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# Diagnostic Utility of Anti-Cyclic Citrullinated Peptide Antibodies Versus Rheumatoid Factor in Libyan Patients with Rheumatoid Arthritis: A Cross-Sectional Study

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## ABSTRACT

**Background:** Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial inflammation and progressive destruction of joint structures. Anti-cyclic citrullinated peptide (anti-CCP) antibodies have emerged as highly specific biomarkers, whereas rheumatoid factor (RF) demonstrates lower diagnostic specificity.

**Objective:** To assess the diagnostic utility of anti-CCP in comparison with rheumatoid factor (RF) in Libyan patients with RA and to investigate their associations with immunological markers, demographic characteristics, comorbid conditions , and clinical manifestations.

**Methods:** A cross-sectional case-control study was conducted involving70 RA patients who met the 2010 ACR/EULAR classification criteria and 70 age- and sex-matched healthy controls. Serum concentrations of anti-CCP antibodies, RF, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and complete blood count parameters were measured. Statistical analyses were performed to evaluate associations between serological markers and clinical variables, including sex, age, family history, disease activity, comorbidities, smoking status, COVID-19 vaccination, and vitamin D levels.

**Results:** Anti-CCP demonstrated a higher positivity rate (78.6%) compared with RF (64.3%) and was negative in all controls, whereas RF yielded false-positive results. Anti-CCP positivity was significantly associated with female sex ($p$=0.026), tender joint count, CRP, ESR, neutrophil-to-lymphocyte ratio, platelet count, and mean corpuscular volume. Additional associations were identified with COVID-19 vaccination, smoking, type 1 diabetes mellitus, family history of RA, higher disease activity, and lower vitamin D levels.

**Conclusion:** Anti-CCP demonstrated superior diagnostic specificity and broader clinical relevance compared with RF. Its strong associations with female sex, family history of RA, comorbidities, and disease activity support the incorporation of anti-CCP testing into routine diagnostic and monitoring protocols for RA in Libya.

**Keywords:** Anti- cyclic citrullinated peptide antibodies, Autoantibodies, Diagnostic value, Inflammatory markers, Rheumatoid arthritis, Rheumatoid factor

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## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by persistent synovial inflammation, progressive joint destruction, and consequent functional impairment. Early and accurate diagnosis is critical, as timely initiation of disease-modifying antirheumatic drugs (DMARDs) substantially improves long-term structural preservation and functional outcomes (1, 2).

Traditionally, rheumatoid factor (RF) has been the primary serological marker for RA. However, its diagnostic performance is limited, with a sensitivity of approximately 60–70% and specificity of around 78%. Eevated levels may also occur in healthy individuals and in various other autoimmune or inflammatory conditions (3, 4).

In contrast, anti-cyclic citrullinated peptide (anti-CCP) antibodies have emerged as a highly specific biomarker for RA. Although their sensitivity is comparable to that of RF (61–75%), their specificity is substantially higher, ranging from 94–99%. This makes anti-CCP testing a valuable tool for early and accurate diagnosis. Their inclusion in the 2010 ACR/EULAR classification criteria underscores their clinical importance (5, 6).

Several studies have demonstrated that combined testing of RF and anti-CCP antibodies enhances diagnostic performance compared with either marker alone, yielding improved sensitivity, specificity, and predictive value, particularly in early RA (7, 8).

Despite strong international evidence, the comparative diagnostic performance of RF and anti-CCP has not been adequately investigated in North African populations. Notably, no robust data are available from Libya. This study therefore aims to evaluate their individual and combined diagnostic value in a Libyan RA cohort, thereby addressing an important regional gap in the literature.

## MATERIALS AND METHODS

*Study Design and Setting*

This cross-sectional, case-control study was conducted at the Rheumatology Department of Tripoli University Medical Center between July and September 2024. A total of 70 patients who fulfilled the 2010 ACR/EULAR classification criteria for rheumatoid arthritis (RA) and 70 age- and sex-matched healthy controls were enrolled. Demographic and clinical information was obtained using a structured questionnaire, including age, sex, disease duration, family history, smoking status, comorbidities (e.g., type 1 diabetes mellitus, hypertension), and COVID-19 vaccination history. Clinical evaluation comprised assessment of joint swelling, tenderness, and morning stiffness, while disease activity was determined using standardized clinical indices.

*Sample Size Justification*

The study included 70 patients with RA and 70 healthy controls, selected based on availability during the study period. Although no formal a priori power calculation was performed, the chosen sample size was consistent with previous regional studies on RA biomarkers, which typically enrolled 50–100 participants per group, thereby ensuring methodological comparability.

*Sample Collection and Ethics*

Venous blood samples (5 mL) were collected from all patients and healthy controls. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and received approval from the Department of Medical Laboratory Science, University of Tripoli (Approval No: 177/2024). Written informed consent was obtained from all participants prior to enrollment.

*Laboratory Investigations*

*Anti-cyclic Citrullinated Peptide (Anti-CCP) Antibodies*

Serum anti-CCP concentrations were

measured using the Chorus Trio automated analyzer (Diesse Diagnostics, Italy), employing an enzyme-linked immunosorbent assay (ELISA ) principle. Results were reported in units per milliliter (U/mL), with values>17 U/mL classified as positive according to the manufacturer's reference range.

*Rheumatoid Factor (RF)*

Serum RF was quantified using the Cobas® Integra® 400 Plus analyzer (Roche Diagnostics, Germany) employing an immunoturbidimetric method. Results were expressed in IU/mL, with values>14 IU/mL considered positive according to the manufacturer's reference range.

*C-reactive Protein (CRP)*

Serum CRP concentrations were determined using the Atellica® CH analyzer (Siemens Healthineers, Germany) employing an immunoturbidimetric method. Value>6 mg/L were classified as elevated according to the manufacturer's reference range.

*Erythrocyte Sedimentation Rate (ESR)*

ESR was measured using an automated analyzer based on the Westergren method (SRS 100, Greiner Bio-one, USA). Values>20 mm/hr were classified as elevated according to standard reference criteria.

*Complete Blood Count (CBC)*

Hematological indices, including white blood cells (WBC), hemoglobin (Hb), platelet count (PLT), and mean corpuscular volume (MCV), were assessed using an automated hematology analyzer (Sysmex XN-Series, Japan).

*Statistical Analysis*

Data were analyzed using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean±standard deviation (SD), while categorical variables were summarized as frequencies and percentages. Associations between categorical variables were assessed using the Chi-square test. For comparisons between two groups, either the independent-sample t-test or the Mann–Whitney U test was applied, depending on distribution of data. Comparisons across more than two groups were performed using one-way ANOVA or the Kruskal–Wallis H test,as appropriate. Correlations between continuous variables were assessed using Spearman's correlation test. A p-value<0.05 was considered statistically significant.

## RESULTS

*General Characteristics of the Study Population*

A total of 70 patients with rheumatoid arthritis (RA) and 70 age- and sex-matched healthy controls were included. Females represented 70% of the RA group and 76% of the control group. The mean age of RA patients was 52.6±13.9 years (range: 20–80), while controls had a mean age of 51.0±14.5 years (range: 26–90). Within the RA cohort, the 41–50-year age group was the most represented (28.6%), with females being predominant (70%), which is consistent with the established epidemiology of RA. Age distribution is illustrated in Supplementary Fig. S1. Supplementary Fig. S1.

Biomarker Profiles Among the evaluated biomarkers, anti-CCP antibodies demonstrated the highest mean concentration (213.0±241.2 U/ml), whereas CRP exhibited the lowest (20.8±32.7 mg/L). These findings highlight a pronounced elevation of anti-CCP levels in the RA group (Table 1).

*Age and Gender Associations*

Mean anti-CCP antibody levels were higher in females, whereas RF and ESR were elevated in males; CRP tended to be higher in females. Across age groups, anti-CCP and CRP peaked in the 41–50 years group, while RF and ESR reached their highest levels in the 61–70 year group. These variations were not statistically significant, except for ESR, which demonstrated

**Table 1. Mean±SD Values of Selected Laboratory Investigations in the Study Population**

| Variables | Mean±SD |
|---|---|
| anti-CCP level | 213.02±241.2 U/ml |
| RF level | 151.4±296.9 IU/ml |
| CRP level | 20.76±32.66 mg/L |
| ESR level | 45.76±23.69 mm/hr |

anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

**Table 2. Association of age and gender with serological biomarkers in RA patients**

| Variable | Mean values across age groups (highest group) | *p*-value (age) | Gender differences (trend) | *p*-value (gender) |
|---|---|---|---|---|
| anti-CCP | Highest mean in 41–50 years (295±351.5 U/ml) | 0.584 | Higher in females (225.1±255.04 U/ml) | 0.302 |
| RF | Highest mean in 61–70 years (290.7±519.3) | 0.069 | Higher in males (239.2±470.9 IU/ml) | 0.102 |
| CRP | Highest mean in 41–50 years (41.3±68.5) | 0.641 | Higher in females (21.21±40.0 mg/L) | 0.103 |
| ESR | Highest mean in 61–70 years (71.5±28.6) | 0.035* | Higher in males (50.52±28.7 mm/hr) | 0.306 |

Values are expressed as mean levels within each subgroup. The Kruskal-Wallis test was applied for age group comparisons, and the Mann-Whitney U test for gender comparisons.

a modest association with age ($p$=0.035) (Table 2). Further stratified details are presented in Supplementary Tables S1 and S2.

*Seropositivity by Gender and Age*

Anti-CCP positivity was significantly more frequent among females ($p$=0.026), whereas RF, CRP, and ESR did not exhibit gender-related variation. With respect to age, RF positivity was significantly associated with older age groups ($p$=0.010), while anti-CCP, CRP, and ESR showed no significant age-related differences. Comprehensive age- and gender-stratified positivity profiles are provided in Supplementary Tables S3 and S4.

*Diagnostic and Prognostic Performance of Anti-CCP and RF*

Both anti-CCP and RF demonstrated highly significant differences between RA patients and healthy controls ($p$<0.001). Anti-CCP was positive in 78.6% (55/70) of RA patients and absent in all controls, yielding 100% specificity. In contrast, RF was positive in 64.3% (45/70) of RA patients and in 5.7% (4/70) of controls, confirming its lower specificity compared with anti-CCP (Table 3).

*Clinical and Inflammatory Correlates*

Anti-CCP–positive patients (n=55) were more frequently female ($p$=0.026) and demonstrated significantly higher values for morning stiffness ($p$=0.002), tender joint count ($p$=0.003), CRP ($p$=0.0001), ESR ($p$=0.005), NLR ($p$=0.013), platelet count ($p$=0.011), and MCV ($p$=0.003). Smoking ($p$=0.005) and type 1 diabetes mellitus ($p$=0.0002) were also significantly more prevalent in this group.

By contrast, RF-positive patients (n=45) were older ($p$=0.010), had longer morning stiffness ($p$=0.003), higher swollen joint counts ($p$=0.02), and elevated ESR ($p$=0.010), but showed no significant associations with CRP, NLR, smoking, or comorbidities (Table 4).

A significant association was also observed between anti-CCP positivity and prior COVID-19 vaccination ($p$=0.004), though this observation should be interpreted with caution given the small subgroup sizes.

**Table 3. Anti-CCP and RF positivity in RA patients compared with healthy controls**

| Test Marker | Variable | Negative N (%) | Positive N (%) | *p*-value |
|---|---|---|---|---|
| anti-CCP | Patient | 15 (21.4) | 55 (78.6) | < 0.001** |
| | Control | 70 (100.0) | 0 (0.00) | |
| RF | Patient | 25 (35.7) | 45 (64.3) | < 0.001** |
| | Control | 66 (94.3) | 4 (5.7) | |

Anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor

**Table 4. Associations of anti-CCP and RF Status with RA Patient Characteristics**

| Variables | anti-CCP Positive (n=55) | anti-CCP Negative (n=15) | *p*-value | RF Positive (n=45) | RF Negative (n=25) | *p*-value |
|---|---|---|---|---|---|---|
| Age (years) | 53±13.88 | 52±12.5 | NS | 53.6±11.1 | 52.3±12.4 | 0.010 |
| Male/female (n) | 13/42 | 8/7 | 0.026 | 17/28 | 4/21 | NS |
| Duration of disease (years) | 1.5±6.4 | 1.5±6.4 | 0.01 | 1.5±6.4 | 1.5±6.4 | NS |
| Morning stiffness (minutes) | 52.0±61.2 | 16.4±29.3 | 0.002 | 52.9±63.9 | 19.6±30.4 | 0.003 |
| Swollen joint count | 1.4±1.4 | 0.7±1.3 | 0.08 | 1.5±1.2 | 0.6±1.3 | 0.02 |
| Tender joint count | 6.0±6.4 | 2.8±4.3 | 0.003 | 5.6±6.0 | 3.2±4.9 | NS |
| CRP (mg/dl) | 41.3±68.5 | 16.3±22.6 | 0.0001 | 18.3±13.4 | 13.4±21.3 | NS |
| ESR (mm/hr) | 38.4±22.1 | 35±23.1 | 0.005 | 53.3±23.05 | 33.8±24.8 | 0.01 |
| WBC (1,000/mm³) | 7.0±2.1 | 7.1±2.3 | NS | 7.5±2.0 | 7.4±2.3 | NS |
| NLR | 2.5±1.93 | 1.23±1.94 | 0.013 | 2.4±1.81 | 1.21±1.83 | NS |
| PLT (1,000/mm³) | 300±117.8 | 259±54.9 | 0.011 | 302±114.8 | 256±58.9 | 0.03 |
| Hb (g/l) | 12.0±1.6 | 12.1±1.3 | NS | 12.0±1.5 | 12.5±1.2 | NS |
| MCV | 88 -102 fL | 80 -100 fL | 0.003 | 82-101 fl | 81-93 fl | NS |
| Smoking (n) | 12/55 | 9/15 | 0.005 | 15/45 | 19/25 | NS |
| T1DM | 5/55 | 3/15 | 0.0002 | 9/45 | 4/25 | NS |
| Hypertension | 3/55 | 9/15 | NS | 11/45 | 5/25 | NS |
| COVID-19 Vaccination | 54/55 | 15/15 | 0.004 | 45/45 | 25/25 | NS |

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cells; NLR, neutrophils to lymphocytes ratio; PLT, platelets count; Hb, hemoglobin level; MCV, mean corpuscular volume; T1DM, Type 1 diabetes mellitus

No significant associations were detected between either biomarker and total white blood cell count, hemoglobin, or hypertension.

*Correlation between Anti-CCP and RF*

Anti-CCP and RF levels demonstrated only a weak, statistically non-significant correlation (r=0.292), indicating that they reflect distinct immunological aspects of RA. In several cases, elevated anti-CCP levels were observed in the absence of corresponding RF increases, underscoring the complementary diagnostic role of these two biomarkers (Figs. 1–2).

*Association with Family History*

Among anti-CCP–positive patients, 74.5% reported a positive family history of RA compared with 26.7% of anti-CCP–negative patients ($p<0.01$). This finding indicates a strong association between anti-CCP seropositivity and familial predisposition (Table 5). A supplementary visualization of family history associations is presented in Supplementary Fig. S2.

*Association with Vitamin D Status*

Vitamin D deficiency (<20 ng/ml) was observed in 56.4% of anti-CCP–positive patients compared with 20% of anti-CCP–negative patients.

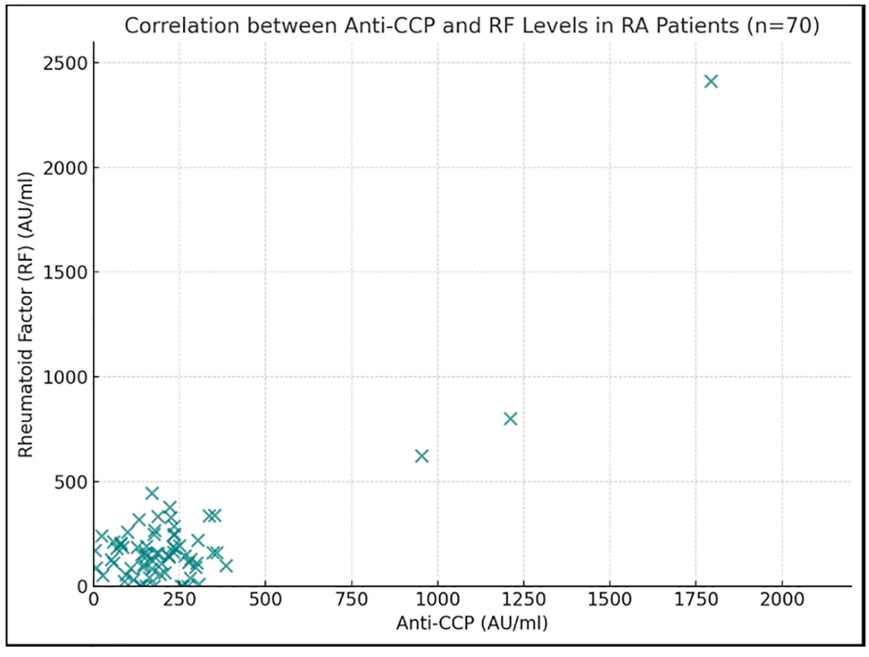


**Fig. 1.** Correlation analysis between anti-CCP and RF levels in RA patients, indicating a weak and statistically non-significant association (r=0.292, $p$>0.05).

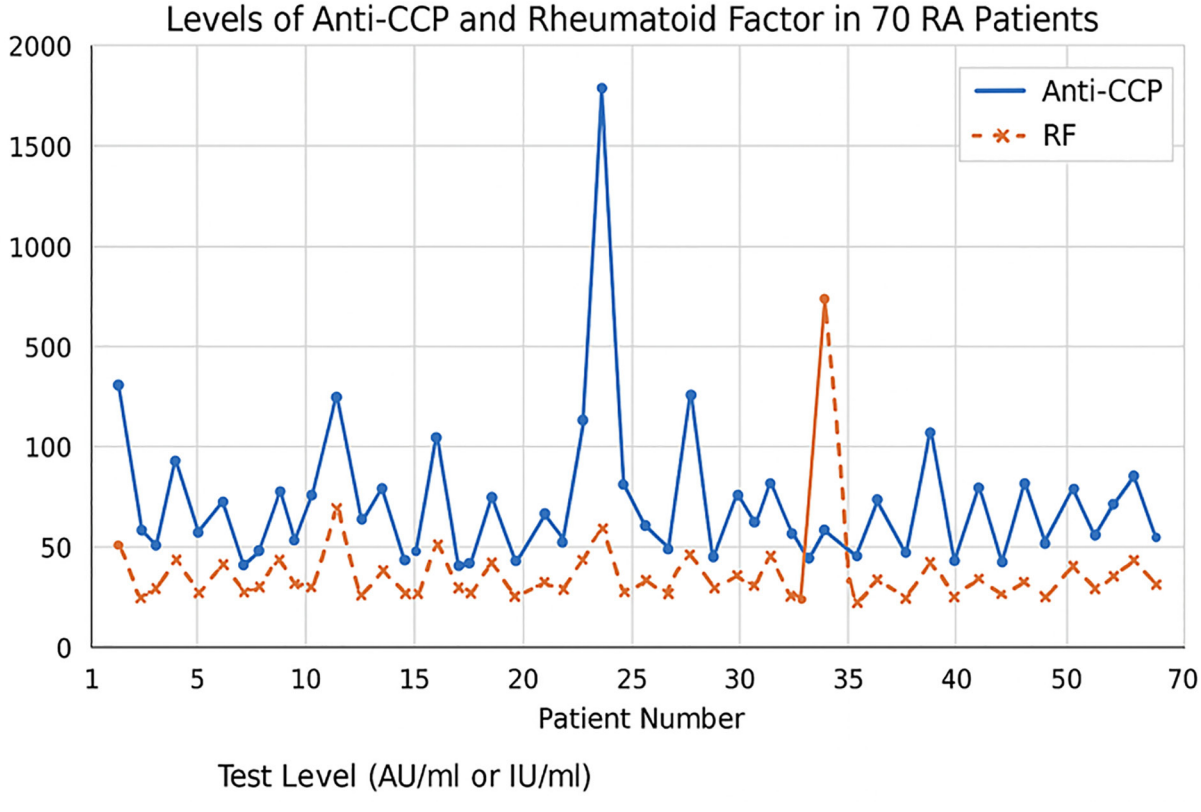


**Fig. 2.** Line chart depicting patient-wise trajectories of anti-CCP and RF values across the study cohort.

Conversely, sufficient or normal vitamin D levels were more frequently detected in the anti-CCP–negative individuals. The association between anti-CCP seropositivity and vitamin D deficiency was statistically significant ($p$=0.044) (Table 6). A supplementary graphical analysis of vitamin D status is presented in Supplementary Fig. S3.

**Table 5. Association Between anti-CCP Antibodies and Familial RA Predisposition**

| Family History | anti-CCP Positive n (%) | anti-CCP Negative n (%) | *p*-value |
|---|---|---|---|
| Yes | 41(74.5%) | 4(26.7%) | <0.01 |
| No | 14 (25.5%) | 11(73.3%) | |

**Table 6. Association Between anti-CCP Serostatus and Vitamin D Levels**

| Vitamin D Status | anti-CCP Positive n (%) | anti-CCP Negative n (%) | *p*-value |
|---|---|---|---|
| Deficient (<20 ng/mL) | 31 (56.4%) | 3 (20%) | 0.044 |
| Insufficient (20–30 ng/mL) | 14 (25.5%) | 7 (46.7%) | |
| Normal (>30 ng/mL) | 10 (18.1%) | 5 (33.3%) | |

**Table 7. Cross-tabulation of anti-CCP titer categories (low, moderate, high) with Disease Activity levels in RA Patients**

| anti-CCP level | Low activity | Moderate activity | High activity | *p*-value |
|---|---|---|---|---|
| Low | 3 (37.5%) | 4 (50%) | 1 (12.5%) | <0.000001 |
| Moderate | 5 (27.8%) | 10(55.6%) | 3 (16.7%) | |
| High | 1 (3.4%) | 0 (0%) | 28 (96.6%) | |

*Anti-CCP Titer Stratification and Disease Activity*

Stratification of patients by anti-CCP titer (low, moderate, high) revealed a strong association with disease activity ($\chi^2$=37.80, $p$<0.000001). Nearly all patients with high anti-CCP titers (96.6%) exhibited high disease activity, whereas those with low or moderate titers were more frequently classified within the low to moderate disease activity categories. These findings underscore the prognostic utility of anti-CCP titration in assessing RA disease act (Table 7, Fig. 3).

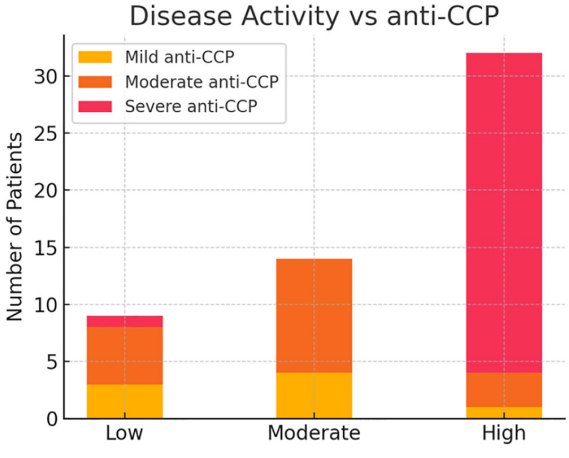


**Fig. 3.** Relationship between anti-CCP antibody titer categories (low, moderate, high) and RA disease-activity level (low, moderate, high).

Numerical distributions are detailed in Tables 1–7 and Figs. 1–3 of the main text. Additional analyses of age- and gender-related variations in biomarker levels and seropositivity, along with supplementary visualizations of age distribution, vitamin D status, and family history, are presented in Supplementary Tables S1–S4 and Supplementary Figs. S1–S3.

## DISCUSSION

In this study, anti-CCP antibodies demonstrated the highest diagnostic performance among the four evaluated markers, consistent with their established role as a reliable biomarker in RA. Compared with RF, CRP, and ESR, anti-CCP showed stronger associations with gender and disease activity, underscoring its diagnostic and prognostic relevance (9-11).

Age-related analysis revealed no significant differences in mean levels of anti-CCP, RF, or

CRP, whereas ESR was significantly higher in older age groups ($p$=0.035), supporting its utility as an indicator of cumulative disease burden. Anti-CCP positivity peaked in patients aged 41–50 years, while RF positivity was more frequent in the 61–70 year group, indicating suggesting possible stage-related differences in biomarker performance (12-14).

Gender-based analysis revealed that anti-CCP positivity was significantly higher in females, consistent with global evidence reporting female predominance in RA. This pattern has been attributed to hormonal influences, particularly estrogen-mediated B-cell activation, as well as genetic predisposition. RF levels tended to be higher in males, although the difference was not statistically significant, suggesting that sex-specific immunological variations may preferentially modulate autoantibody responses such as anti-CCP (15-18).

In our cohort, anti-CCP positivity (78.6%) exceeded RF positivity (64.3%), aligning with findings from Egyptian and Tunisian cohorts, falling within the range reported in Asia (80–85%), and surpassing rates observed in Latin America and Europe. This intermediate prevalence underscores the regional diagnostic value of anti-CCP and reinforces its reliability in North African populations (19-22).

Anti-CCP positivity also demonstrated stronger and more consistent correlations with clinical and laboratory features compared to RF. Both antibodies were associated with key manifestations such as morning stiffness and joint swelling; however, only anti-CCP correlated significantly with tender joint count, reinforcing its role as a marker of inflammatory burden (23-28). Anti-CCP also showed an association with disease duration, suggesting potential utility in early diagnosis and treatment monitoring, whereas RF appeared more age-dependent. Laboratory correlations further supported these findings: anti-CCP was significantly associated with CRP, ESR, NLR, and platelet count, but not with total WBC counts, indicating that leukocyte levels may not adequately capture autoimmune activity. Mild elevation in MCV among anti-CCP–positive patients may reflect treatment-related hematologic changes. Additional associations were observed with smoking and type 1 diabetes mellitus, highlighting the interplay between environmental exposures and autoimmune predisposition (29-33). A preliminary link between anti-CCP positivity and prior COVID-19 vaccination was also noted, though interpretation remains limited by small subgroup size and lack of detailed vaccine data (34-38).

Although RF and anti-CCP levels demonstrated a moderate positive correlation, this relationship was not statistically significant, suggesting partial overlap but also distinct immunopathological pathways. Accordingly, anti-CCP provides added diagnostic value in patient phenotypes where RF may be less informative (39).

Family history of RA was significantly associated with anti-CCP positivity, consistent with international evidence highlighting the genetic contribution to autoantibody-positive RA, particularly among first-degree relatives. Confirmation of this relationship in a Libyan cohort reinforces the role of hereditary predisposition in RA pathogenesis (40, 41).

Vitamin D deficiency was significantly associated with anti-CCP positivity, suggesting that insufficient vitamin D may contribute to autoantibody production through impaired immune regulation. Although potential confounders such as supplementation status, sun exposure, and seasonal variation were not controlled for, this association raises the possibility that vitamin D deficiency represents a modifiable risk factor in RA. Further investigation is warranted to clarify the mechanistic basis and clinical implications of this relationship (42-44).

Finally, patients with higher anti-CCP titers were more likely to present with high disease activity, consistent with international reports linking elevated titers to persistent inflammation and radiographic progression.

However, the cross-sectional design of this study precludes causal interpretation, and longitudinal investigations are required to establish the prognostic significance of anti-CCP levels (45, 46).

Taken together, our findings confirm anti-CCP as a clinically informative biomarker in Libyan patients with RA, with diagnostic performance consistent with global evidence. Importantly, this represents the first study to provide detailed serological correlations in a North African cohort, thereby contributing region-specific insights. While many associations—such as those with family history, vitamin D deficiency, smoking, and comorbidities— mirror international data, their confirmation in this population adds valuable contextual relevance. Routine incorporationof anti-CCP testing into diagnostic protocols is recommended, particularly in early or seronegative RA, with future multicenter longitudinal studies required to validate and extend these observations.

*Study Limitations*

Several limitations of this study should be acknowledged. First, the sample size was relatively small and derived through convenience sampling, which may restrict statistical power and limit generalizability. Second, no formal sample size calculation or power analysis was conducted, reduceing confidence in the robustness of the observed associations. Third, the cross-sectional design precludes causal inference, particularly for associations involving vitamin D deficiency, smoking, COVID-19 vaccination, and anti-CCP positivity. Fourth, potential confounders—including seasonality, vitamin D supplementation, and detailed smoking exposure—were not assessed and may have influenced the findings. Finally, subgroup analyses (e.g., vaccination status, comorbidities) were based on very small numbers and should be interpreted as exploratory. Despite these limitations, the study provides novel region-specific data from a North African cohort, addressing a gap in the literature and supporting the clinical utility of anti-CCP testing in RA diagnosis and patient stratification.

## CONCLUSION AND RECOMMENDATION

In this Libyan cohort, anti-CCP antibodies demonstrated superior diagnostic utility and stronger associations with clinical and laboratory parameters compared to RF. Anti-CCP positivity was significantly linked with female gender, family history of RA, disease duration, inflammatory markers, vitamin D deficiency, smoking, and comorbidities such as type 1 diabetes. While these associations are well-documented internationally, their confirmation in a North African population provides valuable region-specific evidence. Given the cross-sectional design and limited sample size, these findings should be interpreted with caution; nevertheless, they underscore the importance of incorporating anti-CCP testing into RA diagnostic protocols in Libya.

Routine incorporation of anti-CCP testing is recommended, particularly in early or seronegative RA, to enhance diagnostic accuracy and improve patient stratification. Larger, multicenter longitudinal studies are needed to validate these findings, explore their prognostic implications, and clarify the influence of modifiable risk factors such as smoking and vitamin D deficiency within North African populations.

## AUTHORS' CONTRIBUTION

The sole author was responsible for the study conception, data collection and analysis, interpretation of findings, and manuscript preparation.

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Not applicable.

## CONFLICTS OF INTEREST

The author declares no conflict of interest.

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