# Study of Prolactin, Ferritin & Vitamin D as Immunomodulatory Markers in Patients with Rheumatoid Arthritis

*Thesis submitted for partial fulfillment of Master Degree in Endocrinology*

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# Thesis Protocol

## Introduction:

Rheumatoid arthritis (R.A) is a chronic autoimmune inflammatory disease characterized by persistent joint synovial tissue inflammation **(Ruddy *et al*; 2005)[a].**The etiology of R.A is not fully understood. Evidence points to a complex interplay between enviromental and genetic factors **(Goldring,2000).** Multiple cytokines, interleukins (ILs) and growth factors are released, causing further joint destruction and development of systemic complications **(Ruddy *et al*; 2005)[a].**

Prolactin (secreted by lactotrophs in anterior pituitary) has immunomodulatory as well as lactogenic effects. Prolactin is made in extrapituitary tissues including T-lymphocytes, B-lymphocytes and human chorion. Prolactin produced by T-lymphocytes is believed to have autocrine and paracrine functions. The physiologically-significant lymphocyte PRL, particularly in individuals with autoimmune diseases, has been debated **(Chuang and Molitch,2007).**

Serum ferritin protein is an acute phase reactant elevated in any inflammatory state e.g infection, R.A, hepatitis and cancer **(Victor Herbert *et al.*,1997).** Ferritin levels in synovial fluid not in serum are significantly elevated in R.A more than in osteoarthritis (O.A) **(Kumon *et al.*,1999)**.

There is inverse correlation between serum 1,25(OH)2 D3 and disease activity in patients with R.A.

The decrease of active vitamin D levels in those patients may contribute to negative calcium balance and inhibition of bone formation **(Oelzner *et al.*,1998).**

Hypercalcaemia is associated with high disease activity and may contribute to suppression of PTH (parathormone) secretion and vitamin D hormone synthesis. High levels of ionized calcium may be a reflection of disease-activity related systemic bone loss **(Oelzner *et al*, 2006).**

Preventive treatment with vitamin D (for hypercalcaemia) or therapy for HPRL with dopamine agonists, may be considered in certain cases **(Orbach *et al*,2007).** A clinical benefit using bromocriptine (a dopamine agonist) in patients with various inflammatory arthritides (including R.A, Reiter's syndrome and SLE) has been described as well as cabergoline. In contrast, a recent study of prolactin inhibitor quinagolide in nine R.A patients did not show any significant benefit. So, prolactin levels should be checked in patients with persistently active R.A despite treatment. Further studies are required to delineate whether prolactin inhibitors provide clinical benefit additional to that of conventional disease-modifying agents in R.A patients with normal prolactin levels **(Erb *et al*,2001).**

## Aim of the work:

The aim of present study is to assess the relation of prolactin, ferritin and vitamin D levels as immunomodulatory markers in patients with rheumatoid arthritis.

## Subjects and Methods:

Study will be conducted on 50 female subjects: 20 healthy controls and 30 patients with RA selected from Ain-Shams University Hospital. Patients will be subdivided into two groups:

-**Group A**: **15 (fifteen)** patients with active R.A.

-**Group B**: **15 (fifteen)** patients in remission of rheumatoid activity.

## Inclusion Criteria

Diagnosis of patients typically is made when four of seven qualifying criteria established by American Rheumatism Association are met **(Saraux *et al*,2001).**These qualifying criteria are:

1-**Morning Stiffness**: in or around the affected joints for at least one hour after initiating movement.

2-**Arthritis of three or more joint areas**: e.g wrist, PIP, MCP, MTP, elbow, knee, ankle.

3-**Arthritis of the hand**: particularly involvement of PIP, MCP joints, or wrist joints.

4-**Symmetric arthritis**.

5-**Rheumatoid nodules**.

6-**Serum rheumatoid factor positive**: 95% or more.

7-**Radiographic changes**: typical changes of erosions or loss of bone density adjacent to affected joints.

- Diagnostic tests (recommended by American College of Rheumatology Subcommittee on R.A): **(Ruddy *et al*; 2005)ь**

1-**C-reactive protein**: typically increased to more than 0.7 picograms per ml.

2-**Erythrocyte sedimentation rate**: often increased to more than 30 mm per hour.

3-**Hemoglobin/hematocrit**: slightly decreased "i.e. normochromic anemia", may be normocytic or microcytic.

4-**Liver function**: normal or slightly elevated alkaline phosphatase (ALP).

5-**Platelets**: usually increased.

6-**Radiographic findings**: may be normal or show osteopenia or erosions near joint spaces.

7-**Rheumatoid factor**: positive in approximately 70% of patients with R.A.

8-**White blood count**: may be increased.

## Exclusion Criteria

♦ Patients with any cause of hyperprolactinaemia as: prolactinoma, pregnancy, lactation, hypothyroidism, emotional stress, drugs e.g Motilium, Primperan, Cimetidine.

♦ History of intake of vitamin D and calcium within one month.

## **All cases enrolled into study will be submitted to**:

1-Full medical history.

2-Full clinical examination: pulse, blood pressure, weight, careful joint examination, complete drug history.

3-Laboratory investigations:

▪*Routine investigations*: ESR, rheumatoid factor, CBC.

4-Prolactin: measured using ELISA technique.

5-Ferritin: measured using ELISA technique.

6-Vitamin D: measured using ELISA technique.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ACPA** | Antibodies to Citrullinated Peptides or Anti-Citrullinated Protein antibodies. |
| **ACD** | Anemia of chronic disease |
| **ACR** | American College of Rheumatology |
| **ACRSRA** | American College of Rheumatology Subcommittee on Rheumatoid Arthritis. |
| **ADCC** | Antibody Dependent Cell-mediated Cytotoxicity. |
| **ALP** | Alkaline phosphatase. |
| **ANA** | Antinuclear antibody. |
| **Anti-CCP** | Anti-cyclic citrullinated peptide. |
| **Anti-ds DNA** | Anti-double-stranded DNA. |
| **Anti-MCV** | Antibodies against mutated citrullinated vimentin. |
| **APCs** | Antigen-presenting cells. |
| **APRs** | Acute-phase reactants. |

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|---|---|
| **b/min.** | Beat/minute. |
| **CBC** | Complete blood count. |
| **CBL** | Casitas B-Lineage Lymphoma. |
| **CD4+** | $T_H$ (helper) cells. |
| **CDAI** | Clinical Disease Activity Index. |
| **cDNA** | Complementary DNA. |
| **CNS** | Central nervous system. |
| **CRP** | C-reactive protein. |
| **$D_2$** | Ergocalciferol. |
| **$D_3$** | Cholecalciferol. |
| **DA** | Dopamine. |
| **DAS** | Disease Activity Score. |
| **DCH** | Dehydrocholesterol. |
| **dm** | Dermatomyositis. |
| **DMARDs** | Disease-modifying anti-rheumatic drugs. |
| **DNA** | Deoxyribonucleic acid. |

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|---|---|
| **EBV** | Epstein-Barr virus. |
| **EDTA** | Ethylene Diamine tetra-acetate. |
| **ELISA** | Enzyme-linked immunosorbent assay. |
| **ESR** | Erythrocyte sedimentation rate. |
| **EULAR** | European League Against Rheumatism. |
| **Fc** | Fragment, crystallizable. |
| **FDA** | Food and Drug Administration. |
| **GABA** | Gamma-amino butyric acid. |
| **GC** | Germinal center. |
| **HAQ** | Health Assessment Questionnaire. |
| **Hb** | Hemoglobin. |
| **HHV-6** | Human Herpes virus-6. |
| **HLA** | Human leucocytic antigen. |
| **Hp** | Haptoglobulin. |
| **HPRL** | Hyperprolactinemia. |

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|---|---|
| **IDA** | Iron-deficiency anemia. |
| **IFN-¥** | Interferon-gamma. |
| **Ig** | Immunoglobulin. |
| **IL** | Interleukin. |
| **IU** | International unit. |
| **KDa** | Kilodalton. |
| **Kg** | Kilogram. |
| **L** | Litre. |
| **MCHC** | Mean corpuscular hemoglobin concentration. |
| **MCP** | Metacarpophalangeal. |
| **MCV** | Mean corpuscular volume. |
| **MDA** | Minimal Disease Activity. |
| **MHC** | Major Histocompatibility Complex. |
| **mL** | millilitre. |
| **MRI** | Magnetic resonance imaging. |

| | |
|---|---|
| **mRNA** | messenger RNA. |
| **MS** | Multiple sclerosis. |
| **MTP** | Metatarsophalangeal. |
| **MTX** | Methotrexate. |
| **mU** | milli-international unit. |
| **NAB** | Neutralizing antibodies. |
| **NF-©B** | Nuclear factor kappa-light-chain-enhancer of activated B cells. |
| **ng** | nanogram. |
| **NK** | Natural killer. |
| **nmol** | nanomol |
| **NSAIDs** | Non-steroidal anti-inflammatory drugs. |
| **NT** | Neurotensin. |
| **O.A** | Osteoarthritis. |
| **OT** | Oxytocin. |