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The Association of Anti-Cyclic Citrullinated Peptide Positivity with Extra-Articular Manifestations in Patients with Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic, auto-immune disorder that is accompanied by long-term joint illness and systemic redification, which refers to additional-articular manifestations (ExRA). Anti-cyclic citrullinated peptide (anti-CCP) antibodies are RA specific and linked to disease activity. This study attempted to assess the role of anti-CCP positivity as related to ExRA in patients with RA. **Methods:** Non-probability consecutive sampling was utilized to recruit 91 RA patients within the Department of Medicine, BKMC, Swabi, in the form of a cross-sectional study. Demographic, clinical, and serological data were obtained, as well as an anti-CCP level and RA Disease Activity Score-28 (DAS-28). Clinical examination and imaging were used to confirm the presence of ExRA. **Results:** There was a mean age of 45.3 (12.4) years; 71.4 percent were females. ExRA existed in 9.9 percent of patients, rheumatoid nodules (4.4 percent) being in the lead. Eighty one point three percent of participants were found to be anti-CCP positive. Of those having ExRA, 88.9 percent were anti-CCP positive; the relationship was not significant ($p = 0.391$). There was a substantial relationship between high DAS-28 scores (>5.1) and the ExRA presence ($p = 0.032$). **Conclusion:** ExRA was often associated with the presence of Anti-CCP antibodies in the RA patients but the association was not statistically significant. ExRA was significantly associated with higher disease activity. Such results increase the necessity of strict observation of disease activity to mark patients predisposed to system complications.

INTRODUCTION

Rheumatoid arthritis (RA) is a long-term autoimmune disorder whose pathogenesis is uncertain and is characterized by an attack of joint and other body parts and commonly comes along with rheumatoid factor (RF) (1,2). Disease modifying antirheumatic drugs (DMARDs) are also necessary medications that should be given early in the disease so as to control it before it causes joint destruction. Thus, it is important to find a capable serological marker or markers to diagnose early and accurately. Although RF may be positive in several autoimmune and infectious diseases, it is of intermediate sensitivity in the diagnosis of RA (3). The studies indicate that worldwide, the prevalence of rheumatoid arthritis (RA) is estimated at 0.5 to 1 percent of the population, indicating its health-related concern. RA stands as one of the topmost causes of disability after osteoarthritis and gout (4). Historical data on Pakistan show that rheumatoid arthritis (RA) has a prevalence rate in Karachi city of 0.142 percent, and female involvement in this disease is more common (5,6).

IgM rheumatoid factor (RF) is the most widely used one in the diagnosis of rheumatoid arthritis (RA) and realized as one of the classification criteria that has been promoted by the American College of Rheumatology (ACR) (7,8,9), though not too frequently. Even positivity of autoantibodies can be transferred even to up to 20 years before the rheumatoid arthritis (RA) can manifest itself. Moreover, anti-cyclic citrullinated peptide (anti-CCP) has also been proven to be raised in RA patients without an RF. Hence, it can be assumed that the use of anti-CCP may be proposed as a more suitable initial study of the patients with suspected RA (10,11).

Extra-articular manifestation (ExRA) was found in rheumatoid arthritis (RA) patients at a rate of 17.8-40.9% (12). Variables associated with extra-articular symptoms (ExRA) of rheumatoid arthritis (RA) are: Male sex, genetic markers (HLA-DRB1*04 subtype), positive rheumatoid factor (RF), antinuclear antibodies (ANA) and Anti-CCP antibodies, among some environmental factors, smoking

especially (13).

Identification and recognition of the presence of Extra-articular manifestations (ExRA) is very important in clinical practice since it correlates with increased mortality and morbidity (14,15). It has been reported that there were a 2.5-fold higher mortality rates among patients with ExRA than in patients with RA and ExRA (13).

Besides risks of rheumatoid factor (RF) and antinuclear antibodies (ANA), anti-cyclic citrullinated peptide (anti-CCP) antibodies have also been regarded as the possible risk of ExRA. These antibodies are 55-69 percent prevalent and are normally located in the serum of RA patients (16). Anti-CCP antibodies are much more specific to RA than the rheumatoid factor (RF), predictive of severity in the disease (1718). A literature review carried out by investigating Greek patients with RA showed that an increase in anti-CCP2 antibodies could associate with extra-articular vices, including serositis and pulmonary fibrosis (19). An analysis performed to investigate the prevalence of osteoporosis in menopausal women that were diagnosed with RA revealed that the occurrence of osteoporosis in patients with extra-articular manifestations was considerably higher than in patients having RA without manifestations. This was computed using retrospective study of patient records. (20,21).

Sulaiman MD in his study used his tested 159 RA patients as research subjects. The mean age of patients was 48.3 years with 84.3 percent (n=134) being female. There were 83 (52.2) and 99 (62.3) patients who were positive to anti-CCPs antibody and RF, respectively. All of the patients recorded 4.74 (medium and high) on the Disease Activity Score-28 for Rheumatoid Arthritis with erythrocyte sedimentation rate (ESR) (DAS28-ESR). The RA radiological abnormalities were observed in 58 (36.5%) patients and the extra-articular manifestations included rheumatoid nodule (6.3%), pulmonary involvement and anemia (13%).

This study rationale is to investigate the relationships between Anti-CCP and presence of extra-articular manifestations in RA patients. Such studies have not been conducted in our institution before. Through this research, we are in position to understand patients who have RA better. An early determination of whether Anti-CCP positivity was shown in the study to result in an increased risk of extra-articular manifestations could have profound influence on the way RA is managed, patient education and counseling. The findings of this research were employed to generate future research and policy suggestions on how to treat ExRA.

METHODOLOGY

This was a cross-sectional study done between 10 December 2024 to 10 June 2024 at the Institutional review board (IRB), Bacha Khan Medical Complex, Swabi, after the approval of Department of Medicine at Bacha Khan Medical Complex, Swabi. The consecutive sample without probability was not to be employed to enroll 91 patients with rheumatoid arthritis. The sample size has been determined by the WHO sample size tool, upon using a previous reported prevalence rate of rheumatoid nodules (extra-articular manifestations, ExRA) at 6.3%, and a margin of error of 5 percentage points and 95 percent confidence interval.

Our inclusion criteria were 16-70 year old adult patients with rheumatoid arthritis as defined by the 2010 American College of Rheumatology /European League Against Rheumatism (ACR/EULAR) criteria based classification criteria and gender and ethnicity was not considered a factor as long as they satisfy the inclusion criteria of being in a position to fill the cognitive assessment of the prescription trial drug. Patients with a known history of other auto immune diseases (eg, systemic lupus erythematosus or psoriasis), malignancy, or chronic infectious and lung disease (eg, tuberculosis, COPD, or asthma) would not be able to participate. The participants who were willing to take part was also to be excluded in the study.

Data on the patients attending the outpatient department would be collected through a structured questionnaire after getting an informed consent. The demographical and clinical characteristics were age, sex, body mass index (BMI), level of education, socioeconomic status, duration of the disease, rheumatoid arthritis disease activity score (RA DAS-28), anti- cyclic citrullinated peptide (anti-CCP) level and rheumatoid factor (RF). Clinical examination by Consultant Physician and applicable medical imaging such as computed tomography (CT) of the chest, abdomen and pelvis were used to confirm the availability of extra-articular manifestation. To ensure data quality and data confidentiality, all of the data gathered would be recorded in a pre-planned database but this would be confidential.

IBM SPSS Statistics 23 would be used to analyse data. The continuous variables age, duration of the disease, and RA DAS-28 scores would be presented as the mean with its standard deviation. The Shapiro-Wilk test was used to check the normalcy of the continuous variables. Categorical variables such as gender, education level, socioeconomic status, RA factor status, anti-CCP status and ExRA yes/no was be presented in the form of frequencies and percentages. The presence of extra-articular manifestation was stratified on potential effect modifier by age, duration of disease, gender, anti-CCP status, and disease activity scores. A chi-square test or fisher exact and significance at or below a p-value of 0.05 would be used accordingly and post-stratification was to be applied.

RESULTS

The study conducted included 91 patients with rheumatoid arthritis. Mean respondent age was 45.3 (12.4) and the percentage female (71.4, n = 65) predominates. The mean disease duration was 6.7 +/- 3.9 years and mean RA DAS-28 score was 4.9 +/- 1.3 suggesting that moderate or high disease activity occurred. Demography and clinical characteristics of participants are tabulated in Table 1.

There were 9 (9.9%) patients with extra-articular manifestations (ExRA). Rheumatoid nodules (n = 4, 4.4%), interstitial lung disease (n = 3, 3.3%) and episcleritis (n = 2, 2.2%) were the most prevalent ExRA. The prevalence of types of ExRAs is listed in Table 2.

The rheumatoid factor was positive in 78 (85.7%) patients and anti-CCP antibodies positive in 74 (81.3%). Of the patients with ExRA, 8 (88.9%) were positive with anti-CCP

and 9 (100%) with RA factor.

Stratification analysis is shown in Table 3 and has demonstrated significant association (p = 0.032) between the appearance of ExRA and the high RA DAS-28 score. There was no such correlation with gender (p = 0.479), age group (p = 0.562), or anti-CCP status (p = 0.391).

Table 1

Demographic and Clinical Characteristics of Patients (n=91)

Variable		Mean ± SD / n (%)
Age (years)		45.3 ± 12.4
Gender	Male	26 (28.6%)
	Female	65 (71.4%)
BMI (kg/m^2)		26.7 ± 4.1
Education Status	No formal education	20 (22.0%)
	Primary	23 (25.3%)
	Secondary	30 (33.0%)
	Graduate/Postgrad	18 (19.8%)
Socioeconomic Status	Low	41 (45.1%)
	Middle	38 (41.8%)
	High	12 (13.2%)
Duration of Disease (years)		6.7 ± 3.9
RA DAS-28 Score		4.9 ± 1.3
RA Factor Positive		78 (85.7%)
Anti-CCP Positive		74 (81.3%)

Table 2

Distribution of Extra-Articular Manifestations (ExRA)

Type of ExRA	n (%)
Rheumatoid nodules	4 (4.4%)
Interstitial lung disease	3 (3.3%)
Episcleritis	2 (2.2%)
None	82 (90.1%)

Table 3

Stratification of ExRA with Clinical and Demographic Variables

Variable	ExRA Present (n=9)	ExRA Absent (n=82)	p-value
Age ≥ 50 years	5 (55.6%)	41 (50.0%)	0.562
Female Gender	6 (66.7%)	59 (72.0%)	0.479
Disease Duration ≥ 5 years	7 (77.8%)	52 (63.4%)	0.264
RA DAS-28 ≥ 5.1	6 (66.7%)	25 (30.5%)	**0.032**
Anti-CCP Positive	8 (88.9%)	66 (80.5%)	0.391
RA Factor Positive	9 (100%)	69 (84.1%)	0.126

Statistical significance defined at p ≤ 0.05.

DISCUSSION

The objectives of this cross-sectional study were to assess the incidence and trend of extra-articular manifestations (ExRA) in patients with rheumatoid arthritis (RA) and to determine the relationship of ExRA with clinical and serological variables. We noted that 9.9 percent of RA patients had ExRA, where the most common one was the rheumatoid nodules, then followed by the presence of interstitial lung disease, and episcleritis. This data is in line with the existing literature, as the rates of ExRA were evidenced to be between 6 and 30%, depending upon the study population and the chosen criteria of diagnosis.

Our finding of rheumatoid nodular preponderance is consistent with previous reports that subcutaneous nodules and/or nodules continue to be a hallmark of extra-articular manifestations of RA, especially in patients who are seropositive. Probably, it is worth noting that every patient with ExRA that emerged in our cohort had a positive rheumatoid factor (RF), and the vast majority of them were anti-CCP positive. This provides evidence to the hypothesis that seropositivity, especially anti-CCP antibodies, could be correlated with a more severe phenotype of the disease, including the systemic forms.

Intriguingly, high RA disease activity (DAS- 28 >= 5.1) was also significantly associated with the presence of ExRA (p= 0.032). This finding supports the idea of poor control of systemic inflammation being one of the major contributors of extra-articular involvement in RA. Comparable ties have been documented in both developed and developing environments, articulating on the greatness of strict disease control to deter systemic surprises.

In our study, we did not find any key relationships between ExRA and demographic factors, including age and gender. Though there were some earlier observations of male predominance in some of the ExRA patterns, we did not find such in our results. This can be presumably explained by overall tendency of the female preponderance of patients in our sample that aligns with the existing gender distribution of RA in the world.

The small percentage of pulmonary manifestations (3.3%) we identified may be associated with underdiagnosis, especially in resource-poor areas where high-quality imaging and pulmonary testing might simply not be conducted. It also highlights the importance of close follow-up regarding screening of interstitial lung disease and other systemic findings, even in the ambulatory care.

Our study possesses a number of strengths such as standardized criteria of classification (ACR/EULAR 2010) and systematic evaluation of disease activity and serological variables. Nevertheless, there are certain limitations that should be noted. One is the cross-sectional design, which prevents any support of temporal or causal interpretation. Second, the study was only examined in one tertiary care facility; this could have an impact on the validity of the sample. And lastly, underestimation of some ExRA, and the ones that may need more special diagnostic equipment in particular, may have happened due to the reliance on the available imaging and clinical examination. Finally, the extra-articular manifestation is an important clinical property of RA, whereby an increased disease activity and seropositivity is connected. Regular ExRA screening, especially in individuals who have high RA DAS-28 scores and positive serology, comes in handy in early detection before it becomes severe and expensive to treat. Larger, multicentric study cohorts and prospective study designs will be useful in explaining the pathophysiology or the prognostic significance of ExRA in RA patients.

CONCLUSION

This research found that 9.9 percent of the RA patients had extra-articular presentations with the most frequent ones being rheumatoid nodules and interstitial lung disease. The prevalence of anti-CCP positivity was high in patients with ExRA but this was not statistically significant. Nevertheless, there was a considerable association between increased RA disease activity (DAS-28 > 5.1) and the presence of ExRA meaning the systemic inflammation is a decisive variable in the progression towards extra-articular involvement. Such results emphasize the significance of intensive disease management among RA

individuals not only to avoid joint injuries but also to minimize chances of systemic disorders. Standard disease activity assessment and close surveillance of ExRA should be a part of standard RA treatment. To continue, multicenter research in bigger cohorts is necessary to understand the longitudinal effect of anti-CCP positivity on extra-articular developments.

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