INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disorder characterized by inflammation and destruction of articular structures in association with extra-articular manifestations ***(Aref and Ahmed, 2015).***

RA is characterized by abnormal proliferation of synoviocytes, leukocyte infiltration, and angiogenesis ***(Park et al., 2014).*** Epidemiological studies show that RA affects 1% of the population worldwide ***(Rojas-Villarraga et al., 2009).***

The etiology of RA is largely unknown. However, it is considered multifactorial: genetic predisposition and the presence of MHC class II genes3 and PTPN224 increase the susceptibility of RA; the presence of rheumatoid factor (RF) and anti-citrullinated protein-antibodies (ACPA) point to a contribution of autoimmunity mechanisms. Other environmental factors as smoking and obesity may also play a role ***(De Hair et al., 2013).***

The risk of generalized and localized osteoporosis is increased in RA mainly due to prolonged active disease, immobility and decreased functional capacity, and frequent treatment with glucocorticoids and periarticular demineralization, which is probably due to local release of inflammatory agents ***(Hoes et al., 2015).***

ACPA is a highly specific marker for RA ***(Taylor et al., 2011)*** and although its presence is known to be associated with a more severe disease ***(Shi et al., 2011),*** it's only recently discovered to promote bone loss by binding osteoclast precursor cells and directly promoting their differentiation into bone-resorbing osteoclasts.

Other results have shown that in ACPA-positive individuals bone loss starts even before the onset of clinical disease; this is indicative of the independent effect of these antibodies in initiating skeletal damage ***(Kocijan et al., 2013)*** and thus they may be considered a determinant for bone loss ***(Guler et al., 2008).***

AIM OF WORK

This study aimed to assess the effect of anti-cyclic citrullinated peptide (anti-CCP) antibody on bone mineral density (BMD), in a cohort of Egyptian patients with rheumatoid arthritis.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of synovial tissue that leads to damage of cartilage and bone, resulting in irreversible joint destruction ***(Smigielska-Czepiel et al., 2014).***

Epidemiology:

The prevalence of RA is relatively constant in many populations, at 0.5-1% ***(Uhlig, 2014).*** The prevalence of RA in rural Egypt is 0.29% similar to other oriental rural populations but lower than western populations ***(Abdel Tawab et al., 2009)***.

Women are 3 times more often than men. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected ***(Majithia and Greraci, 2007).***

Mortality rates are more than twice as high in patients with RA as in the general population ***(Choy, 2012).***

Etiology of RA:

The exact cause of RA is unknown. It is the result of an environmental exposure or "trigger" in a genetically susceptible individual ***(Gibofsky, 2012).***

1-Hormonal factors:

The predominance of RA in females suggests a role for hormonal factors, estrogens stimulate the immune system, and Low testosterone levels have been reported in men with RA ***(Tobon et al., 2010).*** Female sex hormones may play a protective role in RA. For example, the use of the oral contraceptives pills and pregnancy are both associated with a decreased risk ***(Alan and Jacqueline, 2012).*** One possible explanation to this finding is that hormone replacement therapy (HRT) protects against the production of anti-citrullinated protein-antibodies (ACPA) ***(Tobón et al., 2010).***

Improvement occurs for 50 to 70% of patients by the end of the first trimester and is usually sustained throughout pregnancy. However, within 3 months of delivery, relapse is observed in 90% of patients ***(Shammas et al., 2010).*** Both female subfertility and the immediate postpartum period after a first pregnancy (especially when breastfeeding) appear to increase the risk of RA ***(McInnes and Schett, 2011).***

Male sex also appears to affect disease phenotype. Compared with female RA patients, male patients have significantly later disease onset, are more likely to be RF-positive ***(Jawaheer et al., 2006).***

2-Genetic factors:

Several genes have been indicated so far in the pathogenesis of RA. The most important region is the Human Leukocyte Antigen (HLA) that contributes to approximately half of the genetic susceptibility for RA. The association seems to be stronger or specific for anti-citrullinated protein antibodies positive disease. Several alleles in the epitope-recognition part of the HLA molecule that show the highest association with RA susceptibility, also share a common string of amminoacid residues (the so-called shared-epitope (SE) hypothesis) ***(McInnes and Schett, 2011).***

Other variants in potentially pathogenic genes located in non-MHC regions have been implicated by recently performed genome wide analysis studies. These genes include PTPN22, TRAF1-C5, PADI4, STAT4. Other polymorphisms seem to be responsible for more aggressive disease phenotype such as those located at TNF, IL-1, IL-6, IL-4, IL-5, OPN, PRF1 ***(McInnes and Schett, 2011).***

Twin studies show concordance rates of 15% to 30% between monozygotic twins and 5% among dizygotic twins, suggesting that 50% to 60% of RA cases are due to genetic factors ***(McInnes and Schett, 2011).***

Single-nucleotide polymorphism genotyping across the MHC has identified additional alleles related to RA risk,

including those found on the conserved A1-B8-DR3 (8.1) haplotype and those near the *HLA-DPB1*gene. Other RA-associated loci are PTPN22, PADI4, STAT4, TRAF1-C5 and TNFAIP3, although non-MHC risk alleles may represent only 3–5% of the genetic burden of RA ***(Perricone et al., 2011).***

Studies on gene polymorphisms may allow to determine a group of patients with a predisposition to higher efficacy of certain drugs like Methotrexate (MTX) or Tumor necrosis factor inhibitor or to the development of adverse effects during the therapy ***(Świerkot et al., 2015).***

3- Infectious agents:

Infections have for a long time been proposed to be potential triggers of rheumatoid arthritis (RA), preceding the clinical onset of disease, but despite decades of studies there is very limited epidemiological evidence to support this hypothesis, apart from the well-studied association with Porphyromonas gingivalis through periodontitis ***(Sandberg et al., 2015).***

More recently, it has been recognized that changes in the composition of the microbiome in the gut and other mucosal surfaces may have a major impact on immune homeostasis and inflammatory diseases ***(Cénit et al., 2014)***.

Hemophilus species were depleted in individuals with RA in fecal, dental and salivary microbiomes and negatively correlated with the levels of serum autoantibodies, whereas Lactobacillus salivarius was over-represented in RA patients at all three sites and was especially elevated in patients with very active RA. The clinical significance of these findings are still under evaluation ***(Zhang et al., 2015).***

4- Smoking:

Peptidyl arginine deiminase, type IV (PADI4), an enzyme responsible for post-translational citrullination of peptide antigens on arginine residues. PADI4 has the ability to alter citrullination of mucosal proteins, and its activity is associated with smoking cigarettes ***(De Pablo et al., 2009)***. Citrullinated peptide antigens leads to production of ACPA ***(Okamoto, 2014).***

Pathogenesis of RA:

Activation of innate immunity is probably the earliest process in RA, followed by citrullination, loading of antigen presenting cells (APCs) with autoantigens in the joint, then migration to central lymphoid organs. Once there, APCs present an array of antigens to T cells, which can then activate B cells and/or migrate back to the synovium ***(Schur and Firestein, 2012).***

RA pathogenesis involves complex humoral and cellular reactions including immune complex (IC) formation, vascular reactions and infiltration of lymphocytes and monocytes into the synovium. These infiltrating cells and synoviocytes release pro-inflammatory mediators including IL-6, which maintains inflammation and destruction through effects on other cell types in the synovium and peri-articular structures ***(Dayer et al., 2010).***

- **T - lymphocytes and initial antigen(s):**

T cells constitute about 50 percent or more of cells in most RA synovium; RA synovial T lymphocytes has high expression of HLA-DR antigens and CD27. CD27+ CD4+ T cells provide B cell help that can potentially increase synovial antibody production. There appears to be a preponderance of T cells of the Th1 and Th17 subset, with deficiency of Th2 and regulatory T cells. A broad spectrum of joint specific antigens, such a type II collagen, or nonspecific citrullinated antigens, is responsible for this preponderance of Th1 and Th17 subset ***(Snir et al., 2010).***

The initiating antigen(s) probably vary from patient to patient, perhaps from joint to joint, and from early to late disease in the same patient ***(Schur and Firestein, 2012).***

The three-dimensional reconstruction of the HLA class II antigen structure suggests that recognition is likely to be peptide-dependent, but not peptide-specific ***(Raychaudhuri et al., 2012).***

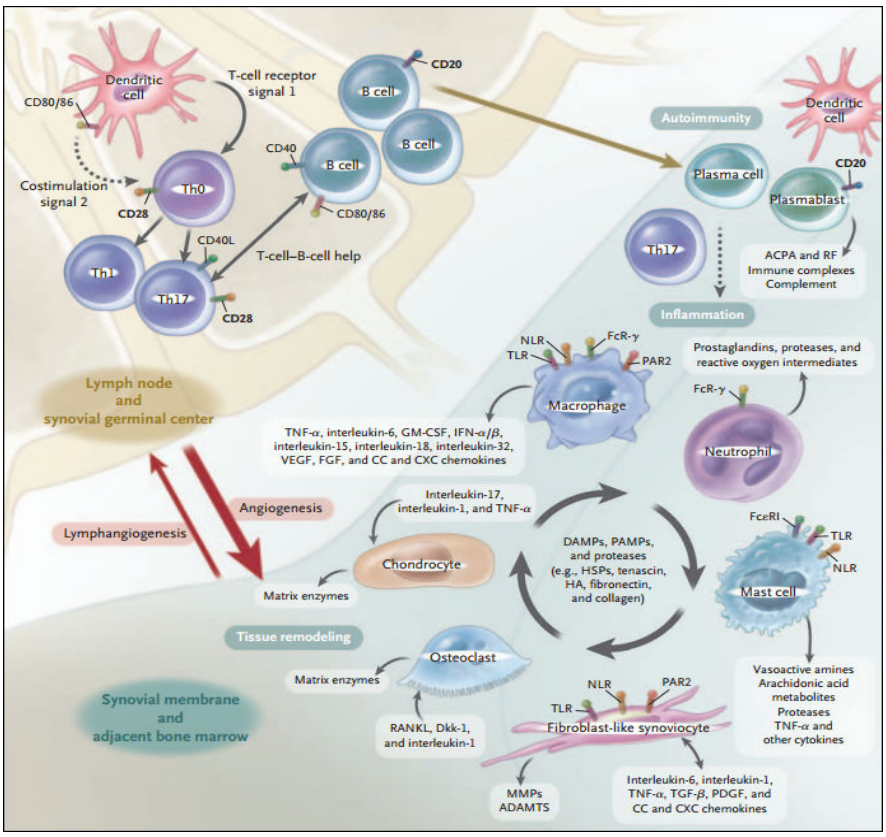

Figure (1): Adaptive and Innate Immune Processes within the Joint in Rheumatoid Arthritis. The costimulation-dependent interactions among dendritic cells, T cells, and B cells are shown. ACPA indicates anti-citrullinated protein antibody; ADAMTS, a disintegrin and metalloprotease with thrombospondin-1–like domains; DAMP, damage-associated molecular pattern; Dkk-1, dickkopf-1; FcR, Fc receptor; FcεRI, high-affinity IgE receptor; FGF, fibroblast growth factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HA, hyaluronan; HSP, heat-shock protein; IFN-α/β, interferon-α/β; MMP, matrix metalloproteinase; NLR, nucleotide-binding oligomerization domain–like receptor; PAMP, pathogen-associated molecular pattern; PAR2, protease-activated receptor 2; PDGF, platelet-derived growth factor; RANKL, receptor activator of nuclear factor κB ligand; RF, rheumatoid factor; TGF-β, transforming growth factor β; Th0, type 0 helper T cell; Th1, type 1 helper T cell; Th17, type 17 helper T cell; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor. From ***(McInnes and Schett 2011)***.

- **B lymphocytes and autoantibodies:**

B-cell play several critical roles in the pathogenesis of RA. They are the source of the RF and Anti-CCP antibodies which contribute to immune complex formation and complement activation in the joints. They are also very efficient antigen- presenting cells (and produce chemokines and cytokines that promote leucocyte infiltration in the joints and formation of ectopic lymphoid structures, angiogenesis and synovial hyperplasia ***(Silverman and Carson, 2003).***

Autoantibodies: as RF and anti-CCP *(discussed later).*

- **Polymorphnuclear leukocytes:**

They are attracted to the joint, penetrate through synovial blood vessels, and quickly move into the joint space. In very active disease, up to one billion cells may gain access to a rheumatoid knee joint each day, while few if any leave the joint and are degraded, these leukocytes produce myeloperoxidase, elastase, lysozyme, collagenase, acid hydrolases, interleukin-1 beta, prostaglandins, platelet activating factor (PAF) and leukotrienes ***(Schur and Firestein, 2012)***.

These products, once released into the synovial fluid, can cause considerable damage and potentiate inflammation in the adjacent synovium. In addition, since leukotriene B4 and PAF are among the most potent chemo attractants known, activated neutrophils are able to recruit additional neutrophils in an autocrine fashion ***(Schur and Firestein, 2012)***.

It has been known that rheumatoid synovial fluid has a low oxygen tension. It has become appreciated that hypoxia is an important factor in aggravating the inflammatory lesion in RA through increased production of COX-2-derived nociceptive eicosanoids and increased production by synovial cells of Matrix metalloproteinases (MMPs) ***(Demasi et al., 2004).***

Complement activation and immune complexes:

These complexes can be found in all tissues of the rheumatoid joint, and may help concentrate additional material within this structure. Cartilage destruction is facilitated by proteolytic degradation of matrix components by enzymes such as neutrophil elastase. In addition, immune complexes isolated from synovial fluids may stimulate the production of TNF from monocytes/macrophages ***(Paula and Alves, 2014).***

In patients with active rheumatoid synovitis, but few extra articular manifestations, C3, C4, and CH50 levels are usually normal or increased ***(Schur and Firestein, 2012)***.

Levels of both C3a is elevated in rheumatoid synovial fluids and correlates with C-reactive protein (CRP) levels, ESR, and disease activity indices ***(Dirmeier et al., 2008)***

Angiogenesis and inflammatory cell recruitment:

- **New blood vessel growths:** One of the earliest histopathologic responses in RA is the generation of new synovial blood vessels. This event is accompanied by the

transudation of fluid and the transmigration of both lymphocytes into the synovium and of polymorphonuclear leukocytes into the synovial fluid ***(Szekanecz et al., 2010).***

- **Cell migration:** as the new vessels develop, cytokines produced in the synovium in response to the driving force of TNF (including IL-1, IL-6, IFNγ, and substance P) activate endothelial cells to produce adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin, and E-selectin ***(Lally et al., 2005).***

These cellular adhesion molecules expedite activation dependent sticking of leukocytes, thereby facilitating diapedesis and extravasation into the synovium. It appears that IL-15 and IL-18 have a major role in stimulating production of TNF, which has a broad capacity to trigger biosynthesis of multiple effector proteins ***(Lally et al., 2005).***

Cytokines network in RA:

- ***Role of cytokines in synovitis:*** Autocrine and paracrine communication through the elaboration of pro-inflammatory cytokines play a key role in initiation and perpetuation of RA. A cascade network of cytokines has a pivotal role in synovitis, including granulocyte macrophage colony stimulating factor (GM-CSF), IL-2, IL-15, IL-13, IL-17, IL-18, IFNγ, TNF, and TGF-β.

Table (1): Key Molecules and Signal Mediators Implicated in the Pathogenesis of Rheumatoid Arthritis ***(McInnes and Schett, 2011)***

Molecule or Signal Mediator Cytokines	Key Disease-Relevant Functions	Status†
TNF-α	Activates leukocytes, endothelial cells, and synovial fibroblasts, inducing production of cytokines, chemokines, adhesion molecules, and matrix enzymes; suppression of regulatory T-cell function; activation of osteoclasts; and resorption of cartilage and bone; mediates metabolic and cognitive dysfunction.	Approved drug
Interleukin-1α and 1β	Activate leukocytes, endothelial cells, and synovial fibroblasts; induce matrix-enzyme production by chondrocytes; activate osteoclasts; mediate fever; enhance glucose metabolism; and reduce cognitive function.	Approved drug
Interleukin-6	Activates leukocytes and osteoclasts ; is involved in B-lymphocyte differentiation; regulates lipid metabolism, acute-phase response, and anemia of chronic disease; and is implicated in hypothalamic–pituitary–adrenal axis dysfunction and fatigue	Approved drug
Interleukin-7 and 15	Promote and maintain T-cell and natural killer–cell activation and T-cell memory, block apoptosis, and maintain T-cell–macrophage cognate interactions	Phase 2trial completed
Interleukin-17A and 17F	Act synergistically to enhance activation of synovial fibroblasts, chondrocytes, and osteoclasts	More than one phase 2 trial with positive results
Interleukin-18	Promotes activation of Th1, neutrophils, and natural killer cells	
Interleukin-21	Activates Th17 and B-cell subsets	
Interleukin-23	Expands Th17	
Interleukin-32	Activates cytokine production by several	

	leukocytes and promotes osteoclast Differentiation	
Interleukin-33	Activates mast cells and neutrophils	
Growth and differentiation factors		
BLyS and APRIL	Activate B cells and have a role in the maturation of B cells and enhancement of autoantibody production	In phase 2 trial
GM-CSF and M-CSF	Enhance differentiation of granulocyte and myeloid-lineage cells in the bone marrow and synovium	In phase 1 trial
RANKL	Promotes maturation and activation of osteoclasts	Phase2trial completed
Intracellular signaling molecules and transcription factors		
JAK	Tyrosine kinase that regulates cytokine-mediated leukocyte maturation and activation, cytokine production, and immunoglobulin production	More than one phase 2 trial with positive results
Syk	Tyrosine kinase that regulates immune-complex–mediated and antigen-mediated activation of B and T cells and other Fc receptor–bearing leukocytes	More than one phase 2 trial with positive results
PI3K	Mediates signals that drive proliferation and cell survival	Phase 1trial planned
BTK	Plays important role in the activation of B cells, macrophages, mast cells, and neutrophils, through regulation of B-cell receptor and Fc receptor signaling as appropriate	Phase 1trial planned
NFκB	Helps integrate inflammatory signaling and is important for cell survival	

* APRIL denotes a proliferation-inducing ligand, BLyS B-lymphocyte stimulator, BTK Bruton's tyrosine kinase, GM-CSF granulocyte–macrophage colony-stimulating factor, JAK Janus kinase, M-CSF macrophage colony-stimulating factor, PI3K phosphatidylinositol 3-kinase, RANKL receptor activator of NF-κB ligand, Syk spleen tyrosine kinase, and Th1 type 1 helper T cells.

† Status indicates the investigational status of agents targeting the molecule or signal mediator. Approved drugs have been approved by the Food and Drug Administration and European Medicines Agency for use in patients with rheumatoid arthritis.