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Evaluation of the renin-angiotensin-aldosterone system in pregnancy complicated by preeclampsia with and without intrauterine growth retardation

Preeclampsia is a major complication affecting 5–10% pregnant women. Etiology of this disease is unclear and it remains a major cause of maternal and fetal morbidity and mortality (4). Preeclampsia is characterized by an increase in vascular tone, hypertension, enhanced platelet aggregation, proteinuria and reduced uteroplacental flow (3, 4, 13). The nature of the hypertension arising in pregnancy suggests that the abnormal increase in blood pressure is due to some humoral factor that mediates vasospasm in the vascular system. Changes observed in pregnancy complicated by preeclampsia may be related to disturbances in the maternal or uterine-placental renin-angiotensin-aldosterone system (RAAS) (4). Angiotensin II seems to mediate some pathophysiologic features including increases in vascular tone, hypertension, reduced uteroplacental flow, and enhanced thromboxane A2 production, which may result in disturbance of the prostacyclin / thromboxane ratio, that are associated with preeclampsia.

The purpose of this study was to evaluate maternal serum angiotensin I, angiotensin II, aldosterone level, plasma renin activity (PRA) and angiotensin converting enzyme activity (ACE) in pregnancies complicated by preeclampsia with and without intrauterine growth retardation (IUGR). The study was given the approval of the Board for Supervising Ethics in Medical Experiments at the Medical University of Lublin in Poland.

MATERIAL AND METHODS

The study was carried out on 26 patients with singleton pregnancy complicated by preeclampsia (group P) and 12 preeeclamptic patients with preeclampsia complicated by intrauterine growth retardation (group PI). Preeclampsia was defined according to ACOG classification. Intrauterine growth retardation (IUGR) was defined as the infant birth weight below the 10th centile for gestational age. The control group consisted of 18 healthy normotensive patients with singleton uncomplicated pregnancies, without any renal, heart and vascular diseases and normal laboratory tests (group K). All arterial blood pressure measurements in the control group were normal and did not exceed 135/85 mmHg. None of the patients from this group suffered proteinuria. All the studied patients were non-smokers.

Maternal angiotensin I, angiotensin II and aldosterone levels and plasma renin activity (PRA) and angiotensin-converting enzyme activity were evaluated. Angiotensin I levels and PRA were estimated by radioimmunoassay from Immunotech Laboratory. The plasma renin activity was calculated as an

amount of angiotensin I (Ang-I) generated in 1 mL per one hour in special condition, using the following equation: PRA=[(ng/mL)Ang-I (37°C) – (ng/mL) Ang-I (4°C)]x2/ incubation time (hour). The plasma angiotensin II level was estimated using double antibody radioimmunoassay from Bühlmann Laboratories AG in extracted plasma. For extraction of plasma samples extraction columns for angiotensin II from Bühlmann Laboratories AG were used. Aldosterone levels were estimated by radioimmunoassay from Immunotech Laboratory.

Serum angiotensin converting enzyme activity was estimated using spectrophotometric analysis. One unit of ACE activity was defined as the amount of enzyme required for releasing one micromole of hippuric acid per minute and per liter of serum at 37°C. ACE activity was estimated both in maternal and umbilical blood. Blood was also obtained for the estimation of serum urea, creatinine, uric acid, alanine and aspartate aminotransferases.

The obtained data were statistically analysed. Elements of descriptive statistics and one-side Student's t-test were employed. The level of statistical significance was established as $p < 0.05$.

RESULTS

There were no statistically significant differences in patient profiles between groups in gravidity, parity, maternal age and mode of delivery. Systolic and diastolic blood pressure and mean arterial blood pressure were higher in both study groups (groups P and PI) in comparison with healthy controls (group K). These differences were statistically significant ($p < 0.001$). The mean systolic blood pressure was 151.603 +/- 12.124 mmHg in group P versus 155.000 +/- 14.720 mmHg in group PI and versus 128.148 +/- 6.500 mmHg in the control group. The mean diastolic blood pressure was 98.974 +/- 7.104 mmHg in group P versus 100.556 +/- 14.413 mmHg in group PI and versus 67.407 +/- 5.666 mmHg in group K. The mean arterial blood pressure values were 116.517 +/- 8.096 mmHg in group P and 118.704 +/- 13.584 mmHg in group PI versus 87.654 +/- 3.197 mmHg in group K.

In preeclamptic patients (group P and PI) mean protein loss from 24-h urine collection was 1.61g +/- 1.89 g on average (range between 0.35g and 8.70g). None of the patients from control group suffered from proteinuria. Creatinine and urea levels were normal in all patients. The elevated level of uric acid was observed in both study groups in comparison with the control group. The mean level of uric acid was 4.88 +/- 1.39 mg/dL (ranged from 2.50 mg/dL to 7.60 mg/dL) in group P versus 5.34 +/ -1.39 mg/dL (ranged from 3.50 to 8.00 mg/dL) in group PI. In the control group urea, creatinine and uric acid were normal (urea below 35 mg/dL, creatinine below 0.9 mg/dL, uric acid below 3.6 mg/dL).

Lower birth weight of infants and lower gestation age at birth were observed in the group of patients with preeclampsia complicated by intrauterine growth retardation in comparison with the control and preeclamptic group without IUGR ($p < 0.004$). The mean birth weight of infants was 3281.54 +/- 818.97g in group P versus 1759.09 +/- 411.28 g in group PI and versus 2945.89 +/- 406.77g in group K. The mean gestational age at birth were 38.58 +/- 5.16 weeks in group P and 34.06 +/- 3.00 weeks in group PI versus 37.73 +/-2.76 weeks in group K.

Decreased Apgar score values at 1st and 3rd minute after birth in patients with preeclampsia complicated by intrauterine growth retardation were found to be lower in comparison with the control healthy patients and in comparison with the preeclamptic patients without IUGR. The mean values of Apgar score minute were 8.5+/- 1.27 points in group P versus 8.89 +/- 0.68 points in the control group at 1st minute and 9.12 +/- 0.82 points versus 9.22 +/- 0.43 points at 3rd minute respectively. The mean values Apgar score in group PI was 7.73 +/- 1.19 points at 1st minute and 8.46 +/- 1.64 points at 3rd minute respectively. It was statistically lower than in group K at 1st minute after birth ($p < 0.013$).

Decreased angiotensin I level in maternal blood in preeclamptic patients were found to be lower in comparison with the control group. The mean values were 0.227 +/- 0.311 ng/ml in group P versus

0.699 +/- 0.775 ng/ml in group K ($p < 0.001$). The mean value in group PI was 0.195 +/- 0.267 ng/ml. It was statistically lower than in the control group ($p < 0.015$). Decreased plasma renin activity in both groups of preeclamptic patients was observed in comparison with the control group. The mean values of PRA were 1.83 +/- 1.86 (ng/ml)/h in group P versus 3.43 +/- 1.77 (ng/ml)/h in the control group ($p < 0.004$). The mean value PRA in group PI was 1.34 +/- 1.08 (ng/ml)/h. It was statistically lower than in group K ($p < 0.002$). There were no statistically significant differences in angiotensin I level and plasma renin activity between groups of patients with pregnancy complicated by preeclampsia and patients with pregnancy complicated by preeclampsia with intrauterine growth retardation ($p = 0.227$ and $p = 0.551$ respectively).

Decreased plasma angiotensin II levels in maternal blood in preeclamptic patients were found to be lower in comparison with the control group. The mean values were 12.069 +/- 7.895 pg/mL in-group P and 12.917 +/- 6.388 pg/mL in group PI respectively versus 53.306 +/- 57.553 pg/mL in the control group. These differences were statistically significant ($p < 0.001$). There are no statistically significant differences in angiotensin II levels between two studied groups of patients with preeclampsia and with preeclampsia complicated by IUGR ($p = 0.4701$).

Maternal aldosterone levels were lower in both study groups in comparison with the healthy controls. These differences were statistically significant ($p < 0.04$). The mean aldosterone levels were 136.077 +/- 82.916 pg/ml in group P versus 122.583 +/- 108.190 pg/ml in group PI and versus 347.944 +/- 333.707 pg/ml in the control group respectively. There are no statistically significant differences in aldosterone levels between the two studied groups of preeclamptic patients with and without intrauterine growth retardation ($p = 0.3303$).

Elevated maternal blood serum angiotensin-converting enzyme activity in preeclamptic patients without IUGR was found to be higher in comparison with the healthy controls and with the preeclamptic patients with IUGR. These differences were statistically significant ($p < 0.004$ and $p < 0.04$ respectively).

Decreased ACE activity in maternal blood in preeclamptic patients with IUGR was found to be lower in comparison with the group P. This difference was statistically significant ($p < 0.04$). Maternal ACE activity in group PI was higher in comparison with the healthy controls, but this difference was not significant ($p = 0.205$). The mean maternal ACE activities were 48.869 +/- 33.676 units in group P versus 27.475 +/- 11.373 units in group PI and versus 23.703 +/- 15.084 in the control group respectively. Decreased umbilical ACE activity in preeclamptic patients with IUGR was found to be lower in comparison with the group P ($p < 0.01$) and with healthy controls ($p < 0.04$). Increased umbilical ACE activity was observed in preeclamptic patients without IUGR. The mean umbilical ACE activities were 45.918 +/- 40.464 units in group P versus 21.310 +/- 10.692 units in group PI and versus 31.805 +/- 14.122 units in the control group.

DISCUSSION

During normal pregnancy the plasma levels of angiotensin II increase, but the pressor sensitivity decreases, protecting the pregnant woman from the potentially hypertensive effects of this elevated hormone concentration (3). In our study decreased angiotensin I, angiotensin II and aldosterone levels and plasma renin activity in all preeclamptic patients were observed.

Similar results were presented by Langer et al. (9). They observed decreased levels of angiotensin I, angiotensin II and aldosterone in preeclamptic patients in comparison with healthy pregnant patients. Also similar results were observed by Hanssens et al. (6), but Gordon et al. (5) and Kingdom et al. (8) did not find any significant changes in angiotensin II levels in preeclamptic patients in comparison with healthy pregnant patients. Different results were presented by Symonds, Broughton-Pipkin and Craven (15). They observed increased angiotensin II levels in first pregnancy complicated by hypertension.

Renin-angiotensin-aldosterone system plays an important role at the beginning of pregnancy. Its role in decidualization processes was suggested (12). Furthermore, inhibition of ACE activity disturbs, whereas angiotensin II infusion helps in normal decidualization. In humans decidualization process starts round the spiral artery and remodelling in spiral artery, which is characteristic of normal pregnancy, may reflect early stages of this process (2). ACE expression in endothelial cells of the spiral artery and in stromal cells around the vessels was observed (12). These data suggest significance of angiotensin-converting enzyme activity and renin-angiotensin-aldosterone system in the normal course of human pregnancy.

Magriples et al. (11) observed elevated serum ACE in pregnant patients with chronic hypertension and vaculopathy, but different results were presented in preeclampsia. There were no differences in women with or without preeclampsia in their studies.

In our study different results of the ACE activity were obtained in both study groups of preeclamptic patients with and without intrauterine growth retardation (groups P and PI). Increased angiotensin-converting enzyme activities in maternal and umbilical blood in preeclamptic patients without IUGR, but lower maternal and umbilical ACE activities in preeclampsia complicated by IUGR were observed. The obtained results of ACE activity in maternal and umbilical blood may additionally suggest increased renin-angiotensin system activity in preeclamptic patients, whereas decreased ACE activity in preeclamptic patients with IUGR may be considered as a consequence of prolonged enhanced renin- - angiotensin -aldosterone system activity or as an attempt to compensate occuring in preeclampsia disorders.

Our results may be the evidence of the possible endothelial cell dysfunction or damage or they may result from this dysfunction, because ACE is attached to the endothelial surface membrane by an anchor peptide and it can be broken down to be released into the blood circulation as a soluble enzyme. Angiotensin-converting enzyme is a marker for the endothelial cell damage (11). It is suggested that a disturbance in the regulation of ACE activity may be one of the factors responsible for the development of preeclampsia (4, 7). Furthermore, our results suggest that the decreased ACE activity in preeclamptic patients with IUGR is similar to that with the intrauterine growth retardation observed in pregnant women who used ACE inhibitors in the treatment of hypertension.

These drugs are contraindicated in pregnant women because of their adverse side-effects and bad pregnancy outcomes. ACE inhibitors result in the uterine-placental flow reduction, intrauterine growth retardation, high fetal morbidity and mortality (14). Fetopathy resulting from the use of these drugs is characterized by renal dysplasia, anuria, IUGR, oligohydramnios, pulmonary hypoplasia, cranial vault defective development and acrocontracture. These changes were observed in patients who used ACE inhibitors in the second and third trimester of pregnancy (14).

Angiotensin II stimulates c-fos, c-myc and c-jun protooncogens, intensifies growth factors and neutrophil chemotactic factor (10), while ACE inhibitors decrease bradykinin degradation and favour nitric oxide (NO) and prostacyclin (PGI2) release from endothelial cells. And maybe because of this decreased ACE activity in preeclamptic patients with IUGR expresses the attempt at compensation mechanism or balance between vasoconstrictors and vasodilatators ratio is observed.

Probably the interaction between maternal and uterine-placental renin-angiotensin-aldosterone system and other regulatory systems may modulate the activity of biological factors activity and tissue function. Whereas disturbances in these interactions might result in dysfunction and disease these changes may play the role in pathomechanisms observed in pregnancy complicated by preeclampsia and preeclampsia complicated by IUGR.

Our results and other reports point out the significant role of RAAS activity in the pregnancy complicated by preeclampsia. The knowledge of the role of renin-angiotensin-aldosterone system in pathogenesis of preeclampsia might contribute to the development of the new method of prophylaxis and treatment of preeclampsia and to improvement of pregnancy outcomes in these patients.

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SUMMARY

The purpose of this study was to evaluate maternal serum angiotensin I, angiotensin II and aldosterone level, plasma renin activity, angiotensin converting enzyme activity in pregnancies complicated by preeclampsia with and without intrauterine growth retardation. The study was carried out on 26 patients with singleton pregnancy complicated by preeclampsia (group P) and 12 preeeclamptic patients with preeclampsia complicated by intrauterine growth retardation (group PI). The control group consisted of 18 healthy normotensive patients with singleton uncomplicated pregnancies, without any renal, heart and vascular diseases and normal laboratory tests (group K). Decreased angiotensin I level and decreased plasma renin activity in maternal blood in preeclamptic patients were found to be lower in comparison with the control group. Decreased plasma angiotensin II levels in maternal blood

in preeclamptic patients were found to be lower in comparison with the control group. Maternal aldosterone levels were lower in both study groups in comparison with the healthy controls. Elevated serum angiotensin-converting enzyme activity in preeclamptic patients without IUGR was found to be higher in comparison with the healthy controls and with the preeclamptic patients with IUGR. Our results and other reports point out the significant role of RAAS activity in the pregnancy complicated by preeclampsia.

Ocena układu renina-angiotensyna-aldosteron w ciąży powikłanej preeklampsją i w ciąży powikłanej wewnątrzmacicznym zahamowaniem wzrastania płodu w przebiegu preeklampsji

Celem badań była ocena matczynego poziomu angiotensyny I, angiotensyny II, aldosteronu, aktywności reninowej osocza oraz aktywności konwertazy angiotensynowej we krwi matczynej i pępowinowej w ciąży powikłanej stanem przedrzucawkowym i stanem przedrzucawkowym z zahamowaniem wewnątrzmacicznego wzrastania płodu. Badaniami objęto 26 pacjentek z ciążą pojedynczą powikłaną stanem przedrzucawkowym (grupa P), 12 pacjentek ze stanem przedrzucawkowym powikłanym zahamowaniem wewnątrzmacicznego wzrastania płodu (grupa PI). Badania wykonano w odniesieniu do grupy kontrolnej 18 zdrowych kobiet ciężarnych z prawidłowym ciśnieniem tętniczym krwi i prawidłowymi wynikami badań laboratoryjnych, bez schorzeń sercowo-naczyniowych i nerkowych (grupa K). Zaobserwowano obniżony poziom angiotensyny I i angiotensyny II oraz aktywności reninowej osocza we krwi matczynej u pacjentek z ciążą powikłaną stanem przedrzucawkowym w odniesieniu do grupy kontrolnej. Poziomy aldosteronu były niższe w obu badanych grupach w porównaniu z wartością obserwowaną wśród zdrowych ciężarnych. Odnotowano podwyższoną aktywność konwertazy angiotensynowej w grupie pacjentek z ciążą powikłaną preeklampsją bez IUGR w porównaniu z ciężarnymi z grupy kontrolnej oraz w odniesieniu do ciężarnych z preeklampsją powikłaną wewnątrzmacicznym zahamowaniem wzrastania płodu. Wyniki naszych badań oraz doniesienia z literatury wskazują na istotną rolę aktywności układu RAA u kobiet z ciążą powikłaną stanem przedrzucawkowym.