Original Article

Association of arterial stiffness, N-terminal pro-brain natriuretic peptide, insulin resistance, and left ventricular diastolic dysfunction with diabetic cardiac autonomic neuropathy

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Abstract

Aim: To investigate the changes of insulin resistance (IR), parameters of arterial stiffness, concentrations of N-terminal pro-brain natriuretic peptide (NT-proBNP), and echocardiographic parameters in patients with cardiac autonomic neuropathy (CAN) and type 2 diabetes mellitus (T2D).

Methods: This study recruited 44 patients with T2D (19 patients without CAN and 25 patients with CAN) and 15 healthy volunteers. Arterial stiffness, immunoreactive insulin, homeostasis model assessment IR, NT-proBNP parameters, and echocardiographic examination were assessed.

Results: Development of CAN is associated with increase in NT-proBNP levels, IR parameters, and arterial stiffening. Among patients with CAN, arterial stiffness parameters were considered as high. We found out that among patients of this group, the value of brachial augmentation index was normal in 52%, elevated in 40%, and pathological in 8%; pulse wave velocity was normal in 16%, elevated in 52%, and pathological in 32% of cases. Obtained results showed that development of CAN is accompanied by more pronounced left ventricular diastolic dysfunction and by formation of left ventricular hypertrophy (LVH), mostly by concentric type. Among patients of this group concentric LVH was diagnosed among 76 % and eccentric LVH among 16% of persons. Multiple

regression analysis, after controlling for age, sex, diabetes duration, blood pressure, HbA1c, and left ventricular mass index, showed an independent association of heart rate response to deep breathing with pulse wave velocity ($P < 0.001$).

Conclusion: Development of CAN is associated with increased IR parameters, increased levels of NT-proBNP, arterial stiffening, left ventricular diastolic dysfunction, and formation of LVH, mostly by concentric type.

Keywords: Arterial stiffness, cardiac autonomic neuropathy, echocardiography, insulin resistance, myocardium, N-terminal pro-brain natriuretic peptide, type 2 diabetes mellitus

INTRODUCTION

Myocardial metabolic remodeling occurred over established type 2 diabetes mellitus (T2D) among adult and retired persons; coronary vessels affection, myocardium changes, diabetic cardiomyopathy (DCM), and diabetic cardiac autonomic neuropathy (CAN) are associated with the definition "diabetic heart"[1,2].

CAN is a significant risk factor for coronary heart disease, including heart attack, stroke, heart failure (HF), and sudden arrhythmic death. The development of CAN plays one of the key roles in the pathogenesis and progression of vessel atherosclerosis[3,4] and is closely connected to metabolic changes, subclinical myocardial dysfunction, and interstitial myocardial fibrosis[5,6]. Nevertheless, the independent connection of CAN with the severity of coronary vessels affections in persons with T2D has not been determined[7].

Several epidemiological studies established that increased parameters of arterial stiffness may increase cardiovascular morbidity and mortality, independently from the presence of several other cardiovascular risk factors. Increase of arterial stiffness parameters by diabetes can be due to changes in structure or type of elastin and/or collagen in the arterial wall, chronic low-grade inflammation, increased oxidative stress (OS), reduced bioavailability of nitric oxide (NO), and increased activity of sympathetic nervous system[8,9].

It was found that cardiac autonomic dysfunction is a significant risk factor in the progression of atherosclerosis and serves as a trigger for cardiovascular and/or cerebrovascular events. The pathophysiology of CAN involves both the endocrine and vegetative nervous system with development of associated abnormalities in metabolic, inflammatory, and hemostatic processes[10]. Autonomic dysfunction and aortic stiffness are pathophysiologically linked processes, but whether impaired cardiac autonomic function leads to arterial stiffening or increased arterial stiffness induces impairment of autonomic function is not clear. Common pathogenetic pathways including chronic hyperglycemia (HG) and hyperinsulinemia, low grade-inflammation, formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), and development of endothelial dysfunction (ED) are involved in the development and progression of cardiac autonomic dysfunction and arterial stiffening[8]. Insulin resistance (IR) and hyperinsulinemia activate the sympathetic part of the vegetative nervous system and renin-angiotensin-aldosterone system, increase systemic metabolic disorders, lead to OS, endoplasmic reticulum stress, and mitochondrial dysfunction, and impair homeostasis of calcium. That leads to development of left ventricular (LV) hypertrophy (LVH), cardiac fibrosis, impairment of the coronary microcirculation, and finally to HF[11]. Moreover, these pathophysiological changes in cardiomyocytes determine the risk factors for development of IR and hyperinsulinemia, resulting in a potentially vicious cycle, which gives rise to the question: does HF cause T2D?[12].

Several investigations found the association between myocardial dysfunction and CAN among T2Dpatients, including LVH and diastolic dysfunction, even in the absence of arterial hypertension and coronary artery disease[13].

The increase of N-terminal pro-brain natriuretic peptide (NT-proBNP) is linked to LV dilatation and volume or pressure overload. An increase in the natriuretic peptides in the blood serum has been shown in persons with impaired and preserved systolic function of the LV. It has been shown that a high level of NT-proBNP is associated with preload, as well as with increased afterload, especially with arterial stiffness and blood pressure (BP)[14].

The aim of our research was to examine the arterial stiffness, insulin resistance, NT-proBNP parameters, and the myocardium functional state in T2D patients with cardiac autonomic neuropathy.

METHODS

Patients

We have enrolled 44 persons with T2D, among them 19 patients without CAN and 25 patients with CAN, and 15 healthy persons. All patients were residents of Lviv and were observed by endocrinologist and cardiologist at the endocrinological department of the Danylo Halytsky Lviv National Medical University, which is located in the Lviv Regional State Clinical Treatment and Diagnostical Endocrinological Center.

Data acquisition and analyses have been approved by the Danylo Halytsky Lviv Medical University Ethics Committee (protocol No. 2 from 18 February 2013). The study protocol was accordant with good clinical practice guidelines and with the Declaration of Helsinki's principles of 1975 as revised in 2014[15]. All persons signed informed consent before their enrollment. The standard hypoglycemic treatment of T2D included sustainable lifestyle modifications (i.e., diet and appropriate physical activity) and oral hypoglycemic drugs. The treatment was unchanged during the study period.

The study inclusion criteria were the following: adults between 50 to 60 years of age diagnosed with T2D based on the American Diabetes Association criteria[16], T2D with optimal or suboptimal glycemic control, body mass index (BMI) within 20-30 kg/m^2, and consent to adhere to the dietary regime and to maintain appropriate physical activity during the study.

The study exclusion criteria were the following: type 1 diabetes mellitus, uncontrolled T2D, ketosis, peripheral vascular diseases, the ischemic diabetic foot, distal neuropathy caused by other factors than T2D (e.g., neurological disease, chronic alcoholism, or pharmacological agents), neoplasms, hypothyroidism, women during pregnancy and lactation, atrial fibrillation or flutter, pacemaker, history of severe liver or kidney disease (i.e., estimated glomerular filtration rate < 30 mL/min/1.73 m^2), HF NYHA III or IV, hypoglycemia, and acute illness in the previous 24 h. To minimize the confounding effect of medications on investigated parameters we excluded persons treated with antiarrhythmic drugs other than beta-blockers, antihistamines, and antidepressants.

Procedures

The examination was done in the morning between 7:30 and 9:30 AM in a stable room temperature (22-24 °C). All participants abstained from drinks (except water), food, and medications for 12 h before the visit and received prescribed drugs after the end of examination. We performed a complete physical examination and used established questionnaires to evaluate previous and current diseases and medication history. The core elements of anthropometry, namely height, weight, and waist circumference were

measured, and BMI was calculated. Triple measurements of BP at the brachial artery were taken at 5-min intervals in the sitting position using an appropriate cuff size. The mean value of the last two measurements was used in the analysis. Current guidelines[17] were used for determination of arterial hypertension: level of systolic BP (SBP) ≥ 140 mmHg and/or level of diastolic BP (DBP) ≥ 90 mmHg, or if patient takes antihypertensive medications.

Examination plan
The examination scheme included measurements of glycated hemoglobin A1c (HbA1c), glucose, five cardiovascular autonomic reflex tests (CARTs), electrocardiography (ECG), Holter-ECG, ambulatory BP monitoring, and echocardiographic examination. Holter-ECG was performed using [ECG "The EC-3H" (Labtech, Hungary)] and ECG-12-channel electrocardiograph "UCARD-200" (UTAS, Ukraine).

Screening for CAN, which included CARTs, time- and frequency-domain heart rate variability (HRV) tests, was performed for all patients[18]. The severity of CAN was determined according to the results of five CARTs. Normal values were evaluated as "0" score, borderline as "0.5" score, and pathological as "1" score. Estimation of autonomic dysfunction severity was performed according to the sum of five CARTs.

Coronary artery disease was defined as a documented history of angina, myocardial infarction (MI), percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. The peripheral artery disease was defined as a history of revascularization procedures at the aorta or the lower limbs, intermittent claudication, or a value of ankle-brachial pressure index less than 0.9[19]. Cerebrovascular disease was defined as a documented history of carotid stenting, carotid arterial disease with 50% stenosis or revascularization, or stroke.

Methods for registration of outcomes
Blood was drawn after an overnight fast (12 h of fasting) in the morning. The glucose concentration in the blood was measured by the glucose oxidase method and HbA1c was determined by the highly sensitive ion-exchange liquid chromatography with BIO-RAD reagents (USA) and D-10 analyzer. Semi-automatic analyzer Humalyzer 2000 with HUMAN (Germany) reagents was used to measure lipid content. Commercial kits from immunogen insulin immunoradiometric assay reagents (Czech Republic) were used to measure serum immunoreactive insulin. Homeostasis model assessment IR (HOMA-IR) was calculated from fasting insulin and glucose as: fasting glucose (mmol/L) × fasting immunoreactive insulin (mcIU/mL)/22.5[20].

NT-proBNP measurement
NT-proBNP level in the blood was determined by solid-phase enzyme-linked assay using kits from DRG (USA) and Biomedica (Austria). Enzyme-linked immunosorbent assays were performed using an enzyme-linked immunosorbent assay (Stat Fax 2100) (Awareness Technology®, USA).

Artery stiffness examination
Evaluation of the arterial stiffness parameters was performed using the TensioMedTM™ Arteriograph [monitor BP "ABPM-04" ("Meditech" Hungary)][21]. To detect arterial wall oscillations in the upper arm using the "stop-flow" principle, the arteriograph cuff was applied on the "non-working" arm over the brachial artery[21]. In the absence of asymmetry of BP, monitoring was performed on the "non-working" arm of the patient. With asymmetry where BP difference is greater than or equal to 10 mmHg, studies were performed on the arm with higher BP. The cuff of the appropriate type was placed on the patient's shoulder so that the lower edge was 2 cm above the elbow. Before starting the measurement, a series of control BP measurements were performed, and the obtained data were compared with hardware data. The standard

measurement frequency interval was set based on the period (15 min in active period of the day and 30 min during the passive period). Upon completion of the examination, the data were transferred for further analysis to a personal computer. The daily parameters of arteriography were determined: aorta augmentation index (AIxao), brachial augmentation index (AIxbr), ambulatory arterial stiffness index (AASI), and pulse wave velocity (PWV). The values were evaluated as following: optimal: AIxbr more than -30%, PWV less than 7 m/s; normal: -30% < AIxbr < -10%, 7 m/s < PWV < 10 m/s; elevated: -10% < AIxbr < 9.8%, 9.8 m/s < PWV < 12 m/s; and pathological values: AIxbr more than 10%, PWV more than 12 m/s[22].

Echocardiography examination
The functional condition and intracardiac hemodynamics of the myocardium were assessed by echocardiography (EchoCG) with "Siemens Sonoline Versa Plus" (Germany), using a sector wide-pole sensor with a frequency of 3-8 MHz in "B" and "M" modes. The recommendations of the American Society of Echocardiography were followed when performing the EchoCG measurements[23]. Cardiac chamber size, interventricular septal thickness (IVS), ejection fraction (EF), end-systolic (LV ESV) and end-diastolic (LV EDV) and were determined. LV myocardial mass (LVMM) and LV myocardial mass indices (LVMI) were calculated. EF was calculated from the LV EDV and LV ESV. The formulas for calculating EF are: EF = LV EDV - LV ESV/LV EDV, or EF = Stroke volume/EDV (multiply by 100). A normal ejection fraction is above 60%, that means that the LV is able to eject 60% of the LV EDV efficiently[24]. Relative wall thickness (RWT) was calculated as 2 times posterior wall thickness divided by the LV diastolic diameter. LVMM was analyzed using sex stratified values of LVMI for the diagnosis of LVH: 110 g/m^2 and more for women and 125 g/m^2 and more for men[23,25].

Diastolic function of LV was evaluated according to the guidelines[23]. Pulsed-wave Doppler assessed mitral inflow from the apical 4-chamber view. The Doppler beam was aligned parallel to the direction of flow. A one- or two-mm sample volume was placed between the tips of mitral leaflets during diastole; peak velocity of early diastolic transmitral flow (E, m/s), peak velocity of late diastolic transmitral flow (A, m/s), deceleration time (DT, ms), and E/A ratio were measured. Isovolumic relaxation time (IVRT, ms) was measured from the end of aortic ejection to the onset of mitral inflow[23-25].

Statistical analysis
Obtained results were expressed as mean ± SEM. One-way ANOVA or repeated-measures ANOVA followed by Tukey post hoc correction for multiple comparisons to localize significant effects were used to assess between-group differences. Multiple logistic regression analysis was performed to analyze various risk factors independently associated with the presence of CAN. Statistical significance was defined as *P* values less than 0.05. Analyses were done using ANOVA (MicroCal Origin v 8.0) software[26].

RESULTS

Anthropometric and clinical characteristics of study participants are presented in Table 1. Obtained results showed, that there were no statistically significant differences between patients with T2D and control group in age, sex, and BMI parameters. As shown in Table 1, patients with T2D were found to have higher SBP, DBP, and heart rate (HR) compared to patients in the control group (*P* < 0.001).

Clinical characteristic of enrolled persons with T2D with and without CAN are presented in Table 2.

As shown in Table 2, persons with T2D and CAN were found to have higher HR compared to patients without CAN (*P* < 0.001). Persons of both groups, namely with T2D without CAN and with CAN, were comparable in age, sex distribution, BMI parameters, diabetes duration, medical history, and treatment. The

Table 1. Anthropometric and clinical characteristics of study participants (M 4 SEM)

Variable	Control group (*n* = 15)	T2D patients (*n* = 44)	P
Age, years	51.9 ± 0.82	53.6 ± 0.57	> 0.05
Male, %	9 (47.4%)	20 (45.5%)	> 0.05
Female, %	10 (52.6%)	24 (54.5%)	> 0.05
BMI, kg/m^2	28.2 ± 0.29	28.2 ± 0.28	> 0.05
HR, bpm	76.5 ± 1.13	91.3 ± 1.74	< 0.001
SBP, mmHg	128.4 ± 1.95	143.4 ± 1.46	< 0.001
DBP, mmHg	75.5 ± 1.18	90.7 ± 1.0	< 0.001
PP, mmHg	52.9 ± 1.52	52.7 ± 1.1	> 0.05

BMI: Body mass index; CAN: cardiac autonomic neuropathy; DBP: diastolic blood pressure; HR: heart rate; M: mean; PP: pulse pressure; SBP: systolic blood pressure; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

Table 2. Clinical characteristic of type 2 diabetes mellitus patients (M 4 SEM)

Variable	T2D patients (*n* = 44)		P
	T2D patients without CAN (*n* = 19)	T2D patients with CAN (*n* = 25)	
Age, years	52.9 ± 0.81	54.2 ± 0.79	> 0.05
Male, %	9 (47.4%)	11 (44%)	> 0.05
Female, %	10 (52.6%)	14 (56%)	> 0.05
Diabetes duration, years	3.53 ± 0.45	3.32 ± 0.33	> 0.05
BMI, kg/m^2	27.7 ± 0.58	28.6 ± 0.21	> 0.05
SBP, mmHg	140.7 ± 1.88	145.4 ± 2.14	> 0.05
DBP, mmHg	88.9 ± 1.44	92.04 ± 1.35	> 0.05
HR, bpm	82.9 ± 1.52	97.8 ± 2.05	< 0.001
History of HT	79.0%	84.2%	> 0.05
History of CAD	42.1%	43.3%	> 0.05
Medications			
ACE inhibitors/ARBs, %	14/73.7%	19/76%	> 0.05
β-blockers, %	5/26.3%	6/24%	> 0.05
Metformin, %	13/68.4%	17/68%	> 0.05
Combined hypoglycemic therapy, %	6/31.6%	8/33.3%	> 0.05
Hypertension, %	14/73.7%	19/76%	> 0.05

ACE: Angiotensin-converting enzyme; ARBs: angiotensin II receptor blockers; BMI: body mass index; CAD: coronary artery disease; CAN: cardiac autonomic neuropathy; DBP: diastolic blood pressure; HR: heart rate; HT: arterial hypertension; M: mean; PP: pulse pressure; SBP: systolic blood pressure; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

therapy of patients was stable for 6 months before inclusion to the study. Persons with T2D and CAN had a statistically significant increase in HR compared to patients without CAN [+17.9%, (*P* < 0.001)]. Levels of SBP, DBP, and PP were comparable for both groups (*P* > 0.05).

Levels of HbA1c, IRI, NT-proBNP concentration, and HOMA-IR parameters in the blood of T2D patients with and without CAN are presented in Table 3.

As can be seen from the results presented in Table 3, patients with T2D had significantly higher levels of HbA1c compared to patients from the control group (*P* < 0.001), but the difference between patients with and without CAN was not statistically significant (*P* > 0.05). We found out, that levels of immunoreactive insulin and HOMA-IR were elevated among T2D patients without CAN (*P* < 0.001) compared to the control group, and further increase of these parameters was found among patients with diagnosed CAN (*P*

Table 3. Concentration of HbA1c, IRI, HOMA-IR, and NT-proBNP in the blood among the studied groups (M 4 SEM)

Variable	Control group (*n* = 15) 1st group	T2Dpatients without CAN (*n* = 19) 2nd group	T2D patients with CAN (*n* = 25) 3rd group	*P*
HbA1c, %	5.45 ± 0.16	6.53 ± 0.14	6.87 ± 0.12	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 > 0.05$
IRI, mU/L	11.23 ± 0.67	16.12 ± 1.15	26.85 ± 1.94	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.001$
HOMA-IR	2.38 ± 0.15	4.36 ± 0.47	8.68 ± 0.8	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.001$
NT-proBNP, fmol/mL	212.3 ± 16.75	258.6 ± 17.37	364.2 ± 22.29	$P_1 > 0.05$ $P_2 < 0.001$ $P_3 < 0.01$

CAN: Cardiac autonomic neuropathy; HbA1c: glycated hemoglobin A1c; HOMA-IR: homeostasis model assessment insulin resistance; IRI: immunoreactive insulin; M: mean; NT-proBNP: N-terminal pro-brain natriuretic peptide. P_1: comparison between 1st and 2nd groups; P_2: comparison between 1st and 3rd groups; P_3: comparison between 2nd and 3rd groups; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

< 0.001). Levels of NT-proBNP were statistically significant higher in T2D patients with CAN compared to the control group [+71.5%, (*P* < 0.001) *vs.* control group] and compared to T2D without CAN group [+40.8%, (*P* < 0.01) *vs.* patients with T2D without CAN], while no significant difference between control group and patients without CAN was found. Table 4 presents the results of the arterial stiffness parameters in T2D patients with and without diagnosed CAN.

As a result of our research, it was found that among T2D patients without CAN, artery stiffness parameters were within normal levels, but this group have a tendency to vascular stiffening: AASI [+33.3%, (*P* < 0.01)], AIxao [+38.3%, (*P* < 0.01)], AIxbr [+33.3%, (*P* < 0.001)], and PWV [+18.7%, (*P* < 0.001)] compared to the control group. It was established that among 60% of patients from the control group the value of AIxbr was optimal and normal in 40%; PWV was optimal in 33.33% and normal in 66.67%. The value of AIxbr was optimal in 15.8%, normal in 78.9%, and elevated in 5.3%, and PWV was optimal in 89.5% and elevated in 10.5% of T2D patients without CAN.

Development of CAN in T2D patients coexist with a further increase in arterial stiffness parameters. In particular, AIxao [+67.9% to the control group (*P* < 0.001)] and [+ 21.4% to the values obtained in patients without CAN (*P* < 0.01)]; IAbr [+76.8% to control (*P* < 0.001)] and [+65.1% to patients without CAN (*P* < 0.001)]; PWV [+48.0% to control group (*P* < 0.001)] and [+24.7% to patients without CAN (*P* < 0.001)], and AASI [+ 59.3% to control (*P* < 0.001)] and [+19.4% to patients without CAN (*P* < 0.05)]. Arterial stiffness parameters exceed physiological values and were considered as high among patients with CAN. We found out that among patients of this group the value of PWV was normal in 16%, elevated in 52%, and pathological in 32%; Aixbr was normal in 52%, elevated in 40%, and pathological in 8% of cases.

The results of the echocardiographic depending on the features of the disease are presented in Table 5.

The obtained results proved that values of left ventricular posterior wall thickness (LV PWT) [+42.4%, (*P* < 0.001)] and IVS [+46.9%, (*P* < 0.001)] in patients with T2D without CAN were higher compared to patients of the control group [Table 5].

Table 4. Arterial stiffness parameters among the studied groups (M 4 SEM)

Variable	Control group (*n* = 15) 1st group	T2D patients without CAN (*n* = 19) 2nd group	T2D patients with CAN (*n* = 25) 3rd group	P
AIxao, %	19.6 ± 1.62	27.1 ± 1.57	32.9 ± 1.33	$P_1 < 0.01$ $P_2 < 0.001$ $P_3 < 0.01$
AIxbr, %	-35.7 ± 2.87	-23.8 ± 1.45	-8.3 ± 2.27	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.001$
PWV, m/s	7.5 ± 0.29	8.9 ± 0.23	11.1 ± 0.31	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.001$
AASI	0.27 ± 0.02	0.36 ± 0.02	0.43 ± 0.02	$P_1 < 0.01$ $P_2 < 0.001$ $P_3 < 0.05$

AASI: Ambulatory arterial stiffness index; AIxao: aorta augmentation index; AIxbr: brachial augmentation index; CAN: cardiac autonomic neuropathy; M: mean; PWV: aortic pulse wave velocity. P_1: comparison between 1st and 2nd groups; P_2: comparison between 1st and 3rd groups; P_3: comparison between 2nd and 3rd groups; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

Table 5. Left ventricular chamber echocardiographic parameters among the studied groups (M 4 SEM)

Variable	Control group (*n* = 15) 1st group	T2D patients without CAN (*n* = 19) 2nd group	T2D patients with CAN (*n* = 25) 3rd group	P
LV EDV, mL	109.4 ± 4.11	112.8 ± 3.03	116.4 ± 3.26	$P_1 > 0.05$ $P_2 > 0.05$ $P_3 > 0.05$
LV ESV, mL	36.2 ± 1.99	33.4 ± 0.73	42.6 ± 1.71	$P_1 > 0.05$ $P_2 < 0.05$ $P_3 < 0.001$
LV PWT, cm	0.85 ± 0.01	1.21 ± 0.01	1.24 ± 0.02	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 > 0.05$
IVS, cm	0.83 ± 0.01	1.22 ± 0.01	1.24 ± 0.02	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 > 0.05$
EF, %	67.3 ± 0.8	70.0 ± 1.07	63.6 ± 0.83	$P_1 > 0.05$ $P_2 < 0.01$ $P_3 > 0.05$

CAN: Cardiac autonomic neuropathy; ESV: left ventricular end-systolic volume; IVS: interventricular septum thickness; LV EDV: left ventricular end-diastolic volume; LV PWT: left ventricular posterior wall thickness; M: mean; P_1: comparison between 1st and 2nd groups; P_2: comparison between 1st and 3rd groups; P_3: comparison between 2nd and 3rd groups; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

Left ventricular concentric remodelling was present among 10.5%, concentric LVH among 31.6%, and eccentric LVH among 15.8% of T2D patients. Among persons with T2D and CAN concentric LVH was diagnosed in 76% and eccentric left ventricular hypertrophy in 16% of cases.

The results of the LVMM, LVMI-1, LVMI-2, and RWT parameters among the studied groups are presented in Table 6.

It was established that among patients with T2D without CAN, parameters of LVMM, LVMI-1, LVMH[2.7], and LV RWT were significant higher for all comparisons ($P < 0.001$) compared to parameters obtained in control group. Development of CAN was accompanied with further increase of LVMI-1 [+10.2%, ($P < 0.05$)] and LVMH[2.7] [+10.7%, ($P < 0.05$)] compared to the group without CAN [Table 6].

Table 6. LVMM, LVMI-1, LVMI-2, and RWT parameters among the studied groups (M 4 SEM)

Variable	Control group (n = 15) 1st group	T2D patients without CAN (n = 19) 2nd group	T2D patients with CAN (n = 25) 3rd group	P
LVMM, g	155.3 ± 5.94	272.4 ± 5.75	296.3 ± 9.16	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 > 0.05$
LVMI-1, g/m^2	78.6 ± 2.79	135.3 ± 3.53	149.1 ± 4.28	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.05$
LVMH$^{2.7}$, g/m$^{2.7}$	36.2 ± 1.35	61.9 ± 1.88	68.5 ± 1.94	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.05$
LV RWT	0.35 ± 0.004	0.49 ± 0.009	0.49 ± 0.01	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 > 0.05$

CAN: Cardiac autonomic neuropathy; LVMH$^{2.7}$: left ventricular mass index for height$^{2.7}$; LVMI: left ventricular myocardial mass index; LVMM: left ventricular myocardial mass; LV RWT: left ventricular relative wall thickness; M: mean; P_1: comparison between 1st and 2nd groups; P_2: comparison between 1st and 3rd groups; P_3: comparison between 2nd and 3rd groups; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

The results of the Doppler-EchoCG changes are presented in Table 7.

Doppler-EchoCG assessment showed, that E/A was decreased in patients with T2D and CAN compared to the control group [-46.0%, (P <0.001)] and compared to T2D patients without CAN [-14.1%, (P < 0.001)]. Simultaneously, development of CAN was accompanied by increased IVRT (+49.9%) and DT (+53.8%) (P < 0.001) compared to the control group, and increased IVRT [+7.8%, (P < 0.05)] compared to T2D patients without CAN [Table 7].

Obtained results showed that development of CAN is accompanied by more pronounced left ventricular diastolic dysfunction and by formation of LVH, mostly by concentric type.

Multiple regression analysis, after controlling for age, sex, diabetes duration, BP, HbA1c, and LVMI, showed an independent association of heart rate response to deep breathing with PWV (P < 0.001).

DISCUSSION

T2D can be associated with two types of autonomic dysfunction, either intrinsic or extrinsic. Intrinsic autonomic dysfunction is related to diseases that directly affect autonomic nerves, whereas the extrinsic autonomic dysfunction arises from diseases that are secondarily induced by cardiovascular dysfunction, such as aortic stiffness and dilated cardiomyopathy. Studies aimed at investigating the major contributors of the development of cardiac autonomic dysfunction among T2D patients have indicated that its formation is primarily intrinsic[24]. A lot of pathophysiological mechanisms responsible for autonomic dysfunction formation among T2D patients are determined by HG, which is associated with increased pro-inflammatory transcription factors activation and OS, leading to development of autonomic neuropathy. Increased levels of AGEs may lead to damage of the important components of the matrix molecules in the vessel wall. Moreover, some studies showed that development of vascular endothelial cells dysfunction is more often among diabetic persons compared to individuals without diabetes, indicating that T2D may both attenuate sensitivity and reduce NO bioavailability. All these pathological ways are involved in development of the HG-associated arterial stiffness. It is assumed that there is a pathophysiological link between cardiac autonomic dysfunction and increased arterial stiffness, and that preserving the elastic properties of the arteries strongly depends on the integrity of the autonomic nervous system[9,27].

Table 7. Doppler-EchoCG parameters in patients among the studied groups (M 4 SEM)

Variable	Control group (*n* = 15) 1st group	T2D patients without CAN (*n* = 19) 2nd group	T2D patients with CAN (*n* = 25) 3rd group	P
E, m/s	0.74 ± 0.02	0.51 ± 0.02	0.46 ± 0.01	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.05$
A, m/s	0.66 ± 0.02	0.72 ± 0.01	0.74 ± 0.01	$P_1 < 0.05$ $P_2 < 0.01$ $P_3 < 0.05$
E/A	1.13 ± 0.02	0.71 ± 0.02	0.61 ± 0.01	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.001$
IVRT, ms	78.0 ± 1.45	108.4 ± 2.33	116.9 ± 2.95	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 < 0.05$
DT, ms	152.7 ± 2.84	221.6 ± 5.2	234.8 ± 6.09	$P_1 < 0.001$ $P_2 < 0.001$ $P_3 > 0.05$

A: Peak velocity of late diastolic transmitral flow; CAN: cardiac autonomic neuropathy; Doppler-EchoCG: Doppler-echocardiography; DT: deceleration time; E: peak velocity of early diastolic transmitral flow; E/A: ratio of peak early to late diastolic filling velocity; IVRT: isovolumic relaxation time; M: mean; P_1: comparison between 1st and 2nd groups; P_2: comparison between 1st and 3rd groups; P_3: comparison between 2nd and 3rd groups; SEM: standard error of mean; T2D: type 2 diabetes mellitus.

Hyperinsulinemia increases synthesis of hepatic very-low-density lipoproteins and cholesterol synthesis/transport in cultured arterial smooth muscle cells, augments collagen synthesis and proliferation of arterial smooth muscle cells, turns on involved in inflammation genes[27].

It is assumed that IR per se has no strong impact on the carotid atherosclerosis development; its effect on the arterial wall is mediated by other hemodynamic, metabolic, and cellular changes related to the T2D and IR, like dyslipidemia, increase of free fatty acids (FFAs), adipocytokines, development of arterial hypertension, and chronic inflammation[27]. It has been reported about the link between arterial stiffness, hyperinsulinemia, arterial hypertension, and CAN in population with T2D signifies their pathogenic roles in the development of cardiovascular diseases (CVDs)[27].

IR relates to development of CVDs in patients with T2D, as well as in non-diabetic subjects, independently of established risk factors. In particular, the risk of CVDs events increased across quintiles of the HOMA-IR; the link between CVDs and HOMA-IR index was found. IR is connected with enhanced generation of reactive oxygen species (ROS) and excessive FFAs and decreased synthesis/release of NO. Elevated levels of FFAs may lead to impairment of endothelial function and through activation of nuclear factor kappa-light-chain-enhancer of activated B cells may induce the development of low-grade inflammation[27-29]. OS has been implicated in the pathogenesis of various CVDs, such as arrhythmia, HF, dyslipidemia, atherosclerosis, diabetic CAN, *etc.*[30]. OS induces DNA damage in the cells, ROS production, protein oxidation, lipid peroxidation that lead to vascular and neuronal damages, and apoptosis[31]. HG-induced cell injury is implicated four main molecular mechanisms: activation of PKC isoforms, increased polyol- and hexosamine pathway fluxes, and formation of AGEs. It is proposed that HG preferentially injures capillary endothelial cells, as they cannot reduce glucose transport inside the cell when they are exposed to HG[3,30].

Recent studies showed an inverse association between activity of autonomic nervous system and levels of inflammatory markers in plasma, which suggested an relationship between inflammation and autonomic dysfunction in patients with CVDs[32]. T2D course represents a spectrum of processes, so drawing clear lines between these stages may be inaccurate, and likely, the microcirculatory dysfunction and endothelial

inflammation start with the first glucose levels elevation[32,33].

Patients with prediabetes have higher E-selectin values as their serum glucose rises, and this becomes highest with the manifestation of T2D. This is consistent with a hypothesis of endothelial inflammation increasing with the diagnosis and progression of T2D[33].

Ulleryd *et al.*[32] (2017) hypothesize that inflammation can be a mediator in the association between atherosclerosis and autonomic dysfunction, leading to the development of CVDs like MI; decreased autonomic function will increase low-grade inflammation.

T2D candidates have increased inflammation and ED with increasing blood glucose levels with the T2D diagnosis. Development of ED may also precede the diagnosis of T2D and may forward the development of glucose dysregulation and IR leading to T2D; however, there is controversy about the exact role of ED in prediabetes and T2D. In T2Dpatients increased arterial wall thickness and impaired endothelial functions potentially lead to development of chronic diabetic micro- and macrovascular complications[2,34].

Chronic hyperglycemia induces vascular smooth muscle cells proliferation and low-grade inflammation, increases the generation of AGEs and enhances collagen cross-linking within the arterial wall, increases the endothelial permeability, augments the generation of angiotensin-2, and up-regulates expression of matrix metalloproteinase-2 and -9[35]. ED is recognized as a key event in the atherosclerotic process initiation, yet it also leads to "functional" arteries stiffening, as a continuous NO release by endothelium contributes to the functional regulation of arterial elasticity[2,27].

It is reported that the level of NT-proBNP and the time and frequency domain parameters were compared between groups of patients with normal and decreased HRV, and the correlation analyses of NT-proBNP and HRV indices was performed. It was found that among patients with abnormal HRV levels of NT-proBNP were significantly higher compared to persons with normal HRV. The NT-proBNP levels were negatively correlated with triangle index, low- and very-low frequency bands of the HRV. Lin *et al.*[36] concluded that the NT-proBNP level is correlated with HRV, and the increase of NT-proBNP indicates CAN in patients with diabetes.

In T2D cardiac autonomic dysfunction, namely decreased vagal activity leads to increase of HR and thus to shortening of diastole, but it appears that CAN may shorten diastole duration *per se*, independently of the impact on HR. As diastole duration strongly impact subendocardial myocardial viability (SVI), CAN and arterial stiffness lead to SVI impairment and may worsen cardiovascular prognosis[37].

Decreased HRV parameters in uncomplicated T2Dpersons highlight the obscure process of CAN in T2D that precedes the development of atherosclerosis. Therefore, screening for CAN should be performed much among patients with T2D, rather than after development of clinical CVDs[28].

Subclinical forms of myocardial disease in T2D patients may progress to HF regardless of presence of arterial hypertension and ischemia. The pathophysiology of DCM has been attributed to a variety of mechanisms, including CAN[38]. Nevertheless, the independent association of DCM and distinct autonomic cardiopathy has been difficult to prove due to these conditions' similar etiologies. Furthermore, the spectrum of LV functional abnormalities identifying this syndrome is not well determined.

Presence of CAN leads to alterations in the pattern of LV diastolic filling with preserved systolic function in patients with T2D. Development of diastole duration in patients with T2D is associated with clinical markers of autonomic neuropathy, as well as with regional markers of sympathetic integrity. However, these findings may reflect the simultaneous diabetic complications development with insufficient compensation, and the independence of the association has not been investigated relative to factors with known involvement in DCM[39].

Arterial stiffening is a frequent indicator for the vascular system's atherosclerotic processes and is known to occur due to presence of risk factors of atherosclerosis, such as dyslipidemia, smoking, arterial hypertension, T2D, and aging. These data suggest that patients with diabetes have an increasing low-grade inflammation and ED with rising blood glucose values through the diagnosis of T2D[33].

Arterial stiffness parameters are increased among T2D persons and arterial stiffening independently predicts mortality level in this cohort of patients. Investigations showed that mean IAxao was similar among T2D and nondiabetic patients; however, after multiple adjustments of several risk factors in the regression model, IAxao was related to diabetes status[8]. Other trials have shown that IAxao, which was similar between T2D and nondiabetic persons, increased in patients with diabetes after HR adjustment[40]. Yeboah *et al*.[40] (2016) hypothesize that BP and impaired cardiac autonomic function are the main determinants of increased PWV in patients with T2D, while the association between CAN and arterial stiffness is not mediated by low baroreflex sensitivity or increased HR. Chorepsima *et al*.[8] (2017) demonstrated that the activity of CAN alter arterial stiffness and it should be investigated in trials aimed to examine factors affecting PWV.

Nevertheless, it should be noted that the pathophysiological features of the relationship between diabetic CAN and arterial stiffening are complex and far from fully understood. The following two hypotheses are proposed: arterial stiffening can lead to myocardial dysfunction, or oppositely, diabetic CAN leads to the development and/or progression of the large arteries wall rigidity. Also, the processes of large arteries elasticity reduction and CAN development are parallel, taking into consideration aging and "toxic" effects of HG[41]. One probable mechanism that can explain the first hypothesis is induced by increased arterial stiffness violation of baroreflex sensitivity. On the other hand, cardiac autonomic nervous system dysfunction can change the elasticity of the artery wall, affecting vascular tone of large arteries[41].

Alternative mechanism that may lead to arterial stiffening and autonomic dysfunction development is HR's increase. Certainly, an increase in HR *per se*, regardless of the autonomic nervous system activity changes, contributes to the development of arterial stiffness. Nevertheless, it was shown that the normalization of HR in type 1 diabetes mellitus patients does not influence on the correlation between PWV and diastolic function of LV. Therefore, it was concluded, that the association between parasympathetic dysfunction and arterial stiffening is not mediated by an increase of HR[41,42]. A significant number of trials showed that HG and IR activate several mechanisms triggering the functional and structural changes in the arterial wall, which are likely to accelerate vascular aging and increased CV risk among T2D patients[43]. Additionally, T2D is accompanying with other systemic and metabolic abnormalities, namely obesity, arterial hypertension, and atherogenic dyslipidemia that may cause development of atherosclerosis, arterial stiffening, or both[2].

Despite the above limitations, our study results suggest that changes in the arterial stiffness, IR parameters, NT-proBNP level, and the myocardium's functional changes are interrelated in diabetes CAN. An independent association of heart rate response to deep breathing with PWV ($P < 0.001$) was found by

performing multiple regression analysis after controlling for age, sex, diabetes duration, BP, HbA1c, and LVMI.

Nevertheless, further studies are needed to clarify whether large arterial stiffness and accompanying pulsatile hemodynamic changes are involved in the pathogenesis of diabetic CAN and whether interventions targeting are interrelated in arterial stiffness are associated with clinical outcomes improvement in diabetic CAN.

DECLARATIONS

Authors' contributions
Concept of the study, general coordination and supervision of the research project, data analysis, statistical analysis, and draft manuscript writing: Serhiyenko VA, Serhiyenko AA
Patients' recruitment, clinical examination, and clinical data acquisition: Segin VB
Concept elaboration, general coordination, and discussion: Serhiyenko VA, Serhiyenko LM

Availability of data and materials
Not applicable.

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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethics approval and consent to participate
The work was done according to the principles of the Declaration of Helsinki of 1975 as revised in 2014 and was approved by an Ethics Committee of the Danylo Halytsky Lviv Medical University, protocol No. 2 from 18 February 2013. All subjects signed an informed consent prior to their inclusion in the study. The research performed corresponded to the generally accepted norms of morality and observance of the rights, interests, and personal dignity of the persons participating in the study.

Consent for publication
Not applicable.

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