

Mutational Analysis in Hypophosphatemic Rickets of Tamil Nadu– A Rare Case Report

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Abstract: Autosomal dominant hypophosphatemic rickets (ADHR) is a rare type of hypophosphatemic rickets characterized by renal phosphate wasting and hypophosphatemia. The genetic analysis revealed with mutation in fibroblast growth factor 23 (FGF23) and it is elevated in several diseases in hypophosphatemia and rickets. The present case study reports a proband of 18 year old male and his mother with ADHR and was examined for mutational analysis in FGF23 gene. The clinical characteristics and biochemical data were analyzed in the proband and the mother. They both had a short stature and a low level of 1,25dihydroxyvitamin D3, elevated alkaline phosphate and a normal serum calcium. The FGF23 level was analyzed using ELISA and it was found out to be elevated in both the patients. The mutational analysis was performed using PCR-RFLP technique. The results were revealed with a heterogenous c.527G>A (p.R176Q) in exon 3 of FGF23 gene in mother as well as in her son. Hence, to the best of our knowledge, this is the first report in India especially in Tamil Nadu with ADHR and it is a necessary measure to investigate the detailed mechanism involving FGF23 in phosphate wasting diseases.

Keywords: Autosomal dominant hypophosphatemic rickets, fibroblast growth factor 23, polymerase chain reaction –restriction fragment length polymorphism, exon 3.

INTRODUCTION

Hypophosphatemic rickets (HR) is the failure of mineralization of osteoid associated with vitamin D deficiency and low level of serum phosphate. For the normal skeletal development and for preservation of bone integrity serum phosphate levels should be maintained. Advanced studies have shed the light in understanding the phosphate homeostasis mechanisms in normal as well as in disorders [1]. Rickets is normally defined as bone mineralization seen in children whereas in adults it is named as osteomalacia [2]. HR was initially termed as vitamin D-resistant rickets to distinguish from nutritional rickets and the causative factor of HR is renal tubular phosphate reabsorption and increased plasma phosphaturic hormone fibroblast growth hormone (FGF23). HR is commonly inherited as X-linked trait with dominant transmission. The other types include autosomal dominant hypophosphatemic rickets (ADHR), hereditary hypophosphatemic rickets with hypercalciuria, primary hyperparathyroidism, idiopathic phosphate diabetes and tumor-induced osteomalacia [3]. In all types, the biochemical characteristics are same were the renal phosphate wasting and low serum

phosphate associated with high level of FGF23 and low 1,25-dihydroxyvitamin D (1,25(OH)₂D) is seen [4].

The autosomal type of HR is a rare disorder and the clinical characteristic is based on the age of patients and importance of hypophosphatemia. The incidence is about 1 in 20000. In children and adults, the diagnosis is based on the confirmation with the dosage of phosphate clearance rate and phosphate reabsorption threshold [5]. The genetic complexity behind ADHR was examined in 2000 that FGF23 is responsible for the disease [6] and it has detected three missense mutations in FGF23 gene in four unrelated families (R176Q, R179Q, and R179W). The protein FGF23 consist of 251 amino acids and it is involved in many disorders associated with hypophosphatemia [7]. The FGF23 concentration is not elevated in all ADHR but the variable phenotype in ADHR result from FGF23 fluctuations [8].

The present case report was investigated for mutational analysis using genomic DNA. The resulted data showed with single heterozygous c.527G>A (p.R176Q) mutation in exon 3 of FGF23 gene. To the

best of our knowledge, this is the first kind of study in Tamil Nadu with an unusual case of hypophosphatemic rickets.

CASE REPORT

An 18 year old boy from Krishnagiri district in Tamil Nadu, India was admitted in Chettinad Hospital and Research Institute, Chennai, Tamil Nadu due to severe pain in joints and difficulty in walking. The mother of the proband was also admitted due to these symptoms. The duration of the symptoms in proband was for the past 2 years and in mother it was about 15 years. The proband showed short stature with a height of 150cms and weight of 43 kgs. The mother was also seen with a height of 148 cms and weight about 54 kgs. The biochemical tests in the proband revealed with serum phosphate of 2.0 mg/dL (normal range, 2.5 to 4.7), serum calcium 9.0 mg/dL (normal range being, 8.7 -10.6), alkaline phosphatase being elevated with 990 IU/L (normal range being, 42 - 98IU/L), parathyroid hormone was 45.68 pg/mL (normal range being, 10.00 - 65.00), 25-hydroxyvitamin D3 was 11.10 ng/mL (normal range, 10.00 to 30.00ng/ml) and 1,25- dihydroxyvitamin D3 was 21.6 pg/mL (normal range, 25.1 to 66.1).

The mother was observed with serum phosphate of 2.1 mg/dL (normal range, 2.5 to 4.7), serum calcium 9.6 mg/dL (normal range being, 8.7 - 10.6), alkaline phosphatase being elevated with 992 IU/L (normal range being, 42 - 98IU/L), parathyroid hormone was 60 pg/mL (normal range being, 10.00 - 65.00), 25-hydroxyvitamin D3 was 12.10 ng/mL (normal range, 10.00 to 30.00ng/ml) and 1,25-dihydroxyvitamin D3 was 22.3 pg/mL (normal range, 25.1 to 66.1) Nephrologic evaluation showed with normal diuresis and normal renal function. The tubular reabsorption rate of phosphate was 90% (normal range, 80 to 100) and the maximum tubular capacity of phosphate per unit volume of glomerular filtrate was decreased to 0.68 mM (normal range, 0.88 to 1.42), which revealed insufficient reabsorption of phosphorus in the kidneys. The FGF23 level was measured using enzyme-linked immunosorbent assay (ELISA) and found to be 197 RU/mL (normal range, <180). She was treated with 0.4 g/day of phosphate and 0.25mcg/day of calcitriol.

The inter-individual coefficient of variation (CV) for the ELISA assay was 3.8% to 6.4%, and the intra-individual CV was 2.5% to 6.1%. The questionnaire and biochemical findings supported the clinical signs of HR and hypocalcaemia. The proband's plain radiograph presented with diffuse bowing deformities in the upper and lower extremities and also in spine and pelvis (Figure 1 and 2). After obtaining pedigree analysis (Figure 4) and informed consent from the patients, peripheral lymphocytes were isolated from the genomic DNA using standard procedure. Using primers from earlier studies the mutational analysis was

carried out [9]. The results were observed in exon 3 with c.527G>A (p.R176Q) mutation in FGF23 gene in both the patients using PCR-RFLP technique. The amino acid substitution at position 176 of protein FGF23 (p.R176W). Hence from the study, it is seen that the FGF23 gene has a significant role in causing HR and the allelic loss is an important prognostic marker. After a follow-up study, the patient has been clinically treated with plate osteosynthesis to recover from bowing deformities (Figure3).

DISCUSSION

Generally in ADHR, the urinary phosphate clearance is increased whereas the phosphate tubular reabsorption and renal threshold phosphate concentration are decreased (10). FGF23 was identified as the disease causing gene of ADHR which is involved in phosphate homeostasis [6]. FGF23 is a representative phosphatonin that stimulates the renal excretion of phosphate in its full-length state by inhibiting sodium-phosphate transport. Physiologically, FGF23 is inactivated by cleavage at the potential recognition or cleavage sites for the enzymes of proproteinconvertase family (RXXR motif) [11]. The ADHR has been reported in six unrelated families [12, 13]. Similar to our study, three family members in a Chinese family carried heterozygous p.R176Q mutation in the FGF23 gene which leads to the processing of proteolysis by converting FGF23 to inactive fragments [14]. Comparative to our data, in three kindred family, the FGF23 level in ADHR showed 9% of gene mutation with elevated plasma FGF23 [8]. In our case, level of FGF23 was high and a normal tubular reabsorption rate of phosphate might contribute to the rachitic symptoms, including generalized bone pain and muscle weakness, despite of gene mutation. Another report revealed that FGF23 level was normal in adult patients with idiopathic phosphate diabetes [15]. From these findings, FGF23 mutations shows the phosphate wasting defect occurred due to the modulating level of FGF23 concentration to the normal level in the serum.

CONCLUSION

From the study, it is known that when serum phosphate changes, there is a modulation of FGF23 concentration. The exact mechanism is unclear. Consequently this is the first study and a novel report in Tamil Nadu population showing a case with ADHR. The clinical and molecular diagnosis revealed with a heterogenous mutation in a family which includes the proband and his mother. The precise mechanism of the disease should be investigated in the phosphate-wasting diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

RUNNING HEAD

Genetic alterations in Rickets

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