AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

The Association of Relaxin and CD40 with Glycemic and Inflammatory Markers in Patients with Atherosclerosis

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Article history
Received: 10/6/2025
Revised: 4/7/2025
Accepted: 20/7/2025

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Abstract: Atherosclerosis is a long-term inflammatory condition that causes plaque to gradually build up in artery walls. It frequently results in ischemic problems and vascular blockage. Vascular remodeling signals and immune-inflammatory mediators are important factors in the development of illness. The purpose of this study was to examine the pathological and diagnostic importance of CD40 and Relaxin levels in atherosclerosis patients as well as their relationships to hematological markers and lipid profiles.
Methods: 90 individuals aged 45 to 65 were divided into three groups for a case-control study: thirty patients with atherosclerosis who also had diabetes and hypertension, thirty pathological controls who had both diseases but no atherosclerosis, and thirty healthy controls. Blood samples obtained while fasting were analyzed for lipid profiles, hematological traits, and inflammatory markers (Relaxin , CD40). ELISA and spectrophotometric techniques were applied. Statistical analysis was done using SPSS v26.
Results: According to the study, CD40 and Relaxin levels were noticeably higher in atherosclerosis patients (P = 0.001). The strong correlation between CD40 and lymphocyte count (r = 0.421, P = 0.046) lends credence to CD40's function in immune-system-induced vascular inflammation . There was a positive connection between VLDL and relaxin levels (r = 0.464, P = 0.026), suggesting a metabolic-compensatory relationship. Furthermore, the patient group's hematological markers (WBCs, PLT, and LYM) were significantly higher. According to ROC analysis, Relaxin performed somewhat well in terms of diagnostic accuracy (AUC = 0.758), whereas CD40 had outstanding diagnostic accuracy (AUC = 0.880, sensitivity = 87%, specificity = 80%).
Conclusions: The findings show that whereas relaxin may be a vascular compensatory response to oxidative and lipid stress, CD40 is a potent inflammatory biomarker of atherosclerosis. The evaluation of these markers in combination may enhance atherosclerotic risk classification and early identification.

Keywords: Atherosclerosis, CD40, Relaxin, Inflammation, Endothelial dysfunction, Lipid profile, Biomarkers, ROC curve, Immune response.

1. Introduction

Atherosclerosis is a chronic progressive disease is defined by the buildup of fibrous materials, lipids, and immune cells inside the artery wall, which results in plaques that harden and constrict the arteries. Cardiovascular diseases (CVDs), such as myocardial infarction and stroke, are mostly caused by it. Endothelial dysfunction, oxidative stress, chronic inflammation, and dysregulation of lipid metabolism are all part of the pathophysiology of atherosclerosis. Over time, these factors lead to vascular problems,

DOI: https://doi.org/10.36320/ajb/v17.i3.20299

thrombosis, and plaque instability, therefore early diagnosis is essential for preventive measures [1,2] Endothelial dysfunction, oxidative stress, and persistent low-grade inflammation are some of the ways that type 2 diabetes mellitus (T2DM) dramatically increases the risk of atherosclerosis. Patients with both diabetes and vascular disease frequently have elevated fasting blood glucose, HbA1c, C-reactive protein (CRP), and white blood cell counts (WBCs), which illustrates how metabolic and inflammatory pathways are intertwined [3] The peptide hormone relaxin, which is a member of the insulin superfamily, has been demonstrated to have anti-inflammatory, anti-fibrotic, and vasodilatory properties that protect the heart. It inhibits the expression of pro-inflammatory cytokines, decreases vascular stiffness, and increases the generation of nitric oxide. According to recent research, it can reduce vascular and cardiac fibrosis, which suggests that it regulates atherosclerotic processes. Increased Relaxin levels may be a biomarker for endothelium healing and plaque stability and have been connected to vascular remodeling [4, 5].

The tumor necrosis factor (TNF) receptor family includes CD40, which is expressed by smooth muscle cells, macrophages, and endothelial cells. Its association with the CD40 ligand (CD40L) sets off inflammatory signaling cascades that facilitate the production of foam cells, leukocyte recruitment, and cytokine release—all of which are essential for the initiation and advancement of atherosclerotic plaque . Both advanced plaque instability and early atherogenesis have been linked to activation of the CD40-CD40L axis.
[6,7]

Relaxin and CD40 are closely linked to changes in the lipid profile, such as higher levels of LDL and triglycerides and lower levels of HDL, which intensify vascular inflammation and plaque development. Their detectable presence in the bloodstream raises the possibility that they could be used as non-invasive biomarkers to determine the risk of atherosclerosis, particularly when compared to conventional lipid markers . Individuals with type 2 diabetes have been found to have elevated CD40 expression, and its signaling pathway is thought to be a major factor in inflammation-induced atherogenesis [8,9].

The pathophysiology of atherosclerosis is largely attributed to lipid imbalances. Increased levels of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cause cholesterol to build up in the artery's intimal layer, which starts the development of plaque. High-density lipoprotein (HDL), on the other hand, offers protection by encouraging reverse cholesterol transfer. Clinical studies have consistently shown that individuals with atherosclerosis had significantly higher LDL, total cholesterol, and triglyceride levels and decreased HDL, which causes oxidative stress and vascular inflammation [10-11].

This dysregulation of lipid metabolism, which also promotes the formation of foam cells and endothelial dysfunction, is strongly associated with the expression of inflammatory biomarkers like CD40 and modulatory hormones like Relaxin, which are implicated in vascular remodeling . These biomarkers and lipid imbalance may interact to offer a more thorough diagnostic method for identifying and tracking the advancement of atherosclerosis [12, 13].

Assessing Relaxin and CD40 levels in addition to glycemic and hematologic parameters may provide more profound understanding of the pathophysiological mechanisms behind atherosclerosis and improve early diagnostic techniques, especially in light of the growing overlap between metabolic dysfunction, inflammatory status, and cardiovascular risk [14].

2. Methodology

In this case-control study, 90 participants were split up into three groups:

• Group 1 (n = 30): Individuals with a history of hypertension, diabetes, and atherosclerosis.
• Group 2 (n = 30): Individuals without atherosclerosis who have been diagnosed with diabetes mellitus and hypertension alone.
• Group 3 (n = 30): Healthy people without a history of diabetes, hypertension, or atherosclerosis who acted as the control group

Participants ranged in age from 45 to 65. The diagnosis of vascular disease and atherosclerosis was confirmed by diagnostic catheterization and ultrasound imaging. Fasting blood samples were obtained from each participant in the morning at the Open Heart Center of Al-Sadr Medical City, which is situated in Al-Najaf Al-Ashraf Iraq.

Each participant underwent a comprehensive medical assessment to rule out any additional underlying conditions that might have affected the study's results,

DOI: https://doi.org/10.36320/ajb/v17.i3.20299

such as thyroid problems, chronic inflammation, or kidney disease .
Each participant underwent a comprehensive medical assessment to rule out any underlying conditions that would have an impact on the study's results, such as thyroid problems, chronic inflammation, or kidney disease.

Samples collection

After a 10-hour fast, five milliliters of venous blood samples were drawn in the morning from both patients and tourniquet-free control subjects using a disposable needle and plastic syringes. The materials were separated by centrifuging them for 15 minutes at 3000 rpm after being transferred into gel tubes.

Serum Level of Cytokines

The enzyme-linked immunosorbent assay (ELISA) method was used to determine the levels of CD40 and relaxin in the serum. To guarantee accuracy and consistency in sample analysis, quantification was carried out using commercial ELISA kits provided by BT LAB (China), adhering to the procedures specified in the manufacturer's instructions.

Biochemical and Hematological Parameters

The levels of triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and cholesterol in the serum were assessed using the spectrophotometric technique. For every parameter, unique test kits were supplied by Spinreact (Spain).
Also, glycated hemoglobin (HbA1c) and fasting blood glucose (FBG) were measured. Using Spinreact kits (Spain), the manufacturer's instructions were followed in order to detect FBG using the glucose oxidase-peroxidase method and quantify HbA1c using a turbidimetric inhibition immunoassay.

A complete blood count (CBC), including white blood cell (WBC) and lymphocyte counts, was performed using an automated hematology analyzer
Statistical analysis
Statistical software (SPSS 26) was used to do statistical calculations on the data gathered from the study of biochemical data. The F-distribution analyses mean, standard deviation, and findings were acquired. Any noteworthy important value or likelihood

3. Results and discussion

Demographic Characteristics

Table 1 shows a summary of the research groups' demographic information. The three groups' ages did not differ significantly (P = 0.948), indicating that age matching was done correctly. The sick group had the highest mean weight, though, and there was a statistically significant difference in body weight between the groups (P = 0.001).

Table 1: Comparative demographic data of the study population

Parameters	Patients =30	Pathological Control= 30	Control = 30	P Value
AGE	55.83 ± 9.23	55.03 ± 10.89	55.76 ± 10.07	0.948
WT	85.48 ± 7.51	66.77± 8.62	76.00 ± 12.80	0.001

Data presented as Mean ± SD; P < 0.05 considered as significant

test below 0.05 (P < 0.05) was found, together with the use of SPSS 26(26) and Microsoft Excel (2019).

Inflammatory Biomarkers: Relaxin and CD40

Table 2 shows the serum concentrations of Relaxin and CD40 in patients with atherosclerosis compared to both pathological and healthy control groups. The results demonstrate a statistically significant elevation in the levels of both biomarkers among the patient group (P = 0.001). Relaxin levels were noticeably greater in the patients, which might be a physiological reaction to endothelial dysfunction and vascular inflammation. The anti-fibrotic and vasodilatory qualities of this hormone point to a potential protective function in the atherosclerotic process.

In a similar vein, the sick group's CD40 levels were noticeably higher than those of the controls. The formation and advancement of atherosclerosis are significantly influenced by endothelial inflammation and immune system activation, both of which may be indicated by this elevation of CD40.

DOI: https://doi.org/10.36320/ajb/v17.i3.20299

Table 2: Enzymatic Biomarker Profile in atherosclerosis patients and controls				
Parameters	Patients =30	Pathological Control= 30	Control = 30	P Value
Relaxin	810.19	1281.63	308.51	383.17
CD40	25.71±10.68	11.62± 9.42	7.86± 7.32	0.001

According to Table 2, patients with atherosclerosis had significantly higher levels of relaxin (P = 0.001) than both the pathological and healthy control groups. In reaction to persistent vascular injury and inflammation, the hormone relaxin, which is generally recognized for its vasodilatory and anti-fibrotic properties, may be elevated in a compensatory way. Several investigations have consistently shown that higher relaxin levels in atherosclerosis patients may represent a compensatory mechanism in response to persistent arterial inflammation and injury. Relaxin, a peptide hormone traditional in th"Relaxin-2 functions as a compensatory reaction during the reduction in blood supply in the elderly, indicating it [15].

Additionally, the atherosclerotic group had considerably greater CD40 levels (P = 0.001). Numerous cytokine pathways are activated by the well-known pro-inflammatory receptor CD40, which is found on immune and endothelial cells. It has a well-established involvement in atherogenesis, especially through promoting foam cell production, leukocyte adhesion, and macrophage activation [13] . CD40/CD40L interactions are crucial to the inflammatory cascade of atherosclerosis, including both T lymphocytes and endothelial cells. They promote leukocyte adherence and transmigration across the vascular endothelium.Furthermore, it was discovered that endothelial-specific CD40 loss leads to higher plaque stability and lower leukocyte accumulation, highlighting the receptor's pathogenic relevance [16-17].

Hematological Parameters

Significant variations in hemoglobin, platelets, WBC, and lymphocyte percentage were observed between the groups (P = 0.001) in Table 4. WBC and Hb levels were higher in atherosclerosis patients than in healthy controls, lymphocyte counts were highest in pathological controls, and platelet counts were higher in both patient and pathological groups.

Table 3: Hematological Parameters in patients and pathological controls and controls				
Parameters	Patients= 30	Pathological Control= 30	Control = 30	P Value
WBC	8.03 ± 1.54	5598.16 ± 3975.22	7.35± 2.31	0.001
PLT	230.96 ± 54.22	267.73 ± 108.21	159.12 ± 43.43	0.001
HB	14.14 ± 2.26	12.10 ± 1.51	13.65 ±2.08	0.001
LYM.	28.92 ± 10.89	37.20± 12.34	16.60 ± 18.56	0.001

As seen in Table 3, the hematological profile in atherosclerosis frequently indicates underlying immunologic and inflammatory dysregulation. White blood cell (WBC) counts were significantly higher in atherosclerosis patients (P = 0.001), which is consistent with multiple studies showing leukocytosis as an indicator of systemic inflammation and cardiovascular risk. (18) It has been shown that both total and differential WBC counts are independently linked to coronary artery calcification and inflammation-driven atherosclerosis progression. Elevated WBC, especially neutrophils and lymphocytes, is highly connected with coronary atherosclerotic burden and plaque instability [19].

Additionally, patients' platelet counts (PLT) were considerably greater than controls' (P = 0.001), supporting the idea that atherosclerosis has a thrombogenic component.found that increased platelet numbers and hyperactivity have a role in atherogenesis by causing thrombus formation and endothelial damage. added that elevated levels of PLT and MPV (mean platelet volume) are predictive of acute vascular events and associated with the severity of coronary artery disease (20).

The atherosclerotic group also had higher hemoglobin (HB) levels (14.14 ± 2.26), which could be a result of compensatory erythropoiesis in response to long-term vascular hypoxia. highlighted that increased blood viscosity and vascular thickening may occur concurrently with elevated hemoglobin levels, causing hemodynamic stress. Furthermore, patients' lymphocyte

DOI: https://doi.org/10.36320/ajb/v17.i3.20299

percentage (LYM) was significantly higher (P = 0.001) than that of healthy controls. Recent research indicates that lymphocyte-mediated adaptive immune responses, despite being historically thought of as a protective immune subset, actually make chronic vascular inflammation worse [21].

Collectively, these hematological changes represent the underlying pathophysiology of atherosclerosis, which includes immunological modulation, inflammation, thrombosis, and hypoxia, in addition to acting as diagnostic indicators

Correlation between Relaxin/CD40 and Glycemic-Inflammatory Markers

The results of the Spearman correlation study between different hematological and biochemical indicators in individuals with atherosclerosis are shown in Table 3. There may be a connection between relaxin and triglyceride metabolism, as evidenced by the substantial positive association between relaxin and VLDL (r = 0.464, p = 0.026). Furthermore, CD40's function in immunological activation was demonstrated by a substantial positive correlation with lymphocyte count (r = 0.421, p = 0.046). Interestingly, HDL and lymphocytes had a positive correlation, whereas LDL and lymphocytes had a negative correlation. Additionally, a strong inverse relationship between hemoglobin and WBC was noted, which may suggest inflammatory consequences. Other correlations, like those involving glycemic or lipid indicators, relaxin, and CD40, failed to achieve statistical significance.

Table 4: Spearman's correlation of variables in atherosclerosis patients

Variables		Relaxin	CD40	WBC	LYM	PLT	HB	HbA1c	B.Sugar
HDL	R	0.197	0.188	-0.017	.466*	-0.017	0.109	0.241	-0.349
	P	0.366	0.391	0.938	0.025	0.938	0.621	0.268	0.103
LDL	R	0.024	0.181	0.041	-.441*	0.041	-0.174	0.022	0.043
	P	0.912	0.408	0.851	0.035	0.851	0.427	0.921	0.844
VLDL	R	.464*	0.118	0.136	0.072	0.136	0.332	-0.035	0.269
	P	0.026	0.592	0.535	0.742	0.535	0.121	0.873	0.215
CHO	R	-0.081	-0.34	-0.144	0.086	-0.144	0.025	0.368	0.088
	P	0.714	0.113	0.513	0.695	0.513	0.91	0.084	0.689
TG	R	-0.046	-0.177	-0.166	0.007	-0.166	-0.072	0.217	0.147
	P	0.836	0.419	0.448	0.976	0.448	0.744	0.241	0.504
Relaxin	R	1	0.021	-0.047	0.018	-0.078	0.156	0.263	0.024
	P Value		0.924	0.831	0.934	0.723	0.477	0.226	0.915
CD40	R	0.021	1	-0.152	.421*	-0.074	-0.051	0.158	-0.041
	P	0.924		0.49	0.046	0.739	0.816	0.471	0.853
WBC	R	-0.047	-0.152	1	-0.072	0.191	-.439*	-0.184	0.011
	P	0.831	0.49		0.745	0.382	0.036	0.4	0.959
LYM	R	0.018	.421*	-0.138	1	-0.138	0.001	0.062	-0.296
	P	0.934	0.046	0.531		0.531	0.995	0.778	0.171
PLT	R	-0.078	-0.074	0.191	-0.138	1	-0.356	0.127	-0.111
	P	0.723	0.739	0.382	0.531		0.095	0.562	0.614
HB	R	0.156	-0.051	-0.356	0.001	-0.356	1	-0.227	0.04
	P	0.477	0.816	0.095	0.995	0.095		0.297	0.855
HbA1c	R	0.263	0.158	-0.184	0.062	0.127	-0.227	1	-0.12
	P	0.226	0.471	0.4	0.778	0.562	0.297		0.585
B.Sugar	R	0.024	-0.041	0.011	-0.296	-0.111	0.04	-0.12	1
	P	0.915	0.853	0.959	0.171	0.614	0.855	0.585	

Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

in Table 4 Relaxin and VLDL levels were shown to significantly positively correlate in the current study among atherosclerotic patients (r = 0.464, P = 0.026). This correlation highlights the possible compensatory function of relaxin in metabolic dysregulation, especially in vascular settings that are overloaded with lipids. The overexpression of relaxin may be a preventive vascular response meant to reduce oxidative stress and enhance endothelial function, since VLDL is increased in insulin-resistant and pro-atherogenic circumstances. This is supported by They showed that relaxin reduces oxidative damage caused by lipids in vascular tissues. A slight positive association between relaxin and HbA1c was also noted, however it was not statistically significant (r = 0.263, P = 0.226), indicating possible endocrine interaction in diseases of the glucose-insulin axis [22].

On the other hand, relaxin did not exhibit any significant correlations with inflammatory cell counts, such as WBC or lymphocytes, suggesting that its function may be more closely related to lipid handling and vascular

DOI: https://doi.org/10.36320/ajb/v17.i3.20299

remodeling than it is to direct leukocyte modulation. Relaxin's selective affinity for triglyceride-rich lipoproteins, such as VLDL, which are known to cause vascular lipotoxicity in insulin-resistant conditions, is reinforced by the fact that it does not correlate with HDL, LDL, or TG [23].

Additionally, the research showed a strong correlation between CD40 levels and lymphocyte numbers (r = 0.421, P = 0.046), indicating that CD40 might be a marker of adaptive immunological activity in the atherosclerotic environment. The function of CD40, a crucial receptor found on immunological and endothelial cells, in fostering T-cell activation, macrophage differentiation, and foam cell generation has been thoroughly studied. This association is consistent with earlier research showing that inflammation triggered by lymphocytes is essential for plaque disintegration and vascular remodeling [24].

This association is consistent with earlier research showing that inflammation triggered by lymphocytes is essential for plaque disintegration and vascular remodeling.

It's interesting to note that CD40 did not significantly correlate with lipid or glycemic indicators (such as LDL, HDL, or HbA1c), confirming its role as an immuno-inflammatory biomarker as opposed to a metabolic one. Given its poor or nonexistent correlations with WBC and PLT, it is likely that CD40 has a more lymphocyte-specific role in atherosclerosis, which is compatible with adaptive immune response characteristics rather than innate ones [25].

Diagnostic Value of Relaxin and CD40 in Atherosclerosis

Table 5: Receiver operating characteristic analysis of Relaxin and CD40 as diagnostic markers for atherosclerosis

Item	Cut off	Sensitivity	Specificity	AUC	95% CI of AUC	P Value
Relaxin	175.87%	73.9 %	63.1%	0.758	0.648-0.867	0.0001
CD40	17.95	87%	80%	0.880	0.808-0.953	0.0001

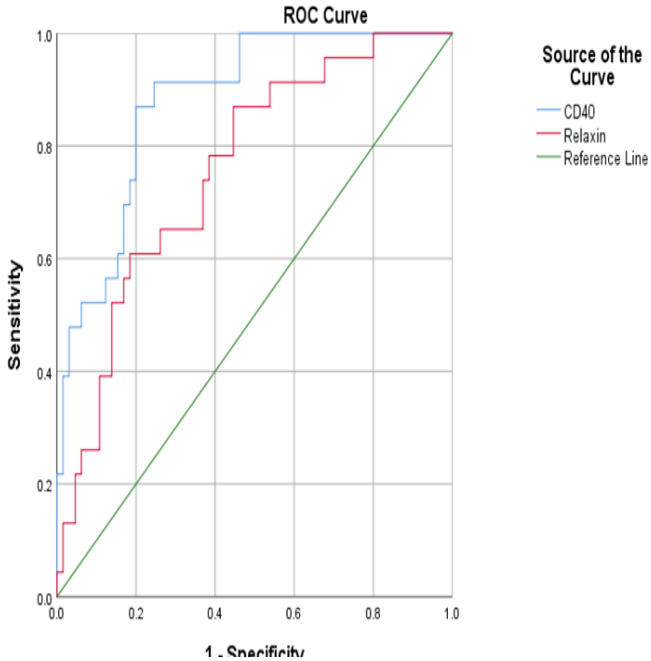

Fig 1: ROC curve demonstrating the diagnostic performance of CD40 and Relaxin in patients with atherosclerosis

The diagnostic performance of Relaxin and CD40 in differentiating atherosclerosis patients from healthy controls was assessed using characteristic (ROC) analysis. With an AUC of 0.880 (95% CI: 0.808–0.953, p = 0.0001), a sensitivity of 87%, and a specificity of 80% at a cut-off value of 17.95, CD40 displayed exceptional diagnostic accuracy, as illustrated in Figure 1 and Table 4. On the other hand, Relaxin also shown a moderate diagnostic value, with a cut-off value of 175.87%, sensitivity of 73.9%, specificity of 63.1%, and an AUC of 0.758 (95% CI: 0.648–0.867, p = 0.0001). These findings imply that whereas CD40 exhibits noticeably more discriminating power, both markers may be clinically useful in the diagnosis of atherosclerosis.

Table 5 Analysis of receiver operating characteristic (ROC) curves is a well-recognized technique for assessing biomarkers' diagnostic efficacy. With an AUC of 0.880 (95% CI: 0.808–0.953, P = 0.0001), excellent sensitivity (87%) and specificity (80%) at a cut-off of 17.95 ng/mL, CD40 showed exceptional diagnostic ability in this investigation. These results are consistent with research that found the soluble CD40 receptor to be

a valid biomarker for the burden of carotid atherosclerosis, highlighting its potential for non-invasive vascular screening [26]. Likewise, using ROC-based models, soluble CD40L was identified as one of the strongest predictive indicators of plaque instability and cardiovascular risk [27].

With an AUC of 0.758 (95% CI: 0.648–0.867, P = 0.0001), sensitivity of 73.9%, and specificity of 63.1% at a cut-off of 175.87 pg/mL, Relaxin, in contrast, demonstrated a modest level of diagnostic competence. Relaxin still has diagnostic significance even though it is not as strong as CD40, especially when considering vascular stress and remodeling. Relaxin signaling pathway components were significantly upregulated in patients with cardiovascular dysfunction, according to a proteomic study, which suggests that multi-marker panels should incorporate them for better risk classification. Additionally, it was observed that the predictive accuracy for cardiac dysfunction was improved by using logistic regression models that combined classical indicators with relaxin-related pathways [28] .

When combined, these findings imply that CD40 might be a stand-alone inflammatory biomarker for atherosclerosis, while Relaxin offers more information about vascular remodeling and metabolic stress, particularly when combined with other analytes in a diagnostic process.

Conclusion

The results of this study, there are notable changes in both inflammatory and compensatory biomarkers in patients with atherosclerosis. Significantly higher CD40 levels suggested active immune-inflammatory engagement in endothelial dysfunction and plaque formation. On the other hand, in reaction to lipid-induced damage and vascular stress, relaxin levels rose. When coupled in diagnostic panels, CD40 exhibited excellent diagnostic accuracy (AUC = 0.880) and Relaxin showed moderate but significant potential (AUC = 0.758). Hematological alterations, such as increased platelets, lymphocytes, and WBCs, further supported the disease's inflammatory profile. These results highlight that whereas relaxin may suggest vascular adaptability, CD40 represents immunological activation. Their dual evaluation may improve risk assessment and early diagnosis. Combining the two indicators could enhance the atherosclerosis detection method beyond conventional lipid profiling.

Acknowledgement

The authors would like to sincerely thank the Open Heart Center staff for their invaluable assistance and collaboration. We also appreciate the willingness and dedication of all the patients who took part in this study.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The study was conducted as part of the requirements for the fulfillment of a Master's degree under the supervision of the College of Faculty of Science, University of Kufa.

Author's Contributions

Zahraa Ismail Abbas, the principal investigator, conducted the experimental work, data collection, statistical analysis, and drafted the manuscript. Dr. Haider Salih Jaffat , as the academic supervisor, provided scientific guidance, supervised the study design and interpretation, and revised the manuscript critically for intellectual content.
Both authors read and approved the final version of the manuscript prior to submission.

Ethics

This study was officially approved by the Department of Health in the Al-Najaf Governorate, Iraq, and was carried out in compliance with ethical standards. An administrative facilitation letter officially authorized the use of the open-heart unit at Al-Sadr Teaching Hospital for clinical sampling and data collecting. Every technique involving human subjects adhered to the Declaration of Helsinki's tenets, and each participant gave their written informed consent before being included in the study.

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