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Original Article

Investigation of Maternal Serum NGAL (Neutrophil Gelatinase-Associated Lipocalin) Levels in Cases of Fetal Pyelectasis

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ABSTRACT

Objective: This study aims to investigate maternal serum NGAL (Neutrophil Gelatinase-Associated Lipocalin) levels in pregnant women diagnosed with fetal pyelectasis during antenatal follow-up and to evaluate the relationship between fetal pyelectasis and maternal serum NGAL levels.

Methods: This prospective study included 39 pregnant women diagnosed with fetal pyelectasis at 20–22 weeks of gestation and 33 pregnant women without pyelectasis as the control group. Fetal pyelectasis was classified based on the anteroposterior diameter of the renal pelvis: 4–7 mm as "mild pyelectasis" and 7-10 mm as "moderate pyelectasis." Maternal blood samples were collected from both groups, and serum NGAL levels were measured using the ELISA method and analyzed statistically.

Results: The fetal pyelectasis group was further categorized into mild and moderate subgroups. NGAL levels showed a significant difference across the groups (p=0.008). The moderate pyelectasis group had significantly higher NGAL levels compared to the control group (p=0.005), whereas the mild pyelectasis group did not show a significant difference. Multinomial logistic regression analysis demonstrated that NGAL levels increased with the severity of pyelectasis.

Conclusion: Maternal serum NGAL levels were found to be associated with the severity of fetal pyelectasis, suggesting its potential as a non-invasive biomarker. However, further studies with larger sample sizes are needed to validate its use in prenatal diagnosis.

INTRODUCTION

Fetal pyelectasis refers to a condition characterized by the dilation of the fetal renal pelvis, measured in the anteroposterior (AP) diameter. According to the Fetal Urology Society, an AP diameter measurement exceeding 4 mm in the second trimester or 7 mm in the third trimester is considered diagnostic.[1] It is one of the most commonly detected anomalies on antenatal ultrasound, occurring in 1–5% of all pregnancies.[2] While unilateral pyelectasis has a higher incidence, it can occur either unilaterally or bilaterally.[3,4]

With the routine use of ultrasound in prenatal follow-ups, cases of fetal pyelectasis can be identified before complications such as renal dysfunction develop.[1] Moreover, pyelectasis detected during the second trimester ultrasound is recognized as a marker for aneuploidy.[5,6] While the condition may arise from numerous pathological states, it can also be entirely physiological.[7] The prenatal and postnatal management of affected patients remains controversial, ranging from close monitoring of all affected individuals to disregarding pelvic dilations smaller than 15 mm.[8,9] Although fetal pyelectasis is defined under a single heading, the reported prevalence and strength of its association with adverse outcomes vary significantly.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa polypeptide produced in various tissues and laboratory animals. It was initially identified as a gelatinase-as-

sociated protein secreted by neutrophils.[10] Lipocalin proteins bind and transport small molecules, and one of NGAL's key functions is believed to involve binding bacterial siderophores and facilitating iron transport into cells.[11] NGAL can be induced in both neutrophils and epithelial cells in response to various stimuli, including inflammation, infections, and neoplasia. It has been shown to have protective roles against infection and ischemic kidney injury.[12]

In healthy individuals, NGAL is produced at low levels across various tissues, filtered in the glomerulus, and subsequently reabsorbed by the proximal tubules. In response to various pathological conditions, NGAL can also be produced by other tissues, including nephrons. For instance, following acute kidney injury, NGAL production is induced in the distal segments of the nephron and released into the blood and lumen, leading to elevated levels in both blood and urine.[13] In addition to the increased NGAL production in the kidneys following injury, nephron damage disrupts proximal tubular reabsorption, further increasing NGAL presence in the urine.[12,14]

This study aims to investigate serum NGAL levels in pregnant women diagnosed with fetal pyelectasis during antenatal follow-up. The objective is to examine the relationship between fetal pyelectasis detected through ultrasound during pregnancy and maternal serum NGAL levels, focusing on its potential utility in prenatal diagnosis and its ability to predict renal pathology. We believe that understanding this relationship could be beneficial in both elucidating the disease's pathophysiology and predicting prognosis during clinical follow-up processes.

MATERIALS AND METHODS

This study was designed as a prospective study conducted with patients who applied to the Perinatology Department at Etlik City Hospital in 2024 for second-trimester ultrasonographic screening. Ethics committee approval was received from the Local Ethics Committee (approval number: AEŞH-BDK-2024-1160). The study was conducted in accordance with the principles of the Declaration of Helsinki. A written informed consent was obtained from the patients.

Maternal blood samples were collected from pregnant women aged 18–45 years who applied to the perinatology clinic and were in the second trimester of pregnancy. Pregnant women who were detected to have fetal pyelectasis at 20–22 weeks of gestation (second trimester) and met the inclusion criteria were included in the fetal pyelectasis group. Pregnant women who did not have fetal pyelectasis and met the inclusion criteria were included in the control group. Patients with known acute or chronic renal failure, pre-existing renal disease, urinary or systemic infections, and those using anti-inflammatory medications were excluded from the study. NGAL serum levels in the second trimester of both groups were compared statistically. Neonatal outcomes and demographic records were obtained from the hospital information system.

During the detailed fetal ultrasound scan, the anteroposterior diameter of the renal pelvis was measured to diagnose fetal pyelectasis. Measurements were taken when the fetus was in a supine position with an empty bladder. The anteroposterior diameter was measured at the midpoint of the kidneys in the coronal plane, and the wider diameter was recorded. Fetal pyelectasis was defined as an anteroposterior diameter of 4 mm or more between 20 and 22 weeks of gestation. It was classified as "mild pyelectasis" for measurements between 4 and 7 mm, "moderate pyelectasis" for measurements between 7 and 10 mm, and "severe pyelectasis" for measurements greater than 10 mm. The classification of fetal pyelectasis is shown in Appendix.

Blood samples were collected from the mothers of fetuses diagnosed with pyelectasis during the anomaly scan. These samples were centrifuged at 5000 rpm for 10 minutes, and the obtained plasma was stored at -80 °C. The analysis of NGAL levels was performed using the ELISA method. A HUMAN brand COMBI WASH model was used for washing, and a NEXT LEVEL brand ALISEI model was used for reading the results. The raw values obtained from the analysis were indicated as "conc." According to the kit instructions, the raw results were multiplied by the dilution factor to obtain the final results. The dilution ratio was 1/100, meaning the raw results were multiplied by 100. The final test results were expressed in ng/mL.

Statistical Analyses

Statistical analyses were performed using SPSS version 29.0. The normality of data distribution was assessed with the Kolmogorov-Smirnov test. Student's t-test was used for normally distributed data, while the Mann-Whitney U test was used for data that did not meet normality criteria. Categorical data were analyzed using the Chi-square test or Fisher's exact test. The comparison of NGAL levels between groups was conducted using the Kruskal-Wallis test, and differences between groups were assessed using the Dwass-Steel-Critchlow-Fligner method. Furthermore, a multinomial logistic regression analysis was performed to evaluate the relationship between NGAL levels and fetal pyelectasis, with a p-value of <0.05 considered statistically significant.

RESULTS

Participants were excluded due to lack of accessible birth and neonatal outcomes, presence of urinary system anomalies accompanying pyelectasis, additional fetal anomalies or chromosomal abnormalities, maternal pyelectasis, or urinary tract infections. Additionally, samples that gave erroneous results due to faulty analysis kits were excluded from the study. There were 39 pregnant women who met the inclusion criteria in the fetal pyelectasis group and 33 in the control group. In the fetal pyelectasis group, there were 33 patients in the mild pyelectasis subgroup and 6 patients in the moderate pyelectasis subgroup.

The demographic data, first trimester screening results, pregnancy complications, and neonatal outcomes of both groups are compared in Table 1. There was no significant difference in maternal age, gravidity, parity, or gestational age at the time of blood sampling between the groups (all p>0.05). The maternal body mass index (BMI) at booking showed a trend toward being lower in the fetal pyelectasis group compared to the controls (28.5 vs. 30.8, p=0.053). First-trimester screening markers, including free beta-hCG MoM, PAPPA MoM, and NT MoM, did not differ significantly between the groups (p=0.193, p=0.685, and p=0.187, respectively). Regarding pregnancy complications, the rates of fetal growth restriction (FGR), preterm birth, and preterm membrane rupture were similar between the groups (p>0.05). The mode of delivery (C-section vs. vaginal delivery) did not differ significantly between the fetal pyelectasis group and controls (p=0.502). Neonatal outcomes, including gestational age at birth, birthweight, and APGAR scores at the 1st and 5th minutes, showed no significant differences between the two groups (all p>0.05).

The outcomes of the Kruskal-Wallis test and pairwise comparisons of NGAL levels between the control group and the mild and moderate fetal pyelectasis subgroups are shown in Table 2. The Kruskal-Wallis test revealed a significant difference in NGAL levels across the three groups (χ^2=9.58, p=0.008, effect size=0.128). Pairwise comparisons using the Dwass-Steel-Critchlow-Fligner method indicated that NGAL levels were significantly higher in the moderate pyelectasis group compared to the control group (W=-4.46, p=0.005). However, no significant differences were observed between the mild pyelectasis group and controls (W=-1.97, p=0.344) or between the mild and moderate pyelectasis groups (W=-2.86, p=0.106).

Table 3 shows the results of the multinomial logistic regression analysis of NGAL levels in fetuses with mild and

Table 1. Maternal, pregnancy, and neonatal characteristics in fetal pyelectasis group and control group

	Fetal Pyelectasis Group (n=39)	Controls (n=33)	P value
Demographics			
Maternal age in years, median (IQR)	27.0 (24.5, 30.5)	28.0 (25.0, 31.0)	0.618
Gravida, median (IQR)	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	0.316
Paritiy, median (IQR)	1.0 (1.0, 2.0)	2.0 (1.0, 3.0)	0.304
Maternal body mass index at booking in Kg/m^2, median (IQR)	28.5 (26.1, 31.2)	30.8 (28.7, 34.3)	0.053
Smoker, n (%)	3 (7.7)	7 (18.9)	0.148
Gestational diabetes, n (%)	3 (7.7)	4 (11.1)	0.620
Gestational age at blood sampling in weeks, median (IQR)	22.0 (21.0, 23.0)	22.0 (21.0, 23.0)	0.560
NGAL Levels (ng/mL), median (IQR)	1.18 (0.81, 2.20)	0.88 (0.72, 1.22)	0.008
First Trimester Screening Results			
fβHcg MoM, median (IQR)	0.760 (0.490, 0.890)	0.890 (0.600, 1.590)	0.193
PAPPA MoM, median (IQR)	0.890 (0.710, 1.150)	0.840 (0.720, 1.100)	0.685
NT MoM, median (IQR)	0.740 (0.620, 0.840)	0.670 (0.560, 0.810)	0.187
Pregnancy Complications			
Fetal growth restriction, n (%)	2 (5.1)	2 (5.6)	0.934
Preterm birth, n (%)	6 (15.4)	4 (12.1)	0.690
Preterm membrane rupture, n (%)	6 (15.4)	7 (19.4)	0.643
Mode of delivery, n (%)			0.502
C Section	17 (43.6)	17 (51.5)	
Vaginal Delivery	22 (56.4)	16 (48.5)	
Neonatal Outcomes			
Gestational age at birth in weeks, median (IQR)	39.0 (37.0, 40.0)	38.0 (38.0,39.0)	0.699
Birthweight in grams, median (IQR)	3390 (3015, 3545)	3120 (2810, 3530)	0.116
Neonatal gender, n (%)			0.813
Female	20 (51.3)	16 (48.5)	
Male	19 (48.7)	17 (51.5)	
APGAR score at 1th minutes	9.0 (8.0, 9.0)	9.0 (8.0, 9.0)	0.458
APGAR score at 5th minutes	10.0 (9.0, 10.0)	10.0 (9.0, 10.0)	0.712

P<0.05 was considered statistically significant. IQR: Interquartile range, fβHcg: Free beta Human Chorionic Gonadotropin; PAPPA: Pregnancy-Associated Plasma Protein-A; NT: Nuchal Translucency; MoM: Multiples of the median.

Table 2. Kruskal-Wallis test and pairwise comparisons of Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels among fetuses with mild and moderate pyelectasis and control group

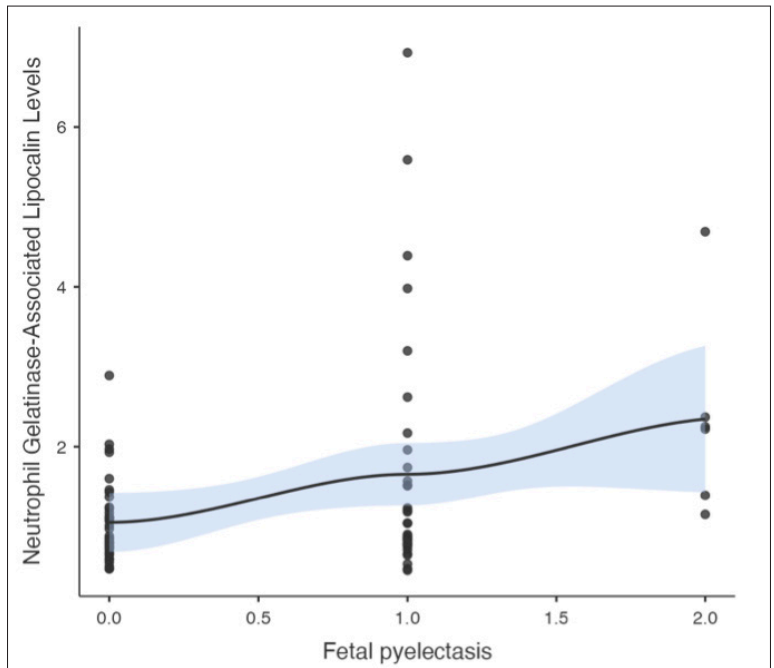
Kruskal-Wallis	χ^2	**P value**	**Effect Size**
Neutrophil Gelatinase-Associated Lipocalin Levels	9.58	0.008	0.128
Pairwise Comparisons (Dwass-Steel-Critchlow-Fligner)	**W Statistic**	**P value**	
Mild Pyelectasis vs. Controls	-1.97	0.344	
Moderate Pyelectasis vs. Controls	-4.46	0.005	
Moderate Pyelectasis vs. Mild Pyelectasis	-2.86	0.106	

p<0.05 was considered statistically significant.

Table 3. Multinomial logistic regression analysis of Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels in fetuses with mild and moderate pyelectasis compared to control group

	Estimate	**OR (95% CI)**	**P value**
Mild Pyelectasis vs. Controls	0.750	2.116 (1.064-4.207)	0.032
Moderate Pyelectasis vs. Controls	1.016	2.762 (1.237-6.165)	0.013

p<0.05 was considered statistically significant. OR: Odds ratio.



Note: *0.0 represents controls, 1.0 represents mild pyelectasis, and 2.0 represents moderate pyelectasis, showing the trend of NGAL levels with increasing severity.*

Figure 1. Relationship between fetal pyelectasis severity and Neutrophil Gelatinase-Associated Lipocalin (NGAL) level.

moderate pyelectasis compared to the control group. Mild pyelectasis was associated with a 2.116 times higher likelihood of elevated NGAL levels compared to controls (OR=2.116, 95% CI: 1.064–4.207, p=0.032). Moderate pyelectasis was associated with a 2.762 times higher likelihood of elevated NGAL levels compared to controls (OR=2.762, 95% CI: 1.237–6.165, p=0.013). Additionally, the relationship between fetal pyelectasis severity and NGAL levels is illustrated in Figure 1, which shows a positive trend of increasing NGAL levels with higher pyelectasis severity. This trend is particularly evident in the moderate pyelectasis group.

DISCUSSION

Recent developments have made biomarkers the focus of many studies aimed at predicting the outcome of renal damage and the need for surgery in fetuses and infants diagnosed with antenatal hydronephrosis. The NGAL biomarker is a glycoprotein that is secreted by renal tubule epithelial cells in stress and trauma situations and has a protective feature that reduces cellular damage.[15-17] It has been shown that patients diagnosed with antenatal hydronephrosis and undergoing surgery in the postnatal period have statistically higher serum NGAL levels.[18] In addition, it has been reported that NGAL levels are high in patients with obstructive ureteropelvic junction stenosis requiring pyeloplasty, and after surgical correction, NGAL levels decrease to the same levels as healthy individuals in the control group.[19,20] It has also been reported that NGAL levels are inversely proportional to split nerve function.[20] In the present study, a correlation was found between NGAL level and pyelectasis severity, which is consistent with the literature. Our findings align with studies reporting elevated NGAL levels in renal impairment. However, unlike previous studies using amniotic fluid or fetal blood samples, we detected elevated NGAL levels in maternal serum, raising questions about its origin and predictive value.

It is known that the 150 kDa Ig G molecule may cross the placenta; however, there is no conclusive evidence in the literature that the 25 kDa NGAL molecule can.[21] The fact that NGAL levels increased in parallel with the increase in the severity of fetal pyelectasis in our study may indicate that the NGAL biomarker molecule can pass through the placenta. It is not yet clear whether maternal NGAL levels truly originate from the fetus. Although our findings suggest a possible transplacental passage, further studies are required to confirm this hypothesis.

Even if we accept that fetal kidney damage can be predicted by NGAL levels measured in maternal serum, the situation may be complicated by increased NGAL levels in cases of maternal kidney damage, trauma, inflammation, or infection. Additionally, one paper highlighted that there was a notable rise in the maternal urine concentration of NGAL when asymptomatic maternal hydronephrosis was present.[22] In the present study, no disease that would naturally raise NGAL levels in pregnant women in the control and fetal pyelactasis groups was found. Therefore, we think that maternal NGAL elevation has been ruled out.

In cases of fetal kidney damage or dysfunction, elevated NGAL levels can normally be demonstrated in amniotic fluid or fetal blood. However, procedures such as cordocentesis or amniocentesis to access fetal blood or urine are invasive and require technical expertise. Our findings offer us optimism that maternal serum NGAL screening can be used to predict pyelectasis because the serum NGAL level in the pyelectasis group was shown to be statistically substantially higher than the control group.

The most important limitation of the study is that the NGAL level measured in the maternal blood sample was not confirmed simultaneously with the NGAL level in the fetal blood/urine. Because the presence of a mild infection or inflammation in the mother that could have gone unnoticed may have incorrectly affected the results of the study. Another limitation is that long-term postnatal follow-up was not performed, and it is not known how many patients went to surgery.

Conclusion

In conclusion, maternal serum NGAL levels were significantly higher in pregnancies with fetal pyelectasis compared to controls. This finding suggests that NGAL could potentially serve as a non-invasive biomarker for detecting fetal renal abnormalities. However, its origin from the fetus and clinical applicability remain uncertain. Future research should focus on confirming these findings and assessing their diagnostic value.

Ethics Committee Approval

The study was approved by the Ankara Etlik City Hospital Scientific Research Ethics Committee (Date: 11.12.2024, Decision No: AEŞH-BDK-2024-1160).

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: C.O.U.; Design: S.C.; Supervision: M.L.D.; Fundings: G.A.; Materials: B.T.C.; Data collection &/or processing: A.K.; Analysis and/or interpretation: C.O.U.; Literature search: U.B.; Writing: C.O.U.; Critical review: S.C.

Conflict of Interest

None declared.

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Fetal Piyelektazi Olgularında Maternal Serum NGAL (Nötrofil Gelatinaz İlişkili Lipokalin) Düzeylerinin Araştırılması

Amaç: Bu çalışma, antenatal izlem sırasında fetal piyelektazi tanısı alan gebelerde maternal serum NGAL (Neutrophil Gelatinase-Associated Lipocalin) seviyelerini araştırmayı ve fetal piyelektazi ile maternal serum NGAL seviyeleri arasındaki ilişkiyi değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: Bu prospektif çalışmaya, Etlik Şehir Hastanesi Perinatoloji Kliniğine başvuran ve 20-22. gebelik haftalarında fetal piyelektazi tanısı alan 39 gebe ve kontrol grubu olarak 33 gebe dahil edilmiştir. Fetal piyelektazi anteroposterior renal pelvis çapına göre sınıflandırılmıştır: 4-7 mm arası "hafif piyelektazi", 7-10 mm arası "orta piyelektazi" olarak tanımlanmıştır. Her iki gruptan alınan maternal kan örneklerinden serum NGAL seviyeleri ELISA yöntemiyle ölçülmüş ve istatistiksel analiz yapılmıştır.

Bulgular: Fetal piyelektazi grubu, hafif ve orta şiddet olarak alt gruplara ayrılmıştır. Gruplar arasında NGAL düzeyleri anlamlı bir farklılık göstermiştir (p=0.008). Orta şiddette piyelektazi grubu, kontrol grubuna kıyasla anlamlı şekilde daha yüksek NGAL düzeylerine sahipti (p=0.005). Hafif piyelektazi grubunda ise bu farklılık anlamlı bulunmamıştır. Çok değişkenli lojistik regresyon analizi, piyelektazi şiddeti arttıkça NGAL seviyelerinin arttığını göstermiştir.

Sonuç: Maternal serum NGAL düzeyleri, fetal piyelektazi şiddeti ile ilişkili bulunmuştur ve bu molekül, invaziv olmayan bir biyomarker olarak umut vaat etmektedir. Ancak, NGAL'nin prenatal tanıda kullanımına yönelik daha büyük hasta gruplarında ileri çalışmalar yapılması gereklidir.

Anahtar Sözcükler: Biyomarker; fetal piyelektazi; nötrofil gelatinazla ilişkili lipokalin (NGAL); prenatal tanı.