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ORIGINAL RESEARCH

Clinical Presentation, Diagnosis, and Genetic Characteristics of Patients with Hypophosphatemic Rickets*

Yasemin DENKBOY ÖNGEN[1], Şehime Gülsün TEMEL[2], Ömer TARIM[1], Şebnem ÖZEMRİ SAĞ[2], Erdal EREN[1]

[1] Bursa Uludağ University, School of Medicine, Department of Pediatric Endocrinology, Bursa, Türkiye.
[2] Bursa Uludağ University, School of Medicine, Department of Medical Genetics, Bursa, Türkiye.

ABSTRACT

Phosphate plays an essential role in bone mineralization. Hypophosphatemic rickets (HR) is a rare phosphate-wasting disorder that leads to rickets, which may be FGF23-dependent or independent. In this study, we aimed to evaluate the clinical features of HR patients with genotypic and phenotypic features. This study included 32 children. The suspected gene was primarily studied in cases meeting the clinical diagnosis. Variants were evaluated according to ACMG criteria. All HR patients' median ages at the onset of complaints and diagnosis were 1.5 and 5 years, respectively ($p<0.001$). The most common complaint is bowlegs. At the time of diagnosis, calcium was 9.70 mg/dl; phosphorus was 2.68±0.64 mg/dl; ALP was 530±245 u/l, PTH was 58.70 ng/l, tubular phosphate reabsorption was 74±17%, and TmP/GFR was 2.58±1.15. Five of 32 patients were diagnosed with McCune-Albright syndrome, and two patients were followed as HR secondary to chemotherapy. Variants of the *PHEX* gene were identified in 15 cases (X-linked HR). In X-linked HR patients, no statistically significant difference was found between the age of presentation and diagnosis. Diagnostic delay was observed in infantile hypercalcemia type 2 and hereditary HR with hypercalciuria patients who were followed with nephrocalcinosis from an early age. In conclusion, HR is rare, and patients with bowlegs, short stature, kidney stones, hypophosphatemia, and renal phosphate wasting should be classified as FGF-23 dependent or FGF-23 independent of initiating treatment as soon as possible. Delayed diagnosis is common in cases of nephrocalcinosis, so measurement of calcium and phosphate levels should be standard for these patients.

Keywords: Hypophosphatemic rickets. McCune Albright Syndrome. *PHEX. SLC34A3. SLC34A1.*

Hipofosfatemik Rikets Hastalarının Klinik Başvuru, Tanı ve Genetik Özellikleri

ÖZET

Fosfat kemik mineralizasyonunda önemli bir rol oynar. Hipofosfatemik rikets (HR), FGF23'e bağlı veya bağımsız olabilen riketse yol açan nadir bir fosfat kaybı bozukluğudur. Bu çalışmada, hipofosfatemik rikets hastalarının klinik özelliklerini genotip ve fenotipik özellikleriyle değerlendirmeyi amaçladık. Bu çalışmaya otuz iki hasta dâhil edildi. Klinik tanıyı karşılayan olgularda öncelikle şüpheli gen çalışıldı. Varyantlar ACMG kriterlerine göre değerlendirildi. Tüm HR hastalarının şikâyetlerin başlangıcındaki ve tanıdaki medyan yaşları sırasıyla 1,5 ve 5 yıl idi ($p<0,001$). En sık görülen şikâyet bacak eğriliğiydi. Tanı anında kalsiyum 9,70 mg/dl; fosfor 2,68±0,64 mg/dl; ALP 530±245 u/l, PTH 58,70 ng/l, tübüler fosfat reabsorbsiyonu %74±17 ve TmP/GFR 2,58±1,15 olarak bulundu. Otuz iki hastanın beşi McCune Albright sendromu tanısı aldı ve iki hasta kemoterapiye sekonder HR olarak takip edildi. On beş vakada *PHEX* gen varyantları saptandı (X'e bağlı HR). X'e bağlı HR hastalarında başvuru yaşı ile tanı yaşı arasında istatistiksel olarak anlamlı bir fark bulunmadı. Erken yaştan itibaren nefrokalsinozis ile takip edilen infantil hiperkalsemi tip 2 ve hiperkalsiürik herediter hipofosfatemik raşitizm hastalarında tanı gecikmesi gözlendi. Sonuç olarak hipofosfatemik raşitizm nadir görülür ve bacak eğriliği, boy kısalığı, böbrek taşı, hipofosfatemi ve renal fosfat kaybı olan hastalar mümkün olan en kısa sürede FGF-23 bağımlı veya FGF-23 bağımsız olarak sınıflandırılmalı ve tedavi planlanmalıdır. Nefrokalsinozis olgularında tanı gecikmesi sık görüldüğünden bu hastalarda kalsiyum ve fosfat düzeylerinin ölçümü standart olmalıdır.

Anahtar Kelimeler: Hipofosfatemik raşitizm. McCune Albright Sendromu. *PHEX. SLC34A3. SLC34A1.*

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Yasemin DENKBOY ÖNGEN, MD
Bursa Uludağ University Hospital. Department of Pediatric Endocrinology. Bursa, Türkiye
Phone : +90 224 295 04 13
E-mail: ydenkboyongen@uludag.edu.tr

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AUTHORS' ORCID INFORMATION
Yasemin DENKBOY ÖNGEN: 0000-0002-5657-4260
Şehime GÜLSÜN TEMEL: 0000-0002-9802-0880
Ömer TARIM: 0000-0002-5322-5508
Şebnem ÖZEMRİ SAĞ: 0000-0002-3948-8889
Erdal EREN: 0000-0002-1684-1053

Phosphate is involved in cell membrane integrity, nucleic acid skeleton, ATP production, cell signaling, buffering of acid-base balance, and bone mineralization. 80-85% of the phosphate filtered from the renal glomeruli is reabsorbed by the proximal tubule, and the remainder is excreted in urine. Serum phosphate levels (P) are influenced by dietary intake and regulated through feedback mechanisms involving parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and 1,25-dihydroxy vitamin D (1,25(OH)2D). FGF23 is a phosphatonin synthesized by a gene located on chromosome 12 in response to hyperphosphatemia and increased vitamin D. Klotho protein acts as a cofactor for the FGF23 to bind to its receptor on the cell membrane[1-2].

Hypophosphatemic rickets (HR) is a group of rare phosphate-wasting disorders with rickets or osteomalacia caused by genetic mutations, drug-induced or a part of syndromes that are FGF23-dependent or FGF23-independent[3-4].

The most common inherited FGF23-dependent HR is X-linked HR (XLH), in which inactivating mutations in the phosphate regulatory endopeptidase homologous X-linked *(PHEX)* gene lead to local and systemic effects[5-6]. Its prevalence is estimated to be 1/20,000-25,000[2].

Autosomal dominant hypophosphatemic rickets (ADHR) due to *FGF23* and *SGK3* mutations, autosomal recessive hypophosphatemic rickets 1 (ARHR1) due to *DMP1* mutations, autosomal recessive hypophosphatemic rickets 2 (ARHR2) due to *ENPP1* mutations, hypophosphatemic rickets with hyperparathyroidism (*KLOTHO*), osteoglophonic dysplasia (OGD) due to *FGFR1* mutations, Raines syndrome (*FAM20C*), tumor-induced osteomalacia, drug-induced HR, intravenous iron infusion, gain-of-function mutations in *GNAS, HRAS, or NRAS and* Neurofibromatosis type 1 are also cause of FGF23-dependent HR[2]. Whilst hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (*SLC34A3*), infantile hypercalcemia type 2 (*SLC34A1*), Fanconi renotubular syndrome type 2 (*SLC34A1*), nephrolithiasis/osteoporosis hypophosphatemia type 1 (*SLC34A1*), nephrolithiasis/osteoporosis, hypophosphatemia type 2 (*SLC9A3R1*), and Fanconi syndrome (Dent disease, cystinosis, or drug-induced) are forms of FGF23-independent HR.

This study aimed to evaluate the clinical features of patients with HR and their genotypic and phenotypic characteristics.

Material and Method

Patient selection and laboratory

The study sample consists of 32 children aged under 18 years old from 27 families who were diagnosed with HR based on clinical and laboratory assessments. The diagnosis of hypophosphatemia was based on at least two P measurements. Patients were excluded from the study if their hypophosphatemia was attributable to calciopenic rickets, or nutritional phosphate deficiency.

The patients' age at which their complaints started, age at diagnosis, gender, diagnostic body weight (BW) standard deviation scores (SDS), height SDS, body mass index (BMI) SDS, calcium (Ca), P, PTH, and alkaline phosphatase (ALP) levels; tubular reabsorption of phosphate (TRP), and renal tubular maximum reabsorption rate/GFR (TmP/GFR) at diagnosis; genetic analysis, consanguinity, presence of a family member with similar complaints, additional findings (cafe au lait, fibrous dysplasia, bone fracture, bone deformities etc.) were evaluated. Height, weight, and BMI SDS were assessed according to Turkish child standards using 'child metrics'[7].

Genetic analysis

Genetic analysis was performed based on the clinical presentation. In patients without hypercalcemia, hypercalciuria, and with normal/high FGF-23 levels, Sanger sequencing of the *PHEX* gene was performed first. Multiplex ligation-dependent probe amplification (MLPA) of the *PHEX* gene was performed in clinically suspicious cases where a pathogenic/likely pathogenic variant was not detected. After that, if no clinically related pathogenic/likely pathogenic variant was detected, patients were included in the targeted next-generation sequencing (NGS) gene panel. In FGF-23 independent HR patients, a targeted NGS panel (nephrocalcinosis and hypophosphatemia) was performed. Whole-exome sequencing (WES) was performed if necessary. The pathogenicity of variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) criteria[8].

In cases where McCune-Albright syndrome (MAS) was considered, the diagnosis was made without genetic analysis, with café au lait, fibrous dysplasia, and additional endocrinological findings.

Statistics

Statistical analyses were performed using IBM SPSS 29.0.2.0 (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp.) statistical package program. Pearson's Chi-square tests were used in the analysis of categorical data. Wilcoxon tests were used to compare tests; $p<0.05$ was considered statistically significant.

Ethics

The study was ethically approved by the local ethics committee (Approval number 2021-17/28) and conducted following the Declaration of Helsinki. The patient's parents provided signed informed written consent.

Results

All HR patients

Data of the 32 children's data were evaluated. The median age at onset of complaints was 1.5 years (0.3-11 years), while the median age at diagnosis was 5 years (0.3-14.5 years). A significant difference was found *($p<0.001$).* The female-to-male ratio was 1.46:1. The most common presenting complaints were bone-related: bowlegs (34.4%), in-toeing (9.4%), bone fractures (6.3%), and hip pain (3.2%). Besides, positive family HR history (15.6%), kidney stones (9.4%), suspicion of precocious puberty (9.4%), hypercalcemia (3.2%), and growth retardation (9.1%) were noted as presenting factors. The median calcium was 9.70 (8.80-15.30) mg/dl; the mean phosphorus was 2.68±0.64 mg/dl; the mean ALP was 530±245 U/L, and the median PTH was 58.70 ng/l (6.60-273.00 ng/l) at diagnosis. The mean tubular reabsorption of phosphate (TRP) was 74±17%, and the mean TmP/GFR was calculated as 2.58±1.15. Of the 32 patients, five were diagnosed with MAS, and two patients were followed up as HR secondary to chemotherapy (drug-induced HR). Genetic analyses were performed on 24 of the remaining 25 cases. Variants of the *PHEX* gene were identified in fifteen cases, variants of the *OCRL* gene in two cases, variants of the *SLC34A3* gene in two cases, variants of the *SLC34A1* gene in two cases, and a variant of the *SLC2A2* gene in one case (Figure 1). The last two patients are the dizygotic twin brothers, who both had hypophosphatemic rickets and normal/elevated intact FGF23 (iFGF23) levels. Their physical examination met the XLH phenotype. The sequencing of the *PHEX* gene, the NGS panel (*PHEX, CLCN5, FGF23, SLC34A1*, and *SLC34A3),* MLPA of the *PHEX* gene, and whole-exome analysis (WES) were studied. However, no variant was detected. 24 patients had been following as FGF23-dependent, and eight patients were FGF23-independent HR.

<u>FGF23-dependent HR</u>

PHEX patients (XLH): Fifteen patients have several *PHEX* gene variants (Table I). No statistical difference was found in the age of onset of complaints and diagnosis. Phosphate and calcitriol treatments (conventional) were started on all patients first.

Others: Dizygotic twin brothers with no *PHEX* variant were diagnosed with FGF23-dependent HR due to normal-elevated intact FGF23 levels (133 and 223 RU/ml- reference value <230). Their calcium was 10.10±0.14 mg/dl; phosphorus was 2.24±0.22 mg/dl; ALP was 415±2 U/L; PTH was 21±0 ng/l. Tubular reabsorption of phosphate (TRP) 63.0±11.3% and TmP/GFR were calculated as 2.78±0.58. Their admission complaint was bowlegs; O-Bain. Although they met the XLH phenotype, Sanger sequencing of the *PHEX* gene, the hypophosphatemia panel (*PHEX, CLCN5, FGF23, SLC34A1,* and *SLC34A3*), MLPA of the *PHEX* gene, and WES were studied.

Treatment: Phosphate and calcitriol treatments (conventional) were started for all XLH patients and dizygotic twin brothers. During follow-up, Burosumab treatment was recommended to them due to the side effects of conventional treatment, such as nephrocalcinosis, inadequacy in increasing phosphorus levels, difficulty in compliance with 6*1 phosphate and 2*1 calcitriol daily, and proven positive results in patients using it. Due to a lack of insurance approval and difficulty obtaining it, burosumab was started in only 8 of 17 patients. On Burosumab treatment, patient compliance and phosphorus levels were more stable, and no significant side effects were observed.

Chemotherapy-induced HR: Long bone fracture and HR were detected in an 11-year-old female patient who received chemotherapy with the diagnosis of Ewing sarcoma. Vertebral compression fracture and HR were detected in a 5-year-old female patient who received chemotherapy for neuroblastoma. Phosphate and calcium treatments were started in patients who were thought to have HR secondary to chemotherapy, and bisphosphonate treatment was started in the first patient because of the DEXA Z score of -3.4.

McCune Albright Syndrome (MAS): The median age at diagnosis of the five patients followed up with MAS was 8 years (6-10 years). Three patients were presented with suspicion of precocious puberty, one with a bone fracture, and one with hip pain. Diagnostic mean Ca 9.42±0.41 mg/dl, P 3.1.4±0.92, ALP 799 U/L (180-1203), and PTH 50.1 ng/l (27.2-84) were found. The TRP and TmP/GFR were calculated as 85.2±10% and 4.26±3.29, respectively. In all cases, café au lait spots and polyostotic fibrous dysplasia were detected at the time of diagnosis, while three cases presenting with precocious puberty had ovarian cysts (peripheral precocious puberty). Two cases were started on bisphosphonate due to recurrent bone fractures and DEXA Z scores of -2.7 and -5.2, and four cases were started on calcitriol and phosphate.

<u>FGF23-independent HR</u>

Fanconi-Reno tubular syndrome: In the case of those who applied with bowlegs at the age of six, there were proximal renal tubular acidosis (RTA) findings other than HR and a second-degree cousin marriage, and there was no similar complaint in the family. Genetic studies could not be performed in this case, who was followed up with a preliminary diagnosis of the "Fanconi-Reno tubular syndrome."

Oculocerebrorenal (Lowe) syndrome: In a 5-month-old male patient, syndromic facial appearance, history of clavicle fracture, bilateral cataract, nephrocalcinosis, and HR were detected. In the

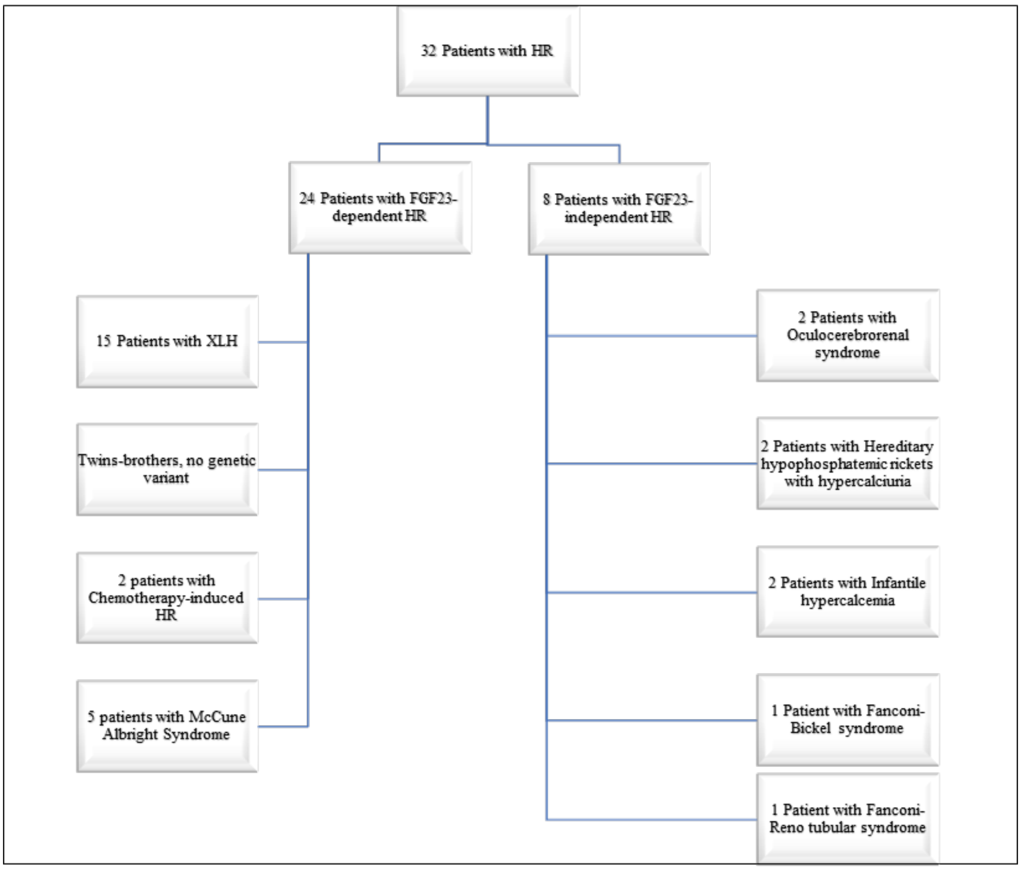

Figure 1:
The distribution of patients according to FGF23-dependent and FGF23-independent groups is shown in Figure 1.

physical examination, weight and height were -3.36 and -4.18 SDS, respectively. Calcium 9.8 mg/dl (8.8-10.8), phosphorus 3.2 mg/dl (3.7-5.6), ALP 767 U/L (156-369), PTH 8.2 ng/L (16-63), TRP: 84%, TmP/GFR: 3.79, and urine Ca/Cre 1.5. A genetic study was performed, and the *OCRL* gene c.1467-1G>C variant was found.

A 14.5-year-old male was admitted with short stature and *OCRL* gene variant results. He was diagnosed with proteinuria at 7 years old. In the physical examination, weight and height were -1.42 and -3.24 SDS, respectively, and no skeletal dysplasia. Hemizygous C.560+1G>A variant detected in the *OCRL* gene. Calcium 8.9 mg/dl (8.8-10.8), phosphorus 2.5 mg/dl (3.7-5.6), ALP 436 U/L (156-369), PTH 25.7 ng/L (16-63), TRP: 88%, TmP/GFR: 4.26, and urine Ca/Cre 0.42.

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH): A 9-year-old girl presented to the outpatient clinic with growth retardation. She had been diagnosed with kidney stones at 6 months of age, and she was receiving hydrochlorothiazide treatment. On physical examination, her weight was -2.97 SDS, and her height was -3.23 SDS. Laboratory tests revealed calcium 10.9 mg/dl (8.8-10.8), phosphorus 2.6 mg/dl (3.7-5.6), ALP 287 U/L (156-369), PTH 9.9 ng/L (16-63), 25-OH vitamin D 22.9 µg/L (>15), 1.25-OH vitamin D 26.44 pg/mL (16-65), Somatomedin-C 70 µg/L (-1.8 SDS), free t4 1.35 ng/dL, TSH 2.931 mU/L, bone age 7 years 2 months, TRP: 51%, TmP/GFR: 1.35 and urine Ca/Cre 0.01 (under treatment). With these findings, genetic analysis was performed for hereditary hypophosphatemic rickets and hypercalciuria. Heterozygous pathogenic variant C.100_1003delGTGC is detected in the *SLC34A3* gene. Oral phosphate (20 mg/kg/day) was started, and dietary calcium was restricted.

An 11.8-year-old female was referred to us with the diagnosis of nephrocalcinosis with the *SLC34A3* gene variant. She had been followed up with kidney stones since the age of two and had a family history of kidney stones. She was receiving hydrochlorothiazide treatment. In her physical examination, her weight was -1.08 SDS, and her height was SDS -0.39, which was within the normal range, with no skeletal deformity. Laboratory tests revealed calcium 10.5 mg/dl (8.8-10.8), phosphorus 3.7 mg/dl (3.7-5.6), ALP 266 U/L (156-369), PTH 8.6 ng/L (16-63), TRP: 93%, TmP/GFR: 4.47 and urine Ca/Cre 0.02 (under treatment). Oral phosphate (25 mg/kg/day) was started.

Table I. Clinical, laboratory, genetic, and family characteristics of patients with *PHEX* gene mutations (XLH).

Patient no	Family no	Gender	Onset of complaints (years)	Age of diagnosis (years)	Weight at diagnosis (SDS)	Height at diagnosis (SDS)	Body mass index at diagnosis (SDS)	Complaints at admission	lower bone deformity	Additional deformities	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (ALP) (U/L)	Parathyroid hormone (PTH) (ng/l)	Tubular reabsorption of phosphate (TRP) (%)	TmP/GFR	DNA description	Protein description	Variant	Pathogenicity: According to ACMG criteria (7)	Clinvar	Franklin	HGMD	Zygosity	Family members with XLH	Consequence
3	2	Male	1.0	2.5	n/a	n/a	n/a	Bowlegs	X-bain	Frontal bossing and pectus excavatum	8.9	2.2	349	273	71	3.2	g.22016715_22056805del		Novel (10)	Pathogenic	0	0	0	Heterozygous	No	No
4	3	Male	1.5	1.5	0.48	-1.72	2.28	In-toeing	O-bain	Frontal bossing	9.8	2.3	707	102.3	66	1.53	c.281_281delTTCCCGAA	p.Ile94Argfs*14	Novel (11)	Likely pathogenic	0	LP	0	Hemizygous	Mother	No
5	4	Female	3.0	6.0	-2.26	-5.56	1.58	In-toeing	O-bain	No	9.8	2.2	374	35.6	72	1.65	c.1645+1G>A	no transcript	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	No	No
6	5	Male	1.0	10.0	-2.29	-4.08	-0.07	Bowlegs	X-bain	No	9.0	1.7	345	69.1	68	1.15	c.2242_2243delCT	p.L748fs*48	Reported	Likely pathogenic	0	P	0	N/a	No	No
7	6	Female	2.0	2.0	-1.68	-2.91	-0.03	Bowlegs	O-bain	No	9.3	1.9	485	109.9	40	1.20	c.1806G>A	p.Trp602*	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	Mother, uncle, grandmother, cousins	No
8	6	Female	0.5	0.5	-0.31	-0.84	0.26	XLH of family members	No	No	10.5	3.1	685	109	85	2.64	c.1806G>A	p.Trp602*	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	Father, aunt, grandmother, sister	No
9	6	Female	0.7	0.7	-0.32	-1.18	0.48	XLH of family members	No	No	10.4	2.5	418	107.8	53	1.49	c.1806G>A	p.Trp602*	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	Father, aunt, grandmother, sister	No
10	7	Male	0.3	0.3	-1.62	-1.68	-0.88	XLH of family members	No	No	9.9	3.1	730	137	91	3.18	c.1899+5G>T	IVS14+1G>A	Reported	Pathogenic	0	VUS	0	Hemizygous	Mother, aunt, grandfather	No
11	7	Female	0.4	0.4	-0.41	1.36	-1.80	XLH of family members	No	No	9.7	3.3	686	104.2	88	2.91	c.1899+5G>T	IVS14+1G>A	Reported	Pathogenic	0	VUS	0	Heterozygous	Mother, aunt, grandfather	No
12	8	Female	1.5	8.0	1.02	-1.19	1.51	Bowlegs	O-bain	No	9.4	2.7	535	72.1	82	2.2	c.1735G>A	p.Gly579Arg	Reported	Pathogenic	0	P	DM Rickets, hypophosphataemic	Heterozygous	Mother	No
13	9	Female	2.0	2.0	n/a	n/a	n/a	Bowlegs	O-bain	No	9.2	2.8	497	118	80	2.08	c.448A>T	p.Lys150Ter	Reported	Pathogenic	P	P	0	Heterozygous	No	No
14	8	Female	2.1	2.1	1.64	-1.29	3.13	XLH of family members	O-bain	No	10.1	2.80	642	54.9	75	2.12	c.1735G>A	p.gly579Arg	Reported	Pathogenic	0	P	DM Rickets, hypophosphataemic	Heterozygous	Sister	No
15	10	Female	1.0	8.1	-0.56	-2.82	1.17	Bowlegs	O-bain	No	9.5	3.0	928	164	79	2.39	c.2247G>T	p.W749C	Novel	Pathogenic	0	LP	0	Heterozygous	No	No
16	11	Male	1.8	3.2	0.28	-1.99	2.20	Bowlegs	O-bain	No	9.1	2.1	570	69.7	84	2.11	Exons 8 and 9 deletions	n/a	n/a	Pathogenic			DM Rickets, hypophosphataemic	Hemizygous	Mother, grandmother	No
17	12	Female	1.5	2.0	-2.73	-2.60	-1.59	Bowlegs	O-bain	No	9.3	2.37	n/a	n/a	84	2.1	c.1461_1465del	p.H487Qfs*2	Novel	Likely pathogenic	0	LP	0	Heterozygous	No	No

Table II. Genetic and diagnostic characteristics of patients with FGF23-independent HR

Patient no	Family no	Disease	Age at presentation (years)	Age at diagnosis (years)	Complaint	Gene	DNA description	Pathogenicity: According to ACMG criteria (7)	Zygosity
18	13	Fanconi-Reno tubular syndrome	6	6	O Bain	n/a	n/a	n/a	n/a
19	14	Oculocerebrorenal (Lowe) syndrome	0.4	0.6	Nephrolithiasis and fracture	*OCRL1*	c.1467-1G>C	pathogenic	Homozygous
20	15	Oculocerebrorenal (Lowe) syndrome	7	14	Family history	*OCRL1*	c.560+1G>a		Hemizygous
21	16	Hereditary hypophosphatemic rickets with hypercalciuria	0.5	9	Growth retardation	*SLC34A3*	c.100_1003delGTGC	pathogenic	Heterozygous
22	17	Hereditary hypophosphatemic rickets with hypercalciuria	9	11.8	Nephrolithiasis	*SLC34A3*	c.846G>A	pathogenic	Homozygous
23	18	Infantile hypercalcemia type 2	0.4	5	Nephrolithiasis and genetic result	*SLC34A1*	c.272_292del c.644+1G>A	pathogenic	Compound Heterozygous Maternal (Heterozygous) *SLC34A1* c.272_292del Paternal (Heterozygous) *SLC34A1* c.644+1G>A
24	19	Infantile hypercalcemia type 2	0.3	14	Hypercalcemia	*SLC34A1*	c.713A>C c.1449G>A	pathogenic	Compound Heterozygous Maternal (Heterozygous) *SLC34A1* c.713A>C Paternal (Heterozygous) *SLC34A1* c.1449G>A
25	20	Fanconi-Bickel syndrome	0.83	1.2		*SLC2A2*	c.835_836del	pathogenic	Homozygous

Infantile hypercalcemia type 2: A 5-year-old boy who had been diagnosed with nephrocalcinosis at 5 months. He was admitted with genetic analysis c.272_292del and c.644+1G>A variants found in the *SLC34A1* gene. His weight is 0.41 SDS, and his height SDS is 0.57, within the normal range, and he has no skeletal deformity. Laboratory tests revealed calcium 11.5 mg/dl (8.8-10.8), phosphorus 4.2 mg/dl (3.7-5.6), ALP 234 U/L (156-369), PTH <3 ng/L (16-63), TRP: 90%, TmP/GFR: 4.22, and urine Ca/Cre 0.3.

A 4-month-old male was admitted with vomiting. In the physical examination, weight and height were -1.98 and -0.26 SDS, respectively. No skeletal deformity. Calcium 15.3 mg/dl (8.8-10.8), phosphorus 1.7 mg/dl (3.7-5.6), ALP 248 U/L (156-369), PTH 39.8 ng/L (16-63), TRP: 86%, TmP/GFR: 3.39, and urine Ca/Cre 0.8. Oral phosphate (40 mg/kg/day) was started immediately. In the follow-up, nephrolithiasis was detected. After a long follow-up period when he was fourteen, *SLC34A1* gene variants (heterozygous c.713A>C and c.1449G>A) were found (In segregation analyses, the father had a heterozygous c.713A>C variant, and the mother had a c.1449G>A variant).

Fanconi-Bickel syndrome: She initially was admitted to an external center at the age of 1 with growth retardation, and since Calcium 9.5 mg/dl (8.8-10.8), phosphorus 2.5 mg/dl (3.7-5.6), ALP 558 U/L (156-369), PTH 85.7 ng/L (16-63), and TPR were 45%. Her cousin was diagnosed with Fanconi-Bickel syndrome; she was diagnosed at the age of 14 months, and calcitriol and phosphate treatments were started immediately (c.835_836del variant homozygous in the *SLC2A2* gene). In her physical examination, weight -2.65 SDS, height SDS -3.55, and O-Bain were detected. FGF23-independent patients were described in Table II.

Discussion and Conclusion

We presented single-center experiences via diagnostic characteristics and genetic analyses of HR in thirty-two children. Most patients were in the FGF23-dependent group; the pathogenic/likely pathogenic *PHEX* gene variants (47%) were the most common, similar to the literature[3-4,9]. While the median age at diagnosis was 5 years in all HR patients, it was 2.05 years in XLH patients, which is very similar to previous Turkish HR surveys[3]. However, a Norwegian study found a median age of 2.1 and 0.9[4]. The very high rate of family history in this study (78%) may explain the earlier diagnosis. The fact that no non-XLH case had a skeletal deformity at presentation in our study suggests that XLH cases may be diagnosed earlier due to their more severe skeletal deformities.

Four novel *PHEX* mutations were found in our study-g.22016715_22056805del, c.281_281delTTCCCGAA, c.2247G>T, c.1461_1465 del- two of which we reported previously[10,11]. There were also many novel *PHEX* variants that had been published before from Türkiye: c.1217G>A, c.2078G>T, IVS13+1G>T, IVS13–2A>G, c.684_685dup, c.1900-6T>A, c.1162G>T, c.1309G>T, c.1392delA, c.1313T>A, c.1173+5G>A, c.1899+5G>T, c.154G>T, c.401_402insGCCAAA, c.1600C>T, c.2242_2243delCT, c.1382_1391del, c.1111_1112insTT, c.983_987dupCTACC, c.1586+2T>G, c.1206delA, c.436+1G>T, c.1217G>T, and g.22,215,887±22,395,767del[11-16].

No significant difference was found between the age of symptom onset and diagnosis in HR cases with *PHEX* mutation. In contrast, a significant difference was found when all cases were considered. Diagnosis may be delayed, especially in HR cases accompanied by hypercalciuria that presents with nephrocalcinosis (esp. *SLC34A1* and *SLC34A3* cases)[4,13]. In another study, Broadman-Pretty speculated on "a diagnostic odyssey" in XLH patients and emphasized the prevention of diagnostic delay and the importance of early initiation of proper treatment[17]. Although symptoms appear early in most cases, diagnosis may be delayed. One study similarly states that HHRH patients were misdiagnosed with FGF-dependent HR before targeted next-generation sequencing, were given the wrong treatment, and received revised treatment immediately[13]. While PTH was normal in patients, ALP was found to be high. In this patient group, especially when PTH is normal, ALP elevation is important; it responds to phosphorus loss[12,18], and despite this, secondary hyperparathyroidism can be seen during treatment. In our study, while skeletal deformities were very evident in XLH cases, it was noted that skeletal findings were not apparent in the remaining group, and short stature was mainly seen. Notably, the fact that non-XLH patients present with non-specific findings such as short stature, nephrocalcinosis, and growth retardation indicate that calcium metabolism tests, including phosphorus, should be evaluated at least once in all these patients. In cases with precocious puberty, hyperthyroidism, and bone fractures, skin findings should be carefully examined, and, if necessary, bone radiographs should be taken to exclude MAS. Calcitriol and phosphate (conventional treatment) are started once the diagnosis is made (only phosphate in hypocalciuric cases)[5,19]. Although it has been known as an initial treatment for a long time, many studies showed that there were complications: nephrolithiasis, secondary hyperparathyroidism, and persistence of renal phosphate wasting and hypophosphatemia were seen[4-5,20]. It has been reported that the new treatment modality, burosumab[5,20,21], increases serum phosphorus by increasing TPR, improves growth, and helps recover rickets[5,9,20,22]. An international multicenter phase 3 study demonstrated that burosumab was superior to conventional therapy in improving rickets scores, growth, limb deformities, and mobility in children ages 1 to 12. This was supported by real-world studies reporting promising improvements in linear growth, rickets scores, and laboratory values in pediatric patients treated with burosumab. The FDA expanded approval to children ages 6 months and older in 2022[20,23]. This treatment is now recommended as a first-line treatment in symptomatic patients[22]. In our cases, due to the risk of nephrocalcinosis, skeletal deformities, and persistent low phosphate levels, Burosumab treatment has been started in most XLH cases. No complications or side effects were seen with this treatment.—FGF-23 independent causes leading to hypophosphatemia are often followed by other clinics, with different findings: nephrocalcinosis, hypercalcemia, kidney failure, developmental delay, and psychomotor delay. Firstly, FGF-23 dependent and independent distinctions should be made in hypophosphatemic cases to use accurate treatment (for example, HHRH patients must not use calcitriol treatment, and Burosumab is effective in XLH patients)[17,19-21]. Gene panels of possible causes should be designed, or NGS should be performed to prevent diagnosis delay and incorrect treatment[12-13]. Eltan et al. have recently reported that MLPA may provide an additional explanatory value of 10% regarding molecular etiology in HR cases[12]. Despite all analyses, this method should be considered when the diagnosis cannot be established.

Hypophosphatemic rickets is a rare disease. When hypophosphatemia and increased renal phosphate wasting are detected in patients presenting with bowlegs, short stature, and kidney stones, it should be determined whether they are in the FGF-23 dependent or FGF-23 independent group, and appropriate treatment should be initiated. Since the delay in diagnosis is particularly evident in the group presenting with nephrocalcinosis, it should be routine to measure calcium and phosphate levels in patients diagnosed with nephrocalcinosis.

Strengths and limitations

Although HR is a rare disease, the number and variety of patients are superior to many studies.

The limitation of this study is that it is a descriptive study conducted through retrospective file scanning.

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Idea and design: Y.D.Ö., E.E.; Data collection and processing: Y.D.Ö.; Analysis and interpretation of data: Y.D.Ö., Ş.G.T., Ö.T., Ş.Ö.S., E.E.; Writing of significant parts of the article: Y.D.Ö., Ş.G.T., Ö.T., Ş.Ö.S., E.E.

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