RESEARCH PAPER

Etiological Profile of Nephrocalcinosis in Children from Southern India

KAGNUR RAMYA, SRIRAM KRISHNAMURTHY AND PALANISAMY SIVAMURUKAN

From Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India.

Correspondence to: Dr Sriram Krishnamurthy, Additional Professor, Department of Pediatrics, JIPMER, Pondicherry 605 006, India. drsriramk@yahoo.com
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Objective: To study the etiological profile and patterns of clinical presentation of nephrocalcinosis. **Methods:** In this observational study, patients 18 years or younger, referred to the pediatric nephrology clinic with nephrocalcinosis were evaluated for etiology. Symptoms/signs at presentation, estimated glomerular filtration rate (eGFR) at presentation and follow-up, and growth parameters were recorded. **Results:** The etiology of nephrocalcinosis (*n*=54) included distal renal tubular acidosis (*n*=18; 33.3%), primary hyperoxaluria (*n*=9; 16.7%), Bartter syndrome (*n*=7; 13%), Dent disease (*n*=4; 7.4%), cystinosis, familial hypomagnesemia with hypercalciuria and idiopathic hypercalcemia of infancy (2 each). Idiopathic nephrocalcinosis was seen in 5 (9.3%) children. Clinical features included failure to thrive (53.7%), polyuria (44.4%), bony deformities (31.5%) and hypokalemic paralysis (11.1%). At a median (IQR) follow-up of 24 (8, 56) months, the mean (SD) eGFR had improved from 59 (25.5) to 77 (31.48) mL/min/1.73m^2 (*P*<0.01). Consanguinity was present in 50% (27/54). Genetic analysis in 5 primary hyperoxaluria cases confirmed AGXT mutations in 4; and GRHPR mutation in 1 child. C**onclusion:** Distal RTA, primary hyperoxaluria and Bartter syndrome were the common etiologies of nephrocalcinosis in our patient population.

Keywords: *Distal RTA, Calculi, Outcome, Tubular disorders*.

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Nephrocalcinosis (NC) is defined as calcium deposition in the renal parenchyma as detected by renal ultrasonogram. Pediatric NC is a rare entity and might occur secondary to inherited renal tubular disorders, vitamin D excess, etc [1-4]. A comprehensive metabolic evaluation of NC would help in specific therapies, prevent progression to end-stage renal disease and enable optimal prenatal counseling.

Most published information on NC is from developed nations [1,5] and there is paucity of information regarding pediatric NC from India [6]. Since NC often has an underlying genetic or metabolic etiology, it can be speculated that its etiological profile is likely to vary with ethnicity. We studied the etiological profile of NC among children from Southern India.

METHODS

This cross-sectional study was conducted at the pediatric nephrology clinic of a referral hospital in Southern India from July, 2017 through July, 2019, after obtaining approval from the institutional ethics committee. Prior written informed consent was obtained from the parents. The primary objective of the study was to evaluate the underlying etiology of NC, while the secondary objectives were to record the patterns of clinical presentation, complications and outcomes (estimated glomerular filtration rate (eGFR) on follow-up) in these children.

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All patients ≤18 years with NC who were referred for diagnostic evaluation were included. NC was defined as calcium deposition in the renal parenchyma as detected and graded by ultrasound. Medullary NC was graded as: grade 1, mild increase in echogenicity of medullary pyramids; grade 2, mild diffuse increase in echogenicity of medullary pyramids without acoustic shadowing; and grade 3, greater homogenous increase in echogenicity of medullary pyramids with acoustic shadowing [1]. Cortical nephrocalcinosis was diagnosed by the presence of calcifications in the renal cortex.

This was an observational study, supplemented by analysis of hospital records. For the prospective component of the study, consecutively presenting children who were referred for evaluation of NC were evaluated. For the retrospective component of the study, data were collected from the records of children 18 years or younger with NC, who had presented to the pediatric

nephrology clinic over the last 10 years and were under follow-up at the pediatric nephrology clinic.

We have been using the following protocol for investigating NC for the last 10 years: (*i*) First-line investigations- Blood pH, blood urea, creatinine, sodium, potassium, magnesium, chloride, serum bicarbonate, calcium, phosphorous, alkaline phosphatase, uric acid; urinalysis for urine pH, crystals, urine culture (if clinically indicated), spot calcium: creatinine ratio and 24-hour urine excretion of calcium, oxalate, uric acid and creatinine, were recorded. Estimated glomerular filtration rate (eGFR) was determined using modified Schwartz formula [7]. (*ii*) Second-line investigations- Urine sodium nitroprusside test was restricted to patients where a cause of NC was not found on first-line investigations. Blood parathyroid hormone (PTH) and 25 hydroxy-cholecalciferol was evaluated in patients with hypercalcemia (serum calcium >11mg/dL on >2 occasions). Urine β2 microglobulin levels were performed in males with suspected Dent disease.

Following definitions were used for defining the etiology of nephrocalcinosis [6, 8-10]: Distal renal tubular acidosis (RTA) was diagnosed in patients with suggestive clinical features (failure to thrive, polyuria, rickets, hypokalemic paralysis etc) and hyperchloremic metabolic acidosis (serum bicarbonate <18 mEq/L), normal anion gap (8-12 mEq/L), normal fractional excretion of bicarbonate (<5%), urine pH >5.5 and hypercalciuria (elevated urinary calcium >4 mg/kg per day in a 24 hour urine sample). Idiopathic hypercalciuria was defined as hypercalciuria with absence of other tubular defects and normocalcemia (9-11 mg/dL). Bartter syndrome was diagnosed in children with suggestive clinical features (failure to thrive, polyuria, etc), metabolic alkalosis (serum bicarbonate >25 mEq/L), hypokalemia (potassium <3.5 mEq/L), normal blood pressure, increased urinary potassium (>20 mEq/L) and chloride (>30 mEq/L), with high plasma renin activity. Primary hyperoxaluria was defined as elevated urinary oxalate excretion (>40 mg/1.73 m^2 per day on a 24-hour urinary specimen) and no history of malabsorption, steatorrhea or intestinal surgical resection. Hyperparathyroidism was diagnosed in those with high serum calcium (>11 mg/dL) and PTH (>50 pg/mL) with or without hypercalciuria. Hyperuricosuria was diagnosed if uric acid excretion was >815 mg/1.73m^2 on a 24-hour urine specimen. Dent disease was diagnosed as per standard definitions [10]. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) was diagnosed in those with low serum magnesium (<1.5 mg/dL), urinary magnesium wasting (fractional excretion ≥5%), hypercalciuria, NC with/without family history of hypomagnesemia with hypercalciuria. Idiopathic hypercalcemia of infancy was diagnosed when hypercalcemia (>11 mg/dL) was noted in the absence of vitamin D toxicity, hyperparathyroidism, absence of calcium supplement intake or subcutaneous fat necrosis.

Determination of the cause of NC was followed by specific therapy. Patients were advised to consume plenty of fluids and to restrict intake of added salts. Long term outcome was assessed in terms of clinical improvement, weight *Z* score, height *Z* score and renal functions.

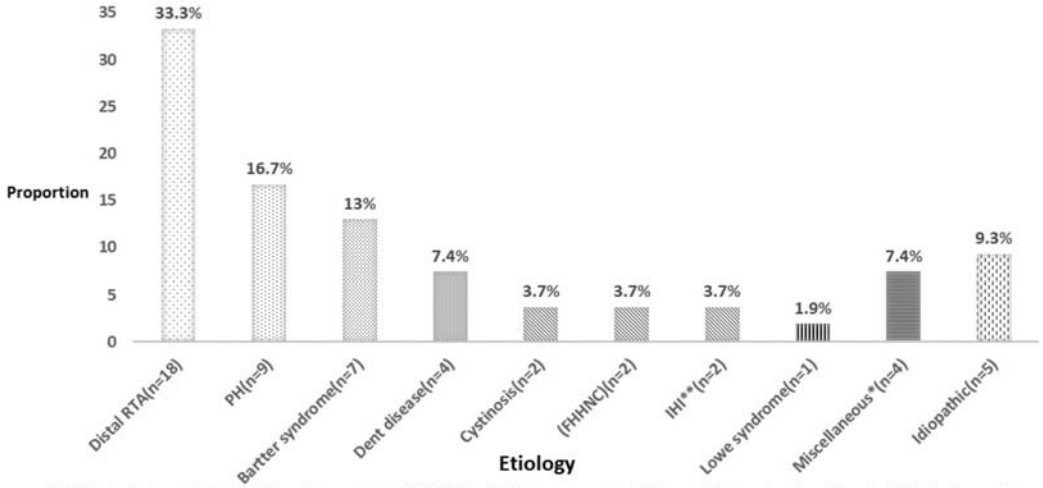
Statistical analyses: The data were analyzed by SPSS 23.0. Normality of data was analyzed by Kolmogorov-Smirnov test. Paired t-test was used to compare means of two dependent sample groups. Median and IQR of two dependent sample groups were compared using Wilcoxon-signed rank sum test.

RESULTS

Of the 54 children with NC (29 males), 18 were recruited prospectively. Fifty-two children had medullary NC. One child with primary hyperoxaluria had both cortical and medullary NC, while 1 child with autosomal recessive polycystic kidney disease (ARPKD) had cortical NC. ***Fig. I*** shows the etiological profile of NC in our study. Distal RTA, primary hyperoxaluria, Bartter syndrome and Dent disease were the most common causes of NC.

Dent disease was diagnosed in 4 cases, of which 2 cases had type 1 phenotype (with no metabolic acidosis), while the other 2 cases had a phenotype consistent with type 2 Dent disease (with metabolic acidosis). Out of two children with FHHNC, one had positive family history of hypomagnesemia and urolithiasis in a maternal uncle. Cystinosis was diagnosed in two cases of NC, who had Fanconi syndrome and cystine crystals in cornea. They were treated with potassium citrate, phosphorus supplements and oral cysteamine (in one case). Another child with medullary NC had global developmental delay, bilateral cataracts, hypotonia and Fanconi syndrome; and was diagnosed as Lowe syndrome. Two infants were diagnosed as idiopathic hypercalcemia of infancy (serum calcium 12.5 mg/dL and 12 mg/dL, respectively) and were treated with bisphosphonates, on which the serum calcium levels normalized.

***Table* I** provides the baseline clinical and biochemical features of the enrolled children. Grade 1, 2 and 3 nephrocalcinosis were noted in 45 (83.3%), 8 (14.8%) and 1 (1.9%) of cases, respectively. All the NC cases (except the 5 idiopathic cases) had hypercalciuria. There was



RTA-Renal tubular acidosis; PH- Primary hyperoxaluria; FHHNC-Familial hypomagnesemia with hypercalciuria and nephrocalcinosis; ARPKD- Autosomal recessive polycystic kidney disease; IHI- Idiopathic hypercalcemia of infancy
*Includes 1 case each of Rabson-Mendenhall syndrome, ARPKD, Familial isolated hypoparathyroidism and Hyperuricosuria

Fig. 1. *Etiology of nephrocalcinosis in the enrolled children (n=54).*

TABLE I Baseline Clinical and Biochemical Characteristics of Children with Nephrocalcinosis (NC) (*N*=54)

Parameters	*Value*
Age at Symptom onset, mo	24 (6, 48)
#Age at diagnosis, mo	36 (11.5, 84)
#Symptom-diagnosis interval, mo	24 (7.6, 59)
‡*Clinical features*	
Failure to thrive (WFA<-2 Z score)	29 (53.7)
Polyuria	24 (44.4)
Polydipsia	17 (31.5)
Rickets	19 (35.2)
Hypokalemic paralysis	6 (11.1)
Short stature	5 (9.3)
Carpopedal spasm	5 (9.3)
Pathological fractures	4 (7.4)
Acute Kidney Injury	3 (5.6)
Hematuria	4 (7.4)
Biochemical features	
*eGFR at presentation (mL/min/1.73m²)	59 (25.5)
Metabolic acidosis	26 (48.1)
#Serum creatinine at presentation (mg/dL)	0.59 (0.49, 0.70)
eGFR <60 mL/min/1.73 m^2 at diagnosis	24 (44.4)
eGFR <60 mL/min/1.73 m^2 at last follow up	15 (27.8)
^Associated urolithiasis	3 (5.55)

*Values in no. (%) except *mean (SD) or #median (IQR); ‡Recurrent vomiting, salt craving, and antenatal detection in 2 each; eGFR-Estimated Glomerular filtration rate, WFA- Weight for age; ^One child each with primary hyperoxaluria, familial hypomagnesemia and hypercalciuria with nephrocalcinosis (FHHNC), and Rabson-Mendenhall syndrome.*

history of consanguinity in 27 (50%) of cases, while there was a family history of nephrocalcinosis in 14 (25.9%) of cases. The mean (SD) 24-hour urinary oxalate in 9 children with primary hyperoxaluria was 85 (31.8) mg/1.73 m^2/day. Four children presented with persistently low eGFR for more than 3 months. During the median (IQR) duration of follow up of 24 (8, 56) months in children with NC, there was improvement in the weight *Z* scores and eGFR (***Table* II**).

Genetic studies were performed in 8 children. Out of these, in five children with primary hyper-oxaluria, AGXT mutation was detected in four cases; and GRHPR mutation in one. One child with distal RTA had ATP6V0A4 mutation, and two children with Bartter syndrome had ROMK and CLCN-KB mutations, respectively.

DISCUSSION

This study is one of the largest single-centre studies on the etiological profile of NC. The study showed that the most common etiologies of NC were distal RTA, primary hyperoxaluria, Bartter syndrome and Dent disease,

Table II Growth and Biochemical Features at Presentation and at Follow-up in Children with Nephrocalcinosis (*N*=54)

Parameter	*At presentation*	*At last follow up†*
*Age (mo)	36 (11.5, 84)	78 (38, 144)
*Weight (Z score)	-3.4 (-2.0, -4.98)	-2.95(-3.75,-1.68)
#Height (Z score)	-3.20 (2.04)	-2.96 (2.02)
#eGFR*(mL/min/1.73m^2)	59.04 (25.5)	77 (31.48)

*†median (follow up) of 24 (8,56) mo; eGFR-estimated Glomerular filtration rate using modified Schwartz formula; values in *median (IQR) or #mean (SD);P<0.01 for all comparisions except age.*

WHAT THIS STUDY ADDS?

- Distal renal tubular acidosis, primary hyperoxaluria, Bartter syndrome and Dent disease were the commonest etiologies of nephrocalcinosis at a referral hospital in Southern India.

together accounting for more than two-thirds of cases. Common clinical presentations included failure to thrive, polyuria and bony deformities. At a median (IQR) follow up of 24 (18, 56) months, the estimated glomerular filtration rate (GFR) had significantly increased, possibly due to resolution of AKI (resulting from a polyuric state).

There have been few studies evaluating the clinico-etiological profile of pediatric NC [1,4-6, 11]. Mantan, *et al*. [6] retrospectively evaluated the etiology of NC in 40 children from northern India, which included d-RTA (50%), idiopathic hypercalciuria (7.5%) and primary hyperoxaluria (7.5%). At a median (range) follow up of 35 (14,240) months, the eGFR had declined from 82.0 (42,114) to 70.8 (21.3, 126.5) mL/min/1.73 m^2. Ronnefarth, *et al.*, [1] retrospectively evaluated 152 children with NC from Germany, which included idiopathic hypercalciuria (34%), hereditary tubular disorders (32%) and vitamin D toxicity (8%). The eGFR had increased from 96 to 103 mL/min/1.73m^2. Dogan, *et al*., [4] in 36 Turkish children with NC, reported distal RTA (30.5%), Bartter syndrome (13.8%), Vitamin D toxicity (8.3%), idiopathic hypercalciuria (5.5%) and primary hyperoxaluria (5.5%). Among 41 children from Italy, hereditary tubulopathies was the single largest etiology (41.4%), of which distal RTA was seen in 17% [5]. During a mean follow up of 4.4 years, eGFR remained stable in 89% [5].

There appear to be some differences in our results when compared to those of the aforementioned studies [1,4,6]. The etiological profile of our enrolled cases is notable for the absence of idiopathic hypercalciuria, which in previous studies ranged from 7.5%-34% [1,4,6]. The hypercalciuria in our enrolled cases was secondary in nature. Furthermore, a cause for NC was not identifiable in 9.3% of enrolled cases in our study. This is comparable to the results of the Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN) survey (6%) [1]. Consanguinity was noted in 50% of our cases; and this, along with ethnic variations could have accounted for high percentage of inherited tubulopathies. Hypercalciuria was commonly noted in our study, highlighting its importance as a major pathogenic factor [12,13]. Primary hyperoxaluria was another important cause, similar to those reported in developed countries [14,15]. We did not encounter any cases of vitamin D excess among the enrolled cases.

Owing to resource constraints, genetic studies could not be performed in all cases. Moreover, we could not perform urine citrate estimation due to logistic reasons. Finally, the etiological profile of patients enrolled in this study might be affected by a referral bias.

To summarize, distal RTA, primary hyperoxaluria, Bartter syndrome and Dent disease were the most common etiologies of NC in our study. Failure to thrive, polyuria, polydipsia and bony deformities were the common presenting features in our patients. With a systematic approach, etiologies of NC could be identified in most of the cases.

Contributors: KR, SK, PS: management of the patients; KR: collected the data, reviewed the literature and drafted the first version of the manuscript; SK: conceptualized the study, collected the data, reviewed the literature, revised the manuscript and critically reviewed the manuscript. All authors contributed to drafting of the manuscript and approved the final version of the manuscript; SK: shall act as guarantor of the paper.

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