INTRODUCTION

Anti-citrullinated protein antibodies (ACPA) have been reported as more specific serological markers of rheumatoid arthritis (RA). They provide a superior alternative to the rheumatoid factor (RF) test in laboratory diagnostics of RA. This autoantibody family is an overlapping group of antibodies dependent on the citrullination of arginine residue. It includes antiperinuclear factor (APF), antikeratin antibody (AKA), antifilaggrin antibodies (AFA), anti-Sa, and anti-cyclic citrullinated peptide (CCP) antibodies ***(XIA et al., 2009).***

Citrullinated vimentin, identified as the antigenic target for anti-Sa, is a new member in the family of ACPA. Vimentin is secreted and citrullinated by macrophage depending on the proinflammatory signals, in active RA anti-MCV antibody has been reported to have a sensitivity of 65–82%, specificity of 80–97%. Moreover, several different types of statistical analysis showed that anti-MCV is more powerful for diagnosing early RA than anti-CCP ***(Bang et al., 2007).***

Tissue inflammation and cell apoptosis lead to changes in the structure of the protein by enzymatic citrullination, and activate the immune system by the increased production of autoantibodies. Different studies suggest that the enzymatic citrullination and the production of ACPAs may also be

associated with other inflammatory arthritis-associated autoimmune diseases ***(Szodoray et al., 2010).***

In systemic lupus erythematosus (SLE) arthritis is one of the most common symptoms, seen in 60–90% of patients. In the majority of cases of SLE, arthritis is nondeforming and nonerosive and thus will not directly cause irreversible functional impairment, the presence of anti-CCP antibodies was strongly associated with erosive arthritis ***(Kakumanu, 2009*)*.***

In Systemic sclerosis (SSc) Polyarthritis: have varied between 36 - 80%. Erosive arthritis have been reported throughout the metacarpophalangeal (MCP), proximal interphalangeal (PIP), and distal interphalangeal (DIP) joints, as well as the wrist. Indeed, at 7 years of SSc, bony erosions (mostly in the hands) have been noted in 4 - 57% of patients ***(Clements et al., 2012).***

Psoriatic arthritis (PsA) is a seronegative spondyloarthropathy that develops in up to 30 % of patients with psoriasis, high levels of ACPA members in psoriatic patients without clinically manifest arthritis may distinguish patients who are more likely to experience a severe disease course, and potentially require biological therapy ***(Liu et al., 2008).***

Ankylosing spondylitis (AS) primarily affects the axial skeleton, peripheral arthritis occurs in up to 35% of cases although anti-CCP antibodies are highly specific for rheumatoid arthritis it was occasionally present in AS, and their presence may be helpful as a serum marker for predicting peripheral arthritis ***(Kim et al., 2012).***

Gouty arthritis is the most common inflammatory arthritis in adults and is characterized by very painful flares. Gouty arthritis can progress to a chronic, deforming and physically disabling disease through the development of disfiguring tophi, bone erosion, joint destruction and persistent pain ***(Schlesinger, 2011***.)

The incidence of arthritis in Behcet disease (BD) ranges from 40 - 70%. The most frequent sites of involvement have been the knees, ankles, wrists, and elbows. Arthritis has been found to be present at the time of diagnosis in about 70% of the BD patients; 9% of the patients had arthritis only as an initial manifestation, and the differential diagnosis was difficult. The diagnosis of BD depends primarily on clinical manifestations of the disease without serological support. Anti-CCP antibody is a useful marker for diagnosis of RA (***Koseoglu et al., 2011).***

Hepatitis C virus (HCV) infection is a worldwide. Besides hepatic complications, polyarthralgia is the most

common rheumatological symptom in HCV infected patients. In about 20–83%of patients arthritis is occured and is characterized by an intermittent, mono- or oligoarticular, non-destructive arthritis or symmetrical polyarthritis mimicking rheumatoid arthritis, HCV infection is commonly associated with the detection of RF with prevalences ranging from 30 - 68% depending on the presence of mixed cryoglobulinaemia. The distinction between HCV associated arthropathy and recent onset rheumatoid arthritis, when articular damage and deformities have not yet occurred, may be difficult ***(Se`ne et al., 2006).***

AIM OF THE STUDY

Aim of the study is to detect the presence of anti CCP and anti MCV antibodies in rheumatological disorders other than rheumatoid arthritis and its correlation to radiological findings and disease activity.

ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES AND ANTI MUTATED CITRULLINATED VIMENTIN

Historical Background:

The first citrulline-binding autoantibodies in RA sera were discovered by **Nienhuis and Mandema (1964)**, as an autoantibody able to bind to perinuclear granules in normal human buccal mucosal cells, and were named antiperinuclear factor. Antiperinuclear factor was found in 48% of patients with RA, and only 1% of healthy controls. The specificity of antiperinuclear factor for citrulline was not appreciated until years later.

In **1979, Young et al**. reported that RA sera contained antibodies that reacted to the keratinized layer of epithelium. These antibodies were called anti-keratin antibodies, and were only found in RA patients. Anti-keratin antibodies and antiperinuclear factor (APF) recognized a similar epitope and were perhaps the same antibody. It was also discovered that the conversion of arginine to citrulline on peptides was essential for anti-keratin antibody and perinuclear factor binding. Therefore, antiperinuclear factor and antikeratin antibodies can be broadly categorized as anti-citrullinated peptide antibodies.

There is evidence for abnormal citrullination of various peptides in a diverse array of human diseases, including RA, psoriasis, and multiple sclerosis. The formation of antibodies to citrullinated peptides seems to be specific for RA patients **(Niewold et al., 2007).**

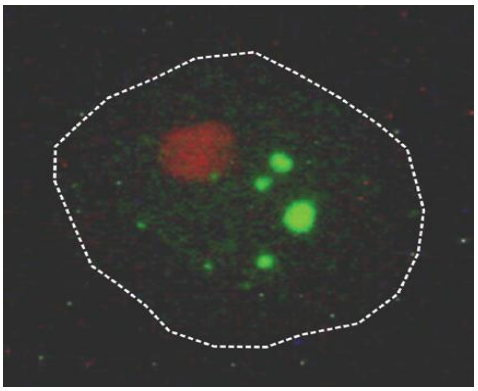

Fig. (1): Typical immunofluorescence staining pattern of a buccal mucosa cell by APF-positive RA serum. The borders of the buccal mucosa cell are marked by a dashed line. The nucleus is counter-stained with ethidium bromide (red). The keratohyalin granules are stained by patient antibodies via a secondary fluorescently labeled anti-human-IgG antibody (green). Note that superficial buccal mucosa cells are actually dead cells that can easily be scraped off from the inside of the human cheek. In such fully differentiated cells, the filaggrin protein is citrullinated adapted from **(Niewold et al., 2007).**

Citrullination:

Citrullination of proteins is a process that occurs almost exclusively in dying cells, the reason being that the enzymes that catalyze the conversion of peptidylarginine into peptidylcitrulline are only active when Ca2+ concentrations are $\geq 10^5$ mol/l. Normal cellular Ca2+ concentrations are about 100 times lower.

During cell death (apoptosis or necrosis) the cell membrane becomes leaky, allowing an unlimited influx of extracellular Ca2+ ions. As a consequence, the peptidylarginine deiminase enzymes become activated and start the modification of peptidylarginine into peptidylcitrulline. The effects of citrullination can be dramatic for the protein: due to the loss of positive charge, the citrullinated protein loses intramolecular and intermolecular interactions, unfolds, and subsequently is rapidly degraded by cellular proteases ***(Venrooij et al., 2011).***

In this way, many cellular proteins will lose their activity and many molecular machines will be destroyed. One of the first cellular proteins to become citrullinated is vimentin. This cytoskeletal protein is important for the structural integrity of the cell, and loss of its function will change the phenotype of the dying cell dramatically. Other intracellular proteins that become rapidly citrullinated are the nuclear histones, which are basic proteins essential for chromatin structure. Indeed, the cell-death process is profoundly accelerated by the citrullination of intracellular proteins. ***(Venrooij et al., 2011).***

Peptidylarginine deiminase (PAD)

PAD is the enzyme that catalyze the formation of peptidyl-citrulline only by post-translation modification of arginine ***(Rus'd et al., 1999).***

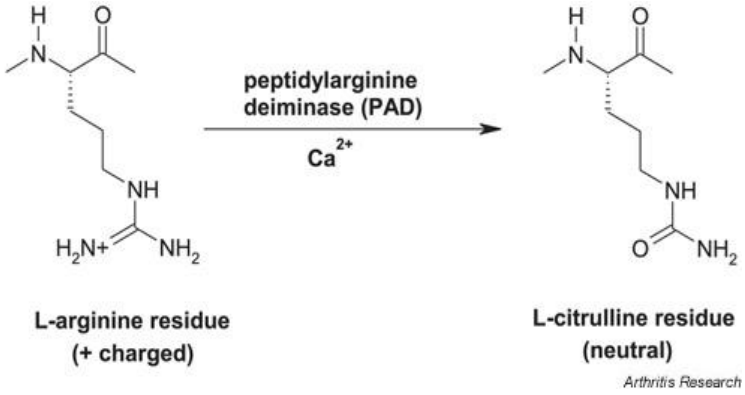

Fig. (2): The enzymatic conversion of peptidyl-arginine to peptidyl-citrulline ***(Adapted by Rus' d et al., 1999)***

Generation of ACPA is MHC dependent :

For over four decades it has been known that certain MHC class II alleles are a major genetic risk factor for RA. Indeed, numerous studies have shown the association between HLA-DRB1 shared epitope (SE) alleles and RA ***(Klareskog et al., 2009)***.

Hill et al., 2003 demonstrated that the conversion of arginine to citrulline at the peptide side-chain position that interacts with the SE significantly increases peptide–MHC affinity and leads to the activation of CD4+ T cells in DR4-IE transgenic mice.

A few years later, these authors showed that citrullinated fibrinogen is able to induce arthritis in these transgenic mice. T-cell epitope scanning and antibody microarray analysis

identified a unique pattern of citrulline-specific reactivity that was not found in DR4-IE transgenic mice immunized with unmodified fibrinogen or in the corresponding wild-type C57BL/6 mice immunized with citrullinated fibrinogen ***(Hill et al., 2008).***

Conversion of arginine into citrulline generates 'altered self ' peptides that can be bound and presented by DRB1*1001, one of several SE alleles that is also strongly associated with RA and ACPA ***(Woude et al.,2010)***.

James et al., 2010 showed that the accommodation of the citrulline moiety can occur in multiple pockets (positions 4, 7 and 9) of DRB1*1001.

Using a new classification of the SE, In particular, the S2 and S3P alleles (both associated with increased risk of RA) predisposed individuals to the production of anti- CCP and anti-MCV (mutated citrullinated vimentin) antibodies ***(Gyetvai et al., 2010).***

Antibodies against citrullinated fibrinogen had a different association pattern, and correlated best with the S1 allele. Thus, from these and many other studies, it can be concluded that the SE significantly increases the risk of developing ACPA ***(Bang et al., 2010).***

The production of ACPA can ultimately lead to the formation of immune complexes and continuation of inflammation, with the final outcome of chronic joint inflammation, which we refer to as RA ***(Klareskog et al., 2008).***

Assays for the detection of anti citrullinated peptide antibodies:

Assays for the detection of Anti-CCP antibodies have become popular in the last years for diagnosing RA. ***(Levesque et al., 2009).*** Several simple and cost-effective tests have been developed to capture the citrullinated epitope. These tests often incorporate modified or synthetic target proteins, such as fillagrin, vimentin, enolase, collagen, and fibrinogen. Adding cysteine residues to create cyclic peptides from disulfide bond formation was found to produce a more antigenic configuration, and this became a common feature of the synthetic peptides used to capture (ACPA***) (Breedveld et al., 2004).***

This lead to the development of the term anti-cyclic citrullinated peptides (anti-CCP) ***(Whiting et al., 2010).***

The first-generation CCP ELISA test (the CCP1 test) used a filaggrin-derived cyclic peptide as the antigenic substrate (***wiik et aL., 2010).***

As filaggrin is not present in the inflamed joint, they hypothesized that better antigenic peptides, possibly derived from citrullinated synovial proteins, could be found. About 12 million peptides from dedicated synthetic peptide libraries were subsequently screened with RA sera, and the best citrullinated peptides were incorporated into a new format, which was referred to in the literature as the second-generation CCP test (CCP2 test). This test first became commercially available in 2002 and has not been changed since then, although CCP2 ELISA tests are nowadays supplied by many companies, and CCP2 tests have been developed for several automated analyzers as well. According to the literature, the CCP2 test is still recognized as the gold standard of testing for (ACPA) ***(Pruijn et al., 2010).***

The third generation of anti-cyclic citrullinated peptide (CCP3) was made available for the laboratory diagnosis of RA. These assays have been reported to recognize additional citrulline epitopes that are not identifiable with the second generation CCP assays. The CCP3 assays have had reported results of up to 5% increased sensitivity compared to the CCP2 assays (**Shidara et al. 2011).**

Anti-CCP antibodies and RF were detected by ELISA in only 4% of patients with non-RA inflammatory disease and in no patient with non inflammatory disease in a cross-sectional

study performed in patients with inflammatory and non inflammatory disease to study the prognostic value of anti-CCP antibodies and RF, alone and in combination, in patients with very early synovitis. In these patients with early arthritis, the combination of anti-CCP antibodies and RF had a specificity, positive predictive value (PPV), sensitivity, and negative predictive value (NPV) for a diagnosis of RA of 100%, 100%, 58%, and 88%, respectively. The specificity, PPV, sensitivity, and NPV of this antibody combination for the development of persistent disease-fulfilling classification criteria for RA were 97%, 86%, 63%, and 91%, respectively. Suggesting that in patients with synovitis of 3 months' duration, a combination of anti-CCP antibodies and RF has a high specificity and PPV for the development of persistent RA. This autoantibody combination can be used to identify patients with disease destined to develop RA who may be appropriate for very early intervention ***(Raza et al., 2005).***

The prevalence, sensitivity and specificity of anti-CCP in patients with advanced RA were found to be similar to those reported in patients with early disease, as anti-CCP was significantly associated with some parameters of both disease activity and severity. Anti-CCP might be a useful parameter in clinical evaluation of patients with advanced RA ***(Samanci et al., 2005).***

Sensitivity and specificity of anti-CCP antibodies for RA:

Unlike RF, anti-CCPs are not commonly found in infectious diseases and do not occur with aging. Anti-CCP is also uncommon in other rheumatic diseases although it can be seen in patients with palindromic rheumatism ***(Willemze et al., 2012).***

Similar to RF, the presence of anti-CCP at early diagnosis predicts more radiographic progression, as demonstrated by many studies showing a strong association between anti- CCP positivity and the development of bone erosions. Furthermore, anti-CCP titers do not reliably change with disease activity. Therefore, like RF, anti-CCP can help identify patients prone to more severe disease, who might benefit from more aggressive treatment ***(Willemze et al., 2012).***

Sensitivity and specificity using anti-CCP1 assay ranged from (44 - 56%) and (90- 97%) respectively ***(Bizzaro et al., 2001)***. Detection of antibodies with anti-CCP2 assays resulted in improved sensitivity (64%-89%), and specificity (88%-99%) for diagnosis of RA ***(Suzuki et al., 2003).***

Rheumatoid factor sensitivity ranged from (80% - 84%) in the same groups ***(Niewold et al., 2007)***.

Whiting et al., (2010) reported that specificity was considerably higher for anti-CCP2 than for rheumatoid factor 96% versus 86% respectively for RA.

The anti–CCP-3 test has been reported to have sensitivities and specificities comparable with those of anti–CCP-2 (69–83% and 93–95%, respectively) ***(Dos Anjos et al., 2009).***

Table (1): Showing difference in sensitivity & specificity of three types of AntiCCP ***(Dos Anjos et al., 2009)***

	AntiCCP 1	**AntiCCP 2**	**AntiCCP3**
Sensitivity	44 to 56%	64-89%	69–83%
Specificity	90 to 97%	88-99%	93–95%
References	**(Bizzaro et al., 2001)**.	**(Suzuki et al., 2003).**	**(Dos Anjos et al., 2009).**

Presence of citrullinated proteins in inflamed synovial tissue and sera:

Search for cell and tissue substrates containing antigens that could be bound by autoantibodies from patients with RA found that granules from differentiating buccal mucosal cells expressed such an autoantigen. The autoantibody system was termed "antiperinuclear factor". A similar screen performed 15 years later showed that rat oesophagus was an ideal and more