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ORIGINAL ARTICLE

Potential Biomarkers of Leptin and Insulin Resistance in Clinical-subclinical Coronary Atherosclerosis and Related With Diabetes Mellitus in Male Patients: Cross-section Study

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Abstract

Background: **Atherosclerosis is an inflammatory disease of the large arteries, a buildup of fatty plaque inside the arteries, leading to narrowing and hardening of the blood vessels.**

Objective: **Find the relation of leptin and insulin resistance in clinical-subclinical coronary atherosclerosis and its relationship with diabetes mellitus.**

Material and method: **The cross-sectional study includes 90 males with a mean age of 33.00 ± 6.131. They were used in the present study and divided into 45 patients with clinical coronary atherosclerosis, with a mean age of 34.500 ± 4.949, and 45 patients with subclinical coronary atherosclerosis, with a mean age of 32.250 ± 7.228, with ages ranging from 20 to 60 years. Serum concentrations of leptin (LEP), insulin (INS), and C-peptide (C-P) were measured using commercially available ELISA kits (Elabscience, Wuhan, China), following the manufacturers' protocols. All assays were based on the sandwich ELISA principle, where specific antibodies pre-coated onto microplate wells captured the target analytes. Subsequently, biotinylated detection antibodies and Avidin-HRP conjugates (or HRP-conjugated antibodies) were added, forming an immune complex. After washing to remove unbound components, a substrate solution was added, producing a colorimetric reaction. The absorbance was measured spectrophotometrically at 450 ± 2 nm. The concentrations of the biomarkers were calculated by comparing the optical density (OD) of the samples to the standard curve. HOMA-IR is calculated by the equation HOMA-IR (Homeostatic Model of Insulin Resistance) = (Insulin (μU) * Glucose (mg/dl)) ÷ 405.**

Result: **The result found a highly significant increase ($p \leq 0.01$) in leptin, C-peptide, and HOMA-IR (223.963 ± 66.507, 123.614 ± 32.939, and 2.137 ± 1.059, respectively) in clinical atherosclerosis patients compared to the subclinical atherosclerosis group (93.112 ± 12.853, 40.291 ± 9.174, and 0.760 ± 0.091, respectively). Significant increases ($p \leq 0.01$) in random glucose, insulin, and HbA1C and lipid profiles revealed significant increases ($p \leq 0.01$) in TC, TG, LDL, and VLDL in clinical atherosclerosis as compared to subclinical atherosclerosis. Leptin showed the highest diagnostic accuracy with an AUC of 99.111 %, sensitivity of 91.121 %, and specificity of 97.678 % at a cutoff value of 125.148 pg/ml. Similarly, HOMA-IR also exhibited excellent predictive performance (AUC 97.037 %, sensitivity 91.111 %, specificity 97.778 %), suggesting its strong association with advanced atherosclerotic changes. In contrast, HbA1c showed lower diagnostic power (AUC 68.370 %, sensitivity 71.111 %, specificity 60.000 %), indicating it may be less reliable as a standalone biomarker for atherosclerosis.**

Conclusion: **The increase in serum Leptin and HOMA-IR in Atherosclerosis patients are related to early predicting cardiovascular complications.**

Keywords: Leptin, Insulin resistance HOMA-IR, Atherosclerosis, Diabetes mellitus

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Introduction

Atherosclerosis is a major cardiovascular condition characterized by the formation of fibrous plaques on arterial walls, leading to morbidity and mortality (Al-Shamma & El-Yassin, 2011; Perrotta, 2022). Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both, it is a significant public health challenge, affecting millions globally and contributing to high morbidity and mortality rates (Abdul-Hussain et al., 2024; Yameny, 2024). Prolonged hyperglycemia is a major contributor to atherosclerosis in diabetes, it leads to non-enzymatic glycation of proteins and lipids, which disrupts their normal function and promotes oxidative stress and inflammation (Al-Fatlawi, 2022a; Beverly & Budoff, 2020) (see Figs. 1 and 2).

HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) is a method used to estimate insulin resistance in the body. It is calculated using fasting blood glucose and insulin levels. Higher HOMA-IR values indicate greater insulin resistance, which is commonly associated with conditions like type 2 diabetes and metabolic syndrome (Wan et al., 2025).

Subclinical atherosclerosis refers to the early stages of atherosclerosis in which structural changes occur in the blood vessels without causing noticeable symptoms. At this stage, fatty deposits (plaques) begin to accumulate in the arterial walls, but they have not yet caused significant narrowing or blockage that would lead to clinical signs like chest pain (angina), heart attack, or stroke (Mszar et al., 2024).

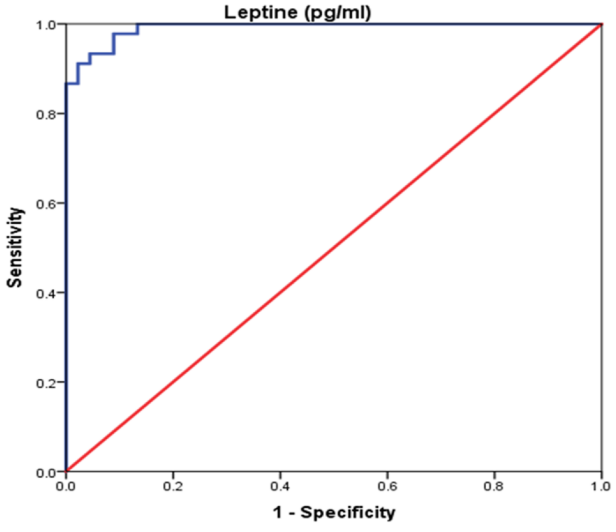

Fig. 1. ROC curve analysis of Leptine predictive accuracy for atherosclerosis detection.

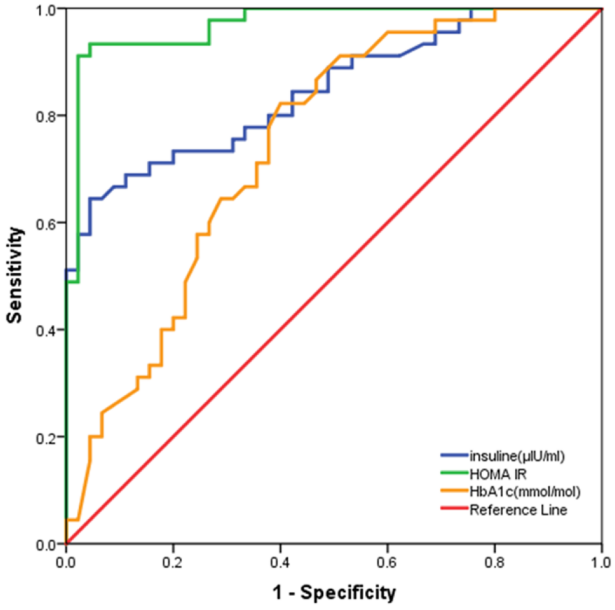

Fig. 2. ROC curve analysis of glucose parameters predictive accuracy for atherosclerosis detection.

Clinical atherosclerosis refers to the symptomatic and advanced stage of atherosclerosis, in which the buildup of fatty plaques inside the arteries has progressed enough to cause measurable blood flow restriction and lead to clinical events or symptoms (Zubirán et al., 2024).

Insulin resistance (IR) plays a significant role in the development and progression of atherosclerosis, a major contributor to cardiovascular disease, the relationship between IR and atherosclerosis is complex, involving multiple pathways and mechanisms that exacerbate vascular damage, insulin resistance (IR) is a complex metabolic condition characterized by the reduced ability of insulin to promote glucose uptake and utilization in tissues, despite normal or elevated levels of insulin in the bloodstream, it is a precursor to several metabolic disorders, including type 2 diabetes mellitus, metabolic syndrome, and nonalcoholic fatty liver disease (Lee et al., 2022).

Leptin is a multifaceted hormone primarily produced by adipocytes, playing a crucial role in regulating energy homeostasis, body mass, and various physiological processes, it acts by binding to leptin receptors (LEPR) distributed throughout the body, influencing functions such as appetite control, immune response, and metabolic regulation (Al-Fatlawi, 2022b; Hryciw & Singh, 2022).

Leptin and insulin resistance are intricately linked to the development of atherosclerosis,

particularly in the context of obesity and type 2 diabetes, elevated leptin levels, often seen in obesity, are associated with insulin resistance and contribute to the pathogenesis of atherosclerosis by promoting pro-inflammatory and pro-atherogenic effects on vascular cells (Dagogo-Jack, 2024).

The aim of the study compare serum Leptin concentration in subclinical –clinical atherosclerosis patients and related with disease.

Material and methods

A Cross-sectional study includes (90) males with range of age (20≥60) mean of age (33.00 ± 6.131) were used in the present study divided into (45) patients with clinical coronary atherosclerosis mean of age (34.500 ± 4.949) and (45) patients with subclinical coronary atherosclerosis mean of age (32.250 ± 7.228). was conducted to evaluate the clinical and sub-clinical coronary atherosclerosis males related with diabetes type 2 in Karbala, Iraq. Diabetes can be classified into three main categories: non-diabetes (normal), prediabetes, and diabetes (Xiong et al., 2025). The data was collected and recorded from September 2024 to December 2024 at the Imam Hussein Karbala Center for Heart Diseases and Surgery Teaching Hospital. Diseases were diagnosed through laboratory tests, monitoring of some clinical symptoms, and all patients diagnostic by conventional coronary angiography or CT scan by cardiologist in addition to collecting information for each group according to the questionnaire. The exclusion of this study includes Female, Patients with a history of an endocrinal disease, cancers, liver disease, nephropathy, alcohol drinking. The inclusion male patients have clinical or sub-clinical coronary atherosclerosis.

Five ml (milliliters) of venous blood samples were obtained from each fasting patients via vein puncture with a sterile syringe, and all samples were identified by their specific numbers (ID) generated by the laboratory information system. 2 ml of blood was put in the EDTA tube (an ethylene diamine tetra acetic acid) to prevent clotting of blood for hematological parameters to measure HbA1C, Fasting blood glucose other 3 ml of blood put in the jell plain tube and let clot (to get the serum) then, centrifuged at (4000 rpm) for 10 min.

Serum levels of insulin by insulin kit (Elabsciences, Wuhana, China)by using ELISA kit uses the Sandwich-ELISA, HbA1C by using (OSN,Korea) measurement by using automated chemistry analyzer by Cobas intgra 400 plus, Lipid profile kit (Linear Chemicals S.L, Spain) was measured by using Enzymatic colorimetric method technique, Homeostatic Model Assessment of Insulin Resistance Calculated by following formula: Homeostatic Model Assessment of Insulin.

Resistance (HOMA-IR = fasting plasma insulin (μU/ml) × fasting plasma glucose (mg/dl)/405) (Wongwananuruk et al., 2025) was calculated as a marker of insulin resistance.

Leptin ELISA kit

Allow samples to clot for 1 h at room temperature or overnight at 2–8°C before centrifugation for 20 min at 1000 × *g* at 2–8 °C. Collect the supernatant to carry out the assay.

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human LEP.

C-peptide test

Principle of the assay The Eagle Biosciences Human C-Peptide ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human C-peptide in serum and/or EDTA-plasma samples. The C-Peptide ELISA Assay Kit utilizes the “sandwich” technique with selected antibodies that bind to various epitopes of C-peptide.

Statistical analysis

Statistical analysis was performed using SPSS software, version 23. The results were presented through descriptive statistics, including the calculation of means and standard deviations. To determine the statistical significance of the findings, a p-value threshold of 0.05 was applied. The normality of the data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. To explore relationships between categorical and numerical variables, chi-square tests and Pearson correlation analyses were employed. Differences between two independent groups were assessed using the Independent Samples t-test and the Mann–Whitney U test, depending on data distribution. For comparisons across multiple groups, one-way analysis of variance (ANOVA) was utilized, followed by Scheffé’s post-hoc test for pairwise comparisons. Additionally, Receiver Operating Characteristic (ROC) curve analysis was conducted to determine optimal cut-off values for identifying critical cases. The predictive accuracy of parameters was evaluated using the Area Under the Curve (AUC), with

Youden's index used to select the optimal thresholds. Data points with a p-value less than 0.01 were marked with asterisks to indicate high statistical significance. Ethical approval for the study was obtained with the Approval Number: IQ.UOK. CAMS. DCL.REC.8.

Result

In the results found a significant decrease in the Random glucose, insulin, HOMA IR, HbA1C, C-peptide, Leptin in sub clinical atherosclerosis patients (119.677 ± 13.521), (2.582 ± 0.238), (0.760 ± 0.091), (7.240 ± 2.747), (40.291 ± 9.174), (93.112 ± 12.853) as compared to clinical atherosclerosis patients (204.484 ± 47.004), (4.175 ± 1.667), (2.137 ± 1.059), (8.893 ± 2.462), (123.614 ± 32.939), (223.963 ± 66.507) while the level of fasting plasma glucose in clinical atherosclerosis non-significant increase (223.629 ± 84.402) compared to Sub-Clinical Atherosclerosis patients (199.222 ± 100.145) as in Table (1).

The results show a significant decrease ($p \leq 0.01$) in Cholesterol, Triglyceride, Low Density Lipoprotein, Very Low Density Lipoprotein Levels in sub clinical (197.785 ± 19.577), (208.802 ± 20.866), (96.370 ± 19.841), (41.760 ± 4.173) respectively, as compared to clinical atherosclerosis patients (247.170 ± 28.155), (249.431 ± 20.182), (156.089 ± 27.699), (49.889 ± 4.036) respectively, while significant decrease in level of high density lipoprotein in clinical atherosclerosis patients (41.195 ± 8.828) as compared to sub clinical atherosclerosis patients (59.654 ± 9.309) as in Table (2).

In the results found a significant decrease in leptin ($p \leq 0.01$) in subclinical age groups, Family history, smoking state, address area, (89.684 ± 12.407) in age group (40–59) and (97.030 ± 12.497) in age group (60–79), (88.638 ± 9.510) in none diabetic, (94.930 ± 13.698) in diabetic, in none smoking (91.121 ± 14.337) and (93.681 ± 12.564) in smoking, (91.828 ± 12.367) in Rural and (94.051 ± 13.359) in Urban, as compared to clinical atherosclerosis patients (137.964 ± 67.853) in age group (40–59)and (214.629 ± 65.184) age group (60–79), in none diabetic (206.077 ± 53.053), (232.041 ± 71.071)in diabetic, (252.272 ± 62.077) in none smoking and (214.804 ± 66.154) in smoking, in Rural (230.206 ± 71.237) and (219.801 ± 64.206) in Urban patients as in Table (3).

In the results found a significant decrease in HOMA-IR ($p \leq 0.01$) in subclinical age groups, Family history, smoking state, address area, (0.738 ± 0.081) in age group (40–59)and (0.785 ± 0.097) in age group (60–79), (0.750 ± 0.091) in none diabetic, (0.764 ± 0.092) in diabetic, in none smoking (0.768 ± 0.077) and (0.758 ± 0.096) in smoking, (0.763 ± 0.094) in Rural and (0.758 ± 0.090) in Urban, as compared to clinical atherosclerosis patients (1.821 ± 0.875) in age group (40–59) and (2.348 ± 1.133) age group (60–79), in none diabetic (1.923 ± 1.100), (2.234 ± 1.045) in diabetic, (1.949 ± 1.010) in none smoking and (2.198 ± 1.083) in smoking, in Rural (2.366 ± 0.861) and (1.985 ± 1.164) in Urban patients as in Table (4).

In the results found a significant decrease in cholesterol, triglyceride, low density lipoprotein, very low lipoprotein ($p \leq 0.01$) in sub clinical atherosclerosis patients with in no diabetic, pre diabetic, diabetic (198.964 ± 23.844), (189.877 ± 15.378), (200.854 ± 17.682), (213.597 ± 17.440), (200.355 ± 24.0855), (209.429 ± 21.290), (94.579 ± 24.995),

Table 1. Comparison of different glucose test, insulin, C-peptide and Leptin between patients with subclinical and clinical atherosclerosis patients.

Parameters	Subclinical (Mean ± SD)	Clinical (Mean ± SD)	P value
Fasting plasma glucose (mg/dl)	199.222 ± 100.145	223.629 ± 84.402	0.21457
glucose (mg/dl)	119.677 ± 13.521	204.484 ± 47.004	0.00005
Insulin (μIU/ml)	2.582 ± 0.238	4.175 ± 1.667	0.00010
HOMA IR	0.760 ± 0.091	2.137 ± 1.059	0.00004
HbA1c (mmol/mol)	7.240 ± 2.747	8.893 ± 2.462	0.00344
C-peptide (pg/ml)	40.291 ± 9.174	123.614 ± 32.939	0.00007
Leptin (pg/ml)	93.112 ± 12.853	223.963 ± 66.507	0.00004

Table 2. Comparison of lipid profile between patients subclinical and clinical atherosclerosis.

Parameters	Subclinical (Mean ± SD)	Clinical (Mean ± SD)	P value
Total cholesterol (mg/dl)	197.785 ± 19.577	247.170 ± 28.155	0.00005
Total triglycerides (mg/dl)	208.802 ± 20.866	249.431 ± 20.182	0.00007
Low density lipoprotein (mg/dl)	96.370 ± 19.841	156.089 ± 27.699	0.00010
High density lipoprotein (mg/dl)	59.654 ± 9.309	41.195 ± 8.828	0.00002
Very low density lipoprotein (mg/dl)	41.760 ± 4.173	49.886 ± 4.036	0.00007

Table 3. Comparison of leptin levels in patients with clinical and subclinical atherosclerosis based on demographic criteria.

Leptin				
Demographic	Groups	Subclinical (Mean ± SD)	Clinical (Mean ± SD)	P value
Age	40–59	89.684 ± 12.407	237.964 ± 67.853	0.00000
	60–79	97.030 ± 12.497	214.629 ± 65.184	0.00000
Family history of diabetes mellitus	No	88.638 ± 9.510	206.077 ± 53.053	0.00000
	Yes	94.930 ± 13.698	232.041 ± 71.071	0.00000
Smoking state	None Smoker	91.121 ± 14.337	252.272 ± 62.077	0.00000
	Smoker	93.681 ± 12.564	214.804 ± 66.154	0.00000
Address area	Rural	91.828 ± 12.367	230.206 ± 71.237	0.00000
	Urban	94.051 ± 13.359	219.801 ± 64.206	0.00000

(93.241 ± 17.816), (99.279 ± 16865), (40.071 ± 4.817), (41.886 ± 4.258) as compared to clinical (252.036 ± 20.884), (249.010 ± 29.543), (245.529 ± 29.634), (238.369 ± 32.163), (253.307 ± 22.472), (250.383 ± 16.141), (157.337 ± 19.156), (156.083 ± 31.249), (155.833 ± 28.738), (50.661 ± 4.494), (50.077 ± 3.228), while In the results found a significant increase in HDL ($p \leq 0.01$) in sub clinical atherosclerosis patients with in no diabetic, pre diabetic, diabetic (61.666 ± 8.281), (56.565 ± 9.784), (59.689 ± 9.784) as compared to clinical (47.025 ± 6.061), (42.266 ± 9.687), (39.619 ± 8.688), while as in Table (5).

The result found high significant increase ($P \leq 0.01$) in most glucose metabolic markers includes: in random diabetic glucose in all clinical groups, no-diabetes, pre-diabetes, and diabetes (148.008 ± 23.694), (183.001 ± 35.115), (223.577 ± 41.827) as compared to subclinical (115.090 ± 10.264), (116.678 ± 13.350), (124.618 ± 14.648) respectively. Also found significant increase ($P \leq 0.01$) in an Insulin for pre-diabetes and diabetes (4.008 ± 1.866), (4.321 ± 1.634) of clinical groups as compared to subclinical (2.594 ± 0.329), (2.585 ± 0.204) respectively. HOMA-IR showed significant increase ($P \leq 0.01$) in all clinical groups (no

Table 4. Comparison of HOMA-IR levels in patients with clinical and subclinical atherosclerosis based on demographic criteria.

HOMA-IR				
Demographic	Groups	Subclinical (Mean ± SD)	Clinical (Mean ± SD)	P value
Age	40–59	0.738 ± 0.081	1.821 ± 0.875	0.00006
	60–79	0.785 ± 0.097	2.348 ± 1.133	0.00001
Family history of diabetes mellitus	No	0.750 ± 0.091	1.923 ± 1.100	0.00153
	Yes	0.764 ± 0.092	2.234 ± 1.045	0.00001
Smoker state	None smoker	0.768 ± 0.077	1.949 ± 1.010	0.00305
	Smoker	0.758 ± 0.096	2.198 ± 1.083	0.00001
Address area	Rural	0.763 ± 0.094	2.366 ± 0.861	0.00001
	Urban	0.758 ± 0.090	1.985 ± 1.164	0.00001

Table 5. Compare serum concentration of lipid profile in Clinical –Sub clinical atherosclerosis patients according to classification of diabetes mellitus groups.

Parameters	Groups	Subclinical (Mean ± SD)	Clinical (Mean ± SD)	P value
Total cholesterol (mg/dl)	No diabetes	198.964 ± 23.844	252.036 ± 20.884	0.00014
	Pre diabetes	189.877 ± 15.378	249.010 ± 29.543	0.00003
	Diabetes	200.854 ± 17.682	245.529 ± 29.634	0.00001
Total triglycerides (mg/dl)	No diabetes	213.597 ± 17.440	238.369 ± 32.163	0.03283
	Pre diabetes	200.355 ± 24.085	253.307 ± 22.472	0.00008
	Diabetes	209.429 ± 21.290	250.383 ± 16.141	0.00001
Low density lipoprotein (mg/dl)	No diabetes	94.579 ± 24.995	157.337 ± 19.156	0.00003
	Pre diabetes	93.241 ± 17.816	156.083 ± 31.249	0.00003
	Diabetes	99.279 ± 16.865	155.833 ± 28.738	0.00001
High density lipoprotein (mg/dl)	No diabetes	61.666 ± 8.281	47.025 ± 6.061	0.00095
	Pre diabetes	56.565 ± 9.877	42.266 ± 9.687	0.00427
	Diabetes	59.689 ± 9.784	39.619 ± 8.688	0.00001
Very low density lipoprotein (mg/dl)	No diabetes	42.719 ± 3.488	47.674 ± 6.433	0.03283
	Pre diabetes	40.071 ± 4.817	50.661 ± 4.494	0.00008
	Diabetes	41.886 ± 4.258	50.077 ± 3.228	0.00001

diabetes, pre diabetes, diabetes) (1.401 ± 0.778), (1.867 ± 1.047), (10.387 ± 1.497) as compared to subclinical (0.727 ± 0.059), (0.747 ± 0.123), (0.791 ± 0.087) respectively. Result found significant increase ($P \leq 0.01$) in HbA1C for pre diabetes of clinical groups (7.035 ± 0.457) as compared to subclinical (6.388 ± 0.395). As well as high significant increase ($P \leq 0.01$) in C-peptide for all clinical groups: (no diabetes, pre diabetes and diabetes) (100.411 ± 47.739), (119.324 ± 38.916), (129.894 ± 25.464) as compared to subclinical (38.617 ± 8.568), (40.023 ± 12.081), (41.681 ± 8.209) respectively. Leptin significant increase ($P \leq 0.01$) in all groups of clinical state (no diabetes, pre-diabetes, diabetes) (213.195 ± 66.448), (244.774 ± 75.771), (219.015 ± 64.126) as compared to sub clinical (88.791 ± 9.517), (96.633 ± 16.670), (94.592 ± 12.673). On other hand – no any significant found ($p \geq 0.05$) in fasting plasma glucose for all clinical groups (no diabetes, prediabetes, diabetes (230.167 ± 103.438), (162.500 ± 45.140), (243.355 ± 82.959) as compared to subclinical (127.667 ± 36.533), (158.000 ± 58.038), (273.500 ± 99.509). no any significant found ($p \geq 0.05$) for insulin of no diabetes clinical groups (3.745 ± 1.687) as compared to subclinical (2.569 ± 0.226). HOMA-IR in no diabetes clinical groups revealed no any significant found ($p \geq 0.05$) (1.401 ± 0.778) as compared to no diabetes subclinical groups (0.727 ± 0.059). No any significant found ($p \geq 0.05$) in serum HbA1C for clinical groups (no diabetes and diabetes) (4.768 ± 0.390), (10.387 ± 1.497) as compared to same groups of subclinical (4.381 ± 0.783), (9.810 ± 1.778), as in Table (6).

In the Table (7) the ROC curve analysis found that Leptin 95 % (CI 0.979–1.000: P-value 0.001: cutoff point: 125.148 AUC: 99.111 %, with Sensitivity to Specificity 91.121 %–97.678 %; and accuracy:

Table 6. Compare serum concentration of glucose parameters in Clinical and Subclinical atherosclerosis patients according to classification of diabetes mellitus groups.

Parameters	Groups	Subclinical (Mean ± SD)	Clinical (Mean ± SD)	P value
Fasting plasma glucose (mg/dl)	No diabetes	127.667 ± 36.533	230.167 ± 103.438	0.05937
	Pre diabetes	158.000 ± 58.038	162.500 ± 45.140	0.84870
	Diabetes	273.500 ± 99.509	243.355 ± 82.959	0.25509
Random glucose (mg/dl)	No diabetes	115.090 ± 10.264	148.008 ± 23.694	0.01777
	Pre diabetes	116.678 ± 13.350	183.001 ± 35.115	0.00003
	Diabetes	124.618 ± 14.638	223.577 ± 41.827	0.00001
Insulin (μIU/ml)	No diabetes	2.569 ± 0.226	3.745 ± 1.687	0.14887
	Pre diabetes	2.594 ± 0.329	4.008 ± 1.866	0.04106
	Diabetes	2.585 ± 0.204	4.321 ± 1.634	0.00001
HOMA IR	No diabetes	0.727 ± 0.059	1.401 ± 0.778	0.08737
	Pre diabetes	0.747 ± 0.123	1.867 ± 1.047	0.00806
	Diabetes	0.791 ± 0.087	2.383 ± 1.048	0.00001
HbA1c (mmol/mol)	No diabetes	4.381 ± 0.783	4.768 ± 0.390	0.26769
	Pre diabetes	6.388 ± 0.395	7.035 ± 0.457	0.00326
	Diabetes	9.810 ± 1.778	10.387 ± 1.497	0.22496
C-peptide (pg/ml)	No diabetes	38.617 ± 8.568	100.411 ± 47.739	0.02450
	Pre diabetes	40.023 ± 12.081	119.324 ± 38.916	0.00001
	Diabetes	41.681 ± 8.209	129.894 ± 25.464	0.00001
Leptin (pg/ml)	No diabetes	88.791 ± 9.517	213.195 ± 66.448	0.00578
	Pre diabetes	96.633 ± 16.670	244.774 ± 75.771	0.00013
	Diabetes	94.592 ± 12.673	219.015 ± 64.126	0.000001

Table 7. Receiver Operating Characteristic (ROC) Curve for evaluation Diagnostic, Accuracy and predictive value for atherosclerosis Biomarkers.

Metrics		Insulin (μIU/ml)	HOMA IR	HbA1c (mmol/mol)	Leptin (pg/ml)
Std. error		0.041	0.016	0.057	0.006
Asymptotic sig.		0.002	0.001	0.003	0.001
Asymptotic 95% confidence interval	Lower bound	0.766	0.939	0.979	0.979
	Upper bound	0.925	1.000	1.000	1.000
Cutoff point		2.793	0.991	7.350	125.148
Area under curve (AUC)		84.568 %	97.037 %	68.370 %	99.111 %
Sensitivity		71.111 %	91.111 %	71.111 %	91.121 %
Specificity		84.444 %	97.778 %	60.000 %	97.678 %
Accuracy		77.778 %	94.444 %	65.556 %	94.444 %
Positive predictive value		82.051 %	97.619 %	64.000 %	97.619 %
Negative predictive value		74.510 %	91.667 %	67.500 %	91.667 %

94.444 %). Also for Insulin 95 % (CI 0.766–0.925 P-value 0.002: cutoff point: 2.793: AUC: 84.568 %, with Sensitivity to Specificity 71.111 %–84.444 %; and accuracy: 77.778 %. HOM-IR 95% (CI 0.939–1.000: P-value 0.001: cutoff point: 0.991: AUC: 97.037 %, with Sensitivity to Specificity 91.111 %–97.778 %; and accuracy: 94.444 %. HbA1C 95 % (CI 0.979–1.000: P-value 0.003: cutoff point: 7.350: AUC: 68.370 %, with sensitivity to specificity 71.111 %–60.000 %; and accuracy 65.556.

Discussion

In clinical atherosclerosis, elevated levels of fasting glucose, insulin, HOMA-IR, and HbA1c are largely attributed to heightened insulin resistance and persistent hyperglycemia, insulin resistance hinders effective glucose uptake, resulting in increased blood glucose concentrations, this metabolic disturbance is more evident in clinical stages of atherosclerosis, where systemic inflammation and endothelial dysfunction further compromise glycemic regulation, together, these pathophysiological mechanisms facilitate the transition from subclinical to clinical atherosclerosis (Kambalapalli et al., 2025; Scott et al., 2024).

Elevated lipid profiles (excluding HDL) are more pronounced in clinical atherosclerosis because the disease is more advanced, with longer and more intense exposure to atherogenic lipids like LDL and triglycerides. Clinical stages involve greater inflammation, metabolic disruption, and more harmful lipid particle types, often alongside comorbidities like diabetes or obesity. These factors contribute to higher lipid levels compared to subclinical (early, asymptomatic) atherosclerosis (Ahmadi et al., 2019; Gigante et al., 2025).

Leptin, levels tend to increase with age due to higher fat stores, but aging can also lead to leptin resistance, smoking and diabetes can similarly raise leptin levels due to increased fat mass and insulin resistance, though leptin resistance reduces its effectiveness in controlling appetite and metabolism (Opalenyk & Patskun, 2020; Vilariño-García et al., 2024).

Elevated HOMA-IR (insulin resistance) is linked to age, smoking, and diabetes. With age, insulin sensitivity decreases; smoking worsens insulin resistance through inflammation and oxidative stress; and diabetes itself impairs insulin function, all contributing to higher HOMA-IR and increased risk for metabolic disorders (Colantoni et al., 2024; Wongwananuruk et al., 2025).

Individuals with diabetes face a significantly higher risk of developing cardiovascular disease (CVD) compared to those without diabetes, consequently, the mortality rate among diabetic patients is elevated, one of the primary contributing factors to this increased risk is diabetic dyslipidemia, which plays a key role in the development of atherosclerosis— a major manifestation of CVD (Al-Fatlawi, 2022b; Farmer, 2007).

In patients with atherosclerosis, glucose parameters are notably higher in those with diabetes and prediabetes compared to nondiabetic individuals due to several interrelated mechanisms, these include Insulin resistance is prevalent in prediabetes and diabetes, leading to elevated blood glucose levels, which contribute to vascular dysfunction, Studies indicate that individuals with prediabetes exhibit a greater atherosclerotic burden than their nondiabetic counterparts, with significant increases in coronary artery plaque and stenosis severity (Açar et al., 2019; Ishida et al., 2024).

Conclusion

The results highlight a clear link between leptin, problems with lipid levels, HOMA-IR, long-term high blood sugar, and insulin resistance, all of which play a major role in the development of atherosclerosis. These findings highlight the crucial role of metabolic dysregulation in atherosclerotic disease advancement and support the potential use of leptin and HOMA-IR as important biomarkers for early detection and management.

Author contributions

The primary supervisor oversaw the study, provided guidance, and reviewed the manuscript. The secondary supervisor assisted with data analysis, provided guidance on practical aspects, and reviewed the manuscript.

Ethical research approvals

This study was approved by the ethics committee of Department of Clinical Laboratories/College of Applied Medical Sciences /University of Karbala (Ref: CLAMSKU/12).

- After the Ministry of Health and Environment granted permission to conduct the study, samples were collected and starting the study.
- The study participants gave their permission to collect socio-demographic data as well as undertake experiments on the selected samples with respecting patient confidentiality.

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Conflict of interest

The authors declare that there is no conflict of interest related to this study.

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