Aim

The aim of this study was to evaluate whether maternal circulating adrenomedullin (AM) values in patients with preeclampsia are different from those in normotensive pregnant women at different gestational ages.

Subjects and Methods

In a prospective clinical study, 90 women aged 17 to 40 years old, were divided into 4 main groups: group I (45 women): Normotensive pregnant women at first trimester (15 women), second trimester (15 women), and third trimester (15 women) of pregnancies. Group II (15 women): Pregnant women with preeclampsia at 25 to 38 weeks of gestation. Group III (15 women): Normotensive healthy nonpregnant women. Group IV (15 women): Hypertensive nonpregnant women. The plasma AM concentration was measured in all women by using enzyme immunoassay kits.

Results

Plasma AM levels in pregnant women with normal blood pressure at different gestational ages (first, second, and third trimesters) were statistically significantly higher than those detected in nonpregnant normotensive women and significantly increased with increasing gestational age ($P < .001$). Moreover, there was significant positive correlation between plasma AM levels and increasing gestational age ($r = 0.915$, $P < .001$). Preeclamptic patients had the highest mean plasma AM levels compared with all other groups, which is statistically significant ($P < .001$) and there was a significant positive correlation between plasma AM levels and systolic blood pressure, diastolic blood pressure, severity of preeclampsia, and proteinuria in pregnant patients with preeclampsia.

Conclusion

Maternal plasma AM concentration increases throughout pregnancy and increases as gestational age progresses. AM production starts very early in gestation, suggesting that it may have an important role in human reproduction, from implantation to delivery. Maternal plasma AM level in preeclampsia appears to be higher than that in normal pregnancy.

Introduction

Adrenomedullin (AM) is a 52-amino acid peptide originally isolated in 1993 by Kitamura and colleagues from a human pheochromocytoma. It is a multifunctional peptide produced by many cells and tissue systems. AM was observed to stimulate adenylyl cyclase activity in a platelet bioassay and shares a slight homology with the calcitonin gene-related peptide, a potent hypotensive peptide,[1] reviewed by Hinson and colleagues.[2] AM has been proposed early as an important hormone in circulation control and maintenance of vascular tone because it regulates endothelial permeability[3] and contributes to the differentiation of bone marrow-derived mononuclear cells into endothelial progenitor cells.[4] AM gene is expressed in most

organs including the reproductive system; however, the main source of plasma AM is considered to be vascular endothelial cells and vascular smooth muscle cells.[2,5] The plasma level of AM is elevated in various diseases that are often associated with pathologic processes of the vasculature. Furthermore, culture studies on vascular smooth muscle cells or endothelial cells have shown that oxidative stress increases AM production. Collectively, a body of evidence suggests that AM participates in the mechanism against progression of vascular damage and remodeling, thereby alleviating the ischemia of tissues and organs.[6]

In normal pregnancy, fetoplacental perfusion is regulated by the local and systemic effects of the different vasoactive compounds produced by the endothelium and consequently the systemic blood pressure, so maintenance of adequate blood supply to the fetus is ensured. In preeclampsia, abnormal function of the endothelium could contribute to the changes in the peripheral resistance and fetoplacental perfusion.[7–9] In the first trimester, improper implantation of the placenta occurs, which predisposes to vasospasm and ischemia of the spiral arteries. By production of vasoactive compounds such as prostaglandins, nitric oxide, and AM, the placenta interacts with the maternal physiology during preeclampsia.[10–12] The roles of AM in the pathogenesis of preeclampsia and in the regulation of fetoplacental perfusion are complex.[13] The aim of this study was to evaluate whether maternal circulating AM values in patients with preeclampsia are different from those in normotensive pregnant women at different gestational ages.

Subjects and Methods

This prospective clinical study was carried out on 90 women who were 17 to 40 years old, attending the antenatal clinic in Banha University Hospitals. Women were divided into 4 main groups according to a statistical basis: Group I (45 women): Normotensive pregnant women at first trimester (15 women), second trimester (15 women), and third trimester (15 women) of pregnancies. Gestational age was calculated from the date of the last normal menstrual cycle and confirmed by conventional ultrasound. Group II (15 women): Pregnant women with preeclampsia at 25 to 38 weeks of gestation with an elevated blood pressure (>/= 140/90 mm Hg) plus proteinuria 2+ or more in a dipstick test. Women were excluded from the study if any of the following were present: Women were in labor or experienced uterine contractions, evidence of intrapartum infection, evidence of other pregnancy complications (multiple gestation, intrauterine growth restriction, diabetes or premature rupture of membranes), prepregnancy medical diseases (chronic hypertension, renal disease, liver disease, or collagen vascular disease), and maternal smoking. Group III (15 women): Normotensive healthy nonpregnant women. Group IV (15 women): Hypertensive nonpregnant women. No woman was known to have a history of liver, renal, or metabolic disease.

All women in the present study were subjected to full history-taking; complete general and abdominal examination; a mid-stream, clean-catch urine sample was examined for proteins; and transabdominal ultrasound for pregnant women by using a New Sonic HQ-LC 2000, plus Doppler with a 3.5-MHz curvilinear probe. Informed consent from all women about the aim of the work and the tests to be done on a protocol approved by the University of Banha, Clinical Pathology Department and the

local Ethics Committee. The plasma AM concentration was measured for all women by using enzyme immunoassay kits (EIA-3418, DRG International). Blood samples, anticoagulated with EDTA and aprotinin (0.6 TIU / mL of blood), were kept on ice and gently rocked several times to inhibit the activity of proteinases. Centrifugation of the blood was done at 1600 × g for 15 minutes at 4 C and the plasma kept at −70 C until assayed.

Principle of Enzyme Immunoassay

The immunoplate in this kit is precoated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample. The biotinylated peptide is able to interact with streptavidin-horseradish peroxidase (SA-HRP), which catalyzes the substrate solution composed of 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxidase to produce a blue solution. The enzyme-substrate reaction is stopped by hydrogen chloride (HCI) and the solution turns yellow. The intensity of the yellow is directly proportional to the amount of biotinylated peptide-SA-HRP complex but inversely proportional to the amount of the peptide in standard solutions or samples. The intra- and interassay coefficient of variation of the results obtained using this kit did not exceed 5% and 14%, respectively.[14] Statistical package for social sciences version 10.0 (SPSS version 10.0) was used for data analysis. The results were analyzed by the suitable statistical methods that include: mean ± SD, student's t-test (t), correlation coefficient “r,” and $P < .05$ is considered significant.[15]

Results

The results of the present study showed that all groups were matched regarding the mean age and mean parity except in the hypertensive nonpregnant group who had significantly higher age and parity compared with normotensive nonpregnant women, pregnant women with normal blood pressure at different gestational ages and preeclampsia (37.5 ± 1.7, 24.4 ± 2.9, 25.1 ± 5.3, 25.8 ± 4.1, 25.0 ± 4.2, and 24.7 ± 4.8, respectively for age, < .001 and 4.66 ± 1.23, 2.0 ± 0.65, 1.26 ± 1.53, 1.33 ± 1.13, 1.27 ± 1.12, and 0.8 ± 1.14, respectively for parity, $P < .001$).

Regarding the mean systolic and diastolic blood pressure among the studied groups, preeclamptic patients had the highest mean systolic and diastolic blood pressure (162.7 ± 13.47 and 104.66 ± 8.12, respectively), which were statistically significant compared with normotensive nonpregnant women (114.0 ± 7.36 and 70.66 ± 7.98, respectively) and pregnant women with normal blood pressure at different gestational ages (111.0 ± 8.70, 70.66 ± 7.28 for first trimester; 110.0 ± 9.81, 70.67 ± 7.76 for second trimester; and 108.0 ± 8.54, 70.33 ± 6.39 for third trimester) and nonpregnant women with hypertension (153.6 ± 8.33, 95.0 ± 5.0, $P < .001$. Significant proteinuria was observed only in patients with preeclampsia (1.47 g/dL ±1.125, $P< .001$).