Original Article

The Relation Between Peripheral Arterial Disease and Diabetic Peripheral Neuropathy in Type 2 Diabetes Mellitus. The Role of Calcitonin Gene-Related Peptide

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Abstract

Background: Among these, peripheral arterial disease (PAD) and diabetic peripheral neuropathy (DPN) stand out to display significant clinical impact. It is worth mentioning that chronic hyperglycemia is considered to be the major aggravating factor for both DPN and PAD. **Objective:** To test the relation between peripheral neuropathy and peripheral artery disease and to assess the impact of CGRP on peripheral artery disease in patients with type 2 diabetes mellitus. **Materials and Methods:** A case–control study which involved 200 diabetic males and females was divided into two groups: Group 1 (94) that includes diabetic patients with peripheral neuropathy (DPN) and Group 2 (106), as control group, that includes diabetic patients without DPN based on electrodiagnosis. There are raged between 30 and 60 years old. Anthropometric measurements including body weight and height, the Doppler study was used to test the lower limbs' arteries and PAD was diagnosed based on Jerker's criteria. CGPR serum level was measured by using an enzyme linked immunosorbent assay (ELISA) kit with a detection range of 2–600 ng/L. **Results:** This study revealed a highly significant increase in glycosylated hemoglobin, while CGRP showed a significant decrease between the study groups. In addition to that PAD showed a significant increase in distribution in the study group than control group. Regarding electrophysiological parameters, changes showed a significant upsurge in latency for motor and sensory nerves and a significant reduction in conduction velocity and amplitude for sensory and motor nerves in addition to a significant prolongation of F wave latency for almost all tested nerves of the study group. The receiver operating curve was used to calculate the cutoff value of CGPR serum level for diagnosing PAD and showed no significant accuracy of 52% with low sensitivity and specificity. There were no significant differences regarding most of the other tested parameters. **Conclusion:** This study revealed an increased rate of PAD in patients with DPN with no significant relation between CGPR level and PAD and, subsequently, no role in diagnosing or predicting PAD.

Keywords: Calcitonin gene-related peptide, diabetic peripheral neuropathy, peripheral arterial disease, type 2 diabetes

Introduction

Many researchers reported that peripheral arterial disease (PAD) is the most frequent macrovascular problem of diabetes mellitus (DM). Atherosclerotic disease of lower limbs is the main manifestation of PAD, which represents systemic atherosclerosis as well as a significant risk factor for diabetic foot sores, gangrene, and even amputation.[1,2]

It is distinguished by stenosis and/or obstruction of the lower-extremity arteries. PAD, like other vascular diseases, is related to the duration and severity of diabetes.[3,4] Most diabetic patients with PAD have diabetic peripheral neuropathy (DPN), so they lack the clinical symptoms of PAD, which are typically not found until symptoms like intermittent claudication, rest pain, and ischemic gangrene appear. Diabetic patients are two to three times more likely than non-diabetic patients to develop this disease.[5] On the other hand,

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some researches pointed out that PAD in diabetic patient manifests earlier, may result in damage of the peripheral nerves, and may progress more rapidly to chronic limb ischemia.[6,7]

The study[8] reports data on the overall prevalence of PAD in DM, as this complication was rarest of other complications, whereas another study demonstrated a high rate of PAD among people with type two diabetes mellitus (T2DM).[9]

These variations in PAD prevalence may be the result of various diagnostic methods being employed. When compared to alternative diagnostic techniques like the ankle-brachial index, which can overestimate the prevalence of PAD, color Doppler ultrasound was frequently employed in studies as the best diagnostic approach.[10]

However, up to the time of conducting this study, there is inconsistency in diabetic patients with PDA prevalence rate. In contrast, PAD by itself elevated the danger of hyperglycemia and DM. Specific signs and symptoms of each of these two illnesses raise the possibility of developing the other.[9,11]

Endothelial cells in the body naturally create nitric oxide (NO), which is crucial for maintaining the integrity of the blood vessel wall. The beginning of vascular disease, including atherosclerosis in humans, and a loss of NO are both closely correlated with endothelial dysfunction in hyperglycemia. It has recently been widely recognized that oxidative stress is a major factor in the development of diabetic vascular problems.[12,13]

The important neuropeptide calcitonin gene-related peptide (CGRP), which is most active in the vascular system, has been linked to vasodilation and vascular protective activity. CGRP also plays a protective role in endothelial cells when high glucose levels cause oxidative injury. Although CGRP is mostly produced by neurons, early research indicated that non-neuronal tissues like endothelial cells can also produce it. Overall, vasorelaxation, angiogenesis, and proliferation are ways that CGRP overexpression could increase NO levels, prevent cell death, and reduce the formation of intracellular reactive oxygen species in vascular smooth muscle cells.[14]

This neuropeptide can be released in response to different physiological conditions and can result in multiple effects. In general, CGPR is more prevalent around arteries than veins and is a powerful local blood flow regulator.[15]

Previous studies demonstrated that CGRP not only protects against factors promoting atherosclerosis,[16] but also antagonizes vessel dysfunction and ischemic injury.[17] The vascular protective mechanism of CGRP may involve suppressing the growth of vascular smooth muscle cells, preventing senescence in endothelial progenitor cells, and reducing the recruitment of monocytes and neutrophils to the vessel wall may account for.[18]

Materials and Methods

During the period from April 2020 to February 2022, a net of 200 subjects suffering from T2DM were included in this case–control study, which was carried out at the diabetic clinic and neurophysiology department at Al Imam Al Sadeq Teaching Hospital and Merjan Medical City in Al-Hilla Governorate. Among them, 94 were patients suffering from DPN confirmed by nerve conduction. The remaining 106 subjects were without DPN, who matched patients in different aspects like age, gender, and body mass index (BMI). All subjects were enrolled in the study after verbal consent was obtained. underwent a full valuation, including history, clinical examination, and a long group of tests like BMI and biochemical investigations (fasting blood sugar, glycosylated hemoglobin, and CGRP). Nerve conduction study, the Nihon Kohden device with its software and required electrodes and accessories had used to assess sensory and motor nerves for both the upper and lower limbs. One radiologist performed color Doppler ultrasonography on each participant according to the standardized scheme using a color Doppler Ultrasonography machine (GE Ultrasound, Volsun S8 with a 9–15 MHz probe) for selective lower limbs arteries including (femoral, popliteal, and posterior tibial) to assess the arterial function in both study groups. The Doppler spectrum is obtained as a graph showing the mixture of frequencies over a short period. The key elements of the time and velocity scales in the resulting Doppler spectrum. Using numerical analysis, the Doppler spectrum, waveform, flow characteristics, peak systolic velocity (PSV), minimum diastolic velocity, end-diastolic velocity (cm/s), and the resistivity index were determined.

The degree of arterial disease and Stenosis was rated based on "Jager's criteria"[19]:

"Normal, triphasic waveform with thin spectral band."

"Grade I, 1% to 19% stenosis: normal triphasic flow with normal PSV with spectral broadening."

"Grade II, 20% to 49% stenosis: triphasic waveform with an increase in PSV ≥30% with respect to the proximal recording site. Marked spectral broadening."

"Grade III, 50% to 99% stenosis: a monophasic waveform with an increase in PSV ≥100% and marked spectral broadening. The distal waveform is abnormal."

Measurement of the serum concentration of CGRP was detected with commercially obtainable assay kits of enzyme-linked immunosorbent "Catalog Number:

CEA876Hu, Cloud-Clone Corp., Wuhan, China." The determination of the quantifications was done using 450 nm analysis spectrophotometry analysis. The enzyme linked immunosorbent assay (ELISA) kit had a detection range of 2–600 ng/L.

Every statistical analysis was carried out utilizing "Statistical Package of Social Science software (SPSS) computer program" (Version 22, SPSS Inc., Chicago, USA). Chi-square testing was done for Categorical variables which as an absolute number with a *t* test was used for continuous variables which were expressed as means ± SD. Receiver operating characteristic (ROC) curve was done to detect the specificity and sensitivity of CGRP for each patient with DNP against the control in the studied group. A *P* value equal to or less than 0.05 is considered significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 88 (including the number and the date of February 11, 2020) to get this approval.

Results

There were statistically significant differences regarding HbA1c % and CGRP serum level. The mean of HbA1c % in group 1 (9.27 ± 2.18) and for group 2 (8.12 ± 2.05) ($P < 0.01$). The CGRP serum level (decreased level of CGRP associated with neuropathy) and between the study groups, the mean of CGPR in group 1 (50.74 ± 14.92) (pg/L) and for group 2 (56.81 ± 18.94) (pg/L) ($P = 0.02$). While fasting blood sugar showed no significant difference between the study groups ($P = 0.22$), as displayed in Table 1.

There were significant differences between the study groups concerning the distribution of PAD in lower limbs ($P = 0.002$), as shown in Figure 1.

Table 1: Comparison in biochemical data between the study groups

Variable Mean + SD	Group 1 (94)	Group 2 (106)	*P* value
HbA1c (%)	9.27 ± 2.18	8.12 ± 2.05	0.01
Fasting Blood sugar (mmol/L)	9.562 ± 3.60	8.964 ± 2.79	0.22
CGRP (pg/L)	50.74 ± 14.92	56.81 ± 18.94	0.02

Group 1 includes diabetic patients with peripheral neuropathy, Group 2 as control group includes diabetic patients without DPN. HbA1c: glycated hemoglobin, CGRP: calcitonin gene-related peptide, $P < 0.05$: significant.

Group 1 includes diabetic patients with peripheral neuropathy, Group 2 as control group includes diabetic patients without DPN. PAD: peripheral arterial disease, $P < 0.05$: significant

Regarding patients with and without PAD's levels of the CGRP in the study groups, there was an insignificant difference in CGPR level in patients with PAD and without PAD ($P = 0.67$). Also, there was an insignificant difference in CGPR level in control group with and without PAD ($P = 0.65$). On the other hand, there was a significant difference in CGPR level in group 1 with PAD when compared with group 2 with PAD. In addition, there was a significant difference in CGPR level in group 1 without PAD when compared with group 2 without PAD ($P = 0.028$), as shown in Table 2.

ROC curve for CGRP serum level was done for PAD group against group without PAD, cutoff value of 55 giving sensitivity of 55% and specificity of 53%, as shown in Figure 2.

Discussion

There were significant differences between the study groups regarding the distribution of PAD and ($P = 0.002$), as in Figure 1. Our finding showed an increase in the rate of PAD in the diabetes patients in a subgroup with DPN as opposed to control group of individuals with diabetes. This study and these findings are extremely important to identify and prevent both entities (PAD and DPN) earliest opportunity aiming to diminish morbidity and mortality and to prevent and control diabetic foot.[20]

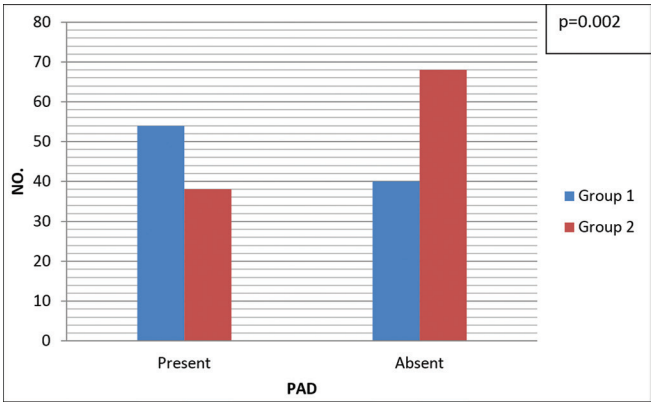
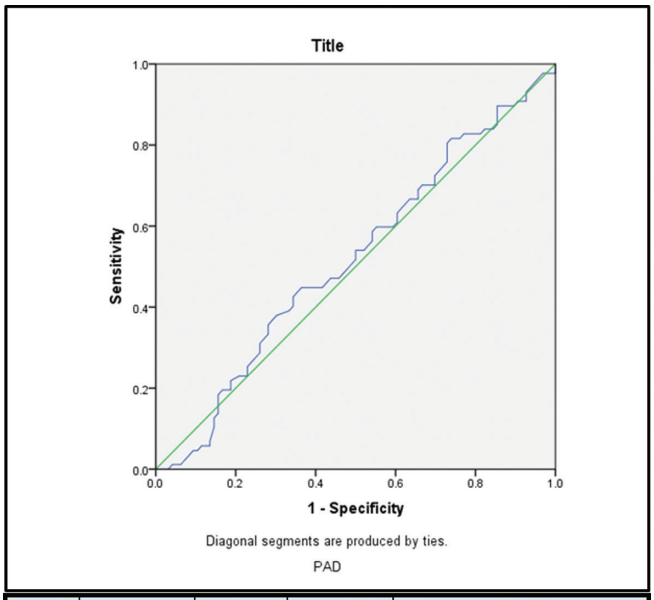


Figure 1: The peripheral arterial disease distribution in the study groups

Table 2: The CGPR level in the study groups with DPN with and without PAD

Study groups with	PAD	No.	CGPR (Mean ± SD)	*P* value
Group 1 (94)	PAD present	54	51.14 ± 16.69[A]	0.67
	PAD absent	40	49.75 ± 14.06[B]	
Group 2 (106)	PAD present	38	55.31 ± 16.24[a]	0.65
	PAD absent	68	57.17 ± 22.75[b]	
P value			0.028	

Group 1 includes diabetic patients with peripheral neuropathy, Group 2 as control group includes diabetic patients without DPN. PAD: peripheral arterial disease, CGPR: calcitonin gene-related peptide and $P < 0.05$ significant



Area	Cutoff value	Sensitivity	Specificity	95% Confidence Interval	
	CGRP			Lower Bound	Upper Bound
0.52	55	55%	53%	.435	.603

Figure 2: Receiver operating characteristic (ROC) curve to CGRP for predicting peripheral arterial disease (PAD) in the positive PAD group against the negative group.

Remarkably, both DPN and PAD are length-dependent rapidly progressive complications in diabetic patients, starting distally and symmetrically. They frequently coexist, further masking PAD symptoms and making their presentation unusual. A ground for worsening peripheral neuropathy in diabetic patients may be provided by interactions between neuropathy and angiopathy, which may be related to blood vessel damage from long-term hyperglycemia, vascular endothelial cell dysfunction, and inadequate nutrition for neurons.[8,21]

This finding agrees with the studies[7,9,22-24] and disagrees with the study of Meena and Manikandan.[25] They explained in their study that in people with T2DM, the prevalence of DPN was higher than that of PAD.

Jayaprakash *et al.*[26] revealed that the rate of both DPN and PAD in diabetic patients is high and they need to screen early in all diabetic patients. On the other hand, PD *et al.*[27] reported that PAD has been discovered to be the most fatal consequence of diabetes, but it usually occurs without any symptoms.

Our result regarding CGPR level in diabetic patients with PAD disagreed with,[12,28,29] who reported that there was significantly lower serum levels of CGRP in diabetic mouse or patients with coronary artery disease than in DM alone and CGPR level lower in diabetic than in control healthy group.

This finding can be explained by study design, sample size, Doppler study readings; late diagnosis of PAD in diabetes, or lab results regarding CGPR serum level. All these might throw light on the need for further studies to study the potential function and potential mechanism of CGRP in the endothelium dysfunction brought on by excessive hyperglycemia in individuals with PAD.

Although there was few published study demonstrating the role of CGPR in diabetic patients with PAD, they suggested that although the nerves are the primary producers of CGRP, initial study indicated that CGRP can also be found in cells that are not neurons like endothelial cells lining vascular system all over the body. It had a vasodilator effect on the vascular system. As CGPR is driving mostly on microvessels rather than large vessels, it played a protective role by improving blood supply to multiple organs and the most site for its action were endothelial cells. Endothelial nitric oxide synthase, which acts as an antioxidant in the vascular system, is stimulated by CGPR to stimulate endothelial cells by producing NO to inactivate superoxide.[30,31] Remarkably, Sohn and his work group demonstrated that the CGRP not only protects against factors promoting atherosclerosis but also antagonizes vessel dysfunction and ischemic injury.[16]

Even though, studies on the cardioprotective effects of CGRP are developing gradually, few studies done on animals, and very little done on humans to explain its role in the heart and aorta. Kee *et al.*[32] and Favoni *et al.*[15] revealed that CGRP plays an important part in cardioprotection, such as mediating vasodilation, refining myocardial contractility, and participating in ischemic preconditioning.

"ROC curve analysis" was done to assess the validity of CGRP serum level in PAD prediction [Figure 1], CGPR level showed an area under the curve with non-considerable accuracy of 0.52 at the cutoff point (55 pg/L), giving sensitivity 55% and specificity 53% respectively which indicate that CGPR level is a weak predictive factor in PAD.

Remarkably, there was no research that agreed or disagreed with our finding reflecting the need for further studies on this field.

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Conflicts of interest
There are no conflicts of interest.

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