**ISSN: 1607-8322, e-ISSN: 2220-5799** **Anaesthesia, Pain & Intensive Care**
Vol 29(1); February 2025 DOI: 10.35975/apic.v29i1.2659

**ORIGINAL RESEARCH** **PERIOPERATIVE MEDICINE**

# The atherogenic indices in people with type 2 diabetes mellitus as predicted by serum levels of SMAD4, ACS, and G-CSF

**Yusra Sebri Abdulsaheb [1], Habiba Khdair Abdalsada [2], Shatha Rouf Moustafa [3], Hussein Kahem Al-Hakeim [4]**

**Author affiliations:**

1. Yusra Sebri Abdulsaheb, Clinical Pharmacy department, College of Pharmacy, Missan University, Iraq; E-mail: YusrSebri Abd33@yahoo.com
2. Habiba Khdair Abdalsada, Al-Muthanna University, College of Pharmacy, Al-Muthanna, Iraq; Email: HabibaKhdair444@yahoo.com
3. Shatha Rouf Moustafa, Clinical Analysis Department, College of Pharmacy, Hawler Medical University, Erbil, Iraq; Email: ShathaRouMou2@gmail.com
4. Hussein Kahem Al-Hakeim, Department of Chemistry, College of Science, University of Kufa, Kufa, Iraq; E-mail: headm201011@yahoo.com

**Correspondence:** Hussein Kahem Al-Hakeim; Email: headm201011@yahoo.com

## ABSTRACT

**Background & objectives:** Atherogenic indices provide a broad picture of the risk of cardiovascular disease (CVD) in people with type 2 diabetes mellitus (T2DM). A broad range of factors influence atherogenic index values. Given the low-grade inflammatory characteristic of T2DM, we assessed three proteins in T2DM patients: 1-aminocyclopropane-1-carboxylate synthase (ACS), granulocyte-colony stimulating factor (G-CSF), and Mothers Against Decapentaplegic Homolog-4 (SMAD-4), and their association with the atherogenic indices.

**Methodology:** The current case-control research included sixty T2DM patients with no severe complications and thirty healthy controls. The chosen proteins were quantified using the ELISA technique, while the remaining biomarkers were measured using a spectrophotometer.

**Results:** T2DM patients exhibited significantly greater levels of ACS, G-CSF, and SMAD4 compared to control participants. After adjusting for all cofounders, serum ACS level may accurately predict a considerable portion of changes in the atherogenic index of plasma (AIP) and Castelli risk index (CRI)-I and II. AIP, CRI-II, and the atherogenic coefficient can all predict serum SMAD4 levels. Glucose levels may predict the amount of inflammatory cytokine (G-CSF), demonstrating that inflammation is dependent on hyperglycemia.

**Conclusion.** T2DM patients had dyslipidemia, high atherogenic indices, and greater levels of SMAD4, ACS, and G-CSF compared to controls. Once confounders are accounted for, serum ACS levels are proven to be a significant predictor of atherogenic indices. These findings contribute to our understanding of these proteins and their role in T2DM consequences including CVD.

**Abbreviations:** ACS - 1-aminocyclopropane-1-carboxylate synthase; AIP - atherogenic index of plasma; CRI - Castelli risk index; G-CSF - granulocyte-colony stimulating factor; SMAD-4 - Mothers Against Decapentaplegic Homolog-4

**Keywords:** Atherogenic Index; SMAD4; ACS; G-CSF; T2DM

**Citation:** Abdulsaheb YS, Abdalsada HK, Moustafa SR, Al-Hakeim HK. The atherogenic indices in people with type 2 diabetes mellitus are predicted by serum levels of SMAD4, ACS, and G-CSF. Anaesth. pain intensive care 2024;29(1):21–31; **DOI:** 10.35975/apic.v29i1.2659

**Received:** April 18, 2024; **Reviewed:** April 19, 2024; **Accepted:** April 20, 2024

## 1. INTRODUCTION

Diabetes is characterized by persistently high blood sugar levels caused by pancreatic β cell loss, among other factors. β cell loss is common across all diabetes types.[1] First. The International Diabetes Federation predicts that 10.5% of persons worldwide will develop type 2 diabetes mellitus (T2DM) by 2021. According to predictions, by 2045, this number will rise to 12.2%, affecting over 783 million people worldwide.

 Open access attribution (CC BY-NC 4.0)

A second. Diabetes is expensive for the global health business and individual healthcare systems, and projected an increase to 12.2% by 2045, which represents more than 783 million adults,[2] costing around 966 billion USD.[3] A high blood glucose level significantly increases the risk of both macrovascular and microvascular complications in T2DM patients. Excessive lipid deposition may cause insulin resistance (IR), diastolic dysfunction, and cardiac fibrosis.[4] Diabetes dyslipidemia, defined by high triglyceride, low-density lipoprotein cholesterol (LDLc), and low-high-density lipoprotein cholesterol (HDLc) levels, is also linked to an increased risk of cardiovascular events in high-risk populations.[5] Metabolic dyslipidemia must be included in the CVD risk categorization for people with T2DM.[6] Metabolic dyslipidemia was shown to be quite frequent (40%) in T2DM patients.[6] Diabetic dyslipidemia is characterized by higher triglyceride (TG) and reduced HDLc levels; LDLc concentrations often remain unchanged.

Many variables in T2DM were investigated as potential lipid profile influencers to prevent CVD. However, a few components, including granulocyte colony-stimulating factor (G-CSF), Sma Mothers Against Decapentaplegia homologue 4 (SMAD4), and 1-aminocyclopropane-1-carboxylate synthase (ACS), have never been explored previously. In this study, the link between these measures, the lipid profile, and other patient characteristics will be investigated. Smad4, a member of the SMAD superfamily, is an intracellular protein that enhances signal transduction via the TGF-β pathway.[7] Smad4 facilitates receptor-regulated SMAD translocation from the cytoplasm to the nucleus, where this complex regulates TGF-response gene transcription.[8]

The enzyme ACS operates on pyridoxal phosphate.[9] and is controlled by transcriptional and posttranscriptional mechanisms.[10,11] in that order. Among many other pathogenic processes, including infection, cancer, and inflammation, ACS (the rate-limiting phase) produces ethylene.[12] The catalytic activity of ACS causes chemotherapy-induced cognitive impairment, which is prevalent in cancer patients and negatively linked with ACC levels.[13] Granulocyte-colony stimulating factor G-CSF) is produced by fibroblasts, granulosa cells, macrophages, natural killer cells, epithelial apical cells, and trophoblasts.[14] Previous investigations[15] showed that G-CSF therapy enhanced left ventricular diastolic function and reduced cardiomyocyte death in diabetic cardiomyopathy. A multivariate generalised linear regression model and multiple regression analysis were used to examine the potential predictive value of these biomarker levels for dyslipidemia in T2DM.

# 2. METHODOLOGY

## 2.1. Study design and subjects

The current case-control research compared the age and gender of sixty T2DM patients to thirty healthy controls. The Al-Sadr Teaching Hospital in Najaf Governorate, Iraq, was recruiting from December 2022 until January 2023. Patients with clear substantial overt diabetic problems, such as heart, hepatic, and renal illnesses, as well as an albumin/creatinine ratio of more than 30 mg/g, satisfied the World Health Organization's T2DM diagnostic criteria.[16] These individuals had fasting plasma glucose levels of 7.0 mM or higher and glycated hemoglobin (HbA1c) values of more than 6.5%. All patients had blood CRP values less than 6 mg/dL, thus those who exhibited obvious evidence of inflammation were excluded.

## 2.2. Ethical considerations

Before participating in the trial, all participants provided written informed permission. The research was authorized by the University of Kufa's Institutional Review Board (IRB) (811/2022) in accordance with the Declaration of Helsinki's International Guidelines for Human Research Protection.

## 2.3. Variables and Scales

We utilized commercial ELISA sandwich kits from Nanjing Pars Biochem Co., Ltd. (Nanjing, China) to assess serum ACS, SMAD4, and G-CSF. Fasting total cholesterol (TC), triglycerides (TG), and glucose were determined spectrophotometrically in serum using a commercially available reagent provided by Agappe Diagnostics Ltd., Cham, Switzerland. Serum HDLc was tested after other lipoproteins precipitated in a solution of sodium phosphotungstate and magnesium chloride. The cholesterol levels in the supernatant were measured using an automated analyzer. CVDLc is calculated as very-low density lipoprotein cholesterol (TG) multiplied by 2.19. LDLc was calculated using Friedewald's formula: TC - HDLc - VLDLc. Castelli's Risk Indices (CRI) is made up of two indices: CRI-I (TC/HDLc) and CRI-II (LDLc/HDLc). The atherogenic index of plasma (AIP) is calculated by logarithmically transforming the molar ratio of TG to HDLc. The Atherogenic Coefficient (AC) is an indirect measure of cholesterol in the VLDLc, IDLc, and LDLc fractions compared to the HDLc fraction. AC may be represented mathematically as (TC-HDLc)/HDLc or (Non-HDLc). The blood CRP level was tested using a Spinreact® reagent from Spain. The test is based on the notion of latex agglutination. The colorimetric examination was carried out using an Apel BR 5100 spectrophotometer from APEL in Japan. The

 Open access attribution (CC BY-NC 4.0)

**Table 1: Demographic, clinical, and biochemical data in healthy controls and T2DM patients.**

| Variables | Control N = 30 | T2DM N = 60 | df | $F/\chi^2$ | p |
|---|---|---|---|---|---|
| Age (yr) | 44.670 ± 9.279 | 46.620 ± 8.279 | 1/88 | 1.023 | 0.315 |
| Gender (Female/Male) | 19/11 | 37/23 | 1 | 0.024 | 0.878 |
| BMI ($kg/m^2$) | 26.778 ± 4.819 | 26.837 ± 3.052 | 1/88 | 0.005 | 0.944 |
| Single/married | 2/28 | 4/56 | 1 | 0 | 1 |
| Residency Rural / Urban | 3/27 | 6/54 | 1 | 0 | 1 |
| Family History of T2DM (No/Yes) | 29/1 | 35/25 | 1 | 14.306 | < 0.001 |
| Exercise (No/Yes) | 8/22 | 11/49 | 1 | 1.092 | 0.311 |
| TUD (No/Yes) | 19/11 | 34/26 | 1 | 0.367 | 0.545 |
| Duration of DM (Yr) | - | 12.411 ± 5.363 | - | - | - |
| Cholesterol (mM) | 4.205 ± 0.799 | 5.103 ± 1.318 | 1/88 | 11.722 | 0.001 |
| Triglycerides (mM) | 1.559 ± 0.304 | 2.306 ± 0.983 | 1/88 | 16.464 | < 0.001 |
| VLDLc (mM) | 0.712 ± 0.139 | 1.053 ± 0.449 | 1/88 | 16.464 | < 0.001 |
| HDLc (mM) | 1.145 ± 0.155 | 0.987 ± 0.192 | 1/88 | 15.261 | < 0.001 |
| LDLc (mM) | 2.349 ± 0.832 | 3.063 ± 1.111 | 1/88 | 9.661 | 0.003 |
| Z score: CRI-I | -0.622 ± 0.532 | 0.311 ± 1.037 | 1/88 | 21.323 | < 0.001 |
| Z score: CRI-II | -0.551 ± 0.633 | 0.276 ± 1.039 | 1/88 | 15.975 | < 0.001 |
| Z score: AIP | -0.687 ± 0.546 | 0.344 ± 1.00 | 1/88 | 27.582 | < 0.001 |
| Z score: AC | -0.621 ± 0.632 | 0.311 ± 1.037 | 1/88 | 21.323 | < 0.001 |
| Glucose (mM) | 5.532 ± 0.686 | 8.182 ± 2.742 | 1/88 | 27.038 | < 0.001 |
| LnSMAD4 (ng/mL) | 4.027 ± 0.704 | 5.050 ± 0.76 | 1/88 | 37.916 | < 0.001 |
| LnACCS (pg/mL) | 1.470 ± 0.413 | 1.874 ± 0.201 | 1/88 | 39.313 | < 0.001 |
| LnG-CSF (pg/mL) | 4.113 ± 0.665 | 4.857 ± 0.575 | 1/88 | 30.207 | < 0.001 |
| Sitagliptin 100 mg (No/Yes) | 30/0 | 42/18 | 1 | 11.250 | 0.001 |
| Amaryl 4 mg (No/Yes) | 30/0 | 42/18 | 1 | 11.250 | 0.001 |
| Daonil 5 mg (No/Yes) | 30/0 | 36/24 | [1] | 16.364 | <0.001 |

***Abbreviations:*** *ACCS: 1-aminocyclopropane-1-carboxylate synthase, AIP: atherogenic index of plasma, BMI: body mass index, CRI-I: Castelli risk index 1, CRI-II: Castelli risk index II, G-CSF: Granulocyte colony-stimulating factor, HDLc: High-density lipoprotein cholesterol, LDLc: Low-density lipoprotein cholesterol, Ln: natural logarithm, Smad4: Sma Mothers Against Decapentaplegia homologue 4, TUD: tobacco-use disorder, VLDLc: Very low-density lipoprotein cholesterol.*

immunoassay measurements were performed using an ELISA reader, namely an American BioTek ELx800.

## 2.4. Data collection

After an overnight fast, between 8:00 and 9:00 am, 5 mL of venous blood were taken from patients and controls. After the blood was fully coagulated, the serum was extracted by centrifuging it for 10 min at 3000 rpm using a Jiangsu Jinyi Instrument Tech. Co., Ltd. Donated the device from China. The serum was stored at -80°C until analysis.

 Open access attribution (CC BY-NC 4.0)

### 2.5. Statistical analysis

Using ANOVA, it was determined if the examined variables differed across study groups. The $\chi 2$-test was used to evaluate the correlations between nominal variables. Associations between biomarkers were explored using correlation matrices constructed using Pearson's product-moment correlation coefficients. We employed multivariate general direct model (GLM) analysis to explore the connection between diagnosis and biomarkers (more specifically, between controls and T2DM). This strategy takes into account complex aspects including age, BMI, gender, education level, and nicotine addiction. We studied inter-subject effects to understand the link between biomarkers and diagnosis. Effect sizes were estimated using partial Eta ($\gamma 2$) values. Using multiple regression analysis, the components of atherogenic indices were predicted by automatically progressively using significant biomarkers and other biomarkers as explanatory variables. All significant explanatory variables have standardized beta coefficients estimated using t-statistics and accurate P-values. The model F statistics and total variance explained (R2) were also computed. A total of 90 samples are required for a covariance analysis with two groups, a P-value of 0.05, an effect size of 0.3, and a power of 0.8. 90 participants were recruited, 30 as controls and 60 as ill. All statistical studies used the Ln transformation to normalize the distributions of SMAD4, ACS, and G-CSF. We performed all statistical analyses using IBM SPSS for Windows version 25.2017.

## 3. RESULTS

The clinical and biochemical data for T2DM patients and healthy controls showed that there were no significant differences between the patient and control groups in terms of age, gender, BMI, marital status, domicile, or exercise frequency (Table 1). Twenty-four patients got Daonil® (5 mg), and eighteen of sixty patients received Sitagliptin (100 mg) or Amaryl (4 mg). When comparing the patient and control groups, the patient group had a significantly higher family history of T2DM (P = 0.001). As predicted, T2DM patients developed substantial hyperglycemia. The group's average sickness duration was 12.411 ± 5.363 years.

T2DM patients exhibited dyslipidemia, as shown by higher levels of atherogenic indices (CRI-I, CRI-II, AC, and AIP), lower HDLc, VLDLc, LDLc, and serum cholesterol (TG), compared to the control group.

**Table 2: Results of multivariate GLM analysis examining the associations between the measured biomarkers with the diagnosis and covariates.**

| Tests | Dependent variables | Explanatory variables | F | df | p | Partial η2 |
|---|---|---|---|---|---|---|
| **Multivariate** | All serum parameters | Diagnosis | 9.657 | 9/75 | < 0.001 | 0.537 |
| | | TUD | 1.408 | 9/75 | 0.200 | 0.145 |
| | | Gender | 1.006 | 9/75 | 0.443 | 0.108 |
| | | Age | 0.935 | 9/75 | 0.500 | 0.101 |
| | | Exercise | 0.768 | 9/75 | 0.646 | 0.084 |
| | | BMI | 0.729 | 9/75 | 0.681 | 0.080 |
| **Between-subject effects** | Diagnosis | LnACCS | 40.722 | 1 | < 0.001 | 0.329 |
| | | LnSMAD4 | 35.426 | 1 | < 0.001 | 0.299 |
| | | LnG-CSF | 30.418 | 1 | < 0.001 | 0.268 |
| | | Z score: AIP | 23.132 | 1 | < 0.001 | 0.218 |
| | | Z score: CRI-I (AC) | 15.382 | 1 | < 0.001 | 0.156 |
| | | TG (VLDLc) | 14.597 | 1 | < 0.001 | 0.150 |
| | | HDLc | 12.562 | 1 | 0.001 | 0.131 |
| | | Z score: CRI-II | 10.849 | 1 | 0.001 | 0.116 |
| | | Cholesterol | 8.550 | 1 | 0.004 | 0.093 |
| | | LDLc | 6.328 | 1 | 0.014 | 0.071 |

***Abbreviations:*** *ACCS: 1-aminocyclopropane-1-carboxylate synthase, AIP: atherogenic index of plasma, CRI-I: Castelli risk index 1, CRI-II: Castelli risk index II, G-CSF: Granulocyte colony-stimulating factor, HDLc: High-density lipoprotein cholesterol, LDLc: Low-density lipoprotein cholesterol, Ln: natural logarithm, SMAD4: Sma Mothers Against Decapentaplegia homologue 4, TUD: tobacco-use disorder, VLDLc: Very low-density lipoprotein cholesterol*

 Open access attribution (CC BY-NC 4.0)

**Table 3: Correlation matrix showing the partial correlations adjusted for age, sex, BMI, smoking, and exercise.**

| Parameters | zCRI-I (AC) | z AIP | z CRI-II | LnACCS | LnG-CSF | LnSMAD4 |
|---|---|---|---|---|---|---|
| Age | 0.176 | 0.170 | 0.125 | 0.088 | 0.054 | 0.028 |
| Sex | 0.013 | -0.050 | 0.032 | 0.118 | -0.006 | 0.115 |
| Smoking | 0.002 | -0.156 | 0.069 | 0.225* | -0.078 | 0.063 |
| Exercise | -0.302* | -0.089 | -0.345** | 0.050 | 0.112 | -0.001 |
| Duration of DM | 0.059 | 0.167 | -0.007 | 0.091 | 0.112 | 0.154 |
| Family history | -0.228 | -0.078 | -0.239 | 0.047 | 0.204 | -0.019 |
| BMI | 0.079 | 0.136 | 0.047 | -0.111 | -0.087 | -0.073 |
| Cholesterol | 0.820*** | 0.530*** | 0.817** | 0.112 | -0.071 | -0.047 |
| TG & VLDLc | 0.624*** | 0.909*** | 0.392** | 0.018 | -0.038 | -0.023 |
| HDLc | -0.566*** | -0.532*** | -0.508*** | -0.084 | 0.000 | 0.078 |
| LDLc | 0.818*** | 0.353** | 0.899*** | 0.140 | -0.069 | -0.060 |
| Glucose | -0.207 | -0.184 | -0.189 | -0.031 | 0.277* | -0.168 |
| z CRI-I | 1 | 0.733*** | 0.956*** | 0.157 | -0.034 | -0.089 |
| z AIP | 0.733*** | 1 | 0.527** | 0.103 | -0.025 | 0.002 |
| z CRI-II | 0.956*** | 0.527*** | 1 | 0.182 | -0.033 | -0.091 |
| z AC | 1.000*** | 0.733*** | 0.956** | 0.157 | -0.034 | -0.089 |
| LnACCS | 0.157 | 0.103 | 0.182 | 1 | 0.110 | 0.199 |
| LnG-CSF | -0.034 | -0.025 | -0.033 | 0.110 | 1 | 0.616*** |
| LnSMAD4 | -0.089 | 0.002 | -0.091 | 0.199 | 0.616*** | 1 |

**: Significant correlation (P < 0.05), **: Significant correlation (P < 0.01), ***: Significant correlation (P < 0.001),*

***Abbreviations:*** *ACCS: 1-aminocyclopropane-1-carboxylate synthase, AIP: atherogenic index of plasma, BMI: body mass index, CRI-I: Castelli risk index 1, CRI-II: Castelli risk index II, G-CSF: Granulocyte colony-stimulating factor, HDLc: High-density lipoprotein cholesterol, LDLc: Low-density lipoprotein cholesterol, Ln: natural logarithm, Smad4: Sma Mothers Against Decapentaplegia homologue 4, TUD: tobacco-use disorder, VLDLc: Very low-density lipoprotein cholesterol.*

Patients had substantially greater levels of SMAD4, ACS, and G-CSF (P < 0.001) compared to the control group.

## 3.1. Interpretation of Multivariate GLM Analysis

The results of a multivariate GLM investigation have been summarized in Table 2. The assessed biomarkers,

**Table 4: Results of multiple regression analysis with atherogenic indices as dependent variables.**

| Dependent variables | Explanatory variables | β | t | p | F model | df | p | R2 |
|---|---|---|---|---|---|---|---|---|
| **#1.** CRI-I and AC | **Model** | | | | 2.257 | 8/81 | 0.048 | 0.169 |
| | Exercise | -0.240 | -2.150 | 0.035 | | | | |
| | LnACCS | 0.244 | 2.130 | 0.036 | | | | |
| **#2.** CRI-II | **Model** | | | | 2.447 | 8/81 | 0.030 | 0.195 |
| | Exercise | -0.256 | -2.279 | 0.025 | | | | |
| | LnACCS | 0.226 | 1.955 | 0.054 | | | | |
| **#3.** AIP | **Model** | | | | 2.242 | 8/81 | 0.032 | 0.181 |
| | LnACCS | 0.245 | 2.154 | 0.034 | | | | |

***Abbreviations:*** *ACCS: 1-aminocyclopropane-1-carboxylate synthase, AIP: atherogenic index of plasma, CRI-I: Castelli risk index 1, CRI-II: Castelli risk index II, Ln: natural logarithm.*

 Open access attribution (CC BY-NC 4.0)

**Table 5: Results of multiple regression analysis with ACCS, G-CSF, and ACCS as dependent variables.**

| Dependent variables | Explanatory variables | β | t | p | F model | df | p | R2 |
|---|---|---|---|---|---|---|---|---|
| **#1.** LnSMAD4 | **Model** | | | | 4.046 | 3/86 | 0.010 | 0.124 |
| | Z score: AIP | 0.869 | 2.767 | 0.007 | | | | |
| | Z score: CRI-II | 1.897 | 2.053 | 0.044 | | | | |
| | Z score: AC | 1.456 | 1.893 | 0.062 | | | | |
| **#2.** LnACCS | **Model** | | | | 4.079 | 3/86 | 0.009 | 0.125 |
| | AIP | 0.663 | 2.113 | 0.038 | | | | |
| **#3.** LnG-CSF | **Model** | | | | 44.142 | 3/86 | <0.001 | 0.511 |
| | Glucose | 0.165 | 2.439 | 0.017 | | | | |

***Abbreviations:*** *ACCS: 1-aminocyclopropane-1-carboxylate synthase, AIP: atherogenic index of plasma, CRI-I: Castelli risk index 1, G-CSF: Granulocyte colony-stimulating factor, Ln: natural logarithm, SMAD4: Sma Mothers Against Decapentaplegia homologue 4.*

except glucose, which was already the type 2 diabetes diagnostic biomarker, served as the study's dependent variables. The diagnosis and covariates (age, BMI, exercise, sex, and TUD) were the primary explanatory variables. The effect size (partial γ2) = 0.537 suggests that the diagnosis has a statistically significant impact on biomarker levels. None of the parameters had a significant effect on the biomarker levels.

Assessments of between-subject effects revealed that the diagnosis had a significant impact on blood biomarker levels. The diagnosis had a significant ($P < 0.001$) impact on six biomarkers: ACS ($F = 40.722$, Partial $\eta^2 = 0.329$), SMAD4 ($F = 35.426$, Partial $\eta^2 = 0.299$), G-CSF ($F = 30.418$, Partial $\eta^2 = 0.268$), AIP ($F = 23.132$, Partial $\eta^2 = 0.218$), CRI-I (AC) ($F = 15.382$, Partial $\eta^2 = 0.156$), and TG (VLDLc) ($F = 14.597$, Partial $\eta^2 = 0.150$).

## 3.2. Matrix of correlations

Table 3 shows the partial correlations between other measured parameters and the atherogenic indices (DMAD4, ACS, and G-CSF). We will not investigate the association between these factors and atherogenic indices since atherogenic indices are composed of cholesterol lipoproteins

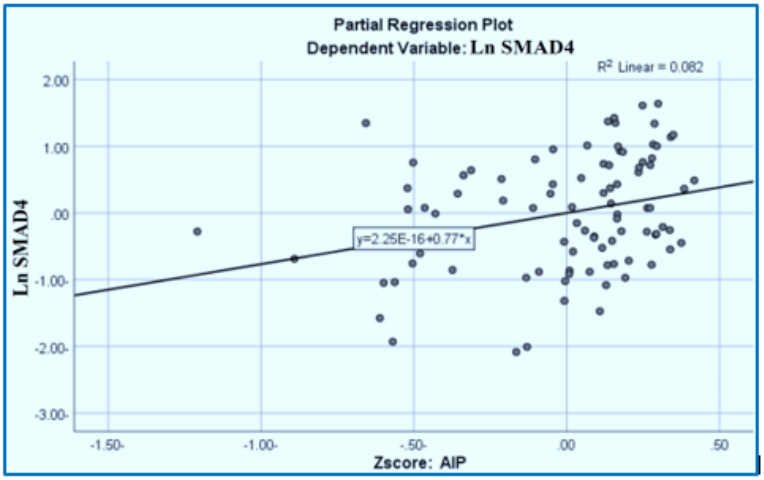


**Figure 1: Partial regression plot of serum SMAD4 on AIP**

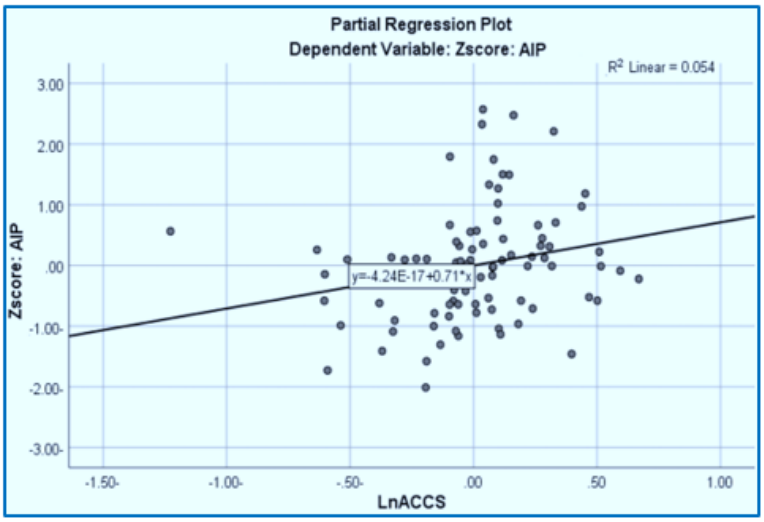


**Figure 2: Partial regression plot of serum AIP on ACCS**

 Open access attribution (CC BY-NC 4.0)

(TC), TG, and TG. Exercise has a substantial and negative correlation with CRI-I (AC) and CRI-II. LnACS is strongly associated with cigarette smoking. LnG-CSF, glucose, and LnSMAD4 are all substantially connected.

### 3.3. Multiple regression analysis

Table 4 presents the findings from many stepwise multiple regression investigations. These studies investigated the effects of age, gender, and education on the independent variable of all measured biomarkers and the dependent variable, atherogenic index scores. According to Regression #1, the exercise inversely explained a considerable amount (16.9%) of the variance in the CRI-I (AC) score. Furthermore, as Regression #2 demonstrates, the regression LnACS could explain the same proportion of the variance in the CRI-II, whilst the exercise could explain the remaining 19.5%. Regression No. 3 shows that LnACS explains 18.1% of the variance in AIP value. Figure 1 depicts the serum SMAD4 partial regression curve on AIP.

Table 5 shows the dependent variables for the second multiple regression analysis, which included the biomarkers G-CSF, ACS, and SMAD4. As shown in Regression #1, the combined effects of AIP, CRI (AC), and CRI-II accounted for an incredible 12.4% of the variance in LnSMAD4. The second regression revealed that the regression on AIP could explain 12.5% of the variance in LnACS. Figure 2 illustrates how AIP regresses on ACS. As seen in Regression No. 3, the glucose regression accounts for 51.1% of the variability in LnG-CSF.

## 4. DISCUSSION

Patients with T2DM had dyslipidemia compared to the control group, as seen by higher levels of blood cholesterol, TG, VLDLc, and LDLc, as well as a reduction in HDLc. These findings are congruent with those of several other researchers who have discovered dyslipidemia in T2DM patients. Alkubaisi and colleagues (2023) discovered that dyslipidemia in T2DM patients is associated with a variety of issues, including diabetic retinopathy and CVD.[18] Furthermore, most studies examining atherogenic and lipid markers in diabetics discovered dyslipidemias; statin therapy is indicated to reduce lipid levels.[19] Aberrant lipid profiles (e.g., high TG and/or low HDLc) are characteristic of metabolic dyslipidemia, which has been associated with ischemic stroke and cardiovascular disease.[6, 20] Surprisingly, diabetics exhibit a shift in the distribution of LDL subfractions towards small dense LDL, which is more prone to undergo several chemical modifications, increasing its atherogenic effects.[21] The logarithmic transformation of the molar ratio of TG to HDLc, also known as AIP, provides a comprehensive view of the balance between proatherogenic and antiatherogenic components. Because the fractional esterification rate of HDL and AIP are positively correlated, AIP may be utilized instead of LDL particles for assessing diabetes risk.

According to prospective studies [22], a combination of a high TG level and a low HDLc level, sometimes known as "atherogenic dyslipidemia," may serve as a clinical indicator to identify people at risk for cardiovascular disease. Because IR directly induces dyslipidemic abnormalities, this may be especially true for those with metabolic illnesses and obesity.[23] Furthermore, it has been shown that the T2DM-specific IR has a substantial and positive connection with AIP.[24] As evidence accumulated, it became obvious that AIP was substantially associated with type 2 diabetes. A prior meta-analysis found that AIP provided a more exact estimate of the risk of T2DM than other lipid markers. In a large cross-sectional study, AIP increased the prevalence of chronic microvascular issues; consequently, it may be beneficial to monitor diabetic patients after that.[25] Given the above, dyslipidemia and high atherogenic indices harm our patient population, making them susceptible to atherosclerosis and cardiovascular disease. As a result, new biomarkers must be discovered to predict atherogenicity early on and explain the etiology by tying it to biomarkers from other pathways.

The research found considerably higher levels ($P < 0.001$) of G-CSF, ACS, and SMAD4 in T2DM patients compared to the control group. Research suggests that high glucose levels might harm β-cells by increasing the production of reactive oxygen species (ROS), leading to impaired insulin secretion and insulin resistance. Thus, elevated levels of the proteins SMAD4, ACS, and G-CSF offer a plausible and acceptable explanation for the inflammation associated with diabetes. [26] Furthermore, SMAD4 has been shown to regulate a wide range of metabolic homeostasis processes, including fibrosis, inflammation, and fibroblast and matrix remodelling.[27] Diabetics have been found to express SMAD4 more than others, and hypoxemia and diabetic acidosis have been linked to increased SMAD4 levels.[28] Elevated plasma SMAD4 levels have been associated with the coexistence of dyslipidemia and diabetes.[28] Previous research has shown that both HIF1α and circadian genes promote TGFβ expression. Furthermore, prospective research found that those with T2DM had relatively high plasma TGF levels.[29] The recent findings show that ROS-induced inflammation may be responsible for the increase in the proteins ACS, G-CSF, and SMAD4 found in T2DM patients. Inflammation is associated with SMAD4;[27] molecules under investigation include ACS,[30] G-CSF,[31] and G-CSF.[31] Inflammation

 Open access attribution (CC BY-NC 4.0)

contributes significantly to the aetiology and effects of diabetes.[32] The multivariate GLM analysis demonstrated that diabetes affected the investigated biomarkers. The factors (age, BMI, activity, sex, and TUD) had no statistically significant effect on the more recent biomarkers ACS, SMAD4, and G-CSF, nor on the lipid profile or atherogenic indices (Table 2).

These data demonstrate that hyperglycemia has a direct influence on the biomarkers studied. Between-subjects effect tests show that the diagnostic factors had the greatest impact on ACS, SMAD4, G-CSF, AIP, CRI-I (AC), and TG (VLDLc). Hyperglycemia and insulin resistance, two characteristics of type 2 diabetes, have a direct impact on the levels of these proteins. Without Smad4, NADPH oxidase 4 (NOX4), the primary kidney generator of reactive oxygen species (ROS), which is increased in diabetic nephropathy and podocytes in response to high glucose, could not express itself.[33] Podocyte-specific Nox4 deletion reduces renal reactive oxygen species (ROS) formation, which is linked to the prevention of diabetic nephropathy in rats.[33] Inhibiting Smad4 in pancreatic β-cells improved glucose intolerance caused by a high-fat diet but did not affect insulin resistance.[34] Exercise and CRI-I (AC) and CRI-II were shown to have the greatest correlation among the evaluated parameters (Table 3). Smoking and LnACS had a statistically significant correlation ($r = 0.225$, $P < 0.05$). Both glucose ($r = 0.277$, $P < 0.05$) and LnSMAD4 ($r = 0.616$, $P < 0.001$) are substantially linked with LnG-CSF. Physical activity may benefit people with dyslipidemia by improving their lipid profile.[35] G-CSF, as a proinflammatory biomarker, is predicted to have a link with glucose levels since type 2 diabetes is primarily caused by hyperglycemia.[36] These findings supported the theory that atherogenicity in diabetics is induced by inflammation. It seems that the mechanisms behind atherosclerosis and IR are comparable and interconnected. It is important to remember that the two most prevalent IR illnesses, obesity and T2DM, have strong underlying inflammatory pathways that promote atherosclerosis.

Anti-inflammatory medications have the potential to prevent atherogenesis by reversing and lowering the intimal-medial thickness of the carotid artery.[37] The multiple regression analysis results shown in Table 4 show a correlation between the measured proteins and the atherogenic indices; this correlation explains a marginally significant variation in serum levels of these proteins. These findings revealed that the impact of these proteins on plasma lipid levels enhanced atherogenic indices and hence the risk of cardiovascular disease. Hyperglycemia causes Smad4 to localise to podocyte mitochondria, reducing oxidative phosphorylation and glycolysis while increasing the creation of reactive oxygen species. A variety of pathophysiological processes, including hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, chronic low-grade inflammation, oxidative stress, endothelial dysfunction, arterial calcification, and hypercoagulability, have contributed to the development of CVD in T2DM.[38] SMAD4 interacts with its receptor to boost signaling pathways, including TGF-β signaling. Nonetheless, the interaction of these processes altered the levels of inflammatory mediators, metabolic hormones, adipokines, and apolipoproteins in the plasma of persons with diabetes and associated cardiovascular disease concerns.[38] Increased C-peptide and insulin levels have been related to an increased risk of coronary artery disease in persons with type 2 diabetes. Interestingly, hyperinsulinemia promotes atherosclerosis in diabetics more than in non-diabetics.[39]

Apart from increasing antiapoptotic proteins, G-CSF prevents cardiomyocytes from committing suicide.[40] As a result, elevated levels of SMAD4, ACS, and G-CSF provide light on the processes that influence the development of dyslipidemia and atherosclerosis in T2DM patients.

# 5. LIMITATIONS

The primary drawback of the current research is the limited sample size. When the sample size increases, the findings become more generalizable.

# 6. CONCLUSIONS

Patients with T2DM had dyslipidemia in this research, and their SMAD4, ACS, and G-CSF levels rose considerably when compared to the control group. Even after correcting for all confounding factors, the serum ACS level may accurately predict a significant amount of changes in the AIP, CRI-I, and CRI-II. In contrast, serum SMAD4 concentrations are predicted using AIP, CRI-II, and AC. These findings provide light on these proteins and their role in T2DM consequences, including cardiovascular disease.

## 7. Acknowledgment

We thank the personnel of Sadr Teaching Hospital in Najaf City for their assistance in sample collection, as well as the highly competent team of Asia Clinical Laboratory in Najaf City for their assistance with ELISA measurements.

## 8. Funding

There was no funding for this particular research.

## 9. Conflict of interest.

The authors have no financial or other conflicts of interest.

## 10. Data availability

 Open access attribution (CC BY-NC 4.0)

The numerical data generated during the conduct of this study is available with the corresponding author.

### 11. Author's Contributions

The text was prepared in collaboration with all of the participating writers.

## 12. REFERENCES


1. Eizirik DL, Pasquali L, Cnop M. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nat Rev Endocrinol. 2020;16(7):349-62. [PubMed] DOI: 10.1038/s41574-020-0355-7
2. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Medicine (Abingdon). 2014;42(12):698-702. [PubMed] DOI: 10.1016/j.mpmed.2014.09.007
3. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119. [PubMed] DOI: 10.1016/j.diabres.2021.109119
4. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia-and insulin-resistance-induced heart disease. Diabetologia. 2018;61(1):21-8. [PubMed] DOI: 10.1007/s00125-017-4390-4
5. Ye X, Kong W, Zafar MI, Chen LL. Serum triglycerides as a risk factor for cardiovascular diseases in type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. Cardiovasc Diabetol. 2019;18(1):48. [PubMed] DOI: 10.1186/s12933-019-0851-z
6. Kaze AD, Santhanam P, Musani SK, Ahima R, Echouffo-Tcheugui JB. Metabolic dyslipidemia and cardiovascular outcomes in type 2 diabetes mellitus: findings from the Look AHEAD study. J Am Heart Assoc. 2021;10(7):e016947. [PubMed] DOI: 10.1161/JAHA.120.016947
7. Itatani Y, Kawada K, Sakai Y. Transforming growth factor-β signaling pathway in colorectal cancer and its tumor microenvironment. Int J Mol Sci. 2019;20(23):5822. [PubMed] DOI: 10.3390/ijms20235822
8. Ullah I, Sun W, Tang L, Feng J. Roles of Smads family and alternative splicing variants of Smad4 in different cancers. J Cancer. 2018;9(21):4018. [PubMed] DOI: 10.7150/jca.20906
9. Bleecker AB, Kende H. Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol. 2000;16(1):1-18. [PubMed] DOI: 10.1146/annurev.cellbio.16.1.1
10. Lincoln JE, Campbell A, Oetiker J, Rottmann W, Oeller P, Shen N, et al. LE-ACS4, a fruit ripening and wound-induced 1-aminocyclopropane-1-carboxylate synthase gene of tomato (Lycopersicon esculentum). Expression in Escherichia coli, structural characterization, expression characteristics, and phylogenetic analysis. J Biol Chem. 1993;268(26):19422-30. [PubMed]
11. Chae HS, Faure F, Kieber JJ. The eto1, eto2, and eto3 mutations and cytokinin treatment increase ethylene biosynthesis in Arabidopsis by increasing the stability of ACS protein. Plant Cell. 2003;15(2):545-59. [PubMed] DOI: 10.1105/tpc.006882
12. Paardekooper LM, van den Bogaart G, Kox M, Dingjan I, Neerincx AH, Bendix MB, et al. Ethylene, an early marker of systemic inflammation in humans. Sci Rep. 2017;7(1):6889. [PubMed] DOI: 10.1038/s41598-017-05930-9
13. Juan Z, Chen J, Ding B, Yongping L, Liu K, Wang L, et al. Probiotic supplement attenuates chemotherapy-related cognitive impairment in patients with breast cancer: a randomised, double-blind, and placebo-controlled trial. Eur J Cancer. 2022;161:10-22. [PubMed] DOI: 10.1016/j.ejca.2021.11.006
14. Ding J, Yang C, Zhang Y, Wang J, Zhang S, Guo D, et al. M2 macrophage-derived G-CSF promotes trophoblasts EMT, invasion and migration via activating PI3K/Akt/Erk1/2 pathway to mediate normal pregnancy. J Cell Mol Med. 2021;25(4):2136-47. [PubMed] DOI: 10.1111/jcmm.16191
15. Shin JH, Lim Y-, Song YS, So BI, Park JY, Fang CH, et al. Granulocyte-colony stimulating factor reduces cardiomyocyte apoptosis and ameliorates diastolic dysfunction in Otsuka Long-Evans Tokushima Fatty rats. Cardiovasc Drugs Ther. 2014;28:211-20. [PubMed] DOI: 10.1007/s10557-014-6519-8
16. Organization WH. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation; 2006.
17. Al-Hakeim HK, Al-Rammahi DA, Al-Dujaili AH. IL-6, IL-18, sIL-2R, and TNFα proinflammatory markers in depression and schizophrenia patients who are free


 Open access attribution (CC BY-NC 4.0)

of overt inflammation. J Affect Disord. 2015;182:106-14. [PubMed] DOI: 10.1016/j.jad.2015.04.044

18. Alkubaisi MR, Tahir NT, Abdilya R. Dyslipidemia poses a significant risk of many complications for Type 2 Diabetes Mellitus Patients: Article Review. NTU J Pure Sci. 2023;2(3).DOI: 10.56286/ntujps.v2i3.562
19. Feingold KR, Grunfeld C. Diabetes and dyslipidemia. In: Diabetes and Cardiovascular Disease. Springer; 2023. p. 425-72.
20. O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, et al. INTERSTROKE investigators: risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet. 2010;376(9735):112-23. [PubMed] DOI: 10.1016/S0140-6736(10)60834-3
21. Zhou K, Qin Z, Tian J, Cui K, Yan Y, Lyu S. The atherogenic index of plasma: a powerful and reliable predictor for coronary artery disease in patients with type 2 diabetes. Angiology. 2021;72(10):934-41. [PubMed] DOI: 10.1177/00033197211012129
22. Andersson C, Lyass A, Vasan RS, Massaro JM, D'Agostino Sr RB, Robins SJ. Long-term risk of cardiovascular events across a spectrum of adverse major plasma lipid combinations in the Framingham Heart Study. Am Heart J. 2014;168(6):878-83.e1. [PubMed] DOI: 10.1016/j.ahj.2014.08.007
23. Soran H, Schofield JD, Adam S, Durrington PN. Diabetic dyslipidaemia. Curr Opin Lipidol. 2016;27(4):313-22. [PubMed] DOI: 10.1097/MOL.0000000000000318
24. Kou H, Deng J, Gao D, Song A, Han Z, Wei J, et al. Relationship among adiponectin, insulin resistance and atherosclerosis in non-diabetic hypertensive patients and healthy adults. Clin Exp Hypertens. 2018;40(7):656-63. [PubMed] DOI: 10.1080/10641963.2018.1425414
25. Li Z, Huang Q, Sun L, Bao T, Dai Z. Atherogenic index in type 2 diabetes and its relationship with chronic microvascular complications. Int J Endocrinol. 2018;2018. [PubMed] DOI: 10.1155/2018/1765835
26. Jain C, Bilekova S, Lickert H. Targeting pancreatic β cells for diabetes treatment. Nat Metab. 2022;4(9):1097-108. [PubMed] DOI: 10.1038/s42255-022-00618-5
27. Massagué J, Blain SW, Lo RS. TGFβ signaling in growth control, cancer, and heritable disorders. Cell. 2000;103(2):295-309. [PubMed] DOI: 10.1016/s0092-8674(00)00121-5
28. Díaz-García E, Jaureguizar A, Casitas R, García-Tovar S, Sánchez-Sánchez B, Zamarrón E, et al. SMAD4 overexpression in patients with sleep apnoea may be associated with cardiometabolic comorbidities. J Clin Med. 2020;9(8):2378. [PubMed] DOI: 10.3390/jcm9082378
29. Herder C, Zierer A, Koenig W, Roden M, Meisinger C, Thorand B. Transforming growth factor-β1 and incident type 2 diabetes: results from the MONICA/KORA case-cohort study, 1984–2002. Diabetes Care. 2009;32(10):1921-3. [PubMed] DOI: 10.2337/dc09-0476
30. Abaffy T, Möller M, Riemer DD, Milikowski C, DeFazio RA. A case report: volatile metabolomic signature of malignant melanoma using matching skin as a control. J Cancer Sci Ther. 2011;3(6):140. [PubMed] DOI: 10.4172/1948-5956.1000076
31. Xiao BG, Lu CZ, Link H. Cell biology and clinical promise of G-CSF: immunomodulation and neuroprotection. J Cell Mol Med. 2007;11(6):1272-90. [PubMed] DOI: 10.1111/j.1582-4934.2007.00101.x
32. Barbu E, Popescu MR, Popescu AC, Balanescu SM. Inflammation as a precursor of atherothrombosis, diabetes and early vascular aging. Int J Mol Sci. 2022;23(2):963. [PubMed] DOI: 10.3390/ijms23020963
33. Jha JC, Thallas-Bonke V, Banal C, Gray SP, Chow BS, Ramm G, et al. Podocyte-specific Nox4 deletion affords renoprotection in a mouse model of diabetic nephropathy. Diabetologia. 2016;59(2):379-89. [PubMed] DOI: 10.1007/s00125-015-3796-0
34. Li H, Oh Y, Lee YJ, Lee EK, Jung H, Jun HS, et al. Amelioration of high-fat diet-induced glucose intolerance by blockade of Smad4 in pancreatic beta-cells. Exp Clin Endocrinol Diabetes. 2014:221-6. [PubMed] DOI: 10.1055/s-0034-1395583
35. Wang Y, Xu D. Effects of aerobic exercise on lipids and lipoproteins. Lipids Health Dis. 2017;16(1):132. [PubMed] DOI: 10.1186/s12944-017-0515-5
36. Ebtehaj S, Gruppen EG, Parvizi M, Tietge UJ, Dullaart RP. The anti-inflammatory function of HDL is impaired in type 2 diabetes: role of hyperglycemia, paraoxonase-1 and low-grade inflammation. Cardiovasc Diabetol.

 Open access attribution (CC BY-NC 4.0)

2017;16(1):132. [PubMed] DOI: 10.1186/s12933-017-0613-8

37. Chistiakov DA, Melnichenko AA, Grechko AV, Myasoedova VA, Orekhov AN. Potential of anti-inflammatory agents for treatment of atherosclerosis. Exp Mol Pathol. 2018;104(2):114-24. [PubMed] DOI: 10.1016/j.yexmp.2018.01.008
38. Poreba M, Rostoff P, Siniarski A, Mostowik M, Golebiowska-Wiatrak R, Nessler J, et al. Relationship between polyunsaturated fatty acid composition in serum phospholipids, systemic low-grade inflammation, and glycemic control in patients with type 2 diabetes and atherosclerotic cardiovascular disease. Cardiovasc Diabetol. 2018;17(1):29. [PubMed] DOI: 10.1186/s12933-018-0672-5
39. Wang L, Lin P, Ma A, Zheng H, Wang K, Li W, et al. C-peptide is independently associated with an increased risk of coronary artery disease in T2DM subjects: a cross-sectional study. PLoS One. 2015;10(6):e0127112. [PubMed] DOI: 10.1371/journal.pone.0127112
40. Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med. 2005;11(3):305-11. [PubMed] DOI: 10.1038/nm1199

 Open access attribution (CC BY-NC 4.0)