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Research Article

STUDY OF ANTI-PEPTIDYL ARGININE DEIMINASE 4 AUTO ANTIBODIES IN RHEUMATOID ARTHRITIS PATIENTS AND THEIR CORRELATION WITH ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN TELANGANA POPULATION

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ABSTRACT

Objectives: This study aimed to evaluate the role of anti-peptidyl arginine deiminase 4 (PADI4) autoantibodies in the early diagnosis of rheumatoid arthritis (RA) and their association with Disease Activity Score 28 (DAS28) and anti-cyclic citrullinated peptide (anti-CCP) antibodies.

Methods: A case–control study involving 196 subjects, 98 patients of confirmed RA patients and 98 healthy controls matched in age and sex, was carried out at Rheumatology department of Chalmeda Anand Rao Institute of Medical Sciences (CAIMS), hospital. Detailed history is taken. Patients were included after following the inclusion and exclusion criteria, getting informed consent from patients, and approved by the institutional ethical committee of CAIMS. Appropriate statistical analysis will be done after collecting data. Statistical Package for the Social Sciences version 25 was used for further statistical analysis. $p<0.05$ was considered as statistically significant, DAS28, rheumatoid factor (RF), Erythrocyte sedimentation rate, anti-CCP, antibodies, and anti-PADI4 antibodies were measured in RA patients and controls BY ELIAS sandwich method.

Results: Anti-PADI4 and anti-CCP antibody levels were significantly elevated in RA patients ($p<0.001$). Anti-PADI4 showed 68% sensitivity and 98% specificity, whereas anti-CCP had 84% sensitivity and 98% specificity. Combined testing increased diagnostic sensitivity to 90%. Anti-PADI4 levels showed positive correlations with DAS28 ($r=0.54$, $p=0.006$), anti-CCP ($r=0.79$, $p<0.00001$), and RF ($r=0.62$, $p=0.0001$). RA patients positive for anti-PADI4 had significantly higher DAS28 scores, RF, and anti-CCP levels than those negative for anti-PADI4. Anti-PADI4 also identified some anti-CCP-negative RA cases.

Conclusion: Serum anti-PADI4 Auto antibodies give an important diagnostic value with correlation with anti-CCP antibodies and DAS28.

Keywords: Rheumatoid arthritis, Anti Peptidyl arginine deiminase auto antibodies, Disease activity score 28, Anti-cyclic citrullinated peptide.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease and represents a common form of inflammatory polyarthritis. RA develops when the body's immune system mistakenly attacks the tissues surrounding the joints. This immune response triggers the release of certain chemicals and enzymes that gradually damage the cartilage and bones, leading to pain, swelling, and joint deterioration [1]. The global prevalence of RA is approximately 0.8% among adults. It is characterized by irreversible joint damage, which typically occurs within the first 3 years following disease onset and diagnosis, potentially leading to disability and significant deterioration in the patient's quality of life. Early diagnosis is, therefore, critical to prevent irreversible joint destruction [2].

In addition to rheumatoid factor (RF) and disease activity score 28 (DAS28), serological tests play a crucial role in the early detection and monitoring of disease progression. The identification of autoantibodies targeting citrullinated antigens has made a significant contribution to the diagnosis of RA [3].

Citrullination is a post-translational modification, in which the basic amino acid arginine is converted to citrulline, a non-standard amino acid. This process is catalyzed by calcium-dependent peptidyl arginine deiminase (PADI) enzymes, which lead to citrullinated proteins that may trigger autoantibody production [4].

There are five known isoforms of PADI enzymes in humans: PADI1 to PADI4 and PADI6. These isoenzymes are expressed in various tissues and cell types [5].

PADI4, in particular, is localized in the cytoplasm of monocytes, T-cells, B-cells, neutrophils, eosinophils, macrophages, fibroblast-like synoviocytes, and endothelial cells within the RA synovium. It is also found in natural killer cells and can translocate to the nucleus upon cell activation. PADI4 plays a physiological role in gene regulation by catalyzing histone citrullination. In RA, it contributes to the generation of anti-citrullinated peptide antibodies (ACPA) by modifying specific substrates and may itself serve as an autoantigen [6].

Anti-cyclic citrullinated peptide (CCP) antibodies tend to appear early in the course of RA and are considered more specific to the disease. This makes them a more reliable marker for both diagnosing and predicting the progression of RA when compared to RF, which has lower specificity for the condition [7]. Autoantibodies against PADI4 have also been identified as specific markers in patients with clinically evident RA [8].

Early studies have reported an association between *PADI4* gene polymorphisms and RA, including links with anti-CCP antibodies and disease activity. However, these findings have not been consistent across different populations, ethnicities, and geographic regions [9-12].

Several researchers have demonstrated the presence of anti-PADI4 antibodies in RA patients and suggested a potential association with disease activity [13]. In our study, we evaluated the presence of anti-PADI4 antibodies in 98 RA patients from Telangana to assess their role in the early course of disease. This study aimed to explore the association of anti-PADI4 antibodies with anti-CCP antibodies and DAS28 scores.

METHODS

A case–control study was conducted in the Department of Biochemistry at Chalmeda Anand Rao Institute of Medical Sciences (CAIMS), Karimnagar, Telangana, over a period of 14 months. A total of 196 subjects were included in the study, comprising 98 confirmed RA patients and 98 age- and gender-matched healthy controls (HC) with no family history of RA.

RA diagnosis was established based on the revised 2010 American College of Rheumatology (ACR) criteria for the classification of RA. In addition, only patients fulfilling at least four of the revised 1988 ACR criteria were included (Arnett *et al*., 1988) [14]. All participants were screened for ankylosing spondylitis (AS) to rule out confounding conditions. Detailed clinical histories were obtained from all subjects. Informed consent was obtained, and the study protocol was approved by the Institutional Ethical Committee of CAIMS (Ref. No. CAIMS/IEC/PhD/002/2020, Dated. February 27, 2020).

Inclusion criteria

- Patients previously diagnosed with RA and attending the Departments of General Medicine and Orthopedics at CAIMS
- Healthy individuals serving as controls
- Participants aged 25–65 years, including both males and females.

Exclusion criteria

Subjects were excluded if they had any of the following conditions:

- Known cases of AS
- Pregnancy
- Chronic liver failure
- Chronic renal failure
- Other autoimmune diseases, malignancies, or acute infections.

Sample collection

Venous blood samples were collected after an overnight fast of 12 h. All samples were analyzed for the following biomarkers:

- RF
- Anti-CCP antibodies
- C-reactive protein (CRP)
- Anti- PADI4 antibodies.

Detection of anti-PADI4 autoantibodies

Serum levels of anti-PADI4 autoantibodies were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) method with a commercial kit (Cayman Chemical, Ann Arbor, MI, USA). The reference values were as follows:

- Negative: <20 U/mL
- Weak Positive: 20–50 U/mL
- Moderate Positive: 51–100 U/mL
- Strong Positive: >100 U/mL
- Cut-off value: 220 U/mL.

Detection of anti-CCP antibodies

Anti-CCP antibodies were quantified using a second-generation ELISA kit (Immunoscan RA, EURO-Diagnostica, Sweden). Subjects with anti-CCP antibody levels ≥25 IU/mL were considered positive. The assay involved antigen-coated wells treated with calibrators, controls, and patient sera, followed by incubation. Wells were washed and then incubated with a horseradish peroxidase-conjugated anti-human immunoglobulin G (IgG). After further washing and substrate addition, the reaction was stopped, and absorbance was measured at 450 nm.

Detection of CRP

CRP levels were measured using a particle-enhanced immunoturbidimetric method. A value <5.0 mg/L was considered normal, and values ≥5.0 mg/L were considered positive. Samples exceeding the assay's linearity limit of 225 mg/L were diluted appropriately for accurate quantification.

DAS28 calculation

The DAS28 was calculated based on tender joint count, swollen joint count, and erythrocyte sedimentation rate (ESR) values using a standard DAS28 calculator. The disease activity was classified as:

- Low: DAS28 <3.2
- Moderate: DAS28 3.2–5.1
- High: DAS28 >5.1.

ESR was measured using the Westergren method, with normal ranges being 5–15 mm/h for males and 5–20 mm/h for females.

RF determination

RF was estimated by an immunoturbidimetric method using latex-bound, heat-inactivated IgG as the antigen. Antigen-antibody complexes formed through agglutination were measured turbidimetrically. RF levels >14 IU/mL were considered positive. Samples exceeding the assay's upper limit of 125 IU/mL were diluted and retested for accurate values.

Statistical analysis

All collected data were entered into Microsoft Excel and subsequently analyzed using Statistical Package for the Social Sciences version 25.0. Qualitative variables were expressed as proportions, and quantitative data were summarized as mean±standard deviation. The association between categorical variables was tested using the Chi-square test. Mean differences between groups were evaluated using the independent t-test, and correlation analyses were conducted for continuous variables. A $p<0.05$ was considered statistically significant at a 95% confidence level.

RESULTS AND OBSERVATION

This study included 196 subject, 98 patients 98 matched HCs, diagnosed with RA, and their clinical and serological parameters were analyzed to assess the correlation between DAS28, anti-PADI4 auto antibodies (PADI4), and anti-CCP antibodies.

The subjects in controls and RA patients with matched age, sex groups, there was in RA patients highly significant increase in DAS28 score, RF, ESR, and CRP, anti-CCP, anti-PADI4 auto-antibodies than HCs (Table 1).

This table summarizes the demographic and key laboratory parameters in both RA patients (n=98) and HCs (n=98). The mean age of RA patients was slightly higher (44.1±15.91 years) than that of controls (40.1±15.91 years). The gender distribution revealed a female predominance in both groups, with a higher number of females among RA patients (82 out of 98) (Figure 2).

Table 1: Demographic and laboratory data of study population

Demographic data	RA patents (n=98)	Control (n=98)	t-test/ Chi-square	p-value
AGE (mean±SD) years	44.1±15.91	40.1±15.91	1.75	0.08
Gender Distribution (Male/Female)	16/82	12/86.	0.34	0.559
Laboratory data (Mean±SD)				
ESR (mm/hour)	76.1±4.91	9.2±0.50	134.18	<0.001
CRP (mg/dL)	45.0±3.10	4.0±0.25	130.5	<0.001
Uric acid (mg/dL)	4.0±0.19	4.1±0.43	−2.1	0.036
Urea (mg/dL)	35.1±0.91	30.1±1.42	29.34	<0.001
FBS (mg/dL)	92.3±4.1	79.1±1.90	28.91	<0.001

RA: Rheumatoid arthritis, SD: Standard deviation, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, FBS: Fasting blood sugar

Inflammatory markers such as ESR and CRP were significantly elevated in RA patients (76.1±4.91 mm/h and 45.0±3.10 mg/dL, respectively) compared to controls (9.2±0.50 mm/h and 4.0±0.25 mg/dL), indicating systemic inflammation. Metabolic parameters such as uric acid and urea were slightly raised in the RA group but not substantially different. Fasting blood sugar was also higher in RA patients (92.3±4.1 mg/dL) compared to controls (79.1±1.90 mg/dL), possibly reflecting stress-related hyperglycemia or underlying metabolic dysregulation.

This table details the diagnostic and clinical markers observed among RA patients. The mean DAS28 score was 4.12±1.98, indicating moderate to high disease activity. RF and anti-CCP levels were elevated (79.2 U/mL and 59.1 U/mL, respectively), supporting seropositive RA classification. Elevated ESR and CRP reaffirm the ongoing inflammation. The average morning stiffness duration was 35.9±2.01 min, and the average disease duration was 15.2±1.01 months, indicating subacute to chronic disease presentation (Table 2).

This table evaluates the sensitivity of anti-CCP and anti-PADI4 antibodies as diagnostic markers in RA. Anti-CCP showed a sensitivity of 84% (83/98), anti-PADI4 68% (66/98), and when both tests were used in combination, the sensitivity increased to 90% (89/98). Notably, no patients tested negative for both markers simultaneously, underscoring the utility of combining these assays for enhanced diagnostic accuracy.

This table compares the specificity of both antibodies in HCs. Anti-CCP and anti-PADI4 both demonstrated 98% specificity, as only 2 out of 98 controls tested positive for either marker. Importantly, no individual tested positive for both antibodies, suggesting high specificity when both tests are combined.

This table compares RA patients based on their anti-PADI4 antibody status. Those with positive anti-PADI4 antibodies (n=66) had higher DAS28 scores (6.41±1.10 vs. 5.92±0.62), indicating greater disease activity. They also showed higher RF (119.1±28.1 vs. 80.1±10.12 U/mL), anti-CCP (88.1±2.10 vs. 79.10±5.12 U/mL), and inflammatory markers like ESR and CRP. Morning stiffness and disease duration were also slightly elevated. This suggests that anti-PADI4 positivity may correlate with increased disease severity and inflammation.

This table distinguishes RA patients based on anti-CCP positivity. Patients with positive anti-CCP (n=83) had markedly elevated levels of anti-PADI4 antibodies (78.1±21.1 vs. 33.1±6.12 U/mL), RF, DAS28 scores, ESR, and CRP, compared to anti-CCP-negative patients (n=15). These findings reinforce the relationship between anti-CCP and disease activity and show that anti-PADI4 is also elevated in more active and seropositive cases.

This figure visually demonstrates a significantly higher level of anti-PADI4 autoantibodies in RA patients compared to HCs. It supports the role of anti-PADI4 as a potential biomarker for RA diagnosis and disease activity.

This bar graph or scatter plot (depending on format) shows a sharp elevation of anti-CCP antibodies in RA patients versus controls. It visually confirms anti-CCP as a sensitive and specific serological marker in RA diagnosis.

This scatter plot shows a moderate positive correlation (r=0.54, p=0.006) between anti-PADI4 (n=98) antibody levels and DAS28 scores (n=98). This suggests that higher anti-PADI4 levels are associated with increased disease activity.

A strong positive correlation (r=0.79, p=0.00001) is observed between anti-PADI4 and anti-CCP levels. This indicates that both antibodies tend to be co-expressed in RA patients and may serve as complementary diagnostic and prognostic markers.

This figure presents a significant positive correlation (r=0.62, p=0.0001) between anti-PADI4 levels and RF. The correlation indicates that patients with elevated RF are also more likely to exhibit elevated anti-PADI4 levels, further supporting its potential role in disease stratification.

DISCUSSION

Our study clearly demonstrates that serum levels of anti-Peptidylarginine deiminase 4 (anti-PADI4) autoantibodies were significantly elevated in patients with Rheumatoid Arthritis (RA) compared to HCs. Among the RA patients, 68% tested positive for anti-PADI4 autoantibodies, whereas only 2% of the HCs showed positivity, reflecting high disease-specific prevalence. This finding was supported by Fig. 1, which visually depicts elevated anti-PADI4 titers in the RA group. When evaluating diagnostic performance, anti-PADI4 antibodies showed a sensitivity of 68% and specificity of 98% (Tables 3 and 4), confirming their high diagnostic value, particularly in seropositive patients.

The diagnostic utility was further enhanced when anti-PADI4 antibodies were combined with anti-CCP testing, increasing sensitivity to 90% (Table 3). The correlation analyses strengthen this association: a moderate positive correlation (r = 0.54, p = 0.006) was observed between anti-PADI4 levels and DAS28 scores (Fig. 3), suggesting a link between antibody titers and disease activity. Moreover, anti-PADI4 levels showed a strong positive correlation with anti-CCP (r = 0.79, p = 0.00001, Fig. 4) and a significant correlation with RF levels (r = 0.62, p = 0.0001, Fig. 5).

These findings are consistent with the observations of Kolfenbach *et al*. [15], who reported that anti-PADI4 antibodies may precede the clinical diagnosis of RA and are strongly associated with anti-CCP positivity. The presence of these antibodies up to 3 years before diagnosis aligns with other predictive markers such as anti-CCP and RF [16-20]. In a study by Takizawa *et al*. [21], although anti-PADI4 showed a sensitivity of only 45%, its specificity was notably high at 93.5%, paralleling our findings of 98% specificity. This specificity underlines anti-PADI4's value in excluding non-RA cases in clinical settings.

Notably, when we stratified RA patients based on anti-PADI4 status (Table 5), individuals who were positive exhibited significantly higher DAS28 scores (6.41±1.10 vs. 5.92±0.62), RF (119.1±28.1 vs. 80.1±10.12 U/mL), anti-CCP (88.1±2.10 vs. 79.10±5.12 U/mL), and CRP levels (45.0±3.10 vs. 3.60±3.01 mg/dL), suggesting a strong association with disease severity. These patients also reported more prolonged morning stiffness and elevated inflammatory markers, reinforcing the clinical relevance of anti-PADI4 in monitoring disease activity.

Interestingly, our data showed that 15 of 98 RA patients were negative for anti-CCP antibodies, yet two of these anti-CCP negative

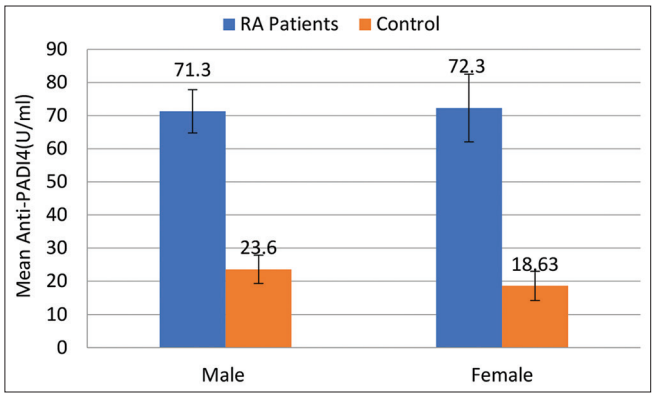

Fig. 1: Serum level of anti-peptidyl arginine deiminase 4 in healthy control subjects, rheumatoid arthritis (RA) patients. Number of RA patients (n=98, M/f: 16/82) Control patients (n=98, M/F: 12/86)

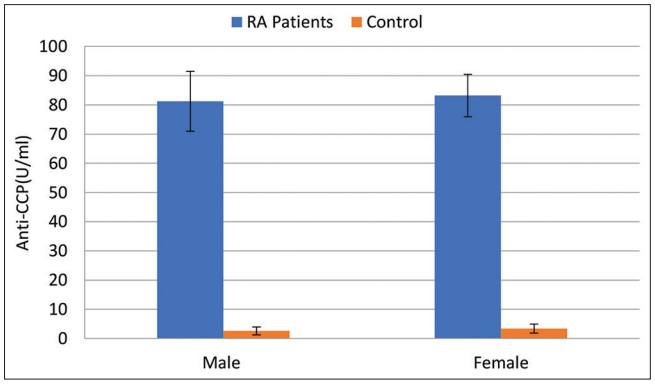

Fig. 2: Serum level of anti-cyclic citrullinated peptide in healthy control subjects, rheumatoid arthritis (RA) patients. Number of RA patients (n=98, M/f: 16/82) Control Patients (n=98, M/F: 12/86)

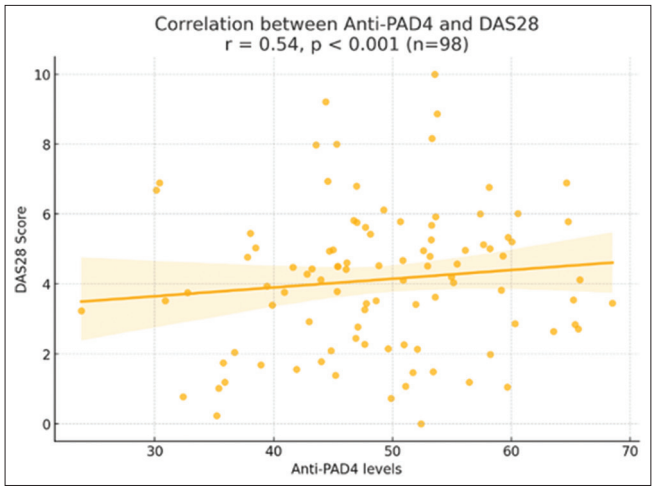

Fig. 3: Correlation between anti-peptidyl arginine deiminase 4 and disease activity score 28 score in rheumatoid arthritis patients (r=0.54, p=0.006)

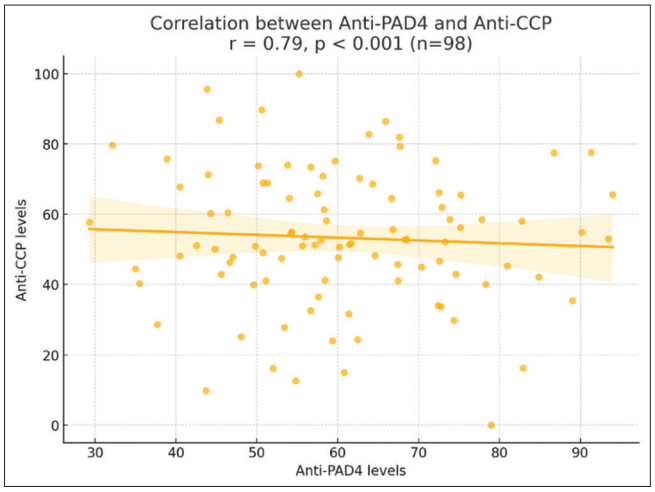

Fig. 4: Correlation between anti-peptidyl arginine deiminase 4 and anti-cyclic citrullinated peptide in rheumatoid arthritis patients (r=0.79, p=0.00001)

individuals were positive for anti-PADI4 (Table 6). This implies that anti-PADI4 antibodies may aid in identifying RA cases that are missed by traditional markers. The combined sensitivity of anti-CCP and anti-

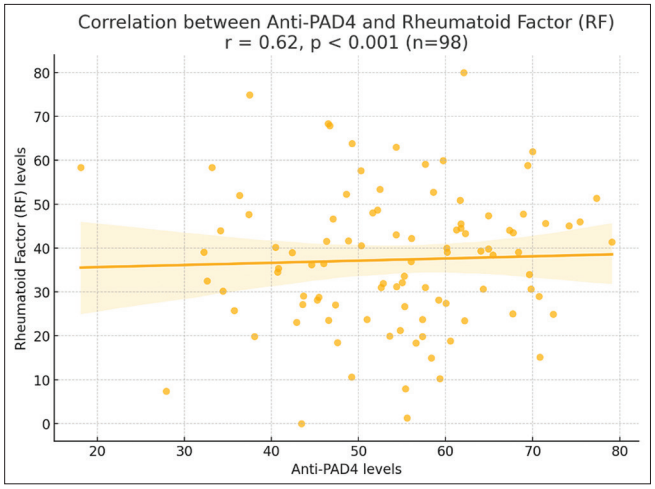

Fig. 5: Correlation between anti-peptidyl arginine deiminase 4 and rheumatoid factor in rheumatoid arthritis patients (r=0.62, p=0.0001)

Table 2: Diagnostic features of RA

Parameter	RA patients (n=98)
DAS28 (Mean±SD)	4.12±1.98
Rheumatoid factor (U/mL)	79.2
Anti-CCP (U/mL)	59.1
ESR (mm/hour) (Mean±SD)	75.9±6.10
CRP (mg/dL)	
Morning stiffness (minute)(Mean±SD)	35.9±2.01
Disease duration (month) (Mean±SD)	15.2±1.01

RA: Rheumatoid arthritis, SD: Standard deviation, CCP: Cyclic citrullinated peptide, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, DAS28: Disease activity score 28

Table 3: Sensitivity of anti-CCP and anti-PADI4 auto antibodies

Components	Anti-CCP	Anti-PADI4 antibodies	Both
Positive	83	66	89
Negative	15	32	0
Total no of RA Patients	98	98	
Sensitivity (%)	84	68	90

CPP: Cyclic citrullinated peptide, PADI4: Peptidyl arginine deiminase 4, RA: Rheumatoid arthritis

Table 4: Specificity of anti-CCP and anti-PADI4 auto antibodies for healthy control

Components	Anti-CCP	Anti-PADI4 auto antibodies	Both
Positive	2	2	0
Negative	96	96	96
Total no of healthy control	98	98	98
Specificity (%)	98	98	98

CPP: Cyclic citrullinated peptide, PADI4: Peptidyl arginine deiminase 4

PADI4 increased to 94.3%, highlighting the additive diagnostic benefit of this dual approach.

The pathophysiological basis of anti-PADI4 antibody production is not fully elucidated. However, it is hypothesized that overexpression of the PADI4 enzyme in RA may lead to loss of immunological tolerance, triggering an autoimmune response. This, in turn, may lead to increased protein citrullination and the development of anti-citrullinated peptide antibodies (anti-CCP). Our results, along with others [21,22], indicate

Table 5: The clinical data of RA-positive and negative anti-PADI4 auto-antibodies

Parameter	Negative anti-PADI4 auto antibodies (n=32)	Positive anti-PADI4 auto antibodies (n=66)	t-test/ Chi-square	p-value
Age (Mean±SD)	35.1±1.10	29.1±1.92	16.39	<0.001
Sex (M/F)	8/32.	10/66.	0.93	0.333
DAS28 (Mean±SD)	5.92±0.62	6.41±1.10	−2.34	0.021
Rheumatoid factor (U/mL) (Mean±SD)	80.1±10.12	119.1±28.1	−7.59	<0.001
Anti-CCP (U/mL) (Mean±SD)	79.10±5.12	88.1±2.10	−12.34	<0.001
ESR (mm/hour) (Mean±SD)	60.19±8.10	73.12±6.32	−8.64	<0.001
CRP (mg/dL) (Mean±SD)	3.60±3.01	45.0±3.10	−62.57	<0.001
Morning stiffness (minute) (Mean±SD)	55.0±3.10	60.1±4.10	−6.22	<0.001
Disease duration (month) (Mean±SD)	16.12±0.50	16.3±1.40	−0.70426	0.48

ESR: Erythrocyte sedimentation rate, DAS28: Disease activity score 28, CPP: Cyclic citrullinated peptide, CRP: C-reactive protein, SD: Standard deviation, PADI4: Peptidyl arginine deiminase 4, RA: Rheumatoid arthritis

Table 6: The clinical data of RA-positive and negative for anti-CCP

Parameter	Negative anti-CCP (n=15)	Positive anti-CCP (n=83)	t-test/Chi-square	p-value
Age (Mean±SD)	32.19±1.23	31±1.10	3.78	0.003
Sex (M/F)	03/09	12/71	0.87	0.349
Anti-PADI4 auto anti bodies (U/mL) (Mean±SD)	33.1±6.12	78.1±21.1	−8.16	<0.001
DAS28 (Mean±SD)	4.92±0.12	6.4±0.91	−6.26	<0.001
Rheumatoid factor (U/mL) (Mean±SD)	69.1±14.1	107.1±10.1	−12.56	<0.001
Anti-CCP (U/mL) (Mean±SD)	30.1±6.10	81.12±18.12	−10.75	<0.001
ESR (mm/hour) (Mean±SD)	55.10±6.24	79.12±6.12	−13.94	<0.001
CRP (mg/dL) (Mean±SD)	35.10±4.12	45.10±3.10	−10.9	<0.001
Morning stiffness (minute) (Mean±SD)	43.10±3.10	61.1±3.25	−19.87	<0.001
Disease duration (month) (Mean±SD)	20.1±2.10	16.1±1.19	10.47	<0.001

ESR: Erythrocyte sedimentation rate, DAS28: Disease activity score 28, CPP: Cyclic citrullinated peptide, CRP: C-reactive protein, SD: Standard deviation, PADI4: Peptidyl arginine deiminase 4, RA: Rheumatoid arthritis

that anti-PADI4 positivity is linked with higher anti-CCP levels and greater radiographic progression of joint damage.

Onder *et al*. [23], in a Turkish population-based study, similarly reported that anti-CCP levels correlated significantly with higher DAS28 scores, reinforcing the prognostic value of serological markers in disease activity assessment. Moreover, studies suggest that anti-PADI4 antibodies may modulate the activity of the PADI4 enzyme. Auger *et al*. [24] provided insight into this mechanism, showing that these antibodies could inhibit PADI4-mediated citrullination. This dual functionality – either inhibiting or enhancing enzyme activity – may influence disease progression by altering substrate specificity and protein modification, as hypothesized in other investigations [24-26].

The exact implications of these immunological dynamics remain an area of active research. It is also noteworthy that several small-molecule PADI4 inhibitors are being developed for potential therapeutic application in RA and even cancer [27]. Understanding how these inhibitors interact with naturally occurring autoantibodies could lead to novel therapeutic strategies.

Medical science is constantly evolving and adapting, and it is possible that future advancements may take us further into the realm of genetics [28].

CONCLUSION

Overall, our study corroborates previous findings while also contributing novel insights into the diagnostic and prognostic utility of anti-PADI4 autoantibodies. These antibodies are not only markers of seropositive RA but are also associated with disease activity, inflammatory burden, and may provide diagnostic value in anti-CCP negative cases. Further research is warranted to explore the functional consequences of anti-PADI4 autoantibody binding and to investigate its potential as a therapeutic target in RA.

AUTHOR CONTRIBUTION

All the authors have contributed equally.

CONFLICT OF INTERESTS

None.

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None.

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