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Review Article

AN ELABORATED VIEW OF RA CYCLE, LATEST WITH HIGHLY SPECIFIC DIAGNOSTIC TECHNIQUES AND THE EFFECT OF FREE RADICALS AND ANTIOXIDANTS ON RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid Arthritis (RA) is a systematic disorder whose exact origination is not known till now, but in this review we will discuss about the most suitable cycle of Rheumatoid Arthritis which is majorly based on the formation of citrullin by the action of Peptidylarginine deiminase (PAD) in inflamed joints, which later shows the mechanism of the degradation of synovial fluid of joints and the origination of Rheumatoid Arthritis which is explained in 5 process. Along with this emphasis is laid on the diagnostic techniques like RA factor, Anti CCP, Anti MCV along with their sensitivity and specificity, and it is found that Anti CCP in combination with RA factor can give best specific results and are used most commonly for diagnostic purpose. Finally this review will focus on the aspects of free radicals and antioxidants and will try to understand the good and bad effect of free radicals and antioxidants in Rheumatoid Arthritic patients.

Keywords: RA Factor, Anti CCP, Anti MCV, Citrullination , Peptidylarginine deiminase (PAD)

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic disorder which is chronic in nature and is an inflammatory disease of the joints, which often causes destruction of joints, deformity and functional impairment[1]. Its severity varies from less to higher, and as it is a systemic disorder possibility of extra-articular manifestations is there, with involvement of the skin, blood vessels, and internal organs. If inadequately treated; means right medication at the right time not given, RA leads in the long term to a significant impairment of the quality of life; morbidity and mortality increase. Rheumatoid arthritis is an autoimmune disorder which involves multiple molecules and pathways. Autoantibodies and cytokines represent classes of immune cell secreted proteins which plays variety of roles in rheumatoid arthritis, from regulating the initiation and perpetuation of chronic inflammatory responses to joint destruction[2, 3, 4]. Tumor necrosis factor (TNF)Alpha and interleukin (IL)1 which are proinflamattory cytokines probably play important roles in regulating immune activation, driving the inflammatory process and promoting joint destruction in a variety of inflammatory diseases of joints [5] .Today it is well established fact that continued disease activity leads to joint damage, resulting in reduction of physical functioning of the patient – and if damage is progressive, it can lead to irreversible disability and multiple organ disorders[6].

Along with facts mentioned above it has been found that matrix metalloproteinase (MMP) up regulation ultimately results in destruction of articular cartilage and underlying subchondral bone in Rheumatoid Arthritis. Firstly low partial pressure of oxygen in the microenvironment of synovial fluid in RA patients was recorded [7], and subsequent studies somewhat clearly demonstrated decreased oxygen tension and glucose levels alongside raised lactate, carbon dioxide and acetate levels, consistent with anaerobic metabolism [8,9].

The discovery of macrophage-derived proinflammatory cytokine tumor necrosis factor alpha (TNFα) played a central role in the pathogenesis of RA [10] which later led to the introduction of anti-TNFα drugs, a new biological DMARD class (Anti-TNFα drugs should be restricted to patients who do not respond sufficiently to DMARD combinations until experimental evidence demonstrates that the new biological drugs have greater efficacy in earlier stages of RA.).Scientists have recently developed and applied antigen microarrays for the diagnosis and classification of rheumatoid arthritis and early rheumatoid arthritis detection[11, 12.] For this they described 1536-feature arthritis antigen arrays containing 225 peptides and proteins representing candidate autoantigens in rheumatoid Arthritis. Rheumatoid arthritis[11]. Antigens included a wide variety of native and *in vitro* citrullinated proteins and peptides, which were robotically printed to the surface of microscope slides, where the binding of serum autoantibodies was detected [11,13].

Actually RA is considered to have a complex etiology: environmental and genetic factors contribute to the disease development [14, 15, 16] .whereas layman emphasize mostly on environmental factors. The genetic component of RA is widely investigated [17]: the strongest gene association is considered to be the one with the human leukocyte antigen (HLA) region, particularly the HLA-DRB1 genes accounting for about two-thirds of the genetics of RA. Certain HLADRB1 alleles (DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*0101, DRB1*102, DRB1*1001 and DRB1*1402), encoding the so-called shared epitope (SE) at amino acid positions 70 to 74 in the third hypervariable region of the DRB1 molecule, are associated with a higher susceptibility for RA [18].

Cycle of Rheumatoid Arthritis-[19]

The stages of Rheumatoid Arthritis can be explained in 5 stages.

STEP 1

Initiation of Rheumatoid Arthritis takes place when unknown inflammation of the joint or any other tissue in the body leads to infiltration of inflammatory cells (lymphocytes , granulocytes , monocytes),whereas in normal situations many of the infiltrating cells will die by the process of apoptosis and will be removed by phagocytes. But due to the effect of toxicant or infection or some genetic defect in clearance system massive apoptosis takes place and due to this some apoptotic cells may become necrotic. Inflammatory cells like Granulocytes and monocytes, and macrophages emerging from monocyte differentiation, contain citrullinating peptidylarginine deiminase (PAD) enzymes. Peptidylarginine deiminase (PAD) enzymes are activated by the elevation of cytosolic Ca2+-concentrations, for example when cells undergo apoptosis.[19]

STEP 2

Then uncleared left necrotic cells release the intracellular citrullinated proteins (for example, histones, vimentin) and the activated Peptidylarginine deiminase (PAD) enzymes. These enzymes, PAD2 and/or PAD4, can then citrullinate extracellular synovial proteins like fibrinogen. Whereas, the mere presence of citrullinated proteins will not necessarily lead to chronic inflammation because these citrullinated proteins are degraded

without a (humoral) reaction of the immune system in most of the persons [20]. However various groups have showed excess number of citrullinated proteins in the inflamed synovium.

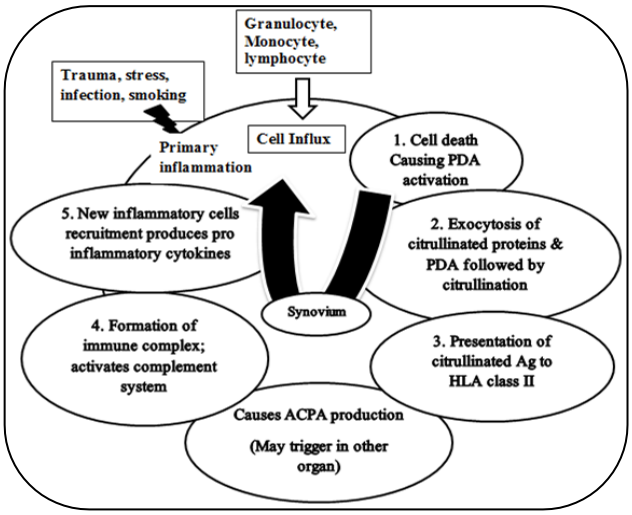

FIG 1: The rheumatoid arthritis (RA) cycle. Model for the role of protein citrullination in the pathophysiology of RA. The various aspects of the five major steps are depicted. Step 1, entry and death of inflammatory cells in the synovium; step 2, peptidylarginine deiminase (PAD) activation and protein citrullination; step 3, immune response to citrullinated antigens; step 4, formation of citrullinated immune complexes and their effects; step 5, recruitment of new inflammatory cells. ACPA, anti-citrullinated protein/peptide antibody.[19]

STEP 3

An immune response to citrullinated antigens might be generated in those individuals who are able to present citrullinated fragments of proteins to T cells via certain HLA molecules, which results in the production of high affinity IgG ACPAs [21].Occurance of autoreactive B cells may activate locally in the inflamed joint, but may also occur in other inflamed tissues. Via the circulation, these ACPAs or the plasma cells producing them will ultimately enter the joint[22].There is experimental evidence that ACPAs are produced in RA joints and may mediate tissue injury, irrespective of the site of B cell activation [23,24].

STEP 4

After their production in/entry into the inflamed synovium, ACPAs can react with the citrullinated antigens which are present in abundant number in the microenvironment of synovium. Then in the circulating plasma some Immunocomplexes are found as well as in the inflamed synovium. The inflammatory process is stimulated by these Immunocomplexes by activation of the complement system and further recruitment and activation of granulocytes, monocytes, and macrophages via both complement receptor and Fcγ receptor-dependent pathways. In this way, ACPAs contribute to the joint inflammation and to the chronicity and severity of RA.[19]

STEP 5

New monocytes and granulocytes will enter the synovium by the process of step 4,where they will be activated, subsequently die and release another load of activated PAD enzymes. A new round of citrullinated antigens and ACPA production will take place, leading to a new flare of inflammation. It has been noted that novel IgM-producing B cells are continuously recruited to the inflamed RA joint, demonstrating that the ACPA response is continuously reactivated during the course of arthritis [25].The continuation of this vicious circle for years, along with various environmental events that stimulate inflammation, will ultimately lead to a chronic inflammation that finally manifests as the disease we know as Rheumatoid Arthritis.

Tools, Techniques and concepts necessary for the diagnosis of Rheumatoid Arthritis-

Previously the techniques which were used for the detection of Rheumatoid factor was Rheumatoid Arthritis Factor, and at that time only rheumatoid factor were considered useful but now a day's anti-citrullinated protein/peptide antibodies (ACPAs) are also considered clinically useful. Presently for specific detection both the measurement of antibodies directed against rheumatoid factor and cyclic citrullinated peptides (CCP) determinations are commonly used in daily clinical practice. ACPAs (antibodies against citrullinated peptides/protein) along with RA factor are performed because of extreme specificity for the maximum confirmation of Rheumatoid Arthritis disorder[19].

RA Factor (RF)-

Many years back rheumatoid factor was mentioned as an autoantibody, which was suggested to be IgA, IgG or IgM [26]. RA Factor is used because it recognizes domains CH2 and CH3 of the Fc segment of human IgG and is a component of the classification criteria for RA detection[27]. Rheumatoid factor (RF) can be determined by various test methods; ELISA (enzyme-linked immunosorbent assay) and nephelometry are standardized methods. Some years back Rheumatoid factor was the main diagnostic criteria for Rheumatoid arthritis but now RA Factor alone is not considered sufficient for the diagnosis of rheumatoid arthritis because the specificity of rheumatoid factor is 79% as well as its sensitivity is 60% by employing all current analytical methods.

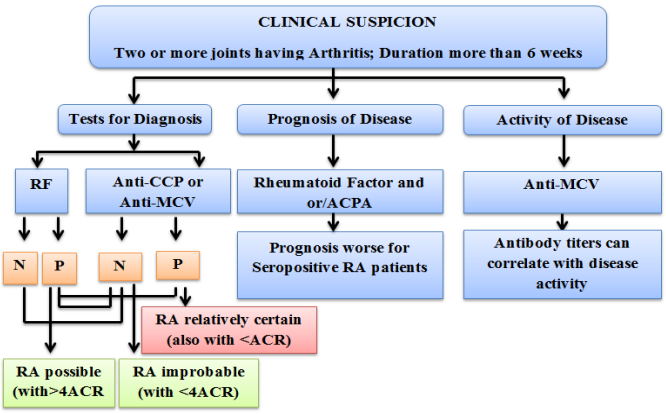

FIG 2.Role of ACPA in diagnostic testing for rheumatoid arthritis (RA), with consideration of the ACR criteria (modified from [14]).

RF, rheumatoid factor; a-CCP, antibodies to cyclic citrullinated peptides; a-MCV, antibodies to mutated citrullinated vimentin; ACPA, anticitrullinated protein/peptide antibodies; P-positive; N-negative; ACR-criteria of the American College of Rheumatology.

Anti-CCP

Latest serological discoveries in rheumatology have been the characterization of autoantigens in RA containing the amino acid citrulline[28]. There are many citrullinated proteins present in the inflamed RA synovium[29] - for example, vimentin, histones, α-enolase, and collagen types I and II- but citrullinated fibrin(ogen) is certainly one of the most abundant and important antigens[30]. Citrullination of proteins happens by enzymatic deimination of arginine residues, to leads to give citrulline residues. Citrullination has an essential physiological and biochemical role in cell differentiation and in programmed cell death (apoptosis) which alters the charge of a protein, leading to changes in its three dimensional structure, which in turn result in changes in antigenic properties.Citrullination is a posttranslational modification [29].The use of these cyclic citrullinated peptides (CCP) has improved the specificity of Rheumatoid Arthritis detection between 96% and 98%, without changing the sensitivity [30]. Several studies now accurately suggests that anti-CCP antibodies are not only highly specific, but also of high predictive value for an erosive course of the disease and are thus of prognostic value [31].

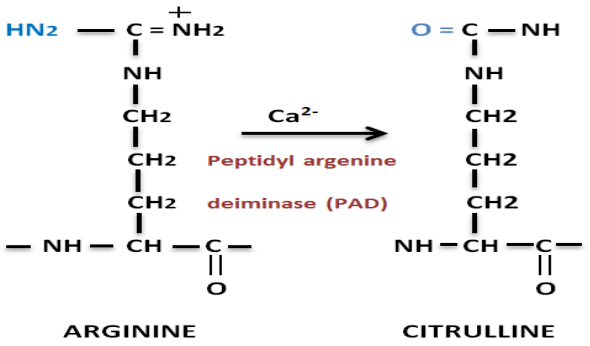

Figure 3: Citrullination: When positive charge loses on arginine it leads to a change in tertiary structure; it is considered the possible structure of CCP.

Anti MCV

Anti MCV is anti-Mutated Citrullinated vimentin has been described as a relevant autoantigen expressed in synovial tissue. Subsequently, it was clearly clarified that citrullinated vimentin is identical to the formerly known antigen Sa. Anti-Sa antibodies provide a limited sensitivity of 22% to 40% and high specificity of >98%, but only for patients with rheumatoid arthritis [32]. Although there is currently no commercial assay for detecting anti-Sa antibodies, Moreover, anti-Sa antibodies have the high predictive value of about 84% to 99% for rheumatoid arthritis and are closely associated with severe joint involvement and extra articular manifestations [32]. The most recent studies have shown that both mutation and citrullation can influence the antigenicity of vimentin. The process of mutated citrullinated vimentin (MCV) an ELISA based technique which has been commercially available for the diagnosis of rheumatoid arthritis for some time and has about the same diagnostic specificity and sensitivity as anti-CCP antibodies [33,34,35]. By research it appears that anti-CCP antibodies is less advantageous than anti-MCV antibodies as Anti MCV correlates better with disease activity of RA patient[32].

Table 1: Antibody diagnostic testing in patients with Rheumatoid Arthritis. The table demonstrates that anti-MCV and anti-CCP2 are tests of equal value for diagnostic testing for RA.(32).

	RF-IgA	RF-IgM	Anti-MCV	Anti-CCP2
Early RA sensitivity	29-39%	15-30%	51-71%	25-58%
RA Sensitivity	44%	60-80%	69.5-82%	39-94%
RA Specificity	84%	80-95%	90.3-98%	81-100%
Correlation with activity	Yes	Doubtful	Yes	No
Correlation with outcome	Yes	Yes	Yes	Yes
Association with extra-articular manifestation	Yes	Yes	Unknown	Yes

RF: Rheumatoid Factor

Effect of Antioxidants on Rheumatoid Arthritis

Antioxidants are the compounds which are exogenous or endogenous in nature that either prevents the generation of toxic oxidants (which are produced by the reaction of free radicals) or intercept any that are generated and inactivate them and thereby block the propagation of chain reaction produced by these oxidants. Antioxidants are classified in many types as enzymatic antioxidants**,** superoxide dismutase, catalase, alpha-tocopherol,glutathione peroxidase, glutathione reductase, non-enzymatic antioxidants like (nutrient antioxidants) beta-carotene, ascorbic acid, bioflavonoids and (metabolic antioxidants) like glutathione, bilirubin, ferritin, ceruloplasmin, albumin, transferrin, uric acid and lactoferrin. Although the initiation and cycle of rheumatoid arthritis is still unknown, the inflammation resulting from the immunological reaction is quite well described by scientists. It is known that neutrophil granulocytes, macrophages and lymphocytes are activated, and that reactive oxygen and nitrogen species (RS) are produced [36, 37]. These RS can react with lipid, protein and nucleic acids and are thought to be of importance for the chronicity and aetiology of the inflammatory Rheumatoid Arthritic diseases [38,39]. The aspect of damage by RS and loss of critical functions can be analysed by use of 'molecular markers; as these markers may include antioxidant status, products which arise as a consequence of oxidative damage to DNA, proteins and lipids and tissue damage (as assessed by lactic dehydrogenase release) or cytokine.

Effect of Vitamin E

Very less studies support the therapeutic or preventive activity of antioxidants in the pathogenesis of RA. These studies were all conducted with an oral intake of 1,200 mg/ day of vitamin E reported that the antioxidant a-tocopherol significantly reduced pain parameters in a placebo-controlled double-blind trial following a 3-week supplementation period [40]. Results from a randomized double-blind parallel group comparison study with a-tocopherol and diclophenac showed that the clinical parameters assessed after the use of antioxidants , e.g. morning stiffness, Ritchie joint index, grip strength and pain, were significantly reduced by vitamin E, with similar effectivity and less side effects as compared to regular drug therapy by diclofenac [41]. Vitamin E seems to show beneficial effect on joint destruction and it uncouple joint inflammation and joint destruction in the transgenic KRN/NOD mouse model of RA.[42].

Effect of Vitamin C, Retinol and Uric acid

In a randomized, after the intervention of controlled Mediterranean dietary study in patients with RA, it was observed that plasma levels of vitamin C, retinol and uric acid inversely correlated with variables related to disease activity. Thus proposing even dietary antioxidant interventions in patients of RA. [43]

Problems associated with antioxidant supplementation-

The biggest doubt, which antioxidant rises is that various antioxidants induce suicidal oxidative stress[44, 45, 46]. These antioxidants can act as pro-oxidants in certain conditions like presence of transition metals [45, 46] or at high concentrations and can cause the cell to undergo severe oxidative stress finally resulting in suicidal cell death. Hence, antioxidants must not be used prior to doctor advice.

So clinical trials of antioxidant on Rheumatoid Arthritis patients suggests a preventive and an adjuvant role or intake of natural dietary antioxidants. However, therapeutic efficacy, dose, duration and appropriate timing of administration of antioxidant supplementation to derive best possible results are still not established till now. Therefore, there is a great need of conducting larger, adequately powered clinical trials in this direction to find out answers for all these unsolved questions.

Role of smoking generated free radical in RA

Firstly, Scientists Vessey and colleagues described an association between hospitalization due to RA and cigarette smoking, which was an unexpected finding of their gynecological study [47].

Cigarette smoke represents a mixture of 4,000 toxic substances including nicotine, organic compounds (unsaturated aldehydes such as acrolein), solvents, gas substances (carbon monoxide) carcinogens (polycyclic aromatic hydrocarbons), and free radicals which is the causes oxidative stress[48]. Exposure to cigarette smoke results plays a vital in the depression of phagocytic and antibacterial functions of alveolar macrophages (AM) [49, 50]. Although AMs from smokers are able to phagocyte intracellular bacteria, they are unable to kill the bacterium-which consequently implies the deficiency of these cells in smokers [51]. Chronic smoking leads to results in T-cell anergy by impairing the antigen receptor-mediated signaling [52]. One study has proved that tobacco smoking was related to an increased risk of anti-CCP-positive RA [53]. A great investigation of consecutive sera of RA patients in a rheumatology clinic has shown that anti-CCP titers were associated with tobacco i.e. by smoking exposure [54].

CONCLUSION

Rheumatoid Arthritis is a systemic disorder which is said to be found in 1% of the world population, and majorly affects females as compared to males. It affects the psychological amenity along with the degradation of mobility and general health of a being as it is a

systemic disorder. But Extensive works by scientists have improved the condition a lot, and now it is proved that remission of this disease can takes place if it is diagnosed within 3-6 months of orignation.Rheumatoid Arthritis is majorly found between the age of 40-50 but sometimes it can be seen in children's also which is called as Juvenile Rheumatoid Arthritis.

Till now the exact mechanism and the genes associated with Rheumatoid Arthritis is unclear, and it is understood that its origination belongs to lifestyle and genetic problems. But the treatment of this disorder have improved a lot from last 20-30 years; now remission of this disorder can be done if diagnosed within 3-6 months of orignation.Many medicines are present now a days which are very effective out of which monoclonal antibodies derived medication is the landmark achievement for the treatment of this disorder.

In this review it is tried to understand the most common and most accurate mechanism of Rheumatoid Arthritis which is majorly based on the formation of citrullin by the action of Peptidylarginine deiminase (PAD) in inflamed joints, which later shows the mechanism of the degradation of synovial fluid of joints and the origination of Rheumatoid Arthritis which is explained in 5 process. Along with this emphasis is laid on the diagnostic techniques like RA factor,Anti CCP,Anti MCV along with their sensitivity and specificity, and it is found that Anti CCP in combination with RA factor can give best specific results and are used most commonly for diagnostic purpose. Now a days antioxidant therapy has proved to be a good tool to overcome various disorder associated with free radicals and due to this a discussion about affect of various antioxidant on Rheumatoid Arthritis is done here and how free radicals produced by smoking can lead to Rheumatoid Arthritis, and by that we found that Antioxidant can deal with many disorders but it must be taken carefully by consulting a doctor, because specific antioxidant works for specific disorders.

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