IJKD KIDNEY DISEASES

Distal Renal Tubular Acidosis and Its Relationship With Hearing Loss in Children

Preliminary Report

Mostafa Sharifian,[1] Nasrin Esfandiar,[1] Samira Mazaheri,[1] Ariana Kariminejad,[2] Masoumeh Mohkam,[1] Reza Dalirani,[1] Rana Esmaili,[1] Mitra Ahmadi,[1] Mehrnoush Hassas-Yeganeh[1]

[1]Pediatric Infectious Research Center and Department of Nephrology, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Kariminejad-Najmabadi Pathology-Genetic Center, Tehran, Iran

Original Paper

Keywords. renal tubular acidosis, hearing loss, child, mutation

Introduction. In autosomal recessive distal renal tubular acidosis (DRTA), a substantial fraction of the patients have progressive bilateral sensorineural hearing loss. This coexistence is due to the mutations of a gene expressed both in the kidney and in the cochlea. The aim of this study was to assess the correlation between hearing loss and DRTA.

Materials and Methods. In this study, 51 children diagnosed with renal tubular acidosis were evaluated. Diagnosis of DRTA was based on clinical manifestations and detection of normal anion gap metabolic acidosis, urine pH higher than 5.5, and positive urinary anion gap. Audiometry was performed in children with DRTA and sequencing of the *ATP6V1B1* gene was done for those with sensorineural hearing loss.

Results. Twenty-seven patients (52.9%) had DRTA, of whom 51.9% were younger than 1 year old, 55.6% were boys, and 44.4% were girls. Eleven patients (40.7%) had bilateral sensorineural hearing loss, consisting of 5 of 15 boys (33.3%) and 6 of 12 girls (50.0%). There was no correlation between hearing loss and gender. Three patients with hearing loss had mutation in the *ATP6V1B1* gene (11.1% of patients with DRTA and 27.3% of patients with DRTA and hearing loss).

Conclusions. This study indicated that a significant percentage of the children with DRTA had sensorineural hearing loss and mutation in *ATP6V1B1* gene. It is recommended to investigate hearing impairment in all children with DRTA.

IJKD 2010;4:202-6
www.ijkd.org

INTRODUCTION

Primary distal renal tubular acidosis (DRTA) is characterized by metabolic acidosis of various severity accompanied by inappropriately alkaline urine.[1-3] Other features include low serum potassium due to renal potassium wasting and elevated urinary calcium. Primary DRTA is inherited as either an autosomal dominant or an autosomal recessive trait. Patients with autosomal recessive DRTA are severely affected, presenting with either acute illness or growth failure at a young age.[4]

Mutations in solute carrier family 4, anion exchanger, member 1 (SLC4A1) gene that encodes bicarbonate/chloride anion exchanger 1 protein cause autosomal dominant DRTA.[5] Mutations in ATPase, H+ transporting, lysosomal V1 subunit β1 (ATP6V1B1) or the ATP6V0A4 (subunit α4) genes are responsible for syndromes of DRTA which are

often associated with progressive sensorineural deafness due to the extrarenal expression of these subunits in the inner ear. Impaired cochlear endolymph pH maintenance leads to sensorineural deafness.[6] Missense, nonsense, frame-shift, and splice junction mutations in the β1 subunit of the *ATP6V1B1* have been shown to be responsible for autosomal recessive DRTA with sensorineural deafness.[7]

This study aimed to assess the correlation between hearing loss and DRTA and to determine if hearing loss had an association with gender or mutation in the *ATP6V1B1* gene in pediatric patients with DRTA at our center.

MATERIALS AND METHODS

This study was carried out on 51 children diagnosed with RTA who were hospitalized in Mofid Children Hospital, in Tehran, Iran, between 2001 and 2006. Patients with secondary or acquired types of DRTA, such as pyelonephritis, and those taking medications known to cause DRTA, eg, amphotericin B, were excluded.

The information on children suffering from RTA were collected, including gender and age, RTA type, urine pH and specific gravity, blood pH, bicarbonate content before and after treatment, serum blood urea nitrogen, and serum creatinine. Clinical parameters including developmental delay, failure to thrive, rickets, and mortality rate, and paraclinical parameters including urine anion gap (AG), nephrocalcinosis, and kidney calculus were also taken into consideration.

Diagnosis of DRTA was based on clinical manifestations and detection of normal AG metabolic acidosis, urine pH which was never under 5.5, and positive urine AG. If the patient had normal AG metabolic acidosis without any other causes, RTA was considered, and sodium and potassium in plasma, urine pH, urine AG, and chromatography were measured. Finally, renal ultrasonography, response to treatment, and dose of alkali needed, were used to confirm the type of RTA.

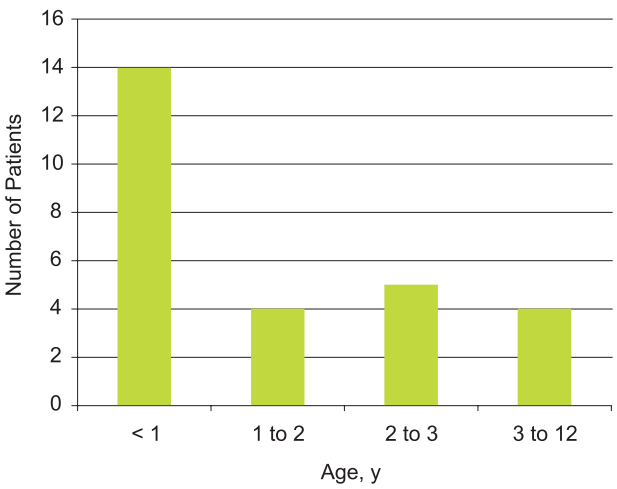
Audiometry was performed in all of the patients with DRTA who were cooperative and evoked potential in infants for hearing evaluation. To perform genetic evaluation, DNA was extracted from peripheral blood using salting out procedure. DNA analysis was performed by polymerase chain reaction amplification and sequencing of the *ATP6V1B1* gene. Direct sequencing of the *ATP6V1B1* gene was performed in Prof Fiona Karet's Laboratory, in Cambridge, United Kingdom.

Statistical analyses were performed using the Student *t* test and the paired *t* test. *P* values less than .05 were considered significant.

RESULTS

The mean age of the patients with RTA was 33.5 ± 42.0 months (range, 8 days to 16 years). Twenty-eight of the patients (54.9%) were boys and 23 (45.1%) were girls. Of 51 children with RTA, 27 (52.9%) suffered from DRTA. Proximal RTA, hyperkalemic RTA, and type 3 RTA were found in 14 (27.5%), 5 (9.8%), and 1 (2.0%) patients, respectively. In 4 cases (7.8%), the RTA type could not be detected due to lack of follow-up information.

The majority of the children with DRTA (n = 14; 51.9%) were younger than 1 year old (Figure). In terms of gender, 15 (55.6%) were boys and 12 (44.4%) were girls. The mean urine pH was 7.40 ± 0.7, ranging from 6 to 9. The mean blood pH before treatment was 7.30 ± 0.1 (range, 6.89 to 7.52) in patients with mixed metabolic acidosis and respiratory alkalosis, and the mean blood pH at discharge from hospital was 7.38 ± 0.1 (*P* = .005). The mean blood bicarbonate prior to treatment was 12.8 ± 4.6 mmol/L (range, 3.0 mmol/L to 18.4 mmol/L), which increased to 20.5 ± 4.7 mmol/L (range, 11.8 mmol/L to 33.9 mmol/L) at the time of discharge from hospital (*P* = .003). The mean serum creatinine level was 0.7 ± 0.6 mg/dL (range,



Age distribution at diagnosis of 27 children presented with distal renal tubular acidosis at Mofid Children Hospital.

0.2 mg/dL to 3.9 mg/dL), and the mean blood urea nitrogen level was 16.8 ± 16.7 mg/dL (range, 3 mg/dL to 90 mg/dL). Urine AG was evaluated to 52.1 ± 40.1, revealing positivity of urine AG in the patients.

Of the 27 children suffering from DRTA, 7 (25.9%) and 11 (40.7%) had nephrocalcinosis and kidney calculi, respectively. Seven patients (25.9%) and 13 (48.1%) suffered from growth delay and developmental delay, respectively, and 3 (11.1%) had rickets. The mortality rate was 3 (11.1%).

Eleven patients with DRTA (40.7%) showed bilateral sensorineural hearing loss, of whom 6 (54.5%) were girls. Hearing loss in the patients had been detected before the age of 6 years. Genetic analysis showed that 3 patients (2 boys) with DRTA and hearing loss had an *ATP6V1B1* mutation (27.3%).

DISCUSSION

In our study of patients with RTA, we found that 11 of 27 with DRTA (40.7%) had bilateral sensorineural hearing loss. Rodriguez Soriano[8] studied 31 nonrelated families with recessive DRTA among whom 20 were born to consanguineous marriages. The patients with hearing loss had been detected before the age of 6 years. All of patients had normal kidney function. In this study, 15 afflicted patients came out of 10 families showed bilateral sensorineural hearing loss, and 20 patients out of these 15 families showed normal sensorineural hearing, ruling out the role of chance in hearing loss among patients with DRTA.

Our genetic analysis showed that 3 of our 11 patients with DRTA and hearing loss had an *ATP6V1B1* mutation. In a study performed in Turkey by Ruf and coworkers,[9] there were 6 families with consanguineous marriage and recessive DRTA, in whom 10 candidate positions for mutations were determined on chromosomes 1 and 2. The *ATP6B1* gene was located on one of them and was the responsible gene for recessive DRTA accompanied by sensorineural hearing loss. According to this study, these gene mutations were found in 8 cases of the 15 studied families. Another study carried out by Karet and colleagues[3] revealed that the dominant form of DRTA resulted from mutations in the anion exchanger 1 gene, and also most patients with DRTA and sensorineural hearing loss had mutations in the *ATP6V1B1* gene. The authors proved that a large amount of sporadic or autosomal recessive DRTA cases were associated with sensorineural hearing loss. Distribution variety in presentation time of hearing loss could be seen from birth to the end of childhood. Sporadic or autosomal recessive DRTA with sensorineural hearing loss often manifest as a primary defect. Karet also showed that this form might be due to mutations in *ATP6V1B1* gene coding the 116-KD subunit of H+ ATPase.[10] With acquired experiences, one can expect that these patients might experience sensorineural hearing loss after the second decade of their life.

In a cohort study of related families originating from the Middle East and Turkey, the genome was analyzed and 2 genes for DRTA were determined on the p arm of chromosome 2 and q arm of chromosome 7 as the cause of DRTA accompanied by hearing loss and normal hearing, respectively. These two genes were the *ATP6V1B1* and *ATP6V0A4* coding β1 and α4 subunit of H+ ATPase pump in the apical membrane of α intercalated cells and were specific for the kidney. Screening tests in order to confirm mutations in *ATP6V1B1* gene revealed 15 different mutations in patients who almost all suffered from confirmed bilateral sensorineural hearing loss and all but one were homozygote.

In mutation analysis of our patients, we found 3 cases of *ATP6V1B1* mutations (27% of patients with DRTA and hearing loss). Our study indicates that this mutation is present in our country as well. Also, the *ATP6V1B1* gene in human and murine cochlea have been shown which is present in the apical surface of interdental cells and epithelium of endolymphatic sac and is necessary for maintaining endolymph pH and increasing sensitivity of hair cells.[11] The *ATP6V1B1* gene in male genital lumen has been observed, as the other location requires being acidic for maturation of human spermatozoids. Long-term follow-up of cohort studies performed on patients with recessive DRTA and mutation in *ATP6V0A4* confirmed the presence of mild hearing loss with delayed onset among these patients. Recently, it has been shown that the *ATP6V0A4* could be emerged in the internal ear of matured human and embryo, too.

In a study by Stover and colleagues, 26 families recently affected with recessive DRTA (23 related) were genetically analyzed, observing 7 new single nucleotide polymorphism and 5 polymorphic

markers in *ATP6B1* and *ATP6V0A4*, and it was found that 4 families had no relation with loci as strong evidence for confirming the genetic heterogeneity.[12] One new and 5 previously described mutations in the *ATP6V1B1* were found in 10 families. In 12 families, 7 of 10 mutations in the *ATP6V0A4* were new. Nine other mutations in the *ATP6V0A4* were found in sporadic cases. The relation of *ATP6V1B1* defects with hearing loss still have remained to be confirmed. A number of patients with mutations in the *ATP6V0A4* show advanced hearing loss that is usually seen in young adolescents. In the abovementioned studies, it has been shown that *ATP6V0A4* emerges in human inner ear. These findings confirm other evidence indicating genetic heterogeneity in recessive DRTA. Increasing illness range due to *ATP6V1B1* and *ATP6V0A4* for the first time showed the emergence of the *ATP6V0A4* inside the cochlea.

In Gil and coworkers' study,[13] the *ATP6V1B1* gene mutations were shown in 5 children with DRTA and sensorineural hearing loss. One of the mutations is the known mutation in exon 1 C/T (R31X), the other three were homozygous for a splicing mutation in intron 6 +1 G/A, and the remaining one was a compound heterozygote that showed this mutation and a new mutation in exon G/A (E330K):10. Zakzouk and colleagues[14] followed up 7 patients with autosomal recessive DRTA and sensorineural hearing loss. In 5 patients, primary diagnosis of DRTA was accompanied by rickets. Four patients showed severe sensorineural hearing loss at the level of 80 db. Two patients were brothers, 2 other patients suffered from secondary DRTA and a genetic disorder, osteopetrosis. These patients were brothers, too, and their audiograms revealed a mild bilateral conducive hearing loss in 35 db. All of the patients showed growth and developmental delay. Hearing loss showed no recovery with alkali treatment. According to the pedigrees for the two families in which half of the children were affected, consanguineous marriage caused the disease in the family. Five cases of the 7 studied patients were related.

In their study on *ATP6V1B1* in mice,[11] Finberg and associates indicated that the β1 isoform was the major β subunit isoform that incorporated into functional plasma membrane H+ ATPase in intercalated cells of the cortical collecting duct that was required for maximal urinary acidification. In the abovementioned studies, different mutations in *ATP6V1B1* were considered as the etiology of hearing loss in DRTA. In our study, 3 patients with hearing loss had a mutation in the *ATP6V1B1* gene. This comprised 11% of the patients with DRTA and 27% of the patients with DRTA and hearing loss, which is comparable with most of the findings in other studies. In patients with recessive DRTA and hearing loss who have a mutation in the *ATP6B1*, administering alkali cannot correct or prevent progression of hearing loss.[10]

CONCLUSIONS

A significant percentage of the children with DRTA had sensorineural hearing loss and mutation in *ATP6V1B1* gene. We recommend investigation of hearing status in all children with DRTA.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We would like to thank Prof Fiona Karet for her utmost cooperation and contribution.

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Correspondence to:
Mostafa Sharifian, MD
Pediatric Infectious Research Center and Department of Nephrology, Mofid Children Hospital, Shariati Ave, Tehran, Iran
E-mail: mostafasharifian@yahoo.com

Received July 2009
Revised November 2009
Accepted January 2010