# Peptidyl Argenine Deiminase- 4 (PADI4) as Diagnostic and Prognostic Marker in Rheumatoid Arthritis

Thesis

*Submitted in Fulfillment of Master Degree of Physical Medicine Rheumatology and Rehabilitation*

By

***Nada Hamdi Hussein***
*M.B.,B.Ch., 2013*
*Faculty of Medicine-Ain Shams University*

Under supervision of

**Prof. Dr. Nadia Salah Kamel Abd el Bar**
*Professor of Physical Medicine*
*Rheumatology and Rehabilitation*
*Faculty of Medicine - Ain Shams University*

**Prof. Dr. Neven Mahmoud Taha Fouda**
*Professor of Physical Medicine*
*Rheumatology and Rehabilitation*
*Faculty of Medicine - Ain Shams University*

**Dr. Dina Abou Bakr Farrag**
*Lecturer in Physical Medicine*
*Rheumatology and Rehabilitation*
*Faculty of Medicine - Ain Shams University*

**Faculty of Medicine - Ain Shams University**
**2017**


سورة البقرة الآية: ٣٢

# Acknowledgment

*First and foremost, I feel always indebted to ALLAH, the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to Prof. Dr. Nadia Salah Kamel Abd el Bar, Professor of Physical Medicine Rheumatology and Rehabilitation - Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to Prof. Dr. Neven Mahmoud Taha Fouda, Professor of Physical Medicine Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

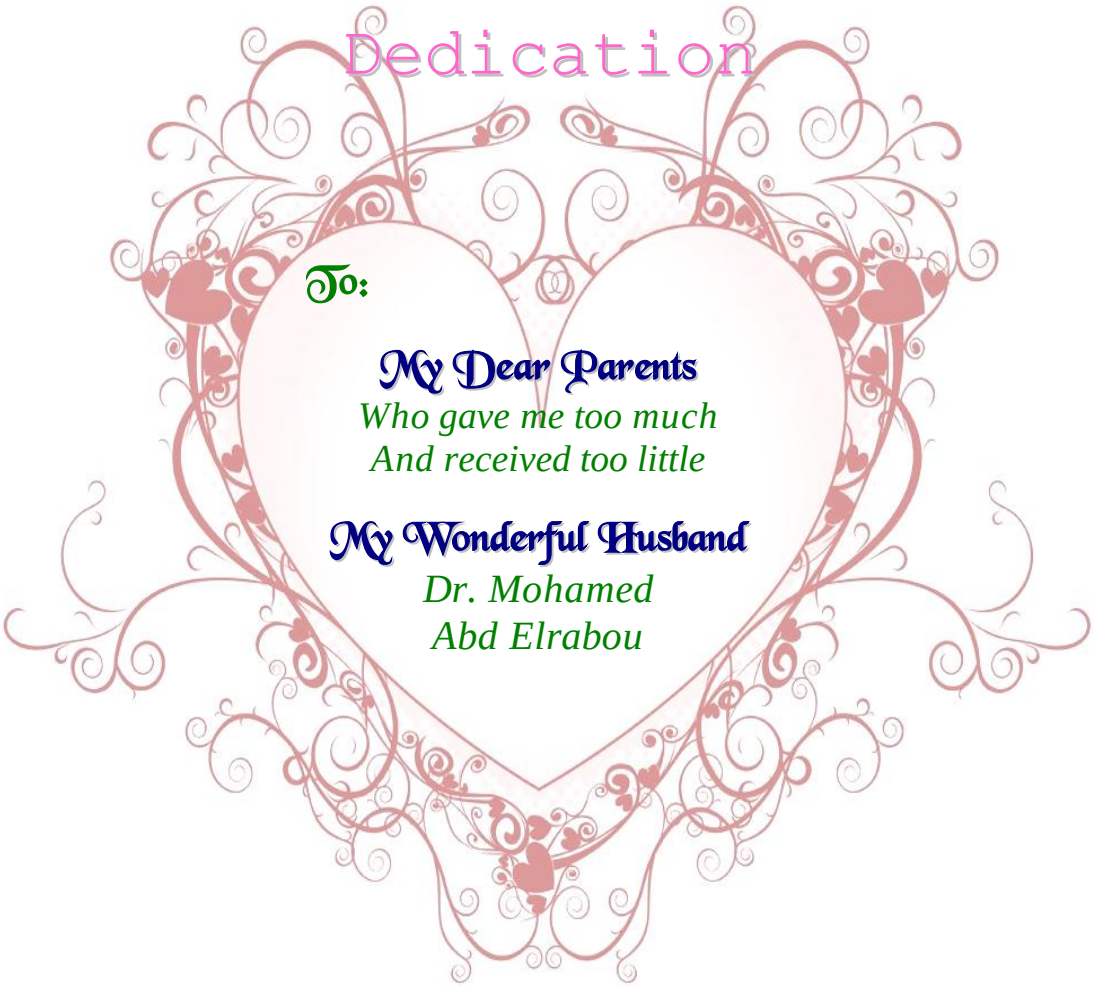
*I am deeply thankful to Dr. Dina Abou Bakr Farrag, Lecturer in Physical Medicine Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

*Special thanks goes to the team of immunology laboratory, Ain Shams University Hospital for their help and co-operation.*

*Last but not least my sincere thanks and appreciation to all patients participated in this study.*

**Nada Hamdi Hussein**

# Dedication



To:

**My Dear Parents**

*Who gave me too much*
*And received too little*

**My Wonderful Husband**

*Dr. Mohamed*
*Abd Elrabou*

# List of Contents

# List of Tables

# List of Tables (Cont…)

# List of Figures

# List of Abbreviations

| Abb. | Full term |
|---|---|
| *ACPA* | *Anti-citrullinated peptide antibodeis* |
| *AKA* | *Anti-keratin autoantibodies* |
| *Anti-CCP* | *Antibodies to a cyclic citrullinated peptide* |
| *APF* | *Anti-perinuclear factor* |
| *AFA* | *Ani –Fillagrin autoantibodies* |
| *Anti –MCV* | *Anti -Mutilated Citrullinated Vimentin* |
| *CCP* | *Cyclical citrullinated peptides* |
| *DAS28* | *Disease activity score* |
| *DMARDS* | *Disease modifying antirheumatic drug* |
| *ELISA* | *Enzyme linked immune sorbent assay* |
| *ESR* | *Erythrocytes Sedimentation Rate* |
| *ICs* | *Immune complexes* |
| *IP* | *Interphalyngeal* |
| *MBP* | *Myelin basic protein* |
| *MCP* | *Metacarpophalyngeal* |
| *MHAQ* | *Modified health assessment questionnaire* |
| *MS* | *Multiple sclerosis* |
| *MTP* | *Metatarsophalyngeal* |
| *MTX* | *Methotrexate* |
| *NETs* | *Neutrophil extracellular traps* |
| *PAD* | *Peptidyl Arginine Deaminase* |
| *PAD4=PADI4* | *Peptidyl arginine deiminase 4* |
| *PDCs* | *Plasmacytoid dendritic cells* |
| *PGA* | *Patient global assessment* |
| *PIP* | *Proximal interphalyngeal* |
| *RA* | *Rheumatoid Arthritis* |
| *RFs* | *Rheumatoid factors* |
| *SF* | *Synovial fluid* |
| *SLE* | *Systemic lupus erythrymatosis* |
| *TNFα* | *Tumor necrosis factor α* |
| *VEGF* | *Vascular endothelial growth factor* |

**Abstract**

**Introduction:** Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint destruction, functional impairment, disability, and reduced life expectancy. The bone and cartilage destruction rarely heals, the damage accumulates over time, thus early interfering with the inflammatory cascade before it is fully established is most effective.

**Aim of the Work:** His study is designed to assess the role of serum PADI4 level as diagnostic and prognostic marker in RA patients.

**Patients and Methods: T**his study was conducted on 31 RA patients diagnosed according to the American College of Rheumatology (ACR) new classification criteria. Ten healthy individuals matched for age and sex who served as a control group.

In conclusion, our study highlighted that PAD4 sensitivity was 90.3% and specificity was 100% while anti-CCP sensitivity was 93% and its specificity was 100%. According to these findings serum PAD4 provide additional diagnostic value not over the already established anti-CCP, but it is correlated with disease activity and can be used in follow up remission in RA patients.

# INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint destruction, functional impairment, disability, and reduced life expectancy. The bone and cartilage destruction rarely heals, the damage accumulates over time, thus early interfering with the inflammatory cascade before it is fully established is most effective ***(Ideguchi et al., 2006).***

Disease modifying drugs (DMARDS) and particularly new biological agents have been shown to control both clinical and laboratory signs. Therefore, it is evident that therapeutic intervention will have greater effect on the outcome if started early, and ideally, if commenced even before damage has occurred ***(Mitchell et al., 2007).***

Rheumatoid factors (RFs) and other autoantibodies have been associated with RA. Antibodies to a cyclic citrullinated peptide (anti-CCP) were found to be specific for RA, including the early form. They have a possible prognostic value, either individually or in combination with RF, as a marker of a more serious disease. Anti-CCP antibodies were incorporated into diagnostic criteria for RA (American College of Rheumatology /European League Against Rheumatism) ACR-EULAR, and their positivity showed association with erosive arthritis ***(Manivelavan et al., 2012).***

In addition to the widely used RF and anti-CCP autoantibody assay, several tests have emerged to help in diagnosis of RA such as serum anti PAD4 ***(Karlson et al., 2016),*** Anti-carbamylated protein antibodies ***(Koppejan et al., 2016),*** and recently serum PAD4 which is the ezyme that cause citrullination of proteins leading to production of autoantibodies ***(Umeda et al., 2016).***

Peptidyl Arginine Deaminase (PAD) enzymes consist of five PAD isoforms (PADs 1 to 4 and PAD6), with differential cellular and tissue distribution, that have been described in humans. They are responsible for post-translational modification of the amino acid arginine to citrulline, a process known as citrullination. This process regulates homeostatic processes such as keratinocyte differentiation and maintenance of myelin sheath insulation. Also it is involved in the innate immune response and regulation of chemokine activity ***(Harauz and Musse, 2007).***

In addition PADs have been linked also to the pathogenesis of an increasing number of chronic inflammatory diseases, including neurodegenerative conditions such as multiple sclerosis and Alzheimer's disease ***(Mortier et al., 2011)***.

The process of citrullination is likely to be of significance in patients with RA as PADs are expressed in synovial tissues, also neutrophils express high levels of PAD

and accumulate in the synovial fluid (SF) during disease flares. One isoform of these PAD family is PAD4 which has been found extensively expressed in T cells, B cells, macrophages, neutrophils, fibroblast-like cells and endothelial cells in the lining and sublining areas of the RA synovium. The immunoreactivity of citrullinated fibrin with IgA and IgM in the RA synovium supports the notion that citrullinated fibrin caused by PAD 4 enzyme activity is a potential antigen of RA autoimmunity ***(Vossenaar et al., 2004)***.

However, the role of PAD 4 in the development of RA and in disease evolution has not been fully elucidated. Subsequent studies showed that PAD 4 may also act as an antigen, generating antibody responses in subjects with RA and these Anti PAD antibodies can be used as an additional marker with anti CCP and RF ***(Yang et al., 2015).***

While ***Quian et al. (2011) and Umeda et al. (2016)*** had studied the role of serum PAD4 enzyme level as an additional diagnostic marker of RA and for monitoring disease activity in addition to anti-CCP and RF.

# Aim of the Work

This study is designed to assess the role of serum PADI4 level as diagnostic and prognostic marker in RA patients.

# RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic, systemic, progressive autoimmune disease, that principally attacks the joints in a systemic pattern producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints ***(Hekmat et al., 2011)***.

RA affects almost 1-2% of the population. It has an impact on health causing pain, fatigue, radiological damage, functional disability and reduced life expectancy ***(Cohen et al., 2007).***

Extra-articular complications also often occur, leading to a worsening of the prognosis ***(Okuda, 2008).***

RA is considered an autoimmune disease ***(Firestein., 2003).*** Autoimmunity and the overall systemic and articular inflammatory load drive the destructive progression of the disease. However, structural changes can be visualized by conventional radiography or other imaging techniques and distinguish RA from other arthritic disorders ***(Bohndorf et al., 1996).***