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

Background: The aim of this study was to investigate the relationship between umbilical cord blood neutrophil gelatinase-associated lipocalin and neonatal diseases, such as acute kidney injury. Materials and Method: The neutrophil gelatinase-associated lipocalin levels were measured in umbilical cord blood of 180 babies born in the department of Obstetrics and Gynecology between 2015-2016. Patients were classified according to maternal diseases, neonatal diseases, and demographic features. The obtained data were compared with umbilical cord blood neutrophil gelatinase-associated lipocalin levels. Results: There was a statistically significant difference between umbilical cord blood NGAL levels and premature rupture of membranes ($p<0.05$), ABO incompatibility ($p<0.05$), meconium aspiration syndrome ($p<0.001$), ventricular septal defect ($p<0.001$), and breech presentation birth ($p<0.001$). Conclusion: neutrophil gelatinase-associated lipocalin can be useful as a diagnostic biomarker in the evaluation of maternal and neonatal diseases. However, further studies on larger patient populations are needed.

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Conclusion: neutrophil gelatinase-associated lipocalin can be useful as a diagnostic biomarker in the evaluation of maternal and neonatal diseases. However, further studies on larger patient populations are needed.

What's already known about this topic?

The studies, it has been observed that NGAL plays a critical role in the transformation of the leading cells in the developing kidney into epithelial and tubule cells. It has been shown that the concentration of this protein in serum increases dramatically if epithelial organs are damaged by ischemia-reperfusion or sepsis.

What does this article add?

The NGAL levels in umbilical cord blood, which may be a marker for the diseases that occur in the neonatal period. Umbilical cord blood NGAL levels may also be related to maternal diseases and perinatal and neonatal morbidities.

Introduction

Neutrophil gelatinase-associated lipocalin (NGAL), also known as Lipocalin 2, is a protein that belongs to the lipocalin protein family and weighs 25 kDa. It is covalently bonded to gelatinase in neutrophils, takes part in iron transport, and is released at very low levels from many other tissues such as kidney, lung, stomach, colon as well as neutrophils.[(1)]

According to the experimental animal studies conducted recently, NGAL is released in the kidneys in the early stages of ischemic injury. With these studies, it has been observed that NGAL plays a critical role in the transformation of the leading cells in the developing kidney into epithelial and tubule cells. It has been shown that the concentration of this protein in serum increases dramatically if epithelial organs are damaged by ischemia-reperfusion or sepsis.

To date, elevated serum and urine NGAL levels in neonates have been associated with intrauterine hypoxia, sepsis, bronchopulmonary dysplasia, and acute kidney injury (AKI). [(2-6)]Although data on umbilical cord blood NGAL concentrations are insufficient, it was demonstrated that NGAL measurements could be predictive for certain diseases, such as neonatal infections.[(7)]

Until now, no accepted reference NGAL values have been determined for neonates. Different serum and urine NGAL levels have been reported in studies by different researchers.

In our study, we aimed to investigate NGAL levels in umbilical cord blood, which may be a marker for the diseases that occur in the neonatal period. Umbilical cord blood NGAL levels may also be related to maternal diseases and perinatal and neonatal morbidities.

Material and Methods

We used the umbilical cord blood of 180 babies born in Gynecology and Obstetrics Clinic in 2015-2016. This prospective study was approved by the Dicle University medical school ethics committee.

Umbilical cord blood samples were collected from neonates who born in Dicle University Obstetrics and Gynecology Clinic. The blood samples were centrifuged for 10 minutes at 4000 rpm and were kept at -20 °C. Serum NGAL was studied on these 180 blood samples obtained. Serum NGAL levels were measured by the enzyme-linked immunosorbent assay (ELISA) technique via a commercial kit (SunRed® Biotechnology Company).

The gestational ages of the babies were determined according to the last menstrual period or the New Ballard Score. Those with a gestational age below 37 weeks were classified as preterm, those with a gestational age between 37-42 weeks were classified as term, and those with a gestational age over 42 weeks were classified as postterm. There were no babies in our study older than 42 weeks. The babies were divided as 28 weeks below, 28-31 weeks, 32-37 weeks, and 37 weeks and over according to their gestational ages. They were also divided as 750 grams and below, 750-1200 grams, 1200-1800 grams, 1800-2500 grams, and 2500 grams and over according to their birth weights.

Exclusion criteria:

1- Having chromosomal disorders (such as trisomy 13, 18, 21) 2- Having major congenital malformations 3- Stillbirth 4- Preterm babies born at <22 gestational week.

Statistical Analysis

In the statistical evaluation of our research data, IBM SPSS 21.0 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 standard deviation. Hypotheses were two-sided; p[?]0.05 was considered as statistically significant, p[?]0.01 highly significant, and p[?]0.001extremely significant.

Results

In total, 180 babies were included in the study. 58.9% of the babies were boys, and 41.1% were girls; 96.7% were delivered via cesarean section, and 3.3% were delivered via normal birth. Of the babies included in the study, 74.4% were 2500 grams and over in birth weight, 60% were born at 37 weeks gestation or later, and 26.1% were born between 32-36 weeks.

The mean NGAL value of the boys was 1283.99 ng/mL, and the mean NGAL value of the girls was 1306.52 ng/mL. There was no statistically significant difference between gender and umbilical cord blood NGAL levels. There was also no statistically significant difference between childbirth type, birth weight, and gestational age and umbilical cord blood NGAL levels. The demographic characteristics of the babies included in the study and the relationship between these characteristics and umbilical cord blood NGAL levels are shown in table 1.

The most common postnatal disease of the babies included in the study was respiratory distress syndrome (RDS)(15.6%). Other common postnatal diseases were *neural tube defects(* NTD)(4.4%), congenital heart diseases(CHD)(3.3%), and blood incompatibility(2.2%).

The mean NGAL value of 28 babies diagnosed with RDS was 1083.36 ng/mL, and those without RDS had a mean NGAL value of 1331.92 ng/mL. There was no statistically significant difference between RDS and umbilical cord blood NGAL levels. There were statistically significant differences in umbilical cord blood NGAL levels between babies diagnosed with meconium aspiration syndrome or ventricular septal defect or ABO incompatibility and babies who had not been diagnosed with these diseases. The relationship between neonatal diseases and umbilical cord blood NGAL levels is shown in table 2.

The most common maternal disease of the mothers of the babies included in the study was oligohydramnios (8.9%). Other common maternal diseases were gestational diabetes mellitus (GDM) (6.1%) and hypertension (6.1%). 39.4% of the mothers were healthy.

There were no statistically significant differences in umbilical cord blood NGAL levels between mothers diagnosed with preeclampsia or placenta previa or GDM or oligohydramnios and mothers who had not been diagnosed with these diseases. However, there was a statistically significant difference between premature rupture of membranes (PROM) and umbilical cord blood NGAL levels. The relationship between maternal diseases and umbilical cord blood NGAL levels is shown in table 3.

There was no statistically significant difference between Apgar scores and umbilical cord blood NGAL levels. The relationship between Apgar scores and umbilical cord blood NGAL levels is shown in table 4.

Discussion

High NGAL levels in body fluids have been associated with many diseases especially acute kidney injury.[8,9,10] The risk of acute kidney injury (AKI) is higher in neonates than in other groups.[11,12,13] For this reason, early detection of AKI in neonates is vital. Current biomarkers in use are too late to show kidney injury. Studies have shown that NGAL can be used as a biomarker for early detection of AKI.[14] In addition, it was found valuable in other cases with ischemia and related tissue damage.

In the study of Krawczeski et al.[15],urine NGAL levels of neonates were significantly increased at the second hour after cardiopulmonary bypass surgery, while there were no changes in serum creatinine levels. If the cutoff value was taken as 185 ng/ml, the sensitivity and specificity of urine NGAL for AKI were 100% and 93%, respectively. It was stated that urine NGAL was an early and reliable marker in the diagnosis of neonatal AKI compared to serum creatinine. In another study conducted in critically ill neonates, serum NGAL levels increased earlier than serum creatinine in neonates who developed AKI. It was shown that, with or without sepsis, NGAL could be used as an early marker in neonatal AKI cases.[16] However, in a more recent study, Reiter et al.[17] stated that plasma and urine NGAL levels were not reliable markers for developing AKI in neonates after cardiac surgery with cardiopulmonary bypass. In our study, there was no patient diagnosed with AKI. They attributed increased NGAL levels to inflammation following cardiac bypass. As supported by these studies, NGAL increases in ischemic and inflammatory events, and this is only noticed when organ functions are affected. However, NGAL can be used in the early detection of organ damage. In our study, there was no statistically significant difference, such as low birth weight, prematurity and low Apgar score, and umbilical cord blood NGAL levels.

Sepsis is one of the major causes of AKI in neonates. In a study conducted by Pynn et al.[18], urine NGAL was pointed out as a non-invasive biomarker with high negative predictive value at the time of late-onset sepsis assessment in neonates. According to a study conducted by Parravicini et al.[4], on 91 very low birth weight infants, urine NGAL is a promising candidate as an early biomarker for sepsis and it was also shown that urinary NGAL levels was an early biomarker to detect sepsis. In our study, there was no statistically significant difference between the risk factors of sepsis, such as male gender, gestational age below 37 weeks and umbilical cord blood NGAL levels; however, there was a statistically significant difference between PROM, which is also a risk factor in sepsis, and umbilical cord blood NGAL levels. [18]

In a group of neonates with intrauterine hypoxia, Essajee et al.(20) found that urine NGAL is a predictor of mortality and hypoxic encephalopathy. Similar results were found in another study conducted by Fiala et al. [21]. According to this study, NGAL has the potential to be a good marker for perinatal hypoxia and has high sensitivity and specificity. In our study, there was no statistically significant difference between the risk factors of asphyxia, such as cesarean section, low birth weight and preterm labor, and umbilical cord blood NGAL levels.

In a study conducted by Chen et al. [22] on 24 preterm and 38 term infants, it was found that there was no statistically significant difference between gestational age and birth weight and urine NGAL levels in preterm infants. The same study found that urine NGAL values of the female term infants were higher compared to male term infants. Another study reported that NGAL concentrations were higher in preterm female infants and decreased with higher gestational age.[23] In our study, there was no statistically significant difference between gestational age, gender, birth weight and umbilical cord blood NGAL levels.

In clinical practice, there is always a need for markers that predict complications and divide patients into risk categories so that they can be diagnosed and treated early. If NGAL takes its place in routine use as a diagnostic biomarker, it will contribute greatly to the development of new treatment approaches. In our study, the desired number of patients could not be reached in some groups. Also, the limited number of similar studies on this biomarker reduces the comparability of our study. In order to accurately determine the effects of specific perinatal risk factors on umbilical cord blood NGAL levels, multivariate analyses and comprehensive studies in large numbers of neonates with specific risk factors isolated are required.

In this study, the relationship between umbilical cord blood NGAL levels and neonatal diseases was examined. As there are only a few studies in the literature on umbilical cord blood NGAL levels, this study was compared with studies that examined urine and serum NGAL levels and their relationship to certain diseases. However, further studies on larger patient populations are needed.

Table 1: The relationship between demographic characteristics and NGAL levels

		NGAL (ng/mL)	**NGAL (ng/**
Demographic characteristics of the babies	Demographic characteristics of the babies	**n(%)**	**Mean±SD**
Childbirth types	Normal birth	6(3.3)	1345.77±1740
	Cesarean section	174(96.7)	1291.44±1496
Gender	Male	106(58.9)	1283.99±1405
	Female	74(41.1)	1306.52±1634
Birth weight (grams)	750 grams below	2(1.1)	509.38±189.08
	750-1199 g	5(2.8)	1342.53±1512
	1200-1799 g	9(5)	1362.35±1836
	1800-2499 g	30(16.7)	1657.33±1884
	2500 g and over	134(74.4)	1216.96±1391
Gestational age at birth (weeks)	28 weeks below	5(2.8)	1151.96±1579
	28-31 w	20(11.1)	1348.89±1395
	32-36 w	47(26.1)	1360.55±1687
	37 w and over	108(60)	1260.20±1447

Table 2: The relationship between neonatal diseases and NGAL levels

	NGAL (ng/mL) With(mean±SD)	**NGAL (ng/mL) Without(Mean±SD)**	**NGAL (ng/mL) p**
Preterm	1311.95±1560.36	1280.79±1465.05	0.892
Term- Neonates with any problems	1659.17±1918.02	1228.68±1411.11	0.170
Term- Neonates without any problems	1177.76±1276.76	1383.59±1653.91	0.362
Macrosomia (Over 4000 grams)	1205.85±749.97	630.50±370.03	0.907
RDS	1083.36±1171.19	1331.92±1552.76	0.422
NTD	1050.91±1567.15	1304.52±1500.26	0.641
Rh incompatibility	727.58±618.33	1306.11±1512.07	0.447
ABO incompatibility	3013.76±2570.63	1264.09±1470.01	**0.045**
IUGR	1913.46±2833.54	1275.53±1455.22	0.350
Renal pathology	584.97±-.	1297.21±1502.93	-
Thrombocytopenia	607.64±129.42	1304.87±1509.63	0.426
MAS	762.97±15.50	1299.21±1507.05	**0.000**
VSD	598.45±130.01	1305.03±1509.56	**0.000**
ASD	1683.79±1837.32	1286.63±1498.77	0.650
Other CHDs	1468.02±1184.73	1287.23±1511.73	0.772
Breech birth	411.81±177.45	1334.25±1521.47	**0.000**
Multiple birth	924.89±583.49	1308.16±1524.10	0.442

ASD: *Atrial septal defect,* ***CHD:*** *Congenital heart disease,* ***IUGR:*** *Intrauterine growth restriction,* ***MAS:****Meconium aspiration syndrome,* ***NTD:*** *Neural tube defect,****RDS:*** *Respiratory distress syndrome,* ***VSD:*** *Ventricular septal defect.*

Table 3: The relationship between maternal diseases and NGAL levels

	n(%)	NGAL (ng/mL) With(mean±SD)	NGAL (ng/mL) Without(Mean±SD)	NGAL (ng/mL) p
Preeclampsia	31(17.2)	1635.97±1856.55	1221.94±1411.26	0.163
Placenta previa	27(15)	882.43±759.34	1365.75±1585.74	0.123
Oligohydramnios	16(8.9)	1470.87±1496.03	1275.92±1503.45	0.621
Hypertension	11(6.1)	1056.62±891.19	1308.65±1531.36	0.591
Gestational diabetes mellitus	11(6.1)	1289.74±1133.50	1293.48±1523.07	0.994
Anhydramnios	6(3.3)	1706.05±2589.66	1279.01±1458.44	0.494
Premature rupture of membranes	6(3.3)	2594.19±2037.13	1248.39±1465.03	**0.034**
Polihydramnios	5(2.8)	506.26±119.33	1315.73±1514.91	0.235
HELLP syndrome	4(2.2)	1322.06±1523.55	1292.59±1503.52	0.969
Hypothyroidism	4(2.2)	801.94±375.49	1304.41±1514.04	0.509
Thrombocytopenia	4(2.2)	1134.94±1139.88	1296.85±1509.15	0.832
Nephrolithiasis	4(2.2)	1224.54±1024.30	1294.81±1510.72	0.926
Hepatitis B	3(1.7)	726.58±416.31	1302.85±1509.89	0.511
Splenectomy	3(1.7)	605.39±128.86	1304.91±1509.61	0.425
Heart disease	2(1.1)	531.01±299.03	1301.81±1505.74	0.471
Chorioamnionitis	2(1.1)	3100.19±3320.83	1272.95±1474.71	0.087
Renal ectasia	1(0.6)	495.46±-	1297.71±1502.67	0.595
Ventriculoperitoneal Shunt	1(0.6)	1592.64±-	1291.58±1503.70	0.842
Other diagnoses	36(20)	1139.14±1233.31	1331.78±1560.57	0.492

Table 4: The relationship between Apgar scores and NGAL levels

	NGAL (ng/mL) With (mean±SD)	NGAL (ng/mL) Without(Mean±SD)	p
Apgar (1^{st}minute) <5	1608.03±1744.42	1244.83±1458.76	0.271
Apgar (1^{st} minute) 5-7	1228.68±1473.83	1361.05±1538.45	0.562
Apgar (1^{st} minute) 7>	1269.98±1431.04	1302.71±1532.04	0.895
Apgar (5^{th} minute) <5	-	1293.25±1499.66	-
Apgar (5^{th} minute) 5-7	1327.73±1537.66	1279.99±1490.62	0.849
Apgar (5^{th}minute) 7>	1278.27±1496.30	1331.14±1522.40	0.832

The captions to tables

Table 1. The relationship between demographic characteristics and NGAL levels

Table 2. The relationship between neonatal diseases and NGAL levels

Table 3. The relationship between maternal diseases and NGAL levels

Table 4. The relationship between Apgar scores and NGAL levels

References

1. American Society of Nephrology. Renal Research Report. J Am Soc Nephrol 2005;16:1886–1893.
2. Bellos I, Fitrou G, Daskalakis G, Perrea DN, Pergialiotis V. Neutrophil gelatinase-associated lipocalin as predictor of acute kidney injury in neonates with perinatal asphyxia: a systematic review and meta-analysis. *Eur J Pediatr.* 2018;177(10):1425-1434.
3. Raggal NE, Khafagy SM, Mahmoud NH, Beltagy SE. Serum neutrophil gelatinase-associated lipocalin as a marker of acute kidney injury in asphyxiated neonates. *Indian Pediatr.* 2013;50(5):459-462.

4. Parravicini E, Nemerofsky SL, Michelson KA, et al. Urinary neutrophil gelatinase-associated lipocalin is a promising biomarker for late onset culture-positive sepsis in very low birth weight infants. *Pediatr Res.* 2010;67(6):636-640.
5. Ertuğrul S, Annagur A, Kurban S, Altunhan H, Ors R. Comparison of urinary neutrophil gelatinase-associated lipocalin, C-reactive protein and procalcitonin in the diagnosis of late onset sepsis in preterm newborns. *J Matern Fetal Neonatal Med.* 2013; 26(4): 430-3.
6. Inoue H., Ohga S., Kusuda T., Kitajima J., Kinjo T., Ochiai M., Takahata Y., Honjo S., & Hara T. (2013). Serum neutrophil gelatinase-associated lipocalin as a predictor of the development of bronchopulmonary dysplasia in preterm infants. *Early human development*, *89*(6), 425–429.
7. Kisiel A, Roszkowska-Blaim M, Pańczyk-Tomaszewska M, Stelmaszczyk-Emmel A, Górska E, Borszewska-Kornacka M. Effect of perinatal risk factors on neutrophil gelatinase-associated lipocalin (NGAL) level in umbilical and peripheral blood in neonates. *Cent Eur J Immunol.* 2017;42(3):274–280.
8. Medić B, Rovcanin B, Vujovic KS, Obradovic D, Duric D, Prostran M. Evaluation of Novel Biomarkers of Acute Kidney Injury: The Possibilities and Limitations. *Curr Med Chem.* 2016;23(19):1981-1997.
9. Suchojad A., Tarko A., Smertka M., Majcherczyk M., Brzozowska A., Wroblewska J., & Maruniak-Chudek I. (2015). Factors limiting usefulness of serum and urinary NGAL as a marker of acute kidney injury in preterm newborns. *Renal failure*, *37*(3), 439–445.
10. Al-Ismaili Z, Palijan A, Zappitelli M. Biomarkers of acute kidney injury in children: discovery, evaluation, and clinical application. Pediatr Nephrol. 2011;26:29–40.
11. Selewski D. T. Charlton, J. R. Jetton, J. G. Guillet, R. Mhanna, M. J. Askenazi D. J., & Kent A. L. (2015). Neonatal Acute Kidney Injury.*Pediatrics*, *136*(2), e463–e473.
12. Andreoli SP. Acute renal failure in the newborn. Semin Perinatol. 2004;28:112–123.
13. Malyszko J. Biomarkers of Acute Kidney Injury in Different Clinical Settings: A Time to Change the Paradigm. Kidney Blood Press Res 2010;33(5):368-82.
14. Koralkar R, Ambalavanan N, Levitan EB, McGwin G, Goldstein S, Askenazi D. Acute kidney injury reduces survival in very low birth weight infants. Pediatr Res. 2011;69(4):354–358pmid:21178824
15. Krawczeski CD, Woo JG, Wang Y, Bennett MR, Ma Q, Devarajan P. Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass. *J Pediatr.* 2011;158(6):1009-1015.e1.
16. El-Farghali OG, El-Raggal NM, Mahmoud NH, Zaina GA. Serum neutrophil gelatinase-associated lipocalin as a predictor of acute kidney injury in criticallyill neonates. Pak J Biol Sci 2012;15(5):231-7.
17. Reiter K., Balling G., Bonelli V., Pabst von Ohain, J., Braun S., Ewert P., & Ruf, B. (2018). Neutrophil gelatinase-associated lipocalin reflects inflammation and is not a reliable renal biomarker in neonates and infants after cardiopulmonary bypass: A prospective case–control study. Cardiology in the Young, 28(2), 243-251.
18. Pynn JM, Parravicini E, Saiman L, Bateman DA, Barasch JM, Lorenz JM. Urinary neutrophil gelatinase-associated lipocalin: potential biomarker for late-onset sepsis. *Pediatr Res.* 2015;78(1):76–81.
19. Murthy S, Godinho MA, Guddattu V, Lewis LES, Nair NS. Risk factors of neonatal sepsis in India: A systematic review and meta-analysis.*PLoS One.* 2019;14(4):e0215683.
20. Essajee F, Were F, Admani B. Urine neutrophil gelatinase-associated lipocalin in asphyxiated neonates: a prospective cohort study.*Pediatr Nephrol.* 2015;30(7):1189–1196.
21. Fiala M, Baumert M, Surmiak P, Walencka Z, Sodowska P. Umbilical markers of perinatal hypoxia. *Ginekol Pol.* 2016;87(3):200–204.
22. Chen C-N., Chou C-H., Jeng S-F., Tsai I-J., Chen P-C., Chen C-Y., Chou H-C., Tsao P-N., & Hsieh W-S. Urinary Neutrophil Gelatinase-Associated Lipocalin Levels in Neonates. *Pediatr Neonatol.* 2016;57(3):207-212.
23. Saeidi B, Koralkar R, Griffin RL, Halloran B, Ambalavanan N, Askenazi DJ. Impact of gestational age, sex, and postnatal age on urine biomarkers in premature neonates. *Pediatr Nephrol.* 201