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Comprehensive Assessment of Immunological (Anti-CCP, TNF-α, and ADA) and Biochemical (ESR and CRP) Biomarkers in Correlation with Disease Activity Scores in Rheumatoid Arthritis Patients from Kirkuk City

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Abstract

Rheumatoid arthritis (RA) is a persistent autoimmune disorder marked by joint inflammation and permanent damage. This study examined the clinical relevance of immunological, biochemical, and demographic biomarkers—Anti-Cyclic Citrullinated Peptide (anti-CCP), Tumor Necrosis Factor-alpha (TNF-α), Adenosine Deaminase (ADA), Erythrocyte Sedimentation Rate (ESR), C-reactive Protein (CRP), and Gender—in connection with disease activity evaluated by the Disease Activity Score 28 (DAS28). This case-control study, conducted in Kirkuk City, Iraq, comprised 90 rheumatoid arthritis patients categorized into untreated and treated groups, alongside 45 healthy controls matched for age and gender. The results indicated substantial post-treatment decreases in anti-CCP, TNF-α, ADA, ESR, and CRP levels, signifying therapeutic efficacy. Notwithstanding clinical advancements, anti-CCP and TNF-α levels continued to be higher relative to controls, reflecting ongoing autoimmune activity. ADA normalized post-treatment, indicating restored T-cell functionality. ESR and CRP shown strong responses to treatment, corresponding effectively with clinical results. Gender analysis indicated substantial disparities in biomarker profiles, highlighting hormonal influences on illness development. association tests revealed a robust negative association between pre-treatment TNF-α and DAS28 ($r = -0.729$, $p < 0.001$), which dissipated post-treatment, while anti-CCP exhibited a moderate correlation post-treatment ($r = -0.454$, $p < 0.001$). ADA demonstrated a low association, signifying its negligible contribution to the evaluation of joint-specific activity. These findings confirm the critical importance of these biomarkers in assessing disease activity, determining treatment effectiveness, and customizing personalized RA management approaches. Consistent assessment of these markers may enhance clinical results in rheumatoid arthritis patients.

Keywords: Rheumatoid arthritis, Anti-CCP, TNF-α, ADA, ESR, CRP, Gender, DAS28, Biomarkers.

INTRODUCTION

Rheumatoid arthritis (RA) is a prevalent chronic inflammatory disease characterized by symmetrical joint inflammation, bone erosion, and cartilage deterioration, significantly affecting life quality and functionality [(1)]. Approximately 1% of the global population suffers from RA, with a higher prevalence among women [(2)]. Timely diagnosis and effective therapeutic interventions are critical to preventing irreversible joint damage

[3,4]. Anti-cyclic citrullinated peptide antibodies (anti-CCP), Tumor Necrosis Factor-alpha (TNF-α), Adenosine Deaminase (ADA), Erythrocyte Sedimentation Rate (ESR), C-reactive Protein (CRP), and Gender have emerged as significant biomarkers in assessing disease activity and therapeutic responses in RA patients. [5-7]

Materials and Methods:

This case-control study, sanctioned by Tikrit University College of Medicine, was executed at Kirkuk Teaching Hospital and private clinics from December 2024 to April 2025. The study cohort consisted of three groups: untreated RA patients (n=45), treated RA patients (n=45), and healthy controls (n=45), all matched for age and gender. Blood samples were examined for anti-CCP, TNF-α, ADA, CRP, ESR, rheumatoid factor (RF), hemoglobin (Hb), and body mass index (BMI). ELISA was utilized to quantify biomarkers, and statistical analyses were conducted to evaluate associations with DAS28.

Results:

The demographic data indicated a notable female majority (62%) among RA patients and a higher average BMI relative to controls ($p<0.001$). Rituximab was recognized as the primary treatment (48.9%) (table 1).

Table (1): Demographic and Clinical Characteristics of the Study

Characteristic	RA Group	Control Group	p-value
Total Participants	45	45	
Gender (Male)	17	17	< 0.05*
Gender (Female)	28	28	< 0.05*
Mean Age (Years)	42	42	> 0.05
Mean BMI (kg/m²)	26.2	21.56	< 0.05*
Mean RA Duration (Years)	3.64	-	-
Family History (Positive)	17	21	< 0.05*
Family History (Negative)	28.0	24	< 0.05*
Drugs types			
Drug types	No.	Percentage	
RTX	22	48.9%	
NSAID	10	22.2%	
Simponie	7	15.6%	
Remical	5	11.1%	
Rnfluximumb	1	2.2%	

*p-values < 0.05 indicate statistically significant differences between groups.

Immunological markers demonstrated substantial post-treatment decreases: anti-CCP ($p<0.0001$), TNF-α ($p<0.0001$), and ADA ($p<0.0001$). Nonetheless, anti-CCP and TNF-α levels persisted at increased levels relative to controls, signifying ongoing autoimmune and inflammatory processes despite clinical amelioration. ADA normalized with treatment, indicating effective regulation of T-cell activation. Both ESR and CRP exhibited a considerable reduction during treatment, corresponding with clinical enhancements (table2).

Table (2): Paired Sample Comparison of Immunological and Markers Before and After Treatment in Rheumatoid Arthritis Patients (n = 45).

RA associated parameters			**No.**	**Mean**	**Std. Deviation ±**	**P-Value**
Anti-CCP	before		45	531.2	91.4	< 0.0001**
	after		45	273.4	93.5	
	before		45	531.2	163.0	< 0.0001**
	control		45	91.2	11.3	
	after		45	273.4	93.5	< 0.0001**
	control		45	91.2	11.3	
TNF-α	before		45	849.0	24.0	< 0.0001**
	after		45	120.4	8.0	
	before		45	849.0	24.8	< 0.0001**
	control		45	64.3	7.3	
	after		45	120.4	8.0	< 0.0001**
	control		45	64.3	7.3	
Adenosine Deaminase	before		45	14.9	7.5	< 0.0001**
	after		45	1.6	1.3	
	before		45	14.9	7.5	< 0.0001**
	control		45	1.4	0.3	
	after		45	1.6	1.3	> 0.05

	control	45	1.3	0.3	

*Significant at level ($p < 0.05$)، ** Highly significant, no Significance at level ($p>0.05$). TNF-α: Tumor Necrosis Factor, Anti-CCP: Anti-Cyclic Citrullinated Peptide.

association analysis demonstrated a robust negative association between pre-treatment TNF-α levels and DAS28 ($r=-0.729$, $p<0.001$), which was absent post-treatment. Anti-CCP had a moderate negative association with DAS28 post-treatment ($r=-0.454$, $p<0.001$), indicating its effectiveness in assessing long-term immune responses. ADA exhibited no significant correlation with DAS28, highlighting its restricted utility in joint-specific clinical evaluations. The analysis of gender revealed significant disparities in biomarker levels, highlighting hormonal influences on the etiology of rheumatoid arthritis (table 3).

Table (3): Correlation of TNF-α, Anti-CCP and ADA with DAS28

Variable Pair		Correlation Coefficient (r)	p-value	Significance
DAS28 TNF α	before	-0.729	< 0.001	Significant
DAS 28 TNF α	after	-0.014	0.9281	Not Significant
DAS 28 Anti-CCP	before	-0.135	0.3783	Not Significant
DAS28 Anti-CCP	after	-0.454	< 0.001	Significant
DAS28 ADA	before	-0.13	0.3961	Not Significant
DAS 28 ADA	after	-0.156	0.3076	Not Significant

DISCUSSION:

This study's findings demonstrate substantial therapeutic effects and emphasize the clinical importance of immunological and biochemical indicators in the treatment of rheumatoid arthritis (RA). Significantly, biomarkers including anti-CCP, TNF-α, ADA, ESR, and CRP demonstrated substantial alterations post-treatment, underscoring their relevance in evaluating therapy effectiveness and monitoring disease activity.

Anti-CCP, a highly specific biomarker for rheumatoid arthritis, markedly diminished following treatment, confirming its use in assessing autoimmune activity. The continued elevation after treatment relative to controls underscores persistent subclinical autoimmune activity, indicating the need for continuous therapeutic measures and regular monitoring to prevent long-term joint injury. [8]

The significant decrease in TNF-α levels following treatment corresponds with the established pro-inflammatory function of this cytokine in the pathophysiology of rheumatoid arthritis. The significant negative connection between pre-treatment TNF-α and DAS28 further substantiates TNF-α as a vital marker of disease severity before intervention. The lack of this correlation after treatment indicates that medication substantially reduces TNF-mediated inflammation, hence lessening its association with clinical symptoms, in agreement with other recent investigations. [9].

Notably, ADA levels returned to normal following therapy, indicating successful regulation of immunological responses and restoration of T-cell functionality. ADA's restricted connection with DAS28 highlights its wider immunological function rather than its specificity to joint inflammation. This discovery aligns with prior research indicating that ADA serves as a marker of overall immune dysregulation rather than a direct indicator of joint inflammation. [10]

Biochemical indicators ESR and CRP exhibited significant reductions post-treatment, closely corresponding with clinical enhancements evaluated by DAS28. Their responsiveness to medication substantiates their role as acute-phase reactants for assessing RA disease activity and therapeutic effectiveness, validating previous research findings. [11]

This study highlights gender-related differences that underscore the impact of hormonal and genetic variables on the development and course of rheumatoid arthritis (RA). The increased incidence and biomarker variability in females align with global epidemiological data, indicating possible hormonal influences on autoimmune mechanisms. [12].

The correlation between elevated BMI and increased inflammatory markers highlights obesity's exacerbating role in RA inflammation and clinical outcomes. Addressing obesity through targeted interventions is therefore crucial for comprehensive RA management, aligning with findings suggesting that lifestyle interventions can positively influence RA prognosis [13,14].

The study's results support the regular integration of anti-CCP, TNF-α, ESR, and CRP in clinical practice, offering significant insights into persistent inflammatory activity despite clinical remission. Ongoing surveillance and personalized therapeutic strategies informed by these biomarkers may markedly enhance patient outcomes and disease prognosis, highlighting the advancing relevance of precision medicine in rheumatoid arthritis treatment. [15,16,17].

Future longitudinal studies are advised to investigate ADA's extensive clinical importance and to evaluate the long-term consequences of persistent biomarker increases despite evident clinical remission. These investigations will improve comprehension of the fundamental immunopathology in rheumatoid arthritis and guide the development of more effective individualized therapy regimens.

CONCLUSION:

This study underscores the therapeutic significance of incorporating immunological and biochemical biomarkers—specifically anti-CCP, TNF-α, ESR, and CRP—into the standard care of rheumatoid arthritis. These markers provide precise disease monitoring and enable customized therapy methods that target subclinical activity and specific patient profiles. The normalization of ADA and notable correlations between biomarker levels and disease activity endorse their combined efficacy in informing clinical decision-

making. Furthermore, acknowledging the influence of fat and gender on RA progression reinforces the need for a comprehensive, individualized approach to treatment. The evolution of RA care necessitates the integration of precision medicine, guided by comprehensive biomarker analysis, to enhance patient outcomes and ensure sustained disease control.

RECOMMENDATIONS:

1- Healthcare providers should incorporate a combination of anti-CCP, TNF-α, ESR, and CRP in standard RA assessment protocols to improve detection of subclinical inflammation and monitor therapeutic efficacy more precisely.

2- Clinicians are encouraged to customize RA treatment regimens based on patients' biomarker profiles, with particular attention to persistent elevations despite symptomatic improvement, in alignment with precision medicine principles.

3- Given the strong correlation between elevated BMI and inflammatory activity, weight reduction and lifestyle interventions should be considered integral components of RA management programs.

4- Clinical protocols should account for gender-based immunological differences, which may influence disease severity and biomarker expression, to ensure equitable and effective patient care.

5- Further longitudinal and mechanistic studies are warranted to determine the utility of ADA as a biomarker of systemic immune dysregulation, especially in the context of disease remission.

6- Frequent evaluation of biomarker levels—especially anti-CCP and TNF-α—both before and after treatment initiation is vital for anticipating flares and adjusting therapy proactively.

7- Researchers should design long-term cohort studies to evaluate the prognostic value of sustained biomarker changes and their association with radiographic progression and functional outcomes.

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