

Diverse Expression of Alkaptonuria: A Case Series

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ABSTRACT

Alkaptonuria is a rare autosomal metabolic disorder that predominately affects the male population. In infants, it is usually asymptomatic except darkening of urine on exposure, while in adults it starts appearing in 3rd and 4th decade of life. We received 4 cases of this rare disease at the Department of Toxicology, Armed Forces Institute of Pathology Rawalpindi, Pakistan, from Jan 2020 to Feb 2023 presenting in different age groups ranging from early childhood to middle age with a common clinical finding of darkening of urine on prolonged standing. Urine samples of cases were analyzed by gas chromatography-mass spectrometry (GC-MS) and matched with the reference library of GC-MS for the peak of homogentisic acid (HGA) on urine organic acid (UOA).

Keywords: Alkaptonuria, Gas Chromatography-Mass Spectrometry, Homogentisic Acid.

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INTRODUCTION

Alkaptonuria, also called endogenous ochronosis, is a rare metabolic autosomal recessive disease with a prevalence of 1:1,000,000.¹ It arises from total or partial inhibition of hepatic enzyme homogentisate 1, 2-dioxygenase (HGO). The gene for homogentisate oxidase has been mapped to chromosome 3q2. Any mutation in the homogentisate 1, 2-dioxygenase (HGD) gene cause alkaptonuria.² Deficiency of enzymes results in the binding of the oxidized polymers of homogentisic acid (HGA) to connective tissue and its excretion in the urine. This deposition of HGA results in the dark pigments deposition in various connective tissues eventually leading to cartilage destruction.³

Alkaptonuria presentation and diagnosis varies from accidental findings in children to symptomatic presentation in adult age. It is mainly observed with complications in the joints of the cervical spine, osteoarthritis along with hyperpigmentation of other connective tissues like skin, cartilage, and sclera.^{2,4} Alkaptonuria is diagnosed on the peak of HGA on urine organic acid (UOA) analyzed by gas chromatography-mass spectrometry (GC-MS).⁵

METHODOLOGY

This case series of 4 cases, was presented to the Department of Toxicology, Armed Forces Institute of Pathology, Rawalpindi from January 2020 to February

2023 for the analysis of (UOA). Ethical clearance was obtained from the Head of Department. The samples were analyzed on gas chromatography-mass spectrometry (GC-MS) using ethyl acetate and derivatization by N, O-Bis (trimethylsilyl, TMS) trifluoroacetamide (BSTFA) compound to make them more volatile.⁵ The mass spectra were then identified by comparing the retention time, ions spectrum, and peak shapes with the reference library of (GC-MS). Patients were diagnosed with Alkaptonuria in the presence of homogentisic acid peak on the chromatogram of urine organic acid appearing at the retention time (RT) of 16.5 minutes as shown in Figure.

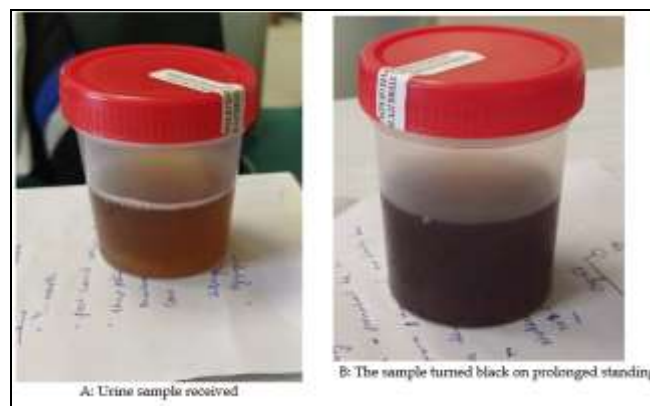


Figure: Alkaptonuria initial sample which turned black on prolonged standing (n=4)

A comprehensive proforma for workup of inherited metabolic disorders having details of the patient regarding demography, gender, age, presenting complaints, and any significant past history

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is required to be filled in at the time of sample collection. The biochemical profile includes plasma lactate, plasma ammonia, plasma glucose levels, renal function tests arterial blood gas analysis, and liver function tests which should also be done in parallel with this test if not done previously to rule out other metabolic diseases.

CASE 1: An infant, 9 months of age, was brought with complaints of smelly urine which turns dark on prolonged exposure to air along with staining of diapers. The child was exclusively on the mother's feed. Parents had a consanguineous marriage. A spot urine sample was collected for organic acid analysis to rule out alkaptonuria. The child had no history of rashes, fever, vomiting, diarrhea, and seizures. There was no history of use of drugs by the mother during pregnancy. The sample was analyzed on GC-MS and the levels of homogentisic acid turned out to be markedly raised confirming the diagnosis of alkaptonuria.

CASE 2: A six-year-old boy reported to AFIP with a history of staining of diapers in childhood and blackening of urine since then, along with a sharp pungent smell. The child was otherwise active and had no other significant past history. Parents had a consanguineous marriage. There was no history of use of any medicine by the child, and no history of fever, rashes, or bone deformities. However, a small hyperpigmented area was noticed on the face around the left side of the nose and cheek area. There was no history of any discoloration of the sclera and no other significant clinical findings.

CASE 3: A 34-year-old male patient presented with a clinical history of smelly urine and darkening of urine on long standing. Moreover, he has been suffering from backache, pain in his legs, and limited knee joint movements for the last 04 years. His MRI lumbosacral spine showed nerve root compression, enlarged disk spaces, calcification, and vertebral fusion. He had dark pigmentation on his skin and sclera. The family history of alkaptonuria was also positive. The pain in joints especially in the big joints was also affecting his normal routine activities which he reported and on analysis he came out to be positive for alkaptonuria.

CASE 4: Another male patient, aged 43 years, presented with complaints of a pungent smell in urine and darkening of urine since childhood. He had his initial checkups but he remained undiagnosed. Radiography of the vertebral column showed calcification of lower and lumbar intervertebral discs.

His MRI lumbosacral spine report showed spine ankylosis spondylitis. He too had joint pains and backache on and off. On analysis of the urine for organic acid homogentisic acid peak was noted confirming alkaptonuria.

The demographic, clinical, and biochemical findings of these patients are shown in Table showing mildly raised levels of ammonia and lactate in childhood while the common positive finding of a history of consanguineous parents and darkening of urine were found in all four cases. Family history was positive in two cases and all patients were on symptomatic treatment only.

Table: Demographic, Clinical, and Biochemical investigations of Alkaptonuria cases (n=4)

Presenting Age	Clinical Findings		Biochemical Investigations	
	Joint Pain	Urine darkens on Standing/ staining of diapers	Ammonia (12-47 umol/L)	Lactate (0.5-2.2mmol/L)
9 months	No	Yes	60	3.2
6 years	No	Yes	75	2.5
34 years	Yes	Yes	40	2.2
43 years	Yes	Yes	32	2.3

DISCUSSION

Due to reasons including missed diaper staining in infants, and few laboratories offering diagnostic tests for Alkaptonuria, patients are often diagnosed late, which leads to their poor quality of life.^{4,5} Cases go undiagnosed and they are reported in late age with complaints of joint pains and restricted movements, later on diagnosed on x-ray and ultrasound findings.

Flexion contractures of the knee along with non-articular features of ochronosis include bluish discoloration of the ear pinnae, triangular pigmentation of the sclera, pigmentation over the nose, axillae, and groin can be observed. Prostatic calculi are also common in men, and cardiac murmurs sometimes occur due to the deposition of pigment in cardiac valves.⁶ Up till now there is no defined pharmacological treatment for this disease. The restricted intake of some proteins like tyrosine and phenylalanine is suggested in most cases. Vitamin C has an oxidant effect that prevents the oxidation of homogentisic acid.^{1,7} Nitisinone is also significantly helpful by diminishing the production of harmful metabolites by inhibiting the enzyme (4-hydroxyphenylpyruvate dioxygenase).^{8,9} Early diagnosis and treatment can prevent the developmental complications often appearing in 3rd 4th decades and get advanced in 5th and 6th decade.¹⁰

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MR & MUM: Data acquisition, data analysis, critical review, approval of the final version to be published.

MQAK & NZ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MA & NA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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