

Early diagnosis of co-existent β -thalassemia and alkaptonuria

Moushumi Lodh, Joshi A. Kerketta¹

Departments of Biochemistry, and ¹Paediatrics and Neonatology, The Mission Hospital, Durgapur, West Bengal, India

Since the aggregate incidence of inborn errors of metabolism is relatively high, a high degree of suspicion is essential to correctly diagnose an inborn error of amino acid metabolism. We report a case of alkaptonuria an autosomal recessive disorder that occurs due to deficiency of homogentisic acid oxidase in a β -thalassemia infant presenting with reddish discoloration of nappies and clothes, breath holding spells, and microcytic hypochromic anemia. Born to consanguineous cousins, to our knowledge, the combination of β -thalassemia and alkaptonuria, which we have described in this baby, has not been reported earlier.

Key words: Alkaptonuria, β -thalassemia, blackening of urine, homogentisic acid

from India. One was a 4-month-old female baby born of a non-consanguineous marriage^[2] and another early presentation was reported at the age of 10 years with the complaint of bluish discoloration of sclera.^[3]

Case Report

Our case was a 1-year 5-month-old male child, brought with complaints of reddish discoloration of the nappies and clothes and breath-holding spells. There was no abnormal odor of the skin or urine. There was no history of crying while passing urine, poor urinary stream or bleeding from skin or mucus membrane. There was no history of fever, rash, abdominal pain, constipation or alteration of sensorium. The mother of the baby was a known case of thalassemia trait; however, had never received transfusion. The baby's parents were consanguineous cousins. The father's thalassemia status could not be elicited.

On examination, the baby weighed 8.5 kg with head circumference 44 cm and length 81 cm and the baby was conscious and alert. There was no jaundice or dehydration or abnormal odor. Arterial oxygen saturation (SpO₂) was 94%. The capillary refill time was between 3 s and 4 s. The heart rate was 146/min. The respiratory rate was 26/min. Non-invasive blood pressure was 73/44 (54) mm Hg. Respiratory system examination revealed normal chest movements with equal air entry on both sides. Abdomen was soft. The liver was palpable 1 cm below the costal margin. The spleen was not felt. There were no other masses felt. Examination of the cardiovascular system showed normal heart sounds. There were no added heart sounds, murmurs or rubs. Reflexes were normal, and he

Introduction

In India, the prevalence of inborn errors of metabolism is one in 2497 newborns and Alkaptonuria have incidence of about 1/250, 000.^[1] A delay in diagnosis and treatment of this disease can result in arthritis and ochronosis (darkening of the tissues) due to the slow accumulation of the dark polymer of homogentisic acid (HA) in cartilage and other mesenchymal tissue; this leads to the dark blackened spots in the sclera, cornea, ear cartilage, and arthritis with advancing age. Literature search revealed two documented reports of alkaptonuria

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.116104

Address for correspondence: Dr. Moushumi Lodh, Department of Biochemistry, Institute of Lab Medicine and Research, The Mission Hospital, Immon Kalyan Sarani, Sector 2C, Bidhannagar, Durgapur - 713 212, West Bengal, India.
E-mail: drmoushumilodh@gmail.com

had hypotonia. There was no neck stiffness. The spine and gait were normal. The eyes, musculoskeletal system, skin, and cardiovascular systems were normal.

The baby was admitted for observation and investigations, and the initial differential diagnosis included alkaptonuria, myoglobinuria, hemoglobinuria, porphyria, and hemochromatosis. He had an under-current viral illness, which was managed symptomatically. The urine routine examination and ultrasound abdomen were normal.

The hemoglobin levels were low with microcytic hypochromic anemia. Hemoglobin was 10.2 g/dl (normal 10.5-14 g/dl), packed cell volume, mean corpuscular volume (MCV) and mean corpuscular hemoglobin were low at 31.9% (normal 32-42%), 68 fl (normal 72-88 fl), and 21.8 pg (normal 24-30 pg), respectively. MCV concentration was 32% (normal 31.5-34.5%) and red cell distribution width 17.1% (normal 11-16%). Serum iron, total iron binding capacity, and ferritin levels were within the reference range. Hemoglobin analysis by cation exchange high-pressure liquid chromatography (HPLC) revealed Hb A0 to be 80.4% and Hb A2 to be 6.1% (normal 2.40-3.60). The HPLC picture on correlation with the complete hemogram suggested β -thalassemia trait. DNA studies confirmed the beta globin gene defect of β -thalassemia.

The serum electrolytes and serum bicarbonate were normal. The renal function and liver function were within the reference range. The child's urine porphyrin, estimations were within normal levels, and serum lead levels and δ -amino levulinic acid were not tested. Myoglobin and hemoglobin levels were undetected in urine. No abnormalities were detected on the electroencephalogram, leading us to believe that the breath holding spells were due to anemia.

Urine was of normal color when voided but turned black over variable periods spontaneously; furthermore, turned black with Benedict's reagent, strong alkali (filter paper impregnated with 10% sodium hydroxide turned black within 5 min when dipped into the urine) and ferric chloride (addition of dilute ferric chloride solution drop by drop showed an evanescent violet blue color). The urine of an alkaptonuric individual usually appears normal when passed. However, it starts to darken upon standing, and this is caused by oxidation and polymerization of the homogentisic acid, and it is enhanced with an alkaline pH.

Benedict's test was strongly positive with red brown precipitate at the bottom and black colored supernatant. Glucose oxidase test (with multistix) was negative excluding the role of glucose as reducing substance. For the qualitative assay of HA, to 0.5 ml of sample, a few drops of 10% ammonia was added followed by the addition of 3% silver nitrate solution. A greenish black color developed, signifying the presence of a substantial amount of HA. Quantitative examination of urine by tandem mass spectrometry revealed that concentration of HA in urine was 91 mg/dl (normally HA is not present in urine). Parents' urine was negative for HA. Due to the financial constraints, confirmatory diagnosis by DNA mutation analysis could not be carried out.

The child was treated symptomatically, and he was given vitamin C (50 mg once a day for 3 months), low phenylalanine and tyrosine diet, and advised to monitor counts, get liver and renal function tests carried out every 2 weeks. On the request of his parents, the child was discharged after 3 days and advised to follow-up in the outpatient department (OPD). After 3 months post-therapy, his HA levels had not reduced. Currently, the baby is under follow-up every 6 months in the OPD.

Discussion

Alkaptonuria is due to an inborn error of metabolism of the tyrosine linkage in the process of protein metabolism, the end product being 1, 4-dihydroxyphenyl-acetic (homogentisic) acids, which is passed into the urine. Alkapton urine is normal in color when freshly passed, but on exposure to air and light rapidly darkens from the surface downwards, ultimately assuming a dark-brown or black color. Addition of alkali increases the rate of oxidation. It reduces cupric oxide and also silver nitrate solution (cold). 1, 4-Dihydroxyphenyl acetic acid may be regarded as a derivative of hydroquinone. Therefore, its presence can be detected in urine quite readily by first making the urine strongly alkaline with caustic soda or caustic potash. This color change does not take place with the substances occurring normally in urine, or pathologically except with homogentisic acid. Thus, the diagnosis of alkaptonuria may be made.

Isolated case reports from India are also available.^[4,5] However, our case is probably the first documented case of alkaptonuria co-existing with thalassemia in a 1-year 5-month old baby with no symptom except reddish discoloration of nappies and clothes. The breath holding spells complicated the presentation. Our use of basic biochemical tests supported the heightened index of suspicion to lead us to a diagnosis in 3 days.

The gene encoding homogentisate 1, 2-dioxygenase, is the only gene in which mutations are known to cause alkaptonuria.^[6] It maps to chromosome 3q. Several disease-causing mutations of this gene have been identified.^[7] Various presentations of alkaptonuric patients have been described such as aortic valve regurgitation and inferior myocardial infarction, ochronosis with joint pain, end-stage renal failure and pigmented conjunctival lesions.^[8] The aminoacids tyrosine and phenylalanine are not metabolized beyond the stage of homogentisic acid, which is, therefore, excreted in urine. HA is a strong reducing agent, which on exposure to atmospheric oxygen for some hours, gets converted to an oxidized polymer that is black in color. Urine containing homogentisic acid, therefore, turns black on standing. Levels of HA in the blood are minimally increased because it is rapidly cleared by the kidneys. Since, ascorbic acid impedes oxidation and polymerization of HA *in vitro*, its use has been suggested as a possible means of decreasing pigment formation and deposition.^[9]

Estimates show that 1.5% of world populations are carriers of β -thalassemia, and about 50% of incidence is from South-East Asian population, which mainly includes countries such as India, Thailand, and Indonesia. The carrier rate for β -thalassemia varies from 1% to 17% in India with an average of 3.2%.^[10] Curtin and Kan^[11] have described the genetic defects seen in β -thalassemia. Although, co-incidence of more than one inherited disease in highly consanguineous kinships has been described, the novel combination of alkaptonuria and β -thalassemia has not yet been reported in the literature. Further, genetic studies are required to analyze any possible linkage between thalassemia and alkaptonuria.

We would however like to stress that in infancy, a history of dark-stained diapers should alert the physician to alkaptonuria, and they can be evaluated with simple urine

testing on an outpatient basis. Medical therapy is used to ameliorate the rate of pigment deposition. This minimizes articular and cardiovascular complications in later life. A mild dietary restriction of phenylalanine and tyrosine reduces HA excretion, thus, avoiding or minimizing later complications. The mild antioxidant nature of ascorbic acid helps to retard the process of conversion of homogentisate to the polymeric material that is deposited in cartilaginous tissues. Although limited use of nitisinone, an inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, which mediates the formation of HA, has been reported, safety of prolonged use is still an open question. Therefore, it was not given to our patient.

References

1. Latheef SA. A database for inborn errors of metabolism in the Indian state of Andhra Pradesh. *Bioinformation* 2010; 4:276-7.
2. Datta AK, Mandal S, Dasgupta A, Ghosh TK. Alkaptonuria diagnosed in a 4-month-old baby girl: A case report. *Cases J* 2008;1:308.
3. Verma SB. Early detection of alkaptonuria. *Indian J Dermatol Venereol Leprol* 2005;71:189-91.
4. Biswas TK, Nayek G, Chatterjee A, Bhattacharjee D, Halder R, Roy DN. Alkaptonuria. *J Indian Med Assoc* 1985; 83:208-11.
5. Chaudhary HR, Gokhroo RK, Arora SK, Bhardwaj B, Bhati RS, Mathur MS. Alkaptonuria – 2 cases. *J Assoc Physicians India* 1983;31:676-8.
6. Fernandez Canon JM, Granadino B, Beltrán-Valero de Bernabé D, Renedo M, Fernandez-Ruiz E, Peñalva MA, *et al.* The molecular basis of alkaptonuria. *Nat Genet* 1996;14:19-24.
7. Elçiöğlü NH, Aytuğ AF, Müller CR, Gürbüz O, Ergun T, Kotiloğlu E, *et al.* Alkaptonuria caused by compound heterozygote mutations. *Genet Couns* 2003;14:207-13.
8. Al-Mefraji SH. Alkaptonuria in a 5-year-old boy in Iraq. *East Mediterr Health J* 2008;14:745-6.
9. Sexton JP, Boehm CD, Goff SC, Waber PG, Rosenberg LE. Storage diseases at amino-acid metabolism. In: Wilson JD, Braunwald E, Isselbacher KJ, editors. *Harrison's Principles of Internal Medicine*. 12th ed. Humberg: McGraw Hill Book Company; 1991. p. 1875.
10. Agarwal MB. The burden of haemoglobinopathies in India: Time to wake up. *J Assoc Physicians India* 2005; 53:1017-8.
11. Curtin PT, Kan YW. The inactive beta globin gene on a gamma delta beta thalassemia chromosome has a normal structure and functions normally *in vitro*. *Blood* 1988; 71:766-70.

Cite this article as: Lodh M, Kerketta JA. Early diagnosis of co-existent β -thalassemia and alkaptonuria. *Indian J Hum Genet* 2013;19:259-61.

Source of Support: Nil, **Conflict of Interest:** None declared.