SHORT COMMUNICATION

Association of nitric oxide levels and lipid profile with endothelial dysfunction in type 2 diabetic patients

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Background: Evidence-based screening is crucial to detect myocardial ischemia in high-risk diabetics. We explored the relationship between nitric oxide (NO) levels, lipid profile indices, and atherogenic index of plasma (AIP) in type 2 diabetics with coronary artery disease (CAD) and to determine their potential as prognostic markers. **Materials and Methods:** A case–control study included 50 diabetics with CAD (cases), 30 diabetics without CAD (control 1), and 23 healthy controls (control 2). Biochemical parameters were determined using standard protocols; plasma NO was measured via the Griess reaction. **Results:** Cases had the highest levels of NO, fasting blood sugar, glycated hemoglobin (HbA1c), and triglycerides, and the lowest total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. Cases exhibited the highest TC: HDL-C, LDL-C: HDL-C, and AIP ratios. A significant positive correlation between NO and HbA1c (r = 0.328, P = 0.020). **Conclusion:** Chronic hyperglycemia could enhance NO overproduction driven by inducible isoform, suggesting a potential role for chronic hyperglycemia in endothelial dysfunction and vascular complications in diabetes.

Key words: Diabetes mellitus, endothelial dysfunction, lipoproteins, nitric oxide

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INTRODUCTION

Patients with type 2 diabetes (T2DM) have a 2–4 times higher risk of coronary artery disease (CAD) compared to healthy individuals, contributing to over 50% mortality rates in T2DM patients.[1] Nitric oxide (NO), a crucial signaling molecule, regulates blood vessel dilation. NO is synthesized from L-arginine to L-citrulline by NO synthase (NOS), which includes neuronal NOS (nNOS), endothelial NOS (eNOS), and the inducible (iNOS) isoforms.[2] Extreme rates of morbidity and mortality in T2DM are associated with endothelial dysfunction and the development of atherosclerotic plaque.[3]

Chronic hyperglycemia in T2DM causes endothelial and autonomic nerve damage, leading to asymptomatic myocardial infarction (silent MI). This often goes undiagnosed, necessitating evidence-based screening to detect myocardial ischemia in T2DM patients at risk for CAD.[4]

This study aimed to investigate the relationship between NO levels, lipid profiles, and atherogenic index of plasma (AIP) as prognostic indicators in T2DM and to propose preventive measures for T2DM patients at risk of developing CAD.

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MATERIALS AND METHODS

Study design and population

This case–control study, approved by the Qassim Regional Ethics Committee (No. 1242768/144, 2020), adhered to the Helsinki Declaration and ensured participant confidentiality. It was conducted between March 2020 and March 2021 and included a total of 103 participants. The participants were divided into three groups: (1) the cases group which included 50 diabetic patients with CAD; (2) the control 1 group which included 30 diabetic patients without CAD or other diabetes complications; and (3) the control 2 group which included 23 healthy controls without diabetes or CAD. Cases were recruited from the Cardiology Department at Prince Sultan Cardiac Center, King Fahad Specialist Hospital, Buraidah, Qassim, KSA. Control 1 subjects were recruited from AlBassam Diabetes and Endocrinology Center, King Saud Hospital, Unizah, Qassim, KSA. Both groups included patients needing preventive measures and cholesterol-lowering therapies. Subjects in the control 2 group were healthy individuals who were recruited from the community.

Measurement of body mass index, blood collection, and laboratory experiments

Weight and height were recorded to compute body mass index (BMI) (kg/m^2). A fasting venous blood sample was collected in fluoride and EDTA vacutainers. Plasma was extracted by centrifuging at 2500 RPM for 10 min. Blood glucose and lipid profiles were measured on Roche Cobas 6000 auto analyzers. Glycated hemoglobin (HbA1c) was analyzed using a Siemens Dimension Xpand auto analyzer. Ratios total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL)-C/HDL-C, and AIP were calculated. NO was measured according to Griess reaction using the NO Colorimetric Assay Kit (Elabscience, USA; Catalog No. E-BC-K035-S).

Statistical analysis

The continuous data were presented as mean ± standard deviation, minimum–maximum, and median (interquartile range), whereas categorical data were shown as *n* (%). ANOVA was used for the continuous data comparison and Chi-square for the categorical variables. *Post hoc* tests identified groups with significant differences ($P < 0.05$). Pearson correlation assessed relationships between NO levels and selected variables. Significance was set at $P < 0.05$. Data analysis used Statistical Package for the Social Sciences (SPSS) software (version 25), IBM, Armonk, NY, USA.

RESULTS

Participants' ages varied across groups, with cases having the highest mean age; while the control 1 group had the highest BMI. Fasting blood sugar levels were the highest in the case group, followed by control 1 and control 2, with significant differences between cases and controls ($P < 0.001$). This suggests an increased susceptibility to cardiovascular problems, emphasizing the importance of maintaining glycemic control in managing both T2DM and CAD. HbA1c levels were the highest in cases compared to control 1 and control 2, highlighting the need for optimal HbA1c levels to reduce cardiovascular risk. As illustrated in Figure 1, plasma NO levels were significantly higher in cases (8.18 ± 3.93 μM/L) compared to control 1 (6.25 ± 1.77 μM/L) and control 2 (5.07 ± 2.23 μM/L). Moreover, cases had higher TC: HDL-C, LDL-C: HDL-C, and AIP ratios, indicating a dysregulated lipid profile and higher atherogenic potential. Detailed demographic and biochemical data are provided in Table 1.

A Pearson correlation analysis found a significant positive, but low correlation between NO levels and HbA1c in the case group ($r = 0.328$, $P = 0.020$), suggesting a link between glycemic management and NO generation in CAD patients [Table 2].

DISCUSSION

Consistent with previous findings,[5,6] our study showed that T2DM patients with CAD had significantly elevated NO levels compared to control 1 ($P = 0.008$) and control 2 ($P < 0.001$) groups. Elevated NO may indicate endothelial dysfunction and oxidative stress, linked to CAD progression. It is reported that patients with T2DM had significantly higher LDL/HDL ratio and AIPs than nondiabetics.[7] This study showed imbalanced NO levels and altered lipid profiles which both may contribute to vascular complications. We can deduce that lipid profile screening and management are crucial in reducing cardiovascular risk and improving strategies for T2DM patients with CAD.

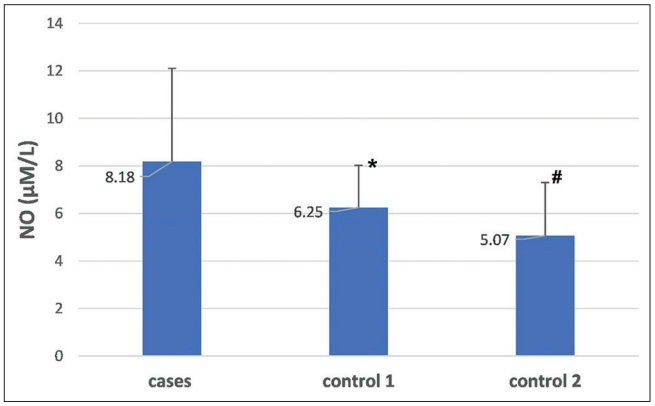

Figure 1: The bar chart represents the mean and standard deviation of plasma nitric oxide (NO) levels (μM/L) among the study participants across the three groups. The cases group (type 2 diabetics with coronary artery disease [CAD]) showed significantly higher NO levels compared to both the control 1 group (type 2 diabetics without CAD) ($P = 0.008$) and the control 2 group (healthy individuals) ($P < 0.001$). NO = Nitric oxide

Table 1: The sociodemographic and laboratory analytes among the participants enrolled in the study

Variables	Mean±SD (minimum–maximum)			Statistical analysis		
	Cases (*n*=50)	Control 1 group (*n*=30)	Control 2 group (*n*=23)	χ^2/F	*P*	*Post hoc* (*P*)
Gender, *n* (%)						
Male	35 (70.0)	6 (20.0)	14 (60.9)	19.501	<0.001*	0.008[a,*] 0.440[b] 0.002[c,*]
Female	15 (30.0)	24 (80.0)	9 (39.1)			
Age (years)	62.02±12.72 (34–93)	46.3±14.43 (17–73)	35.22±9.16 (18–55)	39.332	<0.001*	*P*<0.001[a,*] *P*<0.001[b,*] 0.002*[,c]
BMI (kg/m^2)	27.82±3.85 (19.53–36.21)	30.56±6.65 (19.82–46.17)	27.11±4.74 (19.57–36.5)	3.883	0.024*	0.020[a,*] 0.575[b] 0.015[c,*]
FBG (mmol/L)	10.34±3.39 (4.42–19.34)	6.16±1.47 (4.77–11.84)	5.71±0.54 (4.8–7.04)	37.972	<0.001*	*P*<0.001[a,*] *P*<0.001[b,*] 0.525[c]
HbA1c (%)	8.76±2.27 (1.5–13.7)	6.6±1.66 (4.9–10.5)	5.4±0.5 (4.5–7)	30.165	<0.001*	*P*<0.001[a,*] *P*<0.001[b,*] 0.020[c,*]
TC (mmol/L)	3.87±1.1 (1.73–6.9)	4.49±0.75 (2.85–5.62)	4.68±0.97 (2.9–6.39)	6.742	0.002*	0.008[a,*] 0.002* 0.490[c]
TG (mmol/L)	2.07±1.83 (0.88–10.5)	1.5±0.74 (0.36–3.63)	1.93±1.77 (0.96–9.59)	1.235	0.295	-
HDL-C (mmol/L)	0.98±0.31 (0.6–2)	1.26±0.32 (0.74–2.18)	1.29±0.38 (0.76–2.36)	10.221	<0.001*	*P*<0.001[a,*] *P*<0.001[b,*] 0.696[c]
LDL-C (mmol/L)	2.49±0.94 (0.34–4.53)	2.94±0.76 (1.73–4.42)	3.12±0.86 (1.38–4.52)	5.059	0.008*	0.025[a,*] 0.005[b,*] 0.474[c]
NO (μM/L)	8.18±3.93 (2.29–20.57)	6.25±1.77 (4.57–10.29)	5.07±2.23 (1.14–9.14)	8.979	<0.001*	0.008[a,*] *P*<0.001[b,*] 0.173[c]
AIP	0.17±0.34 (−0.6–1.2)	0.004±0.31 (−0.63–0.53)	0.02±0.37 (−0.63–0.99)	2.823	0.064	-
TC: HDL-C	4.26±1.72 (1.5–10.45)	3.74±0.95 (2.2–5.99)	3.83±1.12 (1.58–6.52)	1.522	0.223	-
LDL: HDL-C	2.73±1.26 (0.37–6.2)	2.46±0.83 (1.05–4.18)	2.59±0.92 (0.58–4.44)	0.557	0.575	-

*A statistically significant difference at *P*<0.05; [a]Compare cases versus control 1 group; [b]Compares cases versus control 2 group; [c]Compares control 1 group versus control 2 group. *n*=Number of subjects; χ^2=Chi-square; *F*=One-way ANOVA; BMI=Body mass index; FBG=Fasting blood glucose; HbA1c=Glycated hemoglobin; TC=Total cholesterol; TG=Triglycerides; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; NO=Nitric oxide; AIP=Atherogenic index of plasma; SD=Standard deviation

Table 2: Correlation between nitric oxide levels and numerical variables among study participants

Variable	No (μM/L)					
	Case (*n*=50)		Control 1 group (*n*=30)		Control 2 group (*n*=23)	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	−0.268	0.060	−0.067	0.725	−0.276	0.203
BMI (kg/m^2)	−0.160	0.268	−0.138	0.468	−0.006	0.978
FBG (mmol/L)	0.122	0.400	0.021	0.916	0.274	0.205
HbA1c (%)	0.328	0.020*	0.053	0.780	0.323	0.133
TC (mmol/L)	0.090	0.534	−0.636	<0.001*	−0.416	0.048
TG (mmol/L)	0.040	0.783	−0.250	0.183	0.008	0.970
HDL-C (mmol/L)	0.029	0.839	−0.050	0.792	−0.075	0.735
LDL-C (mmol/L)	0.118	0.413	−0.660	<0.001*	−0.357	0.095
AIP	0.125	0.388	−0.283	0.130	0.039	0.861
TC: HDL-C	0.041	0.776	−0.381	0.038*	−0.166	0.450
LDL: HDL-C	0.064	0.657	−0.466	0.009*	−0.156	0.477

*A statistically significant difference at *P*<0.05. *n*=Number of subjects; *r*=Correlation coefficients; BMI=Body mass index; FBG=Fasting blood glucose; HbA1c=Glycated hemoglobin; TC=Total cholesterol; TG=Triglycerides; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; NO=Nitric oxide; AIP=Atherogenic index of plasma

The imbalance between eNOS and iNOS significantly influences cardiovascular disease progression.[8] Upregulation of iNOS increases NO production, reacting with superoxide radicals to generate reactive nitrogen species (RNSs). These RNSs oxidize lipoproteins in atherosclerotic lesions, promoting atherosclerosis by oxidizing LDL.[9] Hence, prolonged hyperglycemia alters vascular endothelium balance, favoring iNOS expression, and elevating NO levels.[10]

Recommendation and limitation

This study highlights the necessity for future research to directly measure iNOS activity in similar patient cohorts to explicitly validate this biochemical pathway.

The total sample size of 103 participants may be small. Groups of 50, 30, and 23 were determined based on feasibility, availability, and previous literature. Smaller studies can still offer valuable insights and guide future research.

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Conflicts of interest
There are no conflicts of interest.

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