Residência RP Pediátrica
Publicação Oficial da Sociedade Brasileira de Pediatria
ISSN-Online: 2236-6814

Submitted on: 02/22/2020
Approved on: 06/05/2020

CASE REPORT

Alkaptonuria: diagnostic odyssey unraveling a rare metabolic disease

Daniely Passos Camara[1], Isabela Cristina Silva Lima[1], Julia Marques Verissimo da-Silva[1], Tharcila Borges Castro da Rocha[1], Fabiana Schneider[2], Jacqueline Harouche Rodrigues Fonseca[3], Zumira Aparecida Carneiro[1], Charles Marques Lourenco[1]

Keywords:
Alkaptonuria, Ochronosis, Homogentisic Acid, Tyrosine, Drug Therapy.

Abstract

Introduction: Alkaptonuria is a rare autosomal recessive disorder due to the deficiency of the enzyme homogentisic-oxidase, produced by the liver and kidneys, which is involved in phenylalanine and tyrosine metabolism. In the absence of this enzyme, the accumulation of ochronous pigment occurs. The most important clinical manifestations of ochronosis are arthropathy, ocular and cutaneous pigmentation, dark urine and cardiovascular involvement. Nitisinone (NTBC) is a benzoylcyclohexane-1,3-dione that reversibly inhibits the activity of the enzyme step immediately before the homogenized dioxygenase, thereby reducing the production of homogentisic acid (HGA). Thus, it is considered that nitisinone may be a potential treatment for alcaptonuria. Here we report the first Brazilian patient treated with NTBC after a misdiagnosis of congenital porphyria **Case Report:** Male patient, referred for evaluation of urine darkening at two years of age, was diagnosed with alkaptonuria at 3 years of age. At 8 years of age, he was started on NTBC therapy with markedly homogentisic acid decrease. Tyrosine increase and ocular pain were noticed and were managed reducing NTBC dosage. **Discussion:** Although considered a typically adult disease, the symptoms of alkaptonuria begin in childhood and have a progressive and devastating course. Early diagnosis of this disease allows the implementation of therapeutic measures, such as the use of NTBC, preventing the progression of disease manifestations and the appearance of irreversible sequelae.

[1] Estácio University Center of Ribeirão Preto, Faculty of Medicine - Ribeirão Preto - SP - Brazil.
[2] UNIC - University of Cuiabá, Faculty of Medicine - Cuiabá - MT - Brazil.
[3] DLE Laboratory, Genetic Biochemistry - Rio de Janeiro - RJ - Brazil.

Correspondence to:
Charles Marques Lourenco.
Estácio University Center of Ribeirão Preto. Rua Abrahão Issa Halach , 980 - Ribeirânia , Ribeirão Preto -SP. Rio de Janeiro - RJ. Brazil. CEP: 14096-160. E-mail: charlesgenetica@gmail.com

Residência Pediátrica; 2022: Ahead of Print.
DOI: 10.25060/residpediatr-2022.v12n1-274

INTRODUCTION

Alkaptonuria (AKU) is an autosomal recessive hereditary disease resulting from a disorder of phenylalanine and tyrosine metabolism, caused by the mutation of the HGD gene, located in the chromosomal region 3q21-q23, which encodes the enzyme homogentisate-1,2-dioxygenase. . The decrease in this enzyme, which is expressed mainly in the kidneys and liver, is accompanied by the accumulation of homogentisic acid in various tissues and by its elimination in the urine [1-3], where it can be spontaneously oxidized into a blackened pigment [3].

It is a genetic disease that is already present at birth, although the ochronotic manifestations of the disease typically appear after the age of 30. The reason, however, for the late onset of ochronosis remains unknown and the natural history is not yet fully characterized [4,5].

Alkaptonuria is a rare disease . It affects between 1 in 250,000 to 1 in 1,000,000 people, although in some areas, such as Slovakia and the Dominican Republic, the incidence is much higher [4,6]. The risk of recurrence of this pathology in the couple's next pregnancies is around 25% [4,7].

When the catabolic pathway is interfered with by an enzymatic deficiency of HGD, homogentisic acid accumulates in the blood and is eliminated in large quantities in the urine. In contact with air, homogentisic acid oxidizes and polymerizes, giving rise to a black pigment, alkaptone , which gives color to the urine of individuals with this disease. This pigment is also deposited in the connective tissue, giving it a grayish appearance [8,9]. The conversion of this acid to a pigmented, melanin-like polymer, and its binding to connective tissue, especially cartilage, called ochronosis , are the main basis for the pathophysiology of the disease. The deposition of excess acid in different intra and extra-articular structures with a high content of connective tissue causes brownish-black pigmentation and weakening, resulting in tissue degeneration and, finally, osteoarthrosis [10,11].

The most common clinical features are discoloration of urine, pigmentation of the skin, sclera and auricular cartilage, and arthropathy . ochronotic affecting mainly vertebral discs and large joints. Less common manifestations include kidney, urethral and prostate stones and cardiovascular abnormalities such as valvular heart disease , in addition to muscle, tendon, ligament ruptures and fractures [1,4]. Acute metabolic decompensation can occur with fatal complications, such as hemolysis and/or methemoglobinemia [1,7].

Nitisinone (NTBC) is a benzoylcyclohexane-1,3-dione that reversibly inhibits the activity of the enzymatic step immediately before dioxygenase . homogentised , thus reducing the production of homogentisic acid (HGA). Therefore, nitisinone is considered to be a potential treatment for alkaptonuria . A side effect of NTBC therapy, however, is the elevation of plasma tyrosine levels in a manner analogous to tyrosinemia type 2 [12] , another related condition that causes painful palmoplantar hyperkeratosis and ocular symptoms described as conjunctivitis and herpetic-like corneal ulceration [11].

We report here the first case of a Brazilian patient with alkaptonuria with a diagnosis confirmed both by biochemical tests and by molecular genetic analysis and its response to treatment with NTBC.

CASE REPORT

Patient, male, 10 years old, white, son of healthy and non-consanguineous parents, around one and a half years old, was referred to the pediatrician because of abnormal urine color (Figure 1). Urine pigmentation was also observed on the patient's clothing (Figure 2). He was referred for evaluation because of the signs presented, however, given the normality of the general biochemical tests, he was referred for evaluation with a nephrologist. The evaluation with a nephrologist did not point to the cause of the changes observed in the patient's urine.

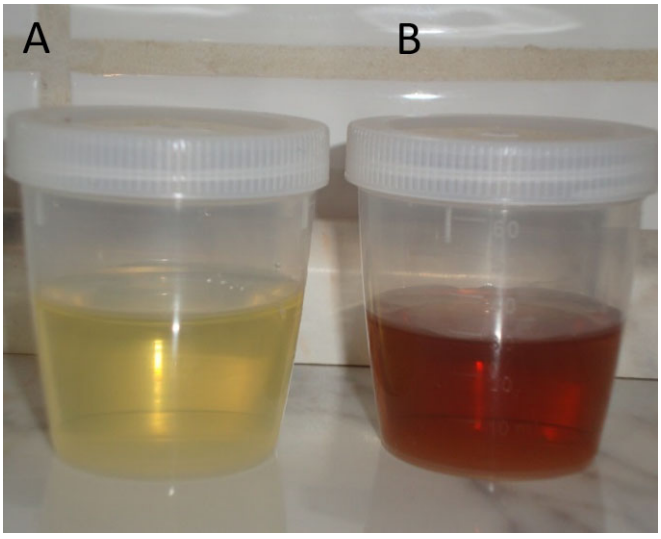


Figure 1. A. Urine sample before light exposure; **B.** Abnormal pigmentation of the patient's urine after exposure to light. Source: The authors (2019).

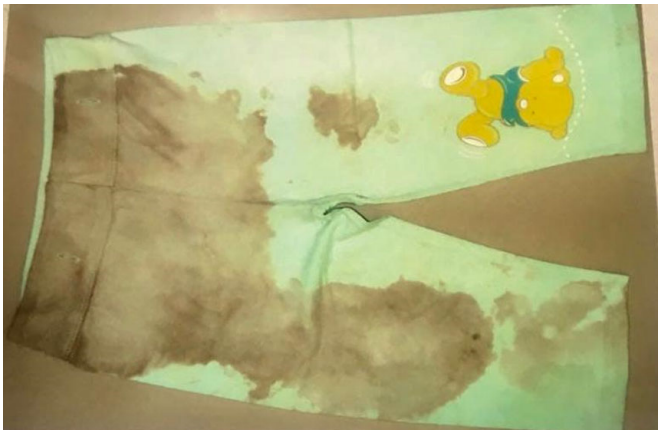


Figure 2. Pigmentation of tissue used by the patient after urination and exposure to light. Source: The authors (2019).

In a subsequent medical evaluation, a diagnostic suspicion of congenital porphyria was made, which was later ruled out after performing targeted biochemical tests (Table 1), in addition to the fact that, clinically, the patient did not report cutaneous photosensitivity , a common finding in congenital cutaneous porphyria.

Table 1. Biochemical tests for congenital porphyria.

Biochemical tests	Sample Result	Reference Values
porphobilinogen	Negative	Negative
Uroporphyrins	Negative	Negative
tyrosinuria	Negative	Negative
Coproporphyrins	Negative	Negative
porphyrins	Negative	Negative
Delta aminolevulinic acid	6.2mg/g	Up to 4.5mg/g
protoporphyrins	Negative	Negative
Organic acid analysis	Chromatographic profile not characteristic of organic acidemia	

Source: The authors (2019).

Therefore, the patient was referred to the genetics unit for diagnostic elucidation, as he continued to present urinary pigmentation alteration. Alkaptonuria was hypothesized based on the patient's clinical findings, and tests directed to this baseline hypothesis were requested (Table 2). The biochemical diagnosis of alkaptonuria was performed by measuring homogentisic acid in urine and was supported by molecular analysis of the HGD gene. Direct sequencing of all the coding exons of the HGD gene revealed the presence of two missense mutations , inherited from their parents: the c.808 G>A (p.G270R) mutation in exon 11 and the c.557 T>A mutation (p. M186K) in exon 9.

After elucidating the patient's clinical condition, additional tests were performed to assess whether there was involvement of other organs associated with the disease, such as cardiac exams, otorhinolaryngology, radiography of hands, wrists, chest and sinuses, polysomnography, ultrasound of total abdomen. Complementary exams were all normal, with the exception of an echocardiogram that showed mild mitral regurgitation.

Faced with the diagnosis and in the absence of other specific therapies, it was decided, together with the family, to use NTBC as a therapy for the disease, understanding the risks and benefits of this therapeutic strategy.

Patient started treatment with NTBC at 8 years of age, following more frequent clinical follow-up since then. Plasma tyrosine levels were within the reference range prior to initiation of NTBC.

NTBC was started at an initial dose of 2mg/day. In the first week of treatment, the patient complained of "eye pain" and photophobia (treated with analgesics), but slit lamp ophthalmologic evaluation showed no abnormal findings. NTBC was stopped for one month and the patient did not report any symptoms after one week.

Table 2. Quantitative determination of amino acids by HPLC and qualitative urine test.

Amino Acids	Sample Result (micromoles /L)	Reference Values (micromoles /L)
aspartic acid	10.1	1 – 24
glutamic acid	74.8	5 – 150
serine	69.1	69 – 187
histidine	50.3	41 – 125
glutamine	472.2	254 – 823
Glycine + Threonine + Arginine	327.2	172 – 707
Alanine	299.8	152 – 547
Tyrosine	38.4	24 – 115
tryptophan	39.1	0 – 79
methionine	12.7	7 – 47
valine	134.1	74 – 321
phenylalanine	33.7	26 – 91
isoleucine	32.6	22 – 107
leucine	65.6	49 – 216
ornithine	69.9	10 – 163
lysine	106.6	48 – 284
Qualitative urine test	Presence of homogentisic acid	

Source: The authors (2019).

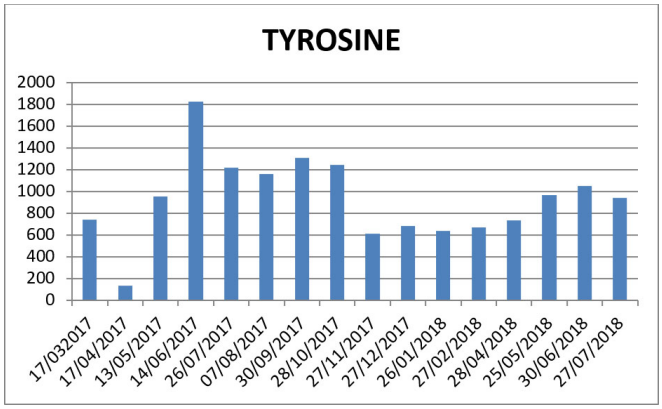
The drug was started again at a lower dose (1.5mg/day) the following month. Tyrosine levels after one month of treatment increased rapidly, but accompanied by a decrease in urine HGA. Urine HGA and plasma tyrosine levels were then measured longitudinally at this dose (1.5mg/day) for one year.

The patient had no other eye complaints and was evaluated by the ophthalmologist every 3 months. Tyrosine concentrations were observed to fluctuate and subsequently remain at significantly higher levels (although NTBC doses decreased to 1.5mg/day) (Graph 1), while a significant drop in HGA continued and reached a plateau. No dietary restrictions were performed, but the patient was closely monitored with monthly tyrosine measurements (tyrosine levels remained high but stable over the past 6 months).

DISCUSSION

Alkaptonuria (AKU) is a rare hereditary metabolic disorder of tyrosine metabolism that results from a defect in an enzyme called homogentisate1,2-dioxygenase [11], as observed in this patient, which revealed the presence of two missense mutations , inherited from his parents. and as a result of this, homogentisic acid (HGA) accumulates in the body. Although typically considered a disease of adults, the symptoms of alkaptonuria begin in childhood and have a progressive and devastating course [12] .

HGA is fundamental for the pathophysiology of this disease and the observed consequences of the accumulation



Graph 1. Tyrosine levels after initiation of NTBC treatment.
Source: The authors (2019).

of this substance (with the consequent formation of polymers that are deposited in the connective tissue) are manifested in the form of spondyloarthropathy , rupture of ligaments/ muscle/tendons, valvular heart disease (including aortic stenosis) and kidney stones [11] , however, the patient in the report presented only mild mitral regurgitation, which can be explained by the fact that it is still a pediatric patient and that the connective tissue lesions are increasingly present as the patient progresses. age.

The early diagnosis of this disease allows the realization of therapeutic measures. The most effective treatment to reduce HGA and its polymers is with the use of NTBC [13] , preventing the progression of disease manifestations and the emergence of irreversible sequelae. Although studies show an improvement in serum HGA levels in adult patients, as a consequence of preventing ochronosis [13] , there is, for example, no regression of already established joint and valvular lesions, which reinforces the need to start therapy as early as possible. Thus, it is worth noting that, in the patient in the report, there was a significant improvement in serum HGA levels with the introduction of NTBC, with no progression - so far - of the symptoms reported in this disease, despite the fact that it is still a period short period of therapeutic observation.

Nitisinone (NTBC) is a benzoylcyclohexane-1,3-dione that reversibly inhibits the activity of the enzymatic step immediately before dioxygenase . homogentised , thus reducing the production of homogentisic acid (Figure 3). Therefore, nitisinone is considered to be a treatment for alkaptonuria . NTBC doses of 0.5 to 2mg/day have been shown to reduce HGA levels by approximately 95% [11] .

Usually, when starting the use of NTBC, there is a decrease in homogentisic acid [14] , which was similarly observed in our patient, with a drastic reduction of this metabolite in the first months of treatment.

In the same way, after starting NTBC, an increase in serum tyrosine is observed [14] , which was also evidenced in our patient and may have been one of the reasons that contributed to the symptoms of eye pain presented by him during the first month of treatment. therapy.

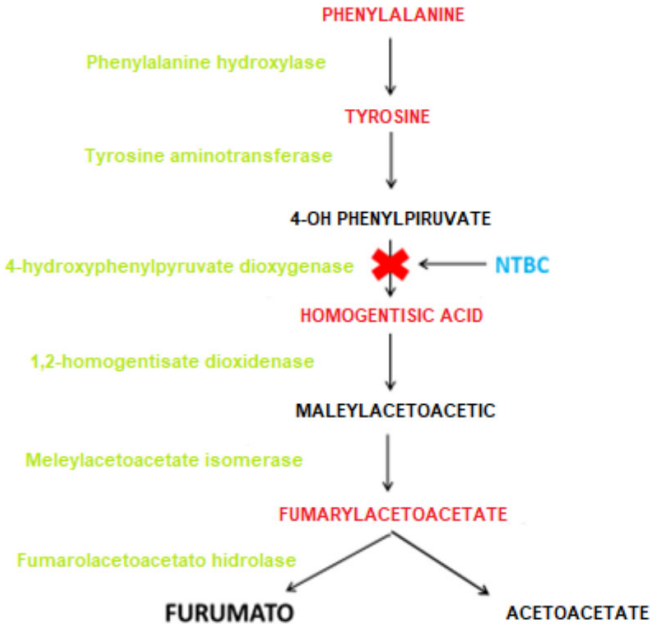


Figure 3. Effect of nitisinone on the tyrosine metabolic degradation pathway.
Source: The authors (2019)

A side effect of NTBC therapy is elevation of plasma tyrosine levels in a manner analogous to type 2 tyrosineemia . Excessive tyrosine elevation leads to the formation of corneal crystal deposits and ocular complications, although several studies have shown that high tyrosine alone may not necessarily be related to ocular symptoms and that some patients are able to tolerate plasma tyrosine levels of more than 1,000Mm [13] . In this case, the patient, after the first week of treatment, complained of “eye pain” and photophobia (treated with analgesics), but the ophthalmologic evaluation with a slit lamp showed no abnormal findings. NTBC was discontinued for one month and the patient , after returning from medication, did not report any more ocular symptoms.

Alkaptonuria is a progressive metabolic disease whose symptoms initially appear in childhood, although the diagnosis is usually made in adulthood when the patient has multiple sequelae of the disease. In view of the new therapeutic possibilities presented by the use of NTBC, it is essential to start therapy as soon as possible, in order to avoid irreversible sequelae of target organs, especially joints, cartilaginous tissues and heart valves, commonly found in adult patients with the disease and that can be prevented with the reduction of homogentisic acid from the use of NTBC.

REFERENCES

1. Ventura-Ríos L, Hernández-Díaz C, Gutiérrez-Pérez L, Pichardo-Bahena R, Cedeño-Garcidueñas AL, Pineda C. Ochronotic arthropathy as a paradigm of metabolically induced degenerative joint disease. A case-based review. Clin Rheumatol. 2016 Mai;35(5):1389-95.
2. Miflin BJ, Lea PJ. Amino acid metabolism. Ann Rev Plant Physiol. 1977;28(1):299-329.

3. Stone JR. Diseases of small and medium-sized blood vessels. In: Buja LM, Butany J, ed. Cardiovascular pathology. 4a ed. London: Academic Press; 2016. p. 125-68.
4. Fisher AA, Davis MW. Alkaptonuric ochronosis with aortic valve and joint replacements and femoral fracture. A case report and literature review. Clin Med Res. 2004 Nov;4(2):209-15.
5. Khatu SS, More YE, Vankawala D, Chavan D, Gokhale NR. Alkaptonuria: case report. Med J DY Patil Univ. 2015 Nov;8:84-6.
6. Aquaron RR. Alkaptonuria in France: past experience and lessons for the future. J Inherit Metab Dis. 2011 Dez;34(6):1115-26.
7. Roberts NB, Curtis SA, Milan AM, Ranganath LR. The pigment in alkaptonuria relationship to melanin and other coloured substances: a review of metabolism, composition and chemical analysis. JIMD Rep. 2015;24:51-66.
8. Braconi D, Milucci L, Bernardini, G, Santucci A. Oxidative stress and mechanisms of ochronosis in alkaptonuria. Free Radic Biol Med. 2015 Nov;88(Pt A):70-80.
9. Arnoux JB, Sang KHLQ, Brassier A, Grisel C, Servais A, Wippf J, et al. Old treatments for new insights and strategies: proposed management in adults and children with alkaptonuria. J Inherit Metab Dis. 2015 Set;38(5):791-6.
10. Zatkova A. An update on molecular genetics of Alkaptonuria (AKU). J Inherit Metab Dis. 2011 Dez;34(6):1127-36.
11. Davison AS, Milan AM, Gallagher JA, Ranganath LR. Acute fatal metabolic complications in alkaptonuria. J Inherit Metab Dis. 2016 Mar;39(2):203-10.
12. Silva J, Lima I, Rocha T, et al. Nitisinone-induced ocular pain in alkaptonuria: a challenging diagnosis despite clinical suspicion. In: 2019 ACMG Annual Clinical Genetics Meeting, April 2-6, 2019, Seattle, Washington.
13. Ranganath LR, Milan AM, Hughes AT, Dutton JJ, Fizgerald R, Briggs MC, et al. Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1): an international, multicentre, randomised, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone on 24-h urinary homogentisic acid excretion in patients with alkaptonuria after 4 weeks of treatment. Anna Rheum Dis. 2016;75(2):362-7.
14. Gertsman I, Barshop BA, Panyard-Davis J, Gangoiti JA, Nyhan WL. Metabolic effects of increasing doses of nitisinone in the treatment of alkaptonuria. JIMD Rep. 2015;24:13-20.