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Association of apelin, endoglin and endocan with diabetic peripheral neuropathy in type 2 diabetic patients

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Abstract. – OBJECTIVE: Diabetic peripheral neuropathy is a common complication of type-2 diabetes mellitus. Endocan, apelin and endoglin are thought to be associated with endothelial dysfunction, angiogenesis and inflammation. In this study, we planned to evaluate these markers in diabetic peripheral neuropathy patients.

PATIENTS AND METHODS: This single-blind, controlled clinical study was conducted on 99 type 2 diabetic patients with or without diabetic peripheral neuropathy and 53 healthy volunteer controls. Physical and laboratory examinations were done in all groups. In these groups, Endoglin, apelin and endocan levels were measured with ELISA method.

RESULTS: Endoglin, apelin and endocan concentrations in diabetic peripheral neuropathy patients were higher than other diabetes mellitus patients and healthy controls. Similarly, diabetes mellitus patient's endoglin, apelin and endocan levels were higher than healthy controls. The differences were statistically significant. We detected a significant positive correlation between endoglin, apelin and endocan levels in all groups.

CONCLUSIONS: Endoglin, apelin and endocan may reflect angiogenesis and endothelial dysfunction in diabetic peripheral neuropathy and they may be used as a marker in the future.

Key Words:
Apelin, Endocan, Endoglin, Diabetic peripheral neuropathy, Type-2 diabetes mellitus.

Introduction

In recent years, diabetes mellitus (DM) has become a serious public health problem in the developing world. There are now 415 million adult DM patients worldwide in 2015, and this number is projected to grow to 642 million adults by the year 2040[1] DM may cause severe microvascular and macrovascular complications that impair the quality of life of diabetic patients. Diabetic neuropathy is a mostly neglected complication of DM. It is associated with severe morbidity, mortality, and imposes a serious economic burden[2].

The pathogenesis of diabetic peripheral neuropathy (DPN) which can be overt or subclinical is associated with many factors such as duration of diabetes, advanced age, hypertension, hypo/hyperinsulinemia, hyperglycemia. Hyperglycemia, increased advanced glycation end (AGE) products, increased pro-inflammatory response, up-regulation of the polyol pathway, an altered blood flow and oxidative/nitrative stress are supposed to cause endothelial dysfunction and impaired angiogenesis in DPN etiology[2-4].

Endocan, is a novel protein encoded by endothelial cell-specific molecule-1 gene, plays an important role in endothelial damage and neovascularization. Endocan is secreted from vascular endothelial cells, mostly inflamed endothelium. Endocan might affect the atherosclerosis process by its effects on inflammatory and vasculoprotec-

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tive signals. In the literature endocan was evaluated in different conditions such as systemic inflammation, cardiovascular disease and some cancers (e.g. brain, lung, liver, kidney and bladder)[5,6].

It has been suggested that endoglin, a type-1 membrane glycoprotein which is a part of transforming growth factor beta complex and located on cell surface, has an important role in angiogenesis[7]. Endoglin gene expression in resting endothelial cells is generally lower than activated endothelium. The expression increases after activation in endothelium of inflamed tissues, tumor vessels and vascular injury[8].

Another peptide apelin, which is widely expressed in organs such as the kidney, heart, lung, adipose tissue, liver, endothelium, and human plasma, leads to the endothelial cell proliferation and angiogenesis[9]. In addition, it was reported that apelin knockout mice had an interruption in the retinal vasculature development[10].

DPN is a serious microvascular complication of DM and its pathogenesis has not been elucidated yet. The microvascular effects of endothelial dysfunction, inflammation and angiogenesis play an important role in the development of neuronal damage[11]. In this study we aimed to evaluate the role of endothelial dysfunction and angiogenesis in patients with diabetic neuropathy through the serum endocan, endoglin and apelin levels. Whether these markers might accelerate the development of DPN in diabetic patients was elucidated.

Patients and Methods

The biochemical analyses were carried out by the same researchers. The researcher making ELISA analyses did not know which serum belonged to which participant, so it was blind. The biochemical analyses were repeated at least three times.

Materials

Glycated hemoglobin (HbA1c) autoanalyser, Primus Premier-Hb9210 (Cat≠09-00-0001/200855), was Trinity Biotech trademark (Jamestown, NY, USA). A Bio-Tech Microplate Readers (Winooski, VT, USA) brand ELISA device was used. Biochemistry (≠Cobas e6000-e501) and hormone devices (≠Cobas e6000-e601) were Roche Diagnostics (Chiyoda, Tokyo, Japan). High-density lipoprotein cholesterol (HDL-C) (Cat≠04399803), total cholesterol (Cat≠03039773), Triglyceride (TG) (Cat≠29767107-322), C-Reactive protein (CRP) (Cat≠20764930), and fasting blood glucose (FBG) (Cat≠04404483) kits were Roche brand (Chiyoda, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula[12]. Sunred human endothelial cell-specific-molecule-1/endocan (Cat≠201-12-1978), apelin (Cat≠201-12-2015), and endoglin (Cat≠ 201-12-3704) ELISA commercial kits were purchased from Sunred Biological Technology Co (Baoshan District, Shanghai, PRC).

Ethical Permission

All procedures were in conformity with the ethical standards of the responsible Committee on Human Experimentation (institutional and national) and with the Declaration of Helsinki. This study was permitted by the local Ethics Committee of Namik Kemal University (26.02.2015/09-23.02.11). Informed consent was obtained from all groups before inclusion in the study.

Study Design, Eligible Criteria and Analyses

All diabetic patients were evaluated for diabetic complications and all medical data were documented. Cases with clinical evidence of cardiovascular disease (coronary, peripheral, carotid artery), any other major disease (hepatic or renal failure, malignancy, autoimmune diseases, acute or chronic infections), history of recent trauma or surgery and females with polycystic ovary syndrome or pregnancy were excluded.

Demographic data, arterial blood pressure, serum lipid profile, fasting blood glucose and HbA1c of all participants and pharmacotherapy of the patients were recorded.

For diagnosis of DPN, clinical symptom history, neurological examination, electrophysiological tests, quantitative sensory testing and autonomic function tests were performed. The diagnoses were based on the patients' clinical symptoms and results of the entire electromyography[3,13-15].

The healthy control group (Group I, n=53) was age, sex and body mass index (BMI) matched with the DM groups. Group II (n=46) included DM patients with DPN and group III (n=53) consisted of DM patients without DPN. Diabetic patients were also grouped according to the usage of oral antidiabetic drug (OAD group n=57) or insulin (insulin group n = 42).

Blood samples were obtained after a resting period of 30 min between 8:00 and 10:00 a.m. from the cannulated antecubital vein. Serum was obtained by centrifugation at 2000 g for 15 minutes at +4 °C. All samples were stored at -80 °C before analysis.

After dissolution for antibody detection, serum samples were transferred to wells in the well plate. The measurements were made in an automated manner in a suitable absorbance (450 nm) after the addition of a suitable chromogenic substrate to the kit's human globulin antiserum.

The sensitivity of the endocan, apelin, and endoglin commercial kits were 7.5 ng/L-1 ng/L-0.25 ng/mL while the intra-assay CV < 10%; inter assay CV<12%. Assay ranges of endocan, apelin, and endoglin were 8-2000 ng/L, 1 ng/L-200 ng/L, 0.25 ng/mL-70 ng/mL respectively.

Statistical Analysis

Statistical analysis was performed with SPSS version 17 (SPSS Inc., Chicago, IL, USA). Homogeneity of groups' data was analyzed with Shapiro-Wilk test. Results were shown as a mean ± standard deviation or median and range depending on data distribution. Normally distributed data were analyzed using independent *t*-test. Abnormally distributed data were analyzed with Mann-Whitney U test. The Pearson test was used for correlation analysis. The alpha significance level was set as < 0.05.

Results

The control group of healthy volunteers (group I: 24 male, 29 female), the group of diabetic patients with DPN (Group II: 23 male, 23 female), and the group of DM patients without DPN (Group III: 24 male, 29 female) were evaluated. Demographic data of groups were noted. No significant difference was observed in terms of age, gender and BMI between all groups ($p > 0.05$).

Biochemical test results showed that fasting blood glucose and HbA1c of Group II were found to be higher than Group III ($p = 0.005$ and $p = 0.004$ respectively).

Endocan levels of Group II and group III were found to be higher than Group I ($p < 0.001$ and $p < 0.001$ respectively). Endocan levels of Group II were significantly higher than those of Group III ($p = 0.001$).

Endoglin levels of Group II and group III were found to be higher than Group I ($p = 0.001$ and $p = 0.014$ respectively). Endoglin levels of Group II were significantly higher than those of Group III ($p = 0.028$).

Apelin levels of Group II and group III were found to be higher than Group I ($p < 0.001$ and $p < 0.001$ respectively). Apelin levels of Group II were significantly higher than those of Group III ($p < 0.001$). Biochemical test results were described in Table I and Figure I.

Table I. Demographic data and inter-group comparisons of biochemical measurments.

		Group I (healthy controls; n = 53)	Group II (patients with DPN; n = 46)	Grup III (patients with non-DPN; n = 53)
Age	(Year)	57.31 ± 8.41	56.91 ± 7.44	58.85 ± 9.04
BMI	(kg/m^2)	28.04 ± 3.28	31.99 ± 6.14	33.07 ± 5.33
SBP	(mm-Hg)	120 ± 3.71	131.62 ± 14.25	128.97 ± 11.73
DBP	(mm-Hg)	80 ± 1.18	76.62 ± 9.84	76.56 ± 6.86
FBG	(mg/dL)	90.09 ± 4.16	210.87 ± 89.15	163.08 ± 59.11
HbA1c	(%)	4.5 ± 1.18	9.16 ± 2.25	7.95 ± 1.62
TC*	(mg/dL)	192.00 (127-240)	205.62 (130-310)	223.85 (125-415)
TG*	(mg/dL)	135.00 (52-160)	186.00 (47-623)	185.28 (36-423)
HDL	(mg/dL)	51.19 ± 17.38	44.02 ± 15.24	47.69 ± 12.50
LDL	(mg/dL)	119.00 ± 36.65	122.18 ± 38.52	138.84 ± 54.15
CRP	(mg/L)	1.09 ± 3.17	3.95 ± 5.89	4.55 ± 10.43
Endocan*	(ng/L)	781.8 (213.3-1433.1)	1227.1 (575.9-1862.3)	1043.0 (429.9-1678)
Apelin*	(ng/L)	55.8 (27.7-106.0)	93.3 (42.6-145.4)	76.2 (46.0-76.2)
Endoglin*	(ng/mL)	20.6 (11.1-58.9)	25.2 (14.5-53.1)	20.7 (10.0-40.6)

Unless otherwise noted, data are presented as mean ± standard deviation, *Median (min-max) BMI: Body mass index; SBP: systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HbA1c: Glycated hemoglobin; TC: Total cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein CRP: C-reactive protein; DPN: Diabetic peripheral neuropathy; Non-DPN: Non-diabetic peripheral neuropathy with type-2 diabetes mellitus.

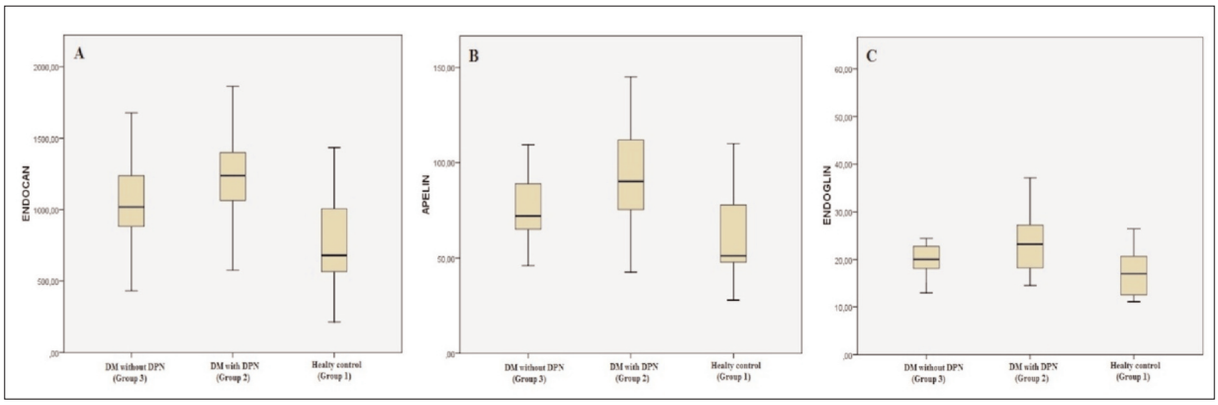

Figure 1. ***A,*** Endocan. ***B,*** Apelin. ***C,*** Endoglin levels of all groups.

Significant positive correlations were detected between Endocan, Apelin and Endoglin levels (Table II).

When diabetic patients have been grouped according to the treatment modality, apelin and endocan levels were higher in insulin group than OAD group ($p = 0.001$ and $p=0.004$ respectively) No statistically significant difference was detected between these two groups in terms of endoglin levels ($p = 0.146$).

Discussion

DPN prevalence is correlated with diabetes duration and severity. While 40% of patients had DPN by 10 years, 70% of patients have been affected by 25 years[16]. DPN pathogenesis studies focus on hypoxia and metabolic theories. Important roles of metabolic factors and hyperglycemia in DPN progression are known but the exact mechanism has not been elucidated yet. Endoneurial microvascular circulation defects, capillary basal membrane thickness, endothelial dysfunction, angiogenesis and capillary thrombosis are reported as main reasons of neuropathy[2,17,18]. Decreased nutritive blood flow to sensory nerve fibers due to impaired angiogenesis and neurotrophic factor deficiency are also suggested in diabetic neuropathy pathogenesis and angiogenesis-neuropathy studies gained speed. For example in a study of Han et al[19], local transplantation of mesenchymal stem cells improved diabetic neuropathy through the promotion of direct peripheral nerve angiogenesis, neurotrophic effects and myelination restoration. Also, Quattrini et al[20] reported that reduced vascular endothelial growth factor expression, a mediator which regulates angiogenesis and neuronal survival by stimulating neurons and glial cells to survive and grow, and intra-epidermal nerve fiber loss were found in diabetic neuropathy patients' foot skin. So microvascular degeneration and impaired angiogenesis have some role in DPN pathogenesis.

Endocan, Apelin and Endoglin are novel markers and there are few studies about these markers in diabetes mellitus in the literature. To our knowledge, there is no study evaluating endocan, apelin and endoglin all together in DPN.

In the report of Rodrigues et al[21], endocan levels of 39 type 2 diabetic patients and 39 controls were evaluated. They found that endocan concentrations of T2DM patients were significantly lower than the control group. However, Arman et

Table II. Correlations of endocan, apelin and endoglin levels with each other.

		Endocan	Apelin	Endoglin
Endocan	r	1	0.502	0.337
	p		0.000	0.000
Apelin	r	0.502	1	0.381
	p	0.000		0.000
Endoglin	r	0.337	0.381	1
	p	0.000	0.000	

al[22] found that serum endocan levels are elevated in a study group of 77 DM patients and decrease following after 3 months of lifestyle change and anti-hyperglycemic treatment. They suggested that endocan levels are correlated with the stage of DM and they supposed that endocan might be a prognostic marker in patients with type 2 DM. In our study including 99 DM patients, diabetic patient's endocan levels were found to be higher than control group like Arman et al[22]. Our findings supported that higher endocan levels might be related to endothelial dysfunction and increased angiogenesis in diabetic patients.

The study evaluating endocan levels in a diabetic microvascular complication was performed by Abu El-Asrar et al[23] with vitreous fluids of 44 proliferative diabetic retinopathy patients. Endocan levels of active proliferative diabetic retinopathy patients were found to be higher than DM patients with inactive proliferative retinopathy and the control group. They suggested that endocan expression could be associated with endothelial cell activation and angiogenesis.

In the present study evaluating DPN, we found endocan levels of DPN group were higher than the DM patients without DPN. This result might indicate an association between endocan level and progression of complications. We supposed that endocan might be a prognostic marker for DM complication development in the future.

Several studies[24,25] have shown that apelin plays an important role in the pathophysiology of some diseases, including hypertension, heart failure, cardiovascular disease, DM, and obesity. The angiogenesis is the result of apelin effect on the migration and proliferation of endothelial cells.

Ma et al[26] assessed the relation of plasma apelin levels and future development of DM. They suggested that plasma apelin level can independently predict incident diabetes in men but not in women and plasma apelin is a new marker to predict incident diabetes in men.

Du et al[27] performed a study about serum level of apelin in diabetic retinopathy patients and they found apelin levels in proliferative retinopathy patients significantly higher than DM patients without retinopathy.

In the present study, apelin levels were found to be higher in DM patients than healthy controls. When diabetic patients were evaluated, apelin levels of DPN group were found to be significantly higher than DM patients without DPN. We supposed that elevated apelin levels may indicate the metabolic state causing diabetic complications. Apelin might reflect endothelial dysfunction and microangiopathic changes in diabetic patients.

Endoglin is a homodimeric transmembrane glycoprotein that has a regulatory role in transforming growth factor-beta signaling and mostly expressed on proliferating endothelial cells. It is especially observed in endothelial cells, activated macrophages, smooth muscle cells and fibroblast. Endoglin expression in vessels increases during various pathological situations like hypoxia or vascular injury[28].

There are several studies with endoglin in DM and its complications. Blázquez-Medela et al[29] evaluated the relationship of endoglin-plasma levels with endothelial dysfunction, hypertensive retinopathy and cardiovascular risk in 288 patients (64 with type 2 diabetes, 159 with hypertension and 65 healthy controls). They reported that endoglin was an indicator of vascular pathologies related with DM and hypertension as endothelial dysfunction and cardiovascular damage. Kovacs et al[30] reported that endoglin concentrations of diabetic patients are higher than the control group. Additionally endoglin levels of proliferative retinopathy patients were found to be higher than DM patients without retinopathy even though it had not reached statistical significance.

We observed that endoglin levels were found to be higher in DM patients than healthy controls and endoglin levels of DPN patients were significantly higher than the non-DPN group. Our results support the association of endoglin with endothelial dysfunction and angiogenesis. So, we suppose endoglin might be used as a predictor of DPN development in DM patients.

Conclusions

Apelin, endoglin and endocan might be associated with endothelial dysfunction, angiogenesis and inflammation mechanisms which all play some role in DPN pathogenesis. Even though further studies with larger sample size are needed to confirm these results, the endocan, endoglin and apelin might be used as markers of DPN.

Some limitations should be considered in this study: the number of patients included was relatively small. The study design was cross-sectional and it is a single center study. New studies, with larger sample size and multicenter designed are needed to confirm these results.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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