P R A C E O R Y G I N A L N E

położnictwo

The possible role of adrenomedullin in the etiology of gestational hypertension and preeclampsia

Możliwy udział adrenomedulliny w etiologii nadciśnienia ciążowego oraz stanu przedrzucawkowego

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Summary

Introduction: *Nowadays the possible role of vasoactive peptide adrenomedullin (ADM) is considered in the etiology of preeclampsia (PE), where ADM is indicated to be a protective factor decreasing blood pressure. The aim of this study was to evaluate the role of –1984A>G ADM gene polymorphism and its connection with ADM plasma level in women with gestational hypertension (GH) and preeclampsia.*

Material and methods: *63 hypertensive (30 with GH and 33 with PE) and 94 healthy pregnant women were included into the study. The frequency of genotypes and alleles of –1984A>G ADM gene polymorphism was examined by PCR/RFLP method. ADM concentration was measured by ELISA method.*

Results: *In GH subgroup higher frequency of heterozygous AG genotype (16.67% vs. 8.50%, O.R.=2.68, p=ns) and G allele (11.67 vs. 4.30%, O.R.=2.97, p=0.043) was observed. In PE subgroup overrepresentation of heterozygous AG genotype (15.15% vs. 8.5%) and slightly higher frequency of G allele (p=ns) were noted. In AA genotype subgroup of hypertensive women in comparison to the AG+GG genotype group higher proteinuria value (212.1 vs. 90.9 mg/dl, p<0.0001), lower systolic (171.1 vs. 177.3 mmHg), as well as lower diastolic blood pressure level (107.1 vs. 111.4 mmHg) were noted. The highest ADM plasma level was observed in the group of women with PE (1.817 vs. 1.692 ng/ml, p=ns). Moreover, higher ADM plasma concentration in patients with AA genotypes in comparison to the carriers of AG and GG genotypes (1.844 vs. 1.402 ng/ml, p=ns) was noted.*

Conclusions: *Higher ADM plasma concentration in women with PE suggests possible correlation between ADM level and pathological changes in cardiovascular system during pregnancy. Overrepresentation of genotypes containing at least one mutated G allele of the -1984A>G ADM gene polymorphism in women with GH and PE suggests participation of this allele in pathogenesis of these conditions. Higher ADM concentration in carriers of homozygous AA genotype found in GH and PE groups indicates the possible important role of A allele in prevention of GH/PE appearance.*

Key words: **adrenomedullin** / **gestational hypertension** / **preeclampsia** / / **gene polymorphism** /

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Streszczenie

Wstęp: *Obecnie rozważa się możliwe znaczenie adrenomedulliny (ADM – adrenomedullin) w etiologii stanu przedrzucawkowego (PE – preeclampsia), gdzie ADM może być czynnikiem protekcyjnym obniżającym ciśnienie krwi. Celem pracy była ocena znaczenia polimorfizmu –1984A>G genu ADM oraz jego związku ze stężeniem ADM w osoczu krwi kobiet z nadciśnieniem ciążowym (GH – gestational hypertension) oraz ze stanem przedrzucawkowym.*

Materiał i metody: *Do badania włączono 63 ciężarne z nadciśnieniem (30 kobiet z GH i 33 z preeklampsją) oraz 94 zdrowe kobiety ciężarne. Częstość występowania genotypów i alleli polimorfizmu –1984A>G genu ADM oznaczano z zastosowaniem metody PCR/RFLP. Stężenie ADM mierzone było metodą ELISA.*

Wyniki: *W podgrupie GH obserwowano wyższą częstość występowania heterozygotycznego genotypu AG (16,67% vs. 8,50%, O.R.=2,68, p=ns) oraz allela G (11,67 vs. 4,30%, O.R.=2,97, p=0,043) w porównaniu do grupy kontrolnej. W podgrupie PE obserwowano przewagę heterozygotycznego genotypu AG (15,15% vs. 8,5% w grupie kontrolnej) oraz słabą przewagę częstości występowania allela G (p=ns). W całej grupie ciężarnych z nadciśnieniem u nosicielek genotypu AA w porównaniu do kobiet nosicielek genotypów AG+GG odnotowano wyższy poziom białkomoczu (212,1 vs. 90,9 mg/dl, p<0,0001), niższe ciśnienie skurczowe (171,1 vs. 177,3mmHg), jak również niższe ciśnienie rozkurczowe krwi (107,1 vs. 111,4 mmHg). Najwyższe stężenie ADM w osoczu obserwowano w grupie kobiet z preeklampsją (1,817 vs. 1,692ng/ml, p=ns). Co więcej obserwowano również wyższe stężenie ADM u pacjentek nosicielek genotypu AA w porównaniu do nosicielek genotypów AG+GG (1,844 vs. 1,402ng/ml, p=ns).*

Wnioski: *Wyższe stężenie ADM w osoczu kobiet z PE wskazuje na możliwy udział ADM w nieprawidłowych zmianach w układzie krążenia podczas ciąży. Przewaga częstości występowania genotypów zawierających co najmniej jeden zmutowany allel G polimorfizmu -1984A>G genu ADM u kobiet z GH oraz PE sugeruje udział tego allela w patogenezie powyższych powikłań. Wyższe stężenie ADM u nosicielek homozygotycznego genotypu AA obserwowany w grupie GH oraz PE wskazuje na ważną rolę allela A badanego polimorfizmu w prewencji występowania obydwu powikłań.*

Słowa kluczowe: **adrenomedullina** / **nadciśnienie ciążowe** / **stan przedrzucawkowy** / / **polimorfizm genetyczny** /

Introduction

In recent years much attention was devoted to vasodilatator peptide – adrenomedullin (ADM) and its possible role in the etiology of preeclampsia. ADM is a 52-amino acids chain, first isolated and described by Kitamura et al. in 1993 from pheochromocytoma cells [1]. The peptide is similar to calcitonine gene related peptide (CGRP) and belongs to the calcitonine, amylin and CGRP family of proteins [2]. The major expression of ADM gene was described in endothelium and smooth muscle cells [3, 4]. ADM is also regarded as biologically active substance found in lungs, brain, kidneys, spleen, small intestine, pancreas, thyroid gland and ovaries. Concentrations of ADM in plasma of healthy subjects ranged from 1 to 10 pmol/l (not correlated with gender or age) [5]. Two forms of ADM, mature (biologically active) and immature (main part of ADM present in plasma), were described. ADM is inactivated by metalloprotease mainly in lungs [6, 7, 8].

Many studies indicated that adrenomedullin is a long-acting vasodilatator with a strong hypotensive effect [9]. ADM acts mainly by specific receptors, increase of cAMP level in smooth muscle cells and affecting the regulation of nitric oxide synthesis. In the endothelial cells ADM inhibits endothelin-1 synthesis, and decreases migration and proliferation of vascular smooth muscle cells. It has been also proven that ADM affects cardiovascular system through the central nervous system path leading to increase of blood pressure and heart rate. Higher concentration of that protein is observed at patients with hypertension, heart failure, myocardial infarction, renal failure and primary hiperaldosteronizm, where ADM is probably involved in the response to an increased blood pressure [9, 10]. ADM is considered to be the protective factor decreasing blood pressure according to the concentration of that protein increased in the course of hypertensive diseases [9, 10, 11, 12].

In 1997 increased concentration of ADM in the plasma of pregnant women, amniotic fluid and umbilical blood was observed. It was established that ADM level increases significantly in the course of normal pregnancy and in the third trimester is much higher (3-5 fold) than in non-pregnant patients. ADM peptide concentration in plasma and in amniotic fluid increases during the first weeks of gestation, stays elevated and increases even further till the delivery [13]. It may prove a possible role of ADM in changes of cardiovascular system in the course of normal pregnancy. Several studies also focus on ADM role in hypertensive disorder development during pregnancy as an answer for pathological changes in cardiovascular system [14, 15]. The changes in blood pressure and vascular tension were connected with endothelium injury so much attention was paid to the gene encoding ADM. In humans ADM gene is located in the short arm of chromosome 11 (11p15.1-15.3, 4 exons, 3 introns, 2400 base pairs long). One of genetic polymorphisms described in the ADM gene is variable numbers of short tandem repeats (VNTR). These variants (11-, 13-, 14-, 19-nucleotide of cytosine-adenine repeats) are indicated to be connected with an increased risk of arterial hypertension and diabetes nephropathy [16]. In last years the attention was paid to the other single nucleotide variants: *+223A/C* polymorphism in intron 1, *+1100C/G* polymorphism in intron 3 and *-1984A>G* genetic variant in promoter of ADM gene [17].

The aim of this study was to evaluate the role of *–1984A>G* ADM gene polymorphism and its connection with ADM plasma level in women with gestational hypertension and preeclampsia.

Material and methods

Patients: The study involved two groups of women: 63 hypertensive and 94 healthy pregnant women. The patients were enrolled into research at Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences from January 2008 to January 2009. All women were informed about the study goal and gave consent to participation in the research. The Bioethical Committee of Poznań University of Medical Sciences approved the study (03/08).

The study group (63 hypertensive pregnant women, mean age 30,4±4,6 years, mean gestational age at delivery 34.9±3.9 week of pregnancy) was divided into two subgroups: 30 pregnant women with gestational hypertension (GH group, mean age 30.5±4.7 years, mean gestational age at delivery 36.9±3.2 week of pregnancy) and 33 preeclamptic pregnant women (PE group, mean age 30.4±4.7 years, mean gestational age at delivery 33.1±3.7 week of pregnancy). (Table I).

Gestational hypertension and preeclampsia were recognized according to ACOG criteria (blood pressure ≥ 140/90 mmHg, and in PE cases the presence of proteinuria ≥ 300 mg/24h). In all women blood pressure, laboratory tests (urea, uremic acid, blood urea nitrogen, total protein blood level, level of electrolytes: Na, K, Cl), proteinuria, the newborn status, the course of pregnancy and obstetrical history were analyzed. The women with diabetes mellitus, chronic hypertension, other cardio-vascular and renal diseases were excluded from the study.

The control group involved 94 healthy pregnant women (mean age 29.5±4.9 years, mean gestational age at delivery 39.1±1.8 week of pregnancy). The inclusion criteria were as follow: blood pressure < 140/90 mmHg, lack of proteinuria, absence of internal diseases and physiological course of pregnancy.

Genetic analysis: In the both study groups frequency of genotypes and alleles of *–1984A>G* ADM gene polymorphism and ADM plasma concentration were estimated. The frequency of genotypes and alleles of *–1984A>G* ADM gene polymorphism was examined by polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method. For amplification 2 starters were used: F-CAA GTG GAA GCT GGC GAC AAG, R-CGG ACC TGA ATT CCA TCT GAG G (Tib MolBiol, Poland). PCR reaction was performed in Dyad DNA Engine Thermocycler (MJ Research Inc., USA). The initial denaturation was performed in 95ºC for 3 min., then through 30 cycles the following conditions were used: denaturation in 94ºC for 30 s, annealing in 63ºC for 30 s, and elongation in 72ºC for 1 min. The final elongation was performed in 72ºC for 10 min. PCR products (377 bp) were hydrolysed with restriction enzyme *Taa*I (*Tsp*4CI) (Fermentas, Lithuania). Analysis of received digested fragments was conducted with in agarose gel by electrophoresis (2.5 % agarose gel, voltage 210 V, for 1h 40 min.). Products of the electrophoresis were stained with ethidium bromide and evaluated through the visualization in UV light, using the system of documentation (KS 4000i/ImagePC, USA, Syngen Biotech, USA). The *AA* genotype was identified at the presence of 377 bp long band, heterozygous *AG* genotype in presence of 377 bp, 241 bp, and 136 bp bands and homozygous *GG* genotype - 241 bp and 136 bp bands.

ADM concentration: Evaluation of ADM plasma concentration was executed with competitive enzyme-linked immunosorbent assay (c-ELISA, Phoenix Pharmaceuticals, USA).

Statistical analysis: For statistical analysis, after all data collection, the Statistical Package for Social Science v. 17.0 (SPSS Inc., Chicago, Illinois, USA) was used. Mean values for clinical and biochemical parameters were compared by *U*-Mann-Whitney test and one-way ANOVA. The *p* value lower than 0.05 was considered statistically significant. Frequencies of genotypes were compared by chi-square test. Expected genotype frequencies were calculated from allele frequencies applying Hardy-Weinberg equation.

Table I. Comparison of the study group and the control group.

		GH + PE n = 63	GH n = 30	PE n = 33	Control group n = 94
Age (years)	mean ± SD	30.4 ± 4.6	30.5 ± 4.7	30.4 ± 4.7	29.5 ± 4.9
	range	18 - 40	18 - 39	21 - 40	18 - 39
	median	31	30,5	31	30
Gestational age at delivery (week)	mean ± SD	34.9 ± 3.9	36.9 ± 3.2	33.1 ± 3.7	39.1 ± 1.8
	range	25 - 42	28 - 42	25 - 40	37-42
	median	36	37,5	33	39
Systolic blood pressure (mmHg)	mean ± SD	172.1 ± 16.8	164.8 ± 15.9	178.8 ± 14.9	113.1 ± 11.1
	range	140 - 200	140 - 200	150 - 200	90 – 135
	median	170	160	180	110
Diastolic blood pressure (mmHg)	mean ± SD	107.8 ± 11.6	105.2 ± 11.5	110.3 ± 11.3	71.4 ± 8.2
	range	90 - 150	90 - 150	90 - 140	60 – 85
	median	110	100	110	70

GH – gestational hypertension, PE – preeclampsia

Table II. Frequency of genotypes and alleles of -1984A>G ADM gene polymorphism in investigated groups.

ADM	Study group (PE + GH) n = 63		PE n = 33		GH n = 30		Control group n = 94	
	observed n (%)	expected (%)	observed n (%)	expected (%)	observed n (%)	expected (%)	observed n (%)	expected (%)
Genotypes								
AA	52 (82.54)	81.85	28 (84.85)	85.41	24 (80.00)	78.02	86 (91.50)	91.60
AG	10 (15.87)	17.24	5 (15.15)	14.01	5 (16.67)	20.62	8 (8.50)	8.20
GG	1 (1.59)	0.91	0 (0.00)	0.58	1 (3.33)	1.36	0 (0.00)	0.20
Total	63 (100.00)	100.00	33 (100.00)	100.00	30 (100.00)	100.00	94 (100.00)	100.00
Alleles								
A	114 (90.47)	-	61 (92.42)		53 (88.33)	-	180 (95.70)	-
G	12 (9.53)	-	5 (7.58)		7 (11.67)	-	8 (4.30)	-
Total	126 (100.00)	-	66 (100.00)	-	60 (100.00)	-	188 (100.00)	-

Results

In all hypertensive pregnant women the frequency of heterozygous *AG* genotype was much higher if compared to the control group (15.87 *vs.* 8.50%, O.R.=2.27, *p*=ns, respectively). Also the frequency of mutated *G* allele was higher in hypertensive women (9.53 *vs.* 4.30% in healthy subjects, O.R.=2.36, *p*=0.052, respectively). The mutated *GG* genotype was observed only in hypertensive pregnant patients (1.59 *vs.* 0.00%). In all analyzed cases the observed values were in accord with the Hardy-Weinberg equilibrium.

In the GH subgroup higher frequency of heterozygous *AG* genotype (16.67% *vs.* 8.50%, O.R.=2.68, *p*=ns) and statistically significant higher representation of *G* allele (11.67 *vs.* 4.30%, O.R.=2.97, *p*=0.043) than in controls was observed. In the PE subgroup overrepresentation of heterozygous *AG* genotype (15.15% *vs.* 8.50% in controls) and slightly higher frequency of *G* allele were noted. Observed differences in PE group were not statistically significant. (Table II).

For further analysis the whole study group (n=63) was divided into two groups depending on the genotype: the carries of homozygous *AA* genotype (n=52) and the subject with both *AG*+*GG* genotypes (n=11). Both groups were compared with regard to the clinical and biochemical parameters. In the *AA* genotype subgroup we noted higher proteinuria values (212.1±236.3 mg/dl *vs.* 90.9±147.2 mg/dl, $p<0.0001$), lower systolic blood pressure level (171.1±16.9 *vs.* 177.3±16.2 mmHg), as well as lower diastolic blood pressure level (107.1±12.1 *vs.* 111.4±8.4 mmHg) in comparison to the *AG*+*GG* genotype group. In newborns of the patients with the *AA* genotype higher Apgar score in the first minute of life (7.6±2.7 *vs.* 6.7±3.4, *p*=ns) was observed. (Table III).

The ADM plasma concentration was higher in the entire study group (GH+PE) than in the control group (1.768±0.921 ng/ml *vs.* 1.692±0.643 ng/ml). Observed differences were not statistically significant (*p*=ns). The highest adrenomedullin plasma level was observed in the group of women with preeclampsia (1.817±0.923 ng/ml *vs.* 1.692±0.643 ng/ml in the control group, *p*=ns). (Figure 1).

Table III. Comparison of clinical and biochemical parameters in the group of AA genotypes (n=52) and in the group of AG+GG genotype (n=11).

	AA n = 52	AG+ GG n = 11	p
maternal status			
age (years)	30.6 ± 4.3	29.7 ± 6.2	*p*=ns
gestational age (week)	34.9 ± 3.8	35.3 ± 4.6	*p*=ns
systolic BP (mmHg)	171.1 ± 16.9	177.3 ± 16.2	*p*=ns
diastolic BP (mmHg)	107.1 ± 12.1	111.4 ± 8.4	*p*=ns
urea (mg/dl)	27.2 ± 10.1	29.8 ± 16.6	*p*=ns
uremic acid (mg/dl)	6.3 ± 1.5	6.4 ± 1.6	*p*=ns
total protein (g%)	5.9 ± 0.7	5.9 ± 0.8	*p*=ns
BUN (mg/dl)	13.4 ± 4.7	13.7 ± 7.7	*p*=ns
Na (mEq/l)	136.6 ± 2.1	135.4 ± 1.4	*p*=ns
K (mEq/l)	4.3 ± 0.4	4.44 ± 0.4	*p*=ns
Cl (mEq/l)	105.0 ± 2.7	104.1 ± 1.9	*p*=ns
proteinuria(mg/dl)	212.1 ± 236.3	90.9 ± 147.2	**p<0.0001**
newborn status			
birth weight (g)	2378.0 ± 1096.4	2743.6 ± 1184.9	*p*=ns
Ap 1	7.6 ± 2.7	6.7 ± 3.4	*p*=ns
Ap 5	8.8 ± 1.7	8.7 ± 1.8	*p*=ns
pH venous	7.28 ± 0.09	7.27 ± 0.10	*p*=ns
pH arterial	7.23 ± 0.09	7.21 ± 0.12	*p*=ns
placental weight (g)	474.1 ± 208.2	530.0 ± 195.7	*p*=ns

BP – blood pressure, BUN – blood urea nitrogen

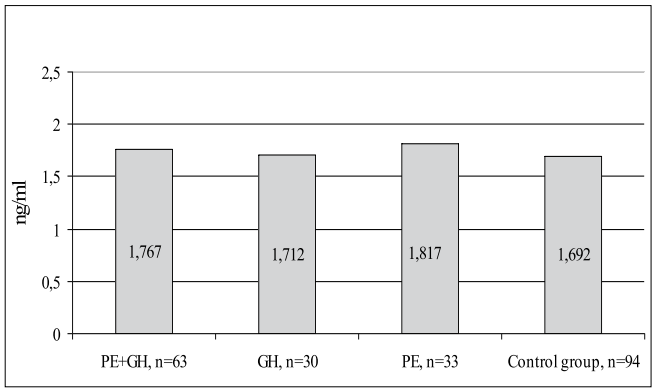

Figure 1. ADM plasma concentration in the study (GH, PE) and in the control groups.

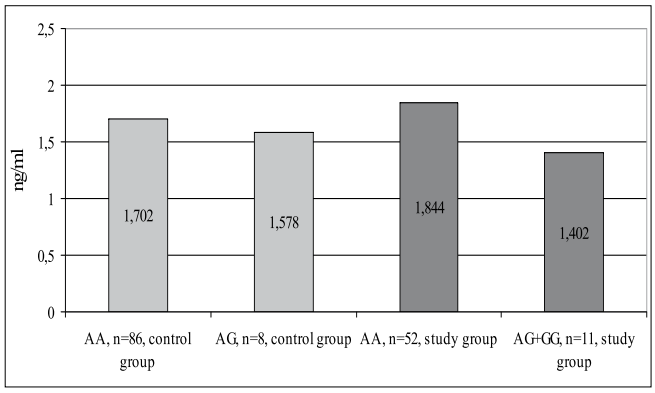

Figure 2. ADM plasma concentration in the control and the study groups in correlation with genotypes of patients.

Moreover higher ADM plasma concentration at patients with *AA* genotypes in comparison to the carriers of *AG* and *GG* genotypes (1.844 *vs.* 1.402 ng/ml, *p*=ns) was been noted. (Figure 2).

Discussion

ADM gene polymorphism

Nowadays much attention is directed to the role of genetic variants of ADM in the development of cardio-vascular diseases, as well as in pregnant women in pathomechanism of gestational hypertension and preeclampsia. One of the most often studied ADM gene polymorphisms is *CA*-repeat polymorphism. In 2001 Ishimitsu *et al.* analyzed the frequency of this polymorphism in general Japanese population of patients with hypertension. The authors observed positive correlation between this genetic variant and hypertension [18]. The other study concerned the frequency of ADM gene haplotypes of *CA*-repeat polymorphism in patients with hypertension and proteinuria. The study involved 205 Japanese patients without anti-hypertensive drugs, while the control group involved 210 healthy persons with negative family history of hypertension. The research did not show connection between two SNP polymorphisms (rs4399321, rs7944706), ADM gene *CA*-repeat polymorphism and hypertension but the *CA*-repeat polymorphism was indicated to be useful as a marker of proteinuria in course of hypertension [19].

Li *et al.* evaluated significance of *-1984A>G* ADM gene polymorphism in general Chinese population with hypertension. The study was executed with the participation of 427 persons with hypertension: 223 women, 113 men (47 were treated with anti-hypertensive drugs). The authors observed lower blood pressure in patients with mutated *G* allele in comparison to *AA* homozygotes ($p<0.03$). It was connected with lower systolic blood pressure in patients with at least one mutated *G* allele (*AG+GG*), although the difference between *AA* homozygotes and *AG+GG* group was not statistically significant ($p=0.22$). The analysis indicated connection between genotype, age and systolic blood pressure. In parents of the analyzed patients lower systolic blood pressure was observed in case of mutated *G* allele in comparison to *AA* homozygotes ($p<0.01$). In children of the studied patients systolic blood pressure was similar for both ADM genotypes [20].

To the best of our knowledge the presented study is the first to correlate *–1984A>G* ADM gene polymorphism and PE/GH appearance. Results obtained in this study are intriguing and important, although the indication of proper role of this genetic variant in etiology of PE/GH conditions brings difficulties because of the lack of comparison with other researches. In our study the high overrepresentation of heterozygous genotype *GA* in both study groups (GH 16.67% and PE 15.15% groups compared to the controls 8.5% with high O.R. in GH 2.68 and in PE 1.92, respectively) is particularly noteworthy. Moreover, statistically significant higher representation of *G* allele ($p=0.043$) in GH group and overrepresentation of this allele (p=ns) in PE was observed. These remarks may suggest participation of mutated *G* allele in the etiology of gestational hypertension and preeclampsia [21].

ADM plasma concentration

In our study we also evaluated the ADM plasma level in healthy pregnant and preeclamptic women. The elevated ADM plasma level in the GH/PE group (1.768 *vs.* 1.692 ng/ml in controls, p=ns) was found with the higher ADM level in preeclamptic women (1.817 mg/ml). Moreover, we divided the entire study group into subgroups: women carrying homozygous *AA* genotype and two other *AG+GG* genotypes. The most interesting finding was higher ADM concentration in the GH/PE women with *AA* genotype (1.844 *vs.* 1.402 ng/ml) as well as in healthy pregnant women with *AA* genotype (1.702 *vs.* 1.578 ng/ml). These observations suggest the significance of ADM concentration in etiology of GH/PE and possible important role of *AA* genotype in prevention of GH/PE appearance. These results may also explain possible role of adrenomedullin gene polymorphism in the changes of the peripheral ADM concentration [21].

Our results are similar to the findings of others. Di Iorio *et al.* observed increase of ADM level in plasma, amniotic fluid and umbilical blood during preeclampsia. The study involved 12 healthy non-pregnant women, 13 hypertensive non-pregnant women, 29 preeclamptic women and 30 healthy pregnant women. ADM plasma concentration was higher in hypertensive non-pregnant women in comparison to the healthy non-pregnant subject. In pregnant women ADM plasma level was higher than in the non-pregnant ones, although ADM concentration did not differ in preeclamptic women and the healthy pregnant women. In patients with preeclampsia ADM level in amniotic fluid and in umbilical blood was higher than the healthy pregnant women. The authors also observed that in preeclampsia the local ADM

production is increased. It has been suggested that the fetus may be involved in the elevated production of that protein [14]. Similar results were confirmed by many other studies [22, 23, 24].

An interesting study was conducted by Senna *et al.* The authors evaluated ADM plasma concentration from normal and preeclamptic pregnancies. That prospective study involved 90 women divided into subgroups: healthy pregnant women, pregnant women with PE, healthy non-pregnant women and non-pregnant women with hypertension. The highest ADM plasma concentration was observed in preeclamptic patients if compared to all other groups (differences statistically significant, $p<0.001$). ADM plasma level in non-pregnant hypertensive patients was statistically significantly higher in comparison to healthy non-pregnant patients and healthy pregnant women in the first trimester of gestation ($p<0.001$). Additionally, ADM blood concentration in healthy pregnant women was statistically significantly higher than in healthy non-pregnant ones ($p<0.001$). In healthy pregnant women in the third trimester of gestation that level was much higher than in non-pregnant women with hypertension. Moreover, ADM level in healthy pregnant patients increased in the course of gestation ($p<0.001$). The authors revealed positive correlation between ADM plasma level and the gestational age in healthy pregnant women, as well as between ADM concentration and systolic/diastolic blood pressure, proteinuria and clinical stage of preeclampsia ($p<0.05$, $p<0.05$, $p=0.01$, $p<0.01$, respectively) in preeclamptic patients [25].

Ulman-Włodarz analyzed 31 women with PE, 24 healthy pregnant women and 14 non-pregnant women. The results showed 3-4 fold times higher ADM blood concentration in healthy non-pregnant women and 7-8 fold times higher at preeclamptic patients. The study revealed that the increase of ADM level was in positive correlation with the increase of blood pressure values. It may be indirect proof of ADM involvement in the hypertension and preeclampsia etiology [26].

There are also some opposite findings to the above results. Several studies did not show any differences in ADM concentration between pregnant and non-pregnant women. The ADM level was also significantly lower in patients with PE than in healthy pregnant women. The authors suggested that ADM production is decreased in preeclampsia [27]. Jerat *et al.* investigated ADM influence on placental arteries in normal and preeclamptic pregnancies. That study also did not show significant difference between ADM plasma concentration in women with GH/PE and the control group of normotensive pregnant women [28].

The results of our study focus on the significance of ADM in pathomechanism of hypertensive disorders during pregnancy and suggest the essential function of this peptide in gestational hypertension and preeclampsia etiology. ADM may be strongly involved in regulation of blood pressure and vascular tension. It is also important to notice that ADM gene expression may be modulated by many different factors. At present it is known that ADM gene is expressed in answer to angiotensin II, carcinogenesis, inflammation, hypoxia, oxidative stress, activation of rennin-angiotensin and sympathetic nervous system [10]. Thus ADM is probably only an element of composed mechanism in GH/PE development. Furthermore, it is possible that other genetic variants, such as the polymorphism of the gene encoding its receptor, may be involved in the ADM related etiology of GH and PE [29].

Conclusions

To the best of our knowledge only a few researches focus on the role of adrenomedullin in the etiology of gestational hypertension and preeclampsia, so formulating clear conclusions from our study is extremely difficult. We are also aware of the fact that more comprehensive study is needed. However, although statistically significantly higher representation of *G* allele has been observed only in the GH subgroup, some important and very probable assumptions may be made:

1. Higher ADM plasma concentration in women with PE suggests a possible correlation between ADM level and pathological changes in the cardiovascular system during pregnancy, however this observation requires further studies,
2. Overrepresentation of genotypes containing at least one mutated *G* allele of the *-1984A>G* ADM gene polymorphism in women with GH and PE suggests participation of this allele in the pathogenesis of these conditions,
3. Higher ADM concentration in carriers of homozygous *AA* genotype found in GH and PE groups indicates the possible important role of *A* allele of investigated polymorphism in prevention of GH/PE appearance.

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