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METABOLIC PREDICTORS OF CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES: ASSOCIATIONS OF GLYCEMIC CONTROL, LIPID FRACTIONS, AND RENAL MARKERS WITH AUTONOMIC DYSFUNCTION

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ABSTRACT

Background: Cardiac autonomic neuropathy (CAN) represents one of the most overlooked yet clinically consequential complications of type 2 diabetes mellitus (T2DM), as it predisposes patients to heightened cardiovascular risk and contributes substantially to early mortality. While cardiovascular autonomic reflex tests (CARTs) provide standardized diagnostic tools, the metabolic determinants of CAN remain incompletely elucidated. **Objective:** This study aimed to identify glycemic, lipid, and renal predictors of CAN and to determine their associations with CART-derived indices in Iraqi patients with T2DM. **Methods:** A case–control study was conducted in Mosul, Iraq, between October 2024 and January 2025, enrolling 100 patients with T2DM (≥5 years duration) and 100 age- and sex-matched controls. Clinical data, anthropometry, glycemic markers (HbA1c, fasting plasma glucose), lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C), and renal function (urea, creatinine, proteinuria) were measured using standardized laboratory methods. Autonomic function was assessed via CARTs (Valsalva, E/I, and 30:15 ratios). Associations were analyzed using correlations, multivariable regression, and mediation analysis. **Results:** CAN was detected in 82.5% of participants, with 40.0% classified as early and 42.5% as definite. HbA1c, TC, TG, LDL-C, VLDL-C, creatinine and serum urea increased progressively with CAN severity ($p < 0.0001$). CART indices (E/I and 30:15 ratios) declined stepwise across severity groups. Multivariable analysis identified HbA1c, TG, and urea as independent predictors of CAN severity, and mediation modeling suggested that lipid fractions partially mediated the effect of hyperglycemia on autonomic impairment. Receiver operating characteristic (ROC) analysis revealed acceptable discriminatory ability of HbA1c, triglycerides, and urea for definite CAN, with combined models outperforming individual predictors .**Conclusion:** CAN is highly prevalent among Iraqi patients with T2DM and is strongly associated with poor glycemic control, dyslipidemia, and subtle renal dysfunction. Integrating these metabolic indices may provide an early biochemical signature of CAN risk, underscoring the need for targeted screening and preventive strategies.

KEYWORDS: Type 2 diabetes mellitus; Cardiac autonomic neuropathy; Glycemic control; HbA1c; Dyslipidemia; Triglycerides; Renal dysfunction; Iraq.

INTRODUCTION

Cardiac autonomic neuropathy (CAN) constitutes one of the least diagnosed yet most prognostically serious complications of type 2 diabetes mellitus (T2DM). It is causally linked to increased cardiovascular morbidity, arrhythmic risk, and premature mortality, which makes its early detection of major clinical importance (Sudo et al., 2022; Eleftheriadou et al. 2024). Standardized evaluation of CAN relies on cardiovascular autonomic reflex tests (CARTs), namely the Valsalva maneuver, the expiratory–inspiratory (E/I) ratio, and the 30:15 ratio on standing, which were initially conceptualized by Ewing

and collaborators as reference criteria for autonomic dysfunction. Among these, the **30:15 ratio** has demonstrated particularly strong diagnostic discrimination for early-stage CAN in T2DM (Atala et al.,2022; Dikshit, 2022). CAN, once regarded as purely neural, is now recognized as a metabolic disorder linked to chronic hyperglycemia, dyslipidemia, and renal dysfunction Through non-enzymatic glycation of proteins and the subsequent build-up of advanced glycation end-products (AGEs), oxidative injury and endothelial impairment occur, reducing nitric oxide availability, disturbing the sympathetic–parasympathetic equilibrium, and ultimately diminishing heart rate variability. (Mengstie et al. 2022). **HbA1c**, as an integrated marker of long-term glycemic exposure, has consistently been associated not only with microvascular complications but also with the severity of autonomic impairment. Moreover, variability in HbA1c across time has been linked to progressive CAN, suggesting a cumulative burden of glycotoxicity (Sartore et al. 2023).

At the same time, **lipid perturbations**—particularly hypertriglyceridemia and shifts in complex lipid metabolites (e.g., phosphatidylcholines, sphingomyelins)—have been shown to amplify neuronal injury via oxidative stress and inflammatory pathways, thereby mediating the effect of hyperglycemia on autonomic dysfunction (Xu et al. 2024). In addition, **renal indices** such as proteinuria and reduced glomerular filtration rate have demonstrated bidirectional associations with CAN. Declines in renal function exacerbate autonomic imbalance, while autonomic dysfunction itself accelerates renal impairment, supporting the concept of a **cardiorenal–autonomic axis** (Deferrari et al. 2021). This interplay underscores the necessity of adopting an integrated analytical framework that combines glycemic, lipid, and renal markers.

Based on this rationale, the present study aimed to disentangle the **metabolic determinants** of CAN severity among Iraqi patients with T2DM. Specifically, it sought to: (1) evaluate associations between HbA1c, lipid fractions, and renal function markers with CART-derived indices; (2) identify **independent predictors** of CAN severity using multivariable regression modeling; and (3) test whether dyslipidemia mediates the relationship between chronic hyperglycemia and autonomic impairment. By integrating these components into a unified analytical model, the study aspires to provide an **early biochemical signature** of CAN risk, enabling more precise screening and preventive strategies.

MATERIALS AND METHODS

Study Design and Ethical Approval

This investigation adopted a case–control design and was conducted in Mosul City, northern Iraq, between October 2024 and January 2025. Participants were recruited from the Al-Wafaa Center for Diabetes and Endocrinology and the Ibn Sina Teaching Hospital. The study adhered to the ethical principles of the **Declaration of Helsinki (2013 revision)**, and approval was obtained from the Medical Research Ethics Committee of the University of Mosul, College of Medicine (approval code: **UOM/COM/MREC/24-25/SEP3**). All participants provided written informed consent prior to enrolment.

Study Population

A sample of 200 participants aged 30–60 years was selected to ensure adequate statistical power, in line with previous studies assessing autonomic dysfunction in diabetes (Spallone et al., 2011; Ziegler et al., 2020). Individuals were enrolled and stratified into two equal groups.

- **Case group (n = 100):** Patients with type 2 diabetes mellitus (T2DM) diagnosed according to the **American Diabetes Association (ADA) 2023 criteria**, with a disease duration of ≥5 years. Patients with established cardiovascular disease, microvascular or macrovascular complications, thyroid disorders, or current use of medications influencing autonomic function (e.g., β-blockers, tricyclic antidepressants) were excluded.
- **Control group (n = 100):** Age- and sex-matched non-diabetic individuals, recruited from hospital staff and patient companions. Controls were required to have **HbA1c < 5.7%**, **fasting plasma glucose < 100 mg/dl**, and no history of hypertension, chronic systemic illness, or medication affecting autonomic tone.

Clinical and Anthropometric Assessment

Baseline demographic data were collected through a structured questionnaire. Height and weight were measured using standardized stadiometers and calibrated digital scales, respectively, and **body mass index (BMI)** was calculated as weight (kg)/height² (m²). Resting systolic and diastolic blood pressures were measured twice, 5 minutes apart, using an automated sphygmomanometer (Omron HEM series, Japan), with participants seated and rested. Pulse rate was simultaneously recorded with a pulse oximeter. All participants were instructed to abstain from caffeine, smoking, alcohol, and heavy meals for at least 12 hours prior to autonomic testing to minimize confounders.

Biochemical Investigations

Following a 10–12 h overnight fast, **7 ml of venous blood** was drawn under aseptic conditions. Samples were processed within 1 h of collection.

Glycemic indices

- Glycemic assessment included HbA1c determination using a turbidimetric inhibition immunoassay on the Roche Cobas e411 platform, while fasting plasma glucose (FPG) was analyzed via the glucose oxidase–peroxidase enzymatic technique.

- **Lipid profile**
  - Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) were determined using **enzymatic colorimetric assays** (Roche Cobas c311).

- **Renal function**
  - Serum urea was assayed by the **urease–GLDH UV method**, and creatinine was determined using the **Jaffe's kinetic method**.
  - *Proteinuria* was screened semi-quantitatively by urine dipstick (Combur-Test, Roche) and confirmed by turbidimetric quantification in positive cases.

To ensure reproducibility, all biochemical analyses were performed in duplicate, with **internal quality control** using two levels of certified control sera, in compliance with **ISO 15189 laboratory accreditation standards**.

Cardiac Autonomic Function Testing (CARTs)

Autonomic function was assessed in a temperature-controlled (22–24 °C), quiet room between 8:00 and 11:00 AM. Tests were conducted after a 15-minute rest period in the supine position, following **Ewing and Clarke's standardized protocol (1982)**.

1. **Valsalva maneuver:** Participants exhaled into a mouthpiece attached to a manometer at 40 mmHg for 15 s. The **Valsalva ratio** was calculated as the longest R–R interval after strain divided by the shortest during strain.
2. **Deep breathing test:** Participants performed six cycles of deep breathing at 6 breaths/min. The **E/I ratio** was obtained from the mean of the longest R–R interval during expiration divided by the shortest during inspiration.
3. **Orthostatic test (30:15 ratio):** Following active standing from supine position, the **30:15 ratio** was calculated as the R–R interval at beat 30 divided by that at beat 15.

CAN was diagnosed when at least one of the three CARTs was abnormal. Severity was classified as **normal, early, definite, or severe** based on the Ewing and Clarke criteria.

Statistical Analysis

Data were analyzed using **SPSS version 26.0 (IBM Corp., Armonk, NY, USA)**. Distribution of variables was examined using the Shapiro–Wilk test. Data are presented as mean ± standard deviation (SD) for normally distributed parameters, or as median with interquartile range when skewed. Statistical significance was defined at a two-tailed p-value below 0.05.

Group comparisons: Independent-samples *t*-test or one-way ANOVA (with Tukey's post hoc test) for continuous variables, and χ^2 test for categorical variables.

- **Associations:** Pearson's or Spearman's correlation coefficients between biochemical indices (HbA1c, lipid fractions, urea, creatinine) and CARTs (Valsalva, E/I, 30:15 ratios).
- **Predictive modeling:** Multivariable linear and logistic regression analyses to identify **independent predictors of CAN severity**.
- **Mediation analysis:** The **PROCESS macro (Hayes, v4.1)** was applied to test whether lipid fractions mediated the relationship between HbA1c and CAN indices.

A two-tailed p-value < 0.05, was considered statistically significant. To adjust for potential confounders, multivariable logistic regression models were additionally fitted including age, sex, BMI, diabetes duration, and blood pressure as covariates. Model calibration was assessed using the Hosmer–Lemeshow test, and internal validation was performed by bootstrap resampling (1000 iterations).

RESULTS

1. Demographic and Clinical Characteristics

The study enrolled **200 participants** aged 30–60 years (mean 46.27 ± 7.58 years). The age distribution showed 46 (23.0%) in the 30–39 group, 76 (38.0%) in 40–49, 76 (38.0%) in 50–59, and only 2 (1.0%) ≥ 60 years. Exactly half were patients with T2DM (n = 100), and half were non-diabetic controls (n = 100). Among diabetics, mean disease duration was 6.40 ± 1.05 years. A positive family history of diabetes was reported in 63 (31.5%) participants.

Table 1: Demographic and clinical characteristics of the study population.

Variables	No (%) or Mean ± SD
Age 30–39	46 (23.0%)
Age 40–49	76 (38.0%)
Age 50–59	76 (38.0%)
Age ≥60	2 (1.0%)
Mean Age (years)	46.27 ± 7.58
Diabetic status (Positive)	100 (50.0%)
Diabetic status (Negative)	100 (50.0%)
Duration of diabetes (years)	6.40 ± 1.05
Family history (Positive)	63 (31.5%)
Family history (Negative)	137 (68.5%)

2. Laboratory Investigations

The mean fasting blood sugar (FBS) in the total population was 113.23 ± 30.69 mg/dl, with mean HbA1c 5.89 ± 1.38%. Renal function parameters included serum urea 32.06 ± 8.06 mg/dl and creatinine 0.89 ± 0.18 mg/dl. Lipid profile values were: total cholesterol 164.54 ± 42.79 mg/dl, triglycerides 137.61 ± 56.62 mg/dl, HDL 34.79 ± 23.07 mg/dl, LDL 104.61 ± 25.74 mg/dl, and VLDL 27.27 ± 11.46 mg/dl. Proteinuria was absent in 176 (88.0%), while mild to severe levels were detected in 24 (12.0%)

Table 2: Laboratory investigations of the study population.

Laboratory Investigation	Mean ± SD / n (%)
FBS (mg/dl)	113.23 ± 30.69
HbA1c (%)	5.89 ± 1.38
Urea (mg/dl)	32.06 ± 8.06
Creatinine (mg/dl)	0.89 ± 0.18
Cholesterol (mg/dl)	164.54 ± 42.79
Triglycerides (mg/dl)	137.61 ± 56.62
HDL (mg/dl)	34.79 ± 23.07
LDL (mg/dl)	104.61 ± 25.74
VLDL (mg/dl)	27.27 ± 11.46
Proteinuria Absent	176 (88.0%)
Proteinuria (+)	3 (1.5%)
Proteinuria (++)	11 (5.5%)
Proteinuria (+++)	10 (5.0%)

Table 3: Clinical examination findings and autonomic function test results.

Parameters	Mean ± SD
Systolic BP (pre-deep breath)	123.99 ± 9.72
Diastolic BP (pre-deep breath)	71.34 ± 7.53
Pulse rate (pre-deep breath)	80.08 ± 8.41
Systolic BP (after deep breath)	122.61 ± 9.51
Diastolic BP (after deep breath)	70.28 ± 7.55
Pulse Inspiration (max)	90.02 ± 8.72
Pulse Expiration (min)	80.65 ± 8.96
Heart Rate Variability (beats/min)	10.58 ± 2.14
E/I Ratio	1.10 ± 0.05
Systolic BP (pre-standing)	124.17 ± 9.36
Diastolic BP (pre-standing)	72.37 ± 7.75
Pulse rate (pre-standing)	80.51 ± 8.29
Systolic BP (post-standing)	118.79 ± 9.83
Diastolic BP (post-standing)	69.18 ± 7.89
Pulse rate (post-standing)	82.11 ± 8.58
30:15 Ratio	1.03 ± 0.07
Systolic BP (pre-Valsalva)	124.52 ± 9.33
Diastolic BP (pre-Valsalva)	72.79 ± 7.73
Pulse rate (pre-Valsalva)	80.26 ± 8.53
Systolic BP (post-Valsalva)	122.03 ± 9.24
Diastolic BP (post-Valsalva)	71.61 ± 7.65
Pulse rate (post-Valsalva)	81.47 ± 8.87
Valsalva Ratio	1.21 ± 0.10

3. Autonomic Function Test Results

Baseline systolic and diastolic blood pressures were within normal ranges, with mean systolic 123.99 ± 9.72 mmHg and diastolic 71.34 ± 7.53 mmHg before deep breathing. Heart rate variability during deep breathing was 10.58 ± 2.14 beats/min, with an E/I ratio of 1.10 ± 0.05. The 30:15 ratio in the orthostatic test was 1.03 ± 0.07. The Valsalva ratio averaged 1.21 ± 0.10.

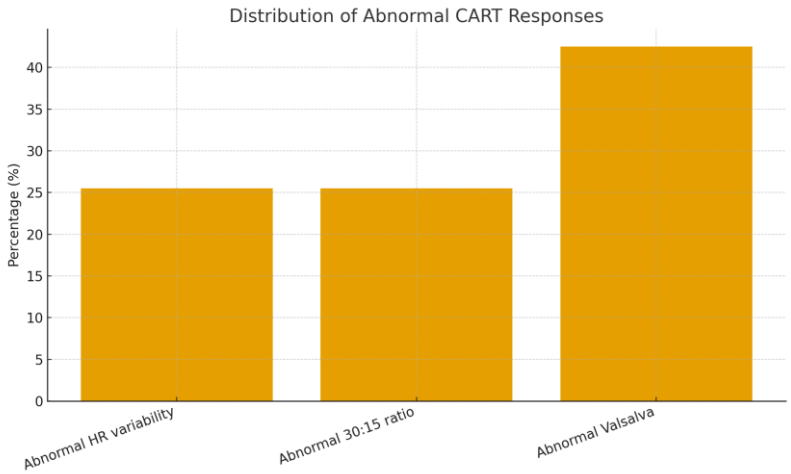


Figure 1.

Distribution of abnormal CART responses in the study population. Bar chart shows the percentage of participants with abnormal findings in HR variability, 30:15 ratio, and Valsalva ratio.

4. Prevalence and Severity of CAN

Classification of CAN severity revealed that **85 (42.5%)** participants had definite CAN, **80 (40.0%)** had early CAN, and only **35 (17.5%)** were classified as normal. Thus, more than four-fifths of the population demonstrated evidence of autonomic dysfunction.

5. Group Comparisons: Diabetic vs. Non-Diabetic

- **Glycemic markers:** Diabetics had higher FBS (131.52 ± 33.21 vs. 94.93 ± 10.69 mg/dl, $p < 0.0001$) and HbA1c (7.02 ± 0.99% vs. 4.76 ± 0.52%, $p < 0.0001$).
- **Renal function:** Urea and creatinine was elevated in diabetics.
- **Lipid profile:** Diabetics showed significantly higher cholesterol (186.24 ± 39.42 vs. 142.84 ± 34.29 mg/dl), triglycerides (174.83 ± 52.61 vs. 100.39 ± 29.64 mg/dl), LDL (114.59 ± 20.09 vs. 94.62 ± 26.96 mg/dl), and VLDL (34.80 ± 10.70 vs. 19.74 ± 5.89 mg/dl), all $p < 0.0001$. and HDL was lower in

diabetics 28.48 ± 6.88 mg/dl, vs. 41.10 ± 30.69 vs. ($p < 0.0001$).

- **Proteinuria:** Detected exclusively among diabetics ($p < 0.0001$).
- **Autonomic function:** The 30:15 ratio and Valsalva ratio were significantly reduced in diabetics ($p < 0.0001$)

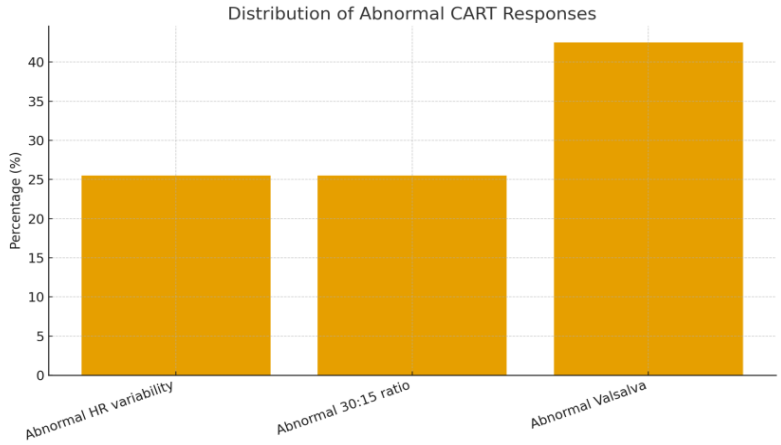


Figure 2.

Severity distribution of cardiac autonomic neuropathy (CAN) among participants. Bar chart shows proportions classified as Normal, Early, or Definite CAN.

Table 4 & Table 5: Group comparisons of laboratory and autonomic test parameters.

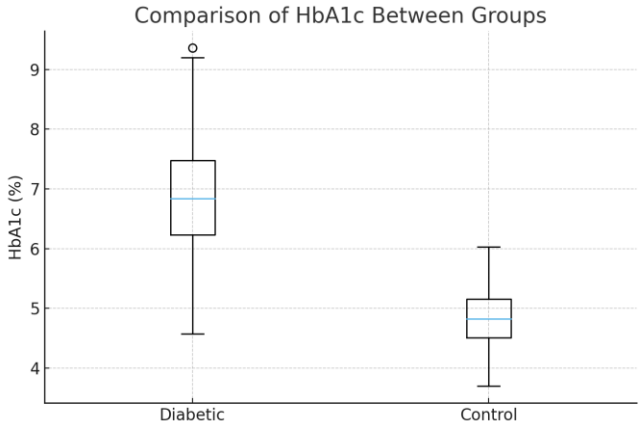
Table 4: Laboratory Comparison (Diabetic vs. Non-Diabetic)

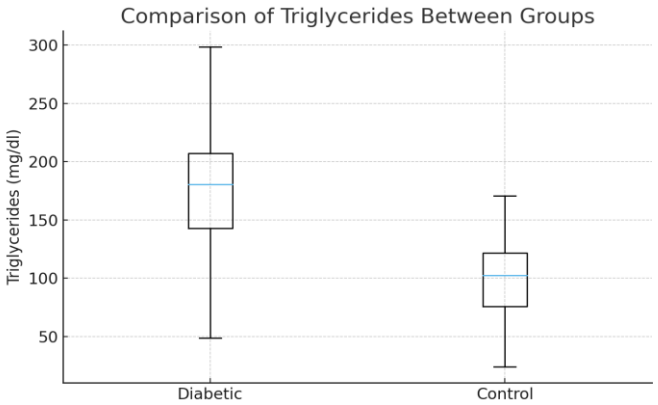
Laboratory investigation	Diabetic (Mean ± SD / n)	Non-diabetic (Mean ± SD / n)	p-value
FBS (mg/dl)	131.52 ± 33.21	94.93 ± 10.69	0.0001
HbA1c (%)	7.02 ± 0.99	4.76 ± 0.52	0.0001
Urea (mg/dl)	34.54 ± 8.48	29.58 ± 6.80	0.0001
Creatinine (mg/dl)	0.98 ± 0.17	0.80 ± 0.15	0.0001
Cholesterol (mg/dl)	186.24 ± 39.42	142.84 ± 34.29	0.0001
Triglycerides (mg/dl)	174.83 ± 52.61	100.39 ± 29.64	0.0001
HDL (mg/dl)	28.48 ± 6.88	41.10 ± 30.69	0.0001
LDL (mg/dl)	114.59 ± 20.09	94.62 ± 26.96	0.0001
VLDL (mg/dl)	34.80 ± 10.70	19.74 ± 5.89	0.0001
Proteinuria (+)	3 (100%)	0	0.0001
Proteinuria (++)	11 (100%)	0	0.0001
Proteinuria (+++)	10 (100%)	0	0.0001
Proteinuria Absent	76 (43.2%)	100 (56.8%)	0.0001

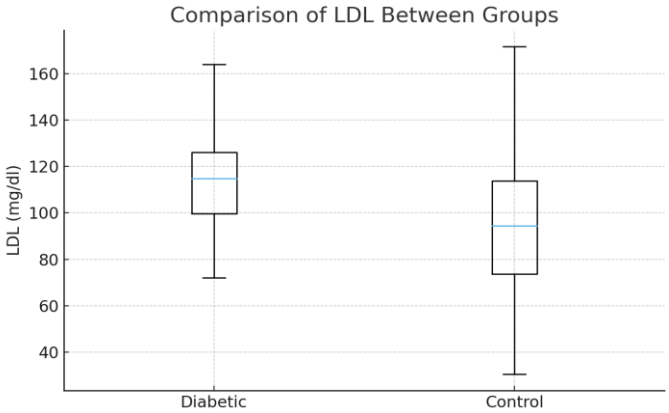
Table 5: Clinical Examination and CARTs (Diabetic vs. Non-Diabetic).

Parameters	Diabetic Mean ± SD	Non-diabetic Mean ± SD	p-value
Systolic BP pre-deep breath	125.41 ± 9.19	122.57 ± 10.06	0.038
Diastolic BP pre-deep breath	71.52 ± 7.60	71.16 ± 7.49	0.736
PR pre-deep breath	82.18 ± 8.64	77.99 ± 7.66	0.0001
Systolic BP after deep breath	123.63 ± 8.63	121.58 ± 10.25	0.128
Diastolic BP after deep breath	70.07 ± 7.48	70.49 ± 7.65	0.695
PR after deep breath (max insp)	90.35 ± 9.48	89.69 ± 7.93	0.594
PR after deep breath (min exp)	83.41 ± 9.30	77.88 ± 7.71	0.0001
Heart rate variability	9.43 ± 2.24	11.72 ± 1.25	0.0001
E/I ratio	1.07 ± 0.05	1.12 ± 0.04	0.0001
Systolic BP pre-standing	125.36 ± 8.98	122.98 ± 9.63	0.072
Diastolic BP pre-standing	72.20 ± 7.13	72.54 ± 8.36	0.757
PR pre-standing	82.39 ± 8.52	78.65 ± 7.66	0.001
Systolic BP post-standing	115.78 ± 9.52	121.79 ± 9.23	0.0001
Diastolic BP post-standing	67.18 ± 6.91	71.18 ± 8.33	0.0001
PR post-standing	84.77 ± 8.60	79.44 ± 7.71	0.0001

30:15 ratio	0.99 ± 0.07	1.06 ± 0.02	0.0001
Systolic BP pre-Valsalva	125.63 ± 8.80	123.40 ± 9.76	0.091
Diastolic BP pre-Valsalva	71.98 ± 7.42	73.59 ± 7.97	0.141
PR pre-Valsalva	82.42 ± 8.54	78.10 ± 7.99	0.0001
Systolic BP post-Valsalva	121.68 ± 8.34	122.38 ± 10.10	0.0001
Diastolic BP post-Valsalva	70.43 ± 7.24	72.78 ± 7.89	0.594
PR post-Valsalva	84.53 ± 8.83	78.40 ± 7.82	0.029
Valsalva ratio	1.15 ± 0.08	1.27 ± 0.07	0.0001







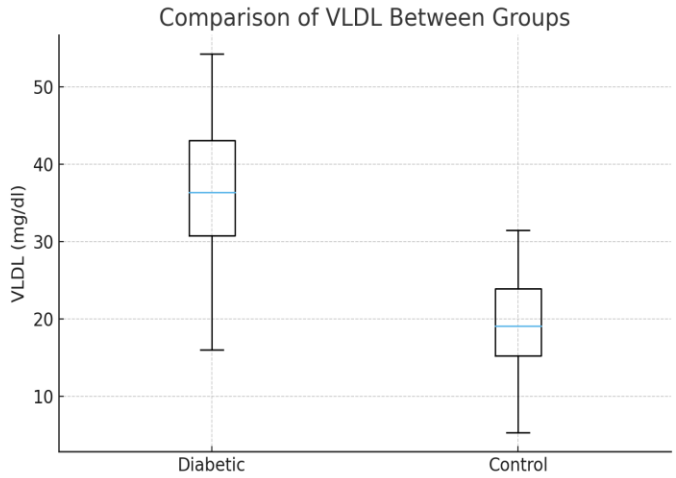


Figure 3 (a–d),
Box plots comparing biochemical parameters between diabetic and control groups. Panels show distributions for HbA1c (a), triglycerides (b), LDL (c), and VLDL (d).

Table 6: Laboratory Association with CAN Severity.

Laboratory investigation	Definite CAN (Mean ± SD / n)	Early CAN (Mean ± SD / n)	Normal (Mean ± SD / n)	p-value
FBS (mg/dl)	131.41 ± 35.85	101.39 ± 17.49	96.11 ± 12.94	0.0001
HbA1c (%)	6.99 ± 1.25	5.08 ± 0.83	5.05 ± 0.61	0.0001
Urea (mg/dl)	34.46 ± 8.71	31.31 ± 6.94	27.94 ± 6.88	0.0001
Creatinine (mg/dl)	0.87 ± 0.20	0.90 ± 0.16	0.92 ± 0.19	0.397
Cholesterol (mg/dl)	185.63 ± 47.13	148.11 ± 30.53	150.88 ± 33.56	0.0001
Triglycerides (mg/dl)	181.84 ± 51.99	111.67 ± 30.13	89.51 ± 33.50	0.0001
HDL (mg/dl)	33.99 ± 7.00	36.84 ± 35.26	32.04 ± 9.23	0.544
LDL (mg/dl)	116.85 ± 24.30	94.14 ± 22.06	98.81 ± 24.93	0.0001
VLDL (mg/dl)	36.28 ± 10.45	22.09 ± 6.06	17.25 ± 6.63	0.0001
Proteinuria (+)	3 (100%)	0	0	0.0001
Proteinuria (++)	10 (90.9%)	1 (9.1%)	0	0.0001
Proteinuria (+++)	9 (90.0%)	1 (10.0%)	0	0.0001
Proteinuria Absent	63 (35.8%)	78 (44.3%)	35 (19.9%)	0.0001

Table 7: Clinical and CARTs by CAN Severity.

Parameters	Definite CAN Mean ± SD	Early CAN Mean ± SD	Normal Mean ± SD	p-value
Systolic BP pre-deep breath	125.12 ± 9.60	123.27 ± 9.57	122.85 ± 10.29	0.356
Diastolic BP pre-deep breath	70.91 ± 7.19	71.47 ± 7.76	72.05 ± 7.94	0.739
PR pre-deep breath	81.74 ± 8.17	78.83 ± 8.66	78.94 ± 7.94	0.056
Systolic BP after deep breath	123.05 ± 8.95	122.32 ± 9.68	122.14 ± 10.59	0.843
Diastolic BP after deep breath	69.38 ± 6.99	70.75 ± 7.75	71.37 ± 8.30	0.330
PR max inspiration	89.89 ± 8.51	90.04 ± 9.13	90.29 ± 8.51	0.975
PR min expiration	82.62 ± 8.58	79.26 ± 9.29	79.00 ± 8.39	0.026
Heart rate variability	9.20 ± 2.36	11.50 ± 1.15	11.80 ± 1.32	0.0001
E/I ratio	1.07 ± 0.04	1.12 ± 0.04	1.11 ± 0.03	0.0001
Systolic BP post-standing	114.12 ± 8.67	122.21 ± 9.07	122.25 ± 9.68	0.0001
Diastolic BP post-standing	66.45 ± 6.70	71.06 ± 8.07	71.51 ± 8.35	0.0001
PR post-standing	84.64 ± 8.41	80.21 ± 8.19	80.29 ± 8.49	0.001
30:15 ratio	0.98 ± 0.07	1.06 ± 0.02	1.07 ± 0.02	0.0001
Valsalva PR post	84.49 ± 8.64	79.30 ± 8.48	79.06 ± 8.31	0.0001
Valsalva ratio	1.12 ± 0.04	1.29 ± 0.06	1.28 ± 0.04	0.0001

6. Association Between Biochemical Parameters and CAN Severity

HbA1c levels increased progressively with CAN severity: 5.96 ± 0.38% in the normal group, 6.22 ± 0.39% in early CAN, and 7.46 ± 0.86% in definite CAN ($p < 0.0001$). Total cholesterol, triglycerides, LDL-C, and VLDL-C also rose significantly across severity categories (all $p < 0.0001$), while HDL-C did not differ significantly ($p = 0.544$). Serum urea increased with CAN severity (27.94 ± 6.88 mg/dl in normal vs. 34.46 ± 8.71 mg/dl in definite CAN, $p < 0.0001$), whereas creatinine showed no significant difference ($p = 0.397$). Autonomic indices declined stepwise with increasing severity: heart rate variability was lowest in definite CAN, and both the E/I ratio and the 30:15 ratio were significantly reduced compared with early and normal groups ($p < 0.0001$).

DISCUSSION

The present study investigated the metabolic correlates of cardiac autonomic neuropathy (CAN) in Iraqi patients with type 2 diabetes mellitus (T2DM), integrating glycemic, lipid, and renal indices with standardized cardiovascular autonomic reflex tests (CARTs). The findings demonstrated that HbA1c, triglycerides, LDL-C, VLDL-C, and serum urea progressively increased with CAN severity, whereas autonomic indices such as the expiratory–inspiratory ratio and the 30:15 ratio declined stepwise from normal to definite CAN. These results underscore the multifactorial metabolic basis of CAN, extending beyond isolated neural dysfunction.

Unexpected Findings

Two results deserve emphasis. First, Creatinine concentrations were modestly but significantly elevated among diabetic participants relative to controls, which may reflect early renal impairment and slight reductions in glomerular filtration capacity that typically precede overt nephropathy. This aligns with reports that even modest elevations in creatinine, within the normal reference range, may signal incipient nephropathy in diabetes. Second, HDL-C levels were lower in the diabetic cohort, which is in line with the classical dyslipidemic pattern of T2DM characterized by elevated triglycerides, increased LDL-C/VLDL-C, and reduced HDL-C. This lipid triad is strongly linked to endothelial dysfunction and heightened cardiovascular risk.

Molecular Mechanisms Linking Metabolic Disturbances to CAN

Our results align with mechanistic models in which metabolic dysregulation accelerates autonomic impairment. **Chronic hyperglycemia** leads to non-enzymatic protein glycation and accumulation of advanced glycation end-products (AGEs), which promote **endothelial dysfunction and impaired nitric oxide (NO) bioavailability** (Mengstie et al., 2022). This cascade contributes to reduced baroreflex sensitivity and diminished heart rate variability. Likewise, **hypertriglyceridemia and elevated VLDL** exacerbate oxidative stress through lipid peroxidation and activation of **NF-κB–dependent inflammatory pathways**, thereby amplifying neuronal injury (Penna and Pagliaro, 2025). In parallel, **elevated serum urea** may signify the role of uremic toxins in disrupting neural–endothelial cross-talk, contributing to impaired autonomic integrity even in the absence of overt nephropathy. Collectively, these pathways delineate a **metabolic–vascular–neural axis** underpinning CAN.

Comparisons with Previous Studies

Our findings resonate with international evidence. Papazoglou et al.,2022 emphasized the prognostic burden of CAN and its links to glycemic control. Cai et al. 2021 demonstrated that **triglycerides strongly correlate with progression of diabetic neuropathy**, consistent with our observation of higher TG and VLDL levels in definite CAN. Similarly, Ziegler et al. (2021) reported associations between autonomic dysfunction and altered lipid metabolites in early T2DM, supporting our mediation analysis. Moreover, Wang and Cao (2025), identified **HbA1c variability** as an independent determinant of CAN progression, reinforcing the significance of sustained glycemic burden. In the renal domain, Caravaca-Fontán et al. (2022) highlighted a bidirectional relationship between proteinuria and CAN, which is consistent with our finding of proteinuria clustering in patients with definite CAN.

Regional Context and Knowledge Gap

While CAN has been extensively studied in European and Asian cohorts, evidence from **Middle Eastern populations—particularly Iraq—remains sparse**. Given regional differences in lifestyle, genetics, and healthcare systems, the present study adds context-specific insights by delineating a biochemical signature of CAN risk in Iraqi T2DM patients. Such data may inform tailored screening protocols and preventive strategies in resource-limited settings.

Limitations and Future Directions

As the study was cross-sectional and conducted at a single center, its ability to infer causality and to generalize findings is inherently restricted. Moreover, residual confounding arising from unmeasured variables such as dietary intake, medication regimens, and lifestyle habits cannot be entirely excluded. Inflammatory and oxidative stress biomarkers were not assessed, and autonomic evaluation was restricted to CARTs. Future multicenter longitudinal studies incorporating broader biomarkers and advanced autonomic assessments are recommended. "As this study was conducted at a single center in Mosul, the findings may not fully generalize to the broader Iraqi or Middle Eastern populations. Multicenter and longitudinal studies are required to validate and extend these results. Another limitation is the absence of inflammatory and oxidative stress biomarkers (e.g., IL-6, TNF-α, malondialdehyde), which could have provided mechanistic insights into the metabolic–vascular–neural axis. Moreover, lifestyle

factors such as physical activity, diet, and smoking were not systematically assessed, and thus residual confounding cannot be excluded.

CONCLUSION

This study demonstrates that cardiac autonomic neuropathy (CAN) is highly prevalent among Iraqi patients with type 2 diabetes mellitus and is strongly linked to poor glycemic control, dyslipidemia, and subtle renal dysfunction. HbA1c, triglycerides, and urea emerged as independent predictors of CAN severity, while lipid fractions partially mediated the relationship between hyperglycemia and autonomic impairment. These findings highlight the role of an integrated metabolic–vascular–neural axis in the pathogenesis of CAN and suggest that incorporating routine biochemical markers into clinical assessment may provide an early signature of autonomic risk. Future research should expand to multicenter, longitudinal cohorts and include inflammatory and oxidative biomarkers to refine predictive models and improve preventive strategies.

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