

Lipoid Congenital Adrenal Hyperplasia with Cholestatic Jaundice

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ABSTRACT

BACKGROUND AND OBJECTIVE: Lipoid congenital adrenal hyperplasia (LCAH) is the most severe form of adrenal hyperplasia and mutations in the StAR gene are the most common cause of the disease. Adrenal insufficiency and cholestasis are reported in few patients. The aim of this study was to report the results of treatment of two sisters with lipoid CAH and cholestatic jaundice.

CASE REPORTS: Here, we present two sisters at the age of 30 and 60 days with conjugated hyperbilirubinemia and elevated liver enzymes and adrenal insufficiency. They had a 46,XY karyotype with external female genitalia without uterus and ovaries. LCAH was detected through electrolyte abnormalities, increased ACTH, decreased levels of cortisol and sex hormones and was confirmed by determination of exome sequencing and Sanger sequencing. In these patients, a homozygous mutation (c.653C>T) in exon 6 of STAR gene was identified. The patients were treated with 10 mg of hydrocortisone IV every 8 hours for 3 days; oral hydrocortisone was then administered at a dose of 2.5 mg every 8 hours and 0.2 mg fludrocortisone daily. One month after the therapy, levels of bilirubin and liver enzymes of these patients became normal. The first patient died 7 months after her mother stopped giving the drugs to the child. The other patient is now 9 years old. She is in good clinical condition as her treatment goes on.

CONCLUSION: Considering the reported cases, adrenal lipoid hyperplasia should be considered as a rare cause of cholestasis with adrenal insufficiency in patients.

KEY WORDS: Lipoid CAH, Adrenal Insufficiency, Neonatal Cholestasis, Karyotype.

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Introduction

Lipoid congenital adrenal hyperplasia (LCAH) is the most severe form of adrenal hyperplasia and is inherited as an autosomal recessive disorder. The steroidogenic acute regulatory protein (StAR) is normally expressed in adrenal glands and gonads. The mutations in the StAR gene and rarely in the gene encoding cholesterol side chain cleavage enzyme (cytochrome P450_{scc}) are responsible for causing LCAH (1, 2). The adrenal and gonadal steroids biosynthesis impairment in LCAH leads to decreased or complete absence of mineralocorticoid, glucocorticoid and sex hormones (3).

The classic form of the disease is presented with severe clinical manifestations from the beginning of life. In non-classical forms of LCAH, 10-25% enzyme activity is maintained, which causes a milder form of the disease (4). Destruction of Leydig cells happens from early fetal period in patients with 46,XY karyotype due to the accumulation of cholesterol and its derivatives lead to elimination of testosterone biosynthesis and natural virilization. As a result, external genitalia in boys are similar to girls with blind vaginal pouch without uterus and ovaries. Affected girls have no sexual ambiguity (5, 6).

Adrenal insufficiency and cholestasis have been reported in a few patients. Neonatal cholestasis is diagnosed by prolonged conjugated hyperbilirubinemia and its prevalence is approximately one in 2500 live births. The increase in direct bilirubin after the 14th day of life is considered as neonatal cholestasis (7, 8). Cholestasis due to adrenal insufficiency appears to be limited to the infancy period, which is also seen in animal models (9-11). The hypothalamic-pituitary-adrenal (HPA) axis effects many liver functions through neuroendocrine signaling pathways. Liver function is regulated by HPA through a molecular component and mechanisms, and HPA-based activity is regulated by the body's needs. StAR is a mitochondrial protein that plays an important role in the production cycle of adrenal steroids (12).

Primary adrenal insufficiency (PAI) is one of the major causes of morbidity in neonates, and congenital adrenal hyperplasia (CAH) is the most common cause of PAI in neonates (9). Improvement of neonatal cholestasis has been reported in many cases following treatment of adrenal insufficiency (12-17). Due to the fact that LCAH is a rare case of adrenal insufficiency and cortisol deficiency in infants and can be associated with cholestatic jaundice, in this study, we reported two patients with LCAH and cholestasis. After treatment

with glucocorticoids, cholestatic jaundice and adrenal insufficiency improved.

Case Reports

This study was reported after approval by Qazvin University of Medical Sciences with the code IR.QUMS.REC.1399.290.

Case 1: A 60 days old infant with female external genitalia admitted to Children Hospital in Qazvin because of failure to thrive, recurrent vomiting and poor feeding. The infant was the first child of her family delivered by cesarean section with birth weight of 3250 grams and height and head circumference of 48 and 34.5 centimeters. The infant had no problem during the perinatal period. The baby's weight, height and head circumference at the time of admission were 3400 grams, 50 and 35 centimeters, respectively. The healthy consanguineous parents were of Afghan origin. The grandparents and grandmothers of the infant also had consanguine marriage. The infant's mother was 17 and his father was 22 years old.

At the time of admission in intensive care unit of pediatric hospital, the infant was ill with severe dehydration, vomiting, hyperkalemia, hyponatremia, jaundice, skin hyperpigmentation and failure to thrive. The blood pressure of the infant was 62/40 mm/Hg with a heart rate of about 135 beats per minutes and a weak pulse. Urine and blood culture were negative. Vein blood gas analysis showed mild acidosis (pH=7.32, HCO₃=15.3 mmol/L, PCO₂=25 mmHg). To rule out other causes of cholestatic jaundice, sweat test, metabolic screening, hepatitis B surface antigen, anti-HIV antibody, alpha-1 antitrypsin level and HIDA scan were performed, all of which were normal. Laboratory tests have been shown in Table 1.

Further evaluations revealed normal male pattern (46,XY) in chromosomal study and enlarged bilateral adrenals in abdominal sonography. In pelvic sonography, the testicles were in the inguinal canal and a small uterus was reported. Karyotype, physical signs and laboratory findings led us to the diagnosis of lipoid congenital adrenal hyperplasia. After that, the infant was treated with 10 mg of hydrocortisone IV every 8 hours for 3 days, then the dose was reduced to 2.5 mg every 8 hours orally and fludrocortisone 0.2 mg daily and sodium chloride (%5) 5 cc/kg/daily. One week after treatment with hydrocortisone, electrolyte abnormalities return to normal limits. The levels of bilirubin and liver enzymes were normalized about one month after taking the treatment. In the periodic visits,

the patient's growth and development were appropriate for age, the bilirubin level, the blood glucose, and liver function tests were within the normal range. The infant height was 65 centimeters, her weight was 6200 grams and head circumference were 40.5 centimeters at the sixth month of age. At the age of 8 months, the mother had not given her the medication arbitrarily and the infant died with adrenal crises due to bacterial gastroenteritis (Family pedigree of patients: Figure 1).

Case 2: The term neonate was the second child of this family by cesarean section. The patient has normal external female genitalia with a birth weight of 2575 grams, a height and head circumference of 48 and 35 centimeters. At the age of 30 days, the infant was admitted to the intensive care unit of the pediatric hospital with a bad general condition, severe dehydration, vomiting, hyponatremia, hyperkalemia, direct hyperbilirubinemia, skin hyperpigmentation and failure to thrive (weight of 2650 grams, a height of 49 centimeters and head circumference of 36 centimeters). The child had a heart rate of about 140 beats per minute, blood pressure 55/40 mm/Hg with a weak pulse. The patient's urine and blood cultures were negative. The infant had metabolic acidosis (pH=7.28, $\text{HCO}_3=11$ mmol/L, $\text{PCO}_2=37$ mmHg.). Other tests were within normal range. Laboratory findings have been shown in Table 1.

Table 1. Laboratory results of patients

Tests	Case 1	Case 2	Normal range
Na (meq/lit)	124	1.16	135-145
K (meq/lit)	9.5	6.96	3.5-5
BS (mg/dl)	109	73	60-99
Ca (mg/dl)	8.4	8.6	2.5-8.10
ALP (IU/L)	485	590	180-1200
Albumin (g/L)	4	4.3	3.5-9
PT	11	12	10-12
AST (IU/L)	269	83	10-50
ALT (IU/L)	64	79	5-40
GGT (IU/L)	119	138	8-120
Total Bilirubin (mg/dl)	7	12.7	0.1-2.3
Bilirubin Direct (mg/dl)	2.7	7.2	<0.3
Cholesterol (mg/dl)	168	128	120-220
TG (mg/dl)	283	157	40-140
ACTH (pg/mL)	>1000	1500	6-60
Renin activity (nmol/lit .h)	18.6	24.1	0.2-2.2
Cortisol (mcg/dL)	2.1	1	5-23
17OHp (ng/ml)	0.23	0.44	<2.5
Androstenedione (nmol/l)	0.1	0.3	1.12-6.4
DHEA (mcg/dl)	3.8	6.9	<40

The chromosomal study of the patient showed 46,XY patterns. Hypertrophic adrenal glands were reported in ultrasound. The testis and uterus were not seen in pelvic sonography. Adrenal crises, karyotype, and other findings in sonography, physical exam, and laboratory tests guided us to a possible diagnosis of Lipoid CAH. So, initially, 10 mg of hydrocortisone was administered intravenously every 8 hours for three days, then the dose was reduced to 2 mg every 8 hours orally, fludrocortisone 0.2 mg daily and sodium chloride solution (%5) 5 cc/kg/daily.

In the first week after the onset of replacement therapy, electrolyte abnormalities were corrected. Gradually the levels of serum bilirubin and liver enzymes were reduced and normalized after one month of treatment. In periodic visits, every month in the first year and then every three months in subsequent years, the patient's growth and neurodevelopment were appropriate for age, the bilirubin level, and the blood glucose, and liver function tests were within the normal range. The infant's height was 65 centimeters, weight was 6200 grams and head circumference were 40.5 centimeters at the age of six months.

At the age of 4 years, the testicles were removed from the abdomen by laparoscopy. So far, the treatment with hydrocortisone and fludrocortisone is continuing. She is now 9 years old and her height and weight are on the 25th percentile CDC growth chart for girls. The last laboratory results of ACTH, Na, K, 17OHP, renin, and aldosterone tests were within normal limits. Gene mutations were determined for this patient and her parents.

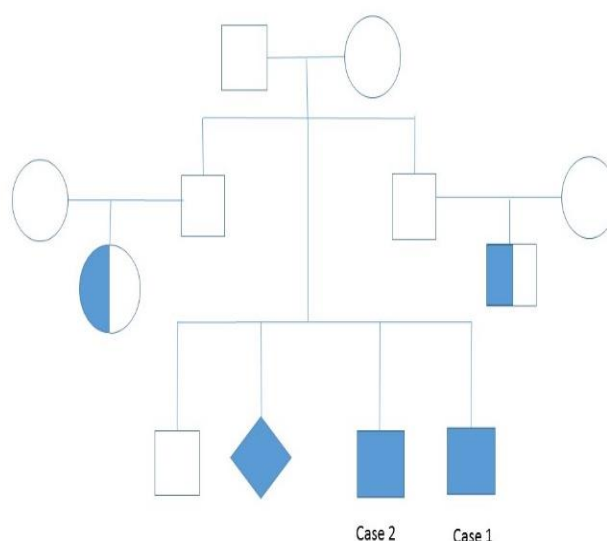


Figure 1. Family pedigree of two cases

Mutational analysis: DNA was extracted from peripheral blood leukocytes of the patient using a commercial kit (High Pure PCR Template Preparation, Roche). Whole-exome sequencing on DNAs was enriched for exonic regions with SureSelect 38Mbp. All exon kits v. 7.0 (Agilent Technologies, Santa Clara, CA, USA) were prepared according to manufacturer protocols and 75×2 bp paired-end sequenced on HiSeq2000 (Illumina Inc.), with an 80-120x mean coverage. The preliminary whole-exome data analysis was performed through Burrows-Wheeler Aligner (BWA) and the Genome Analysis Toolkit (GATK) software to generate a Binary Alignment Map (BAM) and a Variant Call Format (VCF) file, respectively. Annotations of the VCF files were carried out using wANNOVAR software, and the data were manually analyzed for the presence of candidate pathogenic variants. In this patient, a homozygous

missense mutation (c.653C>T) in exon 6 of the STAR gene was identified, changing Alanine 218 to Valine (p. Ala218Val). Sanger sequencing was used to validate the detected variant. Segregation analysis using Sanger sequencing revealed that both parents showed heterozygosity at the mutation position. Detailed computational analysis of p. Ala218Val mutation using prediction methods (PolyPhen-2, SIFT, Mutation Taster) revealed it as disease-causing. The local NGS database, currently consisting of 1406 whole exome sequencing (WES) data from healthy controls, were investigated and no samples exhibited the identified novel mutation in the STAR gene (Figure 2).

The mother's third pregnancy was affected by LCAH, and at the request of the parents, the pregnancy was terminated. A prenatal genetic study was performed for the fourth pregnancy, resulting in a healthy boy.

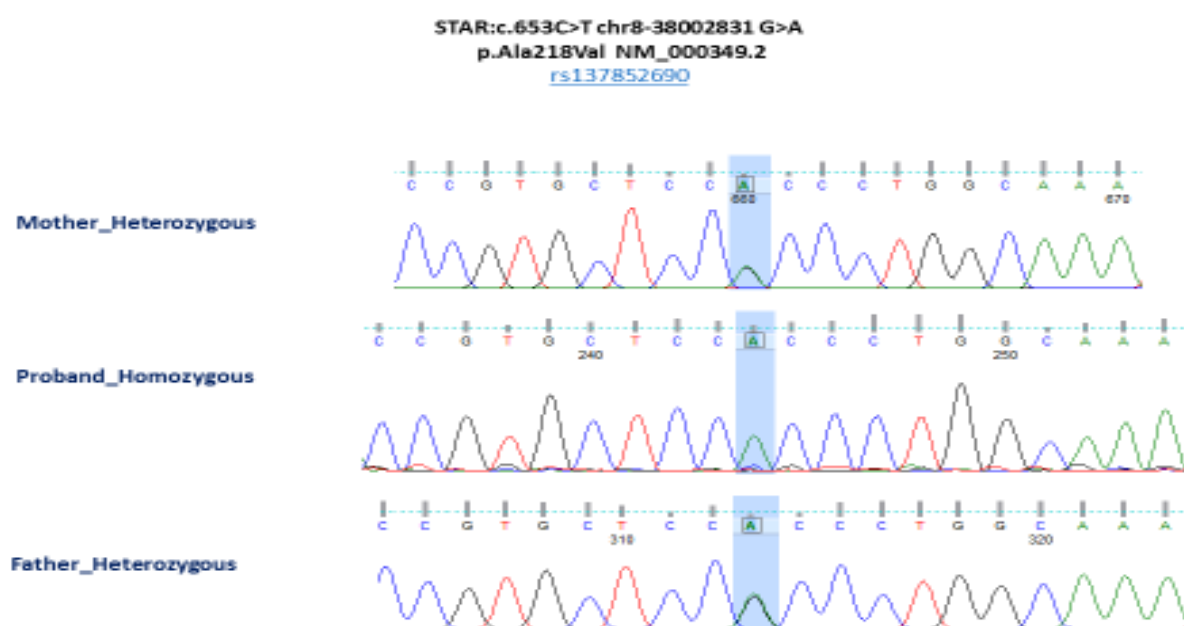


Figure 2. LCAH gene structure and mutation position, Sanger sequencing in case 2 showed a homozygous missense mutation (c.653C>T) in exon 6 of the STAR gene, changing Alanine 218 to Valine (p. Ala218Val).

Discussion

We present two rare cases of lipoid CAH and direct hyperbilirubinemia. The infants had external female genitalia with a male karyotype and they were hospitalized with adrenal insufficiency. After the treatment of adrenal insufficiency, cholestasis improved in these patients. The association between cortisol deficiency and cholestatic jaundice is known since 1965 and has been reported in rare cases of adrenal hyperplasia (15). There are many causes of neonatal cholestasis and lipoid congenital adrenal hyperplasia is

one of the rare causes of neonatal cholestasis (16). Our patients had adrenal crises with electrolyte disturbances, increased liver enzymes, and conjugated jaundice. Hashemipour et al. reported an infant with lipoid CAH. The infant had 46,XY karyotype with female external genitalia. The infant was presented with electrolyte disturbances, hypoglycemia and, direct hyperbilirubinemia (14). Kaplan et al, reported a 26-day-old neonate with lipoid CAH and cholestatic jaundice and hyperlipidemia. This baby had a 46,XY

karyotype with female external genitalia. Cholestasis and adrenal crisis improved after treatment with high doses of hydrocortisone and fludrocortisone (13). In patients with lipoid CAH, destruction of Leydig cells happens from the early fetal period in patients with 46,XY karyotype due to the accumulation of cholesterol and its derivatives lead to the elimination of testosterone biosynthesis and natural virilization, as a result, external genitalia in boys is similar to girls with blind vaginal pouch without uterus and ovaries (6).

Khodadad et al. reported a 45-days-old infant with lipoid CAH. The patient had female external genitalia with a 46,XY chromosomal pattern. The infant was admitted with adrenal insufficiency and cholestasis (17). This case was very similar to our patients. Our patients had 46,XY karyotypes with external female genitalia. In lipoid CAH, the impairment of the production of the testicular steroid during the embryonic period in patients with 46,XY karyotype leads to a complete female genitalia phenotype. Patients with 46,XX genotype also have external female genitalia and no sexual ambiguity (18).

The severity of clinical manifestations in patients may also vary from patient to patient (19, 20). One of our patients was hospitalized at two months of age and the other at the first month of age. Skin hyperpigmentation due to increased ACTH was seen in both patients. Cortisol deficiency leads to hypoglycemia in some patients with lipoid CAH, (21) but none of our patients had hypoglycemia. In patients with LCAH, adrenal steroids are not produced, which leads to an

increase in the secretion of corticotropin from the hypophysis and subsequently causing more accumulation of cholesterol esters in the form of lipid droplets in the adrenal gland, resulting in enlarged adrenal glands (22). In our patients, the adrenal glands were also significantly larger.

We reported two rare cases of Lipoid CAH as the cause of neonatal cholestasis. Both cases had karyotype 46,XY with a female external phenotype. Patients were admitted with manifestations of adrenal insufficiency and cholestatic jaundice, which improved after the diagnosis and treatment of the initial disease. If there is neonatal cholestasis with symptoms of adrenal insufficiency in the patient, we must also consider adrenal hyperplasia.

Conflict of Interest: The authors declare that they have no conflict of interest.

Informed Consent: The authors state that individuals (or parents or guardians) have expressed their written consent to the publication of their file. The study protocol has also been approved by the Human Research Committee of the Children Growth Research Center.

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