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ORIGINAL ARTICLE

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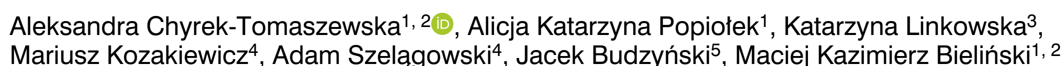

Brain-derived neurotrophic factor serum concentration and BDNF Val66Met polymorphism in patients with peripheral artery disease: the importance of heart failure

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ABSTRACT

Introduction: Brain-derived neurotrophic factor (BDNF) and BDNF Val66Met polymorphism have been associated with cardiovascular diseases such as atherosclerosis, congestive heart failure (CHF), hypertension and ischaemic heart disease (IHD). To the authors' knowledge, such connections have not been described in peripheral artery disease (PAD) yet.

Material and methods: 159 PAD subjects and 57 controls were included. All enrolled subjects underwent evaluation of clinical status. Information on comorbidities such as diabetes type 2, hypertension, IHD and CHF, was gathered. Serum concentrations of BDNF were measured by ELISA. Genotypes of the BDNF-AS SNP rs6265 were determined using TaqMan SNP Genotyping Assay.

Results: PAD patients had significantly lower BDNF serum concentrations compared to controls (median values of 7.2 *vs.* 35.1 ng/mL, $P < 0.001$). Concentrations were significantly lower in patients with concomitant CHF ($P < 0.05$). The CHF subgroup was characterised by a greater prevalence of diabetes and ischaemic heart disease ($P < 0.01$). There was no significant difference between BDNF serum concentrations and other comorbidities, ABI, and medical history including disease duration and past interventions. No important correlations were found for BDNF Val66Met polymorphism.

Conclusions: The present study adds to the body of evidence associating BDNF and atherosclerosis. The serum BDNF concentrations were lower in PAD, especially in a subgroup with comorbid CHF. These results suggest that a larger cardiovascular burden is connected with decreased BDNF serum concentrations. No evidence was found to support the hypothesis that BDNF gene polymorphism may be a contributing factor in the pathogenesis of cardiovascular diseases such as PAD.

Key words: Brain-derived neurotrophic factor, peripheral arterial disease, heart failure, atherosclerosis, Val66Met

Introduction

Brain-derived neurotrophic factor (BDNF) is a widely studied neurotrophin produced principally by neurons. It has an important role in numerous brain functions such as neurogenesis, neuroregeneration, regulation of synaptic transmission and synaptic plasticity. Besides the nervous system, BDNF also acts like exercise-induced cytokine [1]. It is synthesised in e.g. muscle tissues, fibroblasts, macrophages or platelets

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Table 1. Participant characteristics

Parameter	Without CHF group (n = 131)	CHF group (n = 28)	P-value
Age, [y]	65.0 (59.0–72.0)	69.0 (63.5–73.5)	0.02
Gender (♀/♂)	43/88	9/19	0.98
DM, n	45 (34.5%)	20 (71.5%)	< 0.01
HA, n	89 (68.5%)	24 (85.7%)	0.15
IHD, n	42 (32.3%)	24 (85.7%)	< 0.01
BNDF AS polymorphism	C/C n = 86 (65.7%) C/T n = 44 (33.6%) T/T = 1 (0.7%)	C/C n = 19 (67.9%) C/T n = 8 (28.5%) T/T n = 1 (3.6%)	0.71
BDNF, [pg/L]	2.69 (1.11–5.29)	1.38 (0.92–3.1)	0.02
ABI operated leg	0.5 (0.25–0.69)	0.5 (0.2 – 0.66)	0.79
ABI unoperated leg (excluding patients with amputations)	0.66 (0.35 – 0.84)	0.77 (0.5–1.0)	0.25
No of leg revascularization in past	1.0 (0.0–2.0)	1.0 (0.0–2.0)	0.92
Duration from 1st intervention [months]	6.0 (0.0–30.0)	18.0 (0.0–30.0)	0.74
Earlier amputations (yes/~~no~~)	10 (7.7%)	3 (10.5%)	0.55

Data shown as a median or number and per cent. CHF — chronic heart failure; HA — arterial hypertension; DM — diabetes; IHD — ischaemic heart disease; BDNF — brain-derived neurotrophic factor; BNDF AS — brain-derived neurotrophic factor's Val66Met polymorphisms; ABI — ankle-brachial index

and occurs in the heart, vessels or plasma, where other BDNF roles still have been uncovered [2]. As multiple studies have proved, BDNF could be considered a versatile biomarker in various diseases. Decreased peripheral concentrations of BDNF were observed in neuropsychiatric conditions [3–11], but there is a growing number of BDNF studies also in internal medicine. Low concentrations of circulating BDNF have been noted in patients with type 2 diabetes and metabolic syndrome, including obesity and dyslipidaemia [12]. Furthermore, serum BDNF concentrations relate to cardiovascular dysfunction. Consequently, reduced BDNF concentrations were noticed in atherosclerosis [13], chronic heart failure (CHF) [1, 14], hypertension [15] or ischaemic heart disease (IHD) [16]. Moreover, blood BDNF concentrations were inversely associated with the degree of coronary artery calcification [15] and progression of CHF [1]. In addition, reduced serum BDNF concentrations are related to poorer prognosis as an independent risk factor of death and rehospitalization in patients with CHF [14]. Contrastingly, higher BDNF concentrations were found in patients with microvascular angina and ST-elevation myocardial infarction [17, 18].

BDNF is encoded by a polymorphic BDNF gene, found on chromosome 11. A common single nucleotide polymorphism (SNP) in the BDNF gene, in which Methionine (Met) substitutes for Valine (Val) at codon 66 (Val66Met), has also been linked to neuropsychiatric, metabolic or cardiovascular diseases (CVDs) [19, 20].

This study aimed to examine the serum concentration of BDNF and BDNF gene Val66Met variants in a group of patients with peripheral artery disease (PAD) qualified for revascularization and to determine the clinical factors associated with BDNF concentration. To the authors' knowledge, up to now, this topic has not been described.

Material and methods

Patients and control group

A total of 159 adult Caucasian patients of Polish nationality were enrolled in this study, including 62 women and 97 men. All patients suffered from PAD and met the criteria for lower limb revascularization. The diagnoses of PAD and comorbidities such as diabetes, hypertension, IHD, and CHF were made using typical criteria [21–25]. The median age of all participants was 66.0 (range 39–87). Basic demographic and clinical data are presented in Table 1. Exclusion criteria comprised any acute condition, significant neurological or psychiatric disorders and lack of consent.

For the study group, a control group consisting of 57 healthy, chronically untreated people, 25 women and 32 men with a median age of 67.5 (range 45–78) years, was selected. The control group did not differ from the study group in terms of age (p = 0.19) and gender (p = 0.10).

Blood collection and biochemical analyses

BDNF concentrations were assessed in a peripheral blood sample. Tests were performed according to the manufacturer's instructions (Biovendor). BDNF was determined in duplicate using appropriately diluted serum samples. The applied ELISA method involved coating a plate with a monoclonal antibody specific for a BDNF (antigen). To verify the reliability of the results, standard solutions were applied to the plate. Concentrations were measured, and a calibration curve was prepared. Afterwards, plasma samples were added to the plate and incubated for 60 minutes. Once BDNF appeared in the serum, it was bound to a specific antibody. Having washed away unbound antigens, an enzyme-linked antibody specific for a BDNF was added. Samples were incubated for 30 minutes and plates were rinsed. After rinsing plates and removing unbound antibodies, a substrate solution was added to produce a colour reaction. Colour intensity corresponded to the concentration concentrations of BDNF in samples that were read at 450 nm.

Genomic DNA was isolated from blood using GeneMatrix Bio-Trace DNA Purification Kit according to the manufacturer's protocols (Eurx, Gdańsk, Poland). DNA quantity was assessed spectrophotometrically (DeNovix Inc., Wilmington, USA). Genotypes of the BDNF-AS SNP rs6265 were determined using a commercially available TaqMan SNP Genotyping Assay (Assay ID: C_11592758_10) with the ViiA™ 7 Real-Time PCR System (Applied Biosystems, Carlsbad, California) in accordance with manufacturer's instructions.

Ethics

All participants gave informed consent to participate in the study. Permission for the study was obtained from the Local Bioethics Committee (No. 471/2017) and the study conforms to the recognised standards of the Declaration of Helsinki.

Statistical analysis

Computer software Statistica 13 was used to perform statistical analyses. A Shapiro–Wilk test revealed that the distribution of variables was not normal; therefore, nonparametric tests were used in subsequent analyses. Data are presented as median or number and per cent. The statistical significance of the differences between the two groups of data was calculated using Mann–Whitney U tests. The Spearman rank correlation test was used to determine correlations between variables. A logistic regression model was performed to determine factors relevant to BDNF concentration.

Results

The serum BDNF concentration was found to be significantly lower in the study group ($p = 9.28E-26$) with median values of 2.3 *vs.* 31.8 ng/mL. Moreover, the occurrence of CHF was associated with a significantly lower concentration of BDNF (Tab. 1). Patients with CHF have also more comorbidities like diabetes type 2 (71.5% *vs.* 34.5%, $p < 0.01$) and IHD (85.7% *vs.* 32.3%, $p < 0.01$). The subsequent multivariable analysis did not confirm CHF as an independent fundamental differentiating factor ($p = 0.12$). No relevant differences or significant correlations between the BDNF concentration and analysed clinical parameters (illness duration, number of vascular interventions, previous amputations, and ABI) were found. Also, no significant relationships were established for diabetes, hypertension, IHD, CHF and BDNF polymorphism.

Discussion

BDNF, besides its role in the nervous system, participates in energy homeostasis and cardiovascular function. The main mechanism of BDNF action is to bind the tyrosine receptor kinase B (TrkB). The activation of TrkB is essential for coronary vessel development. Besides, BDNF protects endothelium. It is suspected that this protective activity stems from decreasing endothelial oxidative stress, stabilizing the vascular smooth muscle cells, reducing their apoptosis and minimizing vascular calcification [15]. Furthermore, BDNF stimulates neovascularization in response to hypoxia [14, 26]. Similarly, an increase in circulating BDNF fosters the survival of cardiomyocytes [26]. BDNF also regulates myoblast activity and is necessary for the endogenous reparation of the myocardium [2]. BDNF release is highly dependent on blood perfusion [1, 2]. CHF and PAD, due to lower capillary perfusion, are associated with ischaemia-induced myopathy. Dysfunctional skeletal muscles and myocardium are not capable of synthesising and releasing myokines, such as BDNF, in a proper way, which is a part of the vicious circle of adverse cardiac remodelling and myopathy progression [2]. Sympathetic hyperactivity connected with CHF and vasculitis or noninflammatory arteriopathies observed in PAD may also contribute to a reduction of BDNF blood concentration [21, 27]. The dysfunction of BDNF could also be indirectly related to the pathogenesis of CVDs, due to its involvement in the regulation of glucose and lipid metabolism and the development of obesity and dyslipidaemia [12], all of them redounding to CVDs.

The present study duplicated the finding that BDNF serum concentration is lower in patients with CHF. It also confirmed the reduced concentration of

circulating BDNF in atherosclerosis by measuring the concentration of BDNF in patients with PAD, a clinically relevant manifestation of atherosclerosis. A correlation between the serum BDNF concentration and severity of PAD expressed as parameters like ankle-brachial index, a number of past endovascular interventions or illness duration was not observed. Interestingly, in the present study, the co-occurrence of CHF and PAD was associated with a significantly lower concentration of BDNF. Such a dependence was not confirmed among other comorbidities such as diabetes, hypertension, and IHD. It is possible that CHF affects BDNF blood concentrations more than other CVDs. However, it should be noted that the subgroup of people with CHF was characterised by a significantly greater prevalence of diabetes and IHD.

Although the Val66Met polymorphism of BDNF gene in previous studies was associated with changes in circulating concentrations of BDNF and it may be linked to CVDs, no significant correlation was found for BDNF Val66Met polymorphism in the study group.

Growing evidence indicates that BDNF has a positive impact on the cardiovascular system and might prove itself as a risk factor for CVDs. Further experimental studies are needed to prove the importance of BDNF in clinical practice. The accumulated evidence on the BDNF's role in the pathogenesis of atherosclerosis and CHF may help to create an additional therapeutic approach for these diseases' prevention and treatment in the future.

There are limitations to this study that should be noted. The study population was relatively small. Serum BDNF concentrations are influenced by several factors, and not all of them were analysed. There are no detailed data regarding pharmacotherapy, blood pressure, heart rate, smoking, BMI or hypercholesterolemia.

Conclusion

To conclude, serum BDNF concentrations were lower in PAD patients. In addition, serum BDNF concentrations were significantly lower in patients with PAD and CHF. Although this connection may be an effect of the worse health status, which is a result of the coexistence of CHF, diabetes, and IHD.

Ethics statements: *The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Bioethical Commission of the Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz and informed consent was obtained from all individual participants.*

Conflict of interest: *None.*

Funding: *None.*

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