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Immunological Mechanisms Underlying Atherosclerosis in Iraq: A Cross-Sectional Study

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Annotation: **Background:** Atherosclerosis is a leading cause of cardiovascular morbidity and mortality in Iraq, with increasing prevalence in recent decades. While traditional risk factors contribute significantly to disease burden, the role of immunological mechanisms in the Iraqi population remains poorly characterized. This study aimed to investigate the relationship between specific inflammatory biomarkers and atherosclerotic disease severity in Iraqi patients.

Methods: A cross-sectional study was conducted including 240 Iraqi participants (160 with confirmed coronary atherosclerosis and 80 controls). Blood samples were analyzed for inflammatory markers, including high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and selected cell adhesion molecules. Coronary angiography findings were used to classify the severity of atherosclerosis. Demographic

data, lifestyle factors, and comorbidities were recorded.

Results: Patients with atherosclerosis demonstrated significantly elevated levels of hs-CRP (5.2±2.1 mg/L vs. 1.7±0.9 mg/L, $p<0.001$), IL-6 (7.8±3.6 pg/mL vs. 2.9±1.4 pg/mL, $p<0.001$), and TNF-α (18.3±7.5 pg/mL vs. 8.7±3.2 pg/mL, $p<0.001$) compared to controls. These inflammatory markers positively correlated with disease severity ($r=0.68$, 0.71, and 0.63, respectively, $p<0.001$). Multiple regression analysis identified IL-6 as the strongest independent predictor of severe atherosclerosis (OR=3.27, 95% CI: 2.14-5.02, $p<0.001$) after adjusting for traditional risk factors.

Conclusion: This study provides evidence of distinct immunological profiles associated with atherosclerosis in the Iraqi population. IL-6 emerged as a particularly important inflammatory mediator, potentially offering both a biomarker and therapeutic target for addressing the growing burden of atherosclerotic disease in Iraq.

Keywords: Atherosclerosis; immunology; inflammation; interleukin-6; Iraq; cardiovascular disease.

1. Introduction

Cardiovascular diseases (CVDs) are a significant public health issue in Iraq, where the pathophysiological basis for the majority of its clinical manifestations (e.g., atherosclerosis, coronary artery disease, stroke, and peripheral artery disease) is atherosclerosis (1). The disease burden in Iraq has changed dramatically over the past 30 years, with cardiovascular death rates increasing sharply after (1) years of conflict, (2) economic sanctions, and (3) major disruptions to the healthcare systems (2). It is currently estimated that CVDs account for roughly ~33% of all mortality in Iraq, compared to ~22% in the early 1990s, and mark a significant epidemiological transition that warrants thorough exploration, surveillance, and intervention.(3)

Atherosclerosis is fundamentally an inflammatory disease characterized by the slow and

progressive accumulation of lipids, immune cells, and extracellular matrix within arterial walls (4). The first stage of pathogenesis occurs with endothelial dysfunction and retention of oxidized apolipoprotein B-containing lipoproteins within the arterial intima, which initiates a maladaptive immune response (5). Immune activation is a complex process involving both innate and adaptive immune systems, and atherosclerotic plaques themselves are made chiefly of macrophage-derived foam cells, T lymphocytes, dendritic cells, and other immune and inflammatory mediators (6). Established cardiovascular risks for atherosclerosis include dyslipidemia, hypertension, diabetes, and smoking; however, a growing body of evidence supports the idea that immune dysregulation is both an independent risk factor for cardiovascular diseases and a crucial pathophysiological mechanism in atherosclerosis.(7)

The association between inflammation and atherosclerosis has been established through a plethora of epidemiological, clinical, and experimental studies (8). In this regard, elevated levels of inflammatory biomarkers, most notably high-sensitivity C-reactive protein (hs-CRP), have been shown to provide predictive information regarding cardiovascular events, independent of traditional risk factors, in diverse populations (9). Additionally, genetic and pharmacological studies targeting inflammatory pathways have provided strong evidence for a causal link between inflammation and atherosclerosis (10). The CANTOS trial, which demonstrated that interleukin-1β inhibition with canakinumab reduced the rates of cardiovascular events, independent of lipid lowering, represents a seminal moment in establishing a causal link between inflammation and atherosclerotic disease from a treatment perspective .(11)

Despite this global body of evidence, geographic differences in inflammatory markers persist, and inflammatory determinants of atherosclerosis exist that relate to differences in genetics, environmental exposure, lifestyle, and healthcare practices (12). Indeed, studies of neighboring Middle Eastern countries suggest that the inflammatory signature of atherosclerosis may differ from that of Western populations, with evidence of greater elevations of some pro-inflammatory cytokines and relatively higher baseline inflammatory states, even in the absence of clinical cardiovascular disease (13,14). This highlights the need for population-specific studies to delineate the immunological context of atherosclerosis in diverse geographic and ethnic settings.

The Iraqi population embodies an exciting opportunity to study the immune response involved in atherosclerosis. It is subject to unique combinations of stressors from decades of armed conflict, economic sanctions, environmental degradation, and psychosocial distress(15). Many studies have reported significantly heightened markers of inflammation in the general Iraqi population above the internationally accepted reference ranges, suggesting a possible "high-inflammatory background" that may modify the pathogenesis and progression of atherosclerosis(16). In addition, the relatively high burden of infectious diseases that includes (endemic) brucellosis, leishmaniasis, and historical prevalence of tuberculosis, may add to the state of systemic inflammation and potentially create conditions for atherogenesis as a consequence of immune cross-reactivity and associated inflammatory burden(17). The context of traditional cardiovascular risk factors and the inflammatory processes present in Iraq adds even more complexity. Epidemiological studies have reported increasing rates of obesity, diabetes, and metabolic syndrome within the Iraqi population, conditions of chronic low-grade inflammation(18). In a nationwide survey conducted in 2015, about 31% of Iraqi adults were hypertensive, 22% hypercholesterolemic, and 14% diabetic, with these conditions often present together and potentially working with immune inflammation pathways to promote atherosclerosis(19). The combination of these metabolic disorders on the background of these inflammatory contexts could create a particularly pro-atherogenic setting, which would benefit from understanding at the immunological level.

Previous studies on atherosclerosis in Iraq have primarily focused on clinical scenarios, typical risk factor status, and treatment outcomes, while rarely considering the immune effects (20). A small study by Al-Aubaidy et al. reported elevated markers of Oxidative Stress in Iraqi patients with coronary artery disease, which could imply immune activation with oxidation-specific epitopes (21). A study by Mohammed et al. correlated inflammatory markers with carotid intima-

media thickness in Iraqi patients with metabolic syndrome, without systematically characterizing the type of cytokines (22). These modest studies highlight the need to examine the immunological range of the individual Iraqi patients with atherosclerosis.

There are still significant gaps in defining the particular immune mediators relevant to atherosclerosis in the Iraqi patient population. Although hs-CRP and IL-6 have consistently demonstrated associations with atherosclerotic disease in various populations, their relative importance and predictive usefulness in the Iraqi context remain poorly understood (23). Additionally, the influence of other inflammatory mediators, including TNF-α, IL-1β, and various chemokines and adhesion molecules, is underdefined in this population. Defining inflammatory signatures may allow, for example, population-specific biomarkers for risk stratification and targeted treatment.

Beyond indices of circulating inflammation, cellular immune responses ultimately shape the development and progression of atherosclerosis. Central to the atherosclerotic milieu, macrophages adopt different polarization states (M1 pro-inflammatory vs. M2 anti-inflammatory) that are important for plaque formation, stability, and regression (24). T lymphocytes also play a range of roles. Th1 cells are well known to promote atherogenesis by producing interferon-gamma (IFN-γ), while regulatory T cells (Tregs) typically exhibit athero-protective benefits (25). The balance of the pro- and anti-inflammatory immune cell populations in Iraqi atherosclerotic patients is uninvestigated, which is a substantial information gap.

Moreover, considering the possibly complex effects of genetic differences on immune responses in atherosclerosis in the Iraqi population is also warranted. A variety of polymorphisms in the genes that contribute to inflammatory mediators and receptors have been shown in studies of other samples and populations to alter the susceptibility and progression of atherosclerosis (26). For example, polymorphisms in the IL-6 gene have been shown in specific populations to have differential cardiovascular risk (27). The genetic heritage of Iraq is also distinct due to its location at the intersection of migrations of multiple populations in demographic history. Potentially, unique variants may be affecting the immune regulation of atherosclerosis in Iraqi people (28). Characterization of the genetic impact of immune functions may elucidate probabilities for personalized assessments of risk and approaches to treatment.

Environmental factors specific to Iraq may further modulate immunologically mediated parameters related to atherosclerosis. Documented environmental contaminants, able to have pro-inflammatory effects and cause endothelial dysfunction, have been reported to exist in Iraq, including heavy metals and airborne particulate matter from industrial emissions or dust clouds from conflict (29). Psychological stressors from decades of conflict and instability may influence immune regulation through the neuroendocrine system and contribute to a pro-atherogenic immune milieu (30). Environmental impacts should be considered in any holistic approaches to atherosclerosis immunopathology in Iraq.

Understanding the immunological mechanisms that underpin atherosclerosis in Iraq has the potential to enhance prevention, diagnosis, and treatment approaches. If we can determine which inflammatory biomarkers are most pertinent, we can improve risk resource allocations and may even begin to reduce clinical presentations through timely interventions on vulnerable individuals (31). Suppose we characterize the unique immune pathways that predominate in Iraq. In that case, we can ultimately identify appropriate anti-inflammatory therapies, which, if and when they become available, will usher in a return to precision medicine for its atherosclerotic patients.(32)

Given these considerations, this study aimed to comprehensively characterize the immunological profiles associated with atherosclerosis in Iraqi patients and explore relationships between inflammatory biomarkers, immune cell populations, and the severity of the disease, alongside traditional risk factors. Addressing this information gap, we sought to enhance knowledge of the pathophysiology of atherosclerosis in Iraq and identify potential biomarkers and treatment targets for an increasingly urgent public health problem.

2. Methodology

2.1 Study Design and Setting

This cross-sectional study was conducted between March 2023 and February 2024. The study protocol was conducted in accordance with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment.

2.2 Study Population

A total of 240 participants were recruited for this study, comprising 160 patients with angiographically confirmed coronary atherosclerosis and 80 age- and sex-matched controls without evidence of atherosclerotic disease. The case group consisted of consecutive patients referred to the cardiac catheterization laboratory for diagnostic coronary angiography based on clinical indications (stable angina, unstable angina, or non-ST elevation myocardial infarction). Control subjects were recruited from individuals undergoing coronary angiography for evaluation of valvular heart disease, assessment of arrhythmias, or preoperative screening who demonstrated normal coronary arteries.

Exclusion criteria for both groups included: (1) recent acute coronary syndrome (within 4 weeks); (2) active infection or inflammatory condition; (3) autoimmune disease; (4) malignancy; (5) severe renal dysfunction (eGFR <30 mL/min/1.73m²); (6) hepatic impairment; (7) recent major surgery or trauma (within 8 weeks); (8) use of immunosuppressive medications; and (9) inability to provide informed consent.

2.3 Data Collection

Demographic information, medical history, and cardiovascular risk factors were documented using standardized questionnaires administered by trained research personnel. The collected data included age, sex, body mass index (BMI), smoking status, family history of premature cardiovascular disease, and comorbidities such as hypertension, diabetes mellitus, and dyslipidemia. Current medications were recorded with particular attention to statins, antihypertensives, and antidiabetic agents.

2.4 Coronary Angiography and Disease Classification

Coronary angiography was performed according to standard techniques via femoral or radial approach. Angiograms were evaluated by two experienced interventional cardiologists blinded to participants' clinical and laboratory data. Atherosclerosis severity was classified using the modified Gensini score, which considers both the degree of luminal narrowing and the functional significance of the coronary anatomy. Based on the Gensini score, patients were categorized into three groups: mild (score 1-15), moderate (score 16-32), and severe (score >32) atherosclerosis.

2.5 Laboratory Measurements

2.5.1 Blood Sampling

Venous blood samples were collected from all participants after an overnight fast of at least 12 hours. Samples were obtained before coronary angiography to avoid procedure-related inflammatory responses. Blood was collected in appropriate tubes for different analyses: EDTA tubes for complete blood count and flow cytometry, serum separator tubes for biochemical and inflammatory marker analysis, and PAXgene tubes for future gene expression studies.

2.5.2 Routine Laboratory Measurements

Complete blood count, fasting blood glucose, glycated hemoglobin (HbA1c), renal function tests, and liver function tests were performed using standard automated analyzers. Lipid profile including total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides was measured using enzymatic colorimetric methods.

2.5.3 Inflammatory Biomarkers

High-sensitivity C-reactive protein (hs-CRP) was measured using immunoturbidimetric assay on a Roche Cobas analyzer. Plasma levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interferon-gamma (IFN-γ), interleukin-10 (IL-10), and monocyte chemoattractant protein-1 (MCP-1) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Soluble adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were similarly measured using ELISA, All these kits were purchased from Mybiosource company.

2.5.4 Flow Cytometry Analysis

Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. Multicolor flow cytometry was performed to characterize monocyte subsets (classical CD14++CD16-, intermediate CD14++CD16+, and non-classical CD14+CD16++) and T lymphocyte populations (Th1, Th2, Th17, and regulatory T cells) using standardized protocols. Data were acquired on a BD FACSCanto II flow cytometer and analyzed using FlowJo software.

2.6 Statistical Analysis

Sample size was calculated to detect a difference of 1.5 mg/L in hs-CRP levels between cases and controls with 80% power and a significance level of 5%, based on previously published data. Statistical analyses were performed using SPSS version 27.0. Continuous variables were expressed as mean ± standard deviation or median (interquartile range) depending on the normality of distribution. Categorical variables were presented as numbers and percentages.

Comparisons between groups were performed using Student's t-test or the Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables, as appropriate. Correlations between inflammatory markers and Gensini score were assessed using Spearman's rank correlation coefficient. Multiple logistic regression analysis was performed to identify independent predictors of severe atherosclerosis, adjusting for traditional cardiovascular risk factors. A p-value of less than 0.05 was considered statistically significant for all analyses.

3. Results

3.1 Demographic and Clinical Characteristics

The demographic and clinical characteristics of the study population are presented in Table 1. The mean age was 58.7±9.4 years in the atherosclerosis group and 57.3±8.9 years in the control group (p=0.28). Male predominance was observed in both groups (68.1% in atherosclerosis group vs. 65.0% in control group, p=0.62). Traditional cardiovascular risk factors, including hypertension, diabetes mellitus, dyslipidemia, and smoking, were significantly more prevalent in patients with atherosclerosis compared to controls (p<0.001 for all comparisons). BMI was also significantly higher in the atherosclerosis group (29.8±4.3 kg/m² vs. 27.1±3.8 kg/m², p<0.001).

Table 1. Demographic and Clinical Characteristics of Study Participants

Characteristic	Atherosclerosis Group (n=160)	Control Group (n=80)	p-value
Age (years)	58.7 ± 9.4	57.3 ± 8.9	0.28
Male sex, n (%)	109 (68.1)	52 (65.0)	0.62
BMI (kg/m²)	29.8 ± 4.3	27.1 ± 3.8	<0.001
Hypertension, n (%)	112 (70.0)	29 (36.3)	<0.001
Diabetes mellitus, n (%)	87 (54.4)	18 (22.5)	<0.001
Dyslipidemia, n (%)	119 (74.4)	31 (38.8)	<0.001
Current smoking, n (%)	76 (47.5)	21 (26.3)	<0.001
Family history of CVD, n (%)	64 (40.0)	19 (23.8)	0.010

Medications, n (%)			
- Statins	138 (86.3)	19 (23.8)	<0.001
- Anti-platelets	147 (91.9)	17 (21.3)	<0.001
- ACE inhibitors/ARBs	105 (65.6)	23 (28.8)	<0.001
- Beta-blockers	118 (73.8)	14 (17.5)	<0.001
Lipid Profile			
- Total cholesterol (mg/dL)	187.4 ± 42.8	172.1 ± 36.5	0.006
- LDL-C (mg/dL)	117.6 ± 38.2	96.3 ± 31.4	<0.001
- HDL-C (mg/dL)	38.9 ± 10.3	46.7 ± 11.8	<0.001
- Triglycerides (mg/dL)	168.5 ± 72.4	142.6 ± 61.8	0.004

Data are presented as mean ± SD or n (%). BMI: body mass index; CVD: cardiovascular disease; ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blocker; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

3.2 Inflammatory Marker Profiles

Inflammatory marker levels in both study groups are summarized in Table 2. Patients with atherosclerosis demonstrated significantly higher levels of hs-CRP (5.2±2.1 mg/L vs. 1.7±0.9 mg/L, p<0.001), IL-6 (7.8±3.6 pg/mL vs. 2.9±1.4 pg/mL, p<0.001), and TNF-α (18.3±7.5 pg/mL vs. 8.7±3.2 pg/mL, p<0.001) compared to controls. Similarly, IL-1β, IFN-γ, and MCP-1 levels were significantly elevated in the atherosclerosis group (p<0.001 for all comparisons). Conversely, the anti-inflammatory cytokine IL-10 was significantly lower in patients with atherosclerosis (3.1±1.5 pg/mL vs. 5.4±2.3 pg/mL, p<0.001). Soluble adhesion molecules VCAM-1 and ICAM-1 were also significantly higher in the atherosclerosis group (p<0.001 for both comparisons).

Table 2. Comparison of Inflammatory Markers Between Study Groups

Inflammatory Marker	Atherosclerosis Group (n=160)	Control Group (n=80)	p-value
hs-CRP (mg/L)	5.2 ± 2.1	1.7 ± 0.9	<0.001
IL-6 (pg/mL)	7.8 ± 3.6	2.9 ± 1.4	<0.001
TNF-α (pg/mL)	18.3 ± 7.5	8.7 ± 3.2	<0.001
IL-1β (pg/mL)	4.9 ± 2.3	2.1 ± 1.1	<0.001
IFN-γ (pg/mL)	26.4 ± 11.7	14.5 ± 6.8	<0.001
IL-10 (pg/mL)	3.1 ± 1.5	5.4 ± 2.3	<0.001
MCP-1 (pg/mL)	425.7 ± 158.3	276.4 ± 112.6	<0.001
VCAM-1 (ng/mL)	875.3 ± 312.8	572.9 ± 204.7	<0.001
ICAM-1 (ng/mL)	386.5 ± 147.2	248.1 ± 91.3	<0.001

Data are presented as mean ± SD. hs-CRP: high-sensitivity C-reactive protein; IL: interleukin; TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma; MCP-1: monocyte chemoattractant protein-1; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1.

3.3 Relationship Between Inflammatory Markers and Atherosclerosis Severity

Based on the Gensini score, patients in the atherosclerosis group were categorized as having mild (n=42, 26.3%), moderate (n=61, 38.1%), or severe (n=57, 35.6%) disease. Table 3 presents inflammatory marker levels across these severity categories. A significant stepwise increase in pro-inflammatory markers (hs-CRP, IL-6, TNF-α, IL-1β, IFN-γ, MCP-1) and adhesion molecules (VCAM-1, ICAM-1) was observed with increasing atherosclerosis severity (p<0.001 for trend in all markers). Conversely, IL-10 levels demonstrated a significant stepwise decrease with increasing disease severity (p<0.001 for trend).

Table 3. Inflammatory Marker Levels According to Atherosclerosis Severity

Inflammatory Marker	Mild Atherosclerosis (n=42)	Moderate Atherosclerosis (n=61)	Severe Atherosclerosis (n=57)	p for trend
hs-CRP (mg/L)	3.8 ± 1.5	5.1 ± 1.7	6.4 ± 2.2	<0.001
IL-6 (pg/mL)	5.1 ± 2.2	7.4 ± 2.8	10.3 ± 3.7	<0.001
TNF-α (pg/mL)	13.4 ± 5.1	17.8 ± 6.3	22.9 ± 7.8	<0.001
IL-1β (pg/mL)	3.4 ± 1.5	4.6 ± 1.9	6.3 ± 2.5	<0.001
IFN-γ (pg/mL)	19.2 ± 8.1	25.9 ± 9.8	32.9 ± 12.5	<0.001
IL-10 (pg/mL)	4.0 ± 1.6	3.2 ± 1.3	2.3 ± 1.1	<0.001
MCP-1 (pg/mL)	318.6 ± 112.4	418.3 ± 135.2	521.4 ± 172.8	<0.001
VCAM-1 (ng/mL)	687.2 ± 221.5	854.6 ± 274.1	1052.7 ± 338.6	<0.001
ICAM-1 (ng/mL)	302.8 ± 96.7	374.6 ± 127.3	468.5 ± 153.9	<0.001

Data are presented as mean ± SD. hs-CRP: high-sensitivity C-reactive protein; IL: interleukin; TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma; MCP-1: monocyte chemoattractant protein-1; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1.

Spearman correlation analysis revealed significant positive correlations between Gensini score and levels of hs-CRP ($r=0.68$, $p<0.001$), IL-6 ($r=0.71$, $p<0.001$), TNF-α ($r=0.63$, $p<0.001$), IL-1β ($r=0.59$, $p<0.001$), IFN-γ ($r=0.57$, $p<0.001$), MCP-1 ($r=0.62$, $p<0.001$), VCAM-1 ($r=0.60$, $p<0.001$), and ICAM-1 ($r=0.56$, $p<0.001$). A significant negative correlation was observed between the Gensini score and IL-10 levels ($r = -0.54$, $p < 0.001$).

3.4 Immune Cell Population Analysis

Flow cytometry analysis of monocyte subsets and T lymphocyte populations revealed significant differences between atherosclerosis patients and controls, as shown in Table 4. Patients with atherosclerosis demonstrated a higher proportion of intermediate (CD14++CD16+) and non-classical (CD14+CD16++) monocytes compared to controls ($p<0.001$ for both comparisons). Regarding T lymphocyte populations, patients with atherosclerosis showed higher percentages of Th1 and Th17 cells and lower percentages of regulatory T cells compared to controls ($p<0.001$ for all comparisons).

Table 4. Comparison of Immune Cell Populations Between Study Groups

Cell Population	Atherosclerosis Group (n=160)	Control Group (n=80)	p-value
Monocyte subsets (% of total monocytes)			
- Classical (CD14++CD16-)	75.3 ± 8.4	84.6 ± 6.7	<0.001
- Intermediate (CD14++CD16+)	15.8 ± 5.2	9.1 ± 3.5	<0.001
- Non-classical (CD14+CD16++)	8.9 ± 3.4	6.3 ± 2.8	<0.001
T lymphocyte populations (% of CD4+ T cells)			
- Th1 cells	18.7 ± 5.9	12.4 ± 4.1	<0.001

- Th2 cells	7.3 ± 2.5	8.1 ± 2.7	0.027
- Th17 cells	6.4 ± 2.2	3.8 ± 1.7	<0.001
- Regulatory T cells	3.9 ± 1.5	6.7 ± 2.3	<0.001

Data are presented as mean ± SD. Th: T helper.

3.5 Multivariate Analysis

Multiple logistic regression analysis was performed to identify independent predictors of severe atherosclerosis (Gensini score >32) after adjusting for traditional cardiovascular risk factors (age, sex, hypertension, diabetes mellitus, dyslipidemia, smoking, and BMI). As shown in Table 5, IL-6 emerged as the strongest independent inflammatory predictor of severe atherosclerosis (OR=3.27, 95% CI: 2.14-5.02, p<0.001), followed by TNF-α (OR=2.46, 95% CI: 1.62-3.74, p<0.001) and hs-CRP (OR=2.18, 95% CI: 1.43-3.32, p<0.001). Among immune cell populations, the percentage of intermediate monocytes (OR=1.98, 95% CI: 1.31-2.99, p=0.001) and the Th17/Treg ratio (OR=2.84, 95% CI: 1.87-4.32, p<0.001) were independently associated with severe atherosclerosis.

Table 5. Multiple Logistic Regression Analysis for Predictors of Severe Atherosclerosis

Variable	Odds Ratio	95% CI	p-value
Traditional risk factors			
- Age (per 10 years)	1.42	1.15-1.76	0.001
- Male sex	1.69	1.08-2.65	0.022
- Hypertension	1.73	1.12-2.68	0.014
- Diabetes mellitus	2.14	1.36-3.37	0.001
- Dyslipidemia	1.96	1.25-3.08	0.003
- Current smoking	1.85	1.18-2.91	0.007
- BMI (per 5 kg/m²)	1.39	1.11-1.74	0.004
Inflammatory markers			
- hs-CRP (per SD increase)	2.18	1.43-3.32	<0.001
- IL-6 (per SD increase)	3.27	2.14-5.02	<0.001
- TNF-α (per SD increase)	2.46	1.62-3.74	<0.001
- IL-1β (per SD increase)	1.73	1.14-2.62	0.010
- IL-10 (per SD increase)	0.56	0.37-0.85	0.006
Immune cell populations			
- Intermediate monocytes (per SD increase)	1.98	1.31-2.99	0.001
- Th17/Treg ratio (per SD increase)	2.84	1.87-4.32	<0.001

CI: confidence interval; BMI: body mass index; SD: standard deviation; hs-CRP: high-sensitivity C-reactive protein; IL: interleukin; TNF-α: tumor necrosis factor-alpha; Th17: T helper 17 cells; Treg: regulatory T cells.

4. Discussion

This cross-sectional study provides a comprehensive characterization of the immunological mechanisms underlying atherosclerosis in the Iraqi population, demonstrating distinct inflammatory profiles and immune cell distributions associated with disease presence and severity. Our findings reveal significantly elevated levels of pro-inflammatory markers and adhesion molecules, altered monocyte subset distribution, and T lymphocyte polarization toward pro-inflammatory phenotypes in Iraqi patients with coronary atherosclerosis. Notably, IL-6 emerged as the strongest independent inflammatory predictor of severe atherosclerosis, suggesting its potential utility as both a biomarker and therapeutic target in this population.

The robust associations observed between inflammatory markers and atherosclerosis in our Iraqi cohort align with the fundamental understanding of atherosclerosis as an inflammatory

disease(33). However, the magnitude of these associations appears particularly pronounced compared to reports from Western populations. For instance, the mean hs-CRP level in our atherosclerosis patients (5.2 ± 2.1 mg/L) exceeds the threshold of 3 mg/L traditionally considered high risk in Western guidelines and is notably higher than levels reported in comparable European and North American studies (34). This observation supports previous suggestions of a "high-inflammatory background" in the Iraqi population, potentially attributable to unique environmental exposures, a chronic infectious burden, and psychosocial stressors related to decades of conflict and instability (35).

IL-6 demonstrated the strongest correlation with atherosclerosis severity (r = 0.71) and emerged as the most powerful independent predictor of severe disease in our multivariate analysis (OR = 3.27). This finding is particularly noteworthy given the central role of IL-6 in orchestrating both acute and chronic inflammatory responses through multiple mechanisms, including hepatic stimulation of acute-phase protein production, activation of endothelial cells, and regulation of immune cell differentiation(36). The CANTOS trial demonstrated that targeting the IL-1β/IL-6 pathway with canakinumab reduced cardiovascular events independently of lipid-lowering, underscoring the therapeutic potential of modulating this pathway (37). Our results suggest that IL-6-targeted therapies, such as tocilizumab, might warrant investigation specifically in the Iraqi context, where this pathway appears particularly relevant.

The observed elevation of TNF-α levels in our atherosclerosis patients (18.3±7.5 pg/mL vs. 8.7±3.2 pg/mL in controls) represents another notable finding. TNF-α promotes endothelial dysfunction, enhances leukocyte recruitment, and stimulates smooth muscle cell proliferation—all processes central to atherosclerosis progression(38). While anti-TNF therapies have demonstrated mixed results in cardiovascular risk reduction in rheumatoid arthritis patients, our findings suggest that TNF-α might play a more prominent role in primary atherosclerosis in the Iraqi population(39). Further investigation into potential TNF-α genetic polymorphisms prevalent in this population could provide insights into these observations.

The significant reduction in IL-10 levels observed in atherosclerosis patients compared to controls (3.1±1.5 pg/mL vs. 5.4±2.3 pg/mL) highlights the importance of anti-inflammatory counterregulation in atherosclerosis pathogenesis. IL-10 exerts atheroprotective effects through multiple mechanisms, including suppression of pro-inflammatory cytokine production, inhibition of matrix metalloproteinases, and promotion of regulatory T cell function(40). The relatively low IL-10 levels in our atherosclerosis patients suggest a potential deficiency in counterregulatory mechanisms that might normally limit inflammation-driven atherosclerosis progression.

Our flow cytometry analyses revealed significant alterations in both monocyte subsets and T lymphocyte populations in atherosclerosis patients. The observed increase in intermediate (CD14++CD16+) and non-classical (CD14+CD16++) monocytes aligns with emerging evidence regarding the distinct roles of these subsets in atherosclerosis(41). Intermediate monocytes, in particular, express high levels of pattern recognition receptors and produce substantial amounts of pro-inflammatory cytokines upon stimulation, potentially contributing to plaque development and instability(42). The independent association between intermediate monocyte percentage and severe atherosclerosis in our multivariate analysis (OR=1.98) supports the pathophysiological relevance of this subset in the Iraqi context.

The T lymphocyte profile characterized by increased Th1 and Th17 cells and decreased regulatory T cells in atherosclerosis patients reflects the complex interplay between different T cell subtypes in disease pathogenesis. Th1 cells promote atherogenesis through interferon-gamma production, while Th17 cells contribute via IL-17 and other pro-inflammatory cytokines(43). Conversely, regulatory T cells exert atheroprotective effects through various mechanisms, including IL-10 and transforming growth factor-beta production(44). The strong independent association between the Th17/Treg ratio and severe atherosclerosis (OR=2.84) observed in our study suggests that this imbalance represents a particularly important immunological feature in Iraqi patients.

The relationships between traditional cardiovascular risk factors and inflammatory markers in our cohort deserve consideration. While diabetes mellitus, hypertension, dyslipidemia, and smoking all remained significant independent predictors of severe atherosclerosis in our multivariate model, inflammatory markers—particularly IL-6—demonstrated comparable or even stronger associations. This observation suggests that inflammation might contribute substantially to atherosclerosis development in this population, potentially amplifying the effects of traditional risk factors or even serving as an independent pathway. The relatively high prevalence of metabolic disorders in our cohort (54.4% diabetes, 70.0% hypertension, 74.4% dyslipidemia) aligns with nationwide surveys documenting increasing metabolic disease burden in Iraq(45).

Several aspects of our findings may reflect specific characteristics of the Iraqi population. The Middle Eastern region has documented genetic variations influencing both inflammatory responses and cardiovascular risk, including polymorphisms in genes encoding cytokines and their receptors(46). Additionally, the protracted conflict, sanctions, and resulting healthcare system disruption experienced by Iraq over recent decades have created unique environmental and psychosocial exposures that may influence immune regulation(47). The relatively high background inflammatory state observed in our control group compared to international reference ranges supports this hypothesis, though direct comparative studies with other populations would be necessary to confirm these differences.

The clinical implications of our findings are substantial. First, the strong associations between inflammatory markers—particularly IL-6—and atherosclerosis severity suggest potential utility in risk stratification beyond traditional factors. Incorporating these biomarkers into cardiovascular risk assessment could enhance identification of high-risk individuals requiring intensive preventive interventions. Second, the detailed characterization of immune cell populations associated with atherosclerosis provides potential cellular targets for monitoring disease progression and therapeutic response. Finally, the identification of specific inflammatory pathways predominant in this population could guide the selection of anti-inflammatory therapies as they become increasingly available for cardiovascular disease management.

Our study has several strengths, including its comprehensive assessment of multiple inflammatory markers and immune cell populations, the use of angiographic quantification of atherosclerosis severity, and the careful exclusion of conditions that might confound inflammatory profiles. The inclusion of age and sex-matched controls without atherosclerosis allowed for meaningful comparisons and identification of disease-specific inflammatory signatures.

However, several limitations should be acknowledged. The cross-sectional design precludes establishment of causality between inflammatory markers and atherosclerosis development or progression. The single-center nature of the study and the selection of patients referred for coronary angiography may limit generalizability to the broader Iraqi population. While we adjusted for major cardiovascular risk factors and medications in our multivariate analyses, residual confounding cannot be excluded. Additionally, our study did not assess genetic factors that might influence inflammatory profiles and atherosclerosis susceptibility.

These limitations highlight important directions for future research. Longitudinal studies are needed to establish the predictive value of the identified inflammatory markers for cardiovascular events in the Iraqi population. Investigation of genetic variants influencing inflammatory responses could provide insights into the observed associations and potentially identify high-risk subgroups. Intervention studies targeting specific inflammatory pathways, particularly the IL-6 axis, could evaluate the therapeutic potential of anti-inflammatory approaches in this population. Finally, comparative studies with other Middle Eastern and international cohorts would clarify whether the observed inflammatory profiles represent unique features of the Iraqi population or reflect broader regional patterns.

In conclusion, our study provides evidence of distinct immunological profiles associated with atherosclerosis in the Iraqi population, characterized by pronounced elevations in pro-

inflammatory markers—particularly IL-6—and alterations in monocyte subsets and T lymphocyte populations. These findings enhance understanding of atherosclerosis pathophysiology in this specific context and identify potential biomarkers and therapeutic targets for addressing the growing burden of cardiovascular disease in Iraq.

5. Conclusion

This cross-sectional study provides a comprehensive characterization of the immunological mechanisms underlying atherosclerosis in the Iraqi population. Our findings demonstrate significantly elevated levels of pro-inflammatory markers—particularly IL-6, TNF-α, and hs-CRP—in patients with coronary atherosclerosis, with these elevations correlating strongly with disease severity. Flow cytometry analysis revealed altered distributions of monocyte subsets and T lymphocyte populations, with increased intermediate monocytes and a skewed Th17/Treg balance independently associated with severe atherosclerosis.

IL-6 emerged as the strongest independent inflammatory predictor of severe atherosclerosis after adjusting for traditional cardiovascular risk factors, suggesting its potential utility as both a biomarker and therapeutic target in this population. The magnitude of inflammatory marker elevations observed in our cohort supports previous suggestions of a "high-inflammatory background" in the Iraqi population, potentially attributable to unique environmental exposures, infectious burden, and psychosocial stressors.

These findings have important clinical implications for cardiovascular risk assessment and management in Iraq. Incorporating inflammatory biomarkers, particularly IL-6, into risk stratification algorithms could enhance identification of high-risk individuals requiring intensive preventive interventions. Furthermore, the detailed characterization of predominant inflammatory pathways provides a foundation for investigating targeted anti-inflammatory therapies tailored to the specific immunological profile of Iraqi patients with atherosclerosis.

Future longitudinal studies are needed to establish the predictive value of these inflammatory markers for cardiovascular events in the Iraqi population and to evaluate the therapeutic potential of targeting specific immune pathways. Nevertheless, this study advances understanding of atherosclerosis pathophysiology in the Iraqi context and identifies promising avenues for addressing the growing burden of cardiovascular disease in this population.

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