Original paper

Serum level of osteoprotegerin in patients with Parkinson's disease: a preliminary study

Ocena stężenia osteoprotegeryny w surowicy u pacjentów z chorobą Parkinsona: badanie wstępne

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Key words: stroke, Parkinson's disease, osteoprotegerin, cholesterol.

Słowa kluczowe: udar mózgu, choroba Parkinsona, osteoprotegeryna, cholesterol.

Abstract

Introduction: Osteoprotegerin (OPG) has recently been suggested to be involved in the pathophysiology of Parkinson's disease (PD).
Aim of the research: To compare serum OPG levels in PD patients versus controls with mild headache or back pain (CG1) and acute ischaemic stroke (CG2) and evaluate the relationship between serum OPG level and 1) PD duration, 2) parathyroid hormone (PTH), vitamin D [25(OH)D], total calcium and 3) serum total cholesterol (TC), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride levels.
Material and methods: In the included 45 PD patients – 20 with initial PD (iPD) and 25 with advanced PD (aPD) and 50 controls (20 CG1, 30 CG2) – we measured serum OPG levels using a sandwich enzyme-linked immunosorbent assay and analysed data applying Student's *t*, Mann-Whitney, χ^2 tests, Pearson's correlation and linear regression analysis.
Results: OPG serum level was lower in iPD compared to CG2 subjects ($p = 0.026$). OPG level depended on the duration of PD ($p < 0.01$) and TC level ($p < 0.03$) in PD patients, on 25(OH)D level ($p < 0.04$) in CG1 and on TC ($p < 0.05$) and PTH ($p < 0.01$) levels in CG2.
Conclusions: Our results indicate that serum OPG level increases with PD duration and is associated with serum TC level in PD and ischaemic stroke patients.

Streszczenie

Wprowadzenie: Wyniki najnowszych badań wskazują na udział osteoprotegeryny (OPG) w patofizjologii choroby Parkinsona (ChP).
Cel pracy: Ocena stężenia OPG w surowicy u pacjentów z ChP w porównaniu z grupami kontrolnymi: pacjentów z łagodnymi bólami głowy lub kręgosłupa (CG1) i z ostrym udarem niedokrwiennym mózgu (CG2) oraz zbadanie związku między stężeniem OPG w surowicy a: 1) czasem trwania ChP, 2) stężeniami w surowicy parathormonu (PTH), witaminy D [25(OH)D], wapnia całkowitego oraz 3) cholesterolu całkowitego (TC), lipoproteiny o wysokiej gęstości, lipoproteiny o niskiej gęstości i trójglicerydów.
Materiał i metody: Do badania włączono 45 pacjentów z ChP [20 w stadium początkowym choroby (iPD) i 25 w stadium zaawansowanym (aPD)] oraz 50 pacjentów do grup kontrolnych (20 do CG1, 30 do CG2). Stężenia OPG w surowicy oznaczono metodą immunoenzymosorpcyjną typu *sandwich*. W analizie statystycznej zastosowano testy *t*-Studenta, Mann-Whitneya, χ^2 oraz analizę korelacji Pearsona i regresji liniowej.
Wyniki: Stwierdzono niższe stężenie OPG w surowicy pacjentów z iPD w porównaniu z pacjentami z grupy CG2 ($p = 0{,}026$). Stężenie OPG zależało od czasu trwania ChP ($p < 0{,}01$) i stężenia TC ($p < 0{,}03$) u pacjentów z ChP, od stężenia 25(OH)D ($p < 0{,}04$) w grupie CG1 oraz od stężenia TC ($p < 0{,}05$) i PTH ($p < 0{,}01$) w grupie CG2.
Wnioski: Stężenie OPG w surowicy wzrasta wraz z czasem trwania ChP i ma związek ze stężeniem TC w surowicy zarówno u pacjentów z ChP, jak i u pacjentów z ostrym udarem niedokrwiennym mózgu.

Introduction

Osteoprotegerin (OPG) is a member of the tumour necrosis factor receptor family that affects bone (particularly osteoporosis), vascular, immune, and tumour biology. OPG can also be expressed in neurons and dendritic cells and recent studies suggest the involvement of OPG in the pathophysiology of multiple sclerosis, brain ischaemia and Parkinson's disease (PD) [1–4]. PD is a common neurodegenerative disease characterized by progressive motor disability and a wide range of non-motor symptoms. The underlying causes of neurodegeneration in PD remain unclear, although the interaction of many pathomechanisms are proposed, including prion-like spreading of abnormal alfa-synuclein, mitochondrial dysfunction, oxidant stress, excitotoxicity, and inflammation.

Aim of the research

Our study aimed to compare serum levels of OPG in PD patients versus controls (including a stroke control group) and evaluate the relationship between serum OPG concentration and 1) the duration of PD, 2) serum concentrations of selected calcium metabolism indicators: parathyroid hormone (PTH), vitamin D [25(OH)D] total calcium and 3) serum concentrations of selected cardiovascular disease risk factors: total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG).

Material and methods

Subjects

Forty-five PD patients and 50 controls were enrolled. PD was diagnosed following the Movement Disorder Society Clinical Diagnostic Criteria [5]. The duration of PD for at least 3 years was a supplementary inclusion criterion. Twenty patients had initial PD (iPD) and 25 patients had advanced PD (aPD) according to the disease duration (3–5 years for iPD and above 5 years for aPD) and the 5-2-1 criteria [6]. Fifty controls were divided into two groups. One was with 20 subjects diagnosed with a chronic or recurrent mild/benign headache or back pain, without any others diagnosed diseases (CG1), and the second was with 30 subjects with acute ischaemic stroke (CG2) diagnosed according to the definition of the American Heart Association and American Stroke Association [7]. Exclusion criteria were 1) cancer, 2) inflammatory diseases, 3) infectious diseases, 4) diabetes, 5) renal failure, 6) hepatic failure, and 6) circulatory failure for all patients. Additionally, the history of cerebrovascular disease was an exclusion criterion for CG1 patients. All enrolled patients were in the care of the Department of Neurology and Epileptology, Centre of Postgraduate Medical Education, Orlowski Hospital, Warsaw, Poland. The local Bioethics Committee approved the study protocol, and all participants gave informed consent. The baseline characteristics of the groups are presented in the Table 1. There was no difference in sex and age between PD and control groups.

Laboratory assessment

Serum OPG level was measured using a sandwich enzyme-linked immunosorbent assay (Immunodiagnostik AG, Germany) [8]. Serum PTH, 25(OH)D, total calcium, total cholesterol, HDL, LDL and TG levels were determined by routine laboratory blood tests. The blood samples for total cholesterol, HDL, LDL and

Table 1. Baseline characteristics of Parkinson's disease patients and controls

Parameter	PD patients		Control groups		*P*-value
	Initial PD	Advanced PD	Acute ischaemic stroke (CG1)	Mild headache/ back pain (CG2)	
Number of patients	20	25	30	20	-
Sex:					
Female	12	14	15	13	ns*
Male	8	11	15	7	
Age [years] mean ± standard deviation	62.65 ±9.61	64.56 ±7.30	68.43 ±12.04	58.35 ±10.87	ns#
Age at PD onset [years] mean ± standard deviation	59.15 ±9.44	55.08 ±7.69			ns#
Duration of PD [years] mean ± standard deviation	3.40 ±1.05	9.44 ±2.83			0.0001#
Dose of levodopa [mg/daily] mean ± standard deviation	480.63 ±268.63	875.73 ±441.68			0.001#
Hoehn and Yahr scale score mean ± standard deviation	1.97 ±0.75	2.64 ±0.67			0.003#

*PD – Parkinson's disease, ns – non-significant, *χ^2 test, #Student's t-test.*

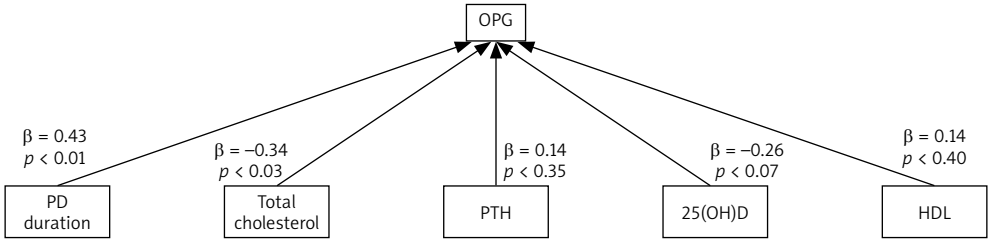

Figure 1. Regression model for serum OPG level and PD duration, serum total cholesterol, PTH, 25(OH)D, HDL levels in PD patients. Values of the model: $F = 3.189$, $p = 0.017$, $R^2 = 0.290$

OPG – serum osteoprotegerin level, PD – Parkinson's disease, PTH – serum parathyroid hormone level, 25(OH)D – 25 hydroxyvitamin D serum level, HDL – serum high-density lipoprotein cholesterol level, F – F-value of the analysis, R^2 – adjusted coefficient of determination.

TG were taken within 24 h and for OPG, PTH, 25(OH)D and total calcium within 7 days from the beginning of symptoms in subjects with acute ischaemic stroke.

Statistical analysis

The χ^2 test was used to compare distribution, and Student's *t*-test or the Mann-Whitney test was used to compare mean values. Pearson's correlation and linear regression analysis were used to determine the relationship between serum OPG level and the examined factors. The statistical significance level was set at $p < 0.05$.

Results

Serum OPG level

The highest mean serum OPG level was found in CG2 (7.59 ±3.45 pmol/l), lower in aPD (6.80 ±2.49 pmol/l) and CG1 (5.68 ±1.75 pmol/l), and lowest in iPD (5.50 ±1.88 pmol/l), but the differences were not statistically significant. Only patients with iPD had significantly lower mean serum OPG levels than CG2 ($p = 0.026$, Mann-Whitney test).

Serum PTH, 25(OH)D and total calcium levels

Mean serum PTH concentration was 53.34 ±23.42 pg/ml in iPD, 42.29 ±15.97 pg/ml in aPD, 40.75 ±16.40 pg/ml in patients with CG1 and 53.59 ±29.26 pg/ml in CG2. The aPD patients had significantly lower mean serum PTH levels than iPD patients ($p = 0.034$, Student's t-test).

Mean serum 25(OH)D level was 15.45 ±7.71 ng/ml in iPD patients, 13.80 ±12.49 ng/ml in aPD patients, 17.48 ±6.72 ng/ml in CG1 and 11.12 ±6.5 ng/ml in CG2. The difference was significant only between the two control groups ($p = 0.002$, Student's *t*-test).

There was no significant difference between groups in mean serum total calcium level.

Serum total cholesterol, HDL, LDL and TG levels

Mean serum HDL level was 59.40 ±14.43 mg/dl in patients with iPD, 52.30 ±13.89 mg/dl in aPD, 53.59 ±29.26 mg/dl in CG1, and 52.20 ±10.30 mg/dl in CG2. Patients with iPD have significantly higher mean serum HDL levels than CG2 ($p = 0.019$, Student's *t*-test).

There was no significant difference between groups in mean total cholesterol, LDL and serum TG levels.

Serum OPG level correlation with PD duration and serum PTH and 25(OH)D levels (Pearson's correlation analysis)

Serum OPG level correlated ($r = 0.38$, $p = 0.011$) with the disease duration in the PD group (including iPD and aPD). Serum OPG level correlated with serum 25(OH)D level ($r = –0.47$, $p = 0.036$) in CG1 and with serum PTH level ($r = 0.52$, $p = 0.003$) in CG2.

Results of linear regression analysis

Serum OPG level was related to PD duration ($\beta = 0.43$, $p < 0.01$) and serum total cholesterol level ($\beta = –0.34$, $p < 0.03$), and unrelated to serum PTH, 25(OH)D and HDL levels in PD (including iPD and aPD) patients (Figure 1).

Serum OPG level depended on serum 25(OH) D level ($\beta = –0.50$, $p < 0.04$) in CG1, without association with PTH, total cholesterol and serum HDL levels. Values of this regression model are *F* (value of the analysis) = 2.566, $p = 0.093$, and R^2 (adjusted coefficient of determination) = 0.0339.

Factors affecting serum OPG level were PTH ($\beta = 0.45$, $p < 0.01$) and serum total cholesterol levels ($\beta = –0.35$, $p < 0.05$) in CG2, without association with serum 25(OH)D and HDL levels. Values of this regression model are $F = 4.684$, $p = 0.010$, and $R^2 = 0.351$.

Discussion

It is well known that increased serum OPG levels are a strong predictor of cardiovascular mortality and morbidity and that the receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL)/OPG system is related to atherosclerotic plaque calcification [9, 10]. However, the function of OPG has not been completely elucidated. On the one hand, it can be

speculated that OPG is a protective, anti-inflammatory mediator for atherosclerosis through the RANK/RANKL/OPG system. On the other hand, OPG might have proinflammatory and proatherogenic features as a decoy receptor for TNF-related apoptosis-inducing ligand (TRAIL) [11]. An increased serum OPG level was likewise observed in patients with cerebral atherosclerosis and is considered a predictor of major disability and long-term mortality in acute ischaemic stroke [2, 12–14]. Additionally, a recent study demonstrated that plasma OPG level correlated with the severity of cerebral small vessel disease [15]. The role of OPG in ischaemic brain injury remains under debate. It is possible that acute ischaemic stress activates OPG proinflammatory and neurotoxic effects during the early stage of brain damage [3, 16]. Increased secretion of OPG may also have a neuroprotective (anti-inflammatory) effect by enhancement of RANK/RANKL/OPG signalling during the later stages of stroke [3, 17, 18].

OPG serum level was significantly lower in patients with iPD compared to patients with stroke. OPG level was associated with the disease duration and total cholesterol level in PD patients and with total cholesterol and PTH levels in controls with stroke.

There have only been a few studies on OPG in PD, and lower serum/plasma and cerebrospinal fluid OPG levels were observed in PD compared to healthy controls [4, 19]. Those results could indicate that the involvement of OPG in PD pathomechanisms is probably related to neuroinflammation [3, 4, 19]. Lin *et al.* (2021) investigated bone-derived factors in PD. They found that osteocalcin (OCN) and osteopontin (OPN), but not OPG, may serve as potential biomarkers for PD and that OCN and OPN plasma levels correlated with motor impairment in PD patients (OPG was not tested) [4]. So far, according to our knowledge, the relationship between OPG and the progression of PD has not been investigated. Our results may suggest that the serum OPG level increase with the progression of neurodegeneration in PD. Our cross-sectional study's results should be interpreted cautiously and treated as preliminary, especially as the low number of enrolled subjects is a limitation and the absence of significant difference in OPG levels between PD patients and controls with mild headache/spine pain is a disappointing finding.

In the context of PD as a risk factor for osteoporosis [20], serum PTH level was surprisingly lower in aPD versus iPD in the current study. Still, serum calcium metabolism indicators levels did not affect OPG levels in PD patients. OPG level was associated with 25(OH)D level in controls with mild headache/spine pain and total cholesterol and PTH levels in controls with stroke. Of note, none of the subjects enrolled in the study was in treatment for osteoporosis.

In our study, serum OPG level was associated with serum total cholesterol levels in PD and stroke groups. The relationship between serum OPG levels and lipid profile has been investigated in a few studies with inconsistent results, probably due to different study populations. A negative correlation of OPG with TG and no correlations with total and HDL cholesterol levels were found in an ageing male Lebanese population [21]. Authors from the same research centre reported no correlations of OPG with lipid parameters (including TG) in obese and non-obese patients [22]. In contrast, OPG level was associated with total and LDL cholesterol levels in healthy Korean females [23].

Conclusions

An increase in serum OPG level occurs in the course of PD. Serum OPG level is associated with serum total cholesterol levels in PD and stroke groups. Serum OPG level may be considered a potential biomarker of PD progression in future studies, whereas the link of OPG with lipid parameters in PD remains unclear. Further studies are needed to elucidate the relationship between OPG and lipid profile in PD and vascular diseases.

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Conflict of interest

The authors declare no conflict of interest.

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