Original Article

Etiology and Biochemical Profile of Rickets in Tertiary Care Centres in Eastern India: A Retrospective Cross-sectional Study

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Abstract

Introduction: We aimed to describe the clinical, biochemical and etiological profile of patients referred with a provisional diagnosis of rickets in tertiary care centres. In addition, we tried to propose a diagnostic algorithm for the evaluation of such patients. **Methods:** This was a retrospective cross-sectional study conducted in two tertiary care centres of West Bengal. Data of patients were retrieved between 2014 and 2021. **Results:** Out of 101 children, 22 had conditions simulating rickets. Renal tubular acidosis (RTA) was the most common (53.2%) etiology of rickets, followed by phosphopenic rickets (PR) (22.8%) and calcipenic rickets (CR) (17.7%). The prevalence of true nutritional rickets (NR) was only 8.9%. Children with RTA had a significantly higher prevalence of chronic ill health (69%) and polyuria (95.2%). Weight standard deviation score (SDS) and body mass index (BMI) SDS scores were significantly lower in the RTA group compared to others. Around 90.5% of children with RTA, and none in the other groups, had hypokalemia. Biochemically, hypophosphatemia and elevated alkaline phosphatase (ALP) were present in all patients with PR and CR. Compared to CR, median serum phosphate was significantly lower in the PR group. A significant difference in ALP values was noticed in patients with hypophosphatemia (815 ± 627 IU/L) compared to those without (279 ± 204 IU/L). Plasma parathyroid hormone (PTH) of 100 pg/ml seemed useful to differentiate CR from other forms. **Conclusion:** NR is uncommon in tertiary care centres. Children with rickets should be approached systematically with the estimation of ALP, phosphorus, creatinine, calcium, PTH and 25-hydroxy vitamin D to reach an etiological diagnosis.

Keywords: Calcipenic rickets, phosphopenic rickets, refractory rickets, renal tubular acidosis, rickets mimickers

Introduction

Rickets, a disorder of epiphyseal growth plates, is characterised by defective maturation and apoptosis of the late hypertrophic chondrocytes along with improper mineralisation of the epiphyseal cartilages and newly formed organic lamellar matrix (osteoid). Rickets is a disease of childhood and adolescence before fusion of the physis (growth plate), and clinical manifestations depend on the age of presentation, underlying aetiology, duration, and severity of the disease. Children typically present with widening of the growing ends of the long bones, skeletal deformities (genu varum, genu valgum, wind-swept deformity), growth retardation, and delayed developmental (motor) milestones.

The literature shows considerable heterogeneity in the annual incidence rate and etiology of rickets. Nutritional rickets (NR) is the most common cause, accounting for about 85% of such cases at the community level; however, genetic disorders are not uncommon.[1] X-linked dominant hypophosphatemic rickets (XLHR) is the most common heritable form of hypophosphatemic (herein termed phosphopenic) rickets, with an incidence of 1 in 20,000 individuals.[2] Regional reports of NR in adolescents suggest that the condition is highly prevalent in several regions, including north India and the

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Middle East, with a female preponderance. The prevalence of NR among adolescents attending general clinics in Saudi Arabia and India in the early twentieth century was 42.2% and 87.9%, respectively.[3,4] In addition, nearly half of the adolescents aged 11–18 years living in Ethiopia were found to be vitamin D deficient.[5] NR, however, is also not uncommon in the developed countries. Analysis of the health registries documented that the prevalence of NR in Alaska and southern Denmark was around 76%.[6,7] Though the prevalence of NR in the Western world declined during the twentieth century, recent reports indicate that the disease is still prevalent due to changes in lifestyle. A recent multicentre, cross-sectional, school-based, observational study conducted in six states of India estimated that 20% of children and adolescents in India are vitamin D deficient (25-hydroxy vitamin D [25OHD]: <12 ng/ml), while 38% are insufficient (12–20 ng/ml).[8]

Children with NR respond dramatically following adequate vitamin D and calcium supplementations with complete clinical, biochemical and radiological recovery; hence, they do not usually attend tertiary care centres. They are often lost to follow-up due to prompt response to therapy offered by their primary care physicians. The majority of children with rickets attending tertiary care centres suffer from the so-called "refractory rickets", rickets not responding to usual dosage of vitamin D. The diagnostic criteria for "refractory rickets" are somewhat arbitrary, with the non-appearance of radiological healing lines 3–6 weeks after 1–2 courses of 600,000 IU of orally administered vitamin D over a 10-day period or two intramuscular doses of vitamin D (600,000 IU) 2–3 weeks apart typically is considered a clinical criterion for diagnosis.[9-11] An underlying pathology should be sought for in such children.

We believe that the etiological spectrum of rickets in tertiary care centres is different from that of primary care centres, and an etiology other than NR should always be searched for in a systematic manner.

Aims and Objectives

This retrospective study was performed (i) to identify the etiology of rickets in tertiary care centres, (ii) to characterise and compare the clinical and biochemical parameters among different forms of rickets in the study population, and (iii) to propose an algorithm to reach a definite diagnosis in refractory rickets.

Materials and Methods

We retrospectively analysed case records of children, referred with a working diagnosis of rickets between 2014 to 2021. We reviewed the case notes for various factors, including age, sex, presenting symptoms, age at onset of symptoms, and any history of similar or related complaints in the family members. We specifically looked for information on conditions such as nocturia, polyuria, graveluria, renal stones, jaundice, gluten sensitivity, and any history of greasy, foul-smelling stools/clay-coloured stools. In addition, we noted details related to housing, clothing, sun exposure, dietary practices, and medication intake. We retrieved data related to height, weight, body mass index (BMI), and the pattern of skeletal deformities. Furthermore, we also retrieved information on dental examinations such as delayed dentition, caries, enamel hypoplasia, amelogenesis imperfecta, abscess, decay, and loss of teeth. In addition, we noted the results of the ophthalmological evaluation, including corneal opacities, keratopathy, cataracts, cystine crystals, and the Kayser–Fleischer (KF) ring. We also included findings from the auditory examination and other systemic examinations.

All patients underwent testing for serum calcium, phosphorus, creatinine, sodium, potassium (K), alkaline phosphatase (ALP), 25OHD, and parathyroid hormone (PTH), as well as arterial blood gas (ABG) analysis, liver function test (LFT), routine urinalysis, skeletal X-rays, and abdominal ultrasonography (USG). The majority of patients had their blood samples collected in the morning after an overnight fast. In addition, simultaneous second-morning void urine samples were collected to estimate K, calcium, creatinine, and phosphorus. Younger children provided random nonfasting serum and urine samples, while toilet-trained children provided a 24-hour urine sample that was sent for calcium and creatinine measurement. Urine samples were also tested for the concentration of β2 microglobulin, a low molecular weight (LMW) protein. Patients with metabolic acidosis (arterial potential of hydrogen [pH] <7.35) underwent further evaluation, including simultaneous urine pH measurement using a pH meter. For patients with arterial pH between 7.35–7.42 and serum bicarbonate (HCO3) >18 mEq/L, but with a clinical suspicion of renal tubular acidosis (RTA), an NH4Cl (0.2 g/kg) challenge test was performed. Calcitriol and fibroblast growth factor-23 (FGF23) were measured in selected patients. All children under 6 years of age with distal RTA (dRTA) underwent otoacoustic emissions (OAE) as a screening test for hearing loss. If OAE results were abnormal, brainstem evoked response audiometry (BERA) was subsequently performed. Pure tone audiometry (PTA) was conducted in children with dRTA who were 6 years of age or older.

The entire cohort was divided into four groups: (1) rickets mimickers (RMs), (2) RTA, (3) phosphopenic rickets (PR), in which renal phosphate loss was the primary pathology, and (4) calcipenic rickets (CR), in which reduced intestinal calcium absorption was the primary inciting event. Appendix Table 1 provides definitions for each of these conditions. Age and method-specific reference ranges of some of the important biochemical parameters (serum phosphorus, tubular maximum reabsorption rate of phosphate [TmP] to glomerular filtration rate [GFR] [TmP/GFR], and ALP) have been summarised in Appendix Tables 2 and 3.

Statistical Analysis

Descriptive data were presented as means and standard deviations (SDs) for normally distributed continuous variables, and as medians and interquartile ranges (IQRs) for non-normally distributed continuous variables. Proportions were expressed

as percentages. The Shapiro–Wilk test was used to test for the normality of continuous variables. For comparison of continuous variables between three groups, one-way analysis of variance (ANOVA) was used for parametric data and the Kruskal–Wallis test was used for nonparametric data. When the difference was found to be significant, Tukey's and Dunn's *post hoc* test was performed respectively for analysing pairwise stochastic dominance. The Chi-square test (χ^2) was used to determine the relationship between proportions. To avoid overestimation, Yates continuity correction was used when calculating χ^2 values in contingency tables with one or more cells containing values <10. Participants with missing data were excluded from the analysis. The statistical analyses were performed using Jeffreys's Amazing Statistics Program (JASP) version 0.17.1 (the University of Amsterdam, The Netherlands).

Ethical Aspect

- Approval from the institutional ethical committee (Reg. No.-ECR/287/Inst/WB/2013/RR-19) was obtained. Consent was waived due to the retrospective nature of the study. Reference Number: MC/KOL/IEC/NON-SPON/1245/12/21 dated 15/12/2021 and IEC/2020/A-2 dated 02/11/2020.
- All study procedures were according to the guidelines laid down in the Declaration of Helsinki. We strictly followed the declaration.

Results

During the 8-year period, we identified a total of 101 children (67 boys and 34 girls) who were referred with a provisional diagnosis of rickets. Among them, 22 children had underlying RMs. Out of the remaining 79 children, 42 (53.2%) were diagnosed with RTA, 18 with PR (22.8%), and 14 (17.7%) with CR. Three were diagnosed with chronic kidney disease (CKD) (one with stage 4 and two with stage 5), of whom one had chronic progressive external ophthalmoplegia, ptosis, pigmentary retinopathy, complete heart block, hypokalemia, glucosuria, and LMW proteinuria. A diagnosis of mitochondrial myopathy with Fanconi syndrome (FS) was considered, but due to underlying CKD, the patient was not included in the RTA group. Definitive diagnosis could not be reached in another two children because of incomplete evaluation.

In the RTA group, 31 out of 42 children (73.8%) were diagnosed with dRTA, 8 (19%) with proximal RTA (pRTA), and 3 (7.1%) with combined dRTA and pRTA (type 3 RTA). Among the 31 children diagnosed with dRTA, 27 underwent an evaluation for proximal tubular dysfunction, which included tests for phosphaturia, glucosuria, and LMW proteinuria. Of these 27 children, 25 (92.6%) had elevated urinary β2 microglobulin. The remaining two patients had normal serum phosphorus, and were not evaluated for phosphaturia. Hypophosphatemia was seen in 51.6% of patients (n = 16) of dRTA, and all of them had phosphaturia. The proximal tubular dysfunction was subsequently resolved in all 25 patients with the correction of acidosis. Three children (9.7%) with dRTA had sensorineural deafness. Within the PR group, one child had McCune–Albright syndrome (MAS), and another child had polyostotic fibrous dysplasia without other features of MAS. In the CR group, nine children had vitamin D deficiency (VDD), four had vitamin D-dependent rickets (VDDR) type 1A (VDDR-1A), and one had VDDR type 2A (VDDR-2A). One girl with VDD was being treated with phenytoin for 7 years and had typical signs of phenytoin toxicity, while one boy had florid signs of severe malnutrition due to malabsorption syndrome. The prevalence of true NR in our cohort was 8.9%. After excluding children with CKD and those in whom a definitive diagnosis could not be made, we further analysed the data of the remaining 74 children with rickets [Figure 1].

The median age of symptom onset and presentation to our clinic was 2.0 (±3.2) and 8.5 (±9.1) years, respectively. The children were found to be short (height SD score (SDS): -4.3 ± 2.3) and lean (weight SDS: -2.7 ± 1.1). The clinical presentations and auxological parameters are summarised in Table 1. Relevant

Table 1: Clinical presentations and auxological parameters of the study population

Parameters	Entire cohort (n=74)	RTA (n=42)	Phosphopenic rickets (n=18)	Calcipenic rickets (n=14)
Boys/Girls	48/26	26/16	13/5	9/5
Age of onset [median (IQR)] (years)	2 (3.2)	2.8 (3.6)	2.2 (1.5)	1.5 (1.3)
Chronic ill health*	34 (45.9%)	29 (69%)	1 (5.6%)	4 (28.6%)
Muscle weakness	37 (50%)	20 (47.6%)	9 (50%)	8 (57.1%)
Polyuria*	41 (55.4%)	40 (95.2%)	1 (5.6%)	0
Height SDS [median (IQR)]**	−4.3 (2.3)	−4.7 (2.2)	−3.5 (2.2)	−3.2 (2.7)
Weight SDS [mean (SD)]*	−2.7 (1.1)	−3.1 (1)	−1.7 (0.7)	−2.1 (1.2)
BMI SDS [median (IQR)]*	−0.3 (1.7)	−1.2 (1.8)	0.3 (1.4)	−0.7 (1.7)
Varus/Valgus/Wind swept	20/49/5	7/31/4	6/12/0	7/6/1
Upper limb involvement	46 (62.2%)	24 (59.5%)	8 (44.4%)	14 (100%)
Spinal deformity	3 (4.1%)	0	2 (11.1%)	1 (7.1%)
Short stature	66 (89.1%)	42 (100%)	16 (88.9%)	8 (57.1%)

BMI=Body mass index, IQR=Interquartile ranges, RTA=Renal tubular acidosis, SD=Standard deviation, SDS=Standard deviation score. *P<0.05 between RTA and other two groups, **P<0.05 between RTA and phosphopenic rickets groups

biochemical parameters are presented in Tables 2 and 3. The RTA group had significantly higher rates of chronic ill health and polyuria and significantly lower weight SDS and BMI SDS compared to the PR and CR groups. The height SDS was also lower in the RTA group compared to the PR group [Table 1].

In children with RTA and VDD rickets, the median serum 25OHD concentration was 26.3 (±18.1) ng/ml and 7.4 (±4) ng/ml, respectively. Those with RTA had an arterial pH of 7.29 (±0.08), serum K of 2.9 (±0.7) mmol/L, and serum HCO3 of 15.1 (±5.5) mmol/L. We compared ALP values in those with hypophosphatemia (815 ± 627 IU/L) with those without hypophosphatemia (279 ± 204 IU/L) in the overall population with rickets and found a significant difference (Mann–Whitney < 0.001). A similar trend was also noticed in the RTA group. The PR group had a significantly lower median serum phosphorus level than the RTA and CR groups. However, all patients with PR and CR had phosphorus values below the age-specific normal range. Calcitriol was measured in 14 children, of whom 6 (pRTA: 2, VDDR-1A: 4) had low, 4 (pRTA: 1, XLHR: 3) had normal, and the remaining 4 (pRTA: 1, dRTA:

Table 2: Comparison of relevant biochemical parameters among the different groups (*n*=74)

Parameter	RTA	Phosphopenic rickets	Calcipenic rickets	*P*
Serum calcium [median (IQR)]	9.0 (1.0)	9.5 (0.4)	7.9 (1.0)	<0.001*
Serum phosphorus [median (IQR)]	2.6 (0.8)	2.2 (0.7)	2.9 (0.8)	0.009
eGFR [mean (SD)]	92 (34)	124 (36)	95 (32)	0.008***
PTH [median (IQR)]	35 (35)	47 (12)	316 (270)	<0.001****
ALP [median (IQR)]	757 (692)	583 (484)	857 (568)	0.166

ALP=Alkaline phosphatase, eGFR=Estimated glomerular filtration rate, IQR=Interquartile ranges, PTH=Parathyroid hormone, RTA=Renal tubular acidosis, SD=Standard deviation. Note: Analysis of variance (ANOVA) was used for eGFR data. For others, Kruskal–Wallis test was used. *Dunn's *P*<0.001between calcipenic rickets with other two groups. **Dunn's *P*=0.035 between Phosphopenic rickets and RTA and 0.008 between phosphopenic and calcipenic rickets. ***Tukey's *P*=0.006 between phosphopenic rickets and RTA. ****Dunn's *P*<0.001 between calcipenic rickets and RTA and 0.005 between calcipenic and phosphopenic rickets

Table 3: Prevalence of biochemical and radiological abnormalities in the study population

	Entire cohort (*n*=74)	RTA (*n*=42)	Phosphopenic rickets (*n*=18)	Calcipenic rickets (*n*=14)
High ALP	68 (91.9%)	36 (85.7%)	18 (100%)	14 (100%)
Hypophosphatemia	64 (86.5%)	32 (76.2%)	18 (100%)	14 (100%)
Hypokalemia	38 (51.4%)	38 (90.5%)	0	0
Hyponatremia	6 (10%)	4 (10.5%)*	2 (15.4%)**	0**
Low bicarbonate	41 (55.4%)	36 (85.7%)	2 (11.1%)	3 (21.4%)
High PTH	52 (70.3%)	30 (71.4%)	8 (44.4)	14 (100%)
Low arterial pH at baseline	38 (51.4%)	35 (83.3%)	2 (11.1%)	1 (7.1%)
Nephrolithiasis/nephrocalcinosis	23 (31.1%)	23 (54.8%)	0	0

ALP=Alkaline phosphatase, PTH=Parathyroid hormone, RTA=Renal tubular acidosis, pH=Potential of hydrogen. *Missing data in 4 patients. **Missing data in 5 patients

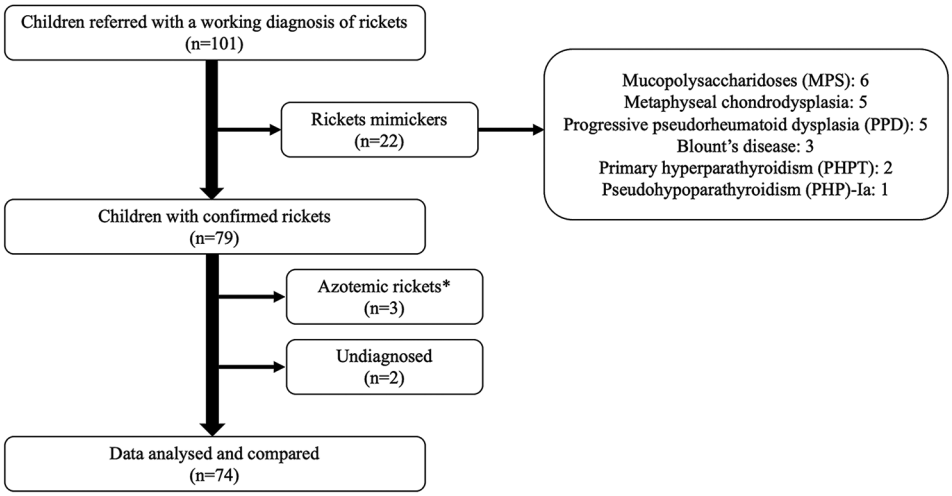

Figure 1: Flow-chart showing the study design (*one of these three patients had mitochondrial myopathy with features of Fanconi syndrome)

1, XLHR: 1, VDDR-2A: 1) had higher values compared to the laboratory-specific reference range. FGF23 was measured in 12 children by C-terminal assay with values ranging between 43.9 and 603 RU/ml. The reference range of the assay was 0–150 RU/ml. Detectable FGF23 in children with hypophosphatemia was considered as inappropriately elevated FGF23, which was found in 11 children with PR and one with dRTA.

Discussion

In addition to the typical skeletal deformities, children with rickets can experience a range of symptoms depending on the underlying cause. In one study in north India, 25% of children aged 10–13 years presenting to orthopedic clinics with musculoskeletal complaints were found to have rickets, with knee pain at night being the most common complaint, followed by leg deformity.[4] In Saudi Arabia, 40% of adolescents with elevated ALP levels (a biochemical marker of rickets and osteomalacia) were found to have NR; of these, 39% presented with bone pain, 18% with limb deformity, 14% with pathological fractures, and 29% were asymptomatic.[3] All of the children in our cohort were referred for joint swelling and/or bony deformities. We found that chronic ill health was common in those with RTA (69%), less common with CR (28.6%), and rare with PR (5.6%). Polyuria appeared to be a crucial clinical indicator of underlying RTA in these children, as 95.2% of our patients with RTA had polyuria. In contrast, only one child with PR and none with CR had this complaint.

A number of skeletal disorders like Blount's disease, metaphyseal chondrodysplasia (Spahr type, Schmid type, metaphyseal anadysplasia), mucopolysaccharidosis (MPS), mucolipidosis (ML) (type II), pseudohypoparathyroidism (PHP), primary hyperparathyroidism (PHPT), progressive pseudorheumatoid dysplasia (PPD) mimic rickets clinically [Appendix Table 4].[10,12-16] X-ray of hands, including the wrist joints and the knees, is an important supplement to clinical examination in patients with suspected rickets. The X-ray not only confirms the diagnosis of rickets, but also provides significant clues to alternative diagnoses [Figure 2].

All three children we encountered with Blount's disease presented with genu varum and had a very high BMI (SDS ranged from +6.2 to +12.3). Children with metaphyseal chondrodysplasia usually present with genu varum, and X-ray shows widening, irregularity and sclerosis of the metaphyses of the bones around knee joints. A "moth-eaten" appearance of the metaphysis should also raise concern about metaphyseal chondrodysplasia. Bones of the spine and upper extremities are often spared, but the involvement of the anterior ends of the ribs and distal ends of the radius and ulna has also been reported.[17-19] Sclerosis of the metaphysis may be mistaken as the healing line of rickets. Three children with metaphyseal chondrodysplasia in our cohort had genu varum, and the remaining 2 had genu valgum. Four children (66.7%) with MPS and 3 (60%) with PPD had genu valgum.

Circulatory ALP level seems to be the best single routine biochemical screening test for rickets and osteomalacia.[20,21] A population-based study performed among the tea-garden community in the Dibrugarh district of Assam, India, noticed that all children with NR had normal calcium and phosphorus but elevated ALP, suggesting that serum ALP is a highly sensitive test to detect nutritional osteomalacia.[22] ALP was found to be elevated in all 81 children with rickets, irrespective of etiology, in a study from Saudi Arabia.[15] Non-elevated ALP in children with clinical suspicion of rickets is a very important

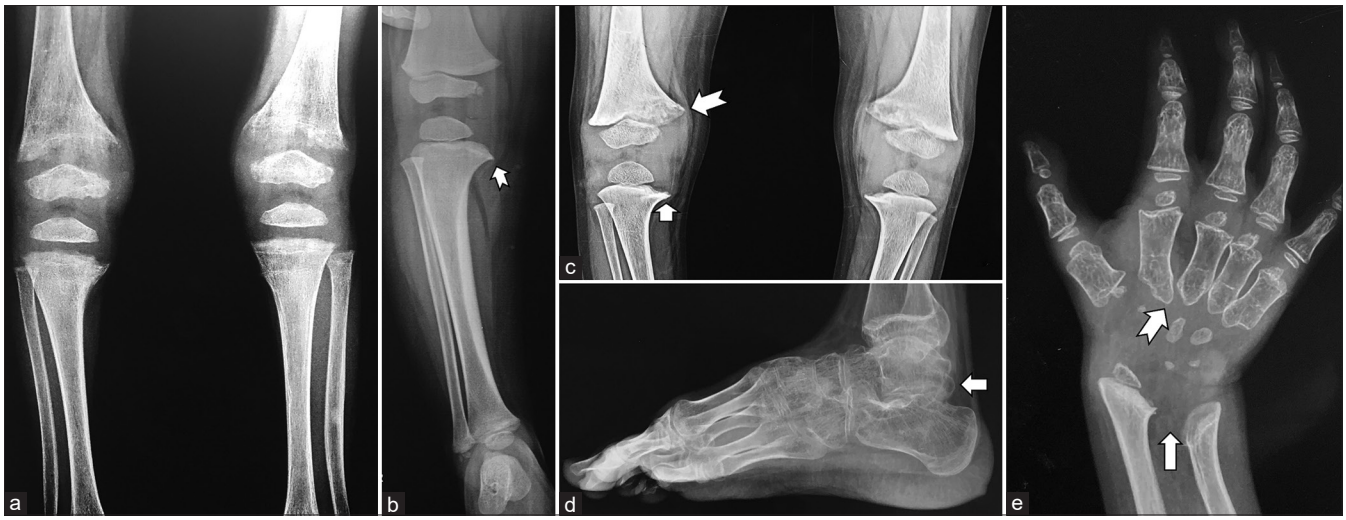

Figure 2: (a) Typical radiological findings in rickets include cupping, fraying and splaying of metaphysis with widened gap between the epiphysis and metaphysis; (b) medial beaking of proximal tibial metaphysis (white notched arrow) in Blount's disease; (c) "Moth-eaten" metaphysis (white notched arrow) and metaphyseal sclerosis (white arrow) in metaphyseal chondrodysplasia; (d) mega os trigonum (white arrow) in progressive pseudorheumatoid dysplasia; (e) "Bullet-shaped" metacarpals (white notched arrow), hypoplastic and irregular carpal bones, and "V"-shaped configuration between the distal radius and ulna (white arrow) in mucopolysaccharidosis

clue for underlying RMs. We encountered elevated ALP only in one child with Blount's disease (25OHD: 13.2 ng/ml), one child with metaphyseal chondrodysplasia (25OHD: 10.5 ng/ml), and two children with PHPT among those 22 patients, who had RMs. The cause of high ALP in the first two children was coexisting VDD. Our findings underline the importance of estimation of serum ALP in all such children; normal serum ALP is helpful to rule out rickets with confidence. ALP typically demonstrates a quadriphasic response; hence, it should always be compared with age and sex-specific reference ranges. Children with hypophosphatasia, pseudohypophosphatasia, and, at times, those with dRTA have low or normal ALP despite typical clinical and radiological features of rickets.[23] On the other hand, patients with ML-type II often demonstrate high ALP and high PTH with normal/low calcium, low/normal phosphorus and low/normal 25OHD.[12] ALP may not be universally elevated in dRTA due to metabolic acidosis-induced suppression in osteoblastic activity. Serum ALP concentrations were found to be significantly lower in dRTA compared to PR (P = 0.04) and VDDR (P = 0.003) in a cohort of non-azotemic refractory rickets, with 64.7% of those with dRTA having ALP levels within the normal range.[10] Normal ALP was encountered in half of the children with dRTA in another cohort of refractory rickets.[11] On the contrary, ALP was elevated in all 81 children (64.3% of the entire cohort) with dRTA within a study population of 126 patients with refractory rickets.[24] All children with PR and CR in our cohort had elevated ALP, while 85.7% of those with RTA had such an abnormality. The difference, however, was not statistically significant. ALP in PR usually ranges between 400–800 IU/L, while the values are much higher, usually up to 2000 IU/L in CR.[25] We noticed a significant overlap between ALP levels in all the three groups of our study [Figure 3a].

The second most important biochemical marker of rickets is hypophosphatemia. Low serum phosphate is considered as the common denominator of almost all forms of rickets, as apoptosis of the terminal cells of the late calcified hypertrophic zone is mediated by circulatory phosphate.[26] Normal or high phosphate in children with rachitic changes suggests one of the following etiologies: RMs, CKD, recent treatment with vitamin D in NR, dRTA, and hypophosphatasia or pseudohypophosphatasia. Children with NR, recently treated with vitamin D and calcium, may demonstrate normal serum phosphorus, as serum phosphorus shows an upward trend as early as 96-hours post-treatment, while complete radiological healing is seen after 24 weeks. Among those 22 children with RMs, only 1 (4.5%) had low phosphorus. We, therefore, propose that serum ALP and phosphorus should be ordered upstream in the diagnostic workup of rickets, and an alternate diagnosis other than rickets needs to be searched for if these values are normal.

The next important biochemical test in the diagnostic algorithm of rickets seems to be serum potassium. It is inexpensive, widely available, and can differentiate a majority of patients with RTA from other forms of rickets. In our study, 38 out of 42 (90.5%) children with RTA had hypokalemia, while none of those with PR or CR showed low serum potassium levels [Table 3]. ABG revealed metabolic acidosis in 35 out of 42 (83.3%) children with RTA, 2 out of 18 (11.1%) with PR, and 1 out of 14 (7.1%) with CR. Elevated PTH and low urinary calcium in CR inhibit sodium-hydrogen exchanger 3 (NHE3) in the proximal tubule and induce HCO3 loss in CR.[27] In addition, severe hypophosphatemia leads to intracellular adenosine triphosphate (ATP) depletion and, at times, results in disordered tubular function with features of FS.[28] Nephrolithiasis/nephrocalcinosis is another crucial clue to underlying dRTA, and pRTA secondary to Dent's disease, cystinosis, tyrosinemia type I, and Fanconi–Bickel syndrome. On ultrasound examination, 54.8% of our children with RTA, and none in the other groups, had such findings.

PTH concentration is higher in CR than in the other forms, which was also evident in our study. We found that plasma PTH of more than 100 pg/ml reliably differentiated CR from other forms, unless the latter group has co-existent VDD [Figure 3b]. In the RTA group, four patients had low PTH, and all of them had dRTA with nephrocalcinosis, and two had hypophosphatemia. Circulatory calcitriol is helpful in differentiating VDDR-1A (low) from VDDR-2 (high) in

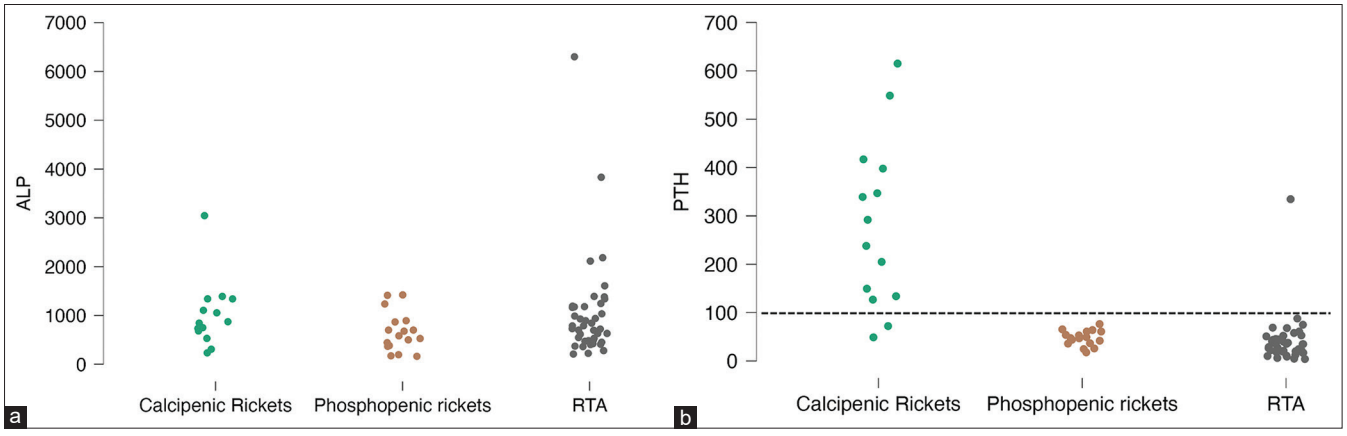

Figure 3: Box plots showing alkaline phosphatase (a) and parathyroid hormone (b) values in three different groups

children with CR and normal 25OHD levels. All four children with VDDR-1A in our cohort had low calcitriol, while the one with VDDR-2A had high calcitriol.

We documented that the prevalence of NR in tertiary care centres is much lower than what is reported in population-based or patient registry-based surveys. Our findings are consistent with similar studies conducted in other tertiary care centres, with the exception of one study from Saudi Arabia [Table 4].[10,11,15,24] Unlike previous studies, we included different conditions that closely mimic rickets and proposed a diagnostic algorithm. In contrast to the previous Indian studies, a higher percentage of children with RTA in our cohort had elevated ALP. We documented reversible proximal tubular dysfunction in at least 93% of patients with dRTA.

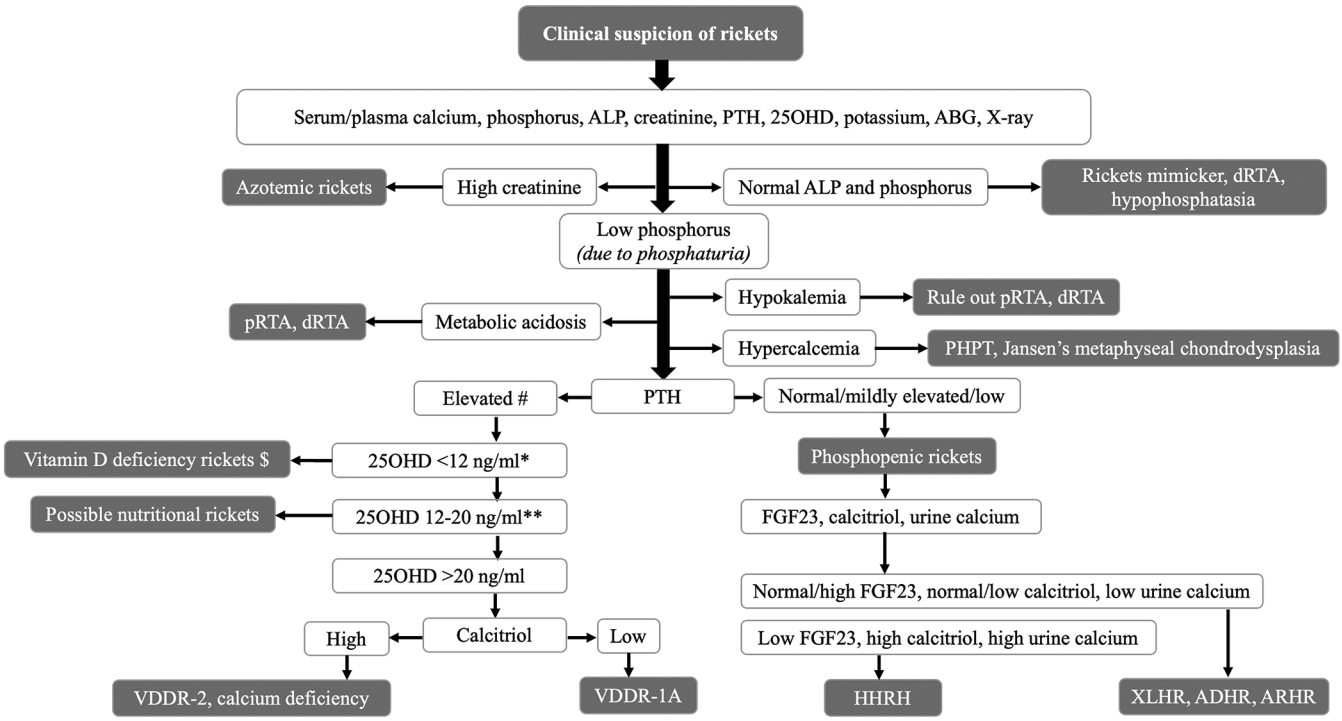

Figure 4: Suggested diagnostic algorithm in refractory rickets ($ there is a time lag between vitamin D supplementation, normalisation of serum 25OHD, and clinical and radiological improvement; *similar findings may be seen in VDDR-1B, VDDR-3 and in those who are on CYP3A4 inducers; ** co-existent calcium deficiency can give rise to rickets; # more than100 pg/ml). 25OHD = 25-hydroxy vitamin D, VDDR-1A = vitamin D-dependent rickets type 1, VDDR-3 = vitamin D-dependent rickets type 3

Table 4: Summary of studies evaluating children with rickets in tertiary care centres

First author (year of publication)	Place of the study	Inclusion criteria	Age	Number of patients	Nutritional rickets
Bajpai A (2005)[10]	AIIMS, New Delhi, India	Non-azotemic refractory rickets	18 days–10 years	131	
Sahay M (2010)[24]	Osmania General Hospital, Hyderabad, India	Refractory rickets	2 months-18 years	126	
Al Jurayyan NA (2012)[15]	King Khalid University Hospital, Riyadh, Saudi Arabia	Rickets	2–18 years	81	58 (71.6%)
Joshi RR (2012)[11]	B.J. Wadia Hospital for Children, Mumbai, India	Refractory rickets	1.5 months–13 years	36	
Current study		Rickets	1.3–25	79	7 (8.9%)

First author (year of publication)	Azotemic rickets	RTA		Phosphopenic rickets	VDDR	Others
		dRTA	pRTA			
Bajpai A (2005)[10]		34 (25.9%)	15 (11.4%)	34 (25.9%)	26 (19.8%)	22 (16.8%)*
Sahay M (2010)[24]	26 (20.6%)	81 (64.3%)	9 (7.1%)	8 (6.3%)	2 (1.6%)	1 (0.8%)**
Al Jurayyan NA (2012)[15]	1 (1.2%)			3 (3.7%)	5 (6.2%)	14 (17.3%)***
Joshi RR (2012)[11]	4 (11.1%)	20 (55.5%)	3 (8.3%)	2 (5.5%)	5 (13.9%)	2 (5.5%)****
Current study	3 (3.8%)	31 (39.2%)	8 (10.1%)	18 (22.8%)	5 (6.3%)	7 (8.9%)*****

*Chronic liver disease (CLD) (9.2%), malabsorption (6.1%). **Malabsorption due to celiac sprue. ***Anti-convulsant use (9.9%), celiac disease (6.2%). ****CLD due to biliary atresia (5.5%). *****Anti-convulsant use (1.3%), malabsorption (1.3%), undiagnosed (2.6%), type 3 renal tubular acidosis (RTA) (3.8%)

Conclusion

The prevalence of NR in tertiary care centres is relatively low, as our study found that only 8.9% of more than 100 children with a working diagnosis of rickets had true NR. RTA was the most common cause of refractory rickets in our study, followed by PR and VDDR. When evaluating a child with clinical and radiological signs suggestive of rickets, the diagnostic algorithm should begin with a detailed history, thorough clinical examination, X-rays, and measurement of serum ALP and phosphorus levels. Chronic ill health and polyuria point towards RTA. A normal ALP level suggests a cause other than rickets, but it is important to note that patients with RTA, particularly those with normal serum phosphorus, may not have elevated ALP levels. Hypophosphatemia is common in rickets, and PR typically presents with much lower levels of phosphorus. In children with high ALP, normal or low phosphorus, and normal eGFR, the next step is to measure serum potassium and PTH levels. Hypokalemia is a key indicator of underlying RTA, while normal PTH levels rule out CR. A PTH concentration of more than 100 pg/ml suggests CR, and the next test would be to measure serum 25OHD followed by serum calcitriol if indicated. Our proposed diagnostic algorithm is shown in Figure 4.

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Authors' contribution

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Conflicts of interest

There are no conflicts of interest.

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Appendix

Table 1: Definitions and calculations

Condition	Definition/calculation
Tubular reabsorption of phosphorus (TRP)	1 – {(urine phosphorus×serum creatinine)/(urine creatinine × serum phosphorus)}
Tubular maximum reabsorption rate of phosphate (TmP) to glomerular filtration rate (GFR) (TmP/GFR)	Serum phosphorus − {(urine phosphorus×serum creatinine)/urine creatinine} when TRP ≤0.85
Hypercalciuria	Urine spot calcium: creatinine more than the age-specific reference range or 24-hour urinary calcium excretion of more than 4 mg/kg of body weight
Phosphaturia	TRP ≤0.85 in the presence of hypophosphatemia or TmP/GFR lower than the age-specific reference range
Polyuria	Daily urine output >50 ml/kg of body weight
Kaliuresis	Urine spot potassium: creatinine >2.5 (mmol: mmol)
Elevated urinary β2 microglobulin	Urine β2 microglobulin ≥300 μg/L in spot sample measured by chemiluminescent immunoassay (SIEMENS IMMULITE ®1000)
FGF23 mediated hypophosphatemia/Inappropriate elevation of FGF23	Detectable FGF23 in the presence of hypophosphatemia
Renal tubular acidosis (RTA)	Normal anion gap metabolic acidosis and eGFR of ≥60 ml/min/1.73 m^2
Distal RTA (dRTA)	Nadir urine pH >5.3 during spontaneous or induced metabolic acidosis
Proximal RTA (pRTA)	Urine pH ≤5.3 during spontaneous or induced systemic acidosis
Azotemic rickets	eGFR calculated by bedside Schwartz formula <60 ml/min/1.73 m^2
Phosphopenic rickets (PR)	Hypophosphatemia (in respect to age-specific cut-offs), low TRP and/or low TmP/GFR, normal calcium, normal or mildly elevated PTH, normal or low calcitriol, and normal or high FGF23 (compared to laboratory-specific reference range)
Calcipenic rickets (CR)	Low/low normal calcium, hypophosphatemia, low TRP and/or low TmP/GFR, and clearly elevated PTH
Nutritional rickets (NR)	Calcipenic rickets with serum 25OHD level of <12 ng/ml
Vitamin D-dependent rickets type 1(VDDR-1A)	Calcipenic rickets with normal/high 25OHD and low calcitriol (compared to laboratory-specific reference range)
Vitamin D-dependent rickets type 2 (VDDR-2A)	Calcipenic rickets with normal/high 25OHD and high calcitriol (compared to laboratory-specific reference range)

25OHD=25-hydroxy vitamin D, FGF23=Fibroblast growth factor-23, pH=Potential of hydrogen

Table 2: Age-specific reference values for serum phosphorus and TmP/GFR

Age	Serum phosphorus (mg/dl)	TmP/GFR (mg/dl)
0–5 months	3.87–7.74	3.16–6.2
6–11 months	3.56–6.66	3.5–5.8
1–5 years	3.25–6.04	3.25–5.5
6–12 years	3.097–5.57	3–5.1
13–15 years	2.94–5.11	2.8–5.2
16–19 years	2.63–4.95	2.6–3.8
Adults	2.63–4.49	2.6–3.8

GFR=Glomerular filtration rate, TmP=Tubular maximum reabsorption rate of phosphate. Serum and urine creatinine measured by modified Jaffe's kinetic method. Serum and creatinine measured by ammonium molybdate method

Table 3: Age and sex-specific reference ranges of alkaline phosphatase (in U/L) (Kinetic photometric test according to the International Federation of Clinical Chemistry and Laboratory Medicine)

Age	Male	Female
Up to 30 days	75–316	48–406
1 month–1 year	82–383	124–341
1 year–up to 10 years	156–369	156–369
10 years–up to 13 years	141–460	141–460
13 years–up to 15 years	127–517	62–280
15 years–up to 17 years	89–365	54–128
17 years–up to 19 years	59–164	48–95

Table 4: Clinical and radiological clues to identify common rickets mimickers in our study

Blount's disease	Obese children with genu varum
	Medial beaking of both the proximal tibial metaphyses without cupping or fraying
Metaphyseal chondrodysplasia	Widened and irregular metaphysis
	"Moth-eaten" metaphysis
	Metaphyseal sclerosis
Mucopolysaccharidoses	Bushy eyebrows, coarse facies, corneal clouding, hepatosplenomegaly
	Metaphyseal fraying
	Anterior beaking of vertebrae
	Oar-shaped ribs
	Bullet-shaped metacarpal bones
	Hypoplastic and irregular-shaped epiphyses and carpal bones
	"V"-shaped configuration between distal ends of ulna and radius
Progressive pseudorheumatoid dysplasia	Involvement of interphalangeal joints
	Enlarged epiphyses
	Widened metaphyses
	Platyspondyly
	Anterior beaking of vertebral bodies
	Enlarged os trigonum