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Direct laboratory evidence that pregnancy-induced hypertension might be associated with increased catecholamines and decreased renalase concentrations in the umbilical cord and mother's blood

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Abstract

Background: Renalase (RNL) is a controversial enzyme as to whether it oxidizes catecholamines (CAs) (as is generally accepted) in the blood or not. CAs (dopamine [DPMN], epinephrine [EPI] and norepinephrine [NEPI]) are associated with hypertension, including pregnancy-induced hypertension, which occurs in 8–10% of all pregnancies. Therefore, the aim of the study was to compare CAs and renalase concentration in (i) normotensive controls (C), (ii) patients with preeclampsia (PE) and (iii) patients with severe preeclampsia (SPE), which is one of the well-known symptoms of hypertension.

Methods: This case-control study involved 90 women divided into three groups – 30 C, 30 PE and 30 SPE – whose age and body mass indexes (BMIs) were similar. A total of 270 blood samples (90 maternal samples, 90 umbilical cord artery samples and 90 umbilical cord vein samples) were obtained. CAs and RNL concentrations of the biological samples were measured by enzyme-linked immunosorbent assay (ELISA).

Results: Comparing the amounts of CAs, RNL and systolic blood pressure (SBP)/diastolic blood pressure (DBP) between healthy control pregnant women and pregnant women with PE and SPE (SBP/DBP was 120/80 mm Hg for

C, above 140/90 mm Hg for PE and above 160/110 mm Hg for SPE), the levels of CAs were significantly increased whereas RNL was reduced. The correlation between SBP/DBP and the amount of RNL in pregnant women with PE and SPE was negative.

Conclusions: These novel results are evidence that hypertension seen in PE and SPE is directly related to increased levels of CAs and reduced RNL concentrations. The use of RNL preparations may be preferred in future to prevent maternal and perinatal morbidity and mortality due to pregnancy-induced hypertension.

Keywords: catecholamines; hypertension; pregnancy; renalase.

Introduction

Preeclampsia (PE), the hypertensive disorder of pregnancy, remains one of the most important causes of maternal and perinatal morbidity and mortality. Pregnancy-induced hypertension affects 5–11% of all pregnancies worldwide [1]. Its frequency is increasing especially in the developed countries and is responsible for the loss of the lives of 76,000 mothers and 500,000 infants worldwide every year. PE, with its current definition, is the presence of proteinuria (>300 mg/24 h) and systolic blood pressure (SBP) and diastolic blood pressure (DBP) of >140/90 mm Hg from at least two measurements with a minimum of 4 h apart after 20 weeks of gestation, for a woman who was normotensive and did not have proteinuria [2–4]. Severe preeclampsia (SPE), besides high blood pressure (BP) and proteinuria, is characterized by the breakdown of erythrocytes, impaired liver and kidney function, a low blood platelet count, swelling, visual disturbance and shortness of breath due to fluid in the lungs.

To date, many agents, such as endothelial cells, spiral arteries [5], antioxidants [6], peptide-structured hormones [7, 8], an anti-angiogenic state [9], systemic dysfunction [10], inflammatory processes [11] and

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placenta insufficiency [12], sympathetic vasoconstrictor activity and increased vascular resistance have been implicated in the pathogenesis of PE, but the underlying mechanism(s) are not fully understood. The condition remains on the agenda as a multisystemic, progressive disease [13].

The kidneys need to function properly to maintain a normal pregnancy. There may be a number of disturbances in renal function both in normal pregnancy and pregnancies with PE [14, 15]. Endothelial dysfunction in preeclamptic pregnancies and glomerular endotheliosis in the kidneys are common pathologies [16]. Therefore, along with impaired glomerular dynamics and barrier functions, this may directly affect the synthesis and release of the renalase (RNL) enzyme, as it is mainly expressed by the kidneys [17–19]. RNL is synthesized in small amounts in the peripheral nerves, hypothalamus, pituitary gland, the heart, skeletal muscle and small intestines [17, 20]. Its expression is enhanced in the gonads during pregnancy [21]. This enzyme is composed of 342 amino acids with a predicted mass of 37.8 kDa. Its function is to oxidize catecholamines (CAs) (dopamine [DPMN], epinephrine [EPI] and norepinephrine [NEPI]) in the blood [19], and to modulate the BP by regulating the myocardial contractility, heart rate and the tonus of resistance vessels [22–24]. However, experimental evidence demonstrates that RNL does not catalyze the oxidation of neurotransmitter CAs [25]. High-performance liquid chromatography results also show that there is no evidence of the consumption of epinephrine by RNL [25]. There seems to be a relation between the high levels of RNL and high BP, regardless of age and gender [26, 27]. One study only measured the RNL concentrations (CAs not measured) in preeclamptic patients, reporting that the RNL concentration is associated with increased BP [28]. Another study also indicated that RNL gene polymorphism is associated with increased BP in PE [29]. RNL levels are regulated by renal function. Renal perfusion and plasma CAs regulate the activity, secretion and synthesis of RNL; and the isolated perfused rat kidney model catecholamine infusions stimulate RNL secretion into the renal vein. The concentration of CAs is increased in PE and eclampsia [30, 31]. Arterial EPI is increased in PE [32]. This increased plasma EPI level has been correlated with increased BP in the development of PE [33].

Hence, from the given information, it appears that no study has yet investigated CAs and RNL levels together insofar as the case of pregnancy-induced hypertension is concerned. Therefore, we set out to determine whether a relationship exists between the amounts of CAs and the amounts of RNL enzymes and CAs in the venous blood

samples of mothers and in the umbilical cord (venous and arterial) blood samples of newborns.

Materials and methods

This retrospective work was approved by the Local Ethics Committee (dated 13/12/2017) of the Faculty of Medicine of Kafkas University, Kars. We have complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects. This case-control study involved 30 normotensive controls, 30 women with PE (without severe features) and 30 women with SPE. All the subjects were admitted to the Obstetrics and Gynecology Clinic, Firat University Hospital, all of whom were Caucasians of Turkish origin. The diagnosis of PE was determined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria [34]. The diagnosis of PE (without severe features) was based on the following criteria [<https://www.acog.org/Clinical-Guidance-and-Publications/Task-Force-and-Work-Group-Reports/Hypertension-in-Pregnancy>]: SBP and DBP of above 140/90 mm Hg after at least two measurements taken at a minimum of 4 h apart, proteinuria of >300 mg per 24 h, and creatine of ≥ 1 + protein (spot urine) [2, 3, 35]. SPE (with severe features) was defined as showing one or more of the following features: SBP ≥ 160 and DBP ≥ 110 mm Hg on two occasions of 4 h or more apart in a pregnant woman on bed-rest and having proteinuria with an excretion ≥ 3 g in the 24-h urine sample, thrombocytopenia $<100,000/\text{mm}^3$, liver function impairment (aspartate aminotransferase [AST]/alanine aminotransferase [ALT] >2 times normal), serum creatinine ≥ 1.1 mg/dL or increase in serum creatinine >2 times the normal values, severe persisting right upper quadrant pain or epigastric pain not responding to medical treatment, fetal growth restriction, headache, visual disturbances and deterioration in the mental condition (a single systemic finding being sufficient). The control group was recruited from the same centers as the normotensive volunteer pregnant women with at least one pregnancy, with their pregnancies continuing on a normal course where no medical problems could be detected medically through routine tests and without a history of PE, hypertension and/or kidney diseases and/or cardiovascular diseases [<https://www.acog.org/Clinical-Guidance-and-Publications/Task-Force-and-Work-Group-Reports/Hypertension-in-Pregnancy>]. Women with hemolysis, elevated liver enzyme levels, low platelet levels (HELLP) syndrome, diabetes mellitus, prior vascular or inflammatory disease and a history of

smoking; women imbibing alcohol and past and present drug abuse; and women who had elective cesareans and no case of labor problems or premature rupture of membranes were all excluded from the study. All participants who had a vaginal delivery were included in this study.

Each group comprised volunteer participants of similar age, body mass index (BMI) and parities. Multiple pregnancies were not included in the study. SBP and DBP were measured 3 consecutive times using a sphygmomanometer after the patients had rested for at least 15 min; the mean of the lowest two readings were recorded in the patients' files. SBP and DBP were obtained and used from the participants' files, and the means of the values obtained 3 days before the initiation of the anti-hypertensive treatment and treatments of magnesium sulfate were recorded. The mean gestational age of the pregnancies in the control, PE and SPE groups was 37.7 ± 1.4 , 36.3 ± 1.6 and 35.4 ± 1.2 weeks, respectively.

Blood samples were drawn from the brachial vein and umbilical cords (artery and vein) at the lithotomy position from the mother at delivery. The samples were centrifuged for 5 min at 4000 rpm and stored at -80°C in the biological fluid archive until analyses. During analysis, we also checked whether the CAs and the RNL concentrations had decreased or not. Briefly, the DPMN and EPI, and the NEPI and RNL levels in eight older control samples stored at -80°C in the biological fluid archive and eight fresh blood samples taken from healthy women giving birth were analyzed by enzyme-linked immunosorbent assay (ELISA) within 2 days. Comparison of the results showed that there was no clear decrease in the concentrations of DPMN, EPI, NEPI and RNL levels.

Measurements of biochemical and hematological parameters

Biochemical parameters, such as ALT, AST and glucose levels were measured using an auto-analyzer. DPMN (catalog no: EU0392), EPI (catalog no: EU2563) and NEPI (catalog no: EU2565; Fine Biotech Co., Ltd., Wuhan, China), and RNL (catalog no: 201-12-5371; Sunred Biological Technology Co., Ltd., Shanghai, China) were analyzed as specified by the manufacturer. Briefly, first the samples were added to specific wells which were coated with the relevant antibodies for incubation. Anti-DPMN, EPI, NEPI and RNL antibodies labeled with biotin were subsequently added to combine with streptavidin-horseradish peroxidase (HRP) and form immune complexes, and the unbound enzymes were removed by washing. At the end of the experiment, quantification of the samples involved

the ELISA method at 540 nm using a ChroMate Microplate (P4300; Awareness Technology Instruments, Palm City, FL, USA). Assay performance (linearity recovery, and inter- and intra-assay values) of the kits used were also tested as described in the methods explained previously in detail before [36] at the same laboratory. Briefly, linearity was reported by diluting the samples for each different kit used (1/2, 1/4 and 1/8), and recoveries for each different kit were detected by adding known amounts of its own standard to the initial concentrations of the samples. The percentage recovery for each different kit was calculated as follows:

$$\text{Recovered value/expected value} \times 100.$$

The minimum (first number before) and maximum (second number after) measured values of the DPMN, NEPI, EPI and RNL kits were indicated by the manufacturer to be 1.56–100 ng/mL, 15.625–1000 pg/mL, 7.813–500 pg/mL and 3–700 ng/mL, respectively, whereas the assay performance of our laboratory indicated that the minimum and maximum measurement values of the DPMN, NEPI, EPI and RNL kits were 0.94–75 ng/mL, 9.4–1500 pg/mL, 4.7–750 pg/mL and 2.16–750 ng/mL, respectively. The DPMN, NEPI, EPI and RNL intra-assay coefficient of variation (CV) values given by the manufacturers were <8%, <8%, <8% and <10%, respectively. The DPMN, NEPI, EPI and RNL inter-assay CV values were noted by the manufacturer to be <10%, <10%, <10% and <12%, respectively. However, our results indicate that, in all the kits throughout this study, the intra-assay CV values were <10%, whereas the inter-assay CV values for all the kits were <12–14%.

Statistical analysis

Statistics were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) 21 package program. A one-way analysis of variance (ANOVA) was used to compare continuous data among the groups, and the Tukey-B tests were used as post-hoc tests. Spearman's correlation test was used to evaluate the intergroup correlation. $p < 0.05$ was considered statistically significant.

Results

There was no difference in the age, parity, gravida and nulliparity among the study groups; the demographic

characteristics are shown in Table 1. There was no statistically significant difference in terms of gestational week. However, when SBP/DBP values of preeclamptic mothers were compared with those of control mothers, the PE group was ~40 points higher, whereas the SPE group was ~60 points higher.

Table 1: Demographic characteristics of preeclamptic mothers and control mothers, with data on the biochemical parameters and newborn birth weights.

Parameters	Control	PE	SPE
Age, year	29.1±1.9	28.9±3.7	29.4±4.2
BMI, kg/m ²	30.1±1.9	29.6±2.1	29.9±2.6
Diastolic blood pressure, mm Hg	67.8±3.6	96.1±6.3 ^a	114.3±8.1 ^a
Gestational period, weeks	37.7±1.4	36.3±1.6	35.4±1.2
Newborn birth weight, kg	3.07±0.2	2.81±0.42 ^a	2.66±0.56 ^a
Parity	3	2	2
Systolic blood pressure, mm Hg	108±2.1	144.2±3.9 ^a	166.7±11.4 ^a
ALT, U/L	16.4±3.4	31.2±5.1 ^a	41.9±5.8 ^a
AST, U/L	18.2±6.2	29.4±6.3 ^a	51.4±6.3 ^a
Creatine, mg/dL	0.7±0.01	1.4±0.08 ^a	1.7±0.1 ^a
Glucose, mg/dL	93.4±2.7	98.1±4.6	108.2±6.7
Hematocrit value, %	32.6±3.5	33.4±4.2	34.1±3.1
Uric acid, mg/dL	3.8±0.2	4.1±0.1	4.4±0.2 ^a

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; PE, preeclamptic; SPE, severe preeclamptic.
^aControl vs. preeclamptic, and control vs. severe preeclamptic.

Comparing the amounts of DPMN (57 ± 6.3 ng/mL), EPI (66 ± 6.2 pg/mL) and NEPI (77 ± 6.9 pg/mL) in the control mothers with those in the preeclamptic mothers showed higher levels of DPMN (73 ± 7.9 ng/mL), EPI (82 ± 8.5 pg/mL) and NEPI (91 ± 8.9 pg/mL) in the preeclamptic mothers, all of which were statistically significant ($p < 0.05$), (Figures 1–3). Similarly, when the amounts of DPMN, EPI and NEPI in the control mothers were compared with those in the severe preeclamptic mothers, the DPMN levels (94 ± 8.9 ng/mL), EPI levels (104 ± 8.8 pg/mL) and NEPI levels (126 ± 10.1 pg/mL) were higher in the preeclamptic mothers than in the control mothers (Figures 1–3), again the differences being statistically significant ($p < 0.05$). When the amounts of DPMN, EPI and NEPI in the preeclamptic mothers were compared with those in the severe preeclamptic mothers, the DPMN levels, EPI levels and NEPI levels were higher in the severe preeclamptic mothers than in the preeclamptic mothers, the differences being statistically significant ($p < 0.05$), (Figures 1–3).

DPMN (36 ± 4.7 ng/mL), EPI (49 ± 4.7 pg/mL) and NEPI (66 ± 6.9 pg/mL) levels in the arterial blood of newborns of the control mothers were compared with the levels of those in the arterial blood of newborns of the preeclamptic mothers; the DPMN levels (42 ± 4.7 ng/mL), EPI levels (54 ± 4.9 pg/mL) and NEPI levels (71 ± 4.6 pg/mL) were higher in the preeclamptic mothers than in the control mothers, with all differences being statistically significant (Figures 1–3). Similarly, DPMN, EPI and NEPI in the control mothers were compared with those in the severe

Figure 1: Changes of dopamine concentrations (ng/mL) in control, preeclamptic and severe preeclamptic mothers, and in their babies' umbilical cord (artery and vein) blood samples.

^aControl vs. preeclamptic, and control vs. severe preeclamptic. ^bPreeclamptic vs. severe preeclamptic. Each data-point is the standard deviation of an average of 30 experiments, $p < 0.05$ being statistically significant.

Figure 2: Changes of epinephrine concentrations (pg/mL) in control, preeclamptic and severe preeclamptic mothers, and in their babies' umbilical cord (artery and vein) blood samples.

^aControl vs. preeclamptic, and control vs. severe preeclamptic. ^bPreeclamptic vs. severe preeclamptic. Each data-point is a standard deviation of an average of 30 experiments, $p < 0.05$ being statistically significant.

Figure 3: Changes of norepinephrine concentrations (pg/mL) in control, preeclamptic and severe preeclamptic mothers, and in their babies' umbilical cord (artery and vein) blood samples.

^aControl vs. preeclamptic, and control vs. severe preeclamptic. ^bPreeclamptic vs. severe preeclamptic. Each data-point is a standard deviation of an average of 30 experiments, $p < 0.05$ being statistically significant.

preeclamptic mothers; the levels of DPMN (76 ± 8.1 ng/mL), EPI (89 ± 7.6 pg/mL) and NEPI (92 ± 7.3 pg/mL) were higher than in the control mothers, all again being statistically significant differences ($p < 0.05$). DPMN, EPI and NEPI levels in the arterial blood of newborns of preeclamptic mothers were compared with those in the arterial blood of newborns of severe preeclamptic mothers, which showed that the DPMN, EPI and NEPI levels in the arterial blood of newborns of severe preeclamptic mothers were higher than in preeclamptic mothers, these elevations being statistically significant ($p < 0.05$), (Figures 1–3).

Comparing the venous DPMN (44 ± 5.1 ng/mL), EPI (52 ± 5.1 pg/mL) and NEPI (74 ± 5.8 pg/mL) levels in the blood of newborns of control mothers with the levels in the venous blood of newborns of the preeclamptic mothers showed higher levels of DPMN (52 ± 4.6 ng/mL), EPI (59 ± 4.7 pg/mL) and NEPI (77 ± 5.8 pg/mL) in the preeclamptic mothers than in the controls, the differences being statistically significant ($p < 0.05$). Similarly, when DPMN, EPI and NEPI levels in the control mothers were compared with those in the severe preeclamptic mothers, DPMN (82 ± 7.4 ng/mL), EPI (97 ± 8.1 pg/mL) and EPI (101 ± 8.9 pg/mL) levels in the

severe preeclamptic mothers were higher than in the controls, the differences again being statistically significant ($p < 0.05$). DPMN, EPI and NEPI levels in the venous blood of newborns of preeclamptic mothers were compared with their levels in the venous blood of newborns of severe preeclamptic mothers; DPMN, EPI and NEPI levels in the severe preeclamptic mothers were higher than those from the preeclamptic mothers, all elevations being statistically significant (Figures 1–3). In a similar fashion, DPMN, EPI and NEPI levels in the arterial blood of newborns in all the three groups were higher than those in the venous blood of newborns, but not significantly so (Figures 1–3).

When the amounts of RNL in the control mothers (514 ± 48 ng/mL) were compared with those in the preeclamptic mothers (447 ± 42 ng/mL), they were lower in the latter, the difference being statistically significant. Similarly, when the levels of RNL in the control mothers were compared with those in the severe preeclamptic mothers (371 ± 36 ng/mL), those in the preeclamptic mothers were significantly lower. Compared to the RNL levels in preeclamptic mothers (447 ± 42 ng/mL), those in severe preeclamptic mothers (371 ± 36 ng/mL) were significantly lower ($p < 0.05$) (Figure 4).

Regarding RNL levels (442 ± 43 ng/mL) in the arterial blood of newborns of control mothers compared with RNL levels in the arterial blood of newborns of preeclamptic mothers, those in preeclamptic mothers were (384 ± 37 ng/mL) lower, this difference being statistically significant ($p < 0.05$). Similarly, RNL in the arterial blood of newborns of control mothers were compared with the levels

(315 ± 29 ng/mL) in the arterial blood of newborns of severe preeclamptic mothers; those in the preeclamptic mothers were lower, which was also a statistically significant difference. RNL levels in the arterial blood of newborns of preeclamptic mothers were compared with the levels in the arterial blood of newborns of severe preeclamptic mothers; those in the severe preeclamptic mothers were lower, with this difference being statistically significant ($p < 0.05$) (Figure 4).

With reference to RNL (419 ± 37 ng/mL) in the venous blood of newborns of control mothers compared with the levels (396 ± 34 ng/mL) in the venous blood of newborns of preeclamptic mothers, RNL levels of preeclamptic mothers were lower. Similarly, RNL in the venous blood of newborns of control mothers compared with the levels in newborns of severe preeclamptic mothers (367 ± 34 ng/mL) showed that the levels in the preeclamptic mothers were lower, a statistically significant difference ($p < 0.05$). RNL in the venous blood of newborns of preeclamptic mothers compared with those in the venous blood of newborns of severe preeclamptic mothers showed that the levels in the severe preeclamptic mothers were lower (Figure 4). No statistically significant difference was found in any of the groups between the arterial and venous DPMN, EPI, NEPI and RNL levels of newborns with respect to gender (56 female, 34 male), and therefore data are not shown.

Given the correlation of the parameters, no significant correlation was found between DPMN and RNL levels in the control group. Moderate negative correlations were found between DPMN and RNL ($p = 0.001$, $r = -0.32$), EPI and RNL ($p = 0.001$, $r = -0.39$), and NEPI and RNL

Figure 4: Changes of renalase concentrations (ng/mL) in control, preeclamptic and severe preeclamptic mothers, and in their babies' umbilical cord (artery and vein) blood samples.

^aControl vs. preeclamptic, and control vs. severe preeclamptic. ^bPreeclamptic vs. severe preeclamptic. Each data-point is a standard deviation of an average of 30 experiments, $p < 0.05$ being statistically significant.

($p=0.001$, $r=-0.43$) levels in the preeclamptic mothers. On the other hand, there was a strong negative correlation between DPMN and RNL ($p=0.0001$, $r=-0.59$, EPI and RNL ($p=0.0001$, $r=-0.48$), and NEPI and RNL ($p=0.0001$, $r=-0.62$) of severe preeclamptic mothers. There was also a negative correlation between SBP/DBP and RNL levels in the preeclamptic mothers ($p=0.0001$, $r=-0.44/p=0.0001$, $r=-0.48$), and severe preeclamptic mothers ($p=0.0001$, $r=-0.52/p=0.0001$, $r=-0.59$). Once again, there was a moderate correlation between the levels of ALT/AST and those of RNL in the PE ($p=0.001$, $r=-0.37/p=0.001$, $r=-0.39$) and SPE ($p=0.001$, $r=-0.41/p=0.001$, $r=-0.37$) groups.

Discussion

Although renal dysfunctions in the gestational period are not always clear, it is generally accepted that the functioning in preeclamptic mothers is impaired [14, 15, 37]. We found a statistically significant increase in the creatinine levels of preeclamptic mothers compared with control mothers. The data for preeclamptic mothers are consistent with the high creatinine levels already reported in preeclamptic mothers [38, 39]. An increase in the extrarenal creatinine degradation in its conversion to carbon dioxide and methylamine by bacteria in the intestine during declining renal function may lead to underestimates in the decline in the glomerular filtration rate [34, 40], as it does not bind to plasma proteins and is easily filtered out from the glomeruli [40]. This is an important indicator in evaluating renal function, but this alone should not be used to assess kidney function. We think that this increase in creatinine in preeclamptic mothers indicates impaired renal functioning. Other findings discussed here support this argument.

Concomitant proteinuria in PE is more complicated than simple gestational hypertension. Urinary protein excretion >300 mg in 24-h urine, a ratio of protein: creatinine in urine ≥ 0.3 or a 300-mg/dL (1+ dipstick) protein persisting in a random sample of urine all indicate proteinuria [2, 3, 41]. We found proteinuria was also present in pregnancy-induced hypertension. An increase in the levels of proteinuria is associated with poor maternal and fetal outcomes. Baba et al. [42] found in their prospective study of 1033 (with 2212 urine specimens) pregnant women that significant proteinuria (2+ protein in dipstick) increases stillbirth, fetal growth restriction and neonatal morbidity rates. As proteinuria increases, clinical outcomes get worse, and fetal and maternal morbidity

increases [43]. In addition, only small amounts of protein are normally detected in urine [44]. We found that the appearance of proteinuria in preeclamptic patients suggests impaired kidney function when we take into consideration the glomerular selectivity of the kidneys of these patients and their increased creatinine values.

We also found that the levels of the RNL enzyme decrease with PE and SPE. That is, when the amounts of RNL enzyme in the control mothers were compared with the amounts of the enzyme in preeclamptic mothers, the lowest RNL levels were detected in the blood of SPE mothers. Thus, the severity of PE increased, and the RNL levels decreased. A decreased RNL level in pregnancy complicated by PE was previously reported [28], but this study only analyzed the RNL levels in the mother's blood in PE and not the catecholamines (CAs) levels in PE, which are the substrates of the RNL enzyme; the concentrations of the umbilical cord RNL and CAs were also not studied. Besides RNL, the levels of CAs were also studied in both mothers' blood and umbilical cords in our analysis. We report that the amounts of EPI, NEPI and DPMN concentrations were the opposite of those of RNL. Reduction in the amount of RNL enzyme may be due to renal cell damage, which secretes RNL [17] because of the hypertension seen in PE. The relationship between the RNL level and hypertension has been well studied [17–20]. Kidney function and RNL levels also decrease in hypertension after cardiopulmonary bypass surgery [45]. One of the most important causes of kidney failure is hypertension. When the BP rises, blood vessels in the kidneys may become damaged, and the blood-filtering function is negatively affected [46]. Kidney damage can be detected in ~30% of people with high BP [47]. Here, the high CAs we detected might be due to the low level of RNL enzyme in the blood. Because kidneys that are damaged due to BP in pregnancy cannot synthesize RNL in sufficient amounts [17] and cannot metabolize CAs unless at physiological doses, the result is an increase in circulating CAs, as noted in this study.

An increase in CAs also leads to higher BP in the later phases of pregnancy. In one study, placental-derived tyrosine hydroxylase activity was found to be increased in patients with PE [48], leading to the suggestion that CA synthesis may increase in preeclamptic patients. Increased CAs in mid-pregnancy have also been seen as a risk for preterm delivery [49]. In this case, we suggest that if PE-induced hypertension is due to CAs, RNL preparations should be given in order to destroy the CAs so that BP can be controlled, and poor maternal and perinatal transmission is avoided. This low level of blood RNL and CAs in PE women were also seen in the umbilical

cord blood of their babies. This means that the levels of RNL and CAs in PE women reflect their babies' umbilical cord blood RNL and CAs levels compared with control RNL and CAs levels. We stress that we are not in agreement with an experimental study that demonstrates that RNL does not catalyze the oxidation of neurotransmitter CAs [25], because we found that if RNL is sufficiently present in the blood, CA levels decrease as in the control mothers' RNL level. If not, CA levels increase as in the PE and SPE mothers' RNL level. This means that CA and RNL levels are inversely related, such that RNL might use CAs as its own substrates. This is why when its level is high, CA levels go down. We therefore support the evidence that RNL metabolizes CAs in the following order of priority: DPMN, EPI and NEPI. Although we have not measured the BP of newborns, any newborn's mothers is preeclamptic. The BP of newborns might be high due to low RNL and high CAs in their umbilical cords. This should be kept in mind regarding the strict control of newborns' BP, which might avoid neonatal death due to high BP related with high levels of CAs and a low level of RNL in the blood.

Conclusions

Although advances are being made, PE still ranks first in terms of maternal morbidity and mortality throughout the world, as in Turkey. Deterioration of kidney function clearly plays a major role in the pathogenesis of PE. We found that the RNL enzyme released from the kidneys decreased in proportion to the severity of PE. Therefore, the increase of CAs due to the decrease in the RNL enzyme is directly related to the hypertension seen in PE. Therefore, to control hypertension due to pregnancy or an unknown reason; CA-induced hypertension can be controlled by administering an RNL preparation that will degrade CAs, which are the hypertension agents in pregnancy-induced PEs.

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