

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

Liliana Celczyńska-Bajew¹, Wanda Horst-Sikorska¹, Bartosz Bychowiec², Andrzej Wykrętownicz², Joanna Wesoły³, Michał Michalak⁴

¹Department of Family Medicine, Poznan University of Medical Sciences, Poznan, Poland

²Department of Intensive Coronary Care and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland

³Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poznan, Poland

⁴Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poznan, Poland

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

Kardiol Pol 2011; 69, 6: 573–578

Address for correspondence:

Liliana Celczyńska-Bajew, MD, PhD, Department of Family Medicine, Poznan University of Medical Sciences, ul. Przybyszewskiego 49, 60–355 Poznań, Poland, e-mail: licelbaj@ump.edu.pl

Received: 05.11.2010 Accepted: 21.02.2011

Copyright © Polskie Towarzystwo Kardiologiczne

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. Świącicki 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 \pm 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/cm² (range: 0.571–1.028 g/cm²). 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 group.

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 <i>OPG</i>	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	Osteopenia	Normal BMD
	T-score ≤ -2.5 SD Mean BMD 0.614 (0.571–0.676) g/cm ²	T-score from -2.5 SD to -1.0 SD Mean BMD 0.764 (0.688–0.833) g/cm ²	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

References

1. Collin-Osdoby P. Regulation of vascular calcification by osteoblast regulatory factors RANKL and osteoprotegerin. *Circulation Res*, 2004; 95: 1046–1057.
2. Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. *JAMA*, 2004; 4: 490–495.
3. Min H, Morony S, Sarosi I et al. Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. *J Experim Med*, 2000; 4: 463–474.
4. Pennisi P, Signorelli SS, Riccobene S et al. Low bone density and abnormal bone turnover in patients with atherosclerosis of peripheral vessels. *Osteoporos Int*, 2004; 15: 389–395.
5. Sennerby U, Melhus H, Gedeberg R et al. Cardiovascular diseases and risk of hip fracture. *JAMA*, 2009; 302: 1666–1673.
6. Schoppet M, Priessner K, Hofbauer L. RANK ligand and osteoprotegerin. Paracrine regulators of bone metabolism and vascular function. *Arterioscl Throm Vasc Biol*, 2002; 22: 549–553.
7. Lorenc R, Kryśkiewicz E, Szlak RANKL/RANK/*OPG* i jego znaczenie w fizjologii i patofizjologii kości. *Terapia*, 2006; 3: 58–63.
8. Schoppet M, Henser S, Ruppert V et al. Osteoprotegerin expression in dendritic cell increases with maturation and is NF-kappa-dependent. *J Cell Biochem*, 2007; 100: 1430–1439.
9. Schoppet M, Sattler A, Schaefer J et al. Increased osteoprotegerin serum levels in men with coronary artery disease. *J Clin Endocrinol Metab*, 2003; 88: 1024–1028.
10. Simonet WS, Lacey DL, Dunstan CR et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*, 1997; 89: 309–319.
11. Brandstrom H, Gerdhem P, Stiger F et al. Single nucleotide polymorphism In the human gene for osteoprotegerin are not related to bone mineral density or fracture in elderly women. *Calcif Tissue Int*, 2004; 74: 18–24.
12. Arko B, Prezejl J, Kocijancic A, Hudler P. Sequence variations In the osteoprotegerin gene promotor in patients with postmenopausal osteoporosis *J Clin Endocrinol Metab*, 2002; 87: 4080–4084.
13. Arko B, Prezejl J, Kocijancic A, Komel R. Association of the osteoprotegerin gene polymorphism with bone mineral density in postmenopausal women. *Maturitas*, 2005; 51: 270–279.
14. Choi J Y, Shin A, Park SK et al. Genetic polymorphism of *OPG*, *RANK*, and *ESR1* and bone mineral density in Korean postmenopausal women. *Calcif Tissue Int*, 2005; 77: 152–159.
15. Langdahl BL, Carstens M, Stenkjaer L, Eriksen EF. Polymorphism In the osteoprotegerin gene are associated with osteoporotic fractures. *J Bone Miner Res*, 2002; 17: 1245–1255.
16. Brandstrom H, Stiger F, Lind L. A single nucleotide polymorphism in the promoter region of the human gene for osteoprotegerin is related to vascular morphology and function. *Biochem Biophys Res Commun*, 2002; 293: 13–17.
17. Hofbauer LC, Schoppet M. Osteoprotegerin gene polymorphism and the risk of osteoporosis and vascular disease. *J Clin Endocrinol Metab*, 2002; 87: 4078–4079.
18. Rhee EJ, Oh KW, Jung CH et al. The relationship between four single nucleotide polymorphism In the promoter region of the osteoprotegerin gene and aortic calcification or coronary artery disease in Koreans. *Clin Endocrinol (Oxf)*, 2006; 64: 689–697.
19. Soufi M, Schoppet M, Sattler A et al. Osteoprotegerin gene polymorphism in men with coronary artery disease. *J Clin Endocrinol Metab*, 2004; 89: 3764–3768.
20. Yamada Y, Ando F, Niino N, Shimokata H. Association of polymorphisms of the osteoprotegerin gene with bone mineral density in Japanese women but not men. *Mol Genet Metab*, 2003; 80: 344–349.
21. Kim JG, Kim JH, Kim JU et al. Association between osteoprotegerin (*OPG*), receptor activator of nuclear factor-kappa B (*RANK*) and *RANKL* gene polymorphism and circulating *OPG*, soluble *RANKL* levels and bone mineral density in Korean postmenopausal women. *Menopause*, 2007; 14: 913–918.
22. Wynne F, Drummond F, o'Sullivan K et al. Investigation of the genetic influence of the *OPG*, *VDR* (*Fok1*), and *COL1A1* Sp 1 polymorphism on BMD in the Irish population. *Calcif Tissue Int*, 2002; 71: 26–35.
23. Garcia-Unzueta MT, Rancho JA, Zarrabeitia MT et al. Association of the 163 A/G and 1181 G/C osteoprotegerin polymorphism with bone mineral density. *Horm Metab Res*, 2008; 40: 219–224.

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

Liliana Celczyńska-Bajew¹, Wanda Horst-Sikorska¹, Bartosz Bychowicz², Andrzej Wykrętowicz², Joanna Wesoły³, Michał Michalak⁴

¹Katedra i Zakład Medycyny Rodzinnej, Uniwersytet Medyczny im. K. Marcinkowskiego, Poznań

²Klinika Intensywnej Terapii Kardiologicznej i Chorób Wewnętrznych, Uniwersytet Medyczny im. K. Marcinkowskiego, Poznań

³Instytut Biologii Molekularnej i Biotechnologii, Uniwersytet im. A. Mickiewicza, Poznań

⁴Katedra i Zakład Informatyki i Statystyki, Uniwersytet Medyczny im. K. Marcinkowskiego, Poznań

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 osteoprotegeryny (*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

Kardiol Pol 2011; 69, 6: 573–578

Adres do korespondencji:

dr n. med. Liliana Celczyńska-Bajew, Katedra i Zakład Medycyny Rodzinnej, Uniwersytet Medyczny im. K. Marcinkowskiego, ul. Przybyszewskiego 49, 60–355 Poznań, e-mail: licelbaj@ump.edu.pl

Praca wpłynęła: 05.11.2010 r. Zaakceptowana do druku: 21.02.2011 r.