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Investigation of Risk Factors for Nephrocalcinosis and Nephrolithiasis in Very Low Birth Weight and Preterm Infants under 32 Weeks at NICU

Nahideh Ekhlasi[1], *Lida Ghorbani[1], Mehrdad Mirzarahimi[1], Khatereh Isazadehfar[2], Zahra Jafari[3]

1. Department of Pediatrics, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
2.Department of Community Medicine, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
3.School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.

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ABSTRACT

Introduction:
Nephrocalcinosis is a common complication in premature infants characterized by calcium deposition in the renal parenchyma. Multiple factors contribute to its development and it can lead to both short- and long-term complications. This study aimed to investigate the risk factors for nephrocalcinosis and nephrolithiasis in very low birth weight infants born before 32 weeks gestation at Alavi and Bu-Ali Hospitals in Ardabil.

Materials and Methods:
In this cross-sectional descriptive-analytical study, a total of 120 premature infants (30 cases and 90 controls) with gestational age less than 32 weeks or birth weight under 1500 g, admitted from April 2022 to August 2024, were assessed. Demographic characteristics, medications, blood and urine parameters, renal ultrasonography, and complications were evaluated. Statistical analysis was performed using Chi-square test, t-test, and multivariate logistic regression. Significance was set at $p < 0.05$.

Results:
Among 120 infants, 30 (25%) had nephrocalcinosis (cases), 12 (40%) of whom also had nephrolithiasis. Gestational age was lower in cases (26.16 ± 1.42 weeks vs. 28.95 ± 1.87 weeks, $p < 0.001$), birth weight was lower (907 ± 152 g vs. 1169 ± 187 g, $p < 0.001$), urine calcium-to-creatinine ratio > 0.8 (OR=8.45[CI:3.76-19.02]), family history of kidney disease (OR=5.12[95%CI:1.85-14.16]), mechanical ventilation > 10 days (OR=2.92[CI:1.71-4.99]), corticosteroid use (OR=3.21[CI:1.31-7.85]), and vancomycin use (OR=2.76[CI:1.14-6.67) were independent risk factors for nephrocalcinosis.

Conclusion:
Nephrocalcinosis is a relatively common complication in premature infants with multiple contributing risk factors. Identifying these risk factors helps in prevention, early diagnosis, and appropriate treatment.

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*Corresponding author: *E-mail: lindaghorbani2020@gmail.com*

Introduction

Nephrocalcinosis is a medical condition characterized by the widespread deposition of calcium salts in the renal parenchyma (the functional tissue of the kidney). It is typically detected through imaging studies and is often associated with disorders that alter calcium metabolism, such as hyperparathyroidism, renal tubular acidosis, or hypercalciuria. Nephrolithiasis refers to the process of forming or the presence of calculi (stones) within the kidneys or urinary tract. These stones are crystalline structures formed from minerals and salts in the urine, such as calcium oxalate or uric acid. Nephrolithiasis can cause symptoms like severe pain (renal colic), hematuria, and urinary tract infections, and it is a common cause of kidney-related morbidity. The most common form in premature infants is medullary nephrocalcinosis, visible on ultrasound as increased echogenicity of the renal pyramids, usually bilateral and symmetrical (1-3). It can result from various renal, infectious, metabolic, or endocrine causes. Systemic or metabolic origins tend to cause generalized involvement, primarily affecting the medulla and corticomedullary junction. Reported prevalence varies widely (7%–64%) depending on populations, ultrasound equipment, and diagnostic criteria (4).

The pathogenesis in preterm infants is multifactorial, involving an imbalance between promotors and inhibitors of stone formation. Predisposing factors include low gestational age, low birth weight, severe respiratory illness, high intake of calcium, phosphate, and ascorbic acid, prolonged total parenteral nutrition (TPN), and use of medications causing hypercalciuria like furosemide, corticosteroids, and methylxanthines.

Other associated factors include metabolic acidosis, hypocitraturia, hypercalciuria, high urinary oxalate-to-creatinine ratio, high urate-to-creatinine ratio, positive family history of kidney stones, and Caucasian ethnicity (5-7).

Identifying etiology and implementing appropriate treatment and prevention are crucial for preserving renal function. Advances in ultrasound technology have increased detection rates, but studies with large pediatric populations remain limited. Most cases resolve spontaneously within the first year of life, but a minority may have persistent disease for years. Short-term complications include urinary tract infection, kidney stones, hydronephrosis, hematuria, and colicky pain, while long-term risks involve impaired renal growth, reduced renal function, and hypertension (8-11). The primary purpose of performing ultrasound in this study was the early detection and monitoring of nephrocalcinosis and nephrolithiasis in very low birth weight preterm infants. As a non-invasive and accessible method, ultrasound allowed for the identification of calcium deposits in the renal parenchyma and the formation of urinary stones, enabling the researchers to assess the progression of this condition at two different time points (1 month of age and 40 weeks corrected gestational age). This study aimed to investigate the risk factors for nephrocalcinosis and nephrolithiasis in very low birth weight infants born before 32 weeks gestation at Alavi and Bu-Ali Hospitals in Ardabil.

Materials and Methods

This descriptive cross-sectional study was conducted in the NICU of Bu-Ali and Alavi Hospitals in Ardabil on 120 preterm infants with gestational age <32 weeks or birth weight <1500 g from April 2022 to August 2024. Census sampling was used.

Infants who died in the first week, had congenital renal anomalies, or known hereditary metabolic diseases were excluded. Demographic characteristics including gestational age, sex, birth weight, delivery method, and Apgar scores were extracted from records.

Family history of kidney diseases, use of nephrotoxic drugs (corticosteroids, surfactant, vancomycin, gentamicin, fluconazole), respiratory illnesses, and ventilator dependency were recorded.

Laboratory parameters included serum calcium, phosphorus, creatinine, BUN, sodium, potassium, urine calcium-to-creatinine ratio, urine analysis (U/A), and urine citrate-to-creatinine ratio.

All infants underwent renal and urinary tract ultrasound at two time-points:

- At 1 month of age
- At corrected gestational age of 40 weeks or at hospital discharge (whichever was earlier)

Ultrasounds were performed by a radiology specialist. Nephrocalcinosis and nephrolithiasis diagnoses were based on official radiologist reports. Infants with positive findings were assigned to the case group, and others to the control group. Data were analyzed using SPSS v25. Normality was checked with Kolmogorov-Smirnov test. All data were normal and we used parametric test. Descriptive statistics included frequency tables, mean, percentages, and SD. Parametric independent t-test was used for analysis data. Chi-square and Fisher's exact tests evaluated qualitative variable associations. Multivariate logistic regression identified independent risk factors. Significance was set at $p < 0.05$.

Ethical Considerations

The study was approved by the Ethics Committee of Ardabil University of Medical Sciences (IR. ARUMS. MEDICINE. REC. 1403. 087) and followed Helsinki Declaration and national bioethics guidelines.

Results

Of 120 infants studied, 30 (25%) had nephrocalcinosis; 12 (40% of cases) had concurrent nephrolithiasis. Gender distribution was 56 males (46.7%) and 64 females (53.3%). Mean gestational age was significantly lower in cases (26.16 ± 1.42 weeks) than controls (28.95 ± 1.87 weeks; $p < 0.001$). Mean birth weight was also lower among cases (907 ± 152 g vs. 1169 ± 187 g; $p < 0.001$). No significant gender difference was found. Apgar scores at 1 and 5 minutes were significantly lower in cases than control ($p < 0.05$). Positive family history of renal disease was significantly more frequent in cases (40% vs. 11%, $p = 0.002$) (Table 1). The diagnostic curve for nephrocalcinosis between the two ultrasound scans shows an increasing trend and reach from 4.4% to 25%. (Figure 1).

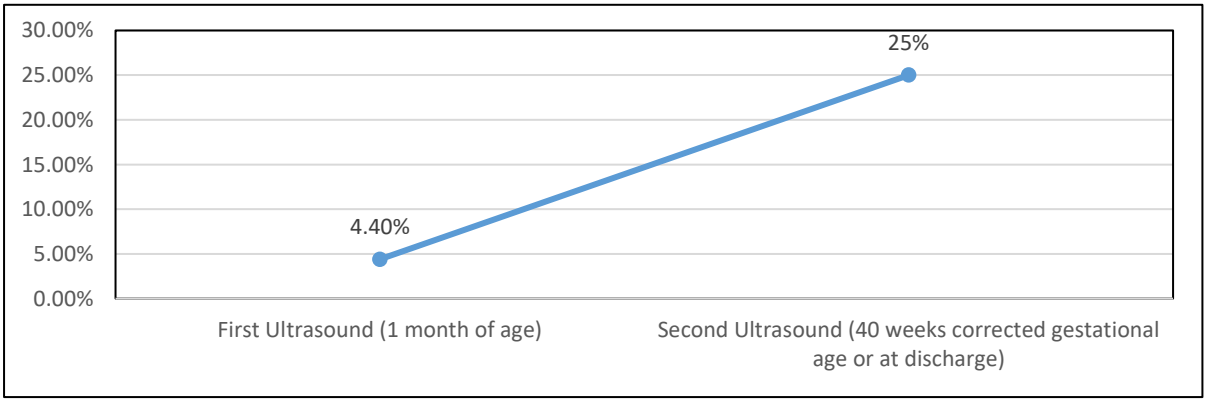

Figure 1. The diagnostic curve for nephrocalcinosis between the two ultrasound scans

Table 1. Demographic and Clinical Characteristics of Study Groups

Variable	Nephrocalcinosis (n=30)	Control (n=90)	p-value
Gestational Age (weeks)	26.16 ± 1.42	28.95 ± 1.87	<0.001*
Birth Weight (grams)	907 ± 152	1169 ± 187	<0.001*
Male Gender, no. (%)	14 (46.7)	42 (46.7)	NS
Apgar Score (1 min)	Lower (significant)	Higher	<0.05*
Apgar Score (5 min)	Lower (significant)	Higher	<0.05*
Family History of Kidney Disease, no. (%)	12 (40)	10 (11)	0.002*
*T-TEST; NS: non-significant			

In this study, 100% of infants weighing between 500 and 750 grams were diagnosed with nephrocalcinosis, whereas no cases were observed in infants weighing between 1251 and 1500 grams. The prevalence of nephrocalcinosis decreased with increasing

gestational age; all infants born at 24–26 weeks had nephrocalcinosis. Prolonged mechanical ventilation was significantly associated with a higher frequency of nephrocalcinosis, with 100% occurrence in those ventilated for over 20 days. Use of corticosteroids, vancomycin, and fluconazole was significantly higher in the nephrocalcinosis group, whereas the use of surfactant and gentamicin showed no significant difference between groups.

Notably, duration of medication exposure was also important. The mean duration of vancomycin use was significantly longer in the nephrocalcinosis group (8.6 ± 3.2 days vs. 1.5 ± 0.2 days, p=0.003).

The effect of diuretics was also significantly notable. With increasing urinary calcium to creatinine ratio, the prevalence of nephrocalcinosis increased, such that 100% of infants with a urinary calcium to creatinine ratio greater than 0.8 had nephrocalcinosis.

Among 30 infants with nephrocalcinosis, 18 cases (60%) had bilateral nephrocalcinosis and additionally, 25 cases (83.3%) had medullary nephrocalcinosis (Table 2).

Table 2. The Clinical and sonographic characteristics of infants with nephrocalcinosis

Variable	Nephrocalcinosis (n=30)
Duration of vancomycin use (days)	8.6 ± 3.2
Duration of Corticosteroid use (days)	7.2 ± 1.8
Bilateral nephrocalcinosis	18 (60%)
Unilateral nephrocalcinosis	12 (40%)
Medullary nephrocalcinosis	25 (83.3%)
Cortical nephrocalcinosis	5 (16.7%)

In ultrasounds performed in the first month after birth, 11 infants (4.4% of the total studied population) were diagnosed with nephrocalcinosis, whereas at 40 weeks corrected gestational age or at hospital discharge, 30 infants (25% of the total population) were diagnosed with nephrocalcinosis, indicating an increase in diagnosis over time.

Respiratory distress syndrome and bronchopulmonary dysplasia were significantly more common in the nephrocalcinosis group compared to the group without nephrocalcinosis. Furthermore, the duration of mechanical ventilation and CPAP support were significantly longer in the nephrocalcinosis group (Table 3).

Table 3. Association of Nephrocalcinosis with Respiratory Diseases and Respiratory Support

Variable	Nephrocalcinosis Group (n=30)	No Nephrocalcinosis Group (n=90)	p-value
Respiratory Distress Syndrome	26 (86.7%)	52 (57.8%)	0.003 **
Bronchopulmonary Dysplasia	13 (43.3%)	14 (15.6%)	0.001 **
Neonatal Pneumonia	7 (23.3%)	15 (16.7%)	0.409 NS
Duration of Mechanical	17.5 ± 8.2	7.5 ± 5.1	<0.001 ***
Duration of CPAP (days)	13.2 ± 7.3	6.8 ± 4.2	0.005 **
NS: Not Statistically Significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001			

Serum calcium levels and urinary calcium-to-creatinine ratios were significantly higher in the nephrocalcinosis group compared to the group without nephrocalcinosis, while serum phosphorus levels and urinary citrate-to-creatinine ratios were significantly lower in the nephrocalcinosis group (Table 4).

Table 4. Association of Nephrocalcinosis with Blood and Urine Biochemical Parameters

Parameter	Nephrocalcinosis Group (n=30)	No Nephrocalcinosis Group (n=90)	p-value
Serum Calcium (mg/dL)	10.64 ± 1.21	9.23 ± 0.86	<0.001 ***
Serum Phosphorus (mg/dL)	4.52 ± 0.78	5.38 ± 0.95	0.009 **
Serum Creatinine (mg/dL)	0.82 ± 0.22	0.65 ± 0.18	0.064 NS
Serum BUN (mg/dL)	18.6 ± 5.4	14.2 ± 4.8	0.037 *
Serum Sodium (mEq/L)	140.5 ± 3.1	141.2 ± 3.5	0.713 NS
Serum Potassium (mEq/L)	4.52 ± 0.41	4.48 ± 0.38	0.842 NS
Urinary Ca/Cr Ratio	1.03 ± 0.28	0.42 ± 0.16	<0.001 ***
Urinary Citrate/Cr Ratio	0.17 ± 0.08	0.38 ± 0.15	0.002 **
NS: Not Statistically Significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001			

The duration of hospitalization, urinary tract infections, and metabolic acidosis were significantly higher in the nephrocalcinosis group compared to the group without nephrocalcinosis (Table 5).

Table 5. Association of Nephrocalcinosis with Complications of Long-Term Hospitalization

Variable	Nephrocalcinosis Group (n=30)	No Nephrocalcinosis Group (n=90)	p-value
Length of Hospital Stay (days)	68.4 ± 15.8	42.5 ± 12.3	<0.001 ***
Urinary Tract Infection	9 (30.0%)	9 (10.0%)	0.006 **
Metabolic Acidosis	20 (66.7%)	30 (33.3%)	0.001 **
Crystalluria	17 (56.7%)	11 (12.2%)	<0.001 ***
Hematuria	10 (33.3%)	7 (7.8%)	0.001 **
NS: Not Statistically Significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001			

Multivariate logistic regression analysis was performed to identify independent risk factors for nephrocalcinosis. Variables that showed a significant association with nephrocalcinosis in univariate analysis ($p < 0.05$) were entered into the logistic regression model. A urinary calcium-to-creatinine ratio greater than 0.8 was the strongest independent risk factor for nephrocalcinosis (OR = 8.45, p = 0.0001), followed by a family history of kidney disease (OR = 12.5, p = 0.003), gestational age less than 28 weeks (OR = 24.3, p = 0.001), mechanical ventilation for more than 10 days (OR = 9.2, p = 0.001), birth weight less than 1000 grams (OR = 8.7, p = 0.002), corticosteroid use (OR = 3.2, p = 0.01), and vancomycin use (OR = 2.8, p = 0.025) were also significant independent risk factors. Metabolic acidosis was not identified as a significant independent risk factor in multivariate analysis (OR = 1.8, p = 0.071) (Table 6).

Table 6. Results of Multivariate Logistic Regression Analysis for Identifying Independent Risk Factors for Nephrocalcinosis

Risk Factor	Odds Ratio (OR)	p-value
Gestational Age < 28 weeks	3.24 (95%CI:1.85-5.67)	0.001 **
Birth Weight < 1000 grams	2.87 (95%CI:1.63-5.04)	0.002 **
Mechanical Ventilation > 10 days	2.92 (95% CI:1.71-4.99)	0.001 **
Urinary Ca/Cr Ratio > 0.8	8.45 (95%CI:3.76-19.02)	<0.001 ***
Family History of Kidney Disease	5.12 (95%CI:1.85-14.16)	0.003 **
Use of Corticosteroids	3.21 (95%CI:1.31-7.85)	0.01 *
Use of Vancomycin	2.76 (95%CI:1.14-6.67)	0.025 *
Metabolic Acidosis	2.18 (95%CI:0.93-5.09)	0.071 NS
Use of Diuretics	4.6 (95%CI:1.6-14.7)	0.01 *
NS: Not Statistically Significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001		

Discussion

In the present study conducted on 120 preterm infants with gestational age less than 32 weeks or birth weight below 1500 grams, 30 infants (25%) were diagnosed with nephrocalcinosis. Among them, 12 infants (40% of nephrocalcinosis cases) concurrently had nephrolithiasis. This prevalence aligns with the findings of Mortazavi et al. (2016) in Iran, who reported a prevalence of 26.6% (12). This similarity suggests that the causative factors for nephrocalcinosis in preterm infants are relatively consistent across different Iranian populations.

However, when compared with some international studies, significant differences are apparent. For example, Fayard et al. (2022) reported a nephrocalcinosis prevalence of approximately 10.8% in very low birth weight infants, which is clearly lower than the rate observed in the current study (13). Such discrepancies may stem from differences in study population characteristics, diagnostic criteria, and treatment protocols. This hypothesis that multiple factors influence the incidence of nephrocalcinosis is consistent with Fayard et al.'s emphasis on the diversity of risk factors across populations (13).

Our study's findings regarding the timing of nephrocalcinosis diagnosis are noteworthy. At the first-month ultrasound, the prevalence was only 4.4%, increasing to 25% at discharge or at 40 weeks corrected gestational age. This gradual increase matches results from Patel et al. (2023), who highlighted the importance of optimal ultrasound timing for early detection following the initial neonatal period (14). This increase may be due to gradual calcium accumulation in the renal parenchyma over time and prolonged exposure to risk factors due to extended hospitalization. This underscores the importance of serial ultrasounds and routine follow-up for early nephrocalcinosis detection in preterm infants (13).

Demographic and clinical data analysis showed significantly lower mean gestational age and birth weight in the nephrocalcinosis group, aligning with Weston et al. (2021) (15). This may be due to immature kidney development in lower gestational age infants and increased exposure to nephrotoxic factors during prolonged NICU stays. Tanaka et al. (2022) introduced a novel pathophysiological mechanism implicating autophagy disruption in renal calcium crystal deposition (16).

Although a higher percentage of females was seen in the nephrocalcinosis group (56.7%) compared to controls (52.2%), this was not statistically significant, consistent with Mortazavi et al. (2016) (12). In contrast, Fayard et al. (2022) identified female sex as a significant risk factor, likely due to sample size or confounding variables (13). A significant association between positive family history of kidney disease and nephrocalcinosis was observed: 40% of cases had such history versus 11% of controls, corroborating Weston et al. (2021) (15). Kawamoto et al. (2023) demonstrated that reduced expression of crystal inhibitory proteins like osteopontin and fetuin-A in renal tissue may underlie genetic susceptibility in affected neonates (11).

Respiratory conditions including respiratory distress syndrome and bronchopulmonary dysplasia were significantly more frequent in nephrocalcinosis cases. These infants also required longer mechanical ventilation and CPAP support, consistent with Fayard et al. (2022) (13). The frequency of nephrocalcinosis reached 100% in infants ventilated for over 20 days.

Medication analysis revealed significantly higher corticosteroid, vancomycin, and fluconazole use in nephrocalcinosis cases, with longer durations for vancomycin and corticosteroids, echoing Mortazavi et al. (2016) (12). Ergonul et al. (2023) linked urinary levels of crystal inhibitory proteins to nephrocalcinosis risk (17).

Laboratory parameters showed significantly higher serum calcium and urinary calcium-to-creatinine ratio and significantly lower serum phosphorus and urinary citrate-to-creatinine ratio in cases, fully consistent with Mortazavi et al. (12) and Fayard et al. (13).

The urinary calcium-to-creatinine ratio above 0.8 emerged as a strong diagnostic indicator for nephrocalcinosis risk.

Nephrocalcinosis cases exhibited significantly longer hospitalization, higher

rates of urinary tract infections, metabolic acidosis, crystalluria, and hematuria, paralleling findings of Fayard et al. (13) and Simoudis et al. (2020). Simoudis et al. underscored the heightened UTI risk and predisposing factors in preterm infants with nephrocalcinosis (18). Multivariate logistic regression identified seven independent predictors: urinary calcium-to-creatinine ratio >0.8 (strongest predictor), family history of kidney disease, gestational age <28 weeks, mechanical ventilation >10 days, birth weight <1000 grams, corticosteroid use, and vancomycin use. These findings reinforce prior research and can aid clinicians in risk stratification, targeted monitoring, and timely preventive interventions.

Based on the findings of Ramanathan et al. (2023), which indicated that children with a history of neonatal nephrocalcinosis are at higher risk of long-term renal impairment, the results of the present study can be interpreted within the framework of long-term outcomes. In our study, infants with nephrocalcinosis had significantly higher rates of complications such as urinary tract infection, metabolic acidosis, crystalluria, and hematuria—all of which may contribute to sustained kidney injury. This aligns with Ramanathan's report of increased risk of renal dysfunction later in life. Furthermore, the independent risk factors identified in our study (e.g., urinary calcium-to-creatinine ratio >0.8, family history, birth weight <1000 g, and corticosteroid use) could help identify neonates who require long-term follow-up. However, unlike Ramanathan's case–control study with long-term follow-up, our study is cross-sectional and did not assess long-term renal outcomes. Thus, our findings underscore the necessity of long-term renal function monitoring in preterm infants diagnosed with nephrocalcinosis (19).

The recognition of CYP24A1-associated nephrocalcinosis expands the etiological spectrum beyond acquired neonatal factors. It reinforces that nephrocalcinosis can be a delayed presentation of a genetic disorder, warranting a different diagnostic and therapeutic approach compared to the multifactorial form commonly seen in NICU graduates. In both populations, however, tailored biochemical surveillance and avoidance of specific triggers remain cornerstone principles of management (20).

This study had limitations including a relatively small sample size, cross-sectional design without long-term follow-up, incomplete biochemical assessments in all subjects, reliance on ultrasound as the sole diagnostic tool, and lack of genetic evaluation. Future prospective cohort studies with larger samples, longer follow-up, and comprehensive biochemical and genetic profiling are needed to better understand the natural history, long-term outcomes, and precise pathophysiological mechanisms of nephrocalcinosis in preterm infants.

Conclusion

Nephrocalcinosis, with a 25% prevalence, is a common complication in very low birth weight preterm infants. Key risk factors include urinary calcium-to-creatinine ratio above 0.8, family history of kidney disease, severe prematurity (gestational age <28 weeks and birth weight <1000 grams), prolonged mechanical ventilation, and exposure to specific medications (corticosteroids and vancomycin). Targeted monitoring of urinary calcium-to-creatinine ratio, cautious use of nephrotoxic drugs with renal function monitoring, regular ultrasound follow-up, evaluation of renal function post-discharge, prospective long-term studies, investigation of preventive interventions (e.g., TPN adjustment), and research into genetic and novel biomarker roles are recommended.

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