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The effects of osteoprotegerin (*OPG*) gene polymorphism in patients with ischaemic heart disease on the morphology of coronary arteries and bone mineral density

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Abstract

Background: The incidence of coronary artery disease (CAD) and osteoporosis increases with age, especially in the elderly. Many studies have shown that vessel calcification is associated with low bone mineral density (BMD) and an increased risk of bone fractures. Experimental studies have shown that osteoprotegerin (*OPG*) gene knockout mice have aortic calcification and osteoporosis at the same time.

Aim: To assess the frequency of *OPG* gene polymorphisms in patients with CAD and to analyse the relationship between the severity of CAD and BMD.

Methods: The study group comprised 31 postmenopausal women (mean age 65.6, range 39–82 years) undergoing elective coronary angiography for CAD symptoms. The BMD was measured at the hip by dual X-ray absorptiometry (DEXA). Clinical data were collected using a questionnaire developed by the authors which addressed CAD risk factors, treatment, previous diagnosis of osteoporosis and the risk factors of osteoporosis. The control group consisted of 30 postmenopausal women attending the osteoporosis clinic without the history of CAD (mean age 70.5, range 56–84 years). Written informed consent was obtained from all the patients. Genotyping of two polymorphisms 209, 245 in the promoter region and 1181 in the exon of the *OPG* gene was performed in both groups.

Results: Coronary angiography in study group revealed normal coronary arteries in 35% (n = 11) of the women. The analysis of 209 C/T polymorphism showed no presence of TT homozygotes in either group. Also, no significant differences between the 209 C/T polymorphic variants, BMD and progression of atherosclerosis in coronary arteries were found. In both groups no CC homozygous variants for 245 A/C were revealed. However, a statistically significant relationship between 245 A/C polymorphism and BMD was shown. The AC carriers had osteoporosis more frequently (57%) than AA carriers (12%) of the *OPG* gene ($p = 0.0382$). There were no significant differences in the *OPG* gene 245 A/C polymorphisms and CAD progression. Homozygotes for CC 1181 were shown to have normal coronary arteries more frequently (60%) than heterozygotes for CG 1181 (29%; $p = 0.0023$). We failed to show significant differences between 1181 C/G polymorphism and BMD in both groups.

Conclusions: 1. This study revealed a significant association between homozygotes for AA 245 and normal BMD in study group. 2. The analysis of 209 C/T and 245 C/T C polymorphisms has shown no presence of homozygotes for TT 209 *OPG* or CC 245 *OPG* in both groups. 3. Carriers of the homozygous CC 1181 *OPG* gene were shown to have normal coronary arteries more frequently when compared to heterozygotes for CG or homozygotes for GG.

Key words: coronary artery disease, osteoprotegerin, polymorphism, bone mineral density

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INTRODUCTION

Progress in the diagnosis and treatment of diseases results in a considerable increase in life expectancy in humans. The demographic changes lead to a higher prevalence of diseases typically affecting the elderly, such as coronary artery disease (CAD) and osteoporosis. Many studies have shown that arterial wall calcification may be associated with reduced bone mineral density (BMD) and an increased incidence of fractures [1–6].

Bone metabolism involves alternate cycles of bone resorption and formation. The RANK/RANKL/*OPG* system is involved in the maturation of osteoclasts [7]. Osteoprotegerin (*OPG*) is synthesised by osteoblasts, cardiac myocytes, cells found in the lungs, kidneys, intestines, arterial and venous walls, endothelium, haemopoietic cells and cells of the immune system [7–10].

Calcification sites in the arterial walls are structurally similar to bone trabeculae, and arterial walls express many proteins involved in bone formation, e.g. *OPG*, osteocalcin, type I collagen, osteopontin. The potential association between *OPG* and calcification in arterial walls was documented in experimental studies, which showed increased osteoporosis and calcification of the aortic and renal artery media in *OPG* knockout mice [1]. Although numerous studies have shown a potential effect of specific *OPG* polymorphisms on BMD and the severity of CAD [2, 4, 11–15], they were inconclusive [9, 16–19].

Finding out whether there is any molecular link between *OPG* expression in the bone and the morphology of blood vessels, particularly coronary arteries, may contribute to determining whether predisposing factors for ischaemic heart disease (IHD) are associated with the risk of osteoporosis.

The aim of our study was to evaluate the frequency of polymorphic *OPG* gene variants in patients with CAD and to evaluate the association between their presence and the severity of CAD and BMD.

METHODS

Study group

The study group consisted of 31 postmenopausal women, defined as women who had their last menses at least 12 months before, (mean age 65.6 years; range 39–82 years) undergoing elective coronary arteriography (performed with the use of Integris Allura Monoplane 12″, Philips) for CAD symptoms.

Densitometry

BMD was measured at the hip by dual-energy X-ray absorptiometry (DEXA) using the Lunar device. Based on the WHO guidelines and due to the patients' age we utilised T-score obtained in the DEXA scan. The patients were divided into group A with T-score values of ≤ 2.5 SD (osteoporosis), group B with T-score values from –2.5 SD to –1.0 SD (osteopenia) and group C with T-score values from –1.0 SD to +1.0 SD (normal range). Coronary angiography and densitometry were performed at the Department of Intensive Coronary Care and Internal Medicine and at the Osteoporosis Clinic of Poznan University of Medical Sciences H. Święcicki Teaching Hospital.

The clinical data were collected using a questionnaire developed by us which addressed the presence of risk factors for CAD, course of the treatment, previous diagnosis of osteoporosis, if any, and the risk factors for osteoporosis (bone fractures, family history).

Control group

The control group consisted of 30 patients with osteoporosis without a history of CAD who were being managed at the Osteoporosis Clinic. The mean age was 70.5 years (age range 56–84 years). In terms of BMD values obtained by densitometry in patients with CAD, the control group was well-matched for this parameter. The patients enrolled in the study provided informed consent (Approval 1493/05 of the Bioethics Committee at Poznan University of Medical Sciences).

The molecular analysis of *OPG* was performed at the Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland. Based on a literature review we selected positions 209 and 245 within the *OPG* promoter and position 1181 within the exon for further analysis. DNA was isolated from peripheral blood leukocytes. The analysis of the PCR product was performed by minisequencing.

Statistical analysis

The results are presented as mean ± SD or numbers and percentages. Statistical analyses included the Shapiro-Wilk test and the Fisher-Snedecor test. The differences were compared using the t-Student test, the Levene test, the *post-hoc* Tukey test or the post-hoc Fisher test, the χ^2 test of independence and the Fisher-Freeman-Halton test. A p value < 0.05 was considered significant.

RESULTS

The mean BMD was 0.805 g/cm^2 (range: 0.571–1.028 g/cm^2). The T-scores obtained by hip densitometry are given in Table 1. Coronary angiography was normal in 35% of patients, one-vessel disease — in 20%, two-vessel disease — in 32% and three-vessel disease — in 13% of 31 studied females. The distributions of individual allelic variants of *OPG* polymorphisms in the study group and the control group are presented in Table 2. There were no significant differences in the study and control groups between the 209 C/T polymorphism and BMD values and the severity of coronary atherosclerotic changes. The results obtained for the 209 C/T *OPG* polymorphism are shown in Table 3. As regards the 245 A/C variants and BMD there were significant associations of reduced BMD in cases of AC *vs* AA carriers (57% *vs* 12%; p = 0.03824). No significant differences were demonstrated in the control gro-

Table 1. T-score values in the study group and the control group obtained in hip densitometry

Hip densitometry results	Study group (n = 31)	Control group (n = 30)
Osteoporosis		
T-score ≤ –2.5 SD	21%	33%
Mean BMD 0.614 (0.571–0.676) g/cm^2		
Osteopenia		
T-score from –2.5 SD to –1.0 SD	38%	34%
Mean BMD 0.764(0.688–0.833) g/cm^2		
Normal BMD		
T-score from –1.0 SD to +1.0 SD	41%	33%
Mean BMD 0.938(0.883–1.028) g/cm^2		

BMD — bone mineral density

up *vs* BMD. No differences in terms of the severity of coronary atherosclerotic changes were observed. The results are presented in Table 4. In the case of the 1181 C/G *OPG* polymorphism normal coronary arteries were demonstrated in the study group in 60% of the patients homozygous for CC *vs* 10% CG and 29% GG (p = 0.0023). No significant association with BMD in the study group or in the control group were shown. The results are presented in Table 5. The occurrence of bone fractures and the T-score values qualifying patients to the groups with osteoporosis, osteopenia or normal BMD, depending on the severity of coronary atherosclerotic changes, are presented in Table 6. A summary of the T-score values depending on the presence or absence of coronary atherosclerotic changes is given in Table 7.

DISCUSSION

We found no TT 209 *OPG* or CC 245 *OPG* homozygotes in the study group or the control group. It is difficult to draw definite conclusions from our study due to the unavailability of information on the population distribution of polymorphic variants of the *OPG* gene. Additional caution results from the small sample size. In other studies of the same fragment of the gene there were carriers of all the alleles or no carriers of one of the homozygous variants (CC, TT or GG, AA) [12, 15, 20].

In the study group, the analysis of the association of *OPG* polymorphisms with the severity of coronary atherosclerotic changes and BMD in patients with IHD showed significant relationship between 245 A/C and BMD and between

Table 2. Distribution of *OPG* polymorphism alleles in the study group and the control group

	209 C/T			245 A/C			1181 C/G		
	CC	CT	TT	AA	AC	CC	CC	CG	GG
Study group	73%	27%	0	76%	24%	0	48%	29%	24%
Control group	83%	17%	0	83%	17%	0	27%	60%.	13%

Table 3. Analysis of the association of the 209 C/T *OPG* polymorphism with bone mineral density (BMD) and the coronary artery status in the study group

Analysed polymorphism 209 C/T *OPG*	Hip densitometry			Coronary angiography	
	Osteoporosis	Osteopenia	Normal BMD	No coronary atherosclerosis	Coronary atherosclerosis
CC (73%)	12%	32%	56%	40%	60%
CT (27%)	50%	13%	38%	25%	75%
TT (0%)	0	0	0	0	0

Table 4. Analysis of the association of the 245 A/C *OPG* polymorphism with bone mineral density (BMD) and the coronary artery status in the study group

Analysed polymorphism 245 A/C *OPG*	Hip densitometry			Coronary angiography	
	Osteoporosis	Osteopenia	Normal BMD	No coronary atherosclerosis	Coronary atherosclerosis
AA (76%)	12%	32%	56%	40%	60%
AC (24%)	57% (p = 0.03824)	14%	29%	29%	72%
CC (0%)	0	0	0	0	0

Table 5. Analysis of the association of the 1181 C/G *OPG* polymorphism with bone mineral density (BMD) and the coronary artery status in the study group

Analysed polymorphism 1181 C/G *OPG*	Hip densitometry			Coronary angiography	
	Osteoporosis	Osteopenia	Normal BMD	No coronary atherosclerosis	Coronary atherosclerosis
CC (48%)	20%	27%	53%	60% (p = 0.0023)	40%
CG (29%)	0%	40%	60%	10%	90%
GG (24%)	57%	14%	29%	29%	71%

Table 6. Summary of T-score values and bone fractures in patients depending on the severity of coronary atherosclerosis

T-score	Severity of atherosclerotic changes in the coronary arteries			
	Three-vessel disease	Two-vessel disease	One-vessel disease	No changes
Osteoporosis				
T-score ≤ –2.5 SD	33%	22%	0	20%
Mean BMD: 0.614 (0.571–0.676) g/cm^2				
Osteopenia				
T-score from –2.5 SD to –1.0 SD	33%	44%	33%	40%
Mean BMD 0.764 (0.688–0.833) g/cm^2				
Normal BMD				
T-score from –1.0 SD to +1.0 SD	33%	44%	66%	40%
Mean BMD 0.938 (0.883–1.028) g/cm^2				
Bone fractures:	66%	55%	33%	20%
Group A patients	50%	20%	0	0
Group B patients	25%	60%	0	50%
Patients with normal BMD	25%	20%	100%	50%

BMD — bone mineral density

Table 7. Summary of T-score values depending on the presence or absence of atherosclerotic changes in the coronary arteries

	Osteoporosis T-score ≤ –2.5 SD Mean BMD 0.614 (0.571–0.676) g/cm^2	Osteopenia T-score from –2.5 SD to –1.0 SD Mean BMD 0.764 (0.688–0.833) g/cm^2	Normal BMD T-score from –1.0 SD to +1.0 SD Mean BMD 0.938 (0.883–1.028) g/cm^2
Atherosclerotic changes in the coronary arteries	19%	38%	43%
No atherosclerotic changes	20%	40%	40%

BMD — bone mineral density

1181 C/G and the severity of atherosclerotic changes. Among the AA carriers of the 245 A/C polymorphism normal BMD was found in 56% of the patients *versus* 29% in AC heterozygotes. These findings differ from those obtained in other studies. Authors who investigated the population of Danish and Japanese women showed a predominance of the GG 245 *OPG* variant in women with reduced BMD [15, 20]. This is in contrast to a Korean study, where none of the above associations was confirmed [21]. Population-related factors are most likely responsible for the above differences [12, 13]. This conclusion, however, requires confirmation in larger studies in a more representative group, especially since no such association has been confirmed in the control population.

It should be emphasised that normal coronary angiograms were found in a significantly larger proportion of carriers of the CC 1181 variant (60%) than in GG homozygotes (29%) or CG heterozygotes (10%). We also showed a trend towards higher BMD values in the CC 1181 *OPG* carriers, similarly to

studies of the Spanish and Korean female populations. On the other hand, in the population of Irish women lower BMD values compared to GG homozygotes were found [14, 21, 23]. It should be emphasised that the differences in the latter study were, however, non-significant [22].

When we examined the 209 C/T *OPG* polymorphism we found no significant differences with BMD and coronary angiograms. This is consistent with the findings of Arko et al. [13], who suggest, however, that despite the lack of statistical significance, the *OPG* polymorphism at position 209 may affect the genetic regulation of BMD.

The search for molecular links between the status of coronary arteries and the status of bones carried out in our study should be regarded as a preliminary attempt. Both osteoporosis and IHD are confirmed social risks to which the contemporary ageing societies of the civilised countries are exposed. Numerous studies documented the fundamental role of hereditary factors in the aetiology of both disease entities. Based on the available studies it seems plausible that there exist shared metabolic points that play a decisive role in the manifestation of the disease. The RANK/RANKL/*OPG* system plays an important role in many metabolic pathways and its involvement in the regulation of bone and endothelial metabolism is very likely. The absence of TT 209 *OPG* and CC 245 *OPG* carriers in the study group and the control group requires wider population analyses to confirm the potential significance of this finding. The predominance of carriers of specific *OPG* gene polymorphisms shown in the clinical observation is of potentially great significance. Our findings are encouraging however, the study sample was small. If our findings are confirmed, a specific variant of the *OPG* polymorphism carrier state may become a valuable molecular marker of the risk of CAD and osteoporosis.

CONCLUSIONS

1. We showed an association of AA 245 *OPG* homozygous variants with normal BMD.
2. We found no TT 209 *OPG* or CC 245 *OPG* homozygotes in the analysed group, which requires further studies to explain the potential significance of this finding.
3. Normal coronary angiograms were observed more frequently in carriers of the CC 1181 *OPG* homozygous variant.

Conflict of interest: *none declared*

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Badanie wpływu polimorfizmu genu osteoprotegeryny (*OPG*) u pacjentów z chorobą niedokrwienną serca na morfologiczny stan tętnic wieńcowych i mineralną gęstość kości

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Streszczenie

Wstęp: Częstość występowania choroby wieńcowej i osteoporozy wzrasta z wiekiem i dotyczy coraz większego odsetka starzejących się społeczeństw. Wiele badań wskazuje na prawdopodobną zależność między wapnieniem ścian tętnic wieńcowych a obniżoną mineralną gęstością kości (BMD).

Cel: Celem pracy była analiza częstości występowania polimorfizmów genu osteoprotegryny (*OPG*) oraz ocena związku między ich występowaniem a zaawansowaniem choroby wieńcowej i wartościami wskaźnika BMD.

Metody: Badaną grupę stanowiło 31 kobiet (średnia wieku 65,6 roku; zakres 39–82 lat), u których wykonano planowe koronarografie tętnic wieńcowych. Grupa kontrolna liczyła 30 pacjentek bez choroby wieńcowej (średnia wieku 70,5 roku; zakres 56–84 lat). Oznaczenia BMD wykonano w obrębie bliższego końca kości udowej. Pacjentki wyraziły zgodę na udział w badaniu. Do analizy wytypowano w obrębie promotora genu *OPG* pozycje: 209, 245 i w obrębie exonu pozycję 1181.

Wyniki: W grupie badanej i kontrolnej nie stwierdzono nosicielstwa homozygotyzmu TT 209 oraz CC 245. Nie zaobserwowano istotnych zależności między wariantami polimorficznymi 209 C/T a BMD i zaawansowaniem zmian miażdżycowych. Wykazano statystycznie istotną zależność pomiędzy wariantami polimorfizmu 245 A/C a BMD. Osteoporoza częściej występowała u heterozygot AC *v*. homozygot AA ($p = 0,03824$). Nie stwierdzono istotnych statystycznie różnic wobec wariantów polimorfizmu 245 A/C a zaawansowaniem zmian miażdżycowych. Istotnie częściej prawidłowy stan tętnic wieńcowych obserwowano u nosicielek homozygotyzmu CC 1181 C/G *v*. heterozygoty CG i homozygoty GG ($p = 0,0023$). Nie stwierdzono istotnych zależności wobec alleli 1181 C/G a BMD.

Wnioski: 1. Wykazano asocjację homozygotyzmu AA 245 *OPG* z występowaniem prawidłowej wartości BMD. 2. Prawidłowy stan tętnic wieńcowych częściej stwierdzano u homozygot CC 1181 *OPG*. 3. Nie zanotowano obecności homozygotyzmu TT 209 *OPG* i CC 245 *OPG*.

Słowa kluczowe: choroba wieńcowa, osteoprotegeryna, polimorfizm, gęstość mineralna kości

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