RESEARCH ARTICLE

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Prevalance of Cardiovascular Autonomic Neuropathy in Patients With Normotensive Type 2 Diabetes Mellitus and Defining Associated Risk Factors *

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Objective: This study aimed to evaluate the prevelance of cardiovascular autonomic neuropathy (CAN), associated risk factors, and the correlation between these risk factors and the progression of CAN in patients with normotensive type 2 diabetes.

Materials and Methods: This study is an observational study including 60 patients with normotensive type 2 diabetes between the ages of 32 and 64 years (mean 49.20±7.44), consisting of 38 women (63.3%) and 22 men (36.7%). All patients are evaluated with standart Ewing Battery tests at baseline and at the end of the first year. Test results are expressed by a scoring system.

Results: CAN prevelance regressed from 78.3% at baseline to 70% at the end of the first year (p=0.245) and Ewing score regressed from 1.50 (1.00-2.50) to 1.00 (0.50-2.00) (p= 0.035). CAN patients were older and had higher SBP at the end of the 12th month (p= 0.037 and p= 0.039, respectively). CAN was associated with age, SBP, and more significantly with lower use of statins (B= 0.12, p= 0.015; B=0.09, p= 0.007; B= -1.83, p=0.052 respectively). Ewing score was significantly correlated with SBP (r=0.38, p=0.034) and near significantly with age, diabetes duration, and HbA1c (r=0.25, p=0.062; r=0.24, p=0.063; r=0.24, p=0.071). Changes in Ewing score was associated only with decreased total cholesterol (B=0.26, p=0.047).

Conclusion: Age, SBP, and total cholesterol levels may be associated with CAN in patients with normotensive type 2 diabetes. There is a need for further studies with different designs.

Key Words: *Cardiovascular autonomic neuropathy, prevalance, risk factors, Type 2 diabetes*

Tip 2 Diyabetes Mellituslu Hastalarda Kardiyovasküler Otonom Nöropati Sıklığı ve Bununla İlişkili Risk Faktörlerinin Belirlenmesi

Amaç: Bu çalışma, normotansif tip 2 diyabetes mellituslu (DM) hastalarda kardiyovasküler otonom nöropati (KON) sıklığını, KON risk faktörlerini ve bu risk faktörlerinin KON'un progresyonu ile ilişkisini araştırmayı amaçladı.

Gereç ve Yöntem: Bu çalışma, normotansif tip 2 DM tanısı olan, yaşları 32 ile 64 arasında değişen (ort. 49.20±7.44 yıl), 38'i (%63.3) kadın, 22'si (%36.7) erkek olmak üzere toplam 60 hastanın dahil edildiği gözlemsel bir çalışmadır. Tüm hastalara başvuruda ve birinci yıl sonunda standart Ewing Battery testleri uygulandı. Test sonuçları bir skorlama sistemi kullanılarak ifade edildi.

Bulgular: Başlangıçta KON prevalansı %78.3 iken, birinci yılın sonunda %70'e gerilerken (p=0.245), Ewing skoru toplamı 1.50 (1.00-2.50)'dan 1.00 (0.50-2.00)'e geriledi (p=0.035). KON grubunda başvuruda hastaların yaşı daha ileriydi ve 12.ayın sonunda sistolik kan basıncı (SKB) daha yüksekti (sırasıyla p=0.037, p=0.039). KON, yaş ve SKB'dan, anlamlılığa yakın olarak da statin kullanımının azlığından etkilenmekteydi (sırasıyla B=0.12, p=0.015; B=0.09, p=0.007; B= -1.83, p=0.052). Ewing skoru SKB ile anlamlı (r=0.38, p=0.034), yaş, diyabet süresi ve HbA1c ile anlamlılığa yakın koreleydi (sırasıyla r=0.25, p=0.062; r=0.24, p=0.063; r=0.24, p=0.071). Ewing skorundaki değişimle sadece total kolesteroldeki azalmanın anlamlı olduğu görüldü (B=0.26, p=0.047).

Sonuç: Yaş, SKB ve total kolesterol seviyeleri normotansif tip 2 DM hastalarında KON ile ilişkili olabilir. Bu konuda farklı şekilde dizayn edilmiş ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler**:** *Kardiyovasküler otonom nöropati, prevalansı, risk faktörleri, Tip 2 diyabet*

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Introduction

Cardiovascular Autonomic Neuropathy (CAN) is a common complication of diabetes and is often overlooked (1, 2). Its prevalence has been reported to range from 1 to 90% in various studies (2). CAN results from the inflammation of autonomic nerve fibers that innervate cardiac and vascular structures. This leads to impaired heart rate control and vascular tone, resulting in exercise intolerance, postural hypotension, syncope, presyncope, prolongation of the corrected QT interval, silent myocardial ischemia, and infarction (3). All these signs indicate that CAN causes higher mortality in diabetic patients.

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Research on this important complication has defined various risk factors. The two most important risk factors are chronic hyperglycemia and diabetes duration. Numerous studies have proven that chronic hyperglycemia is an independent risk factor for diabetic autonomic neuropathy (4). Identifying risk factors allows us to elucidate the etiology of CAN.

However, previous studies have failed to homogenize factors like diabetes and hypertension and have been unable to standardize the tests used for diagnosis and reference intervals, which is considered a crucial limitation. In the present study, we aimed to investigate the frequency of CAN, CAN risk factors, and the correlations of these risk factors with the progression of CAN in patients with normotensive type 2 DM using standardized Ewing Battery tests.

Materials and Methods

Research and Publication Ethics: Approval was obtained from the Konya Meram Medical Faculty ethics committee (numbered 2010-069). All participants provided informed written consent before they were included in the study.

Study Population: It was planned to include patients who applied to cardiology and endocrinology outpatient clinics between March 2010 and September 2010 and were followed up for type 2 DM. The patients were followed prospectively for 1 year after baseline measurements.

Inclusion criteria: The study included normotensive patients aged between 18-90 years, previously diagnosed with Type 2 DM and under oral antidiabetic or insulin therapy.

Exclusion criteria: The study excluded patients with a history of hypertension or receiving medical treatment for hypertension, diagnosis of masked hypertension after 24 hours of ambulatory holter application, Type 1 DM, autonomic nervous system disease, cerebrovascular history, cardiac operation, coronary artery disease, valvular disease that affects hemodynamics, chronic obstructive pulmonary disease, chronic arrhythmia, and left bundle branch block, those using drugs that affect autonomic neuropathy tests (aldosereductase inhibitors, b-blockers, Ca channel blockers), and those with the joint limitation that may cause problems when performing neuropathy-related test maneuvers.

Demographic Characteristics and Biochemical Tests: We recorded the smoking history, diabetes history, and medication use of the patients included in the research. Physical examination included height and weight measurements for anthropometic measurements. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

For biochemical tests, we took 15 cc of blood from the peripheral vein between 08:00 and 11:00 a.m after 10-12 hours of fasting. The samples were analyzed for fasting glucose, HbA1c, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, urea, creatine, Na and K levels, hemoglobin, hematocrit, and white blood cells.

Patients with a serum total cholesterol >200 mg/dL, LDL-cholesterol >100 mg/dL, and triglyceride >150 mg/dl or those with previously proven hyperlipidemia and receiving treatment were considered as hyperlipidemia (5).

Blood pressure measurements were made using an ERKA aneroid sphingomamometer (Kallmeyer Medizintechnik Tölz/GERMANY). Measurements were made twice on each arm with an interval of two minutes, in a quiet environment and in the sitting position, after resting for 15 minutes. SBP and DBP were measured based on Korotkoff phase I and phase V sounds, respectively. Patients with systolic BP <140mmHg and diastolic BP 90 mmHg were evaluated as normotensive. Values above these were accepted as hypertension and were excluded from the study. It was was calculated as Mean BP= DBP + (SBP - DBP) / 3.

Cardiac Autonomic Function Tests: Previously described practical and non-invasive bedside tests were performed to detect cardiovascular autonomic neuropathy (6) (Table 1). The tests were performed between 08:00 and 11:00 a.m and at least 2 hours after breakfast. Attention was paid to ensure that the patients did not smoke or drink coffee on the day of the test and discontinued drugs that may affect test results (decongestants, anxiolytics, antidepressants) at least 8 hours and optimally 24 hours before. The tests were performed in the following order and scored according to results. A score of 0 was given for each normal result, 0.5 for borderline, and 1.0 for abnormal (6) (Table 2).

Table 1. Cardiovascular autonomic neuropathy tests

Test	Position	Test Time (min)
Parasympathetic function tests		
1.Heart rate response to Valsalva Maneuver	Sitting	5
2. Heart rate response to standing up (30:15)	Standing up suddenly	3
3. Heart rate variability during deep breathing (HRV), (E/I)	Sitting	2
Sympathetic function tests		
4. Blood pressure response to standing up (decrease in SBP)	Standing up suddenly	3
5. Blood pressure response during clenching fist (increase in DBP)	Sitting	5

DBP: Diastolic blood pressure, E/I: Expiration/Inspiration, HRV: Heart rate variability, min: minute, SBP: Systolic blood pressure

Tablo 2. Reference Ranges for Cardiovascular Autonomic Neuropathy Tests

Parasympathetic Tests	Normal	Borderline	Abnormal
1. Heart rate response to Valsalva Maneuver	≥1.21	1.11-1.20	≤1.10
2. Heart rate response to standing up (30:15)	≥1.04	1.01-1.03	≤1.00
3. Heart rate variability during deep breathing (HRV), (E/I) *	-	-	-
Sympathetic Tests			
4.Blood pressure response to standing up (mmHg) (decrease in SBP)	≤10	11-29	≥30
5.Handgrip test (mmHg) (increase in DBP)	≥16	11-15	≤10
*** Age-adjusted reference range for E/I ratio**			
Normal: Age 20-24 yr, 1.17; 25-29, 1.15; 30-34, 1.13; 35-39, 1.12; 40-44, 1.10; 45-49, 1.08; 50-54, 1.07; 55-59, 1.06; 60-64, 1.04; 65-69, 1.03; and 70-75, 1.02			
Abnormal: Values below these			

DBP: Diastolic blood pressure, E/I: Expiration/Inspiration, HRV: Heart rate variability, SBP: Systolic blood pressure

Analysis of Test Findings: Total Ewing scores were calculated.

0-0.5: No CAN.

1-5: CAN.

Improved Ewing scores were evaluated as improvement in CAN and increased scores as worsening in CAN.

Nihon Kohden 9022K model, calibrated, digital monitor ECG device was used for ECG recordings.

Follow-up Protocol: We contained 60 type 2 DM patients consisting of 38 (premenopausal women, 25; postmenopausal women 13) (63.3%) females and 22 (36.7%) males. The patients were told that the tests would be repeated one year after baseline measurements. We made no intervention on glucose regulation or medication and the follow-up physician was not informed about the CAN test results. However, the patients were questioned again for medications at the end of the first year. The patients were routinely recommended lifestyle changes like adopting a low-calorie and low-cholesterol diet, walking for 30 minutes at least five days a week, and quitting smoking. CAN tests were repeated at the first year of baseline examination. Biochemical analyses and demographic characteristics were also re-evaluated at the end of this period.

Statistical Analysis: Data are presented as mean±standard deviation (SD), median (25th and 75th percentiles), or numbers and percentages. Group comparison was made using the "Independent-Samples T test" or the "Mann-Whitney *U*test". For baseline and 12th month comparisons, the Paired Samples T-test or the Wilcoxon test was used. Comparison of categorical variables was made using the χ^2 (chisquare) test. The correlations between Ewing score variability and other factors were evaluated using the Nonparametric Spearman correlation analysis. Parameters that are thought to affect CON were evaluated using multivariate stepwise linear regression analysis and multivariate logistic regression analysis. For all tests, $p<0.05$ was considered statistically significant. The SPSS 18.0 package software was used for all statistical analyses.

Results

We included 60 normotensive type 2 DM patients (mean age: 49.20±7.44 years), consisting of 38 (premenopausal women, 25; postmenopausal women 13) (63.3%) females and 22 (36.7%) males. The mean age of the sample was 49.2, female sex rate was 63.3% and smoking rate was 11.6%. Median diabetes duration was 4.5 years. CAN rate was 78.3% at baseline and 70% after 1 year of follow-up (Table 3).

Fasting blood glucose, total cholesterol, LDL-cholesterol, triglyceride, and total cholesterol/HDL-cholesterol ratio decreased significantly at 1-year follow-up (p=0.013, 0.007, 0.015, 0.006, 0.049, respectively), but no statistically significant change was observed for BMI, blood pressure, or antidiabetic treatment (Table 3).

Regarding demographic and laboratory data for patients with and without CAN at baseline and 12-month follow-up, only age was higher at baseline (p=0.037) and only systolic BP was higher at 12 months (p=0.039**)** (Tablo 4). There was no statistically significant difference between the other variables.

The correlation of Ewing scores with other variables (age, gender, smoking, statin use, BMI, DM duration, Systolic BP, Diastolic BP, Mean BP, FPG, HbA1C, Total cholesterol, Triglyceride, LDL-cholesterol, HDL-cholesterol, Total cholesterol/HDL-cholesterol ratio) was investigated separately. Baseline Ewing scores had no statistically significant correlation with any of the variables but were nearly significantly positive with age (r=0.25, p=0.062), DM duration (r=0.24, p=0.063), and triglyceride (r=0.22, p=0.093). 12-month Ewing scores were statistically significantly correlated only with SBP (r=0.38, p=0.034) and nearly significantly positively correlated with HbA1C (r=0.24, p=0.071).

To determine in which variables the difference affected the decline in Ewing scores at the end of one year (and therefore the decrease in CAN ratio), the difference between baseline and 12-month Ewing scores (ΔEwing score), and differences between baseline and 12-month numerical variables (ΔBMI, ΔSBP, ΔDBP, ΔMean BP, ΔFWL, ΔTotal cholesterol, ΔLDL-cholesterol, ΔHDL-cholesterol, ΔTotal cholesterol/HDL-cholesterol ratio, ΔTriglyceride, ΔHbA1C) were subjected to a linear regression analysis; accordingly, only the decrease in total cholesterol affected the decrease in Ewing scores (Beta=0.26, t=2.03, p=0.047).

In our population, the prevalence of CAN was 78.3% at baseline, decreasing to 70% at the end of 12 months. Total Ewing scores decreased significantly at follow-up [from 1.5 (1.0, 2.5) to 1.0 (0.5,2.0)] (p=0.035). The distribution of cardiovascular autonomic reflex test results at baseline and at the end of 12 months is shown in Figures 1 and 2.

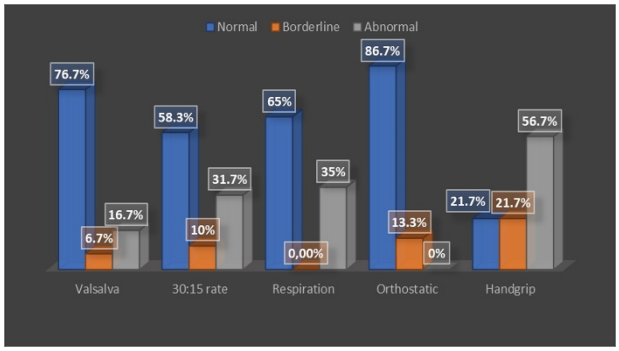

Figure 1. shows the distribution of cardiovascular autonomic reflex test results at baseline

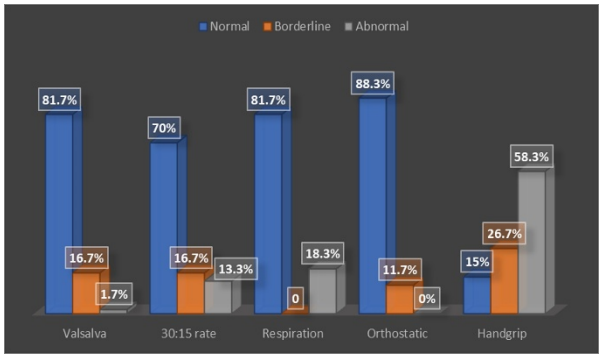

Figure 2. shows the distribution of cardiovascular autonomic reflex test results in 12 months

Table 3. Comparison of Demographic and Laboratory Characteristics of Patients with Type 2 DM at Baseline and 12 Months

Variables	Baseline, n=60	12th Month, n=60	P
Age (years)	49.2±7.4	50.2±7.4	0.382
Female, n(%)	38 (63.3)	-	-
Smoking, n(%)	7 (11.6)	3 (5)	0.562
Diabetes duration (years)	4.5 (2, 10)	5.5 (3, 11)	0.425
BMI (kg/m^2)	29.9±4.4	29.6±4.0	0.816
SBP (mmHg)	122.4±11.8	125.8±11.9	0.072
DBP (mmHg)	79.0±8.4	79.4±8.5	0.476
Mean BP (mmHg)	93.5±8.8	94.9±8.7	0.593
Glucose (mg/dL)	162.3±62.6	144.3±50.2	0.013
HbA1c(%)	7.3±1.4	7.2±1.4	0.274
Total cholesterol (mg/dL)	198.3±38.8	181.7±35.6	0.007
Triglyceride (mg/dL)	176.6±85.5	141.288±80.2	0.006
LDL-cholesterol (mg/dL)	123.5±29.4	111.68±28.8	0.015
HDL-cholesterol (mg/dL)	40.85±10.2	40.748 11.8	0,925
Total Cholesterol/HDL ratio	5.1±1.2	4.7±1.2	0.049
OAD, n(%)	26 (43.3)	28 (46.7)	0.417
Insulin, n(%)	12 (20)	12 (20)	0.435
Statin use, n(%)	2 (3.3)	45 (75)	0.001
Total Ewing score	1.5 (1.0, 2.5)	1.0 (0.5, 2.0)	0.035
CAN (+), n(%)	47 (78.3)	42 (70)	0.384

BMI: Body mass index, CAN: Cardiovascular autonomic neuropathy, DBP: Diastolic blood pressure, DM: Diabetes mellitus, OAD: Oral antidiabetics, SBP: Systolic blood pressure

Table 4. Comparison of Demographic and Laboratory Characteristics of Patients with Type 2 DM with and without CAN at baseline and 12 months

Variables	Baseline CAN No, n=13	Baseline CAN Yes, n=47	P	12th month CAN No, n=18	12th month CAN Yes, n=42	P
Age (years)	46 (36, 52.5)	50.5±6.8	0.037	52.5 (47, 55)	50.3±7.6	0,471
Female, n %)	9 (69.2)	29 (61.7)	0.458	10 (55.6)	28 (66.7)	0.353
Male, n(%)	4 (30.8)	18 (38.3)	0.292	8 (44.4)	14 (33.3)	0.389
Smoking, n(%)	1 (7.7)	6 (12.8)	0.354	1 (5.6)	2 (4.8)	0.372
Diabetes duration (years)	3 (2, 8)	5 (2, 10)	0.631	4.5 (3.0, 8.0)	6 (3.8, 11.0)	0.251
BMI (kg/m^2)	29.4 (26.2, 34.1)	29.8±4.0	0.527	29.3 (26.7, 31.5)	29.6±4.2	0.715
SBP (mmHg)	125 (120, 133)	122.0±12.1	0.442	120 (13.8, 130.0)	128.1±10.7	0.039
DBP (mmHg)	80 (79, 88)	78.3±8.8	0.396	80 (76, 85)	79.7 ± 8.5	0.254
Mean BP (mmHg)	97 (92, 101)	92.9±9.1	0.375	93.3 (86.7, 100.0)	95.8 ± 8.4	0.458
Glucose (mg/dL)	135 (125, 177)	163.7±62.8	0.640	129 (115.3, 163)	145.3±53.8	0.437
HbA1c(%)	7 (6.7, 8.8)	7.2±1.4	0.271	6.7 (5.8, 7.5)	7.3±1.4	0.449
Total cholesterol (mg/dL)	175 (145, 223)	203.7±36.0	0.489	160.5 (151.3, 211.8)	183.1±33.1	0.521
Triglyceride (mg/dL)	141 (107, 201)	185.9±88.9	0.513	131 (96.8, 273.3)	128.7±59.8	0.253
LDL-cholesterol (mg/dL)	116 (91, 143)	125.8±29.8	0.529	99.7 (83.9, 128.1)	113.4±27.7	0.365
HDL-cholesterol (mg/dL)	40 (32, 44)	41.2±9.3	0.872	36.2 (31.3, 42.2)	42.4±12.9	0.387
Total cholesterol/HDL ratio	5.1 (4.1, 5.8)	5.1±1.2	0.922	5.0 (3.9, 6.0)	4.6±1.3	0.422
OAD, n(%)	6 (46.2)	20 (42.5)	0.685	9 (50)	19 (45.2)	0.527
Insulin, n(%)	3 (23.1)	9 (19.1)	0.426	4 (22.2)	8 (19.0)	0.511
OAD + Insulin, n(%)	4 (30.8)	18 (38.3)	0.458	5 (27.7)	15 (35.7)	0.394
Statin use, n(%)	0	2 (4.3)	0.736	15 (83.3)	30 (71.4)	0.489

BMI: Body mass index, CAN: Cardiovascular autonomic neuropathy, DBP: Diastolic blood pressure, DM: Diabetes mellitus, OAD: Oral antidiabetics, SBP: Systolic blood pressure

Discussion

In this 1-year prospective observational research on patients with normotensive type 2 DM, we investigated the frequency of CAN, CAN risk factors, and their association with the progression of CAN. We found that advanced age at baseline and high systolic blood pressure at 1-year follow-up were associated with CAN.

Due to a lack of standardization in the tests to diagnose CAN, we were not precisely able to determine the prevalence of CAN (2). This rate was reported to range between 60-66.5% (9-11). Here, CAN prevalence was 78.3% at baseline and 70% at the end of the first year. The design of the current study was methodologically more detailed than many others for selecting normotensive patients, using all Ewing Battery tests, and considering age-dependent reference ranges for heart rate response to respiration. This may explain the differences in prevalence rates.

Advanced age at baseline and high systolic blood pressure at 1-year follow-up were associated with CAN. We found no correlation between sex and CAN. The literature includes some studies that found no significant correlation between CAN and sex (12), while there are some that found a significant correlation (13, 14). Regarding the conflicting results in these studies, the effect of sex on CON seems to remain unclear.

Again, some previous studies showed no correlation between BMI and CAN (12), while in others, obesity was a risk factor for CAN (15). In the current study, BMI and CAN were not correlated. Because of the inconsistent results in clinical studies, the effect of obesity on CAN remains unclear, as is the case with sex.

In almost all studies, age was defined as a risk factor for CAN (10). Here, the risk of CAN increased with increasing age. Also, age nearly significantly increased Ewing scores. In other words, age was associated with the severity of neuropathy. Numerous studies strongly support that CAN is an early complication that can occur even within two years of the onset of diabetes (16) and that chronic hyperglycemia has a key role in the pathophysiology of CAN (17, 18). Increasing age possibly leads to higher exposure to chronic hyperglycemia, accelerating the formation of CAN. Besides, as age progresses, loss of function develops in both sympathetic and parasympathetic ganglia and nerve conduction decreases in different pathways of the autonomic nervous system for various reasons (oxidative damage, decrease in neuroprotective substances, synaptic deterioration of organelles, changes in the extracellular matrix, etc.) (17, 19). This is why increased age leads to decreased HRV in non-diabetic healthy individuals at 5-year follow-up (20). Remarkably, the correlation between age and CAN

disappeared at the end of the first year. We think the fact that the numerical distributions of the groups changed at the end of 1 year (from 13-47 to 18-42) caused the disappearance of the age difference, which was significant at baseline, although not statistically strong.

Another risk factor that is strongly associated with CON is the duration of diabetes. Previous research indicates an association between diabetes duration and CAN (21, 22) Long-term hyperglycemia is considered the main culprit in the development of diabetic neuropathy (23). Experimental studies have reported changes in polyol-myoinositol metabolism and Na-K ATPase system due to hyperglycemia. Non-enzymatic glycosylation leads to decreased axon diameter and transport, decreased nerve conduction, axoglial junction disorders, microangiopathy, endoneurial hypoxia, and demyelination. These mechanisms are involved in the pathophysiology of neuropathy. The increase in diabetes duration raises exposure to chronic hyperglycemia, as with age. In the present research, we could not find a correlation between diabetes duration and CAN. The difference in diabetes duration between patients with and without CAN was 2 years at baseline and 1.5 years at the end of 1 year. The increased number of patients may possibly reveal the effect of diabetes duration on CAN. On the other hand, here, diabetes duration nearly significantly increased Ewing scores. The severity of neuropathy increases as the Ewing score rises (11). According to long-term data from the Rochester Diabetic Neuropathy Cohort Study, duration and severity of exposure to hyperglycemia were correlated with the severity of neuropathy alone (24).

Some studies suggest that chronic smoking is a risk factor for cardiac autonomic neuropathy in diabetic individuals (25) while others fail to find a correlation between smoking and diabetic neuropathy (26). The rate of smoking was very low in our sample, preventing a definite assessment in this regard.

So far, no correlation has been demonstrated between antidiabetic treatment regimen and CAN (22). In line with previous research, we found that antidiabetic treatment was not associated with CAN.

Another finding obtained here was the correlation between SBP and CAN. Neil HA et al. showed an association between impaired CAN tests and increased SBP [27].Sympathetic baroreflex decreases with an increase in blood pressure and HRV decreases with an increase in norepinephrine levels (28). Endothelial dysfunction develops with decreased NO and prostacyclin levels and increased endothelin-1 level (29). As superoxide levels increase, NO synthesis decreases and direct nerve damage occurs (30). The frequency of CAN increases with all these pathophysiological processes occurring with increased blood pressure. In the current study, Ewing scores were found to be positively correlated with SBP. Thus, SBP was associated with both neuropathy and the severity of neuropathy.

Poor glycemic control has a key role in the progression of CAN and its baseline pathophysiology (31). In the DCCT study, intensive glycemic control slowed the deterioration of autonomic functions, decreasing the incidence of CAN by 53% compared to conventional treatment (23). Possibly, early intensive therapy causes this benefit through various pathological pathways associated with autonomicneural functions. These pathways are the formation of AGEs, increased oxidative and nitrosative stress with increased production of free radicals, activation of polyol and protein kinase C pathway, activation of polyADP ribosylation, and activation of genes involving neural damage (2). The DCCT study reported mean HbA1c of 7.4% for the intervention group, compared to 9.1% for the conventional group. Since our research involved no intervention, there was no significant decrease in HbA1c levels after 1 year compared to baseline. This may be the reason for the absence of such benefit. However, total Ewing scores were nearly significantly positively correlated with HbA1c at the end of 12 months. Given the lack of a correlation between antidiabetics and CAN, the prevention or slowing of the development of CAN may not be correlated with the type of drug used (insulin or OAD) but may be correlated with glycemic control.

Regarding the correlation between hyperlipidemia with CAN, some studies found no significant relationship (32), while others reported that hyperlipidemia was a risk factor for CAN (1) and serum lipid control was more important than glucose control to prevent deterioration in CAN (33). Hypercholesterolemia leads to many changes in vascular hemostasis. It decreases NO bioactivity, increases superoxide production, and increases endothelin reactivity (34).

Besides, it increases adhesion molecules and decreases endothelium-dependent vasodilation (35). Common pathophysiological mechanisms could possibly explain the correlation between hyperlipidemia and CAN. In the present study, we found no significant correlation between total cholesterol, LDL, HDL, and triglyceride levels and CAN at baseline or at follow-up, although at the end of 1 year, improvement in Ewing scores was associated with decreased total cholesterol (hence with decreased CAN ratio). Recent research emphasizes the significance of small and dense LDL cholesterol particles in type 2 DM patients, leading to an increase in oxidative stress and a decrease in NO levels, and ultimately to endothelial dysfunction (36). It has been proven that the serum levels of these small and dense LDL particles may differ, even among patients with similar serum LDL levels (37). Although, their effect on CAN remains unknown. The conflicting results regarding the correlation between CAN and serum LDL levels may stem from the different serum levels of small and dense LDL levels. Here, serum LDL levels and CAN were not correlated, but we believe that well-designed research is needed to shed light on this.

After the first year, statin use was not statistically significant but higher in the non-CAN group (71.4% vs 83.3%) and the presence of CAN was found to be affected by statin use with near statistical significance

according to regression analysis. Statins inhibit the HMG CoA reductase enzyme, the rate-limiting enzyme in cholesterol biosynthesis, and prevent the synthesis of various intermediates with a key role in the pathogenesis of microvascular and macrovascular complications, apart from cholesterol. Hence, independent of its cholesterol-lowering effect, its pleiotropic effects occur (improved endothelial dysfunction, decreased free radicals such as superoxide and hydrogen peroxide, and increased NO synthesis) (38,39). In this study, the pleiotropic effects of statins may have affected the development of CAN through a positive effect on its pathophysiology. It remains unclear whether the decrease in Ewing scores at the end of 12 months is due to decreased total cholesterol or to statin use, given the pleiotropic effects of statins. Since this is observational research, randomized and placebo-controlled studies can shed more light on this matter.

We found a significant rate of CAN in normotensive type 2 diabetic patients by bedside tests. Age and SBP affected the development of CAN and diabetes duration, HbA1c levels, and age affected the severity of CAN with near significance. Also, there was an independent correlation between decreased total cholesterol levels and improved CAN. Thus, correcting lipid profile, ensuring glycemic control, and preventing blood pressure progression may halt or even regress the progression of CAN in patients with type 2 DM. Moreover, statin therapy may show promise in the treatment of CAN. There is a need for further randomized and placebo-controlled research on this subject.

The main limitations of the present study were the small sample size, the one year follow-up period, and lack of recordings for patients' diet and exercise habits.

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

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