INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory auto immune disease that affects articular as well as extra articular structures. It is characterized by an array of immune events with resultant production of inflammatory mediators that if untreated will result in tissue damage and joint deformities ***(Stavropoulos-kalinoglou et al., 2011).***

Citrullination is a process by which arginine residues in a given protein are post-translationally modified ('deiminated') in the presence of high calcium concentrations by an enzyme called (PAD) peptidylarginine deaiminase ***(van Gaalen et al., 2005).***

The presense of anti-citrullinated protein antibodies (ACPA) in serum samples of patients with RA early in the course of the disease even before appearance of the symptoms placed citrullinated proteins at the centre of autoantibody research in RA.

Antigen-antibody complex of human ACPA can activate complement by both the classic and the alternative pathway, and ACPA-containing immune complexes can induce tumour necrosis factor α (TNFα) production by human macrophages ***(Clavel et al., 2008; Trouw et al., 2009).***

Among the different ACPA specificities identified so far, citrullinated vimentin is identical to the previously described and highly RA-specific Serum assays antigen ***(Vossenaar et al.,***

2004). Specific mutations of vimentin have been detected in RA synovial fluid, and serum titres of antibodies targeting these mutated isoforms (called mutated citrullinated vimentin) correlate with disease activity such as Disease Activity Score 28(DAS28), radiographic damage, and with inflammation markers ESR and CRP ***(Rho et al., 2009; Bang et al., 2007).***

Measurement of these autoantibodies will add value to anti cyclic citrullinated peptides (anti-CCP) for diagnostic purposes in RA ***(Mathsson et al., 2008; Raza et al., 2010).***

AIM OF THE WORK

The aim of this work is to study the role of serum Anti-mutated citrurullinated vimentin (Anti-MCV) antibodies as a diagnostic and a disease activity marker in RA and their relation to the remission state of the disease versus anti cyclic citrullinated peptides (Anti-CCP).

REVIEW OF LITERATURE

Introduction:

Rheumatoid Arthritis (RA) is a chronic systemic autoimmune, inflammatory disease it usually affects small and medium-sized joints symmetrically.It affects around 1-2% of the population. It is found in every ethnic gathering. Females are 2.5 times more prone to be influenced than males. The disease top rate occurs within the fourth and fifth decades of life ***(Dorosos, 2004).***

The systemic involvement of the disease may include, the respiratory, cardiovascular and haematopoietic system. Uncontrolled cases of RA are associated with a reduction in life expectancy. Patients with RA have overall mortality rates 2-3 fold in age matched controls ***(Costenbader and Karlson, 2006).***

Diagnosis of RA

Diagnosis of RA is clinically based as it depends on the signs and symptoms that are considered characteristic of RA, and by eliminating other diseases.

Consequently, the 1987 criteria have recently been looked over after researches on patients with undifferentiated arthritis (UA) (persistent arthritis in patients not achieving the 1987 classification criteria). Many studies have proved that UA may develop into RA ***(Wiles et al., 1999)*** and that patients with

UA should also be considered for disease-modifying therapy to prevent this progression ***(van Dongen et al., 2007).***

However, this therapeutic consideration must be based on individual prognostic markers. It is hoped that the new ACR/EULAR classification criteria for RA will help rheumatologists caring for patients with early arthritis to tailor treatment, taking these prognostic markers into account (table 1) ***(Aletaha et al., 2010).***

One has to be aware, however, that some patients may present with typical erosive disease without other features that allow classification. In the absence of another explanation for synovitis, these patients can also be classified as having RA.

Table (1): 2010 ACR/EULAR classification criteria for rheumatoid arthritis (RA) ***(Aletaha et al., 2010)***

Domain	Parameter	Points
A Joint involvement	1 large joint	0
	2-10 large joints	1
	1-3 small joints	2
	4-10 small joints	3
	>10 joints (≥1 small)	5
B Serology	Negative RF *and* negative ACPA	0
	Low positive RF *or* low positive ACPA	2
	High positive RF *or* high positive ACPA	3
C Acute-phase reactants	Normal CRP *and* normal ESR	0
	Abnormal CRP *or* abnormal ESR	1
D Symptom duration	<6 weeks	0
	≥6 weeks	1

Role of anti-CCP in RA pathogenesis

Citrullination: is post-translation modification (deamination) of the arginine residues in any protein by means of peptidylarginine deiminase (PAD) in high calcium concentration (figure 1).

Citrullination is a perfectly physiological process, which is essential for of intracellular proteins degradation during apoptosis.

Citrulline-binding autoantibodies in RA sera were discovered by ***Nienhuis and Mandema (1964)***, as an autoantibody capable of binding to perinuclear granules in normal buccal mucosal cells of humans, and they named them antiperinuclear factor. Interestingly they were found in 48% of patients with RA.

The specificity of antiperinuclear factor for citrulline was truly recognized and appreciated in 1979 by ***Young et al.*** as they reported that the conversion of arginine to citrulline on peptides was essential for anti-keratin antibody and perinuclear factor binding.So, anti perinuclear factor and antikeratin antibodies should be included in anti-citrullinated peptide antibodies.

Anti-keratin antibodies: are antibodies that reacted to the keratinized layer of epithelium., and were only found in RA patients ***(Niewold et al., 2007).***

The discovery of the presence of anti-keratin and antiperinuclear antibodies (they share a common specificity for

citrullinated filaggrin which is a filament-associated protein that binds to keratin fibers) in the sera of RA patients years befor the appearance of the disease focused the attention towards citrullinated proteins autoantibody research in RA. Citrulline-specific reactivity against a number of additional citrullinated proteins (eg, fibrinogen, vimentin and α-enolase) have also been discovered lately. Anti-citrullinated protein antibodies (ACPA) is specific mainly for RA as it is found in 60–70% of patients with RA, but rarely in other diseases or healthy individuals. Despite their unique specificity for the disease, the role of ACPA in RA pathogenesis continues to be the subject of intense debate and further investigation ***(van Gaalen et al., 2005).***

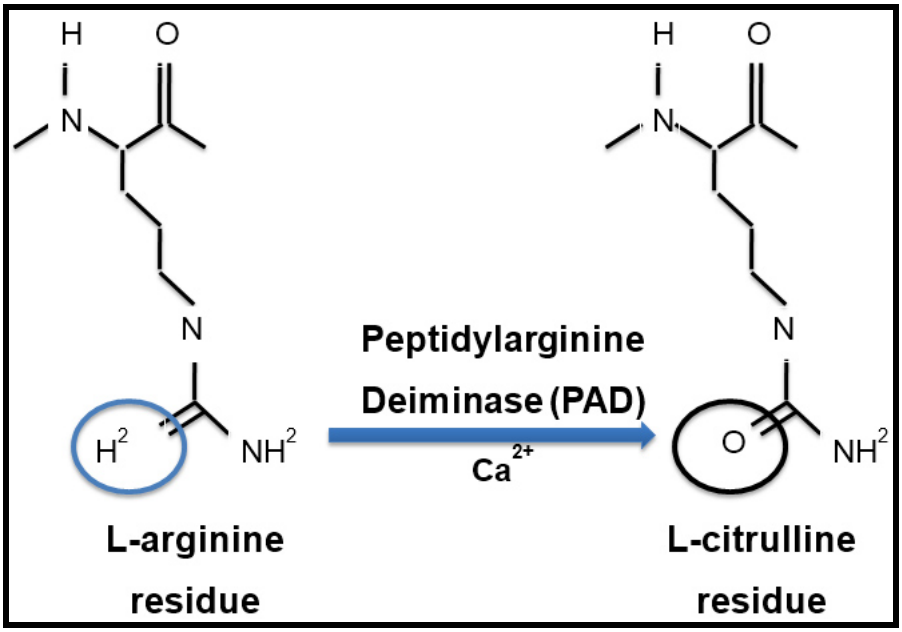

Figure (1): Schematic representation of the enzymatic role of peptidylarginine deiminase (PAD) in the generation of citrulline from arginine residues ***(van Venrooij and Pruijn, 2000).***

The process of autoimmunity in RA was divided into five stepsaccording to the involvement of citrullinated antibodies:

1) Random inflammation together with excessive apoptosis or impaired clearance can increase the level of cytosolic Ca2+.
2) Peptidylarginine deiminase (PAD) stimulation is followed by protein citrullination.
3) Citrullinated antigens become a target for T cells and ACPAs production is initiated as a result of citrulline specific activeT-cell and B-cell cooperation.
4) Immune complexes can be formed if the antigens react with the citrulline autoantibodies.
5) Inflammation is triggerd by immune complexes causing a vicious circle of inflammation resulting in joint destruction for years.

The citrullination of the antigens perfectly fits the model for the development and chronic nature of RA ***(van Venrooij and Ruijn, 2000).***

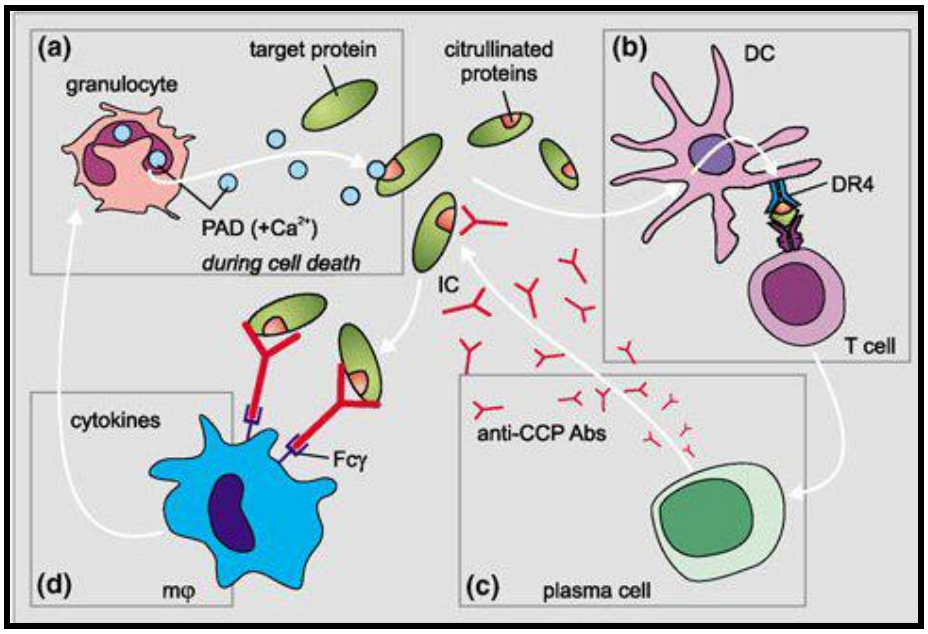

Figure (2): Formation of ACPA in RA ***(Vossenaar et al., 2004 A, B, C, D).***

Studies in healthy relatives of ACPA-positive patients with RA have demonstrated that the number of citrullinated epitopes recognized by ACPA expands in the course of RA, this phenomenon is known as (*epitope spreading*). Additionally the isotype repertoire of these ACPA simultaneously expands ***(Ioan-Facsinay et al., 2008; Brink et al., 2013).***

However, after diagnosis the ACPA epitope recognition profile and the isotype repertoire remain remarkably fixed. Detection of IgM-ACPA in samples of patient with early and late RA means that new antibodies secreting cells are generated all the time and that there is ongoing immune response ***(Verpoort et al., 2006).***

Detection of anti citrullinated peptide antibodies:

Detection of Anti-CCP antibodies has become quite popular in the last years for diagnosing rheumatoid arthritis ***(Levesque et al., 2009).*** Several simple and cost-effective tests have been developed to detect the citrullinated epitope. These tests often include modified or synthetic target protiens, such as fillagrin, vimentin, enolase, collagen, and fibrinogen. Antigenic configuration was increased by adding a cystiene residues which results in creation of cyclic peptide from disulfide bond formation, and this became the basic feature of the synthetic peptides used to capture anti-citrullinated peptide antibodeis (ACPA) ***(Breedveld et al., 2004).***

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

Sensitivity and specificity of anti-CCP antibodies for RA:

Sensitivity and specificity using anti-CCP1 assay ranged from 44% to 56% and 90% to 97% respectively. Detection of antibodies with anti-CCP2 assays resulted in improved sensitivity (64%- 89%), and specificity (88%-99%) for diagnosis of RA. Rheumatoid factor sensitivity ranged from 80% to 84% in the same groups ***(Niewold et al., 2007).***

It was found that anti-CCP2 has specificity higher than Rheumatoid factor 96% versus 86% respectively for RA ***(Whiting et al., 2010).***

A new generation of anti-cyclic citrullinated peptide (CCP3) was developed in 2005 and it's available for the laboratory diagnosis of RA. These assays have the advantage of recognizing an additional citrulline epitopes that cannot be identified with the second generation CCP assays. The CCP3 assays have scored results of up to 5% increased sensitivity compared to the CCP2 assays ***(Shidara et al., 2011).***

Clinical use of anti-CCP antibodies:

1) **Potential predictive value of anti-CCP to detect individuals at risk of RA:** anti-CCP antibodies can appear years before the appearance of the actual disease, and may be able to identify individuals at risk of RA development later on in their lives ***(Niewold et al., 2007).***
2) **Diagnosis of early RA:** RA is often difficult to distinguish from other types of inflammatory arthritis at the begining. Anti-CCP antibody testing helps to differentiate RA from othercauses of arthritis ***(Jansen et al., 2002 and Saraux et al., 2003).***
3) **Correlation of anti-CCP antibodies with disease activity parameters:** Patients with RA experience a great deal of changes in disease activity. Anti-CCP antibodies have the capability to identifying those patients who are likely to develop clinically significant disease activity. A strong correlation was found between greater disease activity and anti-CCP positivity ***(Niewold et al., 2007).***

4) **Prediction of severe disease:** Anti-CCP positive early RA patients is more likely to suffer from more erosive disease course than those without anti-CCP, it's also good predictor to disease radiological progression ***(Glansnoviè et al., 2007).***

5) **Change in anti-CCP titers with treatment:** A decrease in anti-CCP titer can be seen with RA treatment, however Anti-CCP positive patients usually remain positive even after treatment ***(Niewold et al., 2007).***

Anti-Mutated Citrullinated Vimentin (MCV)

Vimentin:

Vimentin, is an intermediate filament, is a protein which has been filtered from a variety of sources ***(Leong et al., 2003).*** Their diameter (8-12 nm) is intermediate between those of microtubules (25 nm) and actin microfilaments (5-8 nm) ***(Kreplak and Fudge, 2007).***

Its name means arrays of flexible rods derived from the Latin word vimentum. It is a protein monomer of highly elongated fibrous molecules with an amino-terminal head, a carboxyl-terminal tail and a central rod domain (fig.3) ***(Leong et al., 2003).***

A coiled-coil dimer is formed by two monomer twisting around each other, two dimers then form a tetramer, which act together with other tetramers creating a sheet ***(Fuchs and Weber, 1994).***

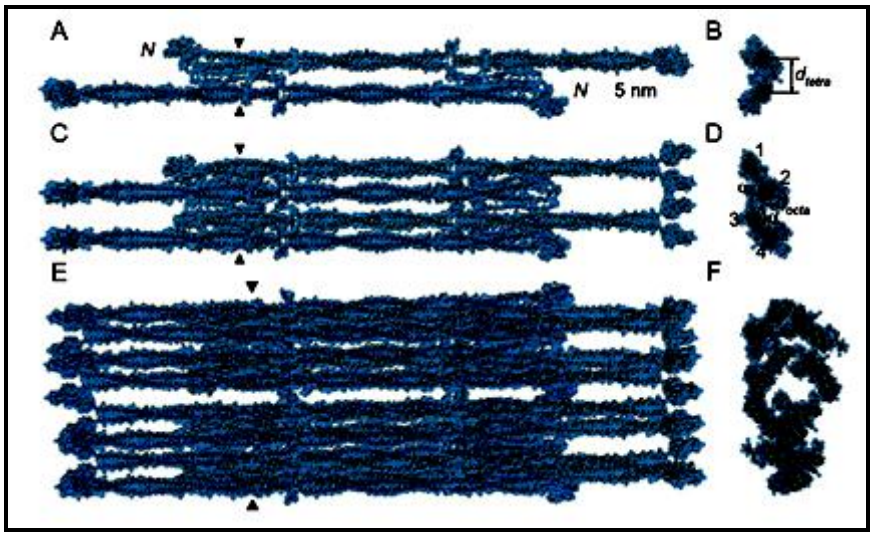

Figure (3): Molecular structures of vimentin dimer and tetramer The structure hierarchy of unit length-vimentin (A, B) the tetramer state (C, D) the dimer of the tetramer (E, F) the asymmetric outcome of the tetramers ***(Sokolova et al., 2006).***

By immunohistochemical staining of fibroblast It was found that vimentin filaments is a part of The wavy network of filaments in the cytoplasm of fibroblasts,and this network is associated with both nuclear and plasma membranes ***(Leong et al., 2003).***

Function of vimentin

1) Vimentin assumes a critical part in supporting and tying down the position of the organelles in the cytosol. It is joined to the core, endoplasmic reticulum, and mitochondria. It is the cytoskeletal segment in charge of keeping up cell structure ***(Katsumoto et al., 1990).***
2) It also regulate low-density lipoprotein LDL-derived cholesterol from a lysosome to the site of esterification. With

the hindering of transport of LDL-determined cholesterol inside the cell, cells were found to store a much lower rate of the lipoprotein than ordinary cells with vimentin. This the only biochemical process in any cell that relies upon a cellular intermediate filament network ***(Sarria et al., 1992).***

3) Also It has been mentioned that vimentin, as other middle fibers, conduct extracellular influence that control calcium flux into the cell and its role in the transcription machinery and may in this way have a part in genes expression.
4) Vimentin fibers are mobile structures, and their great flexible arrangement allow them to sustain mechanical stress between chondrocytes and the surrounding matrix tissue.
5) Moreover, a part for vimentin was described in the control of T cell stimulation ***(Vossenaar et al., 2004d).***

Vimentin expression has been mentioned in carcinomas of the skin, urinary bladder ***(Raymond and Leong, 1989).*** Also, gastric mucosa ***(Takemura et al., 1994).*** A few reports have demonstrated a relationship of vimentin expression with high tumor grades in breast carcinoma and, ovarian epithelial carcinoma ***(Nakopoulpou et al., 1995).***

Anti-MCV antibodies are member of ACPA family, they are antibodies produced against citrullinated vimentin ***(Renger et al., 2010).***