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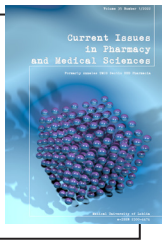

Estimation of the role of growth differentiation factor-15 and atherogenic indices as predictors of disease severity in Iraqi patients with rheumatoid arthritis (Middle Euphrates Region)

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic immune-mediated disease characterized by persistent inflammation, edema, and joint pain. Growth differentiation factor 15 (GDF-15) is a cytokine whose concentration increases in chronic inflammatory states. It has been recognized as both a pro-inflammatory mediator and a potential contributor to the development of cardiovascular disease due to its association with systemic inflammation and metabolic disorders. The aim of this study was to evaluate serum GDF-15 levels and atherogenic indices as predictors of disease activity in patients with RA. A total of 150 RA patients and 150 healthy controls were included. Serum concentrations of GDF-15, rheumatoid factor (RF), C-reactive protein (CRP), and anti-citrullinated protein antibodies (ACPA) were measured using ELISA. Lipid parameters, including total cholesterol, triglycerides (TG), and HDL-C, were assessed spectrophotometrically. Based on the DAS28-CRP calculator, RA patients were classified into high disease activity (HDA; DAS28-CRP > 5.1) and moderate disease activity (MDA) groups. Multivariate general linear model (GLM) analysis and Receiver Operating Characteristic (ROC) curve assessment were performed to identify predictors of RA activity. The results indicated that RA status explained 92.1% of the variance in the measured serum biomarkers. GDF-15 was identified as the most sensitive predictor of elevated disease activity, followed by the atherogenic indices CRI-I, AC, and AIP. Patients in the HDA group demonstrated significantly higher levels of GDF-15, CRP, ESR, and ACPA compared with those in the MDA group. GDF-15 exhibited a strong positive correlation with key inflammatory markers, including ESR and CRP. These findings suggest that GDF-15, CRI-I, AC, and AIP are the most sensitive predictors associated with increased RA activity. The combined use of GDF-15 and atherogenic indices yielded the largest area under the ROC curve, indicating their potential value in assessing RA severity. Moreover, RA patients showed elevated cholesterol levels and adverse atherogenic profiles, placing them at heightened risk for cardiovascular diseases linked to atherosclerosis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by persistent systemic inflammation. The disease initially affects small joints and progressively involves larger joints, resulting in pain and functional impairment. RA may also affect extra-articular organs, including the kidneys, eyes, lungs, skin, and heart [1]. The global prevalence of RA is estimated at approximately 0.45% [2].

Common clinical manifestations of RA include joint swelling, pain, inflammation, fatigue, and stiffness, which may impair joint function and lead to deformities in advanced stages of the disease [3]. RA can substantially

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reduce patients' quality of life due to progressive joint destruction, disability, impaired mobility, and functional limitations [4]. The exact etiology of RA remains unclear [5].

Current evidence suggests that multiple immune cells, including dendritic cells (DCs), monocytes, mast cells, macrophages, B lymphocytes, and T lymphocytes, contribute to inflammatory and immune responses through the secretion of pro-inflammatory mediators, ultimately leading to the degradation of articular cartilage and bone [6].

The hallmark pathological feature of RA is inflammation of the synovial membrane, which leads to progressive joint destruction and deformity [7]. Numerous biological factors involved in RA pathogenesis have been identified, including adhesion molecules, soluble mediators, pro- and anti-inflammatory cytokines [8], trace elements [9], adipokines [10,11], and autoantibodies contributing to internal organ dysfunction, joint inflammation, and structural damage [5].

Several biomarkers have been investigated as potential indicators for disease prognosis and monitoring of RA activity, including growth differentiation factor-15 (GDF-15). GDF-15 belongs to the transforming growth factor-β (TGF-β) cytokine superfamily and is considered a stress-responsive cytokine [12]. Circulating levels of GDF-15 increase with advancing age [13]. Growing evidence suggests that GDF-15 may serve as a clinically useful biomarker in inflammatory and metabolic disorders [14,15].

GDF-15 is produced and released in response to oxidative stress, hypoxia, inflammation, and tissue injury [16]. Under normal physiological conditions, its expression remains low; however, its levels increase with aging and in response to cellular stress across multiple organs and tissues [17]. Due to its widespread upregulation under stress conditions, GDF-15 is considered an important regulator of inflammatory responses with potential anti-inflammatory properties [18,19].

Elevated expression of GDF-15 has been reported in cardiomyocytes, vascular smooth muscle cells, macrophages, adipocytes, and endothelial cells under conditions such as oxidative stress, inflammation, tissue injury, and malignancy [20]. Increased GDF-15 levels have also been detected in macrophages within human atherosclerotic plaques [21]. Circulating GDF-15 concentrations typically range from approximately 200 to 1200 pg/ml and tend to increase with age [12,22].

The most commonly used inflammatory biomarkers for the diagnosis and monitoring of rheumatoid arthritis (RA) activity include C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF) [23]. Elevated levels of these inflammatory markers are associated not only with disease activity but also with an increased risk of comorbid conditions, including cardiovascular disease, atherosclerosis, and anemia [24,25]. Accelerated atherosclerosis, characterized by increased plaque burden, plaque instability, and enhanced thrombogenesis, represents a major contributor to the early development of cardiovascular disease in patients with RA [26,27]. Furthermore, patients with RA frequently develop dyslipidemia during different stages of the disease, as chronic systemic inflammation may significantly alter lipid metabolism and serum lipid profiles [28].

METHODS

Study Design and Subjects

This case-control study included Iraqi patients with rheumatoid arthritis (RA) attending the rheumatology department at Marjan Teaching Hospital in Babylon Province, Iraq. A total of 150 patients with RA (29 men and 121 women) and 150 age-matched healthy controls (31 men and 119 women) were enrolled. The study was conducted between November 2022 and July 2023. Participants ranged in age from 20 to 79 years.

RA diagnosis was established according to the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria. Patients were diagnosed with RA when a total score ≥6 was achieved, based on the number and distribution of affected joints, serological positivity for anti-citrullinated protein antibodies and rheumatoid factor, elevated levels of C-reactive protein and erythrocyte sedimentation rate, and symptom duration. These criteria have demonstrated improved diagnostic accuracy and specificity for predicting the probability of RA [29].

Sample selection and collection

Demographic and clinical data were collected through direct interviews using a structured questionnaire. The recorded variables included age, disease duration, sex, residency, body weight (kg), height (cm), family history, and type of treatment received. In addition, all patients underwent clinical evaluation and physical examination performed by a rheumatologist to assess disease status.

Inclusion criteria

Participants were eligible for inclusion if they fulfilled the diagnostic criteria for rheumatoid arthritis (RA). RA diagnosis was established according to the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria. Patients achieving a total score of ≥6 based on joint involvement, serological findings, acute-phase reactants, and symptom duration were included in the study.

Exclusion criteria

Participants were excluded if they were smokers or alcohol consumers or had a history of diabetes mellitus, hypertension, hyperthyroidism, or liver disease. Individuals with obesity (body mass index [BMI] >30 kg/m²) were also excluded. In addition, subjects receiving lipid-lowering drugs, beta-blockers, thyroxine, vitamin E, estrogen, or progestin therapy were not eligible for inclusion. Patients with malignancies, acute or chronic infections, severe cardiovascular or renal disease, autoimmune disorders other than rheumatoid arthritis, as well as pregnant or breastfeeding women, were excluded. Patients receiving biological therapy were also excluded from the study.

Measurements

After an overnight fast of 12-14 hours, 5 ml of venous blood was collected from both patients and control subjects using sterile disposable syringes. Blood samples were

allowed to clot at room temperature for 15 minutes and were then centrifuged at 1500 × g for 10 minutes. Serum samples were transferred into Eppendorf tubes and divided into three equal aliquots, which were stored at -20°C until analysis.

Approximately 2 ml of fasting venous blood was collected into sterile EDTA tubes for erythrocyte sedimentation rate (ESR) measurement. ESR was determined using the Westergren method [30]. For this purpose, blood samples were mixed with sodium citrate as an anticoagulant and transferred into Westergren tubes up to the 200-mm diameter mark.

Serum concentrations of growth differentiation factor-15 (GDF-15), C-reactive protein (CRP), and anti-citrullinated protein antibodies (ACPA) were measured using enzyme-linked immunosorbent assay (ELISA) kits supplied by Elabscience® Biotechnology Inc. (USA). Rheumatoid factor (RF) levels were measured using a commercial kit supplied by Bioassay Technology Laboratory (BTLAB, China). The inter-assay coefficient of variation (CV) was less than 10%.

Clinical characteristics and sociodemographic data were collected from all study participants. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2) [31-33].

Disease activity was assessed using the Disease Activity Score in 28 joints based on C-reactive protein (DAS28-CRP), calculated using an online calculator available at MDCalc (https://www.mdcalc.com/disease-activity-score-28-rheumatoid-arthritis-crp-das28-crp). According to DAS28-CRP results, patients were classified as having high disease activity (HDA) when DAS28-CRP >5.1 or moderate disease activity (MDA) when DAS28-CRP ≤5.1.

Eligible patients had a disease duration of more than six months, a Visual Analogue Scale (VAS) score >6, positive serological results for anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF), elevated C-reactive protein (CRP) levels (>6 mg/L), and an erythrocyte sedimentation rate (ESR) >20 mm/hour. These criteria demonstrate improved precision and specificity for predicting rheumatoid arthritis [29].

Current treatment regimens included corticosteroids (prednisolone) and disease-modifying antirheumatic drugs (DMARDs), including methotrexate, sulfasalazine, and hydroxychloroquine (HCQ). Some patients also received folic acid supplementation.

The procedures were followed exactly as instructed by the manufacturer. Serum lipid profiles were estimated using commercial analytical kits from BIOLABO SAS (02160, Miazy, France). Enzymatic reactions were used to assess total cholesterol (TC), triglycerides (TG), and HDL-C. HDL-C was estimated using spectrophotometric kits [34-36]. The Friedewald equations were used to estimate VLDL-C and LDL-C levels [37-39].

We used these serum lipid measurements to calculate the atherogenic indexes, which measure cardiovascular risk. The formulas used to calculate the atherogenic indexes in our study are [15,40]:

1. Atherogenic Coefficient (AC): (TC – HDL-C)/HDL-C [41]
2. Atherogenic Index of Plasma (AIP): log10(TG/HDL-C) [42,43]
3. Castelli's Risk Index-I (CRI-I): TC/HDL-C [44]
4. Castelli's Risk Index-II (CRI-II): LDL-C/HDL-C [44].

Statistical analysis

Version 26 of the SPSS software was used to analyze the data. The analysis divided the variables into two groups based on the statistical distribution: normally distributed variables and nonparametric variables, as identified by the results of the Kolmogorov-Smirnov test. The results were given as mean ± SD for a normal distribution. The pooled t-test was used to compare the patient and control groups. The nonparametric variable values are displayed as 25th and 75th percentile medians. The Mann-Whitney U-test was used to compare the patient and control groups. The Kruskal-Wallis test was used to compare three groups based on activity: patients with severe RA, patients with mild RA, and healthy controls. Stepwise multiple regression analysis was used to compare RA activity (DAS-CRP) with moderate and high activity and with healthy controls. A multivariate general linear model (GLM) was used to examine the relationships between the identified biomarkers and RA activity. This model accounts for confounding variables, such as age, BMI, and sex. A between-subject effects test was used to evaluate the impact of each parameter on RA activity. The study used the partial eta-squared (η^2) effect size. We investigated the possibility of using parameters and their ratios as RA diagnostic tools using the Receiver Operating Characteristic (ROC) curve. ROC analysis was used to investigate the capacity of the measured biomarkers for diagnosis. Youden's J statistic, cut-off values, sensitivities, specificities, and the area under the curve (AUC) were computed. When $p < 0.05$, the difference among groups is considered statistically significant.

Ethical approval

Before the start of the research project, informed consent was provided by all patients or their first-degree relatives. The project received ethical approval from the Institutional Review Board (IRB) of the College of Science at the University of Kufa in Iraq (Document No. 8215/2022) and the Training and Human Development Center of the Babylon Health Directorate in Babil, Iraq (Document No. 1502/2023). The study was carried out in accordance with Iraqi, international, and privacy regulations, as well as the Declaration of Helsinki of the World Medical Association.

RESULTS

Role of GDF-15 and atherogenic indices as predictors of RA activity

Table 1 presents the results of the comparison between severe disease activity, moderate disease activity, and the control group.

Table 1 shows no significant differences in age, height, weight, BMI, sex ratio, marital status ratio, or residency between the severe disease activity group, the moderate disease activity group, and the healthy group (all $p < 0.05$). The results indicate that serum C-reactive protein (CRP) levels were substantially higher in high disease activity (HDA) patients compared to moderate disease activity (MDA) patients and the control group, with the highest levels in the severe activity group. Serum HDL-C levels

decreased significantly in both patient groups compared to the control group. There was no significant difference between the two patient groups. There was, however, no substantial difference in disease duration between RA patient groups. HDA patients had higher levels of DAS28-CRP, CRP, ACPA, and GDF-15 than the MDA group. A significant disparity was found in DAS28-CRP levels between the severe and moderate RA patient groups. A significantly higher proportion of patients with HDA took folic acid, methotrexate (MTX), prednisolone, and hydroxychloroquine (HCQ) than the MDA group. However, there were no significant differences in sulfasalazine intake or duration of illness between the two groups. Additionally, patients with HDA had more family history records than the MDA group. There was a significant difference in DAS-CRP levels between the severe and moderate RA patient groups.

The medications administered to the two RA patient groups (moderate and severe) were significantly different (family history, folic acid, methotrexate, prednisolone, and hydroxychloroquine). However, the medications administered to the two RA patient groups (moderate and severe) were not significantly different with respect to sulfasalazine and duration of illness.

Table 1. Comparison of RA patient groups according to disease activity: RA patients with high disease activity (HDA), RA patients with moderate disease activity (MDA), and the control group

Variables	Control [A] (N=150)	Moderate [B] (N=69)	Severe [C] (N=81)	F/χ²	p
Age (years)	44.39±9.99	44.33±9.90	46.88±10.54	1.807	0.166
BMI (kg/m²)	27.59±5.17	28.16±3.44	28.22±3.61	0.743	0.477
Sex (M/F)	31/119	11/58	18/63	1.002	0.606
Married/Single	142/8	66/3	79/2	1.041	0.594
Rural/Urban	27/23	15/54	17/64	0.541	0.763
ESR (mm/h)	10 (9-12) [B,C]	39 (22-50) [A]	30 (20-42) [A]	KWT	<0.001
CRP (mg/dL)	3.33 [B,C] (1.38-4.79) [B,C]	11.39 [A,C] (10.25-12.28)	5.04 [A,B] (3.77-10.32)	KWT	<0.001
DAS-CRP	-	5.30±0.55	3.57±0.85	291.386	<0.001
RF (U/ml)	3.33 [B,C] (3.08-3.79)	27.18 [A] (9.14-34.28)	27.57 [A] (11.03-34.12)	KWT	<0.001
ACPA (IU/ml)	12.34 [B,C] (10.41-13.63)	214.73 [A] (33.12-293.89)	158.55 [A] (24.22-294.81)	KWT	<0.001
GDF-15 (pg/ml)	64.40 [B,C] (47.56-85.47)	312.71 [A] (222.31-375.58)	305.11 [A] (216.70-382.76)	KWT	<0.001
Total cholesterol (TC, mM)	4.46±0.64 [B,C]	5.15±1.09 [A]	5.22±0.96 [A]	27.068	<0.001
Triglycerides (TG, mM)	1.15±0.29 [B,C]	1.64±0.51 [A]	1.68±0.47 [A]	61.855	<0.001
HDL-C (mM)	1.21±0.18 [B,C]	0.89±0.15 [A]	0.93±0.15 [A]	133.073	<0.001
VLDL-C (mM)	0.53±0.13 [B,C]	0.75±0.23 [A]	0.77±0.21 [A]	61.855	<0.001
LDL-C (mM)	2.73±0.64 [B,C]	3.50±1.07 [A]	3.56±0.99 [A]	33.135	<0.001
CRI-I	3.78±0.82 B,C	5.92±1.68 [A]	6.05±1.70 [A]	104.543	<0.001
CRI-II	2.33±0.77 [B,C]	4.06±1.55 [A]	4.16±1.59 [A]	77.521	<0.001
AIP	-0.01 [B,C] (-0.06-0.04)	0.24 [A] (0.14-0.34)	0.26 [A] (0.17-0.36)	KWT	<0.001
AC	2.78±0.82 [B,C]	4.92±1.68 [A]	5.05±1.70 [A]	104.543	<0.001
Folic acid (Yes/No)	-	62/7	73/8	245.456	<0.001
Methotrexate (Yes/No)	-	62/7	73/8	245.456	<0.001
Sulfasalazine (Yes/No)	-	1/68	1/80	2.039	0.361
Prednisolone (Yes/No)	-	16/53	9/72	134.387	<0.001
HCQ (Yes/No)	-	24/45	20/61	54.594	<0.001
Family history (Yes/No)	-	57/12	62/19	197.810	<0.001
Disease duration (yrs)	-	6 (2-9)	6 (3-9)	MWUT	0.387

A, B, C: Pairwise comparisons between groups
Data are presented as mean ± SD for normally distributed variables and as median (interquartile range 25%-75%) for non-normally distributed variables
The binomial data were expressed as ratios and compared using a Chi² test
F/χ² - The F-statistic value is used for continuous variables, while the Chi² statistic value is used for categorical variables
P-values indicate the probability
HCQ - Hydroxychloroquine; KWT - Kruskal-Wallis test; MWUT - Mann-Whitney U test; AC - Atherogenic Coefficient; AIP - Atherogenic Index of Plasma; CRI-I/CRI-II - Castelli's Risk Indices I & II

Receiver Operating Characteristic (ROC) analysis for the diagnosis of RA Severity using atherogenic indices and GDF-15

An ROC analysis was performed to evaluate the diagnostic sensitivity and specificity of atherogenic indices and GDF-15 in identifying RA severity. Figure 1 displays the ROC curves generated from the analysis. Table 2 shows the concentration cutoff and coordinates of the ROC values that yield the highest specificity and sensitivity.

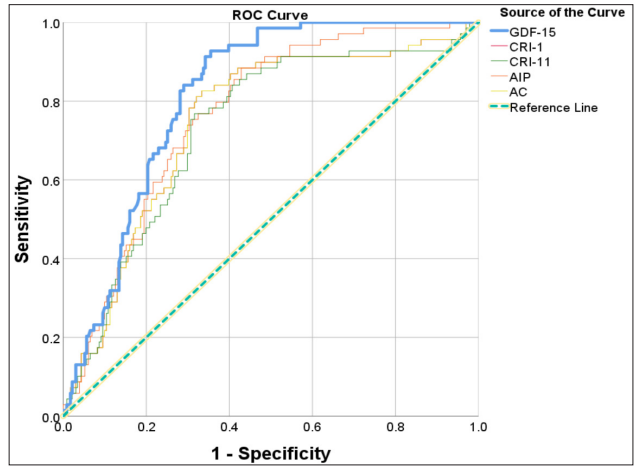

Figure 1. ROC curves using atherogenic indices and GDF-15 to diagnose RA severity show predictors with the largest area under the curve relative to the reference line

Based on an increase in GDF-15 over the cut-off value of 223.275 pg/ml, Table 2 shows that the subjects may be RA patients with respective sensitivities and specificities of 73.9% and 74.0%, respectively. The curve encompasses a high Youden's J statistic and a high area under the curve (AUC) of 0.815.

Table 2 suggests that participants may be RA patients if their CRI-I increases beyond the cutoff value of 5.023. This corresponds to 71.0% sensitivity and 70.1% specificity. The curve encompasses a high Youden's J statistic and a high AUC (0.746). An AIP above the cutoff value of 0.168 suggests that subjects may be RA patients, with respective sensitivities and specificities of 71.0%. The curve encompasses a high Youden's J statistic and a high AUC (0.767). A CRI-II greater than the cutoff value of 3.222 suggests that some subjects may be RA patients, with respective sensitivities and specificities of 69.6% and 69.3%. The curve encompasses a high Youden's J statistic and a high AUC (0.732). Respondents may be identified as RA patients if their AIP value exceeds the cutoff point of 4.023, with a sensitivity of 71.0% and a specificity of 70.0%. The curve encompasses a high Youden's J statistic and a high AUC (0.746).

Table 2. Receiver Operating Characteristic (ROC) analysis of GDF-15 and atherogenic indices for predicting RA severity

Variables	Cut-off	Sensitivity (%)	Specificity (%)	Youden's J statistic	AUC (95% CI)	p
GDF-15 (pg/ml)	223.275	73.9	74.0	0.479	0.815 (0.768-0.862)	<0.001
CRI-I	5.023	71.0	70.1	0.411	0.746 (0.681-0.811)	<0.001
CRI-II	3.222	69.6	69.3	0.389	0.732 (0.665-0.799)	<0.001
AIP	0.168	71.0	71.0	0.420	0.767 (0.709-0.824)	<0.001
AC	4.023	71.0	70.1	0.411	0.746 (0.681-0.811)	<0.001

AUC - area under the curve, CI - Confidence interval; GDF-15 - Growth differentiation factor-15; AC - Atherogenic Coefficient; AIP - Atherogenic Index of Plasma; CR-I, CR-II - Castelli's Risk Indexes

Multivariate Generalized Linear Model study for the effect of confounders on the measured biomarkers

Table 3 presents the results of the multivariate generalized linear model (GLM) analysis assessing the impact of the confounders on the measured biomarkers.

Table 3. Multivariate analysis and the effect of confounders on measured parameters

Type of test	Dependent variables	Explanatory variables	F	p	Partial η^2
Multivariate	ESR, DAS-CRP, CRP, RF, ACPA, GDF-15, IL-6, TC, TG, HDL-C, VLDL-C, LDL-C, CRI-I, CRI-II, AIP, AC	Diagnosis	251.177	<0.001	0.921
		Sex	1.949	0.025	0.082
		Age	8.911	<0.001	0.291
		BMI	1.013	0.438	0.045
Tests of Between-Subjects Effects	Diagnosis	DAS-CRP	2264.649	<0.001	0.885
		RF	403.153	<0.001	0.578
		GDF-15	341.268	<0.001	0.537
		ESR	328.504	<0.001	0.528
		AIP	290.191	<0.001	0.497
		HDL-C	279.736	<0.001	0.488
		AC	205.693	<0.001	0.412
		CRI-I	205.693	<0.001	0.412
		CRP	190.757	<0.001	0.394
		ACPA	204.512	<0.001	0.410
		CRI-II	153.689	<0.001	0.343
		TG	121.971	<0.001	0.293
		VLDL-C	121.971	<0.001	0.293
		LDL-C	66.454	<0.001	0.184
		TC	53.630	<0.001	0.154
	Age	AIP	44.039	<0.001	0.130
		TG	16.133	<0.001	0.052
		VLDL-C	16.133	<0.001	0.052
		HDL-C	15.424	<0.001	0.050
		DAS-CRP	8.571	0.004	0.028
		ESR	3.839	0.050	0.016
	Sex	CRP	6.401	0.012	0.021
		TG	6.052	0.014	0.020
		VLDL-C	6.052	0.014	0.020

We evaluated the impact of RA disease diagnosis using GLM analysis, accounting for confounding variables such as age, BMI, and sex.

The findings revealed that the diagnosis significantly impacted the results ($F = 251.177$, $p < 0.001$) and that RA disease accounted for 92.1% of the variance in blood levels of the studied biomarkers (partial $\eta^2 = 0.921$, $p < 0.001$). Other confounders had no significant effect ($p > 0.05$) on parameter levels, and the impact of each confounder (partial η^2) was low. These results suggest that being a patient is the primary factor contributing to elevated serum levels of the parameters. In the second section of the table, we performed between-subject impact tests to determine which biomarkers are impacted by the diagnosis. Thus, we performed an inter-subject effects analysis to determine the connection between each biomarker and the diagnosis.

The between-subjects effects tests showed that the severity of RA (DAS-CRP) has a highly significant impact on the parameter levels (partial $\eta^2 = 0.885\%$, $p < 0.001$). DAS-CRP level is the most affected factor by the presence of RA, with 88.5% of DAS-CRP variance due to the diagnosis. The other parameters significantly affected by RA after removing the confounding factors ($p < 0.001$) were GDF-15 (partial $\eta^2 = 53.7\%$, $p < 0.001$, ESR (partial η^2=52.8%, p<0.001), ACPA (partial η^2=41.0%, p<0.001), RF (partial η^2=57.8%, p<0.001), CRP (partial η^2= 39.4%, p<0.001), AIP (partial η^2=49.7%, p<0.001), HDL-C (partial η^2=48.8%, p<0.001), AC (partial η^2=41.2%, p<0.001), CRI-I (partial η^2=41.2%, p<0.001), IL-6 (partial η^2=34.8%, p<0.001), CRI-II (partial η^2=34.3%, p<0.001), TG (partial η^2=29.3%, p<0.001,), VLDL-C (partial η^2=29.3%, p<0.001), LDL-C (partial η^2=18.4%, p<0.001), and TC (partial η^2=15.4%, p<0.001).

As seen in Table 3, other parameters were not affected by the presence of RA after the effects of the confounders were removed. A second test of between-subjects effects revealed that age significantly affected AIP levels (partial $\eta^2 = 0.130$, $p < 0.001$), while other biomarkers were minimally affected by age (partial $\eta^2 < 0.06$). Sex had a very small effect on the levels of affected biomarkers (partial $\eta^2 < 0.03$).

Other parameters were not affected by the presence of RA after the confounders were removed, as seen in Table 3. Changes in biomarker levels are significantly due to the presence of the disease. The highest impact was on DAS-CRP, followed by RF, GDF-15, and ESR, respectively.

DISCUSSION

The primary findings obtained from this research are presented in Table 1. HDA patients had higher levels of GDF-15, ESR, CRP, RF, and ACPA than the MDA and control groups. The findings also revealed that increased serum levels of ACPA, CRP, ESR, and RF in RA patients were associated with the onset and progression of their condition. These elevated levels reflected the general status of the inflammatory response in RA patients. Several studies have identified abnormalities in circulating chemokines and cytokines, which are markers of systemic inflammation, during the preclinical RA phase [45,46]. It is believed that cytokines contribute to tissue deterioration by regulating the balance between tissue development and destruction [47, 48]. GDF-15 is one example of a proinflammatory cytokine that plays a substantial role in the pathophysiology of RA [49]. CRP is one biomarker used to diagnose and monitor RA. In addition to being a consequence of the inflammatory response, CRP possesses proinflammatory qualities. CRP

levels have been associated with morning stiffness, discomfort, fatigue, grip strength, articular index, and disability in terms of clinical characteristics. CRP is the most reliable and practical indicator for predicting joint damage, disease progression, and functional outcomes [50]. ACPA is generally accepted as a biomarker for the types and activity of RA [51]. RA has been diagnosed with high specificity using ACPA [52]. ACPA stimulates the production of inflammatory cytokines in RA patients, which accumulate at the citrulline site and damage bone [53,54].

Rheumatoid arthritis (RA) patients can use DAS28, a method for monitoring disease activity, independently of antibody titers [55,56]. While positive increases in acute phase reactants are associated with highly active RA, they are not associated with low-to-moderate activity [56]. GDF-15 has shown diagnostic and prognostic value in RA [57].

At various stages of RA patients may develop significant dyslipidemia due to the inflammatory activity of the illness, which can alter the lipid profile [58]. Exposure to chronic systemic inflammation may account for the accelerated atheromatosis seen in RA patients due to structural and functional changes in lipoprotein related to inflammation. Figure 1 illustrates the results. Examining the ROC curve of the parameters GDF-15, AC, CRI-I, and AIP as predictors for RA shows the largest area under the curve relative to the reference line. GDF-15 is a biomarker associated with inflammation, disease activity, severity, and the progression of RA. More severe illness has been observed to be correlated with elevated GDF-15 levels. The outcomes are displayed in Table 4, showing that elevated GDF-15 levels above the cutoff value indicate a high likelihood of RA in patients with good sensitivity. Serum GDF-15 is the best predictor of RA risk compared with other biomarkers, as shown in Figure 1. Synovitis results from neutrophils producing cytokines, prostaglandins, and reactive oxygen intermediates. Inflammation increases the quantity of proinflammatory markers in various immune cells [59,60]. Mast cells in the synovium also release significant amounts of proteases, cytokines, chemokines, and vasoactive amines, which are detrimental to the surrounding tissue [61,62].

Numerous additional biomarkers have been utilized as diagnostic tools for RA beyond those evaluated in the present study. Although many of these molecules lack definitive cutoff values, they can still provide valuable indications of RA [63]. GDF-15, AIP, CRI-I, and AC demonstrated the strongest predictive performance for RA severity, exhibiting higher sensitivity and specificity than the other evaluated markers, as shown in Table 2.

The findings indicated that GDF-15 had comparatively strong sensitivity and specificity for diagnosing RA, and that it was much greater in RA patients than in patients with other conditions. GDF-15 had the best potential cutoff values (223.275 pg/ml), with 74.0% specificity and 73.9% sensitivity for diagnosing RA. These results suggest that serum GDF-15 levels can accurately predict RA development. GDF-15 level concentrations may be a biomarker for predicting RA, according to ROC analysis [64]. This comparison demonstrated the effectiveness of GDF-15 in diagnosing RA. Therefore, when used with other diagnostic variables, GDF-15 has the potential to be an effective parameter in diagnosing RA.

In pathological conditions such as vascular injury, pressure overload, and a proinflammatory state, GDF-15 production is promoted by adipocytes, vascular smooth muscle cells, endothelial cells, macrophages, and other cells [65]. Depending on the stage of the disease, the microenvironment, and the cellular context, GDF-15 can have various roles (e.g., pro- and anti-angiogenic, pro- and anti-inflammatory, and pro- and anti-apoptotic) [66]. Patients with RA are more likely to develop conventional cardiovascular disease (CVD) in addition to systemic inflammation. Cardiovascular disease is the primary cause of death for people with RA and strikes patients on average ten years earlier than in the general population [67]. AIP values increase with increasing CV risk [68].

A multivariate GLM analysis was performed. This was done to avoid limitations in the efficacy of biomarker detection for diagnosing RA. This analysis is based on the study of the detection limits of the biomarkers. The GLM study is displayed in Table 3. The results indicated that diagnosis has a significant effect ($F = 251.177$, $p < 0.001$), and 92.1% of the variation in observed biomarker serum levels can be attributed to RA. Other confounders had a non-significant effect ($p > 0.05$) on the levels of the parameters, and the impact size of each confounder (partial η^2) was low. These results clearly show that RA is an inflammatory disease. Systemic inflammation can accelerate and worsen the development of atherosclerosis, a hallmark of RA [69]. Inflammation is impacted by numerous cytokines that play a vital role in joint deterioration and enhance cellular infiltration in the synovium by promoting chemokine production [70]. The identified biomarkers are useful tools in clinical practice for diagnosing, monitoring treatment, and prognostication in RA patients [71].

CONCLUSIONS

In conclusion, serum GDF-15 is the most accurate predictor of RA severity. Our research indicates that RA patients are more susceptible to atherogenic indices and exhibit a heightened lipid profile. Individuals with RA have an elevated likelihood of developing cardiovascular illnesses linked to atherosclerosis. The biomarkers GDF-15, CRI-I, and AIP, followed by AC, show the largest area under the curve and the highest sensitivity. They are considered the best predictors of rheumatoid arthritis relative to the reference line.

LIMITATIONS

First, this was a case-control study, which is a limitation of this study. Second, demonstrating an inter-assay CV of less than 10% for the ELISA kits is significant.

ORIGINALITY:

No previous work has been done.

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CONFLICTS OF INTEREST:

No conflicts of interest have been identified.

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