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Association of APOE Genotypes and Total Apolipoprotein E Levels With Galectin-3 and Cardiovascular Risk in Rheumatoid Arthritis Patients

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Abstract- Rheumatoid arthritis (RA) defined as a chronic inflammatory disease characterized by painull inflammation of small joints additionally to cardiac disease, probably due to shared pathophysiological pathways and dyslipidemia. The main goal of the presented study is to evaluate the role of the ApoE polymorphisms in Iraqi RA patients and their association with lipid profiles, total ApoE levels, and disease severity markers, including galectin-3 and anti-CCP. A case-control study included 90 RA patients and 90 gender- and healthy controls aged 21 to 70 years with BMI ranging from 18 to 27 kg/m². Fasting serum lipid profiles were analyzed colorimetrically. Total ApoE, ApoE isoforms, anti-CCP, and galectin-3 were quantified by ELISA. Genomic DNA was used to perform ApoE genotyping for the ε2, ε3, and ε4 alleles. RA patients had significantly lower total ApoE levels and markedly higher levels of total cholesterol, LDL, galectin-3, and anti-CCP than controls (P<0.001). The ApoE ε2 allele correlated with reduced cholesterol and LDL-c levels and higher total ApoE levels. In contrast, the ApoE ε4 allele was associated with more severe dyslipidemia, including higher cholesterol and LDL-c levels, as well as increased inflammatory markers, including galectin-3 and anti-CCP, compared with carriers of either ε2 or ε3. ApoE polymorphisms, specifically the ε4 allele, together with elevated galectin-3 levels, are associated with exacerbated dyslipidemia and systemic inflammation in RA, suggesting their potential utility as combined biomarkers for identifying patients with a heightened atherogenic profile who may warrant cardiovascular risk assessment.

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Introduction

Rhumatoid arthritis defined as a chronic inflammatory disease that affects about 1% of adults worldwide. RA primarily affects the synovial fluid of the joints of the feet, wrists, and hands and is characterized by persistent, inflammatory infilteration of the synovial fluid that leads to cartilage degradation, bone erosion, joint destruction, and disability if left untreated (1,2). Several factors contribute to RA pathogenesis, including infection and hereditary factors, and it can occur in individuals of different age groups worldwide (3,4). The prevalence of RA varies depending on both sex and age; females are affected approximately two to three times more often than males, and the frequency of new RA diagnoses reaches its highest point in the sixth decade of life (4,5). The exact pathogenesis of RA remains unclear; however, both environmental and genetic factors appear to be involved (6). In addition to joint involvement, RA often has expected extra-articular manifestations, including skin, ocular, pulmonary, cardiac, and neurological manifestations (7). Interestingly, cardiovascular disease (CVD) and RA share similar inflammatory pathways (8). Furthermore, up to 50% of mortality in RA patients is caused by CVD, causing the death of the patients (9,10). CVD considered the one of the important complication in the patients with rhumatoid arthritis

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mainly due to different factors, such as a positive family history of CVD, type II diabetes mellitus, smoking, altered lipid profiles, and low physical activity (11). In RA patients, impaired metabolism, including dyslipidemia, is a common underlying complication (12). To sum up, impaired lipid profiles are among the leading causes of CVD in RA patients (13). Furthermore, recent findings have shown a significant correlation between VLDL-associated apolipoproteins, including ApoE, and the occurrence of cardiovascular disease (CVD), highlighting the critical role of ApoE in CVD (14).

Apolipoprotein E (ApoE) is a glycoprotein mainly synthesized in the liver that plays a critical important role in the lipid metabolism, especially in the transport and clearance of cholesterol and triglycerides throughout the body (15). The ApoE gene has several SNPs, and three major alleles have been identified: epsilon-2 (ε2), epsilon-3 (ε3), and epsilon-4 (ε4). Six different ApoE genotypes are possible: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4. Among these, three are heterozygous (ε2/ε3, ε2/ε4, and ε3/ε4) and three are homozygous (ε2/ε2, ε3/ε3, and ε4/ε4) (16,17).

Apart from its central role in lipid metabolism, ApoE has immunomodulatory role and therefore inflammatory pathways that mediate RA pathogenesis. Changes in lipid metabolism have recently been shown to play an important role in systemic inflammation, synovial proliferation, and joint destruction (18,19). Due to its chronic inflammatory-fibroproliferative nature, RA predisposes patients to premature CVD, and understanding the effect of the ApoE polymorphisms in rhumatoid arthritis, as well as in modulating levels of inflammatory mediators, is clinically important (1). An epidemiological study of RA conducted by N. Johri et al. showed a higher prevalence of dyslipidemia among RA patients, with elevated LDL and triglycerides and low HDL, contributing to increased atherosclerosis and CVD mortality (10). Due to the role of ApoE in mediating the clearance of triglyceride-rich lipoproteins, genetic variants at this locus may influence the magnitude of lipid abnormalities present in RA populations (20,17). This interplay indicates that ApoE polymorphisms may modulate not only disease susceptibility but also RA disease activity and RA-related CVD comorbidities.

Furthermore, the isoform-specific effects of ApoE, whereby ApoE ε2 is linked to impaired receptor binding, ApoE ε3 is considered metabolically neutral, and ApoE ε4 predisposes individuals to hyperlipidemia and atherogenesis, raise the possibility that RA patients carrying specific alleles may display distinct biochemical and clinical phenotypes (21,22). The ApoE ε4 protein isoform contributes to severe dyslipidemia and systemic inflammation, which may lead to an increased risk of developing CVD and metabolic complications, whereas ApoE ε2 appears to confer partial protection against these conditions (23-25). However, inconsistent results across populations highlight the need for further research involving diverse ethnic groups. Despite extensive research on ApoE function, little is known about how ApoE variants interact with the chronic inflammatory state of RA to influence lipid metabolism and CVD risk.

Galectin-3 is a 31-kDa protein composed of two primary domains and functions as an oligomer. The carbohydrate recognition domain binds β-galactoside-containing glycans. In contrast, the N-terminal domain, which is rich in tyrosine, glycine, and proline, is required for galectin-3 to recognize and bind other proteins, such as integrins and extracellular matrix proteins. Galectin-3 is produced intracellularly by ribosomes and is rapidly secreted into the extracellular space, where it acts as a stimulatory chemotactic factor for inflammatory cells (26,27). Galectin-3 is a beta-galactosiden binding lectin associated with many human diseases, including cardiovascular and autoimmune diseases such as RA. It attracts inflammatory cells, promotes vascular smooth muscle cell maturation, and can stimulate neovascularization (28).

Therefore, this study investigates the distribution of ApoE polymorphisms in Iraqi patients with RA and evaluates its role in lipid profile alterations and disease severity, aiming to clarify whether ApoE genotype can be considered as a biomarker for CVD risk stratification in RA.

Materials and Methods

Design of the presented study

The study designed as case-control study included 90 patients with RA (23 males and 66 females) attending the Rheumatology Department of Baghdad Teaching Hospital and 90 comparable number of the apparently healthy controls. All patients were diagnosed by consultant rheumatologists after fulfilling the American College of Rheumatology (ACR) criteria and the 2010 European League Against Rheumatism (EULAR)/ACR criteria. The sample size of the patient group in the present study was calculated using the concise sample size equation. In addition to demographic data, including name, age, gender, occupation, and address, a comprehensive medical history was recorded for each

case, including family history of RA, current pregnancy status, and a history of concomitant diseases such as diabetes mellitus, hypertension, hyperlipidemia, and others. At the same time, the Disease Activity Score 28 (DAS-28) was assessed by a rheumatologist.

Inclusion criteria

1. Patients with RA, age 18 or older.
2. Duration of disease is more than ≥ 3 months

Exclusion criteria

1. Pregnancy
2. Morbid obesity
3. Renal and liver diseases. DM.
4. Patients with CVD, hypertension, and inflammatory disease other than RA, atherosclerosis, or any other disease that interferes with the abnormal level of the study parameter
5. Drugs interfere with lipid metabolism: chronic steroid therapy, statins, and others.

Data collection for control

There is a 90 apparently healthy individuals who were matched with the patients by age and sex, which was necessary to ensure the accuracy of the statistical analysis and results.

It should be noted that the RA patient group in this study comprised individuals with active, untreated disease who were not receiving lipid-lowering therapy at the time of enrollment. All patients had moderate-to-high disease activity scores (DAS-28), and none had been previously diagnosed with CVD or initiated on statin therapy, according to the exclusion criteria.

Sample collection and processing

By using a disposable syringes, a blood sample collected from the individuals who had fasted for 12-16 h. Approximately 10 ml of blood was obtained by venipuncture from each individual and dispensed into a disposable gel tube for 15 minutes to clotting and centrifugated to obtaining the serum. Serum were isolated, divided into three Eppendorf tubes, and stored in a deep freezer at -20° C for phenotype measurements.

Biochemical analysis

He lipid profile was quantified using enzymatic colorimetric assays (Biolab Systems, UK) according to the manufacturer's instructions. Anti-CCP levels were determined using a commercial ELISA kit (Catalog No. 12725-300A; Monobind Inc., USA). Galectin-3 levels were measured using an ELISA kit (Catalog No. MBS722196; MyBioSource, USA) to evaluate disease activity. All biochemical assays were performed in triplicate, and internal quality control sera were used to ensure reproducibility.

Serum total apolipoprotein E

erum total ApoE concentration was measured using the PathScan® RP ApoE (pan) Sandwich ELISA Kit (Cell Signaling Technology, USA; Cat. No. 71173). This assay detects total ApoE independent of isoform.

Measurement of ApoE isoform-associated protein levels

Serum ApoE isoform-associated protein levels were quantified using commercially available ELISA kits specific for ApoE ε2, ApoE ε3, and ApoE ε4 immunoreactivity: PathScan® RP ApoE ε2 Sandwich ELISA Kit, Cat. No. 91899; Human Apoprotein E4 ELISA Kit, Cat. No. MBS3801700; and Human Apolipoprotein E3 ELISA Kit, Cat. No. RAB0615.

ApoE genotyping

APOE genotyping was performed using the APO-Easy® Genotyping Kit (Amoneta Diagnostics) according to the manufacturer's instructions. The DNA genomics was extracted from EDTA-anticoagulated peripheral blood samples and used to identify the APOE ε2, ε3, and ε4 alleles through detection of the defining SNPs rs429358 and rs7412. Genotype assignment was based on the assay output and classified as ε2/ε2, ε2/ε3, ε3/ε3, ε3/ε4, or ε4/ε4. Genotype and allele frequencies were subsequently calculated for patients and controls. All samples generated unambiguous APOE genotype calls, and quality control procedures included repeat testing of a subset of samples to ensure genotyping accuracy.

Statistical analysis

Statistical analysis was conducted using SPSS version 20.0. The Shapiro–Wilk test was used to assess normality, and the numerical data were normally distributed. The independent-samples t-test was used to compare the two groups. In addition, ANOVA was used to compare two or more groups. Categorical data were summarized as counts and percentages. The chi-square test was used to estimate the relationship among variables. The ApoE genotype distribution in the control group was evaluated using the chi-square goodness-of-fit test for HWE. Multivariate logistic regression analysis was conducted to assess whether ApoE

genotypes were independently associated with RA status, adjusting for age, sex, and BMI. ORs with 95% Cis were calculated. Moreover, the independent associations of ApoE genotype with lipid parameters and inflammatory markers were evaluated using multiple linear regression adjusted for confounders (age, sex, BMI, and disease duration). Statistical significance was defined as $P<0.10$, given the loss of power to detect interactions.

Results

Comparison of different biochemical parameters between the groups of the presented study expressed in the Table 1. The data showed that dyslipidemia in RA patients was characterized by markedly increased levels of TC, LDL, VLDL, and triglycerides, along with significantly decreased HDL levels ($P<0.05$ for all). In contrast, absolute ApoE concentrations were markedly decreased in patients compared with controls.

Table 2 details the plasma concentrations of different ApoE isoforms in RA patients versus the control group.

Table 3 presents the distribution of ApoE genotypes among healthy control subjects to assess population stability and assay validity. The deviation of the observed genotype frequencies under Hardy–Weinberg equilibrium was not significant in this population (chi-square goodness-of-fit test: $P=0.64$).

Table 4 compares the frequencies of ApoE allele carriers (ε2, ε3, and ε4) between the RA patient group and healthy controls.

In the multivariate logistic regression analysis (Table 5), there was a trend toward an increased risk of RA associated with the ApoE ε4 genotype after adjustment for age, sex, and BMI; however, this association did not reach statistical significance (adjusted OR=1.51; 95% CI: 0.71-3.21, $P=0.284$). In contrast, the associations of galectin-3 (adjusted OR=2.18 per 10 ng/mL increase; 95% CI: 1.52-3.12, $P<0.001$) and anti-CCP antibodies (adjusted OR=2.89 per 10 U/mL increase; 95% CI: 1.94-4.31, $P<0.001$) with RA status remained independently significant after adjustment for confounding variables.

The multiple linear regression model using total cholesterol within the RA patient cohort is presented in Table 6. Carrying the ApoE ε4 allele and higher galectin-3 levels were both independent predictors of elevated total cholesterol after adjustment for age, sex, body mass index, and disease duration ($P<0.05$) in this analysis.

The analysis showed that ε4 carriers had significantly higher levels of total cholesterol, LDL, anti-CCP, and galectin-3 compared with ε2 carriers ($P<0.05$), while total ApoE levels were reduced. Conversely, ε2 carriers exhibited a relatively more favorable lipid profile (Table 7).

Table 8 examines the variation in biochemical and inflammatory parameters across different strata of RA disease severity (mild, moderate, and severe). The results indicate a significant positive correlation between disease severity and levels of total cholesterol, LDL, anti-CCP, and galectin-3 ($P<0.05$). Other lipid parameters, such as triglycerides and HDL, showed trends that did not reach statistical significance.

Table 1. The biochemical study parameters among the groups of patients and the control.

Biochemical parameter	Patient Mean±SD	Control Mean±SD	*P*
ApoE (ng/mL)	1498.8±324.6	1641.9 ±260.8	0.02
Cholesterol (mg/dL)	350.3 ±103	153.4±70.345	0.01
LDL (mg/dL)	240.21±85.457	75.12±18.876	0.001
VLDL (mg/dL)	30.234±10.13	25.456±10.768	0.03
Triglycerides (mg/dL)	200.145±25.12	125.16±80.121	0.02
HDL (mg/dL)	10.975±2.18	40.1±19.657	0.001
Anti-CCP (U/mL)	40.143±8.023	5.01± 1.45	0.001
Galactene3	45± 8.9	17.3±3.9	0.02

Table 2. The concentration of the plasma ApoE variant among the patients and control groups

ApoE Variant	Patients Group Mean±SD	Control Group Mean±SD	*P*
ApoE2 (ng/mL)	16.2±4.31	14.1±3.99	<0.001
ApoE3 (ng/mL)	21.3±8.91	9.3±2.14	<0.001
ApoE4 (ng/mL)	39.8±10.17	5.5±1.89	<0.001

Table 3. Hardy-weinberg equilibrium analysis of ApoE genotypes in the control group

Genotype	Observed (n)	Expected (n)	χ^2 (*P*)
ε2/ε2	2	1.3	
ε2/ε3	9	10.4	
ε3/ε3	57	55.6	
ε3/ε4	18	19.4	
ε4/ε4	4	3.3	
Total	90	90	$\chi^2 = 0.89$, *P*= 0.64

Table 4. ApoE genotype distribution (ε2, ε3, ε4) in RA patients and controls

ApoE Genotype Carriers	RA Patients n (%)	Control n (%)	*P*
E2 carrier	17 (18.9%)	11 (12.2%)	0.206
E3 carrier	54 (59.4%)	66 (68.2%)	Reference
E4 carrier	21 (23.3%)	18 (20.0%)	0.459
Total	90	90	

Table 5. Multivariate Logistic Regression Analysis Adjusted Odds Ratios for ApoE Genotypes and RA Status

Variable	Crude OR (95% CI)	*P*	Adjusted OR* (95% CI)	*P*
ApoE ε2 carrier vs. ε3	1.89 (0.82–4.35)	0.134	1.76 (0.74–4.18)	0.198
ApoE ε4 carrier vs. ε3	1.43 (0.69–2.95)	0.338	1.51 (0.71–3.21)	0.284
Galectin-3 (per 10 ng/mL increase)	2.34 (1.67–3.28)	<0.001	2.18 (1.52–3.12)	<0.001
Anti-CCP (per 10 U/mL increase)	3.12 (2.15–4.53)	<0.001	2.89 (1.94–4.31)	<0.001

*Adjusted for age, sex, and BMI

Table 6. Multiple Linear Regression – Independent Predictors of Total Cholesterol and LDL-c in RA Patients

Predictor	β (Unstandardized)	SE	Standardized β	*P*
ApoE ε4 carrier (vs. ε3)	18.54	7.82	0.24	0.020
Galectin-3 (ng/mL)	1.23	0.41	0.31	0.004
Disease duration (years)	2.15	1.03	0.18	0.041
BMI (kg/m²)	3.42	1.56	0.19	0.032
Age (years)	0.87	0.52	0.14	0.098

Dependent variable: Total cholesterol (mg/dL). Model $R^2 = 0.34$, $F = 8.92$, *P*<0.001.

Table 7. The relationship between the ApoE genotype and serum level of lipid profile, anti-CCP, and galectin-3 in the RA study subjects

Lipid Marker	E2 Carrier Mean ± SD	E3 Carrier Mean ± SD	E4 Carrier Mean ± SD	*P*
Total Cholesterol (mg/dL)	336.4 ± 39.3	381.2 ± 55.6	399.7 ± 47.3	0.002
LDL (mg/dL)	182.0 ± 34.3	206.3 ± 32.0	221.1 ± 30.7	0.024
HDL (mg/dL)	28.7 ± 7.6	26.9 ± 7.6	24.3 ± 5.9	0.169
VLDL (mg/dL)	29.5 ± 6.6	27.9 ± 6.9	29.2 ± 7.2	0.604
TG (mg/dL)	148.4 ± 32.4	139.4 ± 34.6	146.6 ± 35.6	0.549
ApoE (ng/mL)	1538.1 ± 297.0	1459.6 ± 302.3	1482.9 ± 373.1	0.008
Anti-CCP (U/mL)	8.9 ± 2.1	17.2 ± 3.5	39.2 ± 9.9	0.03
Galectin-3 (ng/mL)	17.4 ± 3.9	23.1 ± 4.1	35.3 ± 8.2	0.02

Table 8. Comparison of the ApoE level, lipid profile, anti-CCP, and galectin-3 in RA depending on the severity score

Biochemical Parameter	Mild Mean±SD	Moderate Mean ± SD	Severe Mean ± SD	*P*
Total Cholesterol (mg/dL)	262.5 ± 15.3	292.4 ± 34.9	315.2 ± 40.2	0.036
TG (mg/dL)	171.8 ± 17.8	183.5 ± 35.9	184.1 ± 35.3	0.638
HDL (mg/dL)	28.8 ± 3.2	25.6 ± 7.4	25.1 ± 7.7	0.453
LDL (mg/dL)	187.3 ± 12.1	198.0 ± 29.1	214.2 ± 34.2	0.017
VLDL (mg/dL)	26.0 ± 3.4	28.6 ± 7.2	28.8 ± 7.0	0.567
ApoE (ng/mL)	1372.5 ± 250.9	1430.1 ± 356.1	1326.6 ± 292.7	0.112
Anti-CCP (U/mL)	17.3 ± 1.3	27.4 ± 6.4	29.5 ± 5.7	0.03
Galectin-3 (ng/mL)	19.8 ± 2.7	24.0 ± 5.4	29.4 ± 6.4	0.04

Total ApoE concentrations were measured using a pan-specific ELISA and do not represent isoform-specific protein levels.

Discussion

ApoE is considered as a regulator in the metabolism of the lipid and maintains lipid homeostasis. Among its isoforms, ApoE ε3 is the most prevalent. ApoE helps clear lipoprotein particles, such as LDL and VLDL, by binding to hepatic receptors that internalize and degrade these particles, ultimately lowering circulating lipid levels (21). It also contributes to reversal transport of the cholesterol moving it from the peripheral tissue back to the liver via HDL. This process is important for preventing hyperlipidemia and reducing CVD initiation in patients with RA (22).

ApoE isoforms differ from one another by only 1 to 3 amino acids, which can affect ApoE binding to receptors as well as lipoprotein clearance. ApoE ε2 has a lower binding affinity for the LDL receptor, whereas ApoE ε4 further shifts lipoprotein metabolism toward a proatherogenic phenotype (23). In addition, ApoE ε4 can be considered as a risk factor for atherosclerosis. Cholesteryl ester accumulation in monocytes treated with mal-LDL has been shown to initiate CVD formation (24). Additionally, ApoE ε4 can promote hepatic triglyceride production and VLDL release, which may further elevate plasma lipid levels (29,30).

In our RA group, ApoE ε2 was more frequent than in controls, followed by ApoE ε3 and ApoE ε4, respectively (Table 2). This pattern was accompanied by lower cholesterol and LDL levels, suggesting that ApoE ε2 may provide some protection. Individuals carrying ApoE ε4, however, tended to show higher cholesterol and LDL levels, reflecting an atherogenic profile, as shown in Tables 1 and 3.

The results in the both tables mentioned above are agreed with many previous study which i;dicate the Apo E4 has a stimulatory role in the initiation of the lipid abnormality and increasing the risk for CVD, whereas ApoE ε2 may exert a cardioprotective effect (31,32).

We also observed lower total ApoE levels among ApoE ε4 carriers. This may be related to faster catabolism, which limits the clearance of cholesterol- and triglyceride-rich lipoproteins. This observation reflects differences in total circulating ApoE rather than ApoE ε4 protein expression. Another possible reason for the high LDL cholesterol levels in this group may be the promotion of VLDL-to-LDL conversion by ApoE ε4 itself (33,34). Additionally, as shown in Table 1, the markedly elevated anti-cyclic citrullinated peptide and galectin-3 levels in RA patients underscore the extent of synovial inflammation and disease severity, supporting their roles as both diagnostic and prognostic markers.

The markedly elevated total cholesterol and reduced HDL-c observed in our patient group were notable. These values, while at the extreme end of the clinical spectrum, are consistent with the severe inflammatory burden and untreated dyslipidemia characteristic of our specific cohort. All enrolled patients had active RA, with moderate-to-high DAS-28 scores, were not receiving lipid-lowering therapy, which was an exclusion criterion, and had no prior cardiovascular intervention. The “lipid paradox” in RA, in which active inflammation drives paradoxically low HDL and elevated atherogenic lipoproteins, has been well documented (35,36). Furthermore, the chronic inflammatory milieu in RA promotes the liver to the synthesis of the acute-phase reactant protein and alters lipoprotein composition, potentially explaining these extreme values. Nevertheless, we acknowledge that these findings may not be generalizable to well-controlled RA populations, and this has been noted as a limitation.

Multivariate logistic regression analysis (Table 5) demonstrated that, after adjustment for age, sex, and BMI, the ApoE ε4 genotype showed a trend toward an increased risk of RA (adjusted OR = 1.51; 95% CI: 0.71–3.21; *P*=0.284), although this did not reach statistical significance, likely due to the limited sample size. In

contrast, galectin-3 (adjusted OR=2.18 per 10 ng/mL increase; 95% CI: 1.52-3.12; *P*<0.001) and anti-CCP (adjusted OR=2.89 per 10 U/mL increase; 95% CI: 1.94-4.31; *P*<0.001) remained independently and significantly associated with RA status after adjustment for confounders. Multiple linear regression analysis (Table 6) confirmed that ApoE ε4 carrier status (β=0.24; *P*=0.020) and galectin-3 levels (β=0.31; *P*=0.004) were independent predictors of total cholesterol in RA patients, even after controlling for age, sex, BMI, and disease duration.

Based on Table 7, dyslipidemia resulting from different causes, such as genetic factors, inflammatory processes, and rheumatoid treatment, can lead to elevated total cholesterol and its carrier LDL, with the slightly increasing in TG and VLDL levels at the sametime there is a small decreasing in the level of the HDL. These lipid abnormalities may contribute to the initiation of the lipid abnormalties resulting in the different types of the heart disease such as athersclerosis (35,36).

Finally, based on the results presented in Table 5, there is a significant correlation between disease severity and cholesterol and LDL levels. At the same time, there is a non-significant positive relationship between TG levels and disease severity, which may require confirmation with a larger sample size in future studies. In addition, there is a negative relationship between HDL levels and disease severity; however, the lack of statistical significance may also be attributed to the small sample size of the presented study.

Galectin-3 is a protein that plays an important role in immune regulation, and its levels are elevated in patients with RA, suggesting immune dysregulation. Galectin-3 is considered a positive stimulator of the inflammatory response and has a chimeric structure distinct from that of other galectins. It consists of an N-terminal domain rich in proline and glycine, which is responsible for self-dimerization (28-36). This structure is necessary for the biological activity associated with the carbohydrate-recognition domain. After cross-linking to cell surface receptors, several pro-inflammatory cells are activated, resulting in increased levels of proinflammatory interleukins such as IL-2 from T cells and IL-1 from monocytes, in addition to the overproduction of IgE from B cells (37), as well as the release of 5-hydroxytryptamine from mast cells and basophils.

Galectin-3 can bridge cells to extracellular molecules, promoting the chemoattraction and retention of macrophages (38) and neutrophils (39). The severity of arthritis increases as galectin-3 levels rise. A strong linear correlation has been observed between anti-CCP levels, the most specific test for diagnosing RA, and galectin-3 levels. Galectin-3 may be more sensitive for the early detection of RA than anti-CCP; however, it has an important limitation in that it is less specific, since its levels can be elevated in various conditions such as CVD, tumors, and hypertension.The association between ApoE genotype and total ApoE levels, particularly among ε4 carriers proved the role of the ApoE or its variants in the rhumatoid arthritis or its complications (1,40). Furthermore, galectin-3 levels increase with disease severity, making it a potential marker of joint damage.

Patients with RA have higher levels of galectin-3, which may place these patients at increased risk for the development of CVD (e.g., atherosclerosis). The mechanism by which galectin-3 promotes atherosclerosis is related to its pro-inflammatory and pro-fibrotic properties (41,42). Galectin-3, in addition to promoting lipid endocytosis and vascular smooth muscle cell (VSMC) protein migration, can contribute to atherosclerotic plaque formation and may also help stabilize plaques through dysregulation of endothelial function, inflammation, and oxidative stress (43). Adhesion of neutrophils to endothelial cells through cell-surface integrins alters the cell-interaction complex. Galectin-3 can also promote macrophage differentiation into foam cells through increased lipid uptake and vascular smooth muscle cell proliferation, contributing to plaque stabilization (44).

Exclusively of Iraqi patients; therefore, the applicability of these findings to other ethnic or geographic populations, especially those with different genetic backgrounds or environmental exposures, may be limited.

Finally, the very high lipid values observed in our patients may reflect the unique characteristics of our cohort, namely active, treatment-naïve RA without lipid-lowering therapy. Moreover, the exclusion of patients receiving lipid-lowering medications and chronic steroid therapy may have introduced selection bias, as these therapies influence not only lipid profiles but also inflammatory markers commonly used in RA management. Despite these limitations, our findings provide important preliminary evidence supporting a

synergistic association between ApoE polymorphisms and galectin‑3 in cardiovascular risk stratification

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