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Address: 1 Kraljice Natalije Street, 11000 Belgrade, Serbia
☎ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: srparhiv@bvcom.net, Web address: www.srpskiarhiv.rs

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**Case Report / Приказ случаја**

Aco Kostovski[1,2,†], Nikolina Zdraveska[1,2], Marketa Tesarova[3,4], Jiri Zeman[3,4]

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

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

[1] University Children's Hospital, Skopje, FYR Macedonia
[2] Medical Faculty, University in Skopje, FYR Macedonia
[3] General University Hospital, Department of Pediatrics and Adolescent Medicine, Prague, Czech Republic
[4] First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

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† **Correspondence to:**
Aco Kostovski
University Children's Hospital, Skopje, FYR Macedonia
E-mail: **acokos@gmail.com**

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

**SUMMARY**

**Introduction** Hereditary tyrosinemia type I (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 *FAH* gene are presently reported on Human Genome Mutation Database. To date, no molecular genetic defects of HT1 in Macedonia have been described.

**Case outline** A 2.5-month female infant presented with failure to thrive, anemia and edemas and severe coagulation disturbances. Diagnosis of HT1 was based on high serum α-fetoprotein, increased serum tyrosine and positive succinylacetone in urine. Nitisinone treatment with tyrosine restriction diet was immediately introduced. The patient, currently aged 5 year has a normal growth, psychomotor development and no focal lesions on abdominal MRI. Screening of *FAH* gene revealed two heterozygous mutations c.[1A>G];[784T>A]. c.784T>A is a novel mutation (p.Trp262Arg); by *in silico* analysis this mutation was predicted to be a disease causing.

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

**Keywords**: hereditary; tyrosinemia type 1; nitisinone; mutation

**САЖЕТАК**

**Увод** Хередитарна тирозинемија тип 1 (ХТ1) је озбиљни 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 ни један случај мутација са ХТ1 у Македонији.

**Приказ болесника** Женско одојче, старо 2,5 месеца које не напредује у тежини, са анемијом, отоцима и тешким поремећајима коагулације. Дијагноза ХТ1 је заснована на повишеним вредностима α-фетопротеина и тирозина у серуму, а позитивним сукцинилацетоном у урину. Након постављања дијагнозе уведен је лечење са нитизиноном и ограничење уноса тирозина у исхрани. После пет година, дете има нормалан раст и психомоторни развој, као и уреднан налаз МР абдомена. Молекуларном анализом *FAH* гена откривене су две хетерозиготне мутације c.[1A>G];[784T>A]. c.784T>A је нова мутација (p.Trp262Arg), која се сматра одговорном за појаву болести (*in silico analysis*).

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

**Кључне речи**: наследна; тирозинемија тип 1; нитизинон; мутација

## INTRODUCTION

Hereditary tyrosinemia type I (HT1) is a rare but severe hereditary metabolic disorder of tyrosine metabolism. The worldwide prevalence of HT1 is 1 in 100,000 newborn, but is more common in some regions, notably in Quebec, Canada [1, 2]. It results from fumarylacetoacetate hydrolase enzyme (FAH) deficiency, encoded by *FAH* gene and accumulation of toxic products in many tissues, particularly in liver, kidneys and 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 *FAH* gene are presently reported on 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 *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 2 years of age, although chronic forms are reported allowing longer survival [5].

Biochemical findings include elevated succynilacetone in 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 at diagnosis and start of treatment [6].

Herein we report the first HT1 child from Macedonia which was 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 genetic analysis.

## CASE REPORT

A 2.5-month female infant, second child of healthy nonconsanguineous parents, presented with failure to thrive, anemia and edemas. The infant was born after 39 weeks of gestation with birth weight 3100g and had normal postnatal course. No genetic diseases were reported in the family. The child was exclusively breastfed, but experienced difficulties in gaining weight. Few days before admission swelling of the abdomen, foot and wrists were noticed. Physical examination revealed pale, sick looking infant, with abdominal distension and peripheral edemas. The weight was on the 5$^{th}$ percentile for age. Liver was enlarged 4 cm below the costal margin, non-tender, and had firm consistency. Spleen was palpable 2 cm below the left costal margin.

Laboratory analysis showed anemia, hemoglobin level was 80 g/l and RBC 2.83 x $10^{12}$/l.

There was significant hypoproteinemia and hypoalbuminemia, values of 32 g/l and 18 g/l, respectively. Bilirubin level was slightly elevated, total bilirubin was 39 μmol/l, and conjugated 12 μmol/l. Serum transaminases were within normal limits (AST 65 U/l, ALT 59 U/l), and alkaline phosphatase was 970 U/L (normal 120-450 U/l). Coagulation screening showed prolonged prothrombin time of 46 sec, and partial thromboplastin time was 33 sec. Alpha-fetoprotein was >10.000 IU/ml (normal <87 IU/ml). Blood gases and electrolytes were normal, as well as BUN and serum creatinine. Serum amino acid analysis showed elevated tyrosine of 396 mmol/L (normal value, < 200 mmol/L). Urine organic acid analysis revealed elevated succinylacetone (SA). Ultrasonography of the abdomen showed hepatosplenomegaly, ascites and hypoecchogenic structure of kidneys medulla.

According the findings, the infant was diagnosed with tyrosinemia type I and nitisinone therapy (1.0 mg/kg/day) was initiated combined with tyrosine restricted formula (Tyrex®, Abbott Nutrition formula). The patient received several plasma and abumen transfusions and vitamin K supplementation.



 

Following the NTBC treatment, there was significant improvement of the liver function. Coagulation improved 2-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 between 200-400 mmol/L, as recommended. Succinylacetone was negative two weeks after starting the treatment and was determined yearly afterwards. Alpha-fetoprotein was 8298 IU/ml at age of 6 months, and 312 IU/ml at 12 months. NTBC concentration at age of 3 years was 36,2 μmol/l (target values 40-60 μmol/l); thus nitisinone dose was increased. Annual follow-up liver MRI up till now has shown no focal lesions. The ophthalmological examination was scheduled every 6 months and was always normal. The child is now 5 years old and has normal growth and psychomotor development.

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

## DISCUSSION

Hepatorenal tyrosinaemia or tyrosinaemia 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 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 of the HT1 patients were previously unrecognized.

Recent study from Macedonia included four patients with HT1 diagnosed over 3-year period; 2 of the patients had an unfavorable outcome with death occurring at 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 age of six months typically have acute liver failure with initial loss of synthetic function for clotting factors. Our child presented with a liver dysfunction (edemas, jaundice, bleeding tendency), an important feature for diagnosing hereditary tyrosinemia type I. PT 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 liver function is described in the literature; resistance of affected liver cells to cell death may be possible explanation [9].

Mayorandan et al. in a recent study analyzed 168 patients with HT1 from 21 centers with an average age of diagnosis 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 2–6 months of age [7]. Our patient had preserved renal function. High serum tyrosine in combination with increased α-fetoprotein level and severe coagulopathy raised the suspicion of tyrosinaemia in our patient. Detection of SA in urine is the most reliable biochemical diagnostic for HT1. However, there is a reported unusual case of 4-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 2-(2-nitro-4-trifluoro-methylbenzyol) 1,3 cyclohexanedione (NTBC) a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, 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 few days in more than 90 % of the patients, thus treatment should be started as soon as the diagnosis is confirmed, or even suspected because of liver disease [12].

If coagulation improves within 1 week recovery can be assumed, otherwise increasing 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 requirement of liver transplantation. Mayorandan et al. in their study point out the necessity of newborn screening programs to allow an early diagnosis and access of adequate treatment, as they report 2 to 12 fold higher risk for developing hepatocellular carcinoma depending on age of starting treatment compared to patients treated as neonates. Also, psychomotor impairments, ADHS and behavioral disorders, neurological disturbances or learning difficulties were present in a very few number of 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 3 months after achieving well control and stability, as well as parents understanding and compliance. Metabolic control was assessed by determination of SA concentration in dried blood or urine and the level was always below the detection limit.

The toleration of nitisinone in our child was good without any side effects. Mayorandan et al. reported side effects of NTBC treatment in a 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 [7].



 

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

Simoncelli et al. 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 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 [15].

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 *FAH* gene mutation has been expanded, current knowledge is not adequate for establishing the disease's genotype–phenotype correlation.

Angileri et al. 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 [4].

Our child presents the first case from Macedonia with a genetic confirmed HT1. She was compound heterozygote for two mutations c.[1A>G];[784T>A]. c.1A>G 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. reported a 5-month 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 [18].

Imitaz et al. reported 5 homozygous carriers of c.1A>G mutation in a cohort of 43 HT1 patients originating from the Middle East [19].

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 *in silico* analysis (Mutation Taster, PolyPhen 2, SIFT) this mutation was predicted to be a 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 the possible long term complications, particularly hepatocellular carcinoma.

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



 

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