

Nephrocalcinosis and Placental Findings in Neonatal Bartter Syndrome

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Abstract

Neonatal Bartter syndrome (NBS) is an inherited renal tubular disorder associated with hypokalemic alkalosis. Here we report a case of genetically diagnosed NBS. Polyhydramnios was noted at 26 weeks. A boy was born at 31 weeks and 1 day, weighed 1344 g, and had an Apgar score of 8/8. We initiated indomethacin (IND) at a dose of 0.2 mg/kg/d on day 31, and increased it to approximately 3 mg/kg/d. However, his urinary calcium (Ca) levels remained unchanged. At 4 months of age, nephrocalcinosis was detected by ultrasound. The placenta weighed 700 g (+2.7 standard deviations). Although the proportion of terminal villi was consistent with the gestational age, many of them exhibited poorly dilated capillaries. Hemosiderin pigment was seen throughout the amniochorionic connective tissue and along about 50% of the trophoblast basement membrane (TBM). Von Kossa stain revealed the corresponding area of mineralization along the TBM. In our opinion, urinary Ca levels were high and did not change after IND initiation, indicating that nephrocalcinosis may be inevitable. Enhanced inflow of maternal plasma through the basement membrane would cause Ca deposition, given that the same finding was obtained in the case with polyhydramnios. The same mechanism would also explain the hemosiderin pigment distribution.

Keywords

- ▶ indomethacin
- ▶ nephrocalcinosis
- ▶ neonatal Bartter syndrome
- ▶ trophoblast basement membrane

Bartter syndrome is an inherited renal tubular disorder associated with hypokalemic alkalosis. The symptoms of neonatal Bartter syndrome (NBS) are polyuria, polyhydramnios, and premature birth. Characteristic laboratory findings include hyperreninemia, hyperaldosteronism, and high prostaglandin E₂ (PGE₂) production.^{1–3} In NBS, activation of cyclooxygenase (COX) increases PGE₂ production. Thus, in addition to water and electrolyte supplementation, the COX inhibitor indomethacin (IND) has been used to treat NBS.^{4–7}

Here, we discuss nephrocalcinosis, one of the clinical characteristics and placental findings in a patient with NBS. Written informed parental consent was obtained for this study.

Case Report

The 27-year-old mother had two healthy children from a nonconsanguineous marriage. Polyhydramnios was noted at 26 weeks of gestation. No distinct anomaly was detected by ultrasound. Amniotic fluid was removed three times (1500, 1300, and 2800 mL) and was not bloody. Amniotic fluid analysis revealed a 46,XY karyotype. Labor began just after the third removal of amniotic fluid. Cesarean section was performed as the baby was in the transverse position. The boy was born at 31 weeks and 1 day, weighed 1344 g, and had an Apgar score of 8/8. No anomalies were detected, and we were able to insert a tube into the stomach.

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Table 1 Laboratory Findings at Birth

Blood				Urine		Amniotic fluid	
WBC	5900/ μ L	pH	7.292	Na	112 mEq/L	Na	131 mEq/L
RBC	3.95×10^6 / μ L	pCO ₂	56.1 mm Hg	K	5.0 mEq/L	K	4.1 mEq/L
Hb	15.0 g/dL	pO ₂	34.5 mm Hg	Cl	106 mEq/L	Cl	106 mEq/L
Ht	43.7%	HCO ₃	26.3 mEq/L	Ca	7.9 mg/dL	Ca	6.1 mg/dL
PLT	168×10^3 / μ L	BE	-1.0 mEq/L	P	6.5 mg/dL	P	3.8 mg/dL
CRP	0.05 mg/dL	Na	130.8 mEq/L	Cr	1.67 mg/dL	Cr	0.59 mg/dL
BUN	4 mg/dL	K	3.94 mEq/L	Osm	229 mOsm	Osm	257 mOsm
Cr	0.43 mg/dL	Cl	96.0 mEq/L				
Renin	>400 pg/mL	iCa	1.44 mg/dL				
Aldosterone	2020 pg/mL	Lactate	24 mg/dL				
ADH	1.0 pg/mL	Glu	55 mg/dL				

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; PLT, platelets; CRP, C-reactive protein; BUN, blood urea nitrogen; Cr, creatinine; ADH, antidiuretic hormone; BE, base excess; Osm, osmolality.

At 4 hours of age, polyuria (9.8 mL/kg/h) was detected. We also observed extensive loss of electrolytes into the urine: Na 112 mEq/L; K 5.0 mEq/L; Cl 106 mEq/L; Ca 7.9 mg/dL; and creatinine (Cr) 1.67 mg/dL (**►Table 1**). Similar proportions of electrolytes were detected in the amniotic fluid. He was given sufficient water and electrolytes. NBS was strongly suspected due to the presence of renin at >400 pg/mL, aldosterone at 2020 pg/mL, and antidiuretic hormone at 1 pg/mL in the baby's blood on day 0.

He suffered from neonatal toxic-shock-syndrome-like exanthematous disease on day 5 due to methicillin-resistant *Staphylococcus aureus* and was treated with antibiotics. On day 8, his blood pressure (65/29 to 41/16 mm Hg) and urine output (11.6 to 3.5 mL/kg/h) decreased. There was the absence of diastolic renal artery flow in the ultrasound, and he was diagnosed with late-onset circulatory collapse. We initiated hydrocortisone (HDC) on day 11, and subsequently his urine output improved. We attempted to decrease the amount of HDC twice, but without success. On day 31, IND was initiated at 0.2 mg/kg/d and was gradually increased to 2.7 mg/kg/d. After IND initiation, HDC was reduced and finally terminated on day 73. During his hospital stay, plasma aldosterone levels gradually decreased from 2020 pg/mL on day 0 to 1280 and 935 pg/mL on days 31 and 69, respectively,

whereas the plasma renin levels were over the measurement range on days 0, 31, and 69. Ca was given intravenously from day 0 (600 mg/kg/d) and decreased gradually. Breast milk was started at day 0 and reached at 92 mL/kg/d at day 7. After the late-onset circulatory collapse at day 8, breast milk decreased. Afterward, the breast milk was increased again and became over 100 mL/kg/d at day 13. In addition to fortifier, we added calcium lactate at day 19. We added vitamin D (0.05 μ g/kg/d) at day 20. The serum level of Ca was almost kept from 9 to 11 mg/dL, with the nadir of 8.4 mg/dL at day 21. Urinary Ca (mg/mg Cr) remained high (2 to 3) even after IND administration (**►Fig. 1**). Nephrocalcinosis was not detected by ultrasound during his hospital stay. Urinary Fe was <0.18 mg/d on day 14.

With respect to the auditory brain stem response (ABR), V waves were not detected at 35 dB in either ear at the corrected age of 41 weeks. Brain magnetic resonance imaging showed no abnormal findings at the corrected age of 40 weeks.

He harbored a compound heterozygous mutation in exon 8 c.del1100t and exon 10 p.R471X of the *SLC12A1* gene encoding NKCC2. Both mutations were new to the Human Gene Mutation Database. We did not determine the functional consequences of our mutants. However, two mutations might cause the disease. One mutation, R471X, which was located in exon 10, is subjected to nonsense-mediated decay. The other deletion mutation changes the open reading frame after the deletion site. Thus NKCC2 function might be impaired by amino acids substitutions. We provided genetic counseling for his parents; however, they did not want to get their DNA analyzed.

The placenta weighed 700 g (+2.7 standard deviations).⁸ Although the proportion of terminal villi was consistent with the gestational age, many of them showed poorly dilated capillaries (**►Fig. 2**). Hemosiderin pigment was seen throughout the amniochorionic connective tissue and along about 50% of the trophoblast basement membrane (TBM; **►Fig. 3**). Von Kossa stain also revealed the corresponding area of

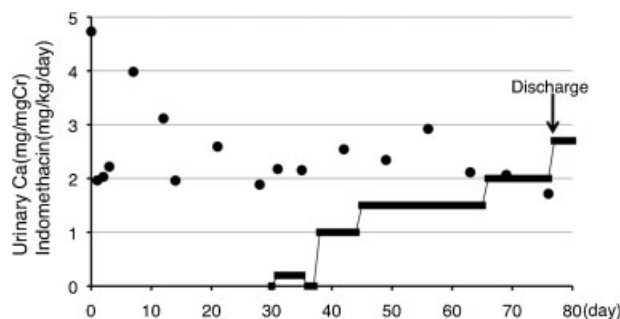


Fig. 1 Urinary Ca (mg/mg Cr, circle marker) levels before and after indomethacin administration (mg/kg/d, square marker).

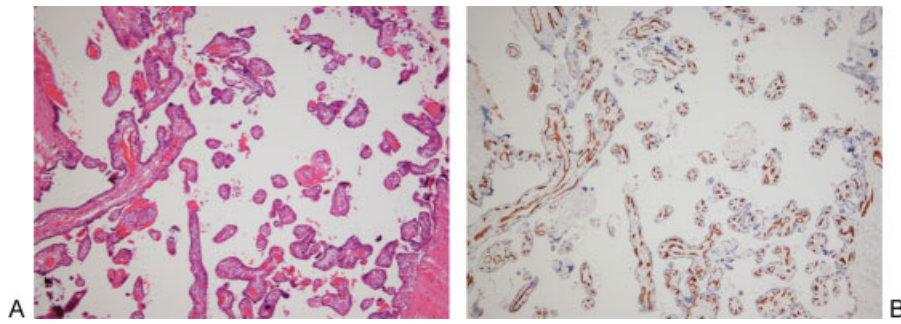


Fig. 2 Hematoxylin-eosin staining of the placenta (A). Although the proportion of terminal villi was consistent with the gestational age, many of them showed poorly dilated capillaries. CD34-positive endothelial cells (B).

mineralization along the TBM (►Fig. 3). No vascular damage or interstitial hemorrhage was detected in the villi and the amniochorionic connective tissue.

On day 77 (42 weeks old), he was discharged with IND, NaCl, and potassium glucuronate. At 4 months of age, nephrocalcinosis was detected by ultrasound. At 7 months of age (corrected age: 5 months), the ABR was normal. Catch-up growth and rolling over were detected.

Discussion

Mutations in ion transport channels lead to decreased Ca reabsorption in patients with NBS, which is similar to that observed in patients treated with loop diuretics. The end result is hypercalciuria, leading to nephrocalcinosis.

Dane et al reported two cases (33 weeks, 1640 g and 35 weeks, 2420 g) where treatment with IND was initiated in the antenatal period. At 18 months of age, the first case had minimal calcification and the second had no calcification,⁵ suggesting that antenatal IND treatment was effective in preventing nephrocalcinosis (►Table 2). In the present case, however, nephrocalcinosis was detected at 4 months of age, and urinary Ca levels did not decrease despite the treatment. Although antenatal IND could prevent nephrocalcinosis, there would be some side effects.⁹ On the contrary, our

findings suggest that nephrocalcinosis may be inevitable because IND was not effective in our case. We checked the usage dose of IND in the previous reports.^{5,6,10} It was around 2 to 3 mg/kg/d, which was too much for us, because we usually use it in dosages of 0.2 mg/kg for patent ductus arteriosus therapy. So we started at a small dose and increased the dose, taking care of side effects.

In the previous reports, the placental weight was high.^{5,11} Small vessels in the villi showed reduced blood circulation. We believe that villi may grow reactively, thereby increasing placental weight. We could not find the cause in the previous reports. The receptors to angiotensin and PGE2 exist in the placenta.^{12,13} We doubt that there would be the influence of them.

According to Dane et al, fetal renal excretion of Ca could lead to dystrophic calcification with placental involvement, particularly in a subtrophoblastic pattern.⁵ Ernst and Parkash hypothesized that a relatively hypocalcemic fetus would require increased transport of Ca ions across the placenta and that excesses of Ca due to overloading of the transport system could be deposited beneath the trophoblastic layer.¹¹

These theories may explain elevated Ca levels in the amniotic fluid and fetal hypocalcemia-induced increase in mother-to-fetus Ca transport. However, the passage of Ca through amniochorionic connective tissue to the TBM as proposed by

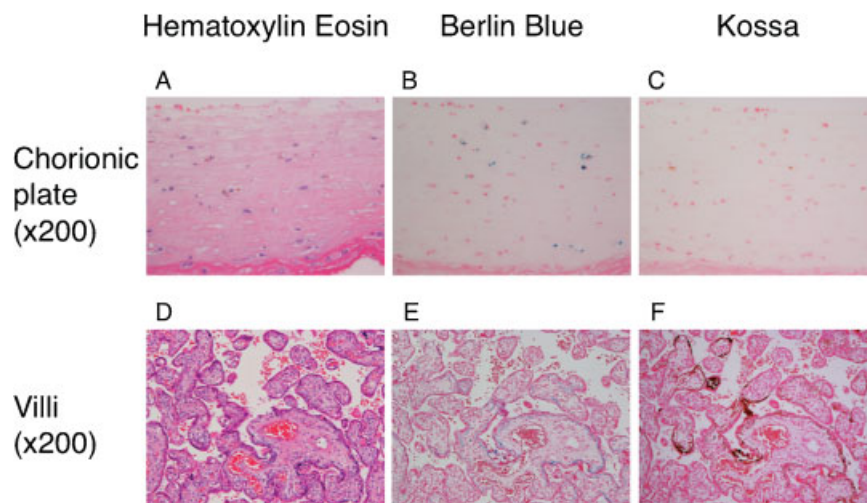


Fig. 3 Placental histology. Hematoxylin-eosin staining (A, D). Hemosiderin pigment deposition seen throughout the amniochorionic connective tissue and along about 50% of trophoblast basement membrane (B, E). Von Kossa staining along the trophoblast basement membrane (C, F).

Table 2 Indomethacin Dosages and Nephrocalcinosis in Neonatal Bartter Syndrome

Author	Indomethacin Dosage	Nephrocalcinosis
Nakagawa ¹⁰	1st case (42 wk): 1 y old, ~3 mg/kg/d (18 mg/d); 2 y old, ~22 mg/d; 5 y old, ~25 mg/d; 16 y old, ~0.5 mg/kg/d	Not mentioned
Konrad ⁶	1st case (28 wk, 750 g): 11 wk old, ~1.6 mg/kg/d, then 3 mg/kg/d 2nd case (35 wk, 2670 g): 7 d old, ~1 mg/kg/d, then 0.2 mg/kg/d due to oliguria, then 0.6 mg/kg/d	1st case: (-) at 2 mo of age 2nd case: mild (+) at 3 mo of age
Ernst ¹¹	1st case (30 wk) and 2nd case (32 wk); Indomethacin was used (dosage not mentioned)	Both cases: (+)
Adachi ⁴	1st case (35 wk, 2340 g): 0.33 mg/kg/d 2nd case (33 wk; 1918 g): 0.1 mg/kg/d	1st case: (+) at 1.5 y of age 2nd case: (+) at 87 d of age
Dane ⁵	1st case (33 wk, 1640 g): 2 mg/kg/d to mother before birth; at 2 wk of age, initiation of indomethacin; spontaneous intestinal perforation at 3 wk of age 2nd case (35 wk, 2420 g): 1 mg/kg/d to mother before birth; at 16 d of age, initiation of indomethacin	1st case: minimal calcification at 18 mo of age 2nd case: (-) at 18 mo of age

Dane et al⁵ is difficult to understand. Only a few studies have specifically examined these linear subtrophoblastic deposits. Krohn et al reported increased membrane calcifications in polyhydramnios.¹⁴ In polyhydramnios, fetal hypovolemia may be caused by increased urine output and intestinal atresia, among others. Accordingly, maternal plasma, including Ca, may pass through the TBM, resulting in Ca deposits.

The hemosiderin deposition pattern was similar to that of Ca, suggesting that the same mechanism may underlie hemosiderin deposition. In other words, increased Fe in the blood and urine caused by enhanced maternal plasma inflow may explain the hemosiderin deposition in the amniochorionic connective tissue. However, increased renal Fe has not been reported, and in our case, the infant did not have much urinary Fe. Furthermore, the amniotic fluid was not bloody. Hemosiderin pigment was seen along about 50% of the trophoblast basement membrane, which was less than 75%.¹¹ But this finding is nonspecific. Thus, the low percentage would not be the problem.

In conclusion, nephrocalcinosis may be inevitable in NBS. Enhanced maternal plasma inflow through TBM could cause Ca deposition, given that the same finding was obtained in the case with polyhydramnios. The same mechanism could account for the hemosiderin pigment distribution.

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