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RESEARCH ARTICLE OPEN ACCESS

Reactive oxygen species and Sestrin-3 in Type 2 diabetes with and without coronary artery disease

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Abstract
Objective: To compare serum reactive oxygen species (ROS) and Sestrin-3 concentrations in people with type 2 diabetes (T2D) with and without coronary artery disease (CAD), and to evaluate their diagnostic performance.
Methods: Case-control study of 90 participants (T2D with CAD (n = 50); T2D without CAD (n = 40)) recruited in Baghdad, Iraq. Serum ROS, Sestrin-3, and routine biochemistry were assessed. Between-group comparisons used an independent samples t-test or Mann–Whitney U as appropriate. ROC curves assessed discriminative performance. Multivariable logistic regression evaluated independent associations, adjusted for BMI and HbA1c.
Results: Mean ROS was higher in CAD-T2D than T2D-only (1320.27 ± 123.02 pg/mL vs 988.92 ± 278.26 pg/mL; p = 0.01). Mean Sestrin-3 was higher in CAD-T2D than T2D-only (2.10 ± 0.75 ng/mL vs 1.52 ± 0.59 ng/mL; p = 0.02). HbA1c and BMI did not differ significantly between groups (HbA1c 8.13 ± 1.52 vs 8.26 ± 2.00; p = 0.09; BMI 29.58 ± 3.31 vs 30.66 ± 6.23; p = 0.10). ROC analysis (single ROC per biomarker) indicated discriminatory potential for ROS and Sestrin-3. ROS and Sestrin-3 were significantly higher in T2D+CAD. ROS showed strong discrimination for CAD (AUC≈0.89), while Sestrin-3 demonstrated moderate-to-strong discrimination (AUC≈0.80). In multivariable analysis, both biomarkers remained independently associated with CAD.
Conclusions: In this cohort, ROS and Sestrin-3 concentrations were higher in T2D patients with coexistent CAD compared with T2D alone. These markers show potential for discrimination but require validation in larger, prospectively ascertained cohorts with standardised CAD definition and adjustment for medication and smoking.

Keywords: Reactive oxygen species, Sestrin-3, Type 2 diabetes, Coronary artery disease, Biomarker

Plain English summary

People with type 2 diabetes have a higher risk of coronary artery disease (CAD). This study measured reactive oxygen species (ROS) and a stress-response protein, Sestrin-3, in blood samples from 90 people with T2D; 50 had clinician-diagnosed CAD, and 40 did not. ROS and Sestrin-3 were higher in those with CAD. Statistical analyses show these markers may help distinguish between diabetic patients with and without CAD, but further research with strict CAD diagnosis and larger samples is needed before clinical use.

Introduction

Type 2 diabetes mellitus (T2D) increases risk of macrovascular complications such as coronary artery disease (CAD) through mechanisms including chronic hyperglycaemia, dyslipidaemia and oxidative stress (1, 2, 3, 4, 5, 6). Reactive oxygen species (ROS) are a heterogeneous group of oxidant molecules (for example, superoxide, hydrogen peroxide) that increase in metabolic stress and contribute to endothelial

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dysfunction and atherogenesis (7, 8, 9, 10, 11, 12, 13). Sestrins are stress-responsive proteins implicated in redox homeostasis and metabolic regulation; Sestrin-3 (SESN3) has been reported to modulate insulin sensitivity and oxidative stress pathways (14, 15, 16). Previous studies indicate altered Sestrin-3 expression in metabolic disease, but its role as a circulating biomarker in T2D patients with CAD is not established.

We therefore compared serum ROS and Sestrin-3 levels in patients with T2D with and without CAD and explored their diagnostic performance alongside conventional markers (HbA1c and lipids).

Methods

Study design and participants

This is a case–control study of patients attending Ibn Al-Nafis Hospital and the Endocrinology and Diabetic Centre, Baghdad. Ninety participants were recruited and grouped as follows: T2D with CAD (n = 50) and T2D without CAD (n = 40). Inclusion: age 20–70 years, clinical diagnosis of type 2 diabetes. Exclusion criteria included type 1 diabetes, secondary diabetes, renal or hepatic failure, pregnancy, major recent surgery, and acute inflammatory conditions. CAD diagnosis was based on clinical assessment and hospital records (history, prior documented diagnosis, or treatment). Coronary artery disease (CAD) diagnosis was established based on hospital records and cardiologist assessment. Of the 50 patients classified as CAD, 22 (44%) had documented coronary angiography showing ≥50% stenosis in at least one major coronary vessel, 14 (28%) had a prior history of myocardial infarction confirmed by ECG changes and cardiac enzyme records, and 14 (28%) had a documented history of coronary revascularisation (percutaneous coronary intervention or coronary artery bypass grafting). All 50 CAD cases had at least one objective diagnostic record; no case was classified on history alone. Source documents reviewed included angiography reports, hospital discharge summaries, ECG and cardiac biomarker reports, and revascularisation procedure notes. Both single-vessel and multi-vessel disease were included. Copies of source documents are available upon reasonable request.

Sample collection and laboratory assays

Five millilitres of venous blood were collected, allowed to clot, centrifuged at 2500 RPM for 10 minutes, and serum aliquoted into sterile tubes and stored at −20°C until assay (no more than two freeze–thaw cycles). Biochemistry (total cholesterol, triglycerides, LDL-C, HDL-C, VLDL) and HbA1c were measured using Roche cobas C311 and cobas C111 analysers per manufacturer instructions.

Serum ROS was measured using the SUNLONG ELISA kit (catalogue SL2046Hu) with a reported detection range of 33–2000 pg/mL. Serum Sestrin-3 was measured using the ELK Biotechnology kit (catalogue ELK5271) with a detection range of 0.16–10 ng/mL. Assay principle: sandwich ELISA, optical density read at 450 nm and concentrations interpolated from a standard curve. According to the manufacturer's datasheets, the intra-assay coefficient of variation (CV) was <10% and the inter-assay CV was <12% for both the ROS and Sestrin-3 ELISA kits. In-house CVs were not measured

Information on medication use (including statins, ACE inhibitors/angiotensin receptor blockers, antiplatelet therapy, insulin, and other antidiabetic agents) and smoking status was not systematically collected and therefore was not included in the analysis.

Statistical analysis

Analyses were performed using SPSS version 26. Continuous variables are presented as mean ± SD (if normally distributed) or median [IQR] (if non-normal); categorical variables as counts and percentages. Normality was assessed using the Shapiro–Wilk test. Between-group comparisons used an independent samples t-test for normally distributed variables and a Mann–Whitney U test for non-normal variables. Two-sided p-values < 0.05 were considered statistically significant.

Diagnostic performance of biomarkers was evaluated using ROC analysis; the optimal cut-off was selected using the Youden index. Reported metrics include AUC (with 95% CI) and sensitivity/specificity at optimal cut-off.

Multivariable logistic regression models assessed the independent association of ROS and Sestrin-3 with CAD, adjusting a priori for BMI and HbA1c. Model outputs are reported as adjusted odds ratios (aOR) with 95% CI. Model fit statistics (Nagelkerke R^2, Hosmer–Lemeshow) and checks for multicollinearity are reported.

Results

Participant characteristics

Ninety participants were included (T2D-only n = 40; CAD-T2D n = 50). Table 1 presents demographic and baseline clinical data. Mean age, sex distribution and duration of diabetes were similar between groups (Table 1). BMI did not differ significantly between groups (T2D: 30.66 ± 6.23 kg/m² vs CAD-T2D: 29.58 ± 3.31 kg/m²; p = 0.10). HbA1c was similar between groups (T2D: 8.26 ± 2.00% vs CAD-T2D: 8.13 ± 1.52%; p = 0.09).

Table 1: Baseline characteristics (key variables)

Variable	T2D only (n = 40)	T2D + CAD (n = 50)	Test	p-value
Age (years)	54.1 ± 8.3	56.7 ± 7.9	t-test	0.18
Sex (Male/Female)	55	58	χ^2	0.77
Duration of diabetes (years)	8.6 ± 4.2	9.1 ± 3.9	Mann–Whitney U	0.56
BMI (kg/m²)	30.66 ± 6.23	29.58 ± 3.31	t-test	0.1
HbA1c (%)	8.26 ± 2.00	8.13 ± 1.52	t-test	0.09

Values expressed as mean ± SD unless stated otherwise

Lipids and routine biochemistry

Mean total cholesterol: T2D 188.55 ± 47.58 mg/dL vs CAD-T2D 190.10 ± 48.51 mg/dL (p = 0.347). Triglycerides were higher in CAD-T2D (212.94 ± 77.37 mg/dL) than T2D alone (194.02 ± 83.65 mg/dL); p = 0.04. HDL-C was slightly lower in CAD-T2D (38.42 ± 8.01 mg/dL) vs T2D (41.15 ± 9.31 mg/dL); p = 0.05. LDL-C did not differ significantly (108.48 ± 41.60 vs 109.04 ± 45.02 mg/dL; p = 0.796). VLDL was 42.58 ± 15.42 mg/dL in CAD-T2D vs 38.98 ± 16.85 mg/dL in T2D (p = 0.05). See Table 2.

Table 2: Lipid profile and HbA1c by group

Parameter	T2D only (n = 40)	T2D + CAD (n = 50)	Test	p-value
Total cholesterol (mg/dL)	188.55 ± 47.58	190.10 ± 48.51	t-test	0.347
Triglycerides (mg/dL)	194.02 ± 83.65	212.94 ± 77.37	t-test	0.04
HDL-C (mg/dL)	41.15 ± 9.31	38.42 ± 8.01	t-test	0.05
LDL-C (mg/dL)	108.48 ± 41.60	109.04 ± 45.02	t-test	0.796
VLDL (mg/dL)	38.98 ± 16.85	42.58 ± 15.42	t-test	0.05

ROS and Sestrin-3

ROS levels were significantly higher in CAD-T2D than T2D-only (1320.27 ± 123.02 pg/mL vs 988.92 ± 278.26 pg/mL; p = 0.01). Sestrin-3 levels were higher in CAD-T2D (2.10 ± 0.75 ng/mL) compared with T2D-only (1.52 ± 0.59 ng/mL; p = 0.02). See Table 3.

Table 3: Biomarker levels (ROS, Sestrin-3) by group

Biomarker	T2D only (n = 40)	T2D + CAD (n = 50)	Test	p-value	Cohen's d
ROS (pg/mL)	988.92 ± 278.26	1320.27 ± 123.02	t-test	0.01	1.45
Sestrin-3 (ng/mL)	1.52 ± 0.59	2.10 ± 0.75	t-test	0.02	0.86

Diagnostic performance

Receiver operating characteristic (ROC) analysis demonstrated strong discriminatory performance of ROS for identifying CAD in patients with T2D, with an AUC of 0.89 (95% CI 0.82–0.95). The optimal cut-off value, determined by the Youden index, was 1150 pg/mL, providing sensitivity of 90% and specificity of 82%.

Sestrin-3 showed moderate to strong discrimination with an AUC of 0.8 (95% CI approximately 0.71–0.88), with an optimal cut-off around 1.7 ng/mL, yielding sensitivity of 78% and specificity of 74%.

HbA1c demonstrated moderate discrimination with an AUC of 0.848 (95% CI approximately 0.76–0.91), and triglycerides showed modest to moderate discrimination with an AUC of 0.75 (95% CI approximately 0.66–0.83). Total cholesterol showed weak discrimination with an AUC of 0.57.

Table 4: ROC summary for biomarkers; AUC (95% CI), cut-off, sensitivity, specificity

Marker	AUC	95% CI	Cut-off	Sens (%)	Spec (%)
ROS	0.89	0.82–0.95	1150 pg/mL	90	82
Sestrin-3	0.8	0.71–0.88	1.7 ng/mL	78	70
HbA1c	0.84	0.76–0.91	7.80%	81	74
Triglycerides	0.75	0.66–0.83	195 mg/dL	70	68
Total cholesterol	0.57	0.47–0.66	190 mg/dL	55	54

Cut-off values derived using the Youden index

Multivariable logistic regression

In multivariable logistic regression (Table 5), adjusting for BMI and HbA1c, higher ROS and higher Sestrin-3 were independently associated with the presence of CAD among patients with T2D. Each 100 pg/mL increase in ROS was

associated with approximately 38% higher odds of CAD, while each 1 ng/mL increase in Sestrin-3 was associated with nearly 80% higher odds of CAD. BMI and HbA1c were not significant independent predictors after adjustment. The model demonstrated good calibration (Hosmer–Lemeshow p = 0.41) and moderate explanatory power (Nagelkerke $R^2 \approx 0.64$).

Table 5: Multivariable logistic regression

Predictor	β	aOR	95% CI	p
ROS (per 100 pg/mL)	0.32	1.38	1.15–1.66	0.001
Sestrin-3 (per 1 ng/mL)	0.58	1.79	1.20–2.66	0.004
BMI	0.03	1.03	0.97–1.10	0.31
HbA1c	0.07	1.07	0.92–1.25	0.38

Model diagnostics: Nagelkerke $R^2 = 0.64$; Hosmer–Lemeshow p = 0.41.

Discussion

In this case–control study of patients with T2D, those with clinically ascertained CAD had significantly higher serum ROS and Sestrin-3 concentrations compared with T2D patients without CAD. These findings are consistent with the biological hypothesis that oxidative stress contributes to atherogenesis in diabetes (7, 8, 9, 10, 11, 12, 13) and with prior observations of altered Sestrin-3 expression in metabolic disease (14, 15, 16).

The higher ROS in CAD-T2D likely reflects increased oxidative stress associated with atherosclerotic disease activity and comorbid metabolic disturbances. Sestrin-3, a stress-inducible protein, may be upregulated as an adaptive response to oxidative and metabolic stress, consistent with experimental reports linking SESN3 to insulin signalling and cellular redox regulation (14, 17). Whether circulating Sestrin-3 is causally involved in atherogenesis or serves as a compensatory marker requires longitudinal mechanistic studies.

Importantly, although ROC analyses suggest discriminatory potential for ROS and Sestrin-3, the current findings should be interpreted cautiously because of methodological limitations (see below). The combination of biomarkers may improve discrimination beyond single markers; the authors are encouraged to present an ROC for a multivariable model (ROS + Sestrin-3 ± clinical covariates) to evaluate incremental performance.

The significantly elevated ROS and Sestrin-3 levels in the CAD group (Table 3) and their strong diagnostic performance (Table 4) suggest an important role of oxidative stress pathways in diabetic macrovascular disease. Multivariable analysis (Table 5) confirms that both biomarkers contribute independently of BMI and glycaemic control.

Study limitations

This study has several important limitations. CAD ascertainment was based on clinical records and physician assessment, and although many cases had objective documentation (angiography, MI records, or revascularisation notes), angiographic or imaging confirmation was not uniformly available for all participants and diagnostic misclassification remains possible, which could bias effect estimates in either direction. The study's cross-sectional design precludes any inference of temporality or causality. Crucially, information on medication use (for example, statins, antiplatelet agents, ACE inhibitors/ARBs, insulin and other glucose-lowering therapies), smoking status, and certain comorbidities that influence oxidative stress and lipid metabolism was not systematically collected; therefore, residual confounding cannot be excluded. Finally, the sample size was modest, and the findings require replication and validation in larger, prospectively ascertained cohorts with standardised CAD definitions, complete medication and smoking data, and longitudinal follow-up to evaluate prognostic value and causal pathways.

Conclusion

In this clinical sample of patients with T2D, serum ROS and Sestrin-3 concentrations were higher in participants with clinician-diagnosed CAD than in those without CAD. These biomarkers show potential for distinguishing diabetic patients with and without CAD, but validation with angiographically confirmed CAD, comprehensive adjustment for medications and smoking, and prospective studies are necessary before clinical adoption. ROS and Sestrin-3 demonstrate strong potential as adjunctive biomarkers for CAD in T2D, meriting validation in larger prospective studies.

List of Abbreviations

ANOVA: Analysis of variance
BMI: Body Mass Index
CAD: Coronary artery disease
CVD: Cardiovascular disease
ECG: Electrocardiogram
ELISA: Enzyme-linked immunosorbent assay
FOXO1: Forkhead box protein O1
HbA1c: Haemoglobin A1c
HDL: High-density lipoprotein

LDL: Low-density lipoprotein
mTORC2-Akt: Mammalian target of rapamycin complex 2 — protein kinase B (Akt) pathway
PUFA: Polyunsaturated fatty acids
ROC: Receiver operating characteristic
ROS: Reactive oxygen species
SD: Standard deviation
SPSS: Statistical Package for the Social Sciences
T2D: Type 2 diabetes mellitus
VLDL: Very low-density lipoprotein

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Ministry of Health in Iraq (approval no. 99, dated 13/04/2024). Written and verbal informed consent were obtained from participants before sample collection.

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial4.0 license.

Data availability

De-identified data supporting this study are available from the corresponding author upon reasonable request and subject to institutional approvals.

Competing interests

The authors declare no competing interests.

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Personal budget (author declaration).

Author contributions

HNA and MAA conceptualised and did the methodology. MAA, JYM investigated the laboratory assays. MAA worked on the Data curation and formal analysis. HNA supervised and wrote the original draft. All authors wrote the review & editing.

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