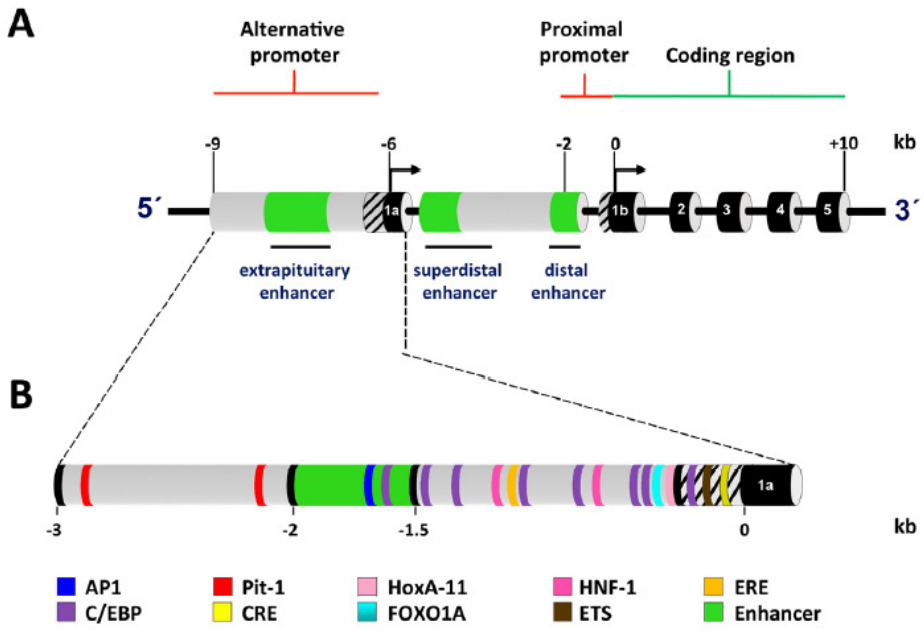
PROLACTIN GENE

Prolactin gene is located in Chromosome 6 . It is 10 kbp with 5 exons and 4 introns.[24,34] This gene has 2 independent promoters. Pituitary specific expression is by the proximal 5000 bp promoter [35] . The upstream promoter is concerned with extra pituitary expression [36.]

PITUTAIRY PROLACTIN SECRETION:

Lactotrophs of anterior pituitary secrete Prolactin. TRH responsive lactotrophs are present in the outer zone of anterior pituitary [37], whereas dopamine responsive acidophils are present in inner zone of anterior pituitary [38] .

Figure 4- structure of PRL gene showing two promoter



Courtesy: Mendez etal- Prolactin in the immune system-2013

AP1: activator protein 1; C/EBP: CCAAT/enhancer binding protein Pit1:pituitaryspecifictranscription factor 1; CRE: cyclic-AMP response element;

HoxA-11: homeobox A11;FOXO1A: forkhead box protein O1A; HNF-1: hepatocyte nuclear factor 1; ETS: E-twenty six; ERE:estrogen response element. Enhancer regions are represented in green.

OTHER SITES OF PRL SYNTHESIS:

Many areas of brain ,caudate nucleus, striatum ,non pregnant uterus, placenta, amnion, mammary gland,synovium,fibroblast,B and T lymphocytes, NK cells,macrophages.

PROLACTIN RECEPTOR:It is a Cell surface receptor [39] .Prolactin receptors are classified as cytokine receptor superfamily classI .

PRL RECEPTOR GENE:The gene is present in chromosome 5.It is made of upof 10 exons [40] .This gene have 3 different tissue specific promoters- for gonads , liver and gonadal& non-gonadal tissues. [41]

PRL RECEPTOR DISTRIBUTION IN VERTEBRATES: [42]

PRL receptors are distributed widely. These receptors are present in the following regions

1. Central nervous system - Brain cortex, hippocampus, choroid plexus, corpus striatum, cochlear duct, corpus callosum ,hypothalamus ,ganglia ,astrocytes ,retina, olfactory ganglia, anterior & posterior lobes of pituitary.

2. Adrenal cortex, Renal cortex, Lungs ,Skeletal muscles, brown adipose tissue, submandibular & submaxillary salaivary glands.

3. Skin- epidermis ,hairfollicle, sweat gland. Bone- osteoblast, cartilage- chondrocytes ,atrial muscles

4. GIT- Duodenum, jejunum, ileum,colon. Pancreas- islets of langerhans . Liver- hepatocytes, kupffer cells

5. Immune system- spleen, thymus ,T&B lymphocytes , MALT ,NK cells , Macrophages

6. Ovary- ova, granulose cells, thecal cells, Sertoli cells corpus luteum, fallopian tubes. Nonpregnant endometrium & pregnant uterus –decidua ,Placenta, amnion

7. Sperm, seminal vesicle, epididymis, testis, prostate, leydig cells

TYPES OF PROLACTIN RECEPTOR:

Many forms of PRL receptors are present. They are formed by alternative splicing [43] .Based on size of the intracellular part the receptors are classified as long , short and intermediate forms. Soluble forms are also available.

STRUCTURE OF PROLACTIN RECEPTOR:

The PRL receptor have extracellular , transcellular and intracellular domains.

The extracellular domain has 3 constitutively expressed regions –

- **CRH**- Cytokine receptor homology region,CRH is divided into D1 & D2.
- **D1 subdomain** – 2 pairs of disulfied linked cysteine present in the N-terminal region.

- **D2 subdomain** – Pentapeptide WS motif-(Trp-Ser-X-Trp-Ser) present in the C terminal region.

Proline rich motif(I-F-P-P-V-P-X-P) proximal to transmembrane domain is necessary for interacting with Janus Activating Kinase 2 . [44]

Single pass transmembrane domain whose function is unknown.

Intracellular region has 2 conserved regions- Box1 and Box2

Box1- It is 8 AA region recognized by transducing molecules. Box1 is rich in proline and hydrophobic residues.

Box2- made of hydrophobic and negatively charged AA [45].

MECHANISM OF PROLACTIN ACTION:

Prolactin acts through JAK-STAT pathway.

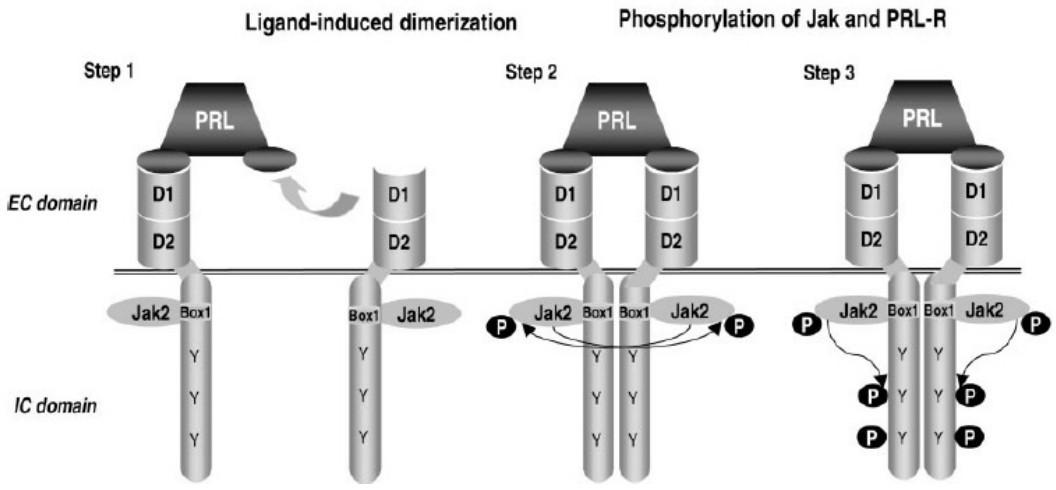
JAK is a family of 4 proteins – JAK1, JAK2, JAK3,TyK2 [46]

An adapter links JAK2 and PRLR through SH3 domain[47].Activation of JAK2 by PRL occurs within 1 minute[48] .

PRLR PHOSPHORYLATION: Dimerisation of the receptor occurs by binding of PRL with its receptor. This causes phosphorylation of Janus kinase and Phosphorylated Janus Kinase transphosphorylates the tyrosine residues in each arm [48] .All the 3 isoforms are capable of activating JAK [48].Usually phosphorylation of 473,479 tyrosine residues occur . But in cells with overexpression of JAK2 in addition to 473,479 tyrosine residues 309, 402, 515 tyrosine residues are also

phosphorylated. Phosphorylation is essential because phosphotyrosine residues are essential for binding of transducer molecules with SH2 domain.

Figure 4 – Activation of PRL receptor



COURTESY:MARC E. FREEMAN, BE´ LA KANYICSKA, ANNA LERANT, AND GYO¨ RGY NAGYPHYSIOLOGICAL REVIEWSVol. 80, No. 4, October 2000

SIGNAL TRANSDUCTION PATHWAYS activated by PROLACTIN

The major pathway activated by PRL is STAT-Signal Transducers and Activators of Transcription. STAT family of proteins are latent cytoplasmic proteins with a molecular weight of 90 to 100 KDa.They are seen bound to intracellular domains of cytokine receptors. . STAT family includes 8 proteins- STAT 1α, STAT β ,STAT2,3,4,5a,5b,6 . STAT5a, STAT5b .STAT1 & STAT3 are the Prolactin induced transducers. STAT 3 is called as Acute Phase response factor .There are 5 conservd domains in STAT. They are DNA binding domain,SH3 like domain, SH2 like domain ,NH2 & COOH terminal domains . Phosphorylated STAT dissociates

from the receptor and form a homo or heterodimer with –SH2 like domain of another phosphorylated STAT molecule.

STAT dimer translocates to nucleus binds with the GAS sequence (TTCxxxGAA) – Gamma interferon activated sequence , a DNA binding motif in the promoter region of target gene and thus enables transcription [49] .

STAT also interacts with other signal transducers thereby initiating cell and cytokine specific response [50].The transactivation potential of Stat are modulated by interaction of STAT with nuclear proteins like p48 , IRF-1 , c- jun , sp1 , Src , nuclear hormone receptors , MCM5 , BRCA1 [51] and with many co- activators [52].Co-activators are agents that enables interaction between transcription factors and basal transcription machinery components. The co-activators also activates HAT- histone acetyl transferase activity, causing chromatin remodeling ;thereby helps in transcription.

STAT 1 interact with 3 regions in the co-activator protein p300/ cAMP response binding protein.

Other Pathways activated

Prolactin also activates MAP kinase pathway. Both JAK-STAT and MAPK pathways are interconnected [53]. Prolactin also acts through Src kinase family, Fyn, IRS-1, Phosphatidylinositol- 3- kinase [54].

TERMINATION OF PROLACTIN SIGNALLING

Inhibition of STAT activation and catalytic activity of Janus Kinase occurs with the help of **SOCS**- suppressors of cytokine signaling . Prolactin itself induces the acute and transient expression of SOCS-1 & 3 [55].

Other inhibitors are **JAB**- JAK binding protein, SSI- STAT induced STAT inhibitor. These inhibitors act by competing with STAT for binding to the receptor.

PIAS3- Protein Inhibitor of activated STAT; another inhibitor blocks the binding of STAT3 to its DNA targets [56-59]

BIOLOGICAL ACTIONS OF PROLACTIN:

The distribution of PRL receptors in many regions and secretion of PRL by many cells describes its n number of functions

LACTATION: PRL is necessary for mammopoiseis and lactogenesis . PRL does this function by activating Jak2-STAT5 pathway [60].

LUTEAL FUNCTION: Prolactin is essential in pregnancy for progesterone synthesis and luteal cell hypertrophy [61]. It has a role in steroidogenesis in granolosa cells [62].High levels of prolactin in humans inhibits luteinization [63].

OSMOREGULATION: Prolactin regulates solute and water transport across cell membrane [64]. PRL has a role in fluid [65]sodium chloride[66],calcium [67]transport across intestinal epithelial membranes.

ANGIOGENESIS: Intact human Prolactin has anti-angiogenic activity which is attributed to 16 KDa fragment of Prolactin .Placental PRL like peptides and Growth hormone has anti-angiogenic properties [68].

ROLE AS A MITOGEN :The essential component of an effective immune response is antigen driven clonal expansion of T-Lymphocytes . Prolactin is a necessary co-mitogen for human T&B Lymphocytes proliferation [69]. Acts as a co-

mitogen for NK cells & macrophages [70] .The cell cycle of lymphocytes is regulated by Prolactin , thereby influence the proliferation of these cells by modulating the gene expression [71,72]. Devinsetal have shown that in humans, decreasing Prolactin levels by dopamine administration results in transient and significant reduction in mitogenic response of isolated peripheral lymphocytes to a mitogen [73].

IMMUNE MODULATION:

Prolactin and other pituitary hormones act as stress adaptation molecules and they are important in immune system homeostasis [74].Prolactin balances the effects of glucocorticoids and other inflammatory mediators at times of inflammation and maintains the steady state. This is supported by an invitro study where glucocorticoid induced death of lymphocytes was prevented by Prolactin [75].Prolactin acts at autocrine, endocrine, paracrine levels in regulating immune response [76] .

Role of Prolactin in immune response has been demonstrated in 1972 by showing that exogenous PRL enhances thymic function in PRL deficient dwarf mice[77]. The significance of Prolactin in immune response has been emphasized and proved by many invitro experimental studies .Immune responses are enhanced by Prolactin[78].Prolactin receptor expression has been seen in spleenocytes, thymocytes, bone marrow cells, PBMC ,lymphocytes and monocytes [61].The constitutive expression of PRL and its receptor in resting T- Cells indicate that Prolactin has a role even in steady state [79]. Granulocytes express PRL transcripts yielding a high molecular weight immunoreactive protein similar to pituitary Prolactin . Prolactin is stored as vesicles inside the monocytes and released during infection.

Immunohistochemical staining of PBMC has shown the presence of Prolactin in vesicles close to the nucleus [80].

IN-VITRO STUDIES:

Prolactin receptors are expressed by thymic epithelium. Thymic epithelial cells on exposure to ovine prolactin secreted thymulin ,the T-Cell differentiation factor [81]. Prolactin enhance thymocytes release from thymus [82].Prolactin induces IL-2 receptors expression on T-Cells [83]. Prolactin stimulates growth of immune cells when used along with a mitogen. This has been demonstrated in proliferation of mouse unfractionated spleen cells, human peripheral mononuclear cells ,Th-T Helper cells. Addition of Anti- Prolactin antibodies inhibited the mitogenic response in these cell cultures [84,85].This indicates that Prolactin acts as a autocrine or a paracrine growth factor [86]. Exogenously added prolactin enhanced the expression of surface activation markers CD69 and CD25 on human peripheral B lymphocyte [87].

LYMPHOCYTE AND PROLACTIN :

Lymphocytes snythesize biologically active form of Prolactin which may act as an autocrine and paracrine growth factor [88]. Dexamethasone inhibits the gene expression of both forms of Prolactin [89] .A patient with Acute Myeloid Leukemia had hyperprolactinemia, her leukamoid cells were isolated and subjected to immunoblotting technique that revealed the presence of Prolactin ,thus Prolactin secreted from lymphocytes can contribute to systemic Prolactin concentration [90] .

IN VIVO STUDIES

Dinitrochlorobenzene induced dermatitis and antibody responses to T-Cell antigen & sheep red blood cells are suppressed in hypophysectomised rats and

reversed by prolactin injections [91].Rats completely deprived of Prolactin by hypophysectomy and anti- Prolactin antibody developed anergy and anaemia, injections of Prolactin reversed spleen and thymic involution by stimulating the c-myc pathway [92].Artificial hyperprolactinemia induced in C57BL/6 mice increased humoral antibody response to sheep RBC [93].Cysteamine ,a sulfhydryl reducing agent reduces serum Prolactin levels. Mice treated with cysteamine developed thymus atrophy and reduced response to T & B Cell mitogens [93].Prolactin is a potent stimulator of macrophage .INF-γ secretion is increased in humans by administration of physiological doses of Prolactin [94] .Hypoprolactinemia was associated with tissue depletion of macrophages. Prolactin increases phagocytic activity, release of nitric oxide and IL-6 secretion from macrophages in mice [95].It inhibits apoptosis of lymphocytes [96].PRL cause proliferation of T- cells either independent of IL-2 or with IL-2 [97].

PROLACTIN and IRF-1:

Interferon Regulatory Factor-1(IRF-1) is a family of nine proteins involved in immune response [98]. IRF-1 regulates expression of genes that mediate antiviral, anti bacterial responses, Th1 responses , macrophage & dendritic cell function , NK-cell function and differentiation , cell cycle progression and apoptosis [99] .IRF-1 can also be considered as a tumour suppressor gene since mutation or deletion in this gene causes myelodysplastic syndromes [99].

Prolactin modulates IRF-1 expression is evidenced by many in-vitro and in-vivo studies. Prolactin stimulates expression of IRF-1 in rat leukocytes and human

granulocytes [100].PRL alters IRF-1 gene transcription at early G1and G1-S transition phase [101] .

This is mediated by activated STAT bound to GAS element at -120bp , constitutive Sp1 binding at -200bp [102] and interaction with co-activator CBP/p300[103].Activation of STAT1 cause expression of IRF1 whereas STAT5 activation causes repression of IRF-1 [104]

PROLACTIN and IL -6:

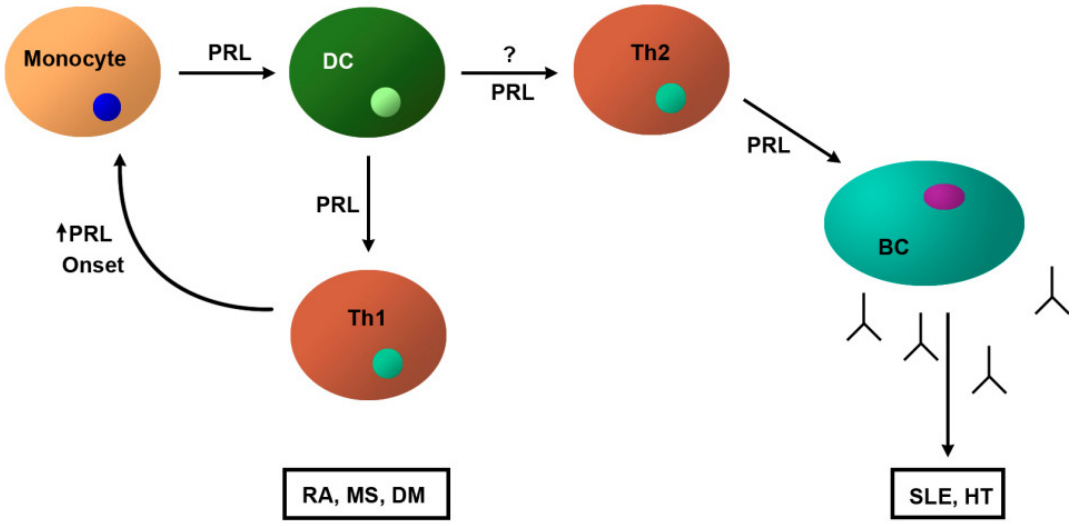
Prolactin along with E2 (estradiol) downregulates glycoprotein 130 component of IL-6, as well as its gene expression in deciduas of uterus. IL-6 expression in decidua cause termination of pregnancy [105].Thus PRL conserves pregnancy by acting as an immune inhibitor.

PROLACTIN AND AUTOIMMUNITY:

The homeostasis of Immune system is maintained by a balance between Th1 and Th2. Prolactin activates both Th1 and Th2 , influence of Prolactin over Th1 is more when compared to Th2. Increased Prolactin levels with either Th1 or Th2 dominance has been documented in many autoimmune diseases. Many animal models and human disorders have suggested that Th1 cytokines like TNF-α,INF-γ, IL-2 are involved in organ specific autoimmune diseases like Rheumatoid arthritis ,multiple sclerosis, insulin dependent Diabetes mellitus. Th2 is associated with SLE, Hashimotos throiditis, allergy[106] .

PRL causes differentiation of monocytes to dendritic cells . Induce T cell activation and proliferation , cause imbalance between Th1 and Th2 leading to autoimmunity. Prolactin enhances the Th1 mediated response [107,108]

Figure 5 – PRL in Autoimmune diseases



Courtesy: Mendez etal- Prolactin in the immune system-2013

RA-Rheumatoid arthritis, MS- Multiple sclerosis, DM- Diabetes Mellitus, HT-Hashimoto thyroiditis, SLE- systemic lupus erythematosus ; DC-Dendritic cell, BC-B-lymphocyte

This figure explains Prolactin involvement in autoimmune diseases.

Prolactin exerts immuno-stimulatory effect.PRL Promotes autoimmunity by mechanisms such as impaired negative selection of self reactive B- lymphocytes during maturation [109].The immune cells are made anti-apoptotic and proliferative response to antigens are enhanced by Prolactin. Thus increased production of immunoglobulins, cytokines increases the propensity towards autoimmunity. In

murine models association between Prolactin levels and disease progression has been seen .Moderate hyperprolactinemia has been seen in Systemic Lupus Erythematosus, Rheumatoid arthritis, sjogren's syndrome ,Hashimoto's thyroiditis ,multiple sclerosis [110-113].

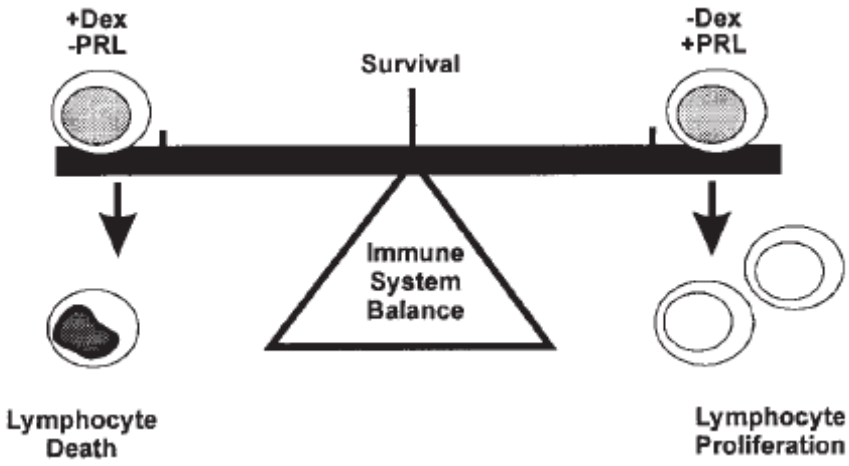
PROLACTIN AND RHEUMATOID ARTHRITIS

In a study Prolactin and Prolactin / Cortisol ratio was elevated in active RA along with elevated proinflammatory cytokines [26]. The diurnal rhythm in disease activity of Rheumatoid arthritis is attributed to diurnal variation of Prolactin secretion [27].Prolactin concentration in synovial fluid and serum of RA patients were elevated . Serum Prolactin in many autoimmune diseases were shown to be high by many studies.

PROLACTIN AND STRESS

Mild elevation in prolactin levels occurs in stress. PRL secretion parallels with corticotropin releasing factor secretion; this is necessary for maintenance of overall metabolism and survival at times of stress[122].Inhibition of immune responses by glucocorticoids is mediated by apoptosis of lymphocytes [123].PRL in culture inhibits (Dex)-Dexamethasone induced apoptosis of normal murine thymocytes. Thus PRL and glucocorticoids appear to antagonize their respective actions.

Figure 6 – PRL antagonizes Glucocorticoid effects and maintains balance.



Courtesy: Prolactin receptor signal transduction in cells of the immune system C V Clevenger, D O Freier and J B Kline, Journal of Endocrinology (1998) **157,** 187–197

Another evidence is reactivation of EBV in students (examination stress) suggested by elevated EBV IgG ,with mild elevation of Prolactin. Since the Prolactin levels are not as high as the rise in cortisol, control of T- cytotoxic lymphocytes over EBV is lost leading to reactivation of EBV[125]. At concentrations of 5 to 30 ng/mL, Prolactin increases the secretion of IFN-γ,IL-12 [124,125].

REGULATION OF PROLACTIN SYNTHESIS IN THE IMMUNE SYSTEM:

Alternative promoter drives expression of Prolactin in extra-pituitary tissues .The distal transcription start site is independent of pit-1 –pituitary transcriptional factor. The extra pituitary Prolactin m-RNA is ~ 150 bp longer than pituitary Prolactin m-RNA [126,127].

In Lymphocytes Prolactin expression is independent of pit-1, progesterone estrogen, TRH, dihydrotestosterone, insulin . PRL expression is stimulated by cAMP, retinoic acid , calcitriol .PRL expression is Inhibited by dexamethasone , IL-2 , IL-4 , IL-β .

cAMP and PRL

Action of PRL through cAMP has been studied in certain Leukemic cell lines and normal human PBMC. The 1st messengers employed were PGE2, β2 adrenergic agonist. It acts through cAMP/PKA pathway – phosphorylates CREB(cAMP response element binding protein [128]

CALCITRIOL and PRL : Calcitriol effects in the immune system occurs at the level of T- helper cells.Calcitriol acts as an immunosuppressor. It mainly curbs Th1. The ability of calcitriol to ameliorate autoimmune diseases and inhibition of allograft rejection responses stand as a evidence for cacitriol action on Th1 cytokines [129.130].Prolactin secretion may be regulated by locally produced calcitriol in some granulomatous diseases, which are documented with elevated Prolactin[131,132]. Calcitriol stimulates PRLm-RNA and protein production in resting PBMC through a VDR – mediated mechanism[133].

CYTOKINES REGULATING PRL SECRETION:

IL- 2, IL-4,IL-1β reduce PRL m-RNA expression in T Lymphocytes. TNF –α stimulates Prolactin release. Stimulation of Prolactin secretion by TNF-α is inhibited by protein kinase C inhibition [134].

DOPAMINE AND PROLACTIN FROM IMMUNE CELLS:

The dopamine receptors – D2, D3, D4, D5 are present in peripheral lymphocytes [135]. D3 receptors are present in secondary lymphoid systems . Dopamine regulates expression of its own receptor and Prolactin in PBMC [136] .

REGULATION OF PITUITARY PROLACTIN SECRETION : Prolactin secretion is pulsatile with 4 to 14 secretory pulses of increasing amplitude after onset of sleep ; declines shortly after awakening with nadir around noon . Prolactin secretion results from removal of Dopamine inhibition and these Prolactin releasing factors act by removing the inhibition over TIDA system[137].

Dopamine is the main regulator of pituitary Prolactin release.It acts by inhibiting the PRL release. All other peptides regulating Prolactin act indirectly via dopamine.

Both central dopaminergic and nor-adrenergic systems(Serotonin , histamine , epinephrine,nor epinephrine) along with estrogen and thyroxine regulates prolactin secretion from the pituitary

Tyrosine hydroxylase enzyme is absent in Anterior pituitary hence dopamine has to reach anterior pituitary through TIDA or Tuberohypophyseal system.

I] Pathways of Dopamine reaching Anterior pituitary

1. Tuberoinfundibular dopamine pathway

ARCUATE NUCLEUS → TIDA neurons → MEDIAN EMINENCE → PORTAL CIRCULATION ↓ ANTERIOR PITUITARY

2. Tuberohypophyseal system:

Nucleus preopticus periventricularis, rostral part of arcuate nucleus ↓ anterior pituitary

II] Beta adrenergic regulation of pituitary prolactin secretion:

Beta adrenergic agonist stimulates prolactin release. The pathway is

Nucleus reticularis lateralis + Nucleus tractus solitaries → Medulla oblongata ↓ portalsystem ↓ Anterior Pituitary [138]

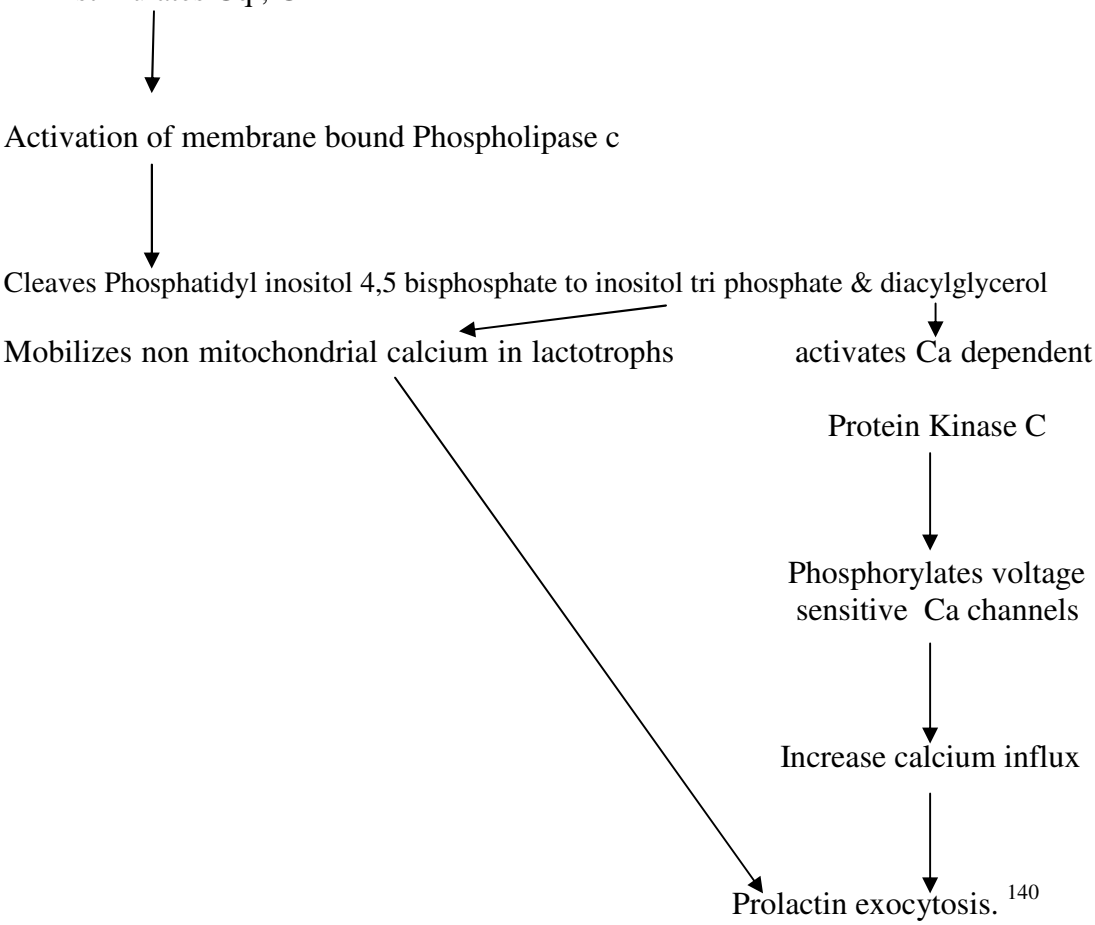
OTHER FACTORS INVOLVED IN REGULATION OF SYNTHESIS

A. **OXYTOCIN** : It increases prolactin levels by inhibiting TIDA system through vaso active intestinal polypeptide[137]

B. **SEROTONIN**: Serotonin affects Prolactin levels through one or more of the Prolactin releasing factors like vaso active intestinal polypeptide(VIP), oxytocin(OT),Vasopressin,Thyroid stimulating hormone . Serotonin exhibits its effect through 5HT1A receptor. It acts by enhancing adenylate cyclase activity, increasing the gene expression of Prolactin.[138]

C. GABA: Acts through Tuberoinfundibular GABA system. Inhibition of TIDA cells occur by serotonergic stimulation of GABA interneurons.[139]

D. TRH: Role of TRH in PRL secretion is unresolved . May act through Protein Kinase C pathway.[137]



E.HISTAMINE: Mediated by dopaminergic aswellas serotoninergic neurons [141]

F.OPIODS : Regulate Prolactin secretion by synchronizing pulsatile pattern of prolactin release[142]

G.ESTROGEN AND PROLACTIN

Estrogen causes increased prolactin by reducing dopamine synthesis.

- Estrogen reduces tyrosine hydroxylase activity
- Decrease gene expression in Tubero infundibiular dopaminergic neurons
- Decrease dopamine concentration in arcuate nucleus.
- Decreases the basal and stimulated Dopamine release from median eminence

HYPERPROLACTINEMIA

Hyperprolactinemia is the most common endocrine disorder of hypothalamic – pituitary axis [143-145].Expected rate of hyperprolactinemia in normal population is upto 3% [106]. Defined as ▪ 20ng/mL in male and ▪ 25ng/mL in female atleast 2 hours after waking up[146].Prevalence ranges from 0.4% in an unselected adult population to as high as 9 to 17% in people with reproductive diseases [144] .About 40% of primary hypothyroid patient, 30% of chronic renalfailure patients, 80% of patients on hemodialysis have elevated Prolactin in serum. Many medications cause Prolactin levels to rise above 100ng/mL[146].

Macroprolactin

The predominant isoform in healthy subject and prolactinoma patients is Monomeric PRL-23 KDa, it contributes 80% of the serum prolactin . The remaining

20% is contributed by the big prolactin 45 to 60 KDa and big-big or macroprolactin 150 to 170 KDa [147-148].Many studies show hyperprolactinemia is due to macroprolactin because of low renal clearance and decreased stimulation of dopaminergic tonus [149].Gold standard for diagnosis of macroprolactin is Gel filtration chromatography . Screening method for macroprolactin detection is by serum precipitation with Polyethyleneglycol(PEG) [150,151].

CAUSES OF HYPERPROLACTINEMIA

I. **Physiological causes**

Pregnancy; lactation; stress; sleep; coitus; exercise

Pregnancy and breast feeding are the most common cause for hyperprolactinemia in females .

Psychological stress causes minimal elevation of serum Prolactin levels .The other forms of stress like physical discomfort, exercise, hypoglycemia ,surgery , fear of venipuncture , myocardial infarction are potent stimulus for Prolactin release

II. **Pathological causes**

a. SYSTEMIC DISEASES

Primary hypothyroidism, adrenal insufficiency, PCOS, renal insufficiency, cirrhosis, pseudocyesis, epileptic seizures

b. HYPOTHALAMIC DISEASES

- Tumors : craniopharyngiomas, dysgerminoma, meningiomas, etc.

- infiltrative disorders : histiocytosis, sarcoidosis, etc.
- metastasis
- cranial radiation
- Rathke's cleft cysts, etc.

c. PITUITARY DISEASES

Prolactinomas; acromegaly; thyrotropinomas; Cushing's disease; infiltrative disorders; metastasis; lymphocytic hypophysitis; emptysella syndrome, etc.

d. STALK DISORDERS

Hastitis; seccion; traumatic brain injury (TBI)

e. NEUROGENIC

- Chest wall lesions: burns, breast surgery, thoracotomy, nipplerings, herpes zoster; etc
- Spinal cord injury : cervical ependymoma, tabes dorsalis, extrinsic tumors, etc.
- Breast stimulation

f. ECTOPIC PROLACTIN PRODUCTION

Renal cell carcinoma, ovarian teratomas, Gonadoblastoma, non-Hodgkin lymphoma, uterine cervical carcinoma; colorectal adenocarcinoma, etc.

III. **Idiopathic**

IV. **Macroprolactinemia**

V. **Drug-induced hyperprolactinemia**

- Antipsychotics:

  Typical – Phenothiazines; butyrophenones; thioxanthenes

  Atypical – Risperidone; molindone; amisulpride; quetiapine; olanzapine

- Antidepressants:

  Tricyclics – Amitriptyline; desipramine; clomipramine

  MAO inhibitors – Pargyline; clorgyline

  SSRIs – Fluoxetine; citalopram; paroxetine

- Antihypertensive drugs: Verapamil; a-methyldopa; reserpine; labetolol

- Anticonvulsivants: Phenytoin

- Prokinetic agents: Metoclopramide; domperidone

- Others: Estrogens; anesthetics; cimetidine; ranitidine; opiates; methadone; morphine; apomorphine; heroin; cocaine; marijuana; alcohol; sibutramine, etc

MECHANISM OF HYPERPROLACTINEMIA BY DRUGS

Drugs act by altering the dopamine or serotonin or GABA metabolism either act directly or via some peptides like VIP, hormones – estrogen etc.

Atypical antipsychotics are the most common cause of drug induced hyperprolactinemia. Antipsychotic drugs decrease dopamine kevels or Dopamine

receptors in the CNS. Antidepressants affect serotonin metabolism. Antihypertensives act by influencing the adrenergic neurons or on calcium fluxes. These are some of the mechanisms through which drugs act.

ESTIMATION OF SERUM PROLACTIN

Though Prolactin concentration varies throughout the day time of the day is not critical in its measurement [152].Supine position prior to sampling is not mandatory. Vigorous exercise and nipple stimulation has to be avoided for atleast 30 minutes before sample withdrawal[153].Even the fear of veinpuncture can cause mild increase in Serum prolactin (40-60ng/mL)[154].

Diagnosis of hyperprolactinemia can be made when prolactin levels on two separate occasions are more than the standard upper limit of normal range 20-25ng/mL[155].Even a single normal value has to be considered as normal ; an isolated raised level is usually spurious [156].

Repeat sampling at 15 to 20 minutes interval for 3 to 4 times another day would account for possible prolactin pulsality [157].To rule out prolactin elevation as a result of vein puncture, sample has to be collected from an indwelling catheter after 2 hours of rest . Sample need to be obtained at 20 minutes interval for the ensuing 2 hours [152].

A single determination is usually sufficient to establish the diagnosis when the levels are more than 100ng/mL [157].

Thus by reviewing the literatures the significance of Prolactin in immune regulation and its role in Autoimmune disease was understood. Hence this study was carried out.

AIMS & OBJECTIVES

AIM OF THE STUDY

The aim of the study is to assess the concentration of Prolactin in serum of the patients with recently diagnosed rheumatoid arthritis and to correlate serum Prolactin levels in those patients with disease activity

The objectives of the study include

- To compare the Serum Prolactin concentration in patients with recently diagnosed Rheumatoid arthritis with apparently healthy individuals.
- To correlate Serum Prolactin concentration levels with Rheumatoid Factor, Anti CCP antibodies and Erythrocyte Sedimentation Rate.

MATERIALS & METHODS

MATERIALS AND METHODS

This study was conducted following ethical committee approval from institutional ethics committee, Madras Medical college held on 11.12.2013 .The approval is enclosed. Volunteers were enrolled only after obtaining informed consent, a copy of the informed consent and the information sheet regarding the study is enclosed .

STUDY DESIGN : CASE – CONTROL STUDY

CASES : 55 recently diagnosed Rheumatoid arthritis patients by the Rheumatologists in the Rheumatology OPD according to ACR criteria 2010.

CONTROLS: Apparently healthy volunteers among the staff and students of Madras Medical College .

STUDY CENTRE: Department of Rheumatology & Institute of Biochemistry, Madras Medical college.

STUDY PERIOD : January 2014 – September 2014

INCLUSION CRITERIA : Recently diagnosed Rhematoid arthritis patients by American college of Rheumatology(ACR) criteria 2010 .

EXCLUSION CRITERIA

1. Rheumatoid arthritis patients already on treatment- steroids, NSAIDS, DMARDS.
2. Pregnant and lactating mothers , infertile individuals .Women on oral contraceptive pills.

3. PCOS, Hypothyroidism, renal failure, diabetes mellitus, hypertension.
4. Other autoimmune diseases, any chronic illness .
5. Individuals with signs and symptoms of hyperprolactinemia, pituitary microadenoma, macroadenoma.
6. Patients on H2blockers, dopamineagonist, antipsychotics, isoniazid, antidepressants, Anticonvulsants ,calcium channel blockers, chemotherapy, hormone replacement therapy, methyl dopa , cannabis abuse
7. People with recent chest wall trauma or irritation/ pain in chest region

PARAMETERS ASSESSED

1. Clinical examination – number of swollen and tender joints.
2. Hemoglobin , ESR , CRP , RF
3. Disease Activity Score DAS (28)3
4. Serum Urea, Serum Creatinine
5. Serum Thyroid stimulating hormone
6. Serum Prolactin, Serum Anti cyclic citrullinated peptide.

STUDY SUBJECTS SELECTION

1. The patients attending Rheumatology OPD with symptoms of early morning stiffness of more than 1 hour , symmetrical joint involvement – pain, tenderness , swelling in the joints above 20 years of age , clinically diagnosed as Rheumatoid arthritis were considered.
2. They were seated , height , weight and blood pressure measured.

3. The swollen and tender joints counted.
4. Hemoglobin , ESR , Rheumatoid factor , CRP of these patients are determined in the Rheumatology department and we have used those data for this study.
5. 5 ml of peripheral venous blood collected from these patients by applying tourniquet.
6. Samples are collected around 9.00 am to avoid diurnal variation of prolactin in fasting state .
7. Subjects with elevated TSH > 5µIU/mL, creatinine , urea were excluded .
8. In controls ; individuals with negative CRP , RF , ESR ≤10 mm in 1 hr are included.

SAMPLE PROCESSING

Serum was separated by centrifugation at 3000 rpm/min for 10 minutes after clot formation. Serum was aliquoted and stored at -20˚c in the deep freezer until analysis of Prolactin and Anti CCP antibody.Remaining analytes are assayed as the samples are collected.

DAS – disease activity score calculation[164]

Disease activity score was calculated using

$$DAS = [0.56*\sqrt{T28} + 0.28*\sqrt{S28} + 0.7*\ln(ESR)]*1.08 + 0.16$$

T- no of tender joints, S- no of swollen joints.

Gradesof Disease activity

< 3.2 low activity

3.2 – 5.1 moderate activity

> 5.1 high activity

ACR / EULAR Criteria 2010 : Diagnostic criteria for Rheumatoid arthritis[170]

Includes clinical & serological variables. The total points need to be more than or equal to 6 to have a diagnosis of Rheumatoid arthritis.

- JOINT INVOLVEMENT

Small joints :- metacarpophalangeal joints, proximal interphalangeal joints, the interphalangeal joint of the thumb, second through fifth metatarsophalangeal joint and wrist

Large Joints shoulders, elbows, hip joints, knees, and ankles

1 large joint	0 point
2–10 large joints	1 point
1–3 small joints (with or without involvement of large joints)	2 points
4–10 small joints (with or without involvement of large joints)	3 points
More than 10 joints (with involvement of at least 1 small joint)	5 points

SEROLOGICAL PARAMETERS – Rheumatoid factor , ACPA – "ACPA" stands for "anti-citrullinated protein antibody":

- Negative RF *and* negative ACPA 0 points
- Low-positive RF *or* low-positive ACPA 2 points
- High-positive RF *or* high-positive ACPA 3 points

Acute phase reactants

Elevated erythrocyte sedimentation rate ESR / elevated CRP value (c-reactive protein) 1 point

Duration of arthritis

For symptoms lasting six weeks or longer – 1 point

SERUM PROLACTIN ESTIMATION

METHOD: ELISA- Sandwich method

Kit Manufacturer : Pathozyme - Omega diagnostics

PRINCIPLE

1. Anti Prolactin antibodies are coated on the microtitre walls.
2. Test sera are applied.
3. Monoclonal anti Prolactin labeled with horse radish peroxidase enzyme-conjugate is added.

4. Human Prolactin present in serum binds with horse radish peroxidase labeled anti Prolactin and anti Prolactin bound with microtitre walls. Thus Prolactin is sandwiched between 2 antibodies.
5. After incubation the unbound materials are washed away.
6. The substrate TMB is added, will be acted upon by horse radish peroxidase enzyme present in sandwich complex and a colour change occurs indicating the presence of Prolactin.
7. The reaction is stopped by the addition of dilute hydrochloric acid.
8. Absorbance is measured at 450 nm, the concentration is directly proportional to the colour developed.

MATERIALS REQUIRED

1. Anti- Prolactin coated microtitre plate – 96 wells.
2. Conjugate – Anti – Prolactin HRP conjugate .
3. Substrate solution – 3,3',5,5' Tetramethyl benzidine in citrate buffer.
4. Stop solution – 1 M HCl, hydrochloric acid dissolved in deionised water.
5. Calibrators:

Cal A	0 ng/mL	Lyophillised human serum free of Prolactin.
Cal B	5 ng/mL	Prolactin diluted in human serum – lyophillised.
Cal C	15 ng/mL	
Cal D	50 ng/mL	
Cal E	100 ng/mL	
Cal F	200 ng/ mL	

6. Micropipettes - 100µL, 200 µL, 1000µL
7. Disposable pipette tips.
8. Absorbent paper.
9. Microtitre plate reader with 450 nm

Reagent preparation

1. All reagents are brought to room temperature.
2. 1 ml of distilled water is added to standard vials and made to stand for 20 minutes, then mixed gently.
3. All other reagents are ready to use.

PROCEDURE

1. 50 µL of standard are pipetted in the1st 6 wells and study subjects serum added in the remaining wells.
2. 100 µL of conjugate added in all wells , mixed for 10 seconds.
3. Incubated for 45 minutes at 20 -25˚c.
4. Machine washing is done 5 times using distilled water, 300 µL distilled water is used per cycle for each well.
5. The remaining fluid present in wells are removed by striking the wells against absorbent paper.
6. 100 µL of substrate solution is added in each well, gently shaken for 5 seconds.

7. Incubated in dark for 20 minutes.

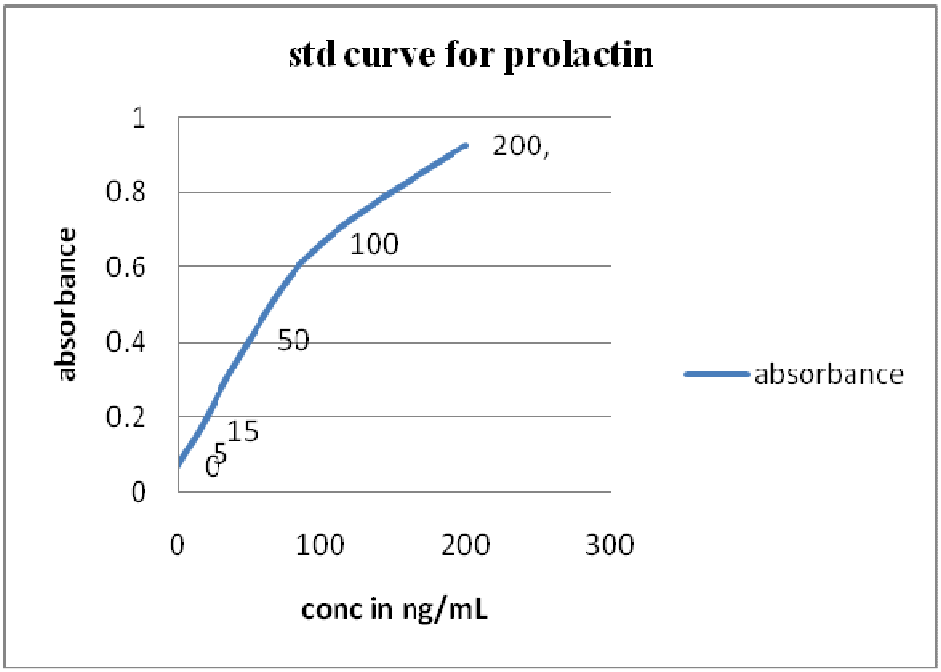
8. 100 µl of stop solution is added in each well, gently shaken for 30 seconds.

9. Optical density is measured at 450nm in ELISA reader .

Calculation of results

1. The standard curve is plotted using concentration along x – axis & absorbance along y- axis

concentration ng/mL, PRL	absorbance
0	0.0738
5	0.1071
15	0.1678
50	0.4129
100	0.6686
200	0.9279



Using the absorbance values for each sample the concentration is calculated with the help of standard curve.

Anti Citrullinated Peptide Antibody(Anti CCP)

METHOD: ELISA- Sandwich

KIT manufacturer : G.E.N.E.S.I.S Diagnostics

Principle

1. Diluted serum samples are incubated with recombinant citrullinated rat fillagrin immobilized on microtitre plate wells.

2. The unbound serum components are washed,rabbit anti-human IgG conjugated to horse radish peroxidase is added which binds with surface bound antibodies on incubation.

3. Unbound conjugate is removed by washing.

4. TMB –substrate is added,incubation allowed for the reaction to take place.

5. Then stop solution is added and optical density is measured at 450 nm, colour developed is directly proportional to concentration of anti ccp.

Materials required

1. Recombinant citrullinated rat fillagrin coated microtitre plate – 96 wells.
2. Sample diluent- 150 mM Tris-buffered saline,pH- 7.2 with antimicrobial agent.

3. Wash buffer – 100 mM,Tris buffered saline with detergent ,pH- 7.2
4. Conjugate – rabbit antihuman IgG conjugated to horse radish peroxidase in protein stabilizing solution and antimicrobial agent.
5. Stop solution- 0.25M sulphuric acid.
6. Standards- 0,6.25,12.5,25,50,100 U/mL in 1mL of 10mM Tris-buffered saline containing human serum IgG antibodies to citrullinated protein.
7. Positive control
8. Negative control
9. Disposable micropipette tips, 10µL & 100µL micropipettes
10. Absorbent paper, de-ionised water.

REAGENT PREPARATION

1. Sample diluent – 1: 14, 14 parts of distilled water.
2. Wash buffer- 1: 9, 9 parts of distilled water.

Sample Preparation

Serum diluted with sample diluent in 1:100, 100 parts of sample diluent.

PROCEDURE

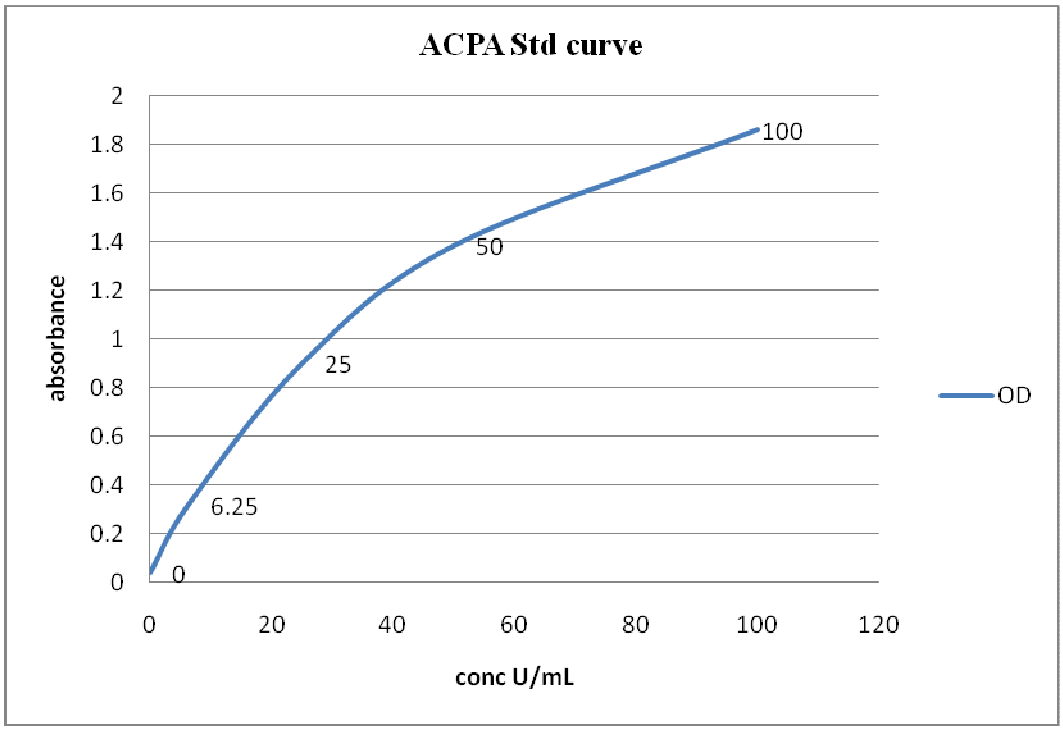
1. The standards, controls , diluted samples - 100µL are pipetted in to appropriate wells.
2. Incubated for 30 minutes at room temperature.

3. After 30 minutes , machine washing is done.
4. Then 100µL of conjugate is added,incubated at room temperature for 30 minutes.
5. Machine washing is done.
6. 100µL of TMB substrate is added, incubated in dark for 10 minutes.
7. 100µL of stop solution is added
8. The optical density is read at 450 nm in ELISA reader.

Calculation

1. The standard curve is plotted using concentration along x – axis & absorbance along y- axis

standards	Conc U/mL	OD
1	0	0.038
2	6.25	0.316
3	12.5	0.580
4	25	0.898
5	50	1.383
6	100	1.860



3. Using this standard curve the concentration is calculated .

THYROID STIMULATING HORMONE

METHOD: - Immuno enzymometric assay

KIT manufacturer : Biotron

PRINCIPLE: The immobilization of antigen occurs during the assay. The well is coated with streptavidin. TSH in the serum forms a complex with Enzyme labeled Polyclonal Anti- TSH and BIOTIN labeled MONOCLONAL Anti TSH.This sandwich of TSH(Ag) between two antibodies binds to streptavidin coated wall by means of biotin labeled monoclonal anti TSH.

1.Ag+ E-poly Ab+B-mono Ab ⟶ B-monoAb-Ag-poly Ab-E.
2.Streptavidin –B-monoAb-Ag-polyAb-E - immobilized complex

Ag- TSH

E-poly Ab- Enzyme labeled polyclonal antibody

B-monoAb- biotinylated monoclonal antibody

3. The unbound fraction are separated.

4. The enzyme activity determined by addition of substrate, the colour developed is directly proportional to the concentration of TSH.

MATERIALS REQUIRED

1. Streptavidin coated plate (96 wells).
2. TSH enzyme reagent
3. Wash solution
4. Substrate A
5. Substrate B
6. Stop solution
7. Micropipettes 50& 100µL
8. Disposable tips, absorbant paper
9. ELISA reader
10. 7 standards 0,0.5,2.5,5,10,20,40 (µIU/mL)
11. Control – 7.7 µIU/mL

REAGENT PREPARATION

1. WORKING SUBSTRATE: equal volumes of substrate A & B are mixed and need to be used immediately.

2. Wash buffer: The wash concentrate is diluted to 1000ml with distilled water.

PROCEDURE

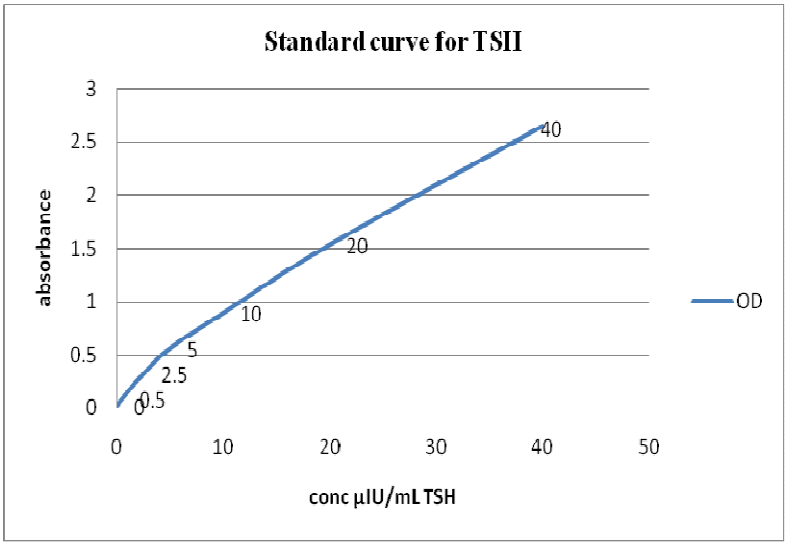
1. 50µL of sample, standard, control are pipetted in to the corresponding wells.
2. 100 µL of TSH enzyme reagent is added.
3. The plate is swirled gently for 12 seconds ,then incubated at room temperature for 60 minutes.
4. Machine washing done- 3 cycles using 300µL of wash buffer per cycle
5. 100 µL of working substrate (freshly prepared) is added.
6. Incubated at room temperature for 15 minutes.
7. 50µL of stop solution is added.
8. Mixed gently for 10 seconds
9. Absorbance measured at 450 nm, refence wavelength -620 nm in ELISA reader.

CALCULATION

1. Graph is drawn using concentration along x axis and absobance along y axis.

2. Using this graph the concentration of individual samples are calculated.

conc	absorbance
0	0.019
0.5	0.088
2.5	0.32
5	0.56
10	0.89
20	1.532
40	2.647



RHEUMATOID FACTOR (semi Quantitative method)

METHOD: Latex Agglutination

KIT Used : ACCUCARE

PRINCIPLE: Rheumatoid factor present in serum agglutinates the latex particles coated with human gamma globulin.If the rheumatoid factor level in serum is greater than 8 IU/mL, agglutination will occur.

MATERIALS REQUIRED

1. RF Latex reagent
2. Positive control
3. Negative control
4. Glass slides
5. Stirrer rods
6. Droppers

PROCEDURE

1. 1 drop of serum,1 drop of positive control, 1 drop of negative control are added in different circles on the slide
2. RF Latex reagent is added in all the circles.
3. Stirrer is used to mix and spread the contents in test circle.
4. The slide is rotated and observed for agglutination with in 2 minutes.

5. The samples showing agglutination are positive for Rheumatoid factor.
6. These samples are quantitated by diluting with normal saline.
7. Samples in these dilutions 1:2,1:4,1:8,1:64 are prepared and subjected to the above described method until there is no agglutination .

CALCULATION

RF = Highest dilution with positive reaction * 8 IU/mL (reagent sensitivity)

C Reactive Protein (Qualitative)

METHOD : Latex Slide agglutination.

KIT used : Pathozyme

PRINCIPLE: CRP in the serum binds with latex particles coated with monospecific anti human CRP. Sensitized to detect levels greater than 6µg/mL.

MATERIALS REQUIRED

1. CRP Latex reagent.
2. Positive control
3. Negative control
4. Disposable droppers, sample applicators.
5. Glass slide

PROCEDURE

1. One drop of serum is added on the slide
2. Then one drop of CRP Latex reagent is added.
3. Gentle to and fro motion is made and watched for macroscopic agglutination with in 2 minutes.

RESULTS

1. Coarse agglutination – strongly positive
2. Finer agglutination - weakly positive
3. smooth suspension/ no noticeable change - negative

ESTIMATION OF CREATININE (auto analyser)

METHOD: Modified Jaffe's reaction.

Kit Used : Erba Mannheim XL System Packs

PRINCIPLE : Creatinine reacts with alkaline picrate to produce a reddish orange colour. This is a non specific reaction .

REAGENT COMPOSITION

R1 Sodium hydroxide – 240 mmoles/L

R2 Picric acid - 26 mmoles/L.

CALIBRATION: Done with serum based XL multical calibrator.

ASSAY PARAMETERS:

Primary wavelength – 505 nm

Secondary wavelength– 570 nm

Assay type – Rate A

Curve type – Linear.

R1 volume – 160 µL

R2 volume – 40 µL

Sample volume – 10 µL.

CALCULATION : Results are calculated automatically by the instrument.

ESTIMATION OF UREA (autoanalyser)

METHOD: Urease – Glutamate dehydogenase.

Kit Used : Erba Mannheim XL System Packs

PRINCIPLE

Urea is hydrolysed by urease enzyme to ammonia and carbon di oxide in the presence of water.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} NH_3 + CO_2$$

Ammonia combines with α – ketoglutarate in the presence of NADH to give glutamate & NAD

$$\backslash NH_3 + \alpha \text{ ketoglutarate} + \text{NADH} \xrightarrow{\text{Glutamate dehydrogenase}} \text{L- Glutamate} + \text{NAD}$$

The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to formation of NAD.

REAGENT COMPOSITION

R1 Tris buffer 100mmol/L

Alpha – ketoglutarate 5.49 mmol/L

Urease (Jack Bean) ≥ 10KU/ L

GLDH (microorganism) ≥2.5KU/ L

R2 NADH 1.66 mmol/L

CALIBRATION : Done with serum based xL multical calibrator.

ASSAY PROCEDURE:

Primary wavelength 340 nm

Secondary wavelength 415 nm

Assay type Rate A

Curve type Linear

R1 volume 160µL

R2 volume 40µL

Sample volume 2 µL

CALCULATION : Results are calculated automatically by the instrument

ERYTHROCYTE SEDIMENTATION RATE.

METHOD

Conventional Westergrens method.

PROCEDURE

1. EDTA anticoagulated blood is drawn upto 200mm mark in the westergrens tube.
2. The tube is placed vertically and left undisturbed for 60 minutes.
3. The cells get sedimented , the nearest 1 mm is read just above the sedimented cells .

The measured reading is ESR, expressed in mm in 1 hr.

STATISCAL ANALYSIS

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software version 20 and the following were carried out

- Tests of significance at 5% significance using Unpaired students't-test was done to compare the Serum Prolactin between cases and controls.

- Pearsons correlation co-efficient was done to measure the linear relation relationship between serum prolactin concentration and other parameters like DAS28(3), ESR , Anti CCP, ACR score , Symptom duration , Age.

- One Way - ANOVA Analysis of Variance to compare more than 2 variables in the same group & between groups was carried out to compare serum prolactin concentration between four groups viz- RF & Anti CCP positive, RF positive Anti CCP negative , RF negative Anti CCP positive , RF & Anti CCP negative.

RESULTS

sym.dur	crp	esr	RF	RF values	ACR score	das	**PRL**	Anti CCP	TSH µIU/ml	hb	menstruation	ureamg/dL	creatinine mg/dl	ht cm	wgt kg	Bp mm of Hg
4 mths	neg	16	pos	5	6	4.5	4	2.27	1.5	9.8	post	27	0.7	152	55	130/80
2 mths	pos	30	pos	8	8	4.8	15.6	3.87	1.3	10	post	24	0.8	156	60	120/70
8mths	pos	10	pos	130	7	4.1	30	192.17	1.8	8.5	11th day	18	0.7	155	62	120/70
9mths	pos	10	neg		6	4.2	30.9	4.64	1.2	9.5		19	1.2	168	70	130/80
2mths	pos	14	neg		7	3.79	32	102	3.32	10.4	6th day	25	1	150	57	130/80
8mths	pos	44	neg		7	5.67	37.7	29.12	3.6	9.5	post	20	1.1	156	65	140/80
12mths	pos	66	pos	138	10	7.73	21.4	182.32	3.2	8.5	3rd day	27	0.7	160	65	122/70
6mths	pos	28	pos	140	6	3.96	16	198.67	1.3	10.2	26th day	28	0.9	161	56	130/80
6mths	pos	26	neg		6	5.49	28	22.6	2	11.6	6th day	26	0.8	164	59	120/80
3mths	pos	34	neg		7	4.64	42	9	1.2	9.8		32	1	170	60	130/80
2 mths	neg	22	neg		6	4.13	33	23.79	1.8	10.2	3rd day	25	0.8	154	63	120/80
3mths	neg	15	neg		6	3.49	27	23.12	3.5	11		21	1.3	158	56	130/80
2 mths	neg	10	neg		6	5.04	28	27.48	1.3	9.8	11th day	26	1	149	58	120/80
3mths	pos	50	neg		7	5.98	16.2	4.38	1.5	9.8	post	28	1.2	157	59	126/80
2 mths	neg	4	neg		7	5.17	12.5	6.5	5.5	10.5	11th day	36	1.1	152	60	130/70
3mths	pos	165	neg		7	6.89	54.9	24.75	1.2	11.6	21st day	32	0.8	154	56	120/70
2 mths	neg	20	neg		7	5.29	28	140.8	2.1	10	11th day	24	0.5	150	54	120/80
4mths	pos	90	neg		7	6.57	50	24.83	1	9.2	post	20	1.2	156	55	120/80
6mths	pos	30	neg		6	5.59	33.6	20.5	1.2	8.5	post	22	1.2	148	43	130/80
4mths	pos	64	pos	32	6	6.6	34	35.4	3.6	11.8	9th day	27	1	151	48	120/70
8mths	pos	60	pos	191.8	10	6.43	55.8	7.6	2.3	9.2	11th day	21	0.7	153	54	130/80
4mths	pos	65	neg		7	6.18	24.6	8.1	1.8	10.4	post	24	0.9	150	49	120/70
3mths	pos	10	neg		6	4.03	22.2	7.54	3.2	9.6	22nd day	29	0.5	152	56	130/80
3mths	pos	38	neg		6	4.72	24.4	19.14	0.9	11.2		24	1.3	169	59	120/80
5mths	neg	60	neg		6	6.94	20.8	3.68	2.2	12		31	1.1	165	61	110/70
3mths	pos	100	neg		7	7.05	29.8	8.89	1	11.2		24	1.2	160	56	126/80
4mths	neg	22	neg		6	4.94	24.1	22.66	1.1	9.4	12th day	22	0.8	154	58	112/70
3mths	neg	30	neg		6	5.89	45.4	58	1.4	10	9th day	20	1	155	59	120/70
6mths	neg	40	neg		7	4.76	11.3	208	1.5	9.2	post	28	1.2	153	60	130/80
5mths	neg	22	pos	128	7	5.74	43.2	51	0.9	13.2		24	1.2	171	65	130/80
6mths	neg	45	pos	32	7	6.82	79	17.15	1.3	8.6	post	29	1.1	168	65	130/80
8mths	pos	33	pos	128	10	5.2	32.5	243.36	1.2	9.3	8th day	31	0.5	150	53	120/80
4mths	pos	50	pos	128	8	5.98	58.2	21.67	2.6	10.4	post	13	1.1	154	49	130/80
2mths	pos	52	pos	16	7	5.54	15.2	1.099	1.3	10.4	21st day	19	0.7	149	50	120/80
6mths	pos	18	neg		7	4.25	17.8	3.18	1.9	11	hystrectomy	27	0.6	152	55	120/70

5mths	pos	35	neg		7	6	57.6	103.33	1.5	9.6	4th day	12	0.9	164	55	110/70
6mths	pos	35	pos	64	10	4.54	17.4	15.75	4.2	9.8	7th day	22	0.7	156	56.5	120/70
2 mths	neg	40	pos	64	7	5.35	44.8	36.78	1.3	9.7	24th day	19	1	153	60	130/80
6mths	pos	40	pos	64	10	5.46	88	211.5	3.4	10.4	18thday	28	1.1	160	49	110/70
2mths	pos	35	pos	256	8	5.96	36	2.14	1.3	9.5	15th day	25	0.6	150	57.5	130/80
7mths	pos	45	pos	16	10	6.73	11.7	139	1.1	10.7	post	20	1	151	59	120/80
3mths	pos	50	pos	256	10	4.59	15.7	14.75	1.5	10.5	post	31	1.2	148	52	122/70
6mths	neg	11	pos	64	6	4.62	48.5	113.8	0.9	9.8	post	25	0.9	156	63	130/80
6mths	pos	20	pos	128	8	4.67	36.8	40	1.3	9.3	22nd day	22	0.7	162	57	120/80
9mths	pos	35	pos	64	7	4.92	14.6	6	1.5	9.2	post	21	0.8	149	55	120/80
5mths	pos	56	pos	128	10	6.37	70.5	9	2.1	10.5	21st day	25	0.5	153	58	130/80
6mths	pos	25	pos	128	10	5.76	25.6	1.72	1.3	10.2	12th day	26	0.8	149	57	120/70
3mths	pos	20	neg		7	5.07	30.5	71.4	5.4	10.3	post	28	0.7	145	59	122/70
6mths	pos	95	pos	64.4	7	5.5	37.4	22.65	1.4	10.9	post	27	0.8	160	56	130/80
6mths	pos	32	neg		7	5.3	22.7	9.1	1.2	9.8	2nd day	29	1	156	60	120/80
2mths	pos	36	neg		6	4.56	29.4	22.5	1.3	11	8th day	17	0.5	149	54	130/80
3mths	pos	40	neg		6	5.08	27.1	1.2	2	10.5	11th day	30	0.8	161	63	120/80
6mths	neg	10	neg		6	3.8	29.9	29.26	1.2	10.4	post	26	0.7	150	60	120/80
6mths	pos	32	neg		10	5.99	76.7	0.81	2.3	9.8	24th day	19	0.5	154	49	120/80
7 mths	neg	38	neg		6	5.42	44.2	1.43	1.1	11.2	post	26	0.9	148	60	120/80

s.no	name	age	sex	rf	crp	esr	TSH µIU/ml	anti ccp	prl	hb	menstruation	ureamg/dL	creatininemg/dL	ht cm	wt kg	BPmmofHg
1	shakila	56	f	neg	neg	12	0.9	4.57	1.6	11	post,10yrs	24	1	153	53	130/80
2	priya	39	f	neg	neg	10	1.3	3.02	10.2	11.2		23	0.6	167	72	120/70
3	faridha	38	f	neg	neg	8	1.2	2.05	20.1	10.6	21stday	22	0.8	155	62	110/70
4	kanan	30	m	neg	neg	12	2.2	16.33	10.7	13		26	1	164	60	120/70
5	viji	25	f	neg	neg	10	1.5	1.8	15	9.8	27thday	18	0.5	150	59	110/70
6	jaya	29	f	neg	neg	12	5.1	2.5	17.5	10.2	25th day	21	0.8	154	60	120/70
7	jerisah	25	f	neg	neg	8	1.3	9.32	15.9	10	15th day	19	0.5	150	53	110/70
8	ravindran	24	m	neg	neg	6	1.9	2.4	17.2	13.2		22	0.8	168	55	120/70
9	suseela	25	f	neg	neg	8	2.9	5	15.2	10.4	3rd day	24	0.8	160	64	122/70
10	raja	53	m	neg	neg	12	1.7	4.4	17.3	12.8		26	1.3	165	75	110/70
11	usha	32	f	neg	neg	8	3.3	5.6	19.1	12	23rd day	20	1	154	64	130/80
12	kajalakshmi	57	f	neg	neg	10	1.3	5.9	7.5	10	post	27	1.1	156	70	130/80
13	bhavani	41	f	neg	neg	10	2.8	5.2	16	11.8	12th day	24	0.8	160	71	120/70
14	revathi	56	f	neg	neg	10	2.5	1.6	5	12.4	post,16yrs	25	0.7	150	52	130/80
15	karthiga	28	f	neg	neg	12	3.1	0.8	19.8	12.8	18th day	21	0.6	150	56	120/80
16	amirtha	34	f	neg	neg	8	2.8	11.2	19.2	13	11th day	25	0.9	162	72	130/80
17	tamilselvi	52	f	neg	neg	12	4.3	1.45	6	10.8	post,8yrs	27	1	160	75	110/70
18	nirmaiadevi	35	f	neg	neg	8	1.9	11.4	20.5	13.2	22nd day	28	1.1	160	76	130/80
19	jaya	42	f	neg	neg	22	3.6	9.8	14.6	10.4	15th day	22	0.6	161	57	120/80
20	lalitha	65	f	neg	neg	22	1.3	2.4	4.5	9.2	post	27	1	156	57	110/70
21	vijayakumari	45	f	neg	neg	12	1.2	2.4	20.8	11.2	24th day	26	1	149	54	120/70
22	govindarajan	67	m	neg	neg	20	1.9	4.8	19.2	12		24	1.3	167	64	130/70
23	rani	30	f	neg	neg	22	3.9	1.36	6.6	12.2	12th day	22	1.2	157	54	120/70
24	rabiya	32	f	neg	neg	12	4.2	12.18	16.4	10.8	8th day	26	1	171	70	120/80
25	jayasudha	31	f	neg	neg	12	2.4	2.6	21.1	12.1	14thday	23	0.7	153	58	130/80
26	kausalya	37	f	neg	neg	16	2.1	6.8	21.1	9.4	16thday	25	0.8	156	50	120/80
27	pushparani	52	f	neg	neg	12	3.2	6.4	10.8	11.2	post	26	0.5	158	51	120/80

RESULTS

Table 1 Distribution of study population based on Gender

GENDER	CASES	CONTROLS	TOTAL
MALE	7(12.7%)	4(14.8%)	11(13.4%)
FEMALE	48(87.3%)	23(85.2%)	71(86.6%)
TOTAL	**55**	**27**	**82**

Table 1 shows the number of males and females in the study population. This table reveals that majority of the study population is females, which implies study is skewed towards females.

Table 2 Distribution of females in study based on menstrual phase.

Phase of Menstruation	CASES	CONTROLS
Bleeding Phase	4(8.3%)	2(8.7%)
Follicular Phase	15(31.3%)	6(26.1%)
Luteal Phase	10(20.8%)	6(26.1%)
Post menopause	19(39.6%)	9(39.1%)
TOTAL	**48**	**23**

Table 2 shows the number of females in each phase of menstrual cycle and the postmenopausal women. Among the premenstrual population, contribution by females in follicular phase was more when compared with luteal phase and bleeding phase.

Table 3 Distribution of age in the study group

AGE in years	CASES	CONTROLS
21-30	10 (18.2%)	8 (29.6%)
31-40	18 (32.7%)	8 (29.6%)
41-50	14 (25.4%)	3 (11.2%)
51-60	10 (18.2%)	6 (22.2%)
>60	3 (5.5%)	2 (7.4%)
Total	**55**	**27**

Table 3 shows that major study population belongs to the age group of 31 to 40 years.

Table 4 –Comparison of Serum Prolactin concentration between cases and control in both males and females.

Variable	Group	N	Mean	Std. Dev	SE Mean	t-Value	P-Value
Prolactin in ng/mL	Cases	55	33.531	17.9264	2.4172	7.161	<0.001 S
	Control	27	14.404	5.9063	1.1367		

S- significant

Serum Prolactin concentration between cases and controls was compared using unpaired students' t- test. The mean Serum Prolactin concentration in cases was 33.531±17.92 ng/mL. The mean Serum Prolactin concentration in controls was 14.4±5.9 ng/mL. The standard error of mean for cases was 2.42. The standard error of mean for controls was 1.14. The 95% confidence interval for mean of serum Prolactin concentration in cases was 50.84 to 16.22 ng/mL. The 95% confidence

interval for mean of serum Prolactin concentration in controls was 22.54 to 6.26 ng/mL. The p value obtained was less than 0.001. It was statistically highly significant.

Figure 1 Mean Prolactin concentration between cases and controls in both males and females.

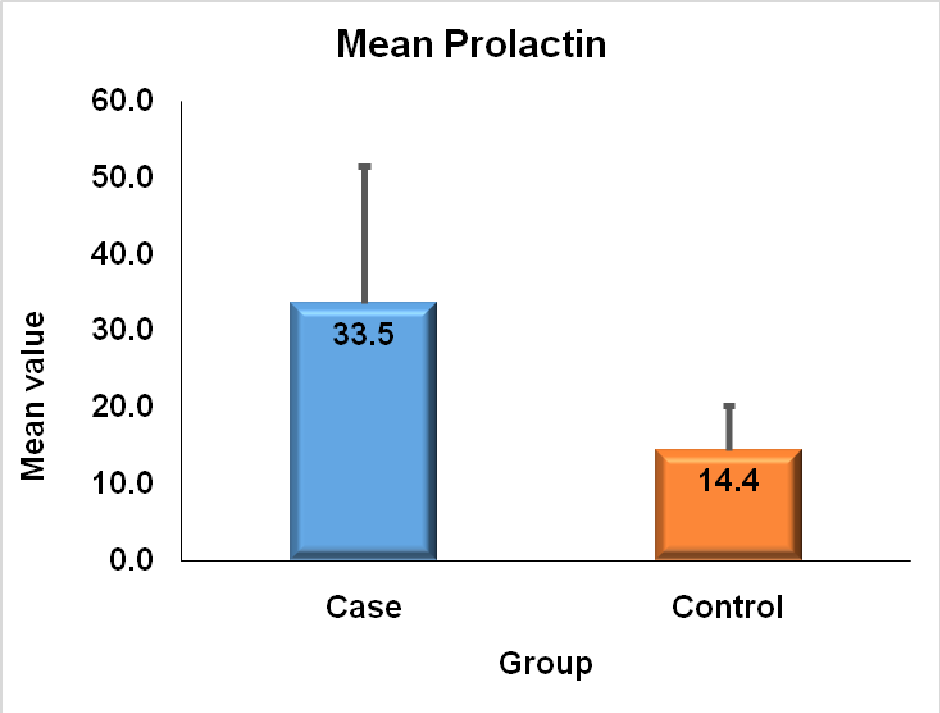


Figure1 shows the mean Prolactin concentration in cases and controls in both sexes.The Serum Prolactin concentration in cases was higher than controls. It is statistically significant.

Table 5 Comparison of Serum Prolactin concentration between cases and controls among females.

Group	Number	Mean PRL ng/mL	Std Dev	SE mean	t-value	p-value
cases	48	33.88	18.75	2.7	4.9174	<0.0001 S
controls	23	14.11	7.9	3		

S - significant

Serum Prolactin concentration between cases and controls among females was compared using unpaired students' t- test . The mean Prolactin concentration in female RA patients was 33.88±18.75 ng/mL. The mean Prolactin concentration in female controls was 14.11±7.9ng/mL. The difference in mean between the two groups was statistically highly significant with the p value <0.0001.

Figure 2- Mean serum Prolactin concentration in female Rheumatoid Arthritis patients and female controls

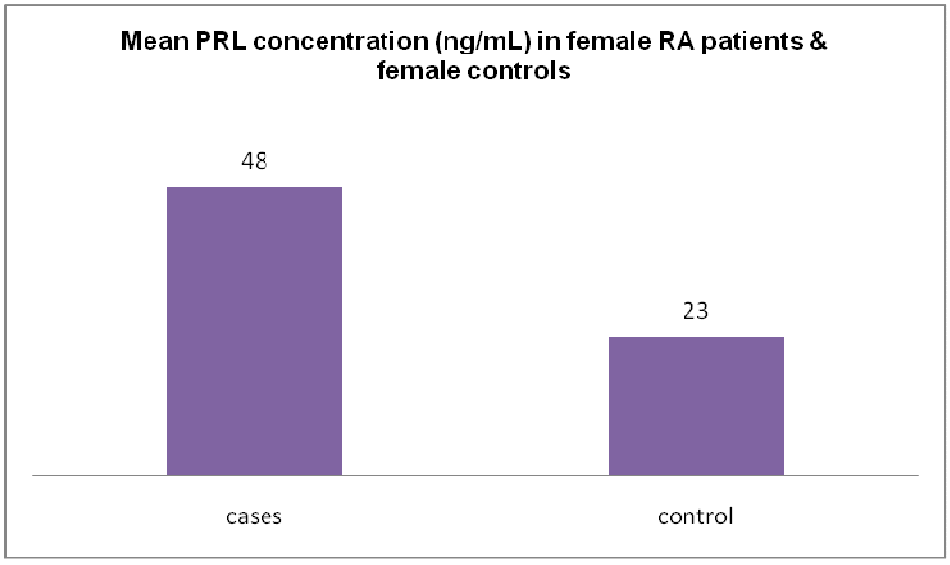


Figure 2 shows the Serum Prolactin concentration in cases and controls among the females in this study group. Serum Prolactin concentration in female RA patients was higher than healthy female controls and it was statistically significant.

Table 6 Comparison of Serum Prolactin concentration between cases and controls among males

Group	Number	Mean PRLng/mL	Std dev	SE mean	t-value	p-value
cases	7	31.16	7.9	3	3.581	0.0059(S)
controls	4	16.1	3.2	1.6		

S - significant

Table6 shows the comparison of mean Serum Prolactin concentration between male RA patients and healthy male controls. The mean Prolactin concentration in male RA patients was 31.16±7.9ng/mL. The mean Prolactin concentration in male controls was 16.1±3.2 ng/mL. The Serum Prolactin concentration in both these groups was compared using unpaired students' t-test . The difference in mean between the groups was statistically significant with the p value- 0.0059.

Figure 3 mean serum Prolactin concentration in male RA patients and apparently healthy male controls.

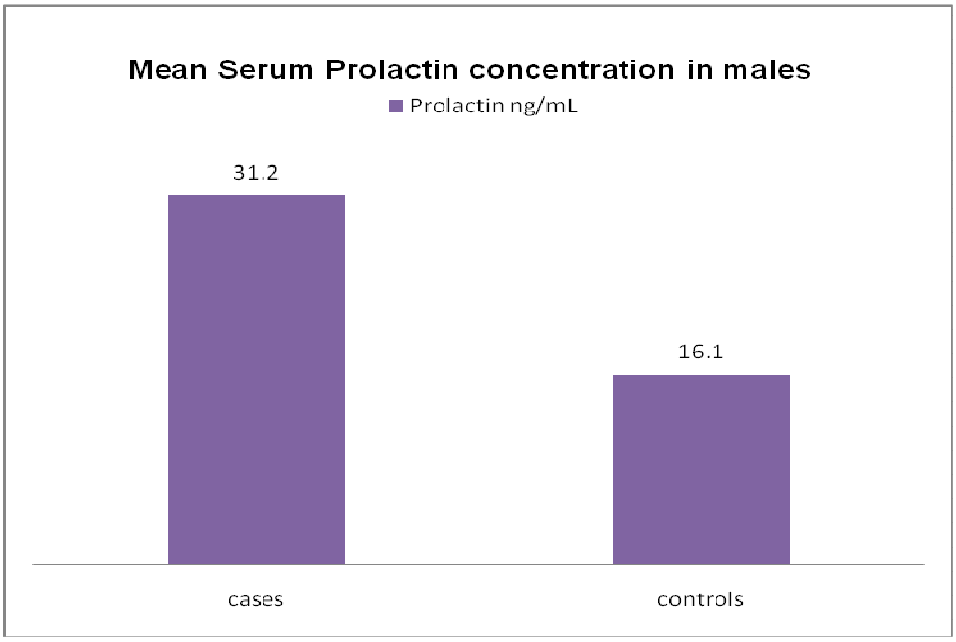


Figure3 shows that Serum Prolactin concentration in male RA patients was higher than apparently healthy male controls. The difference was statistically significant.

Table 7 Mean Serum Prolactin concentration in females in different phases of the menstrual cycle in both cases and controls.

Group	Menstruation phase	Follicular phase	Luteal phase	Post menopausal
Cases	33.7(n=4)	30(n=15)	46.1(n=10)	30.56(n=19)
Controls	10.9(n=2)	17.63(n=6)	18.13(n=6)	9.8(n=9)

Table7 shows the mean prolactin concentration in females in various phases of menstrual cycle . The Prolactin concentration in Luteal phase was higher in both cases and controls . The Serum Prolactin concentration was higher in cases when compared to controls in all the three phases in premenopausal women. The Serum Prolactin concentration was higher in postmenopausal RA patients than the corresponding controls.

Table 8 Mean serum Prolactin concentration & DAS28(3) in both males and female Rheumatoid Arthritis patients.

Group	number	Mean DAS28(3) score	Mean PRL in ng/mL
Moderate activity	22	4.4	25.03
High activity	33	6	39.2

Table 8 shows the mean DAS score and Serum Prolactin concentration in RA patients with moderate and high disease activity. Among cases 22 had moderate disease activity score with a mean DAS score of 4.4±0.4 and mean serum PRL concentration was 25.03±10.3ng/mL in those patients. Among cases 33 individuals

had high disease activity score . The mean disease activity score was 6±0.7 and mean serum PRL concentration was 39.2±19.4ng/mL.

Figure 4 Disease activity in cases based on DAS28(3) in cases.

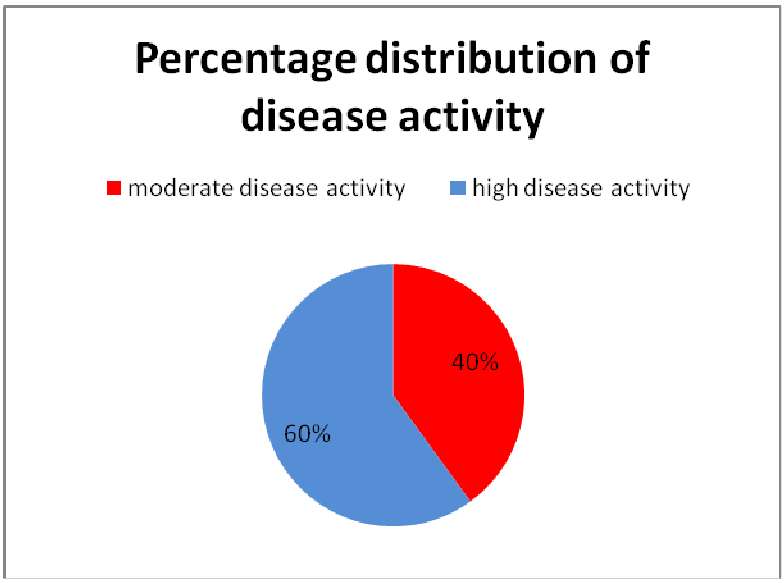


Figure 4 shows among cases 40%(n=22) had moderate disease activity . The DAS score of these individuals varies from 3.2 to 5.1. Among cases 60%(n=33) had high disease activity . The DAS score of these individuals was more than 5.1

Table 9 Comparison of mean Serum Prolactin concentration between moderate and high disease activity RA patients in both males and females.

Group	number	Mean PRL ng/mL	t-value	p-value
Moderate activity	22	25.03±10.3	3.14	0.0028(S)
High activity	33	39.2±19.4		

S- significant

The comparison of mean Serum Prolactin concentration between RA patients with moderate and high disease activity score was done using unpaired students' t-test. The difference in mean between the 2 groups was statistically highly significant with a pvalue- 0.0028.

Figure 5 Mean Serum Prolactin concentration in RA patients with moderate disease activity and high disease activity in both males and females.

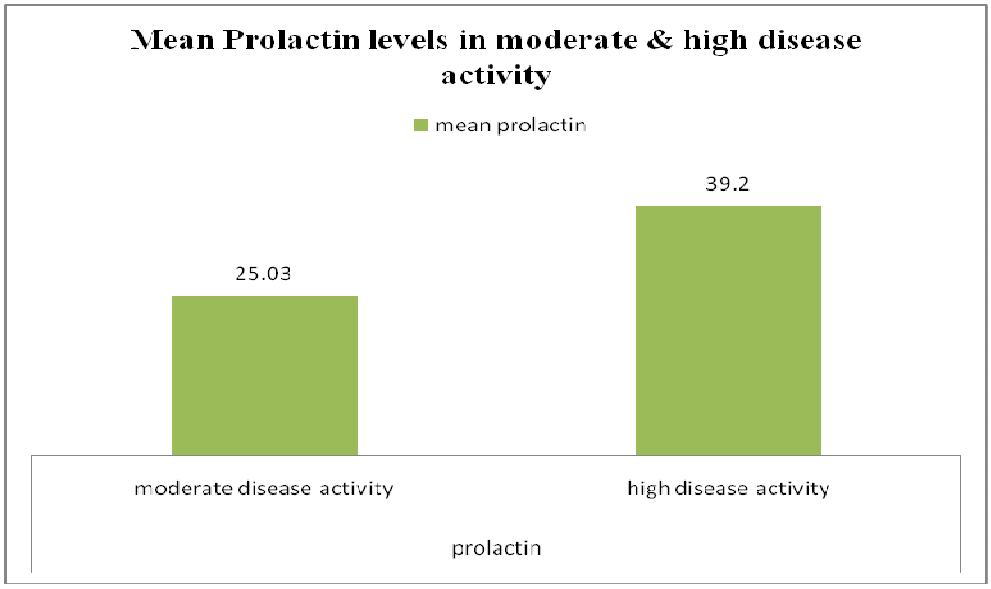


Figure 5 shows the mean serum Prolactin concentration in RA patients with moderate disease activity score and high disease activity score. Mean serum Prolactin concentration in RA patients with high disease activity score was higher than in RA patients with moderate disease activity score.

Table 10 Mean Serum Prolactin concentration in RA patients in association with duration of joint symptoms.

Symptom duration in months	Number	Mean PRL in ng/mL
≤2 months	10	27.45±9.7
3 – 6 months	36	35.86±19.87
>6 momths	9	31±13.2

Table 10 shows the mean Serum Prolactin concentration in RA patients in relation to the duration of joint symptoms.Most of the study population had presented after 2 months of onset of symptoms.

Table 11 Comparison of mean Anti CCP between cases and controls

Group	Number	MeanAnti CCP in U/L	p-value
cases	55	47.5±64.8	0.0012 S
controls	27	5.3±3.9	

S- significant

Table 11 shows the mean Anti CCP levels in cases and controls.The Anti CCP in RA patients was higher than controls. The difference in mean of Anti CCP between cases and control was analysed using unpaired students' t –test and the difference in mean was statistically significant with a p value 0.0012.

Figure 6 Anti CCP status among RA patients in the study group

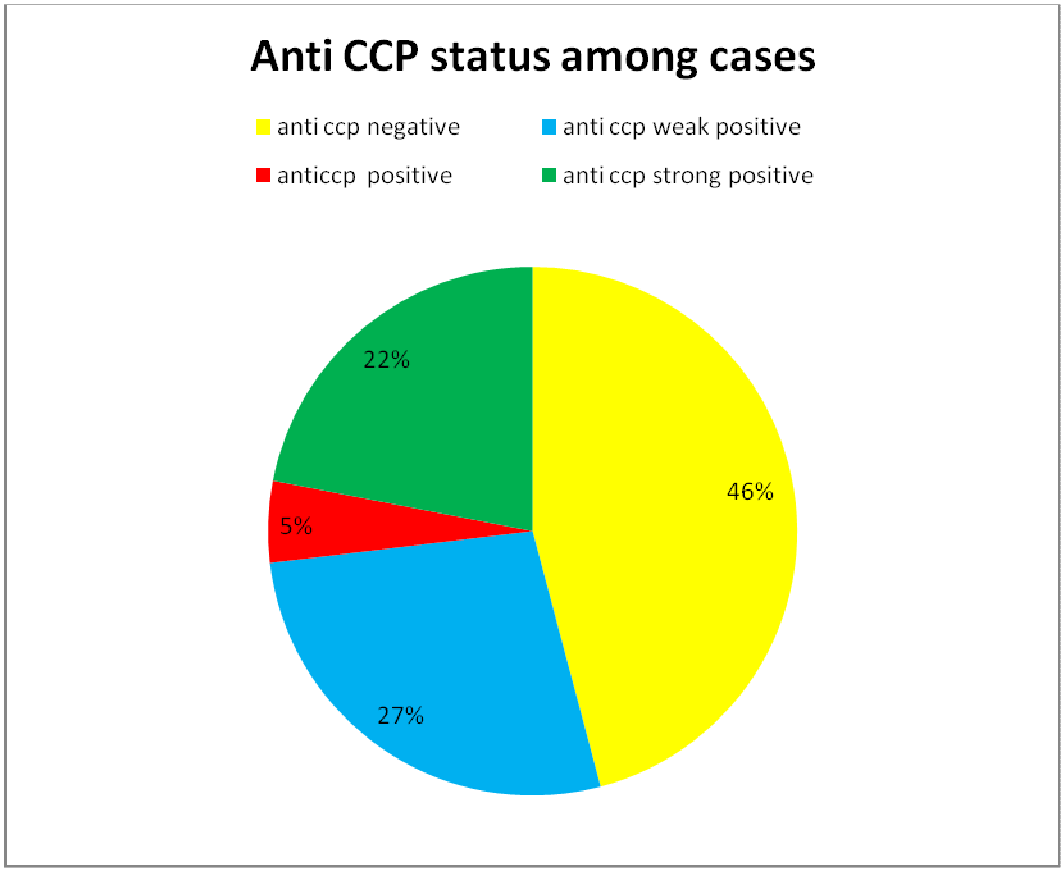


Figure 6 shows the percentage of Anti CCP positivity and Anti CCP negative individuals among the cases. Approximately 50% ie 46%(<20U/L) of RA patients were Anti CCP negative. Among the positive individuals majority were

27% - weak positive (20 – 39.9 U/L).

22% - strong positive (≥60U/L)

5% - positive(40-59.9 U/L).

Table 12 Mean Serum Prolactin Concentration in Anti CCP positive and Anti CCP negative individuals among cases and Mean Anti CCP in each groups

Group	Number	Mean PRL ng/mL	Mean Anti CCP U/mL
Weak positive (20 -39.9 U/L)	15	36.67±10.24	25.8±4.75
Positive (40-59.9 U/L)	3	41.8±3.7	49.7±7.4
Strong positive (≥60 U/L)	12	34±21	158.86±51.87
Negative (<20U/L)	25	30.45±19.9	6.75±5.2

Table 12 shows the mean serum Prolactin concentration in Anti CCP positive and Anti CCP negative individuals. The mean serum PRL in Anti CCP negative RA patients was lower than the positive individuals. In the Anti CCP positive(40 to 59.9U/L) RA patients Serum Prolactin was higher than strong positive, weak positive and negative patients.

The mean Anti CCP in Weak positive patients was 25.8±4.75 U/L .

The mean Anti CCP in Positive Patients was 49.7±7.4U/L.

The mean Anti CCP in Strong positive individuals was 158.86±51.87U/L.

The mean Anti CCP in negative RA patients was 6.75±5.2 U/L.

Figure 7 Mean Serum Prolactin Concentration in Anti CCP positive Patients.

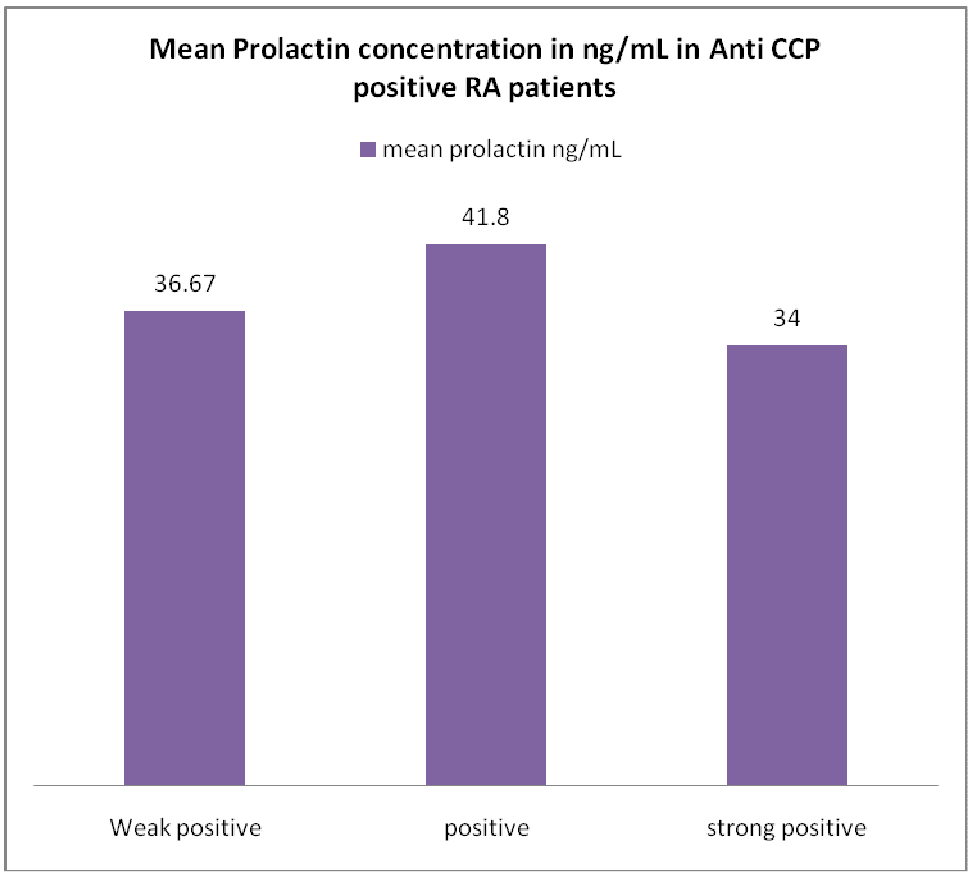


Figure 7 shows the mean Serum Prolactin concentration in weak positive , positive , strong positive Anti CCP RA patients. The mean serum Prolactin concentration(41.8 ng/mL) was higher in the positive group. The mean Serum Prolactin Concentration in the strong positive group was lower than both positive and weak positive Rheumatoid Arthritis patients.

Table 13 Comparison of mean serum Prolactin concentration between Anti CCP positive and Anti CCP negative individuals.

Group	number	Mean PRL ng/mL	p- value
AntiCCP positive	30	36.5±15.3	0.2394 NS
AntiCCP negative	25	30.5±19.9	

NS- Not significant

Table 13 shows the comparison of mean Serum Prolactin concentration between Anti CCP positive and negative individuals. The mean between the two groups was compared using unpaired students' t-test. The difference in mean between the two groups was not significant statistically; p value-0.2394.

Figure 8 Mean Serum Prolactin concentration in Anti CCP positive and Anti CCP negative RA patients.

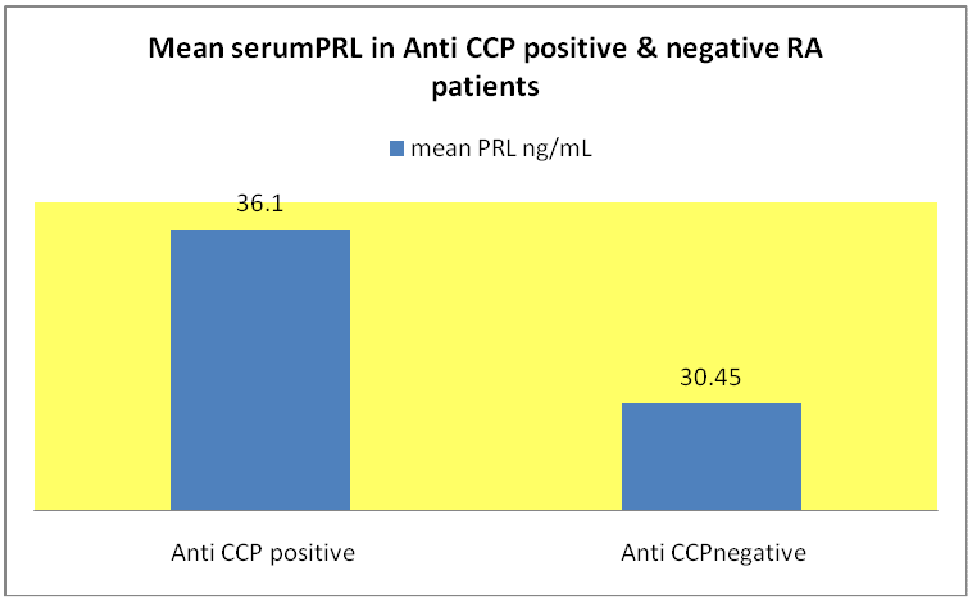


Figure 8 shows the mean Serum PRL concentration between the Anti CCP positive and negative individuals.The serum Prolactin concentration in Anti CCP positive RA patients was higher but the difference was not statistically significant.

Figure 9 Rheumatoid Factor positivity in the cases

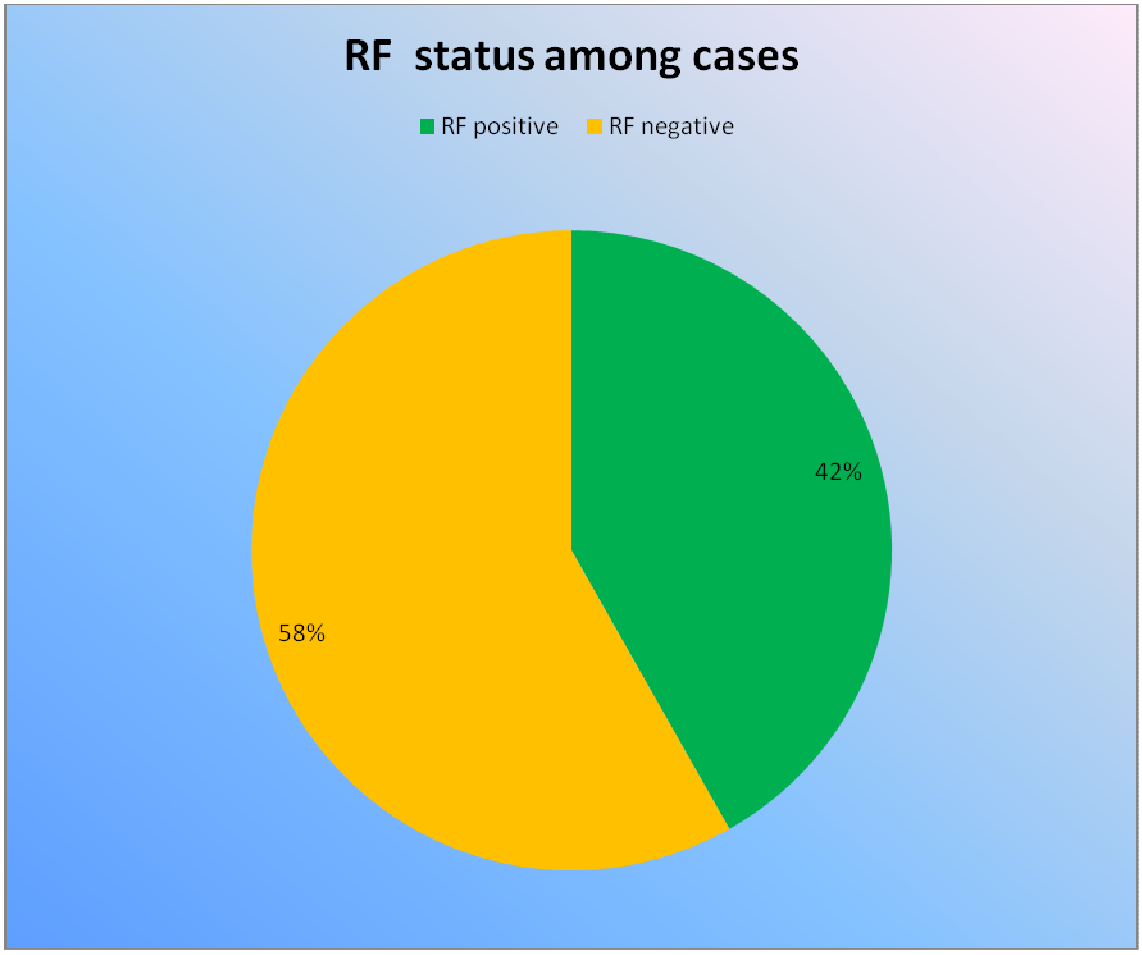


Figure 9 shows the percentage of Rheumatoid factor positive and Rheumatoid factor negative individuals among the cases. Majority of the cases was RF negative 58%. 42% of the cases were RF positive.

Table 14 Comparision of mean Serum Prolactin concentration between RF positive and RF negative RA patients

Group	Number	Mean PRL ng/mL	p- value
RF positive	23	36.58±21.6	0.266 NS
RF negative	32	31.13±14.35	

NS- not significant

Table 14 shows the comparison of Serum Prolactin concentration between RF positive and RF negative patients. The mean between the two groups was compared using unpaired students' t test. The difference in meanserum PRL between the RF positive and RF negative groups was statistically not significant.

Figure 10 Mean Serum PRL concentration in RF positive & RF negative RA patients.

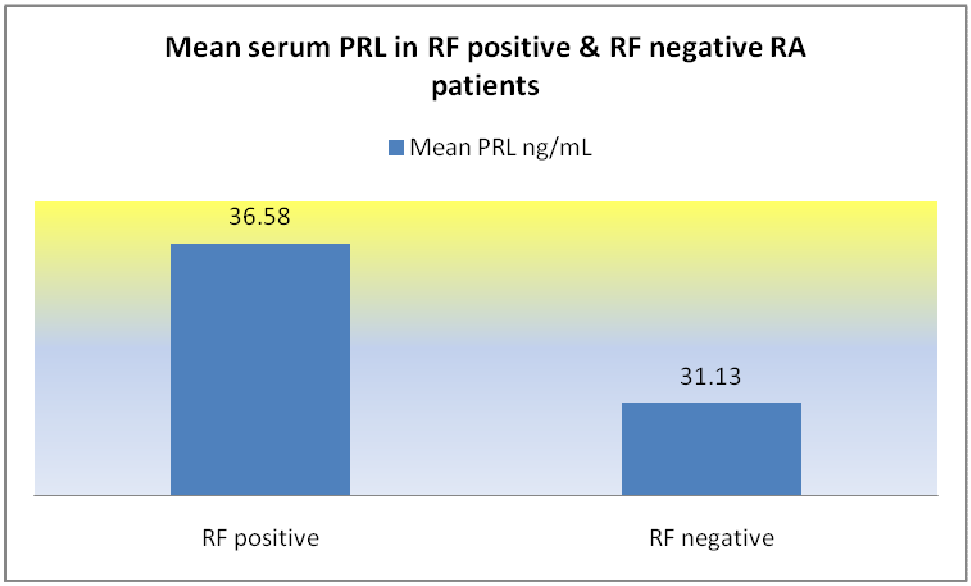


Figure 10 shows the mean serum PRL concentration in RF positive & RF negative individuals. The serum Prolactin in RF positive cases was 36.58ng/mL ;it was higher than the RF negative patients but the difference in mean was statistically not significant; p-value-0.266.

Figure 11 Antibody status among Rheumatoid Arthritis patients.

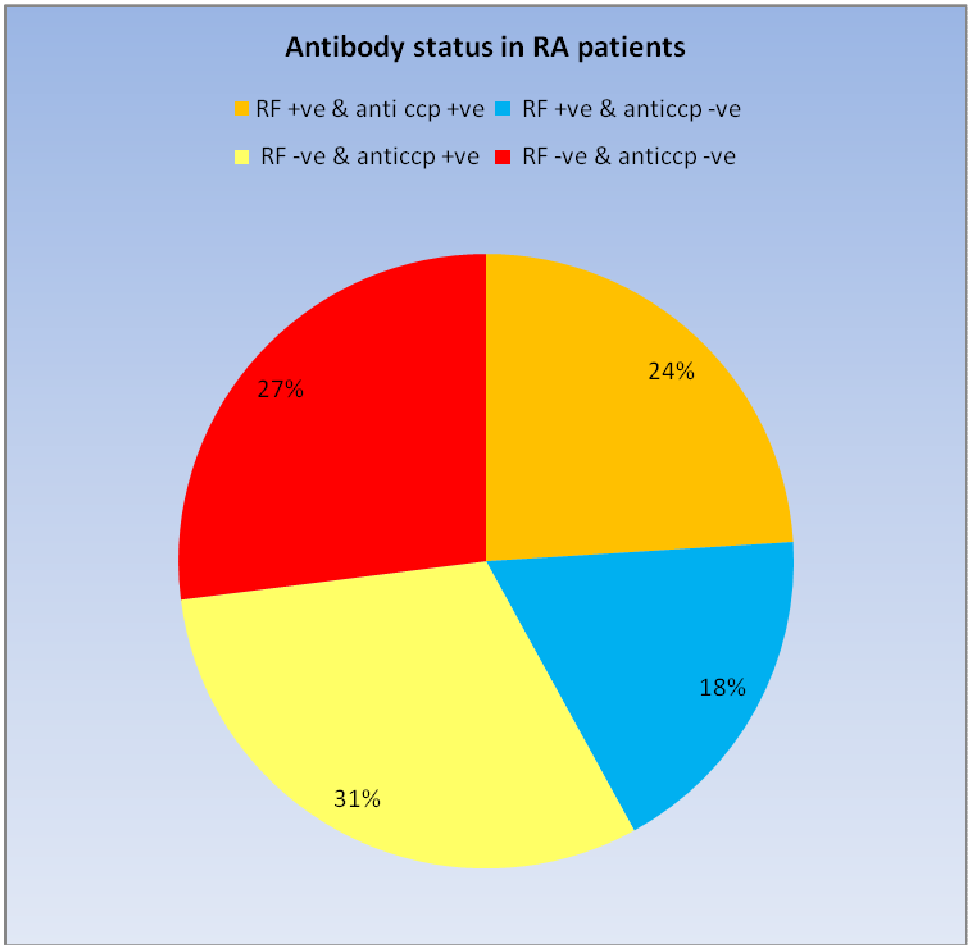


Figure 11 shows the percentage distribution of cases with respect to both RF and Anti CCP. In the cases 24% were positive for both RF & Anti CCP. 27% of cases were negative for both RF & Anti CCP.31% of cases were positive for Anti CCP alone. 18 % of cases were positive for RF alone. Hence majority of the cases were positive for Anti CCP alone.

Table 15 Comparison of mean serum PRL concentration between 4 groups(RF& Anti CCP +ve , RF+ve AntiCCP –ve , RF-ve Anti CCP +ve,RF&Anti CCP negative)

Table 15a Oneway ANOVA to compare mean values between groups.

Variable	Groups	N	Mean	Std. Dev	P-Value
PRL ng/mL	Both RF & Anti CCP positive	13	38.654	19.6836	0.448
	Only RF positive, Anti CCP negative	10	34.540	24.9064	
	Only Anti CCP positive,RF negative	17	34.141	11.7759	
	Both RF & Anti CCP negative	15	27.687	16.8622	
	Total	**55**	**33.520**	**17.8998**	

Table 15b ANOVA Table

Variable	Sum of Squares		df	Mean Square	F-Value	P-Value
PRL	Between Groups	870.013	3	290.004	0.900	0.448 NS
	Within Groups	16431.675	51	322.190		
	Total	**17301.688**	**54**			

NS- not significant

Table 15 shows ANOVA was used to compare the mean serum Prolactin concentration between the 4 groups. The mean serum Prolactin concentration was higher in RA patients with both RF and Anti CCP positive than the other 3 groups viz, RF alone positive, Anti CCP alone positive and both Anti CCP & RF negative. But the difference in mean was not statistically significant ; p value- 0.448.

Table 16 Comparison of mean serum Prolactin concentration between Both RF & Anti CCP positive and both RF & Anti CCP negative RA patients.

Group	Number	Mean ng/mL	p- value
RF& Anti CCP +ve	13	38.654±19.6836	0.1242
RF & Anti CCP -ve	15	27.687±16.8622	NS

NS – Not Significant.

Using Unpaired students' t test the difference in mean serum PRL concentration between RF & Anti CCP positive and RF& Anti CCP negative groups was found to be statistically not significant; p-value – 0.1242.

Figure 12 mean serum PRL levels with respect to antibody status of RA patients.

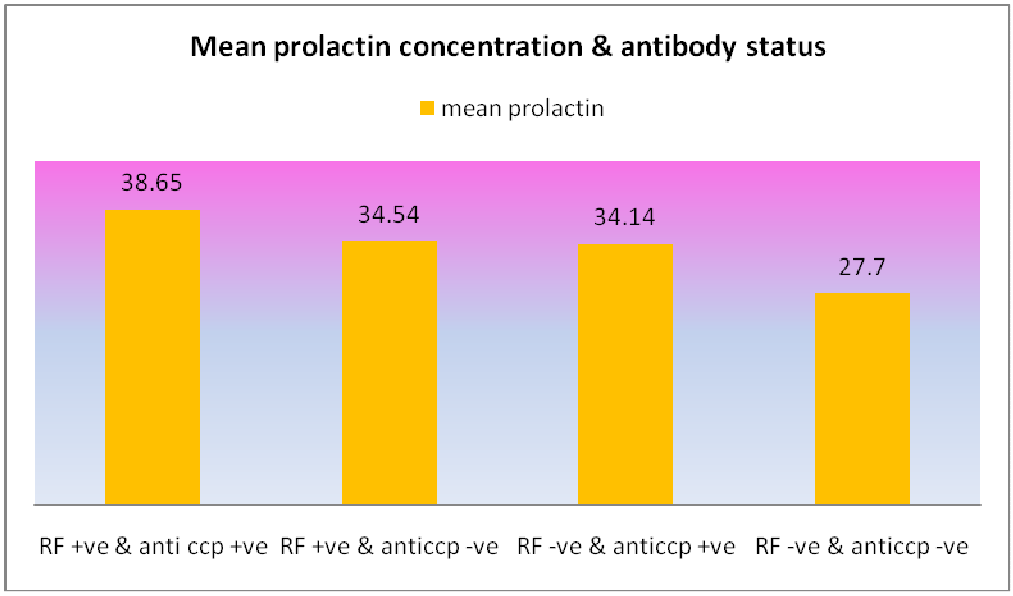


Figure 12 shows the mean serum PRL concentration with respect to their antibody status. The concentration of serum Prolactin was highest (38.65ng/mL) in RA patients with both antibodies RF & Anti CCP positive, lowest (27.7 ng/mL) in RA patients with both the antibodies negative. The mean serum concentration in RA patients with RF positivity alone was 34.54ng/mL . The mean concentration in Anti CCP alone positive RA patients was 34.14ng/mL. The mean prolactin concentration in RA patients with either RF or Anti CCP positive was almost the same .

Table 17 Mean DAS 28(3) with respect to antibody status

Group	Number	Mean DAS28(3) score
Both RF& Anti CCP +ve	13	5.5±1.1
RF alone positive	10	5.6±0.8
Anti CCP alone positive	17	5.1±0.97
Both RF&Anti CCP -ve	15	5.3±0.93

Table 17 shows the mean disease activity score in RA patients with respect to their antibody status(RF & Anti CCP). It was almost similar in all the 4 groups.

Table 18 comparison of mean ESR between cases and controls.

Group	Number	Mean mm at the end of 1 hour	p- value
Cases	55	38.6±27.1	<0.0001(Significant)
Controls	27	12.1±4.4	

Comparison of ESR between cases and controls was done using Unpaired students' t –test the difference in mean of ESR between cases and controls was statistically significant p-value <0.0001

Table 19 Comparison of mean ESR between RA patients with moderate & high disease activity scores.

Group	Number	Mean ESR mm at the end of 1 hour	p- value
Moderate DAS (3.2-5.1)	22	23.36±11.9	0.0004 Significant
High DAS(>5.1)	33	48.76±29.56	

The ESR was high in RA patients with high disease activity score. Comparison of mean ESR between RA patients with moderate and high disease activity score was done using unpaired students' t test. The difference in mean between the two groups was statistically significant; p value 0.0004.

Table 20 Correlation between DAS score and ESR in RA patients with moderate and high disease activity score.

Group	Number	Correlation	p-value
Moderate DAS	22	0.4545	0.033
High DAS	33	0.594	0.0003

Table 20 shows the correlation between ESR and DAS28(3) score in both moderate and high disease activity scores in RA patients. Pearsons correlation coefficient was used to find the association between disease activity score and ESR. A moderate positive linear correlation exists between DAS and ESR in RA patients with moderate disease activity r value 0.4545 and it was statistically significant ;p-value

0.033. A moderate linear positive correlation exists between DAS and ESR in RA patients with high disease activity r value 0.594 and this is statistically highly significant p value 0.0003.

Table 21 Correlation between ESR and Serum PRL in RA patients with moderate and high disease activity.

Group	Number	correlation	p-value
Moderate DAS	22	-0.3737	0.087
High DAS	33	0.1331	0.46

Table 21 shows the correlation between ESR and serum PRL concentration in RA patients with moderate and high disease activity.Pearson correlation coefficient for ESR and serum PRL concentration in RA patients with moderate DAS was -0.3737,fair negative linear correlation but statistically not significant. Pearson correlation coefficient for ESR and serum PRL concentration in RA patients with high DAS was 0.1331, weak positive linear correlation and statistically not significant.

Table 22 Correlation between DAS score and serum PRL in RA patients with moderate and high DAS scores.

Group	Number	Correlation	p-value
Moderate DAS	22	-0.133	0.55
High DAS	33	0.102	0.57

Table 22 showing correlation between DAS28(3) scores & serum PRL concentration in RA patients. Pearson correlation coefficient between DAS score and Serum PRL concentration in RA patients with moderate disease activity scores was -0.133,a weak negative linear correlation and statistically not significant. Pearson correlation coefficient between DAS score and Serum PRL concentration in RA patients with high disease activity scores was 0.102 , a weak positive linear correlation and statistically not significant.

Table 23 Correlation of Serum prolactin concentration with DAS28(3) score in RA patients

DAS	Correlation	0.345
	P-Value	0.010
	N	55

Table 23 shows the Correlation of Serum Prolactin concentration with DAS28(3) score in RA patients .The Pearson correlation coefficient between DAS score and Serum PRL concentration in RA patients was 0.345. It was a fair positive linear correlation and it was statistically significant; p value 0.01.

Figure 13 Correlation between Serum PRL conc & DAS

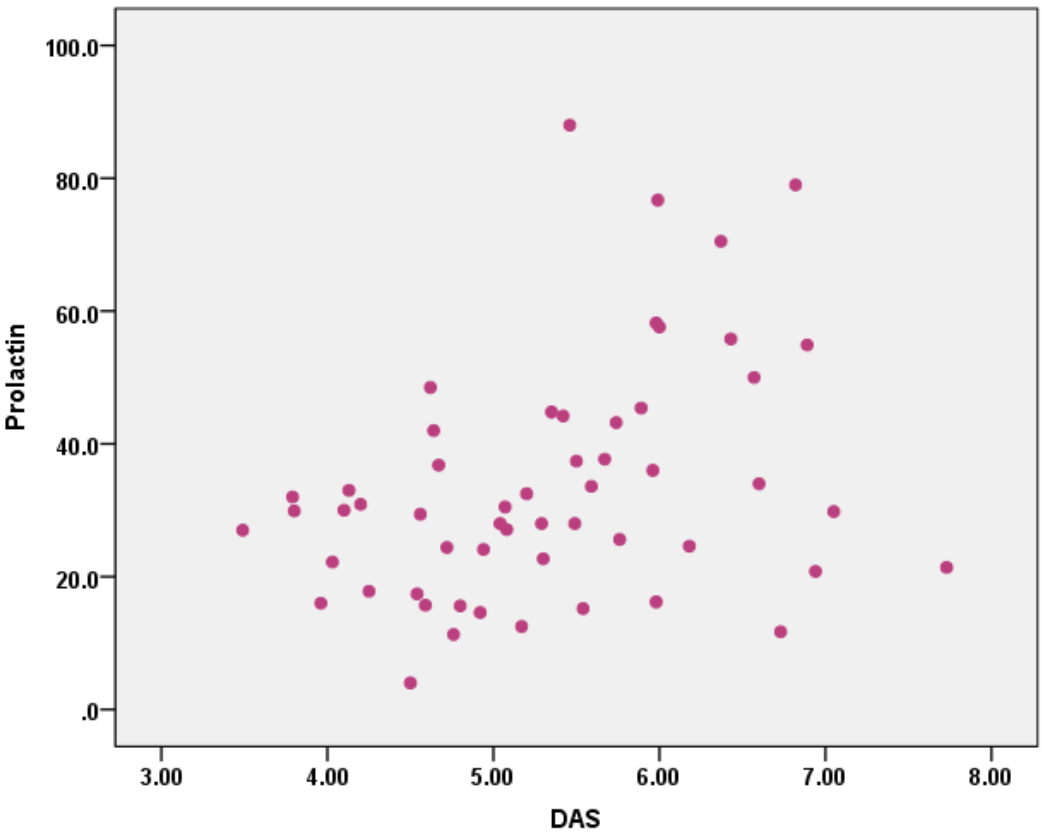


Table 24 Pearson Correlations between prolactin and variables other than DAS among cases

S.NO	Variables		Prolactin
1	Anti CCP	Correlation	0.042
		P-Value	0.760
		N	55
2	ESR	Correlation	0.236
		P-Value	0.082
		N	55
3	RF values in RF positive patients	Correlation	0.089
		P-Value	0.681
		N	24
4	ACR score	Correlation	0.247
		P-Value	0.070
		N	55
5	Symptom Duration (months)	Correlation	0.088
		P-Value	0.521
		N	55
6	Age (years)	Correlation	-0.118
		P-Value	0.392
		N	55

Table 24 shows the correlation of Serum PRL concentration with variables like Anti CCP, ESR, RF, ACR score, symptom duration, Age . The Correlation was found using Pearson correlation coefficient . NO correlation exists between Anti

CCP, RF, Symptom duration , Age with Serum Prolactin concentration. A fair correlation exists between ESR, ACR score with Serum Prolactin concentration but they were not statistically significant.

Table 25 Comparison of mean serum Prolactin concentration in CRP positive and CRP negative RA patients.

Group	number	Mean ng/mL	p-value
CRP positive	39 (70.9%)	33.88±17.83	0.8281 Not Significant
CRP negative	16(29.1%)	32.73±17.56	

Table 25 shows the comparison of mean serum Prolactin concentration in CRP positive and CRP negative RA patients. Among the cases 70.9% were positive for CRP. The mean serum PRL concentration in both these groups are almost similar. The difference in mean between the two groups was calculated using unpaired students' t- test and it was not statistically significant.

Table 26 shows the characteristics of study subjects.

S.NO	VARIABLES	CASES	CONTROLS	P- VALUE
1	AGE in years	41.5±11.6	40±12.7	0.595
2	AGE OF MALES in years	36.14±11.68	43.5±17.36	0.418
3	AGE OF FEMALES in years	42.29±11.37	40.65±11.32	0.571
4	SERUM UREAin mg/dL	24.56±4.7	23.8±2.6	0.437
5	SERUM CREATININE in mg/dL	0.9±0.2	0.8±0.2	0.036
6	SERUM TSH in µIU/mL	2.5±2.2	2.4±1.1	0.82
7	ESR in mm at the end of 1hr	38.6±27.1	12.1±4.4	< 0.0001
8	Anti CCP in U/mL	47.5±64.8	5.3±3.9	0.0012
9	SERUM PROLACTIN in ng/mL	33.53±17.9	14.4±5.9	< 0.001

<0.05 – Significant

<0.01 – Highly Significant

>0.05 – Not Significant

DISCUSSION

DISCUSSION

Rheumatoid Arthritis , a chronic inflammatory Autoimmune disease affecting joint synovium , cartilage ,bursae causes functional disability. This disease present world wide occurs by an interplay of immunological , inflammatory , genetic and environmental genetic factors. However autoimmunity plays a pivotal role in its pathogenesis.[1]

The immune balance is maintained by cytokines released by Th1 and Th2 lymphocytes. Break in immunetolerance or immunedysregulation or cytokine imbalance provokes a autoimmune response. The tissue damage in autoimmune diseases occurs by type III hypersensitivity reactions, damage is caused by immune complex deposition activating classical complement pathway [7]. The pathogenesis has been explained in detail in the review of literature.

Prolactin known as a lactogenic hormone is a cytokine .In addition to secretion from acidophills of anterior pituitary it is also secreted by lymphocytes, decidua of uterus, amnion, chorion of placenta, mammary gland and many regions of brain [42]. The role of Prolactin in immune regulation and autoimmune diseases had been explained by many invitro and invivo animal and human studies.

Prolactin

- regulates the maturation of $CD4^-$ and $CD8^-$ cells CD4+ and CD8+ cells.[69]
- decreases or prevent the apoptosis of lymphocytes.[75]
- Interferes with peripheral tolerance of B cells. [92]

- enhances the proliferative response to certain antigens and mitogens.[83]
- upregulates the expression of Th1 cytokines Th1 cytokines are involved in autoimmune diseases.[84]

Prolactin role in immuneregulation and autoimmune response has been suggested by the above said facts.

Hyperprolactinemia due to various etiologies have been associated with increased incidence of auto antibodies in serum like Anti-Ro , Anti- thyroid , Anti-cardiolipin , Anti- dsDNA , Antinuclear antibodies in individuals without clinical evidences of autoimmune diseases [137,165,167]. Also serum Prolactin is elevated in patients with SLE,RA, Psoariasis,Multiple sclerosis,Reiter syndrome, Uveitis , Sojgren syndrome [2,166-169]. Elevated Prolactin was seen in cardiac allograft transplant rejection patients and in mice with skin allograft rejection [158]. These facts suggest that Certain case reports show that administration of Bromocryptine , cabergoline in Rheumatoid Arthritis patients with elevated serum Prolactin cause disease remission [159-163]. But these evidences were not consistent . Hence the present study was carried out to determine the status of serum Prolactin in patients with recently diagnosed Rheumatoid arthritis.

In this study 55 recently diagnosed patients with Rheumatoid Arthriti s were recruited from the Rheumatology Out Patient department of Rajiv Gandhi Govt.General Hospital Chennai .The diagnosis of RA was based on 2010 American College of Rheumatology criteria[170] . These patients were not under any treatment. Both Rheumatoid factor positive and RF negative RA patients were included in the study. 27 age and sex matched apparently healthy volunteers who had no clinical

evidence of Rheumatoid Arthritis were selected. Serum urea. Serum Creatinine , Serum TSH , Rheumatoid Factor, ESR , CRP , serum Prolactin and serum Anti CCP were determined in all the 55 RA patients and 27 controls.Disease activity was assessed using DAS28(3) score [164] in all the 55 patients.

The cases and control group had been matched with respect to age, sex , serum urea, creatinine, TSH as shown by p value less than 0.05 Among the cases 87.3% (48 out of 55) were females which matches with the literature that autoimmune diseases are common in women [61]. The onset of Rheumatoid Arthritis among the cases in this study was between 30 to 40 years of age which again matches with the literature where it is mentioned that 80% of patients develop the disease between 35 and 50 years of age [1]. The incidence is more common in women older than 60 years of age [1]. In this study the predominant age group is 31 to 40 years of age. Since only recently diagnosed RA patients and not under any treatment were included , the major contribution is by patients in 31 to 40 years of age. In this study premenopausal women were more than postmenopausal women this coincides with the literature [1].

Only 18% (n=10) of cases had presented within 3 months of onset of symptoms Majority of patients 65%(n=36) presented 3 to 6 months of onset of symptoms.

42 %(n=23) of patients with RA were positive for RF & 58%(n=32) were negative for Rheumatoid factor. Although Rheumatoid Factor is not specific for Rheumatoid Arthritis, RF positivity is a cardinal feature of RA. It is mentioned that seroconversion in RF negative patients may occur during the first year of disease

activity, hence these Seronegative RA patients in the present study might turn seropositive for RF later.

Among cases 31%(17) of the patients were positive for Anti CCP antibody alone, 23.6 %(13) were positive for both RF and Anti –CCP antibodies. Anti CCP is 90% specific for Rheumatoid arthritis[1]. 18.2%(10) of cases were positive for RF alone.27.3% (15) were negative for both the antibodies.. According to 2010 ACR/EULAR criteria ,□In a patient with classical features of Rheumatoid arthritis namely morning stiffness for more than 1 hour for atleast 6 weeks, involvement of two or more small joints, RF and anti –CCP antibodies does not exclude the diagnosis of Rheumatoid Arthritis" [170].

ESR was elevated in 49 (89.1%) patients and was within normal limits for 6 patients.

CRP was positive for 70.9% of patients.

60%(33) of the RA patients had a high disease activity score (>5.1). 40%(22) had a moderate disease activity score (3.2-5.1).

In this study the mean PRL concentration among the cases (n=55) was 33.53±17.93 ng / mL which was significantly higher than the control group (n=27) where it was 14.4±5.9ng/mL with p value of <0.001 which correlates with that available in literature

The mean serum PRL concentration in female patients(n=48) was 33.83±18.75ng/mL and was higher than female individuals in the control(n=23) group (14.11±7.9 ng/mL) which is statistically highly significant (p value <0.0001).

Similarly the mean serum PRL concentration in male(n=7) patients (31.16 ±7.9 ng/mL) was higher than male individuals in the contro(n=4)l group (16.1±3.2 ng/mL); which is statistically highly significant (p value 0.0059). All these results correlate with the findings in the literature.[171-180]

The concentration of Serum Prolactin was higher in patients who presented between 3 and 6 months of joint symptoms (35.86±19.9ng/mL) than patients who presented earlier i.e. within 3 months of joint symptoms (27.45±9.7ng/mL) or later than 6 months (31±13.2 ng/mL). However in those patients who presented earlier had higher serum PRL concentration compared to the control group (27.45ng/mL in early presentation as against 14.4ng/mL in the control group)

Although the mean serum PRL concentration in RF negative & Anti –CCP antibodies negative patients was lower (30.45±19.9ng/mL,n=25) than in those patients who were positive for both(36.1±15.3,n=35), it was not statistically significant (p value-0.2394)

An ANOVA comparison of mean PRL concentration between the 4 groups, RF& Anti CCP positive , only RF positive, only Anti CCP positive , both RF & Anti CCP negative did not reveal any statistically significant difference in serum PRL concentration

From the above discussion it can be inferred that serum PRL concentration is higher in patients with RA, which is one of the aim of this study However it did not correlate with other markers of RA namely RF & Anti CCP antibodies positivity

Discussing the second aim of the study correlating serum PRL concentration with disease activity as assessed by DAS 283 score[164], it is seen that the mean serum PRL concentration is higher in patients and a Pearson's correlation coefficient showed a fair correlation with a r value of 0.345 & p-value of 0.01.This correlates with studies in literature [177,179].

The Serum PRL concentration did not correlate with ESR,ACR score, Concentration of Anti CCP antibodies, Symptom duration in RA patients in this study.

Hyperprolactenemia in patients with RA is not likely be due to macroprolact in as the predominant antibody in RA is of IgM class which might not bind with Prolactin [1]. Macro prolactin is a complex of Prolactin with IgG whose renal clearance is slow and hence the hyperprolactinemia . In this study 70.9% of patients were hyperprolatinemic. Hence elevated serum Prolactin in this study is not likey to be due to macroprolactin. Some studies stands as an evidence for this [181,182].

LIMITATION OF THE STUDY

LIMITATIONS OF THE STUDY

1. The number of males in the study group was low and the study is more skewed towards female population .
2. Juvenile Rheumatoid arthritis patients were not included in the study.
3. Screening for Macroprolactin was not done as elevated serum PRL levels in patients and controls could have been due to macroprolctin
4. Pulsality of prolactin secretion was not taken in to account as PRL secretion is pulsatile .

SUMMARY

SUMMARY

The role of Prolactin in autoimmune diseases & use of anti Prolactin drugs in disease remission has been described & investigated in several studies. The role of anti- prolactin drugs in remission of disease activity have been investigated. The present study was carried out to correlate serum Prolactin levels with disease activity in recently diagnosed Rheumatoid Arthritis patients.

A case-control study was carried out in 55 recently diagnosed untreated Rheumatoid factor positive & RF negative Rheumatoid Arthritis patients and 27 age & sex matched apparently healthy individuals. The diagnosis of RA was made using ACR criteria 2010. Serum Prolactin(ELISA) ,Anti CCP(ELISA) , RF (Latex agglutination method) , TSH(ELISA),ESR(Westergrens method),Serum urea & creatinine were assayed. Disease severity was assessed by DAS(28) formula. Statistical evaluation was done byunpaired students' t-test ,Pearson correlation co-efficient,ANOVA

Serum Prolactin levels in RA patients was significantly higher (33.53±17.9 ng/mL) compoared to controls (14.4±5.9) with p value of < 0.001 . A fair correlation was found between disease activity and serum Prolactin levels ($r = 0.345$; $p = 0.01$).

Elevated levels of serum prolactin indicates the immunomodulatory role of PRL and its relationship to diseases activity. Use of Anti Prolactin drugs may be of use in patients with hyperprolactinemia.

CONCLUSION

CONCLUSION

Rheumatoid Arthritis is an autoimmune disease. The role of Prolactin in immune modulation was suggested by its ability to cause T cell & B cell maturation, activation of T- lymphocytes by PRL in the absence of IL-2, ability to break peripheral tolerance. It may provoke autoimmune diseases. This study again stands as an evidence for Prolactin role in Rheumatoid Arthritis. The Serum Prolactin concentration in Rheumatoid Arthritis patients was higher than age and sex matched healthy individuals From this study we can infer that

- Prolactin has a role in Autoimmune diseases.

- Monitoring Serum Prolactin concentration during treatment may be of help since a statistically significant difference in Serum Prolactin concentration was present between patients with high and moderate disease activity.

- Including Dopamine agonist as an adjuvant in treatment of Rheumatoid arthritis patients with elevated Serum Prolactin may be of use in remission.

SCOPE FOR FURTHER STUDIES

SCOPE FOR FURTHER STUDIES

i. As Serum prolactin concentrations are higher in rheumatoid arthritis which has both an autoimmune & inflammatory etiology serum PRL concentrations could be assessed in other autoimmune diseases such as SLE

ii. If Serum Prolactin concentration are measured routinely in all patients with RA, Dopamine agonists such as bromocryptine cabergoline which are inhibit PRL secretion, can be tried as an adjuvant treatment especially in patients with with high serum PRL concentrations .

iii. Serum Prolactin concentration can be monitored to assess disease progression in RA patients with elevated serum Prolactin at the time of presentation and compared with RA patients who had normal serum Prolactin at onset .

iv. Serum Prolactin concentration could be assessed in siblings of patients with Rheumatoid arthritis and elevated serum Prolactin

v. Serum Prolactin concentration could be correlated with inflammatory cytokines and HLA typing

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ANNEXURES

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

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

CERTIFICATE OF APPROVAL

To
Dr. M. Karthiga,
Post Graduate in MD Biochemistry,
Institute of Biochemistry,
Madras Medical College, Chennai-3.

Dear **Dr. M. Karthiga,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Serum prolactin levels in rheumatoid arthritis patients"** No.09122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

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

Sd/Chairman & Other Members

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.

15/12/13

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
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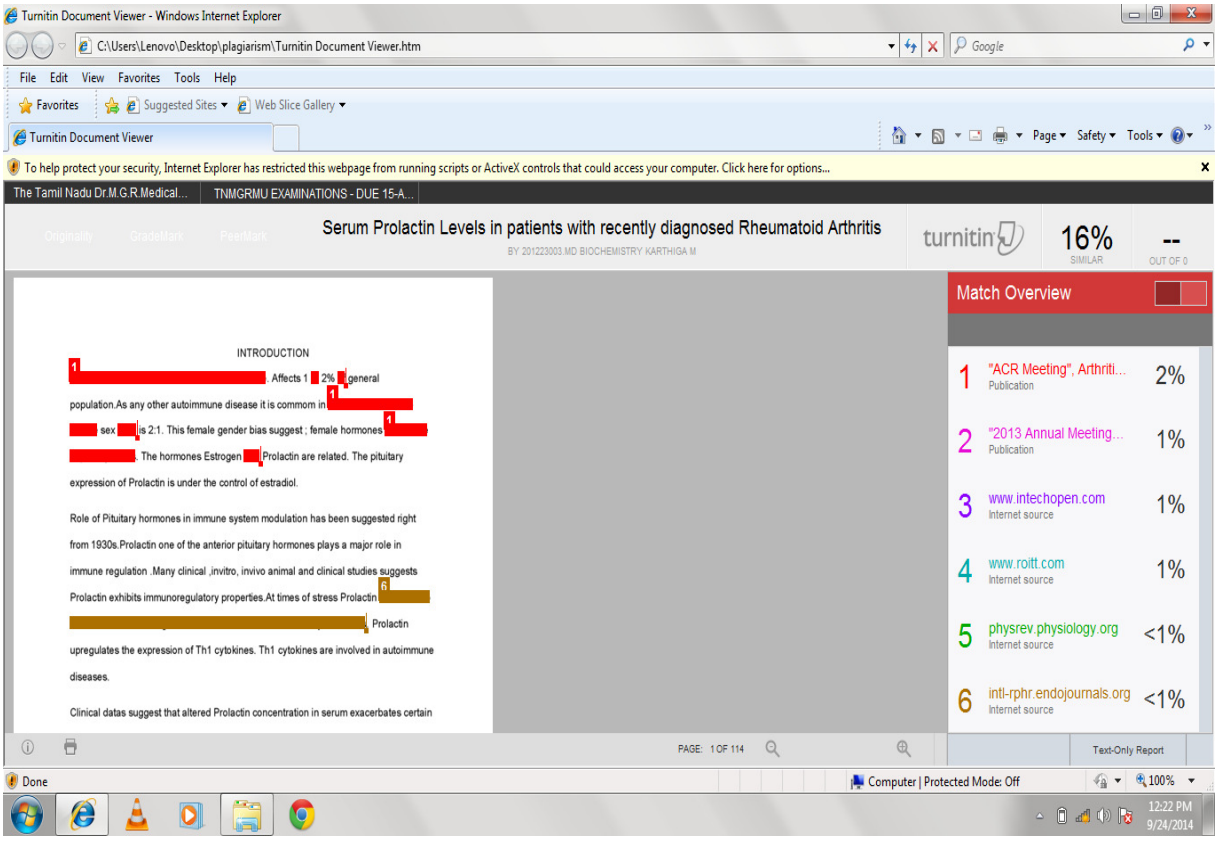
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expression of Prolactin is under the control of estradiol.
Role of Pituitary hormones in immune system modulation has been suggested right
from 1930s.Prolactin one of the anterior pituitary hormones plays a major role in
immune regulation .Many clinical ,invitro, invivo animal and clinical studies suggests
Prolactin exhibits immunoregulatory properties.At times of stress Prolactin
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upregulates the expression of Th1 cytokines. Th1 cytokines are involved in autoimmune
diseases.
Clinical datas suggest that altered Prolactin concentration in serum exacerbates certain
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ஆராய்ச்சி தகவல் தாள்

தலைப்பு : முடக்குவாத நோயாளிகளின் இரத்தத்தில் புரோலேக்டின் நிலை

ஆராய்ச்சியாளர் : **மரு. மு. கார்த்திகா,**
பட்ட மேற்படிப்பு, மருத்துவ மாணவி,
உயிர்வேதியியல் உயர்நிலைத் துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

ஆராய்ச்சி மேற்பார்வையாளர் : **மரு. வி.கே.இராமதேசிகன்,**
துணை பேராசிரியர்,
உயிர்வேதியியல் உயர்நிலைத் துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

புரோலேக்டின் என்கிற ஹார்மோன் மனிதனின் நோய் எதிர்ப்புத் திறனில் பங்கு வகிக்கின்றது எனவும் தன் தடுப்பாற்று நோய்களில் இரத்தத்தில் இந்த ஹார்மோனின் அளவு அதிகமாக உள்ளது என்றும் ஆராய்ச்சிகளின் மூலம் கண்டறியப்பட்டுள்ளது. மனிதர்களின் மூட்டுகளில் பாதிப்பினை ஏற்படுத்தும் முடக்குவாதம் தன் தடுப்பாற்று நோய்களில் ஒன்று. முடக்குவாதத்தில் ஏற்படக்கூடிய குருத்தெலம்பின் அழிவினை புரோலேக்டின் தடுக்கின்றது என ஆராய்ச்சியாளர்கள் கண்டறிந்துள்ளனர். நோயின் வீரியத்தை மருந்தாக அளிக்கப்பட்ட செயற்கை புரோலேக்டின் குறைக்கின்றது என்றும் கண்டறிந்துள்ளனர். புரோலேக்டின் ஹார்மோனுக்கு அழற்சியை ஏற்படுத்தக்கூடிய தன்மை உண்டு எனவும் ஆராய்ச்சிகள் தெரிவிக்கின்றன.

முடக்குவாத நோயில் புரோலேக்டின் நிலைபாடு தற்போதைய சிகிச்சை முறையில் அல்லது நோயின் தன்மையை கண்டறியும் முறையிலும் சில மாற்றங்களை ஏற்படுத்தலாம்.

எனவே சென்னை இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் முடக்குவாத நோயாளிகளின் இரத்தத்தில் புரோலேக்டின் அளவினை கண்டறியும் ஆராய்ச்சியில் நான் ஈடுபட்டுள்ளேன்.

இதற்கு 57 முடக்குவாத நோயாளிகளிடமும், அவர்களின் பாலினம் மற்றும் வயதிற்கு ஏற்றார் போலுள்ள 24 ஆரோக்கியமான (முடக்குவாதம் மற்றும் வேறு எந்த நோயில்லாத) மனிதர்களிடமும் 5மி.லி. இரத்தம் எடுத்து ஆராய்ச்சிக்கு உட்படுத்த உள்ளேன்.

தங்களிடமிருந்து ஊசியின் மூலம் 5 மி.லி. இரத்தம் எடுப்பதனால் எந்த விதமான பக்க விளைவுகளும் ஏற்படாது என உறுதி அளிக்கின்றேன்.

தாங்கள் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதில் பங்கு பெறுவதினால் நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் கூடுதல் செலவீனம் ஏற்படாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயர் மற்றும் அடையாளங்கள் வெளியிடப்படாது என்பதை தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களின் விருப்பத்தின் பேரில் தான் இருக்கிறது. எந்நேரமும் இதிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியின் முடிவுகளை தங்களுக்கு அறிவிப்போம் என்பதை தெரிவித்துக் கொள்கிறோம்.

நீங்கள் இந்த ஆராய்ச்சியில் பங்குபெற விருப்பம் இருப்பின் இந்த தகவல்தாள் மற்றும் ஆராய்ச்சி ஒப்புதல் படிவத்திலும் கையொப்பம் இடுமாறு கேட்டுக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

/ இடது கைவிரல் ரேகை

இடம் :

தேதி :

நோயாளியின் ஒப்புதல் படிவம்

தலைப்பு : முடக்குவாத நோயாளிகளின் இரத்தத்தில் புரோலேக்டின் நிலை

பங்கேற்பாளர் பெயர் : புற / உள் நோயாளி எண் :

வயது : பால் : கைபேசி/தொலைபேசி எண் :

முகவரி ஆராய்ச்சி சேர்க்கை எண் :

..... ஆகிய நான் மரு.மு.கார்த்திகா, பட்டமேற்படிப்பு, மருத்துவ மாணவி, உயிர்வேதியியல் உயர்நிலைத் துறை, சென்னை மருத்துவக் கல்லூரி, சென்னை. மேற்கொள்ளும் ஆராய்ச்சியில் பங்கேற்க எந்தவித நிர்பந்தமின்றி, முழு சுதந்திரத்துனன் சுய நினைவுடன் முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

இந்த ஆராய்ச்சி பற்றிய தகவல் தாளை பெற்றுக்கொண்டு அதன் முழு விவரங்களையும் நோக்கங்களையும் மருத்துவரின் மூலம் புரிந்துகொண்டு எனது மருத்துவ குறிப்புகள், பரிசோதனை முடிவுகள் மற்றும் இரத்தத்தை பயன்படுத்திக் கொள்ளவும் மேலும் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் எனவும் அச்செயலினால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்துக் கொண்டு முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

எனது பரிசோதனை முடிவுகளை அறிவியல் சார்ந்த அமைப்புகள் மற்றும் மருத்துவ இதழ்களில் வெளியிடுவதற்கு முழு மனதுடன் சம்மதிக்கின்றேன்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

/ இடது கைவிரல் ரேகை

இடம் :

தேதி :

INFORMATION SHEET

Title : Serum Prolactin levels in Rheumatoid Arthritis Patients.

Investigator : **Dr. M. Karthiga,**
Post Graduate,
Institute of Biochemistry
Madras Medical College,
Chennai – 600 003.

Guide : **Dr. V.K. Ramadesikan,**
Associate Professor,
Institute of Biochemistry
Madras Medical College,
Chennai – 600 003.

Prolactin, a hormone modulates immune response. Prolactin level is high in many autoimmune diseases and Rheumatoid arthritis is one of the autoimmune disease. Prolactin is proinflammatory in nature but also protects cartilage from destruction. Recent studies show that exogenous prolactin reduces disease activity. Knowledge regarding prolactin in rheumatoid arthritis may alter the current treatment or diagnostic or followup strategy.

Hence I am doing this study Serum Prolactin level in Rheumatoid arthritis patients attending Rajiv Gandhi Govt. Genral Hospital, Chennai. To do this study I need to collect 5 ml blood from 57 Rheumatoid arthritis patients and 24 healthy individuals. While collecting 5 ml of blood there will not be any side effects.

Your identity will be confidential throughout the study and also during publication or presentation in any clinical forums and journals. Participation in this study is purely voluntary. You can withdraw from this study at any time. Your decision will not result in any loss of benefits to which you are otherwise entitled. The results of the study will be intimated to you. If you have willingness to participate in this study, kindly sign in this information sheet and the consent form.

Signature of the Investigator :

Signature of the Participant
/ Thumb Impression

Place :
Date :

PATIENT CONSENT FORM

Title : Serum Prolactin levels in Rheumatoid Arthritis Patients.

Participant's Name : Age : Sex: IP / OP No.

Address : Project Patient No :

Contact No. :

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

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

I agree to use my personal clinical history and 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 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.

Having understood _____ S/o. _____ give my consent to participate in the study conducted by Dr. M. Karthiga, Post Graduate, Institute of Biochemistry, Madras Medical College, Chennai.

Signature of the Investigator : Signature of the Participant

/ Thumb Impression

Place :

Date :

PROFORMA

Date : Sample Id : _____

Name : Age : Sex : Ht : Wgt :

Pre/Post Menopausal:

Ethnicity : Community : Duration of Symptoms:

Pregnancy □ Lactation □ Chest Trauma □

Other Autoimmune Diseases : _____ if any duration _____

Associated diseases with duration :

Renal Failure □ PCOS □ Hypertension □

Cardiac Failure □ Tuberculosis □ CNS Tumours □

Diabetes Mellitus □ Untreated Primary Hypothyrodism □

Drug Intake:

OC Pills □ Antidepressants □ Ergot Derivatives □

H2 Blockers □ Antipsychotics □ Chemotherapy □

Metoclopramide □ Cannabis □ Verapamil □

Alphamethyldopa □ Isoniazid □ Any other medications _____

Smoking :_____ Passive Smoking :_____ Alternative Medicine intake:_____

Clinically :

1. 2/↑ swollen joints : _____
2. Morning stiffness lasting more than 1hr for atleast 2 weeks : _____
3. ↑RF / anti-cyclic citrullinated peptide :
4. Symptoms of hypothyroidism.

DAS 28(3) :

Sample Collection : Date _____ Time _____

Sample Analysis : Date _____

Investigations :

Hemoglobin :

CRP :

Sr.Prolactin :

Anti-CCP :

ESR :

TSH (if available) :

JOINTS		LEFT		RIGHT	
		Swollen	Tender	Swollen	Tender
Shoulder					
Elbow					
Wrist					
MCP	**1**				
	2				
	3				
	4				
	5				
PIP	**1**				
	2				
	3				
	4				
	5				
Knee					
SUB TOTAL					

Total Swollen Joints :

Total Tender Joints :

DAS 28 (3)Score	Activity
< 3.2	Low
3.2 to 5.1	Moderate
> 5.1	High

REVISED ACR CRITERIA – POINTS

- 1 large joint - 0
- 2-10 large joint - 1
- 1-3 small joints with or without Large joint involvement - 2
- 4-10 small joints with or without Large joint – 3
- >10 joints with involvement of atleast 1 small joint - 5

□ Negative RF/ Negative ACPA - 0 □

□ Low positive RF/ Low positive ACPA - 2 □

□ High positive RF / High positive ACPA - 3 □

□ Elevated ESR / Elevated CRP - 1 □

□ Duration of arthritis six weeks or longer - 1 □

TOTAL POINTS : _____