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Original Article

The possible association between neonatal morbidities and amniotic fluid pH and electrolyte levels in infants of preeclamptic mothers

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ABSTRACT

Background. Preeclampsia is a pregnancy-specific syndrome associated with increased perinatal mortality characterized by hypertension and proteinuria. An increasing number of studies have been published on the effect of preeclampsia on neonatal morbidities. However, there is no study regarding the possible effect of preeclampsia on amniotic fluid pH and electrolytes. The aim of this study was to determine the possible role of amniotic fluid pH and electrolytes for the prediction of and/or association with preeclampsia and neonatal morbidities.

Methods. This was a prospective, case-control study. During cesarean section (C/S), 1 ml of amniotic fluid was aspirated before incision of membranes. Amniotic fluid pH and electrolytes were analyzed by blood gas machine and biochemistry laboratory concurrently. Maternal and neonatal demographic features and clinical outcomes, presence of respiratory morbidities were all recorded.

Results. Amniotic fluid pH, sodium and gestational age were found to be independent risk factors for preeclampsia. Subgroup analysis revealed that in early onset preeclampsia group mechanical ventilation duration, duration of 0_2 therapy, sepsis and intrauterine growth retardation (IUGR) were higher than infants in control group born before 32 gestational weeks. Also, in the early onset preeclampsia group pH and potassium were higher compared with the control group.

Conclusions. To the best of our knowledge, this is the first study that reported the value of amniotic fluid electrolyte analysis for the prediction of preeclampsia and neonatal morbidities in term and preterm infants. However, more studies including a larger number of infants are required to confirm the role of amniotic fluid analysis to predict preeclampsia and/or neonatal morbidities.

Key words: amniotic fluid, electrolytes, neonatal morbidities, pH, preeclampsia.

Amniotic fluid (AF) is a complex bio-environment with dynamic content. Amniotic fluid has many immunologic and biochemical properties as well as being a mechanical cushion for the fetus. Additionally, AF has a key role as an early diagnostic tool for fetal/neonatal disease. The pH of AF is proven to be affected by both maternal and fetal conditions such as preterm ruptures of membranes, gestational age and fetal distress.[1] As of the 20th gestational week fetal urine is the main component of AF, nearby amniotic membranous secretion and fetal lung liquid are determined as the other components. The volume of AF is primarily determined by intramembranous absorption of water and solutes from fetal venous structures.[2]

Preeclampsia (PE) is a pregnancy-specific syndrome associated with increased perinatal

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mortality characterized by hypertension and proteinuria. Whereas early onset PE is diagnosed between 20-34 gestational weeks (GW), late onset PE is diagnosed after 34 GW.[3] Affecting 5-8% of the pregnancies worldwide, PE pathogenesis has two phases.[4] At the first phase, disorders of placentation and pseudoangiogenesis induce increased oxidative stress, causing endothelial dysfunction, which leads to maternal systemic inflammatory response syndrome and a clinical picture with typical symptoms and signs of PE.[5] Besides the maternal complications like cerebral edema or hemolysis, elevated liver enzymes, low platelet syndrome (HELLP syndrome), stroke, pulmonary edema; PE harbors severe fetal effects such as fetal distress, oliguria, abruption placenta, intrauterine growth restriction (IUGR). These factors make PE, a global reason for both, fetal and maternal mortality and morbidity.

AF content is related to antenatal steroids, preterm birth and GW, it has also been suggested to be affected from preterm premature ruptures of membranes, fetal distress and PE. Furthermore, AF electrolytes were shown to be predictors for neonatal morbidities.[6] Although PE was thought affect AF content and fetal/maternal conditions, there is no human study about this subject in the literature.

Therefore, the aim of this study was to determine the possible role of PE on AF pH and electrolytes and neonatal morbidities.

Material and Methods

This prospective, single center, case-control study was conducted in four months. The ethics committee of Kanuni Sultan Suleyman Training and Research Hospital approved the study (Ethics Committee approval number: KAEK//2015.7.7). Informed consent was obtained from parents. Preeclamptic mothers and their infants comprised the case group of the study. Pregnant women who did not develop PE, and their infants were taken as the control group. Congenital abnormalities, chromosomal disorders, blood contaminated AF, C/S without labour pain, other abnormalities of the pregnant mother, which might affect the results and those who declined to participate were all excluded. For the subgroup analysis; infants who were born under 34 GW from preeclamptic mothers and infants who were born under 34 GW from mothers who did not develop PE, were compared. To exclude the possible effect of GW on the parameters compared between groups; only the infants, who were born under 34 GW, were included in the group without early onset PE. Blood contaminated AF was determined if hematocrit was measured by the device. The procedure to avoid blood contamination of AF was to aspirate AF before the incision of membranes during the C/S. From all infants included, 1 ml of AF was aspirated during the C/S before incision of the membranes. pH value and electrolytes of AFs were analyzed by the blood gas machine (Siemens RAPIDLab®1200 Systems). Collected samples were analyzed both by the blood gas machine and laboratory of the study hospital with conventional biochemical methods concurrently. Maternal and neonatal demographic features and clinical outcomes, presence of morbidities such as sepsis, respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis (NEC) were all recorded. This study is registered in ClinicalTrials.gov with the number NCT02691559.

Statistical analysis

Clinical data were presented as means ± SD for parametric tests and categorical data displayed as median (interquartile range (IQR)) for nonparametric tests. The distribution of the variables were controlled by Kolmogorov-Smirnov test. Comparisons were performed with the t-test in case of normal distribution of the variable or the Mann-Whitney U test if the distribution is not normal. Mann-Whitney U test was used for the analysis of quantitative and Chi-Square test for qualitative data. A sample size calculation was performed based

on our observed results by using a one-sided McNemar's test. A sample size of 180 infants, at least 69 in each arm, is found to be sufficient to detect a clinically important difference between groups with 80% power and a 5% level of significance.

SPSS version 21.0 (SPSS, Chicago, IL) was used for statistical analysis. Statistical significance was accepted when the probability (P) value was < 0.05 and changes were referred to as significant at this P-value.

Results

A total of 382 infants were born via C/S in the study hospital during the study period. As 202 were excluded due to reasons given in Figure 1, AFs of 180 infants were included. Of these, 72 infants were born from preeclamptic mothers. A flow chart of participants has been given in Figure 1. The two distinct methods of the blood gas machine and conventional biochemistry results did not differ. Receiver operating characteristic analysis showed a statistically significantly lower birth weight and gestational age in the group with PE than the control group ($p<0.001$, $p<0.001$) (Table I). Besides hospitalization rate, RDS, NEC, sepsis, prematurity, IUGR were statistically significantly higher in the PE group when compared with the control group ($P <0.05$) (Table I). AF pH, Na, K were found to be statistically significantly higher in the PE group than the control group ($P <0.05$) (Table II). AF pH, Na and gestational age were found to be independent risk factors for PE in logistic regression analyses (Table III). Subgroup analysis revealed that in the early onset PE (EOP) group mechanical ventilation duration, duration of 0_2 therapy, sepsis and IUGR were higher than control group infants born before 34 GW (p 0.035, 0.012, 0.021, 0.011) (Table IV).

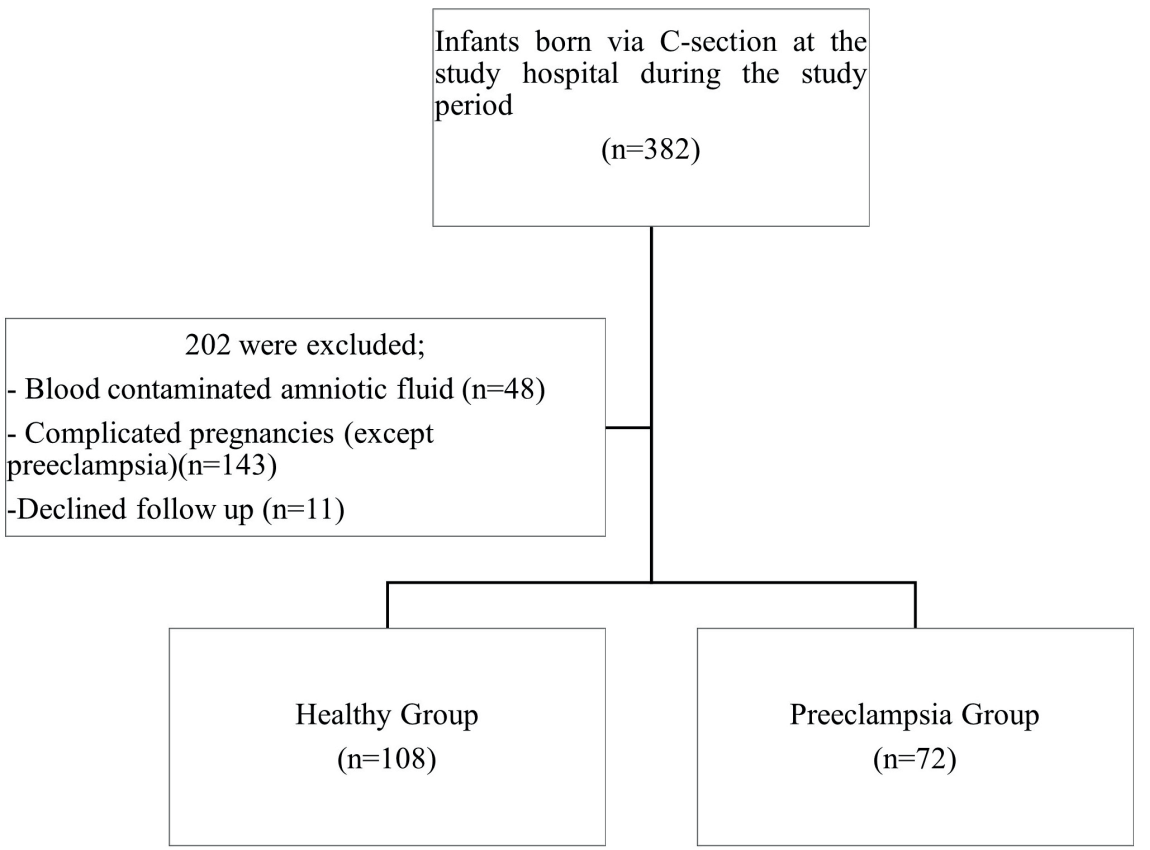

Fig. 1. Flow diagram of the study.

Table I. Comparison of the case and control groups for demographic features and neonatal morbidities.

		Preeclampsia (-) (n=108)		Preeclampsia (+) (n=72)		p
		Mean±SD/n-%	Median	Mean±SD/n-%	Median	
Maternal Age			28.0		27.5	0.288
Sex	Female	53	47.3%	31	43.1%	0.422
	Male	55	49.1%	41	56.9%	
Birth weight (g)			3173		2385	<0.001
Gestational age		37.2 ± 2.3		34.4 ± 3.8		<0.001
Hospitalization		26	23.2%	34	47.2%	0.001
Antenatal steroid		39	34.8%	27	37.5%	0.089
RDS		7	6.3%	19	26.4%	<0.001
TTN		18	16.1%	18	25.0%	0.136
NEC		3	2.7%	13	18.1%	<0.001
Sepsis		14	12.5%	27	37.5%	<0.001
Prematurity		33	29.5%	49	68.1%	<0.001
IVH		1	0.9%	4	5.6%	0.158
IUGR		4	3.6%	10	13.9%	0.043

Mann-Whitney u test / t test / Chi-square test
SD: standard deviation, RDS: respiratory distress syndrome, TTN: transient tachypnea of newborn,
NEC: necrotizing enterocolitis, IVH: intraventricular hemorrhage, IUGR: intrauterine growth restriction

Table II. Comparison of the pH and electrolyte values between case and control groups.

	Preeclampsia (-) (n=108)		Preeclampsia (+) (n=72)		p
	Mean±SD/n-%	Median	Mean±SD/n-%	Median	
pH	7.1 ± 0.1		7.2 ± 0.1		0.007
pC02 (mmHg)	42.6 ± 9.3		39.9 ± 9.2		0.054
HC03 (mmol/l)	13.9 ± 3.4		15.0 ± 5.5		0.955
Lactate (mmol/l)		7.1		7.4	0.588
Na (mEq/l)	118.1 ± 5.5		121.7 ± 5.9		<0.001
K (mEq/l)	4.1 ± 0.5		4.3 ± 0.7		0.019
Cl (mEq/l)	105.3 ± 5.5		103.8 ± 6.9		0.294
Ca (mmol/l)	1.1 ± 0.2		1.1 ± 0.2		0.076

Mann-Whitney u test / t test / Chi-Square test
SD: standard deviation, C02: carbon dioxide, HC03: bicarbonate,
Na: sodium, K: potassium, Cl: chlorine, Ca: calcium

The birth weight of the EOP group was lower than the control group (p 0.031) (Table III). In the EOP group, AF pH and K were statistically significantly higher than the control group (p 0.015, 0.036) (Table V).

Discussion

This study is unique as reveals that Na and pH values of AF might be predictors of PE in association with neonatal morbidities. In utero stress caused by PE was shown to be associated with epigenetic regulations on fetal DNA, amniotic epithelial cells and stromal cells via methylation changes; promising that amniotic membranes as a surrogate fetal tissue for the prediction of adverse intrauterine conditions.[7] However, there are limited studies inquiring about the effects of PE on AF content and neonatal morbidities.[8-10] Recently AF Na was proven to be related to respiratory morbidities in neonates.[6] Fetal urine production, lung liquid

Table III. Logistic regression analyses of data.

	Univariate Model					Multivariate Model				
	OR	%95 CI			p	OR	%95 CI			p
Maternal age	0.983	0.94	-	1.03	0.47					
Sex (Female/Male)	1.28	0.70	-	2.32	0.42					
Birth weight (g)	1.00	1.00	-	1.00	<0.001					
	0.72	0.70	-	0.82	<0.001	0.80	0.67	-	0.88	<0.001
Gestational week										
Hospitalization	2.96	1.56	-	5.60	0.001					
	5.17	1.77	-	15.06	0.003					
Antenatal steroid										
RDS	5.38	2.13	-	13.59	<0.001					
TTN	1.74	0.84	-	3.63	0.14					
NEC	8.01	2.19	-	29.22	0.002					
Sepsis	4.20	2.01	-	8.77	<0.001					
Prematurity	5.10	2.69	-	9.68	<0.001					
IVH	6.53	0.71	-	59.64	0.10					
IUGR	3.37	0.98	-	11.66	0.05					
pH	47.03	3.60	-	620	0.01	23.01	1.34	-	394.2	0.03
pC02 (mmHg)	0.97	0.94	-	1.00	0.06					
HC03 (mmol/l)	1.06	0.98	-	1.16	0.16					
p02 (mmHg)	1.00	1.00	-	1.01	0.30					
Lactate (mmol/l)	0.95	0.81	-	1.20	0.77					
Na (mEq/l)	1.11	1.04	-	1.17	<0.001	1.10	1.03	-	1.15	0.01
K (mEq/l)	1.92	1.13	-	3.31	0.01					
Cl (mEq/l)	0.97	0.90	-	1.02	0.13					
Ca (mmol/l)	0.36	0.08	-	1.97	0.27					

Logistic Regression
SD: standard deviation, OR: odds ratio, CI: confidence interval,
RDS: respiratory distress syndrome, TTN: transient tachypnea of newborn, NEC: necrotizing enterocolitis,
IVH: intraventricular hemorrhage, IUGR: intrauterine growth restriction, C02: carbon dioxide, HC03: bicarbonate,
Na: sodium, K: potassium, Cl: chlorine, Ca: calcium

secretion, swallowing and intramembranous absorption are the four main mechanisms of AF volume regulation.[2] The results of the studies investigating the effects of PE on neonatal morbidities are conflicting. However, it is known that PE is a syndrome, which causes increased inflammation and distress for both the fetus and mother. It was demonstrated that in the AF of preeclamptic mothers' vasoconstrictor mediators, such as endothelin is elevated in content, whereas the content of vasodilators, like nitric oxide is diminished.[11] As a result of this, disrupted placental perfusion in PE was proven to be related to fluctuations in oxygen levels, leading to oxidative stress in AF.[12] As a response to that increased oxidative stress caused by PE, oxygen radical absorbing capacity and Coenzyme Q10 levels of AF were pointed to be in relation to adverse neonatal outcomes including IUGR, preterm labor and preterm premature rupture of membranes due to intraamniotic infection.[13] Depending on those findings, PE is thought to own a two-sided reflection of its pathologies. One

Table IV. Comparison of the demographic features and neonatal morbidities between early onset preeclampsia and control group.

		Early onset preeclampsia (-) (n=15)			Early onset preeclampsia (+) (n=32)			p
		mean±SD/n-%			mean±SD/n-%			
Maternal age		28.8	±	6.8	28.8	±	5.5	0.99
Sex	Female	6		40%	15		20.80%	0.66
	Male	9		60%	17		23.60%	0.74
Birth weight (g)		2057	±	709.0	1044	±	728	0.031
Gestational week		32.4	±	2.0	31.9	±	2.6	0.54
Antenatal steroid		5.0		33%	12		37.50%	0.97
RDS		6.0		40%	18		56.30%	0.3
MV/day		1.5	±	3.9	4.14	±	7.1	0.035
02/day		4.9	±	14	16.51	±	26.57	0.012
BPD		1.0		7%	8		11.10%	0.1
NEC		3.0		20%	12		37.50%	0.2
Sepsis		6.0		40%	24		75%	0.021
IVH		1.0		6.60%	4		12.50%	0.55
IUGR		2.0		13.30%	10		31.30%	0.011

Mann-Whitney u test / t test / Chi-Square test
Standard deviation, RDS: respiratory distress syndrome, BPD: bronchopulmonary dysplasia,
NEC: necrotizing enterocolitis, MV: mechanical ventilation, 02: oxygen, IVH: intraventricular hemorrhage,
IUGR: intrauterine growth restriction

Table V. Comparison of the amniotic fluid pH and electrolyte values between early onset preeclampsia and control group.

	Early onset preeclampsia (-) (n=15)	Early onset preeclampsia (+) (n=32)	p
	mean±SD/n-%	mean±SD/n-%	
pH	7.1 ± 0.2	7.3 ± 0.2	0.015
pC02 (mmHg)	43.9 ± 4.9	40.5 ± 5.3	0.060
HC03 (mmol/l)	13.9 ± 3.4	15.0 ± 5.5	0.960
Lactate (mmol/l)	7.3 ± 2.3	7.2 ± 1.9	0.600
Na (mEq/l)	120.4 ± 6.2	123.2 ± 6.3	0.169
K (mEq/l)	4.2 ± 0.4	4.5 ± 0.6	0.036
Cl (mEq/l)	105.3 ± 5.5	103.8 ± 6.9	0.294
Ca (mmol/l)	1.1 ± 0.2	1.1 ± 0.2	0.076

Mann-Whitney u test / t test / Chi-Square test
SD: standard deviation, C02: carbon dioxide,
HC03: bicarbonate, Na: sodium, K: potassium, Cl: chlorine, Ca: calcium

is on the AF, which acts as a protective tissue for the fetus by re-organizing its antioxidant and secretory capacity, leading the changes in its content. The other reflection is on the fetus and later on the neonate, who is considered to be negatively affected by the oxidative stress and inflammation caused by PE. Therefore, higher hospitalization rate, RDS, NEC, sepsis, prematurity, IUGR, that were observed in the PE group of the study, are compatible with the

recent works concerning the topic/condition.[8-10] In addition, higher mechanical ventilation span, duration of 0_2 therapy, sepsis and IUGR that were detected in EOP group are supportive of this data.

Considering the first effect of PE on the fetus is decreased fetal urine output, for the findings of AF content, as a major content of AF, decreased disproportion of acidotic fetal urine in AF may explain elevated pH in PE group. Although antioxidant enzymes like catalase, glutathione reductase levels were reported to be decreased in AF of preeclamptic pregnancies, prooxidant enzymes such as xanthine oxidase were found to be elevated.[14] Therefore, this precipitous prooxidant capacity of AF can be a result of the inflammation caused by PE and it may be associated with higher AF pH levels in the PE group. Supporting this finding, Banadakoppa et al.[15] proved that especially alternative complement pathway complement activation products in AF at early pregnancy is linked to later development of EOP. Furthermore, higher Na^+ levels in the PE group may be related to increased Na^+ excretion from fetal kidneys that were affected in variable degrees by PE. Otherwise, maternal high serum potassium levels during the first half of pregnancy are demonstrated to be associated with a higher risk for the development of PE.[16] Even before the apparent clinical symptoms of PE, elevated AF K^+ levels, at the second trimester, is proven to be a predictor of PE.[17] Considering AF K^+ levels are correlated with maternal K^+ levels, in present study higher AF K^+ levels in the EOP group may be a result of PE. This also may be a result of the dysregulation of angiotensin converting enzyme (ACE) 2 pathway which is proven to have a role in microvascular endothelial dysfunction of PE.[18] The elevated early onset sepsis rate in the EOP group may also be associated with the endothelial impairment caused by PE.

With all these findings, it is possible to conclude that although the etiopathogenesis of PE and its effect on neonatal morbidities are not completely clarified, AF compositional changes before the development of clinical symptoms may be early biomarkers for both.

Nevertheless, the present study has a few limitations such as a restricted number of cases and lower diagnosis-sensitive numbers for participants with RDS and BPD, etc. Although we tried to choose the subgroups that were similar in terms of gestational age. We were not able to exclude all the factors which might have affected the results.

To the best of our knowledge, this is the first study that reported the value of AF electrolyte analysis for the prediction of PE and neonatal morbidities in term and preterm infants. However, more studies including a larger number of infants are required to confirm the role of AF analysis to predict PE and/or neonatal morbidities. Furthermore, this will create a need for more detailed studies for a better understanding of the molecular mechanisms underlying those changes in AF content and their relation to the maternal-fetal conditions.

Ethical approval

The ethics committee of Kanuni Sultan Suleyman Training and Research Hospital approved the study (Ethics Committee approval number: KAEK//2015.7.7).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SYS, MC; data collection: IME, OSG, SYS; analysis and interpretation of results: SYS, MC, BY; draft manuscript preparation: SYS, MC, BY, IME, OSG. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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