DOI: https://doi.org/10.2298/SARH161013084K
UDC: 616-008.9-056.7-053.2(497.17)

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

First Macedonian child with tyrosinemia type 1 successfully treated with nitisinone and report of a novel mutation in the *FAH* gene

Aco Kostovski[1], Nikolina Zdraveska[1], Marketa Tesarova[2], Jiří Zeman[2]

[1]Ss. Cyril and Methodius University, Faculty of Medicine, University Children's Hospital, Skopje, Macedonia; [2]Charles University, First Faculty of Medicine, General University Hospital, Department of Pediatrics and Adolescent Medicine, Prague, Czech Republic

SUMMARY

Introduction Hereditary tyrosinemia type 1 (HT1) is a severe hereditary metabolic disorder of tyrosine metabolism due to fumarylacetoacetate hydrolase (FAH) deficiency and accumulation of toxic products in tissues. More than 80 mutations in the *FAH* gene are presently reported on the Human Genome Mutation Database. To date, no molecular genetic defects of HT1 in Macedonia have been described.

Case outline A female infant two and a half months old presented with failure to thrive, anemia, edemas, and severe coagulation disturbances. The diagnosis of HT1 was based on high levels of serum α-fetoprotein, increased serum tyrosine, and positive succinylacetone in urine. Nitisinone treatment with tyrosine-restriction diet was immediately introduced. The patient, currently aged five years, has normal growth, psychomotor development, and no focal lesions on abdominal MRI. A screening of the *FAH* gene revealed two heterozygous mutations – c.[1A>G];[784T>A]. The mutation c.784T>A is a novel one (p.Trp262Arg), and was predicted to be the cause of the disease by an in silico analysis.

Conclusion To date, this case is the first and only child with HT1 successfully treated with nitisinone in our country. Also, this is the first report of an HT1 patient caused by the c.784T>A mutation.

Keywords: hereditary; tyrosinemia type 1; nitisinone; mutation

INTRODUCTION

Hereditary tyrosinemia type 1 (HT1) is a rare but severe hereditary metabolic disorder of tyrosine metabolism. The worldwide prevalence of HT1 is 1 in 100,000 newborns, but is more common in some regions, notably in Quebec, Canada [1, 2]. It results from fumarylacetoacetate hydrolase enzyme (FAH) deficiency, encoded by the *FAH* gene and an accumulation of toxic products in many tissues, particularly in the liver, kidneys, and the brain. Molecular genetic testing by targeted analysis for the common *FAH* pathogenic variants and sequence analysis of the entire coding region can detect pathogenic variants in more than 95% of affected individuals [3]. More than 80 mutations in the *FAH* gene are presently reported on the Human Genome Mutation Database (HGMD® Professional 2016.2, http://www.hgmd.cf.ac.uk). Patients from different ethnic groups with HT1 have different common mutations in the *FAH* gene [4].

HT1 patients typically present in infancy with acute liver failure, cirrhosis, neurologic crises, and renal tubular dysfunction with hypophosphatemic rickets. If untreated, death typically occurs before two years of age, although chronic forms allowing longer survival have been reported [5].

Biochemical findings include elevated succinylacetone in the blood and urine; elevated serum concentrations of tyrosine, methionine and phenylalanine, and elevated tyrosine metabolites in urine. The evolution of the disease has improved considerably since the introduction of nitisinone (NTBC) treatment depending on the age of the patient at diagnosis and at the start of the treatment [6].

Herein we report the first HT1 child from Macedonia successfully treated with nitisinone therapy. Due to the low incidence, as well as difficulties in diagnostics of rare diseases in our country, all previous cases were diagnosed with an advanced liver disease and had unfavorable outcome, either lethal or required urgent liver transplantation. Also, this is the first patient in whom the diagnosis of tyrosinemia was confirmed by a genetic analysis.

CASE REPORT

A female infant two and a half months old, the second child of healthy nonconsanguineous parents, presented with failure to thrive, anemia, and edemas. The infant was born after 39 weeks of gestation, with the birth weight of 3,100 g and had normal postnatal course. No genetic diseases had been reported in the family. The child was exclusively breastfed, but experienced difficulties in gaining weight. Several days prior the admission, swelling of the abdomen, feet, and wrists was noticed. Physi-

Примљено • Received: October 13, 2016
Ревизија • Revised: January 10, 2017
Прихваћено • Accepted: February 7, 2017
Online first: March 21, 2017

Correspondence to:
Aco KOSTOVSKI
Department for Pediatric Gastroenterohepatology
University Children's Hospital,
Medical Faculty,
Skopje, Macedonia
acokos@gmail.com

cal examination revealed a pale, unhealthy-looking infant, with abdominal distension and peripheral edemas. The weight was on the fifth percentile for the age. The liver was enlarged 4 cm below the costal margin, non-tender, and had firm consistency. The spleen was palpable 2 cm below the left costal margin.

A laboratory analysis showed anemia, hemoglobin level of 80 g/l, and red blood cell count of 2.83×10^{12}/l.

There was significant hypoproteinemia and hypoalbuminemia, with values of 32 g/l and 18 g/l, respectively. The bilirubin level was slightly elevated, total bilirubin was 39 μmol/l, and conjugated 12 μmol/l. Serum transaminases were within normal limits (65 U/l for aspartate transaminase, 59 U/l for alanine transaminase), and alkaline phosphatase was 970 U/L (normal 120–450 U/l). Coagulation screening showed prolonged prothrombin time of 46 seconds, and partial thromboplastin time was 33 seconds. Alpha-fetoprotein was > 10,000 IU/ml (normal < 87 IU/ml). Blood gases and electrolytes were normal, as well as the blood urea nitrogen and serum creatinine values. Serum amino acid analysis showed elevated tyrosine of 396 mmol/L (normal value < 200 mmol/L). Urine organic acid analysis revealed elevated succinylacetone. Ultrasonography of the abdomen showed hepatosplenomegaly, ascites, and the hypoechogenic structure of the renal medulla.

According to the findings, the infant was diagnosed with tyrosinemia type 1 and nitisinone therapy (1 mg/kg/day) was initiated combined with tyrosine-restricted formula (Tyrex®, Abbott Nutrition, Lake Forest, IL, USA). The patient received several plasma and albumin transfusions and vitamin K supplementation.

Following the NTBC treatment, there was a significant improvement of the liver function. Coagulation improved two days after treatment initiation.

The child was followed-up regularly without further hospitalizations. Parameters remained normal during the follow-up. The serum tyrosine levels were frequently measured and maintained 200–400 mmol/L, as recommended. Succinylacetone was negative two weeks after starting the treatment and was determined yearly afterwards. Alpha-fetoprotein was 8,298 IU/ml at the age of six months, and 312 IU/ml at 12 months. NTBC concentration at the age of three years was 36.2 μmol/l (target values 40–60 μmol/l); thus, the nitisinone dose was increased. Annual follow-up liver MRI has shown no focal lesions to date. The ophthalmological examination was scheduled every six months and has always been normal. The child is now five years old and has normal growth and psychomotor development.

Molecular analysis was performed. Genomic DNA was isolated from the patient's whole blood leucocytes and from her parents' afterwards. Fourteen coding exons of the *FAH* gene (ENSG00000103876) and their flanking intronic regions were amplified in 13 fragments by polymerase chain reaction. The products of the polymerase chain reaction were sequenced in both directions on ABI 3500xL genetic analyzer (Applied Biosystem, Foster City, CA, USA). In the patient's DNA, genetic testing showed two heterozygous mutations: c.1A>G in exon 2, inherited from the child's father, and c.784T>A in exon 10, inherited from the child's mother. The c.784T>**A** mutation has never been reported previously in HT1 patients, is not present in the 1,000 genome database (http://www.1000genomes.org/home), and was predicted to be disease causing (in silico analysis, MutationTaster, Charité – Berlin University of Medicine).

DISCUSSION

Hepatorenal tyrosinemia or tyrosinemia type 1 is a rare autosomal-recessive disorder of tyrosine metabolism with an incidence of 1:125,000 in central Europe [7]. Because of the low global occurrence of HT1, a considerable number of cases may go unrecognized especially in absence of an established newborn screening.

Our case presents the first report and the only HT1 patient from Macedonia diagnosed in early infancy and successfully treated with nitisinone. Due to the limitations of diagnostic tests in our country, many HT1 patients had been unrecognized.

A recent study from Macedonia included four patients with HT1 diagnosed over a three-year period; two of the patients had an unfavorable outcome with death occurring at the mean age of 126 days, and one patient was transferred for a liver transplantation. The authors emphasize the initial promising results of nitisinone treatment started at that time [8].

HT1 children presenting before the age of six months typically have acute liver failure with initial loss of synthetic function for clotting factors. Our child presented with liver dysfunction (edemas, jaundice, bleeding tendency), an important feature for diagnosing hereditary tyrosinemia type 1. The prothrombin time was markedly prolonged and did not correct after vitamin K and plasma supplementation. Paradoxically, serum transaminase levels were normal and serum bilirubin concentration was only slightly elevated, in contrast to most forms of severe liver disease in which there is marked elevation of transaminases and serum bilirubin concentration. This discrepancy in the liver function is described in the literature; resistance of affected liver cells to cell death may be a possible explanation [9].

Mayorandan et al. [7] in a recent study analyzed 168 patients with HT1 from 21 centers with the average age of the diagnosis being 12.9 months; most of them were symptomatic at diagnosis, with a combination of liver and renal dysfunction. In their study, the acute liver failure was significantly higher in the group of patients between two and six months of age. Our patient had preserved renal function. High serum tyrosine in combination with increased α-fetoprotein level and severe coagulopathy raised the suspicion of tyrosinemia in our patient. Detection of succinylacetone in urine is the most reliable biochemical diagnostic method for HT1. However, there is a reported unusual case of a four-month-old infant with HT1 presenting with severe liver disease and negative succinylacetone in urine. Fumarylacetoacetase protein and activity was decreased, but not absent [10].

Nitisinone, or 2-(2-nitro-4-trifluoromethylbenzyol)-1,3-cyclohexanedione (NTBC), a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, a step in the tyrosine degradation pathway, has revolutionized the management of tyrosinemia type 1 [6, 11].

Nitisinone administration usually results in a remarkable clinical improvement within a few days in more than 90% of patients; thus, the treatment should commence as soon as the diagnosis is confirmed, or even suspected because of liver disease [12].

If coagulation improves within one week, recovery can be assumed; otherwise, an increase of the nitisinone dose or liver transplantation should be considered [12]. Our patient showed rapid improvement. Delayed NTBC treatment is associated with an increased risk of liver carcinoma and a requirement of the liver transplantation. Mayorandan et al. [7] in their study point out the necessity of newborn screening programs to allow an early diagnosis and access to adequate treatment, as they report a 2–12-fold higher risk for developing hepatocellular carcinoma depending on the age at the time the treatment was started compared to patients treated as neonates. Also, psychomotor impairments, attention-deficit hyperactivity syndrome and behavioral disorders, neurological disturbances or learning difficulties were present in very few patients when NTBC treatment was initiated in the newborn period [7].

Our patient was monitored regularly in one-month intervals during the first year of life, according to the recommendations, and every three months after achieving good control and stability, as well as the parents' understanding and compliance. The metabolic control was assessed by determining succinylacetone concentration in dried blood or urine and the level was always below the detection limit.

Nitisinone tolerance of in the child was good, without any side effects. Mayorandan et al. [7] reported side effects of NTBC treatment in very few patients: transient thrombocytopenia, leukopenia, and transient ocular symptoms. Patients with side effects seemed to have higher range of NTBC values compared to those with no side effects; however, because of the small sample size, statistical analysis was not possible.

Unfortunately, we were not able to determine the nitisinone level more frequently. Monitoring of nitisinone plasma levels permits individual dosing, minimizing treatment costs and side effects without hampering metabolic control. However, the target level of nitisinone is not well defined and varies among centers [13, 14].

Simoncelli et al. [15] provided a cost–consequence analysis for all children with HT1 treated in Quebec, Canada, between 1984 and 2009, concluding that nitisinone treatment significantly improved the outcomes of patients with tyrosinemia type I, while decreasing the utilization of health care resources by significant reductions in the number and duration of hospital admissions, admissions to a pediatric intensive care unit, and the number of liver transplants.

Although molecular testing is not essential for diagnosing HT1, it has greatly improved the diagnostic power for the disease and is useful for prenatal diagnosis and genetic counselling. Despite the fact that the spectrum of the FAH gene mutation has been expanded, current knowledge is not adequate for establishing the disease's genotype–phenotype correlation.

Angileri et al. [4] in a recent study described the 95 mutations reported so far in HT1 with special emphasis on their geographical and ethnic distributions, concluding that such information should enable a preferential screening for mutations most predominant in a certain region or ethnic group.

Our patient represents the first case from Macedonia with genetically confirmed HT1. She was a compound heterozygote for two mutations – c.[1A>G];[784T>A]. The c.1A>G mutation is a missense previously known mutation in codon 1 which changes the initial Met into Val (p.Met>Val) and negatively affects the initiation of FAH protein translation [16]. This mutation in a homozygous state was also reported in patients with HT1 from Emirates, Greece, and Saudi Arabia [17–20].

Georgouli et al. [18] reported a five-month-old infant with HT1 presenting as Escherichia coli sepsis and severe coagulopathy due to liver dysfunction. The patient was homozygous for c.1A>G. Despite the early diagnosis and NTBC treatment, the patient died from multi-organ failure.

Imtiaz et al. [19] reported five homozygous carriers of the c.1A>G mutation in a cohort of 43 HT1 patients originating from the Middle East.

The other c.784T>A mutation detected in our patient is a novel mutation, which changes highly conserved Trp^{262} into Arg (p.Trp262Arg). By an in silico analysis (MutationTaster; PolyPhen-2 – public domain; SIFT – the University of British Columbia, Vancouver, BC, Canada), this mutation was predicted to be disease causing.

In conclusion, our patient presents the first experience with nitisinone treatment in our country. Despite the excellent results, the child needs further careful monitoring because of possible long-term complications, particularly hepatocellular carcinoma.

Also, reporting of underlying mutations in HT1 patients who belong to different ethnic groups is helpful not just for genetic counseling but also for further research.

ACKNOWLEDGEMENT

This work was supported by project RVO-VFN64165 of the Ministry of Health of the Czech Republic.

REFERENCES

1. Mitchell GA, Grompe M, Lambert M, Tanguay RM. Hypertyrosinemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease. New York, NY: McGraw Hill; 2001:1777–806.
2. De Braekeleer M, Larochelle J. Genetic epidemiology of hereditary tyrosinemia in Quebec and in Saguenay-Lac-St-Jean. Am J Hum Genet.1990; 47(2):302–7.
3. Sniderman King L, Trahms C, Scott CR. Tyrosinemia type 1. Retrieved from GeneReviews® June 2014. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1515/
4. Angileri F, Bergeron A, Morrow G, Lettre F, Gray G, Hutchin T, et al. Geographical and ethnic distribution of mutations of the fumarylacetoacetate hydrolase gene in hereditary tyrosinemia type 1. JIMD Reports. 2015; 19:43–58.
5. van Spronsen FJ, Thomasse Y, Smit GP, Leonard JV, Clayton PT, Fidler V, et al. Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. Hepatology. 1994; 20(5):1187–91.
6. McKiernan PKJ. Nitisinone in the treatment of hereditary tyrosinaemia type1. Drugs. 2006; 66(6):743–50.
7. Mayorandan S, Meyer U, Gokcay G, Segarra NG, de Baulny HO, van Spronsen F, et al. Cross-sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. Orphanet J Rare Dis. 2014; 9:107.
8. Kostovski A, Zdraveska N, Konstantinopoulou V. Patients with tyrosinemia type 1 and first experience with nitisinone treatment in Macedonia. J Inherit Metab Dis. 2012; 35 Suppl 1:S26.
9. Vogel A, van Den Berg IE, Al-Dhalimy M, Groopman J, Ou CN, Ryabinina O, et al. Chronic liver disease in murine hereditary tyrosinemia type 1 induces resistance to cell death. Hepatology. 2004; 39(2):433–43.
10. Cassiman D, Zeevaert R, Holme E, Kvittingen EA, Jaeken J. A novel mutation causing mild, atypical fumarylacetoacetase deficiency (Tyrosinemia type I): a case report. Orphanet J Rare Dis. 2009; 4:28.
11. Santra S, Baumann U. Experience of nitisinone for the pharmacological treatment of hereditary tyrosinaemia type 1. Expert Opin Pharmacother. 2008; 9(7):1229–36.
12. De Laet C, Dionisi-Vici C, Leonard JV, McKiernan P, Mitchell G, Monti L, et al. Recommendations for the management of tyrosinaemia type 1.Orphanet J Rare Dis. 2013; 8:8.
13. Sander J, Janzen N, Terhardt M, Sander S, Gökcay G, Dermikol M, et al. Monitoring tyrosinaemia type I: Blood spot test for nitisinone. Clin Chim Acta. 2011; 412:134–8.
14. Herebian D, Spiekerkoetter U, Lamshöft M, Thimm E, Laryea M, Mayatepek E. Liquid chromatography tandem mass spectrometry method for the quantification of NTBC (2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione) in plasma of tyrosinemia type I patients. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877(14-15):1453–9.
15. Simoncelli M, Samson J, Bussières JF, Lacroix J, Dorais M, Battista R, et al. Cost-Consequence Analysis of Nitisinone for Treatment of Tyrosinemia Type I. Can J Hosp Pharm. 2015; 68(3):210–7.
16. Arranz A, Rigante D, Antuzzi D, Riudor E. Gene symbol: FAH. Disease: tyrosinaemia 1. Hum Genet. 2005; 118(3-4):537.
17. Al-Shamsi A, Hertecant JL, Al-Hamad S, Souid AK, Al-Jasmi F. Mutation spectrum and birth prevalence of inborn errors of metabolism among Emiratis: a study from Tawam Hospital Metabolic Center, United Arab Emirates. Sultan Qaboos Univ Med J. 2014; 14(1):e42–9.
18. Georgouli H, Schulpis KH, Michelakaki H, Kaltsa M, Sdogou T, Kossiva L. Persistent coagulopathy during Escherichia coli sepsis in a previously healthy infant revealed undiagnosed tyrosinaemia type 1. BMJ Case Rep. 2010; 2010:bcr0720103150.
19. Imtiaz F, Rashed MS, Al-Mubarak B, Allam R, El-Karaksy H, Al-Hassnan Z, et al. Identification of mutations causing hereditary tyrosinemia type I in patients of Middle Eastern origin. Mol Genet Metab. 2011; 104(4):688–90.
20. Mohamed S, Kambal MA, Al Jurayyan NA, Al-Nemri A, Babiker A, Hasanato R, et al. Tyrosinemia type 1: a rare and forgotten cause of reversible hypertrophic cardiomyopathy in infancy. BMC Res Notes. 2013; 6(1):362.

Прво дете из Македоније са тиросинемијом тип 1 успешно лечено нитисиноном и приказ нове мутације у *FAH* гену

Ацо Костовски[1], Николина Здравеска[1], Маркета Тесарова[2], Јиржи Земан[2]

[1]Универзитет „Св. Ћирило и Методије", Медицински факултет, Универзитетска дечја клиника, Скопље, Македонија;
[2]Карлов универзитет, Први медицински факултет, Општа универзитетска болница, Одељење за педијатрију и адолесцентну медицину, Праг, Чешка

САЖЕТАК

Увод Хередитарна тирозинемија тип 1 (ХТ1) озбиљан је наследни поремећај метаболизма тирозина који настаје као последица недостатка ензима фумарилацетоацет-хидролазе и нагомилавања токсичних продукта у разним ткивима. До сада је описано више од 80 мутација у *FAH* гену, а ниједан случај мутација са ХТ1 у Македонији.

Приказ болесника Женско одојче старо 2,5 месеца није напредовало у тежини, имало је анемију, отоке и тешке поремећаје коагулације. Дијагноза ХТ1 је заснована на повишеним вредностима α-фетопротеина и тирозина у серуму, а позитивним сукцинилацетоном у урину. После постављања дијагнозе уведено је лечење са нитизиноном и ограничење уноса тирозина у исхрани. После пет година дете има нормалан раст и психомоторни развој, као и уредан налаз МР абдомена. Молекуларном анализом *FAH* гена откривене су две хетерозиготне мутације – c.[1A>G];[784T>A]. Мутација c.784T>A је нова (p.Trp262Arg) и сматра се одговорном за појаву болести (*in silico analysis*).

Закључак Ово је први и једини случај детета са ХТ1 који је до сада у нашој земљи успешно третиран нитизиноном. Такође, ово је први извештај за c.784T>A мутацију код ХТ1 болесника.

Кључне речи: наследна; тирозинемија тип 1; нитисинон; мутација