Significance of Anti-cyclic Citrullinated Peptide Autoantibodies in Immune-mediated Inflammatory Skin Disorders with and without Arthritis

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Abstract

Background: Anti-cyclic citrullinated peptides (CCPs) are autoantibodies directed against citrullinated peptides. Rheumatoid factor (RF), an antibody against the Fc portion of IgG, is known to form immune complexes and contribute to the etiopathogenesis of various skin disorders. C-reactive protein (CRP), an acute-phase protein, increases following secretion of interleukin-6 from macrophages and T cells. Anti-CCP, RF, and CRP are well-established immune-markers, their diagnostic potential in immune-mediated skin disorders remains less widely studied.

Aims and Objectives: To determine the correlation between anti-CCP, RF, and CRP in immune-mediated inflammatory skin diseases. Materials and Methods: About 61 clinically diagnosed cases of various immune-mediated skin diseases (psoriasis [n = 38], connective tissue diseases such as systemic lupus erythematosus and systemic sclerosis [n = 14], and immunobullous disorders including pemphigus vulgaris and pemphigus foliaceus [n = 9]) were included in the study. These patients were subclassified on the basis of presence or absence of arthritis. Arthritis was present in nine cases of psoriasis and seven connective tissue disorder patients. Detection of serum anti-CCP was done using enzyme-linked immunosorbent assay, whereas CRP and RF levels were detected using latex agglutination technique. Results: Of the 61 specimens, 14.75% had elevated serum anti-CCP levels. RF and CRP levels were elevated in 18.03% and 39.34% specimens, respectively. RF was elevated in 13.16% of inflammatory and 42.88% of connective tissue disorders, whereas anti-CCP was raised in 10.53% of inflammatory and 35.71% of connective tissue disorders. CRP positivity was highest in connective tissue disorders (50%), followed by 39.47% in inflammatory and 22.22% in immunobullous conditions. In none of the immunobullous patients, anti-CCP or RF levels were found to be elevated. Association of the presence of arthritis with elevated anti-CCP was found to be statistically significant.

Conclusions: Although anti-CCP, RF, and CRP levels are valuable markers of chronic immune-mediated skin disorders, elaborate studies enrolling a larger number of patients are required to validate these diagnostic markers.

Key Words: Anti-cyclic citrullinated peptide antibodies, C-reactive protein, immune mediated, rheumatoid arthritis factor, skin

Introduction

The skin is a common site of immunopathological responses toward innocuous antigens that may be the result of T cell-dependent and/or autoantibody-dependent mechanisms. A defect in the immune regulation mechanism is increasingly recognized as the underlying pathogenesis in many cutaneous and systemic immune-mediated disorders. Psoriasis is a commonly prevalent immune-mediated chronic inflammatory skin disease affecting 0.5–4.6% of individuals from across diverse populations.[1] The inflammation in psoriasis is mediated through a network of cytokines production, and activated dendritic cells and T cells are central in its pathogenesis. Progressive cutaneous and visceral fibrosis occur in systemic sclerosis or scleroderma.

What was known?
• Immune defects are increasingly recognized as the underlying pathogenesis in several immune-mediated inflammatory skin disorders
• Lack of uniformity exists in the standardized approach to the assessment of such diseases with and without arthritis.

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which is an autoimmune disorder of unknown etiology characterized by cellular and humoral immunological abnormalities. Systemic lupus erythematosus (SLE), with the most severe forms resulting in multiple organ system inflammatory damage in multiple individuals, is another connective tissue autoimmune disorder associated with a chronic activation of the immune system.\(^2\) Pemphigus is an important group of autoimmune blistering diseases with an established immunological basis but unknown etiology.\(^3\) In all these chronic inflammatory cutaneous skin diseases, several immunological, environmental, and multiple genetic components may be responsible for the pathogenesis.

There is no uniformity in the standardized approach to the assessment of such diseases with and without arthritis due to the paucity of data regarding the association of arthritis in such cases. Anti-cyclic citrullinated peptide (anti-CCP) antibodies, very often one of the components of such an assessment, are specific for rheumatoid arthritis (RA) and are useful in identifying major components of patients with RA in collaboration with rheumatoid factor (RF) and C-reactive protein (CRP) as other inflammatory and diagnostic markers. Citrullination of antigens of the synovium in any form of inflammatory arthritis results in the production of anti-CCP antibodies.\(^4\) RF, an antibody to the Fc portion of human IgG, is found in diseases of infectious or autoimmune origin. Another important marker for disease activity is the acute phase reactant-CRP which is sensitive to systemic inflammation and is often elevated after stimuli such as trauma or inflammation. Data on whether there is any association between anti-CCP antibodies and arthritis among patients with psoriasis, connective tissue disorders, or immunobullous disorders are extremely limited and conflicting. Moreover, any correlation with other inflammatory or diagnostic markers such as RF and CRP in such patients is further complicated due to the prevalence of RA and coexistent arthritis in many such diseases.

The aim of this study was to see the prevalence of anti-CCP antibodies in patients of immune-mediated inflammatory skin disorders and further study the correlation of anti-CCP antibodies in such patients with and without arthritis with RF and CRP.

**Materials and Methods**

This case-control study was conducted in the immunology section of the Department of Microbiology and Dermatology, University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi. Sixty-one consecutive patients with immune-mediated inflammatory skin disorders of psoriasis, connective tissue, or immunobullous types admitted in the dermatology ward were enrolled in the study. Each enrolled patient gave written consent before being included in the study. Healthy hospital personnel \((n = 61)\) without any history of inflammatory diseases served as controls. Serum samples were obtained from both patients and controls and were aliquoted and stored at \(-80^\circ\)C until assayed.

Detection of anti-CCP antibodies, RF, and CRP was done with the help of commercially available kits following the manufacturers’ instructions. RF was detected by RHELAX-RF (Tulip Diagnostic, Goa, India) which is a latex agglutination slide test for the detection of RFs of the IgM class with a sensitivity of 10 IU/ml. Anti-CCP antibodies were analyzed with a commercial enzyme-linked immunosorbent assay (ELISA) IMTEC-CCP-antibodies (IMTEC Human, Wiesbaden, Germany) which is a test system for measuring IgG class autoantibodies against CCPs in human serum or plasma. The interpretation of the results was possible by correlating the absorbance of the reference control and the samples. CRP was assayed using RHELAX-CRP (Tulip Diagnostic, Goa, India) which is a slide test for detection of CRP based on the principle of latex agglutination with a sensitivity of 0.6 mg/dl. Chi-square test was used to study the association of arthritis with anti-CCP antibodies positivity in these patients and also to study the association of arthritis with any of the diagnostic markers within the three groups of immune-mediated inflammatory skin disorders.

**Results**

A total of 61 consecutive patients with immune-mediated inflammatory skin disorders were enrolled during the study period. Thirty-two (52.46%) were males, whereas 29 (47.54%) were females. The age of the enrolled patients ranged from 9 years to 65 years with mean age of 35.16 years (standard deviation - 13.46; standard error of mean - 1.723) \[\text{Figure 1}\].

Out of the 61 enrolled patients, 38 (62%) were psoriatic, whereas 14 (47.54%) and 9 (23%) and 9 (15%) had connective tissue and

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**Figure 1: Age and sex distribution of the study population**
immunobullous skin disorders, respectively [Figure 2]. Connective tissue skin disorders included SLE or systemic sclerosis while pemphigus vulgaris or pemphigus foliaceus constituted the immunobullous type.

As far as the association of arthritis with these cases is concerned, 16 of the total 61 (26.23%) patients had arthritis which included 9 (23.68%) cases of psoriatic arthritis and 7 (50%) cases of arthritis associated with connective tissue diseases. None of the nine immunobullous disease cases enrolled during the study period had arthritis.

The positivity of various markers among the three patient groups is shown in Figure 3. RF was present in 11 out of 61 (18.03%) patients that included 5 out of 38 psoriatic patients amounting to 13.16% and 6 out of 14 connective tissue disorders contributing to 42.88%. None of the immunobullous disorders showed the presence of RF. CRP was positive in 24 out of 61 patients (39.34%) with immune-mediated inflammatory skin disorders that consisted of 15 of 38 psoriatic cases (39.47%), 7 of 14 connective tissue disorders (50%), and 2 of 9 immunobullous disorders (22.22%). Anti-CCP antibodies were present in 9 of the total 61 patients (14.75%) out of which 4 of 38 psoriatic and 5 of 14 connective tissue disorders contributing to 10.53% and 35.71%, respectively, whereas none of the immunobullous disorders showed the presence of anti-CCP antibodies. As far as the 38 psoriatic patients were concerned 5 (13.16%), 15 (39.47%), and 4 (10.53%) cases were positive for RF, CRP, and anti-CCP antibodies, respectively. Similarly, 6 (42.88%), 7 (50%), and 5 (35.71%) cases of the 14 connective tissue disorders were positive for RF, CRP, and anti-CCP antibodies, respectively. Among the nine immunobullous disorders, only 2 (22.22%) were positive for CRP, whereas none were positive for RF or anti-CCP antibodies.

The correlation of the positivity of various markers with the presence of arthritis is shown in Figure 4. The correlation of various diagnostic biomarkers with arthritis among the skin disorders under study is depicted in Table 1. Chi-square test for the association of arthritis with anti-CCP antibodies among all the cases of immune mediated inflammatory skin disorders was significant ($P = 0.045$). The association with arthritis was not found significant for RF ($P = 0.457$) or CRP ($P = 0.377$). Within the three groups of psoriasis, connective tissue disorders, and immunobullous disorders, the association of arthritis with any of the three diagnostic markers was not significant.

**Discussion**

The exact prevalence of arthritis in most of these immune-mediated inflammatory skin disorders remains largely unknown due to the paucity of a uniformly accepted diagnostic criteria and the elusive nature of the disease in such cases. As unlike other well-defined forms of inflammatory arthropathies, in arthritis associated with the immune-mediated inflammatory skin disorders, true laboratory diagnostic markers are
lacking, and the guidelines for the same are not uniform. Few markers do exist which however are utilized more often to differentiate other well-recognized forms of arthritis rather than characterize these diseases. On some occasions, arthritis associated with these diseases become clinically indistinguishable from well-classified autoimmune rheumatoid form which may even have features considered hallmarks of another condition. For instance, some patients with RA can have coincidental, preceding or following psoriatic, connective tissue, or immunobullous disorder associated arthritis, and these diseases may ultimately remain hidden or misdiagnosed.

Psoriatic arthritis is defined as an inflammatory, usually seronegative, arthritis, associated with psoriasis.\[5\] Varied presentation of inflammatory arthritis has been reported in 6–42% of patients with psoriasis compared to a prevalence of 2–3% in the general population.\[6\] In our study, 62% of the immune-mediated inflammatory skin disorders were psoriatic though a prevalence of 0.7% has been reported for psoriasis among general Indian population.\[7\] A systematic review of published population-based studies reported the prevalence of psoriasis ranging from 0–2.1% to 0.91–8.5% in children and adults, respectively.\[8\] The association of psoriasis with arthritis in our study was found in 26.23% of cases and is concordant with a similar report of 30% prevalence of chronic arthritis among psoriasis patients.\[6\] Arthritis in SLE is one of the most common disease manifestations with a frequency of 48% in patients followed for 10 years.\[9\] Systemic sclerosis or systemic scleroderma is an autoimmune or connective tissue disease characterized by thickening of the skin caused by accumulation of collagen, and by injuries to the smallest arteries. In a cohort of 58 systemic sclerosis patients, 31% had signs of arthritis, 19% clinically, and 26% radiologically in a recent study.\[10\] In our study, the prevalence of connective tissue disorders was 23% among the immune-mediated inflammatory skin disorders and association of arthritis was seen in 50% of these connective tissue disorders. Immunobullous skin disorders such as pemphigus vulgaris and pemphigus foliaceus are a group of autoimmune, life-threatening diseases that cause blisters and erosions of skin or mucous membranes. In our study, the prevalence of immunobullous disorders among the immune-mediated inflammatory skin disorders was reported as 15%. None of the immunobullous disorder patients enrolled in this study had an associated arthritis as evidenced in the literature as well except of few reports of coexistent pemphigus and RA. The association with arthritis in our study group was found in 26.23% of cases. Out of the 16 cases of arthritis associated with immune-mediated inflammatory skin disorders, 56.25% and 43.75% were of psoriatic and connective tissue disorder type, respectively.

Among the three biomarkers that we used in our patients, the maximum positivity was seen for CRP (39.34%) followed by RF and anti-CCP antibodies in 18.03% and 14.75% of the cases. However, if we consider the individual group of patients that we enrolled, maximum positivity for all the three biomarkers was found in connective tissue disorders, 

**Table 1. Summary of the correlation of various diagnostic biomarkers with arthritis among categories of immune mediated inflammatory skin diseases under study**

| Psoriasis | Category of Immune mediated inflammatory skin diseases |
|-----------|-----------------------------------------------------|
|           | Connective tissue disorders | Immunobullous disorders |
| Proportion out of total Immune mediated inflammatory skin diseases | 38 (62%) | 14 (23%) | 9 (15%) |
| Association of immune mediated inflammatory skin disease with arthritis n (%) : total n=61 | 9 (23.68%) | 7 (50%) | 0 |
| Positivity of diagnostic biomarkers | Rheumatoid Factor 11/61 (18.03%) | 2/9 (22.22%) | 5/38 (13.16%) | 2/7 (28.57%) | 6/14 42.88% |
| P value 0.457 | with arthritis | 3/29 (10.34%) | 4/7 (57.14%) | 0 |
| C reactive protein 24/61 (39.34%) | without arthritis | 5/9 (55.56%) | 15/38 (39.47%) | 3/7 (42.86%) | 7/14 (50%) | 0 |
| P value 0.377 | with arthritis | 10/29 (34.48%) | 4/7 (57.14%) | 2/9 (22.22%) |
| Anti-CCP antibodies 9/61 (14.75%) | without arthritis | 2/9 (22.22%) | 4/38 (10.53%) | 3/7 (42.86%) | 5/14 (35.71%) | 0 |
| P value 0.045 | with arthritis | 2/29 (6.90%) | 2/7 (28.57%) |
50%, 42.88%, and 35.71% for CRP, RF, and anti-CCP antibodies, respectively. As far immunobullous disorders are concerned both the RF and anti-CCP antibodies were absent. In the psoriatic cases, RF, CRP, and anti-CCP positivity were seen in 13.16%, 39.47%, and 10.53% cases, respectively. Arthritis was significantly associated only with anti-CCP antibodies among all the cases of immune-mediated inflammatory skin disorders, whereas the association of arthritis with any of the three diagnostic markers was not significant when the three groups of psoriasis, connective tissue disorders, and immunobullous disorders were analyzed individually. A study done to assess the prevalence of anti-CCP antibodies in psoriatic arthritis and compare it with RA, reports 12.5% positivity in psoriatic arthritis with a higher number of swollen joints in such cases. The study further reported 20% of RF-negative RA patients to be positive for anti-CCP antibodies. 

Another study, however, reported a very low prevalence of anti-CCP antibodies in RF-negative psoriatic polyarthritis and concluded that anti-CCP positivity was more associated with symmetric polyarthritic subset and high disease activity or radiological progression. In a study by Böckelmann et al., anti-CCP antibodies occurred in approximately 18% of psoriasis patients not affected by any form of arthritis, relatively higher than the estimated prevalence within the general population of RA, which may suggest a subclinical or early manifestation of psoriatic arthritis or coincident RA. Among the SLE group, a study reported 6.7% and 15.5% positivity for anti-CCP antibodies and RF and further concluded that anti-CCP antibodies do not associate with SLE arthritis suggesting no pathogenic role in the development of arthritis.

**Conclusion**

The presence of anti-CCP antibodies is associated with immune-mediated connective tissue disorders; however, the association with arthritis is more significant. Anti-CCP antibodies, thought to be a marker for RA till now, are shown in our study to be elevated in other disorders also. Further studies with larger number of patients with and without arthritis and with the general population as controls would help clarify this issue further.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**What is new?**

The presence of anti-CCP antibodies is associated with immune-mediated connective tissue disorders; however, the association with arthritis is more significant.

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