INTRODUCTION

The discovery of the vitamin D receptor (VDR) in the cells of the immune system and the fact that activated dendritic cells (DCs) produce the vitamin D hormone suggested that vitamin D could have immuno-regulatory properties ***(Turhanoglu et al., 2011).*** Therefore, 1, 25-dihydroxyvitamin D3 [1, 25 $(OH)_2$D3] the biologically active metabolite of Vitamin D3, not only regulates bone and calcium metabolism but also exerts immunomodulation via the nuclear VDR expressed in antigen-presenting cells and activated T/B cells ***(Etten and Mathieu, 2005).***

It has been already established that vitamin D has several reported immune-modulatory properties including the reduced generation of pro-inflammatory CD4+Th1 cells and the increase in levels of the anti-inflammatory Th2 subset. Less clear has been the impact of vitamin D on the pro-inflammatory Th17 subset, and whether and how vitamin D may preferentially drive the polarization of one of the T helper subsets ***(Sloka et al., 2011).***

Autoimmunity is driven by Th1, which attack various self-tissues in the body. It is clear that both genetic and environmental factors affect disease prevalence. The fact that vitamin D has been implicated as a factor in several different autoimmune diseases suggests that vitamin D might be an environmental factor that normally participates in the control of

self tolerance. In addition, there may be a higher vitamin D requirement for patients at risk for developing and those that already have an autoimmune disease ***(Cantorana and Mahon, 2004).***

Experimentally, vitamin D deficiency results in the increased incidence of autoimmune disease. Mechanistically, the data point to a role for vitamin D in the development of self-tolerance. The vitamin D hormone (1, 25-dihydroxy vitamin D3) regulates Th1 and DC function while inducing regulatory T-cell function. The net result is a decrease in the Th1-driven autoimmune response and decreased severity of symptoms ***(Cantorana and Mahon, 2004)***.

Low serum levels of vitamin D3 might be partially related, among other factors, to prolonged daily darkness (reduced activation of the pre-vitamin D by the ultraviolet B sunlight), different genetic background (i.e. VDR polymorphism) and nutritional factors, and explain to the latitute-related prevalence of autoimmune diseases such as Rheumatoid arthritis (RA), by considering the potential immunosuppressive roles of vitamin D ***(Cutolo et al., 2007).***

Indeed, major immune system-mediated rheumatic diseases such as RA and systemic lupus erythematosus (SLE), systemic sclerosis or overlap syndromes like undifferentiated connective tissue disease (UCTD) and others are characterized

by low-serum levels of vitamin D that often correlated to the severity of the disease ***(Pelajo et al., 2010).***

Moreover, low vitamin D intake has been implicated as a risk factor in the development of RA, and recent investigations have linked low vitamin D levels with increased disease activity and severity in patients with inflammatory arthritis ***(Liao et al., 2009).***

AIM OF THE WORK

The aim of this work is to establish a relationship between serum vitamin D level and disease activity in RA patients.

Chapter One

RHEUMATOID ARTHRITIS

Introduction:

Rheumatoid arthritis (RA) is a chronic inflammatory disease that manifests predominantly as synovial inflammation leading to cartilage damage and destruction of the joint infra structure**.** Although the joint symptomatology is eventually dominant, the disease is preceded by immune abnormalities that are not joint specific, but systemic and are already apparent many years before onset of the disease ***(Kokkonen et al., 2010).*** It also can affect other tissues, resulting in anorexia, weight loss, fatigue, general itching and stiffiness ***(Lee, 2007*)*.***

Despite intensive work, causes of RA are still not completely known. Clues have been provided by detailed studies of immunogenetics of the class II major histocompatibility complex (MHC) loci and the usage of specific RF genes, but what causes the disease is not known ***(Orozco et al., 2006)***. The role of small-molecule mediators of inflammation, cytokines, growth factors, chemokines, adhesion molecules, and matrix metalloproteinases (MMPs) has been carefully defined ***(Firestein, 2005)***.

Epidemiology:

RA affects approximately 0.8% of the population, is more common in older persons and affects female three times more often than males ***(Pattison et al., 2004)*** Most patients who develop disease are postmenopausal women; indeed the incidence of the disease continues to rise at least into the seventh decade of life and possibly even beyond that ***(Goronzy et al., 2005).***

RA might be a relatively new disease in Europe and Northern Africa. Although still controversial, at least one school of thought suggests that the disease migrated from the New World to the Old World coincident with opening the trade and exploration routes. Because genetic admixture was relatively limited, an undefined environmental exposure potentially caused RA in susceptible Europeans. The most obvious explanation would, of course, be that an infectious agent is responsible ***(Doran et al., 2002)***.

Equally intriguing, the severity and incidence of RA seems to be decreasing. Whereas the former could be related to the advent of new treatments, the latter could be due to a "birth cohort" effect. In certain well-defined populations, including Native Americans, the incidence of RA has gradually declined by as much as half over the last half of the twentieth century. The birth-cohort theory suggests that the earlier high incidence of disease was caused by an etiologic agent, and the exposure

decreased with each succeeding generation. Changes in hygiene and other lifestyle modifications related to industrialization might contribute, and an infectious agent might be less prevalent secondary to these societal changes, as with many other infectious diseases ***(Firestein, 2005).***

Risk factors include female sex, a positive family history, older age, silicate exposure and smoking**.** Consumption of more than three cups of coffee daily - particularly decaffeinated coffee - also may contribute ***(Mikuls et al., 2002).*** On the other hand it was reported that high vitamin D intake ***(Merlino et al., 2004),*** tea consumption ***(Mikuls et al., 2002)*** and oral contraceptive use ***(Kuder et al., 2002)*** are associated with decreased risk.

Pathogenesis:

RA is an autoimmune disease. It is a systemic disorder principally affecting synovial joints ***(Lee, 2007)***. Although the etiology of RA remains a mystery, a variety of studies suggest that a blend of environmental and genetic factors is responsible. A contribution of either one is necessary but not sufficient for full expression of the disease. One or multiple genetic factors probably predispose an individual to developing RA. However, attempts to identify specific infectious agents as the etiology have generally met with disappointment. A guess, based on available data, is that several environmental stimuli, possibly viruses or retroviruses, infect an individual with the appropriate

genetic background, and through some mechanism, the inflammatory response is focused in joints ***(Alamanos and Drosos, 2005).***

Otherwise evidence implicates T and B cells as well as innate immune elements ***(Firestein, 2003).*** The last decade has seen a shift to the model that patients with RA have a fundamental breakdown in self-tolerance and that patients are not able to induce or maintain tolerance to neoantigens ***(Goronzy and Weyand, 2009).*** This breakdown in tolerance occurs in the second half of life suggesting that it is acquired ***(Doran et al., 2002).***

- ***Genetics and environmental factors:***

RA involves a complex interplay among genotype, environmental triggers, and chance. Twin studies implicate genetic factors in RA, with concordance rates of 15 to 30% among monozygotic twins and 5% among dizygotic twins ***(Macgregor et al., 2000; Mclnnes and Schett, 2011).*** Genomewide analysis make it clear that immune regulatory factors underlie the disease ***(Wellcome Trust Case Control Consortim, 2007; Mclnnes and Schett, 2011).***

The long-established association with the human leukocyte antigen (HLA)–DRB1 locus has been confirmed in patients who are positive for rheumatoid factor (RF) or anti-cirtullinated protein antibodies (ACPA); alleles that contain a common amino acid motif (QKRAA) in the HLA-DRB1

region, termed the shared epitope, confer particular susceptibility ***(Mclnnes and Schett, 2011).*** The genetic link between HLA-DR and RA (described in the 1970s) revealed that HLA-DR4 occurred in 70 % of RA patients, compared to about 30 % of controls, giving a relative risk of having RA to those with HLA-DR4**.**Careful study of the MHC using complementary DNA (cDNA) probes directed against specific α- and β-chains of the DR loci have revealed "susceptibility cassettes", or shared epitopes on the β-chains of DR that predispose to the development of RA. It was demonstrated that the susceptibility to RA is associated with the third hypervariable region of DRβ-chains, from amino acids 70 through 74 ***(Orozco et al., 2006).***

These findings suggest that some predisposing T-cell repertoire selection, antigen presentation, or alteration in peptide affinity has a role in promoting autoreactive adaptive immune responses. Other possible Explanations for the link between RA and the shared epitope include molecular mimicry (MM) of the shared epitope by microbial proteins, increased T-cell senescence induced by shared epitope–containing HLA molecules, and a potential proinflammatory signaling function that is unrelated to the role of the shared epitope in antigen recognition ***(De Almedia et al., 2010; Mclnnes and Schett, 2011).***

Association studies support a role for several genes, including Tumor Necrosis Factor-α (TNF-α) (TNFR2), peptidyl

arginine deiminase type IV (PADI4), Solute carrier family 22 member 4 (SLC22A4), Runt-related transcription factor 1 (RUNX1), and Protein Tyrosine Phosphatase (PTPN22) ***(Dieudé and Cornélis, 2005)***.

Other attractive candidates include the genes for cytokines. Cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10) and TNF are important mediators of the inflammatory response and play an important role in the pathophysiology of joint inflammation and destruction in RA ***(John and Worthington, 2001; Nikolopoulos et al., 2008).*** There are various reports showing a relationship between single nucleotide polymorphisms (SNP) in IL-1 gene family and RA ***(Pawlik et al., 2005)***. SNPs have been detected at position -511 C/T in IL-1β gene promoter and at position +3953 C/T in the exon 5 of IL-1β gene ***(Arman et al., 2006).***

SNPs in promoter regions or coding regions have been extensively investigated in RA. SNPs in promoter regions could lead to altered gene regulation due to variable binding of transcription factors to promoters, whereas SNPs in coding regions directly change the amino acid sequence of the encoded protein. A second method for assessing genetic associations involves the evaluation of microsatellite sequences near key genes that are implicated in the disease. Microsatellites are tandem repeated sequences in the DNA that are primarily (but not exclusively) located in noncoding regions. Considerable

heterogenicity exists in the length of each microsatellite, which can indirectly alter gene expression or be in linkage disequilibrium with other undefined genetic polymorphisms. Many SNPs and microsatellites have been studied with RA. The relative contribution of each is poorly defined, and variations in technique, stage of disease, and patient populations result in some disagreement among various reports ***(Firestein, 2005)***.

- ***Infectious agents:***

Many studies on different microorganisms failed to implicate infectious agents in RA by their isolation inside the affected host, or identification by culture or direct microscopic analysis ***(Carty et al., 2003)***. However, despite RA did not fulfill the postulates to consider it as an infectious disease, evidences using polymerase chain reaction (PCR) and other molecular and immunological techniques support this hypothesis and suggest an immune-infectious cause for RA ***(Hyrich and Inman, 2001)***. However, the difficulty of separating pathogens from joints has hampered these studies. On the other hand, the presence of foreign antigens is not specific for RA. Therefore, the role of these organisms in initiating and perpetuating inflammation in RA remains unknown, but continues to be actively investigated. The main theories explaining the biological interaction between microbial antigens, immune system, and RA are MM; superantigens (SA) and heat shock proteins (HSP) (where Foreign antigens are

similar to the host antigens but differ sufficiently to induce an immune response. *SA:* where, bacterial or viral antigens can activate T cells, bypassing the involvement of antigen processing cells, which can lead to proliferation and expansion, anergy, or apoptosis. *HSP:* where, HSP from human and bacteria are expressed in synovial tissue of RA patients) as aconsequence of stress factors (Fig. 1) ***(Jara et al., 2004)***.

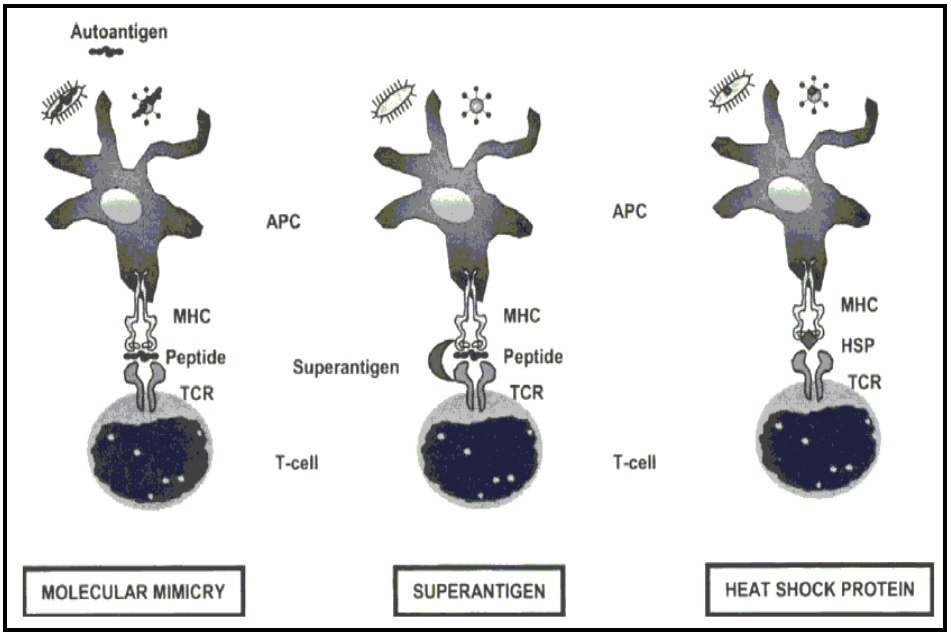

Fig. (1): Molecular mimicry ***(Jara et al., 2004)****:*

Some infectious agents (e.g., Epstein–Barr virus (EBV), cyto-megalovirus, proteus species, and Escherichia coli) and their products (e.g., HSP) have long been linked with RA, and although unifying mechanisms remain elusive, some form of MM is postulated ***(Kamphuis et al., 2005; Mclnnes and Schett, 2011).*** However epidemiological studies have confirmed a

potential association between RA and two herpes virus infections: EBV and *Human Herpes virus 6* (HHV-6) ***(Alvarez-Lafuente et al., 2005).*** The allele HLA-DRB1 * 0404 is associated with low frequencies of T cells specific for the EBV glycoprotein 110 (gp110) and predisposes one to develop RA ***(Balandraud et al., 2004).***The Escherichia coli dnaJ protein, a bacterial HSP, contains the sequence and represents a potential link between gut bacteria and chronic arthritis. RA T cells, especially synovial fluid T cells, but not normal peripheral blood cells, have increased proliferative responses to this protein, perhaps supporting the molecular-mimicry link between a variety of the amino acid motif QKRAA-containing proteins and arthritis ***(Albani et al., 1994).***

Furthermore, RA appears to be associated with periodontal disease caused by: *Porphyromo- nas gingivalis* which expresses *PADI4,* capable of promoting citrullination of mammalian proteins ***(Wegner et al., 2010).*** Finally, the gastrointestinal microbiome is now recognized to influence the development of autoimmunity in articular models, and specific clinical bacterial signatures that are associated with autoantibody positive rheumatoid arthritis are emerging ***(Scher et al., 2010).***

Immuno Pathogenesis:

The costimulation-dependent interactions among dendritic cells, T cells, and B cells are shown as occurring

primarily in the lymph node; these events generate an autoimmune response to citrulline-containing self-proteins. In the synovial membrane and adjacent bone marrow, adaptive and innate immune pathways integrate to promote tissue remodeling and damage. Positive feedback loops mediated by the interactions shown among leukocytes, synovial fibroblasts, chondrocytes, and osteoclasts, together with the molecular products of damage, drive the chronic phase in the pathogenesis of rheumatoid arthritis **(Fig 2)** ***(Mclnnes and Schett, 2011).***

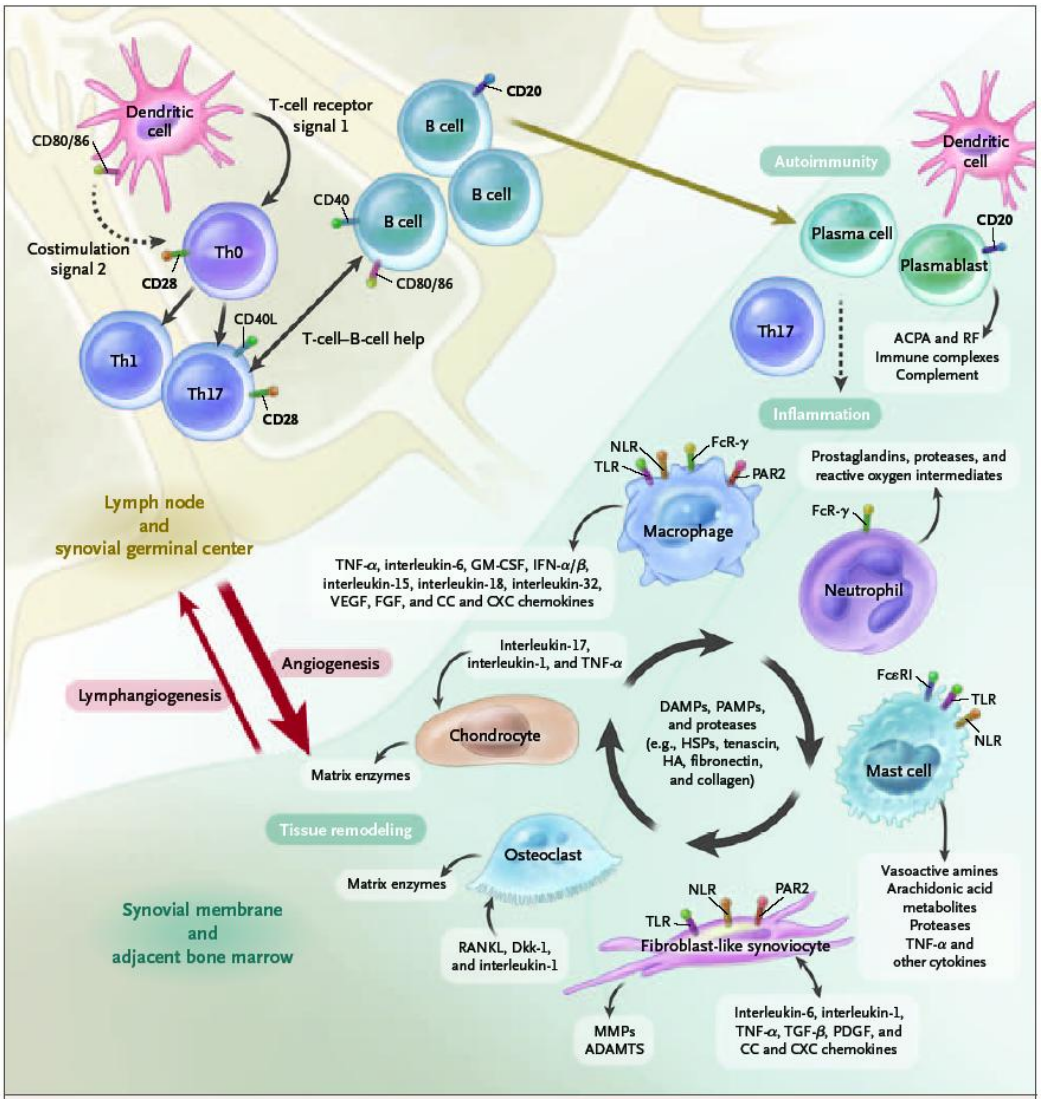

Fig. (2): Adaptive and innate immune processes within the joint in rheumatoid arthritis ***(McInnes and Schett, 2011).***

- ***Activation of the Innate Immune System:***

a) Cellular component:

A variety of innate effector cells, including macrophages, mast cells, and natural killer cells, are found in the synovial