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Original Article / Özgün Araştırma

The Measurement of Neutrophil Gelatinase Associated Lipocalin in Umbilical Cord Blood and the Assessment of Its Relationship with Neonatal Results

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Abstract

Objectives: In this study, the relationship of cord blood Neutrophil Gelatinase-Associated Lipocalin (NGAL) with neonatal diseases was investigated.

Methods: NGAL levels were measured in the cord blood of 180 babies born between 2015 and 2016. Patients were classified according to maternal diseases, neonatal diseases and demographic characteristics. Obtained data were compared with cord blood NGAL levels.

Results: In our study, the mean NGAL levels were 1283.99 ng/mL in boys and 1306.52 ng/mL in girls. Umbilical cord blood NGAL levels of infants diagnosed with intrauterine growth retardation (1913.4±2833.5 ng/mL) and prolonged premature rupture of membranes (2594.2±2037.1 ng/mL) were found to be statistically high ($p<0.05$). There was no statistically significant difference between NGAL levels in infants of mothers with gestational diabetes mellitus, acyanotic congenital heart diseases, meconium aspiration syndrome, infants of mothers with preeclampsia, Apgar scores and infants of mothers with oligohydramnios ($p>0.05$).

Conclusions: Neutrophil Gelatinase-Associated Lipocalin, may be useful as a diagnostic biomarker in the evaluation of maternal and neonatal diseases. However, studies on larger patient populations are needed.

Keywords: Neutrophil Gelatinase Associated Lipocalin, umbilical cord blood, newborn.

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Göbek Kordon Kanında Nötrofil Jelatinaz İlişkili Lipokalin Ölçümü ve Neonatal Sonuçlarla İlişkisinin Değerlendirilmesi

Öz

Amaç: Bu çalışmada, kordon kanı Nötrofil Jelatinaz İlişkili Lipokalin'in (NGAL) yenidoğan hastalıkları ile ilişkisi araştırıldı.

Yöntemler: 2015-2016 yılları arasında doğan 180 bebeğin kordon kanında NGAL düzeyleri ölçüldü. Hastalar anne hastalıkları, yenidoğan hastalıkları ve demografik özelliklerine göre sınıflandırıldı. Elde edilen veriler kordon kanı NGAL seviyeleri ile karşılaştırıldı.

Bulgular: Çalışmamızda intrauterin gelişme geriliği ve uzamış erken membran rüptürü tanısı alan bebeklerin göbek kordonu kanındaki NGAL düzeyleri istatistiksel olarak yüksek bulundu ($p<0.05$). Gestasyonel diyabetes mellitus, asiyanotik konjenital kalp hastalığı, mekonyum aspirasyon sendromu, eklampsili anne bebekleri, Apgar skorları ve oligohidramnioslu anne bebeklerinde NGAL düzeyleri arasında istatistiksel olarak anlamlı fark bulunmadı ($p>0.05$).

Sonuçlar: NGAL, maternal ve neonatal hastalıkların değerlendirilmesinde tanısal bir biyobelirteç olarak faydalı olabilir. Bununla birlikte, daha büyük hasta popülasyonları ile ilgili çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Nötrofil Jelatinaz İlişkili Lipokalin, göbek bağı kanı, yenidoğan.

INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2, is a protein belonging to the lipocalin protein family and weighs 25 kDa. It is covalently bound to gelatinase in neutrophils and is released at very low levels from many other tissues such as kidney, lung, stomach, adipocytes, colon and neutrophils, and is involved in iron transport[1]. In experimental animal studies conducted in recent years, NGAL has been found to occur in the kidneys in the early stages of ischemic damage. The serum concentration of this protein increases dramatically in damage to epithelial organs caused by causes such as ischemia-reperfusion and sepsis.

Neutrophil Gelatinase-Associated Lipocalin is plentifuly expressed in monocytes, macrophages, neutrophils, and adipocytes. NGAL mRNA is expressed in human tissues bone marrow, kidney, lung, liver, stomach and colon[2]. Most of these tissues are exposed to microorganisms and consequently express low levels of NGAL protein[2].

Neutrophil Gelatinase-Associated Lipocalin is transported into the cell after interacting with specific membrane receptors, and especially the 24p3 membrane protein, which is a cell surface receptor, constitutes its important cellular target[2]. Its interaction with this receptor strengthens the structure of the complex NGAL-siderophore, resulting in a significant increase in cytoplasmic iron levels[2]. This mechanism in NGAL is thought to govern the multiple effects attributed to NGAL, including antibacterial activity, neoplastic growth and embryogenesis[2].

In many studies, high serum and urinary NGAL levels in newborns have been associated with intrauterine hypoxia, sepsis, bronchopulmonary dysplasia, and acute kidney injury[3-6]. Although data on umbilical cord blood NGAL concentrations are insufficient, NGAL measurements have been shown to be predictive in some diseases such as neonatal infection[7]. However, there is no accepted reference NGAL level in newborns yet. Different researchers have reported different urine and serum NGAL levels in their studies.

This study was carried out to investigate whether there is a relationship between NGAL levels in umbilical cord blood in diseases that

occur in the neonatal period. In addition, the relationship between maternal diseases, perinatal and neonatal morbidities and NGAL levels in cord blood was investigated.

METHODS

This study was conducted in Dicle University Gynecology and Obstetrics Clinic between 2015-2016 and 180 infants were included in the study. This study is a prospective study. Dicle university Ethics committee for non interventional studies approval was obtained before starting this study. (2015 revision 60 / 13.03.2017). Written informed consent was obtained from the parents of all infants included in the study. This study was supported by Dicle University Scientific Research Projects Coordination Unit (Project Number: TIP.15.027 Year: 2015).

Patient data were obtained through antenatal follow-up, neonatal intensive care unit admission, and outpatient follow-up. Umbilical cord blood of newborn babies included in the study was taken and this samples were centrifuged for 10 minutes and stored in deepfreeze at -86 °C. The NGAL level of these serum samples was studied in the Biochemistry Laboratory of the Faculty of Medicine of Dicle University. Serum NGAL levels were measured by quantitative Sandwich immunoassay method using a commercial kit (SunRed® Biotechnology Company).

The gestational age of the newborns included in the study was determined primarily by the last menstrual period, and for those whose last menstrual period was unknown, the gestational age was determined according to the new Ballard scoring system. Gestational age was classified as ≤32 weeks, 33-36 weeks, and ≥37 weeks. However, babies over 42 weeks of gestation were not included in the study. It was also classified as <2500 grams and ≥2500 grams according to birth weight.

Inclusion Criteria:

1- 24-42 weeks of gestation

Exclusion Criteria:

1- <24 weeks of gestation

2- >42 weeks of gestation

3- Stillbirth

4- Cyanatic congenital heart disease

5- Antenatal or postnatal congenital renal anomalies

6- Hypoxic ischemic encephalopathy

7- Chromosomal abnormalities (such as trisomy 13, 18, 21)

8- Maternal renal disease, heart disease, chronic liver disease, malignancy

Statistical Analysis

In the statistical evaluation of our research data, IBM Statistical Package for the Social Sciences(SPSS) 21 statistical package program for Windows was used. Numeric variables were presented with mean±standard deviation (SD); categorical variables were presented with numbers and percentages (%). The compliance of the data with normal distribution was examined. In the comparison of groups with normal distribution and two measurements, dependent and independent t-tests were used. In the comparison of groups with non-normal distribution and two measurements, Mann-Whitney U and Wilcoxon tests were used. In the comparison of means in groups with normal distribution and multiple measurements, the one-way analysis of variance was used. In the comparison of groups with non-normal distribution and multiple measurements, Kruskal Wallis H and Friedman tests were used. The measured data were expressed as mean and SD. Hypotheses were two-sided; $p \leq 0.05$ was considered as statistically significant, $p \leq 0.01$ highly significant, and $p \leq 0.001$ extremely significant.

RESULTS

In our study, 58.9% of 180 cases were male and 41.1% were female. 96.7% of the cases were delivered by cesarean section and 3.3% by normal spontaneous vaginal delivery. The birth weight of 75.6% of the cases was 2500 g and above. Gestational ages were at or above 37 weeks in 60% of them, and between 32 and 37 weeks in 26.1%. The mean NGAL was 1283.99 ng/mL in baby boys and 1306.52 ng/mL in girls. There was no statistically significant difference between gender and NGAL levels (p> 0.05). There was no statistically significant difference between delivery types, birth weight, gestational week and NGAL levels (p>0.05). Demographic characteristics and delivery types of babies included in the study, the relationship between birth weight and gestational week and NGAL levels are shown in Table 1.

Table I: NGAL levels according to demographic characteristics of infants

Demographic characteristics		NGAL (ng/mL)		*p*
		n(%)	Mean ± SD	
Type of birth	Vaginal	6(3.3)	1345.77±1740.08	0.93
	C/S	174(96.7)	1291.44±1496.46	
Gender	Male	106(58.9)	1283.99±1405.72	0.92
	Female	74(41.1)	1306.52±1634.65	
Birth weight (g)	<2500	44()24.4	1547.60±1810.88	0.03
	≥2500	136(75.6)	1210.96±1381.87	
Gestational age (weeks)	≤32	25(13.9)	1309.50±1401.53	0.99
	33-36	47(26.1)	1291.06±1663.14	
	≥37	108(60)	1290.44±1460.31	

C/S: Cesarean/Section, NGAL: Neutrophil Gelatinase-Associated Lipocalin. SD: standard deviation.

The most common morbidity in the postnatal period was respiratory distress syndrome (RDS) with 15.6%, and the other most common morbidity was neural tube defect (4.4%). While the mean NGAL of 28 infants with RDS diagnosis was 1083.36 ng/mL, the mean NGAL of those without RDS diagnosis was 1331.92 ng/mL, and there was no statistically significant difference between their levels (p> 0.05).

Umbilical cord blood NGAL levels were found to be statistically high in infants diagnosed with intrauterine growth retardation (IUGR) and prolonged premature rupture of membranes (PROM) (>18 hours) (p<0.05). The relationship between neonatal diseases and cord blood NGAL levels is shown in Table 2.

Table II: The relationship between neonatal diseases and NGAL levels in cord blood

	NGAL (ng/mL)				
	Yes		No		
	n	Mean ± SD	n	Mean ± SD	*p*
Healthy term newborn	108	1177.7±1276.7	71	1383.6±1653.9	0.08
RDS	28	1083.3±1171.1	152	1331.9±1552.7	0.17
Neural tube defect	8	1050.9±1567.1	172	1304.5±1500.2	0.99
Blood incompatibility (Rh and ABO incompatibility)	6	1958.4±2033.5	174	1270.3±1480.4	0.15
Gestational DM mother baby	11	1289.7±1133.5	169	1293.4±1523.1	0.68
Acyanotic Congenital heart diseases	14	1516.9±1409.8	166	1274.3±1509.5	0.81
İUGR	5	1913.4±2833.5	175	1275.5±1455.2	0.03
Meconium aspiration syndrome	2	762.9±15.5	178	1299.2±1507.1	0.20
Baby of mother with preclampsia	11	1056.6±891.2	169	1308.6±1531.3	0.30
Baby of mother with oligohydramnios	16	1470.8±1496.1	164	1275.9±1503.4	0.62
Baby of mother with prolonged PROM	6	2594.2±2037.1	174	1248.3±1465.1	0.03

DM: diabetes mellitus, IUGR: intrauterine growth retardation, NGAL: Neutrophil Gelatinase-Associated Lipocalin, PROM: premature rupture of membranes, RDS: respiratory distress syndrome, SD: standard deviation.

When healthy term infants and non-healthy infants were compared, there was no statistically significant difference between cord blood NGAL levels (p>0.05). In our study, there

was no statistically significant difference between NGAL levels in infants of mothers with gestational diabetes mellitus, acyanotic congenital heart diseases (VSD, ASD, and other), meconium aspiration syndrome, infants of mothers with preeclampsia, and infants of mothers with oligohydramnios (p>0.05).

In this study, there was no statistically significant difference between NGAL levels and Apgar scores (p>0.05). The relationship between Apgar scores and cord blood NGAL levels of the babies included in the study is shown in Table 3.

Table III: The relationship between Apgar scores and NGAL levels

	NGAL (ng/mL)		
	Mean ± SD	Mean ± SD	
	Yes	No	p
Apgar (1. min) <5	1608.1±1744.4	1244.8±1458.7	0.27
Apgar (1. min) 5-7	1228.6±1473.8	1361.1±1538.4	0.56
Apgar (1. min) 7>	1269.9±1431.1	1302.7±1532.1	0.89
Apgar (5. min) <5	-	1293.2±1499.6	-
Apgar (5. min) 5-7	1327.7±1537.6	1279.9±1490.6	0.84
Apgar (5. min) 7>	1278.2±1496.3	1331.1±1522.4	0.83

NGAL: Neutrophil Gelatinase-Associated Lipocalin. SD: standard deviation

DISCUSSION

High NGAL levels in body fluids have been associated with many diseases, especially acute kidney injury[8-11]. Parravicini et al.[4] reported that urinary NGAL level is a promising candidate as an early biomarker for sepsis in 91 very low birth weight infants. In this study, it was stated that urinary NGAL level is an early diagnostic and prognostic biomarker in detecting sepsis.

Pynn et al.[11] showed urinary NGAL level as a non-invasive biomarker with a high negative predictive value (NPV) in the evaluation of late-onset sepsis in newborns. In a systematic review and meta-analysis, a statistically significant difference was found between the risk factors for sepsis, male gender, gestational age below 37 weeks, and premature rupture of membranes and cord blood NGAL levels[12].

Prolonged PROM is considered to be risky for sepsis. Therefore, it suggests that NGAL elevation seen in prolonged PROM may be a precursor for the risk of sepsis. In our study, we showed that cord blood NGAL levels were statistically high in infants of mothers with prolonged PROM.

In a study of a group of neonates with a history of intrauterine hypoxia, Essajee et al.[13] showed that urinary NGAL level is a predictor of hypoxic encephalopathy and mortality. Fiala et al.[14] found similar results in another study. According to this study, they found that NGAL has the potential to be a good marker for perinatal hypoxia, as well as having high sensitivity and specificity.

Recent reports have revealed that NGAL concentration is increased in cardiovascular disease and after episodes of hypoxia. Surmiak et al.[15] hypothesized that high plasma NGAL levels might be a result of vascular endothelial damage due to perinatal asphyxia and reported that NGAL measurement in umbilical cord blood could be a valuable biomarker in the diagnosis of perinatal asphyxia in newborns. In our study, we showed that, as in previous studies, NGAL levels increased in hypoxia conditions and cord blood NGAL levels were statistically higher in IUGR patients, which is one of the risk factors for asphyxia. It can be thought that especially cord blood NGAL levels may indicate IUGR fetuses exposed to antenatal hypoxia.

In a study conducted by Chen et al. in 24 preterm and 38 term babies, they found that there was no statistically significant difference between gestational age and birth weight and

urinary NGAL levels in preterm babies[16]. In the same study, it was shown that urinary NGAL levels were higher in female term infants than male term infants. On the contrary, another study reported that NGAL levels were higher in female preterm infants and decreased with increasing gestational age[17]. However, in our study, we showed that gestational age, gender, birth weight and NGAL levels were not statistically high. Similar to our study, Mikulić et al.[18] also showed that there was no significant difference in urinary NGAL concentration in ≥37 older newborns in the first 72 hours postnatally in relation to gender, postnatal age, and birth weight.

In a study, it was shown that maternal serum NGAL levels were significantly higher in the first and second trimesters in pregnancies developing preeclampsia[19]. However, NGAL levels in the umbilical cord blood of babies of preeclamptic mothers were not statistically high in our study.

In clinical use, there is always a need for markers that categorize patients according to risk categories so as to make the diagnosis early, predict complications and adverse outcomes, and guide treatment. In this regard, NGAL's taking its place in routine use as a diagnostic biomarker in a short time will make a great contribution to clinical utility and the development of new treatment approaches.

The desired number could not be reached in some patient groups, in our study. In addition, the limited number of similar studies on this biomarker reduces the comparability of our study. In order to definitively determine the effect of specific perinatal risk factors on cord blood NGAL levels, multivariate analysis or more comprehensive studies with a large number of newborns with isolated specific risk factors are needed.

In our study, we investigated whether there is a relationship between cord blood NGAL levels and neonatal diseases. Since there are few studies examining cord blood NGAL levels, our study has been compared with studies examining urine and serum NGAL levels and their relationship with certain diseases. However, studies with larger patient populations are needed in this regard.

Ethical Committee Approval: Dicle University Ethics committee for non interventional studies approval was obtained before starting this study. (2015 revision 60 / 13.03.2017).

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Declaration of Conflicting Interests: There is no conflict of interest

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