Turkish Journal of Pediatric Disease

Original Article

Evaluation of children with familial hypomagnesemia with hypercalciuria and nephrocalcinosis

Zehra Aydın[1], Fatma Şemsa Çaycı[1], Mihriman İnözü[1], İbrahim Şahin[2], Martin Kondrad[3], Umut Selda Bayrakçı[1,4]

[1]Department of Pediatric Nephrology, Ankara City Hospital, Ankara, Türkiye
[2]Department of Medical Genetics, Diagnostic Center for Genetic Diseases, Dıskapi Yildirim Beyazit Training and Research Hospital, Ankara, Türkiye
[3]Department of Pediatric Nephrology, University Children's Hospital, Muenster, Germany
[4]Department of Pediatric Nephrology, University of Yıldırım Beyazit, Ankara, Türkiye

ABSTRACT

Objective: Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal-recessive renal tubular disorder. It is characterized by renal wasting of magnesium and calcium, which subsequently leads to bilateral nephrocalcinosis, renal stones, and kidney failure. Early diagnosis of FHHNC is important to prevent morbidity and mortality but due to non-specific symptoms it is difficult to diagnose. In this report, pediatric FHHNC patients are presented to raise awareness about the disease.

Material and Methods: We retrospectively analyzed pediatric FHHNC patients in our hospital between 2010 and 2020.

Results: A total of seven patients, five girls (71.4 %) and two boys (28.5 %) with a median age of four years (min: four months, max:13 years) and a mean follow-up time of 4.4±3.5 years were included. Three patients had been diagnosed incidentally. All patients had nephrocalcinosis, hypercalciuria and high parathormone (PTH) level. One patient had normal serum magnesium level. All patients had high urine fractional excretion of magnesium (FEMg). Five patients had CLDN 16 mutation, and two patients had CLDN19 mutation. None of them had ocular findings. Three patients had kidney failure at the end of the follow-up.

Conclusion: Normal serum magnesium levels do not rule out FHHNC. FEMg value is much more significant in patients with FHHNC. FEMg is recommended in all pediatric patients with nephrocalcinosis even if serum magnesium levels are normal.

Keywords: CLDN16, CLDN19, Hypomagnesemia, Hypercalciuria, Nephrocalcinosis

INTRODUCTION

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal-recessive renal tubular disorder. It is characterized by renal wasting of magnesium (Mg) and calcium (Ca) which leads to hypomagnesemia, hypercalciuria, bilateral nephrocalcinosis and kidney failure. This rare disorder is caused by mutation of CLDN16 or CLDN19 genes which encodes claudin-16 and claudin-19 proteins (1,2). Recurrent urinary tract infection, polyuria, polydipsia, enuresis, growth retardation, tetany, and seizures are the most common symptoms of the disease (1). In addition, patients with CLDN19 mutations could be associated with congenital ocular defects (2).

Despite the severe clinical course there is no specific treatment for FHHNC and generally, treatment is supportive such as oral Mg and citrate supplementation, high fluid intake, dietary salt restriction (3). In addition, kidney transplantation is the only curative option for kidney failure. Early diagnosis of FHHNC is important to prevent morbidity and mortality but due to these non-specific symptoms it may be difficult to diagnose. In this report, we present seven pediatric cases of FHHNC to raise awareness about the disease.

MATERIALS and METHODS

Pediatric patients with FHHNC were retrospectively analyzed between 2010-2020 in our hospital. All patients had genetic analysis. Demographic data and medical history were collected from the records of our hospital. Ophthalmologic examinations were also performed in all children.

The fractional excretion of magnesium (FEMg) was calculated using the formula of SeCr×UMg×100/0.7×SeMg×UCr where SeCr = serum creatinine, UMg = urinary magnesium, SeMg = serum magnesium, and UCr = urinary creatinine. FEMg >4% is considered as hypermagnesuria (4). Hypomagnesemia is

Correspondence Address:
Fatma Şemsa Çaycı
e-mail: saltugan2001@yahoo.com

Received : 02.10.2024
Accepted : 17.01.2025
DOI: 10.12956/TJPD.2025.1103

accepted as having serum magnesium lower than 1.46 mg/dL (5). Hypercalciuria is accepted when 24-hour urinary calcium excretion exceeds 4 mg/kg/day, or spot urinary calcium/creatinine ratio > 0.86 mg/mg for children less than seven months of age, and spot urinary calcium/creatinine ratio > 0.2 mg/mg for children older than seven months of age (6,7). Hyperparathyroidism was defined as having serum parathyroid hormone (PTH) level greater than 88 pg/mL according to the references of our laboratory. The 25-OH vitamin D insufficiency is accepted as having levels less than 30 ng/mL (8,9). Hyperuricemia is considered when the serum uric acid level is ≥6 mg/dL in boys and girls younger than 15 years (10). Hypocitraturia is accepted as having urine citrate levels less than 180 mg/g creatinine regardless of gender (11).

Nephrocalcinosis was diagnosed with ultrasonography. The estimated glomerular filtration rate (eGFR) (mL/min/1.73 m^2) was calculated using the original Schwartz equation (12). eGFR levels below 90 mL/min/1.73 m^2 were considered as chronic kidney disease (CKD). Magnesium supplements, usually at a dose of 0.4-0.8 mmol Mg +2 per kilogram of body weight three times a day, and potassium citrate at a daily dose of 0.5-1 mmol/kg were administered. Hydrochlorothiazide was administered at a standard dose of 0.5-1.5 mg/kg/day if the patient has hypercalciuria.

Genetic Analysis -DNA Sequencing and Classification of Variants

In EDTA tubes, peripheral blood samples were collected, and the patients' DNA was isolated using an automated DNA isolation technique (Qiagen Inc. Mississauga, ON, Canada). Sophia DDM software (Sophia Genetics, Saint-Sulp) was used to analyze the data. The variations were evaluated according to the gene, allele frequency, inheritance type, and clinical results of the patient. We removed variants with a MAF greater than 1% on EXAC. The Clinvar and Global Variome LOVD databases were utilized in conjunction with in silico prediction techniques (Mutation taster, SIFT, and Polyphen2). A standardized variant interpretation was undertaken in accordance with the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) recommendations. This research discusses pathogenic and likely pathogenic variants. The Sanger sequencing was used to validate variants.

Statistical Analyses

Descriptive statistics were presented as numbers and percentages for categorical variables, and as mean values with standard deviations for numerical variables.

RESULTS

A total of seven patients (five female, two male) with FHHNC were included. The median age at diagnosis was four years (minimum: four months, maximum: 13 years), and the mean follow-up time was 4.4±3.57 years.

The most common signs and symptoms during disease course were nephrolithiasis, convulsion, and urinary tract infection (28.6%), vomiting (14.3%), macroscopic hematuria (14.3%) and microscopic hematuria (42.8%).

Case 1.2, being the sibling of Case 1.1, was diagnosed during screening. Similarly, Cases 3.2 and 3.3, as siblings of Case 3.1, were also diagnosed during screening.

All patients exhibited normal percentiles for body weight and height, as well as normal ocular results. The CLDN16 mutation was identified in five cases, while the CLDN19 mutation was found in two. The demographic, genetic and extrarenal findings of the patients are summarized in Table I.

Hypermagnesuria, hypercalciuria, nephrocalcinosis, elevated PTH levels, normal urine oxalate levels, and normal blood gas analyses were present in all patients. Hypomagnesemia was detected in 6 patients except one (Case 3.3). Case 3.3 had normal serum magnesium level at diagnosis. Under magnesium replacement, low serum magnesium levels persisted in three patients. During the follow-up period, three patients remained normocalciuric while undergoing HCT therapy.

One patient with renal failure had hypocalcemia and hyperphosphatemia (Case 2). Three patients had less than 90 mL/min/1.73 m^2 eGFR at the end of the follow-up period (Case 2, Case 3.1, Case 3.2). Selected biochemical laboratory data of patients with FHHNC are summarized in Table II.

At the time of diagnosis, five patients had sterile pyuria, three had low urine citrate levels. Selected urine analysis data of patients with FHHNC are summarized in Table III.

DISCUSSION

In this study, we evaluate seven pediatric patients from four different families diagnosed with FHHNC. In general, FHHNC occurs in childhood or before adolescence. While the incidence of FHHNC is unknown, it is one of the most frequent inherited tubulopathies and an important genetic cause of nephrocalcinosis which leads to chronic kidney disease (2,3). The consanguineous parents had a higher risk for FHHNC, and the clinical course of FHHNC is variable. Bilateral medullary nephrocalcinosis is one of the most important diagnostic clues for FHHNC, and nephrocalcinosis may also be accompanied by urolithiasis (1,13). Generally, initial clinical symptoms are usually mild and non-specific, and patients could present with urinary tract infection, hematuria, polyuria, polydipsia, abdominal pain, vomiting, and occasionally tetany (13).

Even though hypomagnesemia is one of the most common symptoms of FHHNC, 34% of patients had normal serum magnesium levels (13). Hypomagnesemia may diminish over time as CKD progresses, making FHHNC difficult to identify (14). It's important to remember that a normal blood magnesium

Table I: Demographic, genetic and extrarenal findings of the patients

	Case1.1	Case 1.2	Case 2	Case 3.1	Case 3.2	Case 3.3	Case 4
Age at Diagnosis	2 years	3 years	13 years	4 months	6 years	3 years	5 years
Gender	F	F	M	F	F	M	F
Consanguinity	+	+	-	+	+	+	-
Initial signs	Vomiting	-	UTI, Nephrolithiasis, Convulsion	UTI	-	-	Macroscopic Hematuria
Last BW (Percentile)	10-25	> 97	> 97	75	50	50	25-50
Last BH (Percentile)	75-90	> 97	10	75	75-90	75-90	25-50
Follow-up period (Years)	10	6	2	9	1.5	1.5	1
Genetic analysis	CLDN16 c.647G>A p.Arg216His Homozygous	CLDN16 c.647G>A p.Arg216His Homozygous	CLDN19 c.269T>C p.Leu90Pro Homozygous	CLDN16 c.211A>G p.Met71Val Homozygous	CLDN16 c.211A>G p.Met71Val Homozygous	CLDN16 c.211A>G p.Met71Val Homozygous	CLDN19 c.269T>C p.Leu90Pro Homozygous

***F:** Female, **M:** Male, **UTI:** Urinary Tract Infectious, **BW:** Body Weight, **BH:** Body Height*

Table II: Selected biochemical laboratory data of patients with familial hypomagnesemia with hypercalciuria and nephrocalcinosis

	Gender	Creatinine (mg/dL)		eGFR (ml/min/1.73 m^2)		Magnesium (mg/dL)		Calcium (mg/dL)		Phosphorus (mg/dL)		PTH (pg/mL)		25-OH Vitamin D (ng/mL)		Treatment
		First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	First	Last	
Case 1.1	F	0.3	0.69	155	116	1.2	1.7	10	9.1	4	4.9	90	152	17	12.7	Mg, potassium citrate, HCT
Case 1.2	F	0.55	0.62	123	102	1.3	1.9	10	9.7	4.9	4.6	137	71	13.9	13	Mg, potassium citrate, HCT
Case 2	M	1.46	3.57	74	30.3	0.96	1.3	8.5	9.2	6.1	5.5	571	523	10	10.3	Mg, potassium citrate, HCT
Case 3.1	F	0.28	0.81	98	83	0.9	1.5	9.6	9.9	5.3	4.4	271	154	35	48	Mg, potassium citrate, HCT
Case 3.2	F	0.55	0.82	120	86	0.8	1.4	8.7	10.1	4.6	4.3	288	181	19	56	Mg, potassium citrate, HCT
Case 3.3	M	0.45	0.53	124	111	1.5	1.5	9.9	10.3	4.8	4.8	259	201	16	32	Mg, potassium citrate, HCT
Case 4	F	0.46	0.55	127	111	0.9	1.3	9.2	9.5	5.4	5.1	188	66	14	31	Mg, potassium citrate, HCT

***F:** Female, **M:** Male, **eGFR:** estimated Glomerular Filtration Rate, **PTH:** Parathormone, **HCT:** Hydrochlorothiazide*

level doesn't rule out FHHNC, and that high FEMg levels (> 4%) are more significant than low serum magnesium levels (13,14).

Furthermore, Sikora et al. (14) reported also hypocitraturia in 60% of patients with FHHNC. Hypercalciuria, hypocitraturia, and urinary acidification defects also reported as a cause of nephrolithiasis and hypomagnesemia as a cause of high PTH secretion which may maintain normocalcemia (15).

In our study, all patients had hypercalciuria and nephrocalcinosis with a male/female ratio of 2/5. One family had two affected siblings, and one family had three affected siblings. Three patients were asymptomatic and diagnosed because of their sibling's diseases. Three patients (42.8%) had hypocitraturia, two patients (28.6%) had urolithiasis. Hypomagnesemia was detected in six patients. One patient (Case 3.3) had normal serum magnesium level. High FEMg level was found in all children.

It is known that FHHNC frequently leads to renal failure during childhood or adolescence. Progressive renal impairment is correlated with tubulointerstitial nephritis and nephrocalcinosis (16). But the pathogenesis of renal function declines in patients with FHHNC remains unclear (17). The risk of CKD was reported to be high in patients with CLDN19 than in patients with CLDN16 mutations (8). Konrad et al. (17) have also reported a phenotype-genotype correlation regarding renal function decline in patients with CLDN16 mutations and Weber et al. (15) reported a median age for end-stage renal failure as 14.5 years. Extrarenal findings such as ocular abnormalities, hearing impairment and neurological manifestations are observed in patients with CLDN19 mutations (18). In addition, various nonspecific ocular abnormalities have also been reported in patients with CLDN16 mutations (19). Up to now, ocular abnormalities or hearing findings were not detected.

Table III: Selected urine analysis data of patients with familial hypomagnesemia with hypercalciuria and nephrocalcinosis at diagnosis

Patient number	Leucocyturia	Hematuria	24h UCa (mg/kg/day)	FEMg (%)	Ucitrate (mg/gr creatinine)
Case 1.1	-	-	12	4.55	510
Case 1.2	+	+	4.7	5.5	532
Case 2	+	+	5.2	16.5	80
Case 3.1	+	-	5.3	20.5	251
Case 3.2	-	-	11.2	39.7	94
Case 3.3	+	+	11.7	23.2	41
Case 4	+	+	7.4	13.9	289

24h UCa: *24-hour Urinary Calcium,* ***Ucitrate:*** *Urinary Citrate,* ***FEMg:*** *Fractional excretion of Magnesium*

Generally, treatment is supportive. Oral Mg and citrate supplementation, high fluid intake, dietary salt restriction and reduction of urinary Ca excretion with hydrochlorothiazide are some of these treatments (20). Zimmermann et al. (21) demonstrated that hydrochlorothiazide (HCT) is effective in reducing hypercalciuria due to CLDN16 mutation. In our patients, hypercalciuria was treated with HCT and three patients were normocalciuric under HCT therapy during the follow-up period. Six of our patients had low serum magnesium levels at the time of diagnosis. Despite magnesium supplementation, three patients continued to have low serum magnesium levels at their final visits. Sikora et al. (14) reported that only 20% of their patients reached normal serum magnesium levels with magnesium supplementation.

In our patients, renal failure was detected in one patient (with CLDN19 mutation) at the time of diagnosis and in 3 patients at the end of the follow-up period (one with CLDN19, two with CLDN16 mutation). It has been reported that in FHHNC patients, chronic kidney disease frequently occurs beyond the first decade of life, and more than 50% progress to kidney failure within the second or third decade of life. However, some patients, particularly those with CLDN19 mutations, require kidney replacement therapy (dialysis or transplant) during the first decade of life (13).

It is known that supportive treatment does not affect progression of renal failure in these patients and kidney transplantation is the only curative option for kidney failure (14). Therefore, early diagnosis of FHHNC is important to prevent morbidity and mortality. In conclusion, we would like to underline the importance of FHHNC awareness in children with nephrocalsinosis. Because a normal serum magnesium level does not rule out FHHNC, high FEMg (> 4%) values are more significant than low serum magnesium levels for the diagnosis.

Ethics Committee Approval

This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by the Ethics Committee No. 2 of Ankara Bilkent City Hospital.(07.04.2021, reference number: E2-21-325).

Contribution of the Authors

AYDIN Z, ÇAYCI FS: collected and recorded the patients' data, were responsible for literature research, ***ŞAHİN İ, KONRAD M:*** performed the genetic analysis, ***ÇAYCI FS, AYDIN Z, İNÖZÜ M, BAYRAKÇI US:*** followed patients, ***ÇAYCI FS, AYDIN Z:*** took the lead in writing the manuscript. All authors discussed the results and contributed to the final manuscript. Funding This study was not supported.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Hou J. Claudins and mineral metabolism. Curr Opin Nephrol Hypertens. 2016;25(4): 308-13. https://doi.org/10.1097/MNH.0000000000000239
2. Wang SB, Xu T, Peng S, Singh D, Ghiassi-Nejad M, A.Adelman R, et al. Disease-associated mutations of claudin-19 disrupt retinal neurogenesis and visual function. Commun Biol. 2019; 25; 2:113. https://doi.org/10.1038/s42003-019-0355-0
3. Claverie Martin F. Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis: Clinical and molecular characteristics. Clin Kidney J. 2015; 8: 656-64. https://doi.org/10.1093/ckj/sfv081
4. Awazu M, Matsumura K. Utility of fractional excretion of magnesium in diagnosing renal magnesium wasting in pediatric nephrology practice. Clin Biochem. 2024 ;131-132: 110807. https://doi.org/10.1016/j.clinbiochem.2024.110807
5. Gragossian A, Bashir K, Friede R. Hypomagnesemia. In: StatPearls (Internet). Treasure Island (FL): StatPearls Bookshelf ID: NBK500003; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK500003/
6. Al Ghali R, El-Mallah C, Obeid O, El-Saleh O, Smail L, Haroun D. Urinary minerals excretion among primary schoolchildren in Dubai-United Arab Emirates. PLoS One. 2021;16(8): e0255195. https://doi.org/10.1371/journal.pone.0255195
7. Sadeghi Bojd S, Narouie B, Mohammadi M. Normal Value of Random Urinary Calcium to Creatinine Ratio in Children in Zahedan, South-East of Iran. Health Scope. 2020; 9(4): e62433. https://doi.org/10.5812/jhealthscope.62433

8. Nwosu IF, Ibeson CE, Olawoye A, Kyaw H, Kumar K, Odigve C, et al. Interpretation of Parathyroid Hormone Levels in Renal Impairment. Cureus. 2022; 14(6): e25819. https://doi.org/10.7759/cureus.25819
9. National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis. 2003; 42(4-3): 1-201. https://doi.org/10.1016/S0272-6386(03)00905-3
10. Fathallah-Shaykh SA, Cramer MT. Uric acid and the kidney. Pediatr Nephrol. 2014; 29: 999-1008. https://doi.org/10.1007/s00467-013-2549-x
11. Srivastava T, Winston MJ, Auron A, Alon US. Urine calcium/citrate ratio in children with hypercalciuric stones. Pediatr Res. 2009; 66(1): 85-90.https://doi.org/10.1203/PDR.0b013e3181a2939e
12. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am. 1987; 34(3): 571-90.https://doi.org/10.1016/S0031-3955(16)36251-4
13. Vall-Palomar M, Madariaga L, Ariceta G. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis. Pediatr Nephrol. 2021; 36(10): 3045-55.https://doi.org/10.1007/s00467-021-04968-2
14. Sikora P, Zaniew M, Haisch L, Pulcer B, Szczepanska M, Moczulska A, et al. Retrospective cohort study of familial hypomagnesaemia with hypercalciuria and nephrocalcinosis due to CLDN16 mutations. Nephrol Dial Transplant. 2015; 30(4): 636-44.https://doi.org/10.1093/ndt/gfu374
15. Weber S, Schneider L, Peters M, Misselwitz J, Rönnefarth G, Böswald M, et al. Novel paracellin-1 mutations in 25 families with familial hypomagnesemia with hypercalciuria and nephrocalcinosis. J Am Soc Nephrol. 2001; 12:(9): 1872-881. https://doi.org/10.1681/ASN.V1291872
16. Marumoto H, Sasaki T, Tsuboi N, Ito T, Ishikawa M, Ogura M, et al. Kidney disease associated with anorexia nervosa: A case series with kidney biopsies. Kidney Med. 2020; 2(4);418-24. https://doi.org/10.1016/j.xkme.2020.03.007
17. Konrad M, Hou J, Weber S, Dötsch J, Kari JA, Seeman T, et al. CLDN16 genotype predicts renal decline in familial hypomagnesemia with hypercalciuria and nephrocalcinosis. J Am Soc Nephrol. 2008 ;19(1): 171-81. https://doi.org/10.1681/ASN.2007060709
18. Faguer S, Chauveau D, Cintas P, Tack I, Cointault O, Rostaing L, et al. Renal, ocular, and neuromuscular involvements in patients with CLDN19 mutations. Clin J Am Soc Nephrol. 2011; 6(2): 355-60. https://doi.org/10.2215/CJN.02870310
19. Prot-Bertoye C, Houillier P. Claudins in renal physiology and pathology. Genes (Basel). 2020; 11: 290. https://doi.org/10.3390/genes11030290
20. Vall-Palomar M, Burballa C, Claverie-Martín F, Meseguer A, Ariceta G. Heterogeneity is a common ground in familial hypomagnesemia with hypercalciuria and nephrocalcinosis caused by CLDN19 gene mutations. J Nephrol. 2021; 34(6): 2053-62. https://doi.org/10.1007/s40620-021-01054-6
21. Zimmermann B, Plank C, Konrad M, Stöhr W, Gravou-Apostolatou C, Rascher W, et al. Hydrochlorothiazide in CLDN16 mutation. Nephrol Dial Transplant. 2006; 21(8): 2127-32. https://doi.org/10.1093/ndt/gfl144