INTRODUCTION

Psoriatic arthritis (PsA) is a seronegative spondyloarthritis that occurs in about 30% of psoriatic patients (National Psoriasis Foundation). It has different clinical phenotypes: oligoarticular, polyarticular, symmetric and asymmetric affection of peripheral joints, enthesitis or axial skeleton involvement ***(Dalmady et al., 2013).***

Although the exact etiology of psoriatic arthritis is not clear, it was found that it results from interplay of genetic and environmental factors and occurs most frequently in HLA-B27 halotype ***(Rahman and Elder, 2005).***

There are different methods and criteria that have been developed to aid in diagnosis and classification of PsA, The most frequently used is the classification criteria of psoriatic arthritis (CASPAR) criteria ***(Taylor et al., 2006).***

Antibodies against mutated citrullinated vimentin (anti-MCV) belong to the group of anti- citrullinated protein or peptide antibodies (ACPAs). The antibodies against citrullinated cyclic peptides (anti- CCPs) are the most widely used members of the ACPAs group. The anti-CCP is found to be of high diagnostic and prognostic value in patients with rheumatoid arthritis (RA) ***(Lee and Shur, 2003).*** Studies showed that anti-MCV is of more sensitivity and same specificity than that of anti-CCP in rheumatoid arthritis patients ***(Mathsson et al., 2008).***

Vimentin is a component of the cytoskeleton intermediate filamments in the cells of mesencymal origin. Tissue inflammation and cellular apoptosis catalyze citrullination of arginine residues of vimentin by the enzyme peptidylarginine deiminase (PAD) found in monocytes and macrophages. The citrullinated vimentin is identified by the immune system as non-self and induces autoantibodies production ***(Vossenaar et al., 2004).***

Aim of the Work

The aim of this work is to investigate the presence of anti-MCVs in psoriatic arthritis patients and psoriatic vulgaris patients without arthritis and its potential correlation with clinical and laboratory parameters.

Chapter (I): Psoriatic Arthritis

Psoriatic arthritis (PsA) is a chronic inflammatory arthropathy, belonging to the spondyloarthritic area, usually associated with skin and/or nail psoriasis or with its familiarity ***(Caso et al., 2014).***

PsA is characterized by inflammation of the distal interphalangeal joints, sacroiliac joints and entheses; it is typically seronegative for autoantibodies and is classed as a spondyloarthritis ***(Bowes et al., 2015).***

Psoriatic arthritis (PsA) is characterised by inflammation of entheses and synovium, eventually leading to joint erosions and new bone formation. It affects approximately 10% to 30% of patients with psoriasis, and has an estimated prevalence of approximately 1% ***(Dolcino et al., 2014).***

Pathogenesis:

A number of studies have suggested genetic, environmental, and immunological aspects in the development of PsA ***(Yamamoto, 2013).***

Bacterial and viral infections have been implicated as a cause or trigger in PsA. Some studies on psoriatic plaque have suggested enhanced humoral and cellular immunity to gram-positive bacteria; however, no direct relationship between bacteria and psoriasis has been proved. Another environmental trigger has been proposed in relation to the Koebner phenomenon, whereby arthritis can develop at sites of traumatized skin ***(Olivieri et al., 2008).***

One study of sera from patients with PsA showed higher levels of antibody to streptococcal exotoxin, which provides some evidence of a link between streptococcal infection and articular inflammation. The possibility that PsA might be virally induced has been proposed, although never confirmed. This association would highlight potential association between innate and specific immunity ***(Cassell and Kavanaugh, 2005).***

A strong genetic component to susceptibility is suggested by the sibling recurrence risk ratio (λs), which is estimated to be 27. Part of the genetic predisposition is likely to be explained by genes within the major histocompatibility complex (MHC) region ***(Ho et al., 2008).***

Genetic variation in the MHC (multiple histocompatibility locus antigen cluster) increases risk of developing psoriasis (PS). However, only ~10% of individuals with this risk factor develop PS, indicating that other genetic effects and environmental triggers are important. Recent approaches using DNA markers known as SNPs (single nucleotide polymorphisms) revealed that the MHC is truly the most important risk factor for PS and that it plays a very major role in PSA, confirmed recently identified associations with interleukin 23 receptor and interleukin 12B in both PS and PSA, and identified new associations. These include a region on chromosome 4q27 that contains genes for interleukin 2 and interleukin 21 that has been recently implicated in other autoimmune diseases, and seven additional regions that include chromosome 13q13 and 15q21 ***(Liu et al., 2008).***

Despite the larger estimated heritability for PsA, the majority of genetic susceptibility loci identified to date are shared with psoriasis. This suggests a substantial difference in the genetic architecture of the two diseases with a heavier genetic burden for PsA. The majority of susceptibility loci identified to date are shared between the two phenotypes, which is expected, and is mediated by the presence of psoriasis in both traits. A well-established example to support genetic differentiation involves the associations to genes in the human leukocyte antigen (HLA) class I region of the major histocompatibility complex (MHC) on chromosome 6. Studies have demonstrated that certain alleles of HLA-B confer risk specifically for PsA (B08, B27, B38), while HLA-C06 is specific for psoriasis. Evidence for a distinct PsA variant was found at the known psoriasis susceptibility locus, *IL23R*, and a new PsA-specific association at chromosome 5q31 was identified. This highlights the important differences in susceptibility to PsA and psoriasis ***(Bowes et al., 2015).***

HLA-A, B, and C antigens were studied in 104 Spanish patients with psoriatic arthritis. Different clinical features were evaluated and the patients divided into disease subsets. HLA-B17, B27, B16, and Cw6 were the most common haplotypes in the total group. The HLA-B17/Cw6 haplotype was increased in patients with oligoarthritis. The increase of antigen B17 correlated with oligoarthritis and spondarthritis, whereas Cw6 was more

significant in oligoarthritis. The prevalence of the B27/Cw1 haplotype was greater in association with spondarthritis and was probably related to the B27.5 subtype linked to Cw1. Lack of one or more HLA factors is thought to be responsible for the different clinical forms of psoriatic arthritis ***(Larrea et al., 1990).***

In a SNP array based genome wide association study (GWAS) of a German case/control collective, *HLA-C* and *IL12B* were confirmed as PsA susceptibility genes and replicated association to intragenic variants of *TRAF3IP2* in various European study groups. Common established susceptibility factors for PsV and PsA are an *HLA-C* risk allele as well as variants in/ near the *IL23R* and *IL12B* genes ***(Hüffmeier et al., 2010).***

Psoriasis has two distinct ages of onset: type I, early onset disease occurring at ⩽40 years of age and type II, late onset occurring at >40 years of age. *HLA-Cw*06* and *HLA-DRB1*07* are associated with patients with PsA having type I psoriasis, suggesting that the primary association is with age of onset of psoriasis. Patients with PsA having type I psoriasis, therefore, have a genetic background different to those with type II psoriasis ***(Ho et al., 2008).***

Patients with PsA carrying both *HLA-Cw6* and *HLA-DRB1*07* alleles have a less severe course of arthritis. This suggests that a protective locus lies on a haplotype marked by these alleles. Patients with type I psoriasis have a stronger family history and genetic predisposition ***Alonso***

et al., 1991 found that *HLA-Cw6* was associated with oligoarthritis in their study, while ***Al Heresh et al., 2002*** found that it was associated with polyarthritis. In addition, other studies have shown that *HLA-DRB1*03* and *HLA-DRB1*04* phenotypes were associated with severe and erosive disease ***(Ho et al., 2007).***

PsA is an autoimmune disease in which the CD8 T cell plays a central role. It appears that the *Cw0602* alleles confer a phenotype with more severe skin disease and, on average, a long interval (≥ 10 years) between the appearance of psoriasis and the development of the musculoskeletal features of PsA. In those with HLA-B alleles *B27* or *B39*, the musculoskeletal component appears more synchronously with the cutaneous component, and PsA is more likely than in the presence of *Cw0602* ***(FitzGerald and Winchester, 2009)***.

Recruitment of T-cells into the synovium may be mediated by chemokines, such as CCL2, CXCL13, CCL21, and CCL22. CCL22 (macrophage-derived chemokine) and its ligand CCR4 play an important role in attracting skin-specific memory T-cells to the synovial tissues. T-cell-derived cytokines such as IL-1β, IL-2, IL-10, interferon-γ (IFN-γ), and TNF-α are dominantly detected in the synovium. Dendritic cells both myeloid DCs and plasmacytoid DCs were present in the synovial fluids of PsA. IFN-α enhances the activation of CD8+ T-cells by antigen-presenting cells. In addition, IFN-α amplifies cutaneous inflammation via the induction of chemokines,

such as CXCL9, CXCL10, and CXCL11, which recruit their receptor CXCR3 expressing lymphocytes, including CD8+ T-cells. Also, plasmacytoid DCs isolated from synovial fluids express CXCR3 and CXCR4, the receptors for CXCL10 and CXCL11, and for CXCL12, respectively. These chemokines could be important in the pathogenesis of PsA ***(Yamamoto, 2013).***

In their study, ***van Baarsen et al. (2014)*** IL-17A, IL-17F, IL-17RA and IL-17RC were abundantly expressed in synovial tissues of all patient groups. Whereas IL-17RA was mostly present in the synovial sublining, IL-17RC was abundantly expressed in the intimal lining layer in arthritis patients. CD4 and CD8 positive cells co-expressed IL-17A and few cells co-expressed IL-17F. Mast cells were only occasionally positive for IL-17A or IL-17F. Interestingly, IL-17A and IL-17F staining was observed in macrophages as well as in blood vessels and lymphatics; this staining probably reflects receptor bound cytokine staining.

Cumulative evidence strongly supports the involvement of Interleukin (IL)-23/IL-17 axis in the pathogenesis of PsA, and a number of compounds that target components of these pathways have been recently used in PsA clinical trials. IL-23 acts synergistically with IL-6 and TGF-β to promote rapid Th17 development and IL-17 release, which, in turn, plays a central role in sustaining chronic inflammation. Both POSTN and ITGB5 have been implicated with this pathway ***(Cretu et al., 2015).***

TNFα affects pathogenesis of psoriasis by activating T lymphocytes, enhancing T cell infiltration, and augmenting the proliferation of keratinocytes in psoriatic plaques. ***Danning et al. (2000)*** examined the immunostaining of a number of different cytokines in PsA synovium including TNFα, which was shown to localise both to the lining layer and to perivascular macrophages.

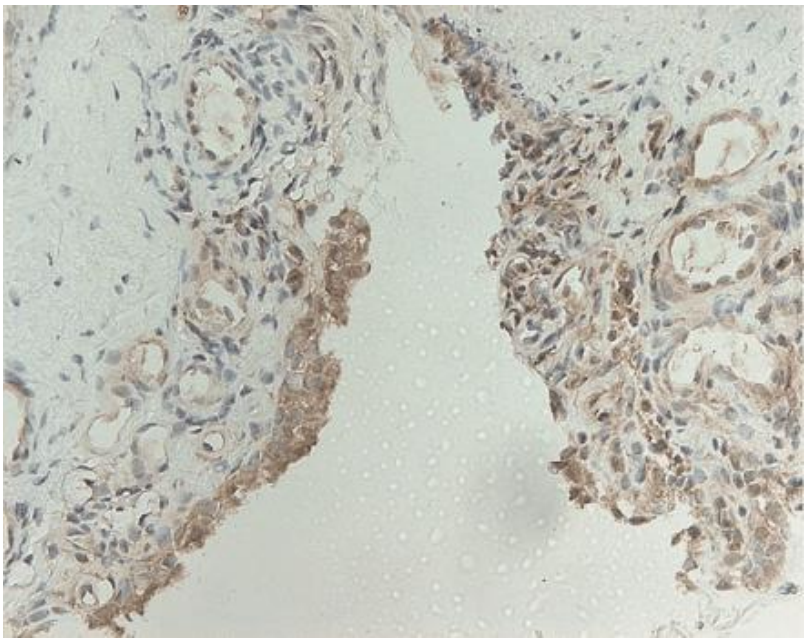

Fig. (1): Immunohistochemical staining of tumour necrosis factor α protein in a section of psoriatic synovial membrane ***(Veale et al., 2005)***.

The important role for TNFα in psoriasis and PsA comes from therapeutic studies of TNFα inhibition. Both etanercept, a fully human fusion protein consisting of two soluble TNF receptor domains linked to the FC portion of human IgG, and infliximab, a chimeric monoclonal IgG1 antibody, have been the subject of a number of clinical

trials. Etanercept achieved a significant improvement in joint symptoms also studies with infliximab improved both skin and joint manifestations ***(Veale et al., 2005)***.

TNF-α enhances endothelial cells to express adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. Angiopoietin-1 and -2 and their receptor Tie-2 are involved in angiogenic processes. Angiopoietin-2 is an effector downstream molecule of VEGF signaling pathway and promotes adhesion by sensitizing endothelial cells to TNF-α. IL-17 stimulates endothelial cell migration and cord formation. Mast cell degranulation in human induces expression of E-selectin on vascular endothelial cells and contribute to the synovial hyperplasia and angiogenesis ***(Yamamoto, 2013).***

TNF-α, a key proinflammatory cytokine, induces the production of other inflammatory cytokines such as IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor, chemokines such as IL-6, degradative enzymes such as several matrix metalloproteinases (MMPs) and other factors. TNF-α mediates a number of biological processes that can result in joint damage including stimulation of bone resorption, inhibition of bone formation, and inhibition of synthesis of proteoglycans. Angiogenic factors such as TNF-α and vascular endothelial growth factor (VEGF) may contribute to vascular proliferation ***(Cassell and Kavanaugh, 2005)***.

Vascular endothelial growth factor (VEGF) is the best characterized angiogenic peptide and its selective inhibition by bevacizumab (Avastin™), a monoclonal antibody against VEGF, has been shown to be effective in different cancers, diabetic retinopathy and retinal macular degeneration. VEGF is also overexpressed in psoriatic lesions, plasma and synovium of inflammatory arthritis and correlates with disease activity. The transformation of synovial tissue to fibrovascular pannus and its tumor-like expansion is related to neovascularization or angiogenesis. Increase in capillary density also induces lymphocytes homing perpetuates inflammation. Moreover, there is no significant difference between the levels of VEGF in between RA and PsA ***(Mitra et al., 2014).***

Recently, association of single-nucleotide polymorphisms (SNPs) within the interleukin-23 receptor gene (*IL23R*) and a gene encoding a subunit of its ligand, *IL12B*, have been reported to be associated with psoriasis. The 2 genes contributed to psoriasis susceptibility independently of each other, and subsequent studies have confirmed association with both genes, which have an additive but not an interactive effect with each other. Psoriasis and PsA occur together and share the skin phenotype, similarity in cytokine profiles, and the response to anti–tumor necrosis factor biologic therapies, suggesting that they are likely to share some etiologic pathways***.(Filer et al., 2008).***

Besides detecting strong association with the HLA class I region in the combined and PSA cohort, and replicating the recently reported associations with IL23R and IL12B, a number of novel associations were identified. These include a region on chromosome 13q13 harboring LHFP and COG6, a region on chromosome 15q21 harboring USP8-SPPL2A-TNFAIP8L3, association with the LCE cluster of genes on chromosome 1q21 from the PSORS4 locus, and a region of chromosome 4q27 recently reported to be associated with several other autoimmune diseases and associated with PSA and potentially PS ***(Liu et al., 2008).***

Although the relative recurrence risk for relatives of patients with PsA is 3.5–13 times higher as compared with those of patients with PsV, indicating an even stronger genetic impact in PsA, most genetic risk factors identified for psoriasis so far; for example, variants in the interleukin 23 receptor (IL23R) pathway and variants in the genes *NAT9*, *SLC9A3R1* and *RAPTOR* at psoriasis susceptibility locus 2 (PSORS2) on chromosome 17q25 do not account for the differences in, for example, sibling recurrence risk, or explain the different organ manifestations. It is of note, though, that frequency differences in the human leucocyte antigen (*HLA*)-*C* risk allele have been observed between patients with PsV and PsA ***(Hüffmeier et al., 2010).***

Ellinghaus et al., 2012 hypothesized that c-Rel, as a member of the Rel/NF-κB family, is associated with PsA in the context of disease pathways that involve other identified PsA and PsV susceptibility genes including *TNIP1*, *TNFAIP3*

and *NFκBIA*. Several susceptibility loci for PsV have been identified with genome-wide levels of statistical significance in populations of European origin, including *HLA-Cw6, IL12B, IL23R, IL4-IL13, IL23A, TNIP1, TNFAIP3, LCE3B-LCE3C, RNF114, TRAF3IP2, NFκBIA, NOS2, FBXL19, TYK2, IFIH1, REL, IL28RA* and *ERAP1*. Many of the susceptibility loci for PsV tested so far are also genome-wide significantly associated with PsA such as *HLA-Cw6, IL12B, TNIP1*, *FBXL19* and *TRAF3IP2*. However, because the causative allele(s) have not been identified within most of these regions, their actual contribution to disease risk remains unknown. They identified genome-wide significant association for SNP rs13017599, which is located between the genes *REL* and *PUS10* and has been already reported to be associated with RA.

Elaboration by cytokines such as IL22 could result in the hyper-proliferative phenotype of keratinocytes and potentially synoviocytes, leading to the vicious cycle of proliferation/inflammation in both the skin and joints. In synovial tissue, disease-related cytokines may also lead to RANK ligand dependent osteoclast formation leading to bone erosion. Noteworthy shared associations in PS and PsA involve components of the IL-23/Th17 pathway, namely, *IL23R, IL12B,* and potentially *IL23A* and *IL21*. As it is known that the different T_h subsets can counter-regulate each other, further dissection of the contribution and interaction of different T_h subsets in PsA is of extreme interest ***(Nograles et al., 2009).***

The cytokine Macrophage migration Inhibitory Factor (MIF) is distinguished functionally by its ability to counter-regulate glucocorticoid immunosuppression and sustain pro-inflammatory activation by inhibiting activation-induced apoptosis. MIF further co-stimulates T and B lymphocytes and upregulates the production of interleukin-6, interferon γ and Tumor Necrosis Factor alpha (TNFα). Two polymorphisms identified in the promoter region of MIF gene: a) the short tandem repeat (STR) -794 $CATT_{5-8}$ *MIF* (rs5844572) which is a microsatellite repetition of Cytosine-Adenine-Thymine-Thymine (CATT) at position -794 bp, in which the repeat length (5 to 8 repetitions) correlates with increased gene expression and with serum MIF circulation levels and b) the another polymorphism is a single nucleotide polymorphism (SNP) -173 G>C *MIF* (rs755622) at position -173 of the *MIF* gene in which there is a change from Guanine (G) by Cytosine (C). The -173*C allele is associated with susceptibility to PsA ***(Zambrano et al., 2014).***

In a recent study, proteomic analysis of synovial fluid (SF) was performed, and identified 137 proteins that were differentially expressed in PsA SF. One of these proteins, alpha defensin 1 (DEFA1), is secreted by neutrophils in response to various antigens and elevated levels have been associated with inflammatory activity in both rheumatoid arthritis (RA) and psoriasis. The enzyme myeloperoxidase (MPO) also seems to play a role in the joint inflammation associated with PsA, as it is elevated in