

Alkaptonuric Ochronosis with Aortic Valve and Joint Replacements and Femoral Fracture

A Case Report and Literature Review

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ABSTRACT

Alkaptonuria is a rare autosomal recessive disorder of metabolism caused by deficiency of homogentisic acid oxidase and resulting in accumulation of homogentisic acid in collagenous structures. It is characterized by homogentisic aciduria, bluish-black discoloration of connective tissues (ochronosis) and arthropathy of large joints. Less common manifestations include cardiovascular abnormalities, renal, urethral and prostate calculi. Bone fractures are unusual in ochronosis.

In this report, we describe a woman, 69 years of age, with a history of dark urine since childhood and progressive pigmentation of the skin, sclera, and auricular cartilages. She had severe arthropathy requiring total joint replacement in both of her knees and right hip. She also had severe aortic stenosis requiring valve replacement, and asymptomatic nephrolithiasis. She presented with a low trauma fracture of the distal femur despite two years of alendroate therapy.

We review the etiology, pathogenesis, clinical presentation, diagnosis and treatment of alkaptonuric ochronosis. Early detection is important for prevention and treatment of multiple systems. Nitisinone, a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, dramatically reduces production and urinary excretion of homogentisic acid; however, the long-term efficacy and side effects of such therapy are unknown. Identifying the gene for alkaptonuria offers the potential for a new therapeutic approach (replacement therapy with a recombinant enzyme) in the treatment of alkaptonuric ochronosis.

INTRODUCTION

Alkaptonuria, a rare autosomal recessive disorder of phenylalanine and tyrosine metabolism caused by deficiency in homogentisate 1,2-dioxygenase activity, leads to accumulation of large amounts of homogentisic acid. Homogentisic acid is excreted in urine, turning dark brown or black upon oxygenation and alkalization. Homogentisic acid is deposited as an oxidized and polymerised pigment (ochronotic pigment) in various tissues and organs binding irreversibly to collagen and causing bluish-black pigmentation (ochronosis).

Alkaptonuria affects between 1 in 250,000 to 1 in 1,000,000 people,¹⁻³ although in some areas such as Slovakia and the Dominican Republic, the incidence is much higher (e.g., up to 1 in 19,000 in Slovakia⁴).

The most common clinical features are discoloration of the urine; pigmentation of the skin, sclerae, and ear cartilage; and ochronotic arthropathy affecting mainly the vertebral discs and large joints. Less common manifestations include renal, urethral and prostate calculi and cardiovascular abnormalities, especially valvular disease. Few reports of bone fracture (mainly vertebral) have been published,⁵⁻⁸ and none have documented distal femur fractures.

We report a case history of a patient with severe multiple system ochronosis who underwent three joint replacements, an aortic valve replacement, and presented with a low trauma fracture of the distal femur. The disease history, etiology, pathogenesis, clinical presentation and treatment are reviewed.

CASE REPORT

A woman, 69 years of age, was transferred from a district hospital with a low trauma fracture of her left distal femur that she reported occurred as she walked past a tennis court and “twisted her body” during an attempt to pick up and throw a stray tennis ball over the fence surrounding the court. She stated that she heard a crack in her left leg and then the knee gave way. She developed severe pain in the leg and was unable to bear any weight on it. X-ray examination showed a fracture of the left distal femur.

The patient’s medical history was remarkable for progressive degenerative arthritis affecting the hips, knees, shoulders, and spine beginning in her late 40s. A diagnosis of ochronosis was made by radiological exam, and alkaptonuria was confirmed 10 years after the first presentation. At the age of 62 years she underwent total replacement of the right hip because of intractable pain, no improvement with non-steroidal anti-inflammatory drugs and physiotherapy, and restricted mobility and sitting tolerance of less than half an hour. Two years later she underwent bilateral knee replacement. Following these operations she was able to walk without walking aids. At 66 years of age, she received an aortic valve replacement (pericardial prosthesis) for severe calcified aortic valvular stenosis with a peak trans-valvular gradient of 90 mm Hg. At surgery, severe annular calcification extending into the left ventricular outflow tract and dark blue discoloration of the valve cusps and aortic wall were noted. Microscopic examination of the aortic valve leaflets revealed nodular calcification, and ochronotic dark pigment within the areas of calcification and focally in the non-calcified valvular tissue (figure 1). Angiography demonstrated minor coronary artery disease; the left ventricle was hypertrophied but functioning well (ejection fraction 60%). A year later, at 67 years of age, she was diagnosed with low-grade papillary transitional cell carcinoma of the bladder. No invasion was identified on repeated biopsies (stage pTa). The same year she sustained a low trauma fracture of the right distal radius. Treatment with alendronate and calcium was initiated at this time. The patient complained of chronic dull pain and stiffness in her lower back and shoulders, and previously noted brown black

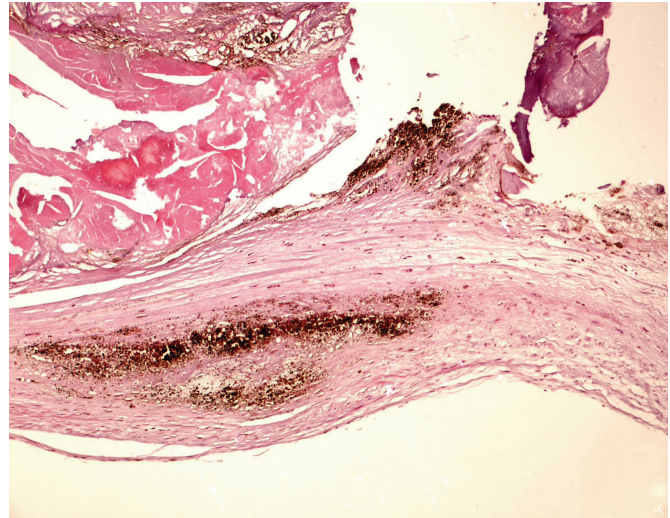


Figure 1. Photomicrograph showing ochronotic pigment deposition in aortic valve leaflet (haematoxylin and eosin stained, magnification = 100X)

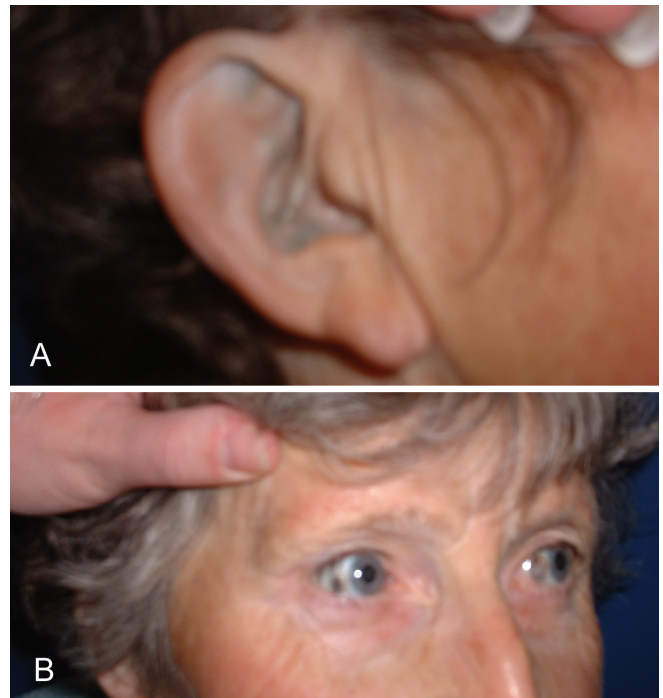


Figure 2. Ochronotic pigmentation of the ear cartilage (A) and the sclera of both eyes (B).

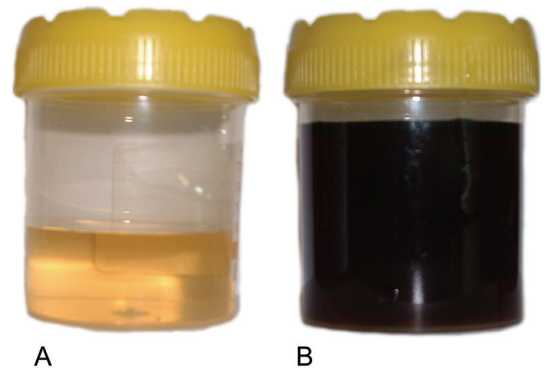


Figure 3. A urine sample before (A) and after (B) sodium hydroxide addition.

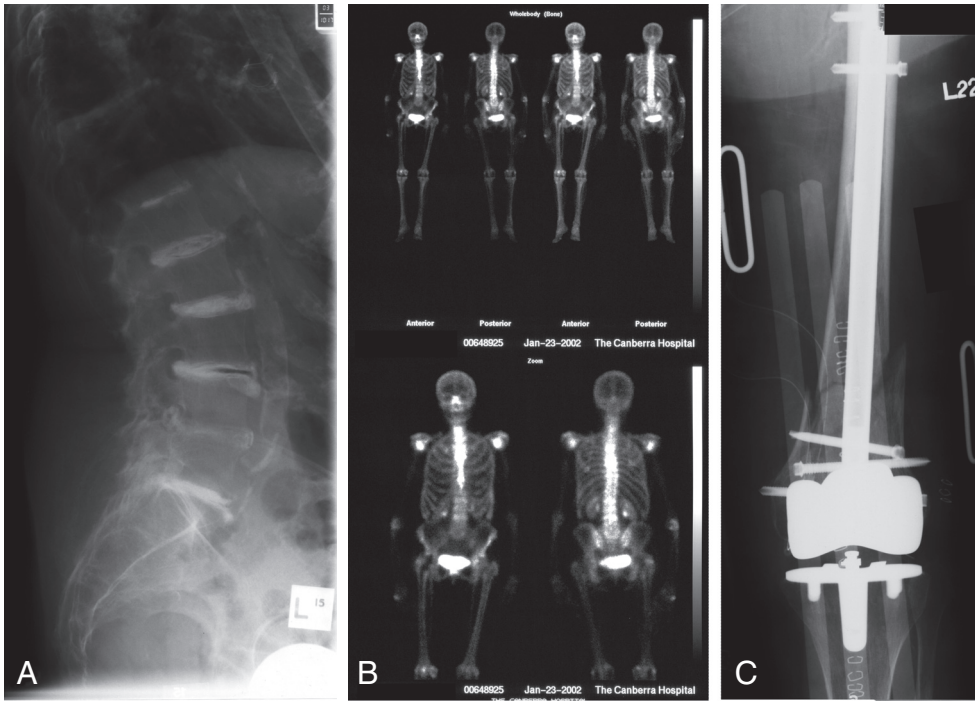


Figure 4. (A) roentgenogram of the spine showing space narrowing and calcification of the intervertebral discs with vacuum phenomena, (B) A whole body scan demonstrating increased uptake in the thoracic, lumbar and manubriosternal regions, arthritic changes in both glenohumeral joints and right hip, and bilateral knee prosthesis, (C) roentgenogram of the fractured left femur.

pigmentation in the sclera of the eyes, face, ears and palms. Black discoloration of her undergarments was noticed since childhood.

The patient's family history was unremarkable for genetic disorders, including alkaptonuria. Her parents were not consanguineous.

Her medications included aspirin, celecoxib, atorvastatin, calcium and alendronate for the previous 2 years. She had never used antimalarial, dopamine or phenolic drugs. Before starting the anti-osteoporotic therapy, bone mineral density T score at the femoral neck measured by dual-energy x-ray absorptiometry was -3.21 , and after 21 months of treatment was -2.60 . Lumbar spine bone mineral density was elevated on both occasions with T score values $+1.92$ and $+1.74$, respectively.

General examination revealed brown pigmentation of the sclera of both eyes (in the nasal and temporal regions), the cartilage of the external ears, the face, thenar, hypothenar, fingertips, and dorsum of both hands and the soles of the feet (figure 2). The pigment deposits were not tender. The visible mucous membranes were normal. Her weight was 49 kg with a body mass index of 21.7 kg/m^2 . She had moderate thoracic kyphosis, loss of lumbar lordosis with limited range of motion and tenderness in the mid-lower thoracic and lumbar spine. Range of motion in both shoulders was also reduced and more painful on the right side. The joints of the hands and feet were not affected.

Cardiovascular examination and echocardiogram were consistent with an artificial aortic valve and left ventricular hypertrophy. The electrocardiogram showed sinus rhythm at 75 beats/min and bifascicular block (right and left bundle

branch block). The respiratory, neurological, and abdominal examinations were normal.

Laboratory analyses included complete blood count, erythrocyte sedimentation rate, C-reactive protein, urea, creatinine, uric acid, liver and thyroid function tests, calcium, phosphate, magnesium, parathyroid hormone (5.6 pmol/l), 25-OH vitamin D (61 nmol/l), vitamin B12, folate, and ferritin, as well as urinalysis. All tests were within normal ranges. Rheumatoid factor and HLA-B27 were negative. The patient's freshly passed urine turned black immediately after sodium hydroxide was added (figure 3).

Radiological examination of the thoracolumbar spine showed calcification in the intervertebral discs at T7/8 and T12 to S1, gas ("vacuum phenomenon") in the L3/4 disc, marked narrowing of intervertebral disc spaces, and endplate osteophytes most prominent in the thoracic and upper lumbar spine, and calcification of the lower costal cartilages (figure 4A). Radiographs of the shoulders disclosed marked narrowing of the joint space and subchondral sclerosis with calcification in the humeral heads.

A whole body scan with technetium 99m dicitronate demonstrated an increased uptake in the mid thoracic, and upper and mid lumbar regions suggestive of degenerative changes. There was also increased uptake in the manubriosternal region. Arthritic changes were present in the glenohumeral joints bilaterally. The right hip prosthesis and bilateral knee prostheses were noted with normal periprosthetic activity, although there was evidence of mild arthritic change in the patellofemoral compartment on the right side (figure 4B). The radiological and

scintigraphic findings were confirmed by computed tomography that also showed a calcific density in the upper pole of the left kidney (a small calculus), but no renal obstruction was noted.

The patient had an open reduction with internal fixation (retrograde nail insertion) of the fractured left femur (figure 4C). Her operation and hospital stay were uneventful, and she was discharged to her home.

DISCUSSION

The earliest verified case of ochronosis was described in an Egyptian mummy dating to 1500 B.C. Radiological and biochemical examination of the intervertebral discs, hips and knees was used to confirm the diagnosis.^{9,10} In 1584, Scribonius reported a boy who passed urine as black as ink.¹¹ The term alkaptonuria was first used in 1859 by Boedeker to describe urine discoloration due to a reducing compound. The compound was identified as homogentisic acid in 1891 by Wolkow and Baumann.³ In 1866, Virchow called the condition ochronosis (meaning "yellow disease" in Greek) because the accumulated pigment in the connective tissues appears as ochre (yellow) when examined microscopically.¹²

At the beginning of the last century alkaptonuria was the first disorder to be found to conform to the applicability of the rediscovered Mendelian laws of autosomal recessive inheritance¹³ and became a cornerstone of the fundamental concept of "inborn errors of metabolism" (Mendelian Inheritance in Man number [McKusick] 203500). A half century later, the specific enzyme defect in the liver of a patient with alkaptonuria was demonstrated to be a deficiency of homogentisic acid 1,2-dioxygenase (HGO) activity, one of six enzymes required for catabolism of the aromatic amino acids phenylalanine and tyrosine.¹⁴ The human HGO gene locus has been mapped to chromosome 3q21-q23.^{2,15,16} In the last decade the HGO gene has been cloned, characterized, and its promoter region identified.^{17,18} A total of 84 mutations impairing this enzyme have been found in the HGO gene from humans and model organisms, and 43 of these mutations result in single amino acid substitutions.¹⁹ More than 40 different mutations have been identified in approximately 100 unrelated patients with alkaptonuria from many different countries.²⁰ The most widespread HGO mutations are probably old mutations that have spread throughout Europe and Asia during human migration.^{21,22}

Alkaptonuria is characterized by a remarkable allelic heterogeneity. Affected persons are either homozygous or compound heterozygous for loss of function mutation(s) in HGO. In a recent series, 46 out of 58 patients were compound heterozygotes and no correlation between the presence or absence of any type of HGO mutation and either level of urinary homogentisic acid excretion or severity of disease was found.³

Inability to convert homogentisic acid to maleylacetoacetic acid results in accumulation of homogentisic acid and a product of its oxidation, benzoquinone, which induces tissue injury. This accumulation causes the classic clinical triad: (1) homogentisic aciduria which presents at birth (pathognomonic sign: urine blackens on standing when oxidized or alkalinized); (2) gradual development of ochronosis after 20 to 30 years of age (deposition of polymers of oxidized homogentisic acid in connective tissues leads to intensive eumelanin-like pigmentation of skin, sclera, cartilages, etc); and (3) degenerative ochronic arthropathies usually in the fourth decade of life. Other important but more rare consequences of alkaptonuric ochronosis are cardiovascular and urinary tract involvement.

Our patient had dark urine and demonstrated extensive, progressive pigmentation of the skin, sclera, and auricular cartilages; advanced spondyloarthropathy necessitating three joint replacements; severe aortic stenosis requiring valve replacement; asymptomatic nephrolithiasis; and low trauma fractures of the radius and distal femur.

It has been suggested that clinical manifestations of alkaptonuric ochronosis are usually delayed, not appearing until the fourth decade of life because with ageing the renal clearance of homogentisic acid decreases.²³ Case reports of ochronotic nephropathy and renal failure further emphasized the role of renal tubular secretion in eliminating homogentisic acid from the body.²⁴⁻²⁶ However, there were no signs of renal impairment in our patient indicating the role of other mechanisms contributing to the severity of the disease. Indeed, of 58 subjects with alkaptonuric ochronosis, reduced creatinine clearance was documented only in one patient with diabetic nephropathy.³

Ochronotic spondyloarthropathy is the most common complication of alkaptonuria affecting large weight bearing joints and later the shoulders. Typically, involvement of the large peripheral joints usually occurs several years after spinal involvement. It is suggested that the characteristic extensive calcification and vacuum phenomena in ochronotic intervertebral discs are pathologically different from degenerative disc disease and are related to cartilage brittleness and fragmentation similar to that in the affected peripheral joints.²⁷ In contrast to rheumatoid arthritis, the small joints of the hands and feet are usually not affected, and in contrast to ankylosing spondylitis, bamboo spine, annular ossification, syndesmophytes, erosion, and fusion of sacroiliac joints do not occur.

It was claimed that arthropathy, especially axial involvement, is more severe in HLA-B27-positive individuals.^{27,28} Coexistence of ochronosis and rheumatoid arthritis,^{29,30} ankylosing spondylitis,^{31,33} or chondrocalcinosis³⁴ has also been reported.

Our patient lacked rheumatoid factor and the HLA-B27 antigen. She did not have signs of rheumatoid arthritis,

ankylosing spondylitis or chondrocalcinosis. She demonstrated the spectrum of clinical and radiological signs typical of severe ochronotic arthropathy with involvement of the spine, knees, hips, and shoulders and had under went total joint replacement of both knees and the right hip. In a series of 58 ochronotic patients, 8 (13.8%) had three or more joints replaced.³ There are two other reports of four total joint replacement arthroplasties in ochronosis,^{35,36} and in one case seven joints had been replaced.²⁵

To our knowledge, the case we present here is the first report of low trauma distal femur fracture in an ochronotic patient. While degenerative changes of the spine and major joints have been frequently reported, there are only three reports of spine fractures,^{5,7,8} one associated with cortisone therapy,⁵ and only one report of femoral neck fracture.⁶

In ochronosis, the changes in the bone are thought to be less severe than those in cartilage.³⁷ The accumulation of oxidized and polymerized products of homogentisic acid reduces the cross-linkage of collagen fibers leading to connective tissue failure, cartilage erosion, and progressive degenerative changes.²³ Although ochronosis in bone induces the same changes as in other connective tissues, the severity appears to be limited by calcification and bone remodelling.³⁷ It is suggested that the detrimental effects of ochronotic pigment on the fibrils of soft connective tissues are avoided by the collagen fibrils of the bones because they are encrusted by a mineral substance and because the newly formed osteoid matrix remains uncalcified for too short of a time to be modified by the pigment. In an ochronotic femoral head, the pigment was not found in osteoblasts but was present in the calcified matrix as well as in the cytoplasmic vacuoles of osteoclasts and in osteocytes, some of which were degenerate or dead.³⁷ In a series of ochronotic patients, the biochemical markers of bone turnover showed increased bone resorption (high urinary excretion of crosslinked N-telopeptides of type I collagen) with an almost normal bone formation in 6 out of 7 patients indicating accelerated bone loss.³⁸ Importantly, these changes were associated with reduced femoral bone mineral density. Moreover, in organ cultures of embryonic chick calvaria it was shown that homogentisic acid inhibits intracellular hydroxylysine formation diminishing intermolecular cross-links that are critically important for the structural function of the newly synthesized collagen.³⁹ It should be mentioned that in our patient, as in other reported cases,³⁸ while femoral neck bone mineral density was markedly reduced, the lumbar spine bone mineral density was normal or increased. This seeming paradox might be due to extensive intervertebral disc calcification.

The case we present demonstrates that in ochronosis bony structures may be severely affected. Our patient had two non-vertebral fractures (distal radius and distal femur) within two years time, but did not have vitamin D deficiency or secondary hyperparathyroidism. She has no other risk factors for osteoporosis such as malnutrition, immobility, smoking, medications (corticosteroids, anticonvulsants), or

family history of osteoporotic fractures. Moreover, she has received alendronate therapy for the last two years with improvement in bone mineral density. It now seems practical that adequate antiresorptive therapy to prevent bone fractures in ochronotic patients should be considered much earlier in the course of treatment.

Our patient has also had a severe aortic stenosis with calcified valves and gross pigmentation of the aorta but no coronary artery involvement. Some case series have showed no increase in frequency of calcification and stenosis of aortic valves or coronary artery disease,^{40,41} but numerous other observations suggest that ochronosis may be associated with pigment deposition in aortic and mitral valves, endocardium, pericardium, aortic intima, coronary arteries and especially with valvular dystrophic calcification, aortic stenosis, and coronary disease.⁴²⁻⁴⁹ In a series of 58 ochronotic patients, 3 (5.2%) had aortic valve replacement, and 50% had computed tomographic evidence of coronary artery calcification by 59 years of age.³

Patients with alkaptonuria are known to be at increased risk of nephrolithiasis. Kidney stones caused by ochronosis were reported in 16 out of 58 patients (27.6%), and more often in males.³ An asymptomatic small calculus in the left kidney was documented in our patient.

It is worth mentioning that clinical variability of alkaptonuric ochronosis, which reflects the spectrum of HGO mutations, may delay the diagnosis and lead to mismanagement including unnecessary biopsies and surgery. In one case ocular ochronotic pigmentation was misdiagnosed as melanoma and one eye was mistakenly enucleated.⁵⁰

Although the diagnostic confirmation of alkaptonuria is easily made by alkalinization of urine with quantitative determination of homogentisic acid in urine available, only 21% of patients are diagnosed before 1 year of age.³ In a recently reported case,⁵¹ despite marked mucocutaneous pigmentation, advanced spondyloarthropathy, unilateral hip replacement, and blackened urine on standing, the diagnosis was not established until the patient was 82 years of age. Moreover, of 755 respondents to a "medical mystery" published in the *New England Journal of Medicine*⁵¹ only 43% correctly diagnosed alkaptonuric ochronosis, while 23% thought of melanoma and 23% of porphyria or porphyria cutanea tarda. Paroxysmal nocturnal haemoglobinuria and ingestion of arsenic or silver were among other responses. Pseudo-ochronosis, which is not an inherited disorder, has been described as a result of argyria⁵² and long term use of levodopa, methyldopa,⁵³ antimalarials, or products containing hydroquinone, phenol, resorcinol, mercury or picric acid.⁵⁴

Currently there is no specific and effective treatment for alkaptonuria. Although some advocate dietary protein restriction (mainly phenylalanine and tyrosine), and ascorbic acid to reduce urinary homogentisic acid excretion and

possibly reverse bone abnormalities,⁵⁵ these observations have not been confirmed in other studies.^{3,56}

A direct pharmacologic reduction of homogentisic acid production could be achieved with nitisinone therapy. Nitisinone is a triketone herbicide and potent inhibitor of 4-hydroxy-phenylpyruvate dioxygenase which is responsible for catalyzing the formation of homogentisic acid from hydroxyphenylpyruvic acid. Nitisinone reduced urinary homogentisic acid excretion by approximately 70% in two patients with alkaptonuria. Long-term side effects of nitisinone therapy are under consideration.³

Understanding the genetic and molecular basis of alkaptonuria has the potential to offer a new therapeutic approach, enzyme replacement therapy with recombinant HGO. However, despite the theoretical advantage, such a strategy may be difficult to employ. Moreover, it is not known whether accumulation of toxic metabolites of tyrosine will occur, thus excluding this as an acceptable alternative therapy. Before human trials can be undertaken, therapies would need to be carefully tested in animal models.

In advanced cases, such as the one we present here, surgical replacement of joints and aortic valves result in significant improvement. Usually the disorder does not affect life span. Physiotherapy, analgesia, and adequate anti-osteoporotic therapy will be continued in our patient to prevent further disability.

In conclusion, diagnosis and management of patients with alkaptonuric ochronosis, a rare inherited disorder, is complex. Advances in orthopaedic and cardiac surgery have enabled many patients to overcome progressive disability. Physicians and surgeons should be aware of multiple system involvement in this disorder, as early recognition and appropriate treatment may significantly improve the quality of life in these patients.

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REFERENCES

1. Kottinen YT, Hoikka V, Landtman M, Saari H, Santavirta S, Metsarinne K, Seegmiller JE. Ochronosis: a report of a case and a review of literature. *Clin Exp Rheumatol* 1989;7:435-444.
2. Janocha S, Wolz W, Srsen S, Srsnova K, Montagutelli X, Guenet JL, Grimm T, Kress W, Mueller CR. The human gene for alkaptonuria (AKU) maps to chromosome 3q. *Genomics* 1994;19:5-8.
3. Phornphutkul C, Introne WJ, Perry MB, Bernardini I, Murphey MD, Fitzpatrick DL, Anderson PD, Huizing M, Anikster Y, Gerber LH, Gahl WA. Natural history of alkaptonuria. *N Engl J Med* 2002;347:2111-2121.
4. Zatkova A, Chmelikova A, Polakova H, Ferakova E, Kadasi L. Rapid detection methods for five HGO gene mutations causing alkaptonuria. *Clin Genet* 2003;63:145-149.
5. Savastano AA, Quigley DG, Scala ME. Spontaneous fractures associated with cortisone therapy in a patient with ochronosis. *Am J Orthop Surg* 1969;11:116-120.
6. Waschulewski H. [Medial femoral neck fracture in ochronotic alkaptonuria]. *Zentralbl Chir* 1969;94:806-810.
7. Millea TP, Segal LS, Liss RG, Stauffer ES. Spine fracture in ochronosis. Report of a case. *Clin Orthop* 1992;208-211.
8. Marsile C, Menozzi C, Menozzi C. [A rare case of high dorsal radicular-medullary compression in a patient with ochronotic arthropathy. Clinico-radiological features]. *Minerva Med* 1995;86:61-66.
9. Stenn FF, Milgram JW, Lee SL, Weigand RJ, Veis A. Biochemical identification of homogentisic acid pigment in an ochronotic egyptian mummy. *Science* 1977;197:566-568.
10. Lee SL, Stenn FF. Characterization of mummy bone ochronotic pigment. *JAMA* 1978;240:136-138.
11. Gaines JJ, Jr. The pathology of alkaptonuric ochronosis. *Hum Pathol* 1989;20:40-46.
12. Vijaikumar M, Thappa DM, Srikanth S, Sethuraman G, Nadarajan S. Alkaptonuric ochronosis presenting as palmoplantar pigmentation. *Clin Exp Dermatol* 2000;25:305-307.
13. Garrod E. The incidence of alkaptonuria: a study in chemical individuality. *Lancet* 1902;2:1616-1620.
14. La Du BN, Seegmiller JE, Laster L, Zannoni V. Alcaptonuria and ochronotic arthritis. *Bull Rheum Dis* 1958;8:163-164.
15. Pollak MR, Chou YH, Cerda JJ, Steinmann B, La Du BN, Seidman JG, Seidman CD. Homozygosity mapping of the gene for alkaptonuria to chromosome 3q2. *Nat Genet* 1993;5:201-204.
16. Fernandez-Canon JM, Granadino B, Beltran-Valero de Bernabe D, Renedo M, Fernandez-Ruiz E, Penalva MA, Rodriguez de Cordoba S. The molecular basis of alkaptonuria. *Nat Genet* 1996;14:19-24.
17. Fernandez-Canon JM, Penalva MA. Molecular characterization of a gene encoding a homogentisate dioxygenase from *Aspergillus nidulans* and identification of its human and plant homologues. *J Biol Chem* 1995;270:21199-21205.
18. Granadino B, Beltran-Valero de Bernabe D, Fernandez-Canon JM, Penalva MA, Rodriguez de Cordoba S. The human homogentisate 1,2-dioxygenase (HGO) gene. *Genomics* 1997;43:115-122.
19. Rodriguez JM, Timm DE, Titus GP, Beltran-Valero De Bernabe D, Criado O, Mueller HA, Rodriguez De Cordoba S, Penalva MA. Structural and functional analysis of mutations in alkaptonuria. *Hum Mol Genet* 2000;9:2341-2350.
20. Goicoechea De Jorge E, Lorda I, Gallardo ME, Perez B, Perez De Ferran C, Mendoza H, Rodriguez De Cordoba S. Alkaptonuria in the Dominican Republic: identification of the founder AKU mutation and further evidence of mutation hot spots in the HGO gene. *J Med Genet* 2002;39:E40.
21. Uyguner O, Goicoechea de Jorge E, Cefle A, Baykal T, Kayserili H, Cefle K, Demirkol M, Yuksel-Apak M, Rodriguez de Cordoba S, Wollnik B. Molecular analyses of the HGO gene mutations in Turkish alkaptonuria patients suggest that the R58fs mutation originated from central Asia and was spread throughout Europe and Anatolia by human migrations. *J Inher Metab Dis* 2003;26:17-23.
22. Beltran-Valero de Bernabe D, Granadino B, Chiarelli I, Porfirio B, Mayatepek E, Aquaron R, Moore MM, Festen JJ, Sanmarti R, Penalva MA, de Cordoba SR. Mutation and polymorphism analysis of the human homogentisate 1,2-dioxygenase gene in alkaptonuria patients. *Am J Hum Genet* 1998;62:776-784.
23. Hamdi N, Cooke TD, Hassan B. Ochrotonic arthropathy: case report and review of the literature. *Int Orthop* 1999;23:122-125.
24. Venkatesheshan VS, Chandra B, Graziano V, Steinlauf P, Marquet E, Irmieri V, Needle MA. Alkaptonuria and renal failure: a case report and review of the literature. *Mod Pathol* 1992;5:464-471.

25. Jagose JT, Bailey RR, Rothwell AG. Alkaptonuria with ochronotic nephropathy and multiple joint replacement for ochronotic arthropathy. *N Z Med J* 1997;110:235-236.
26. Introne WJ, Phornphutkul C, Bernardini I, McLaughlin K, Fitzpatrick D, Gahl WA. Exacerbation of the ochronosis of alkaptonuria due to renal insufficiency and improvement after renal transplantation. *Mol Genet Metab* 2002;77:136-142.
27. Borman P, Bodur H, Ciliz D. Ochrotonic arthropathy. *Rheumatol Int* 2002;21:205-209.
28. Kabasakal Y, Kiyici I, Ozmen D, Yagci A, Gumusdis G. Spinal abnormalities similar to ankylosing spondylitis in a 58-year-old woman with ochronosis. *Clin Rheumatol* 1995;14:355-357.
29. Kihara T, Yasuda M, Watanabe H, Suenaga Y, Shiokawa S, Wada T, Nonaka S, Suzuki T, Nobunaga M. Coexistence of ochronosis and rheumatoid arthritis. *Clin Rheumatol* 1994;13:135-138.
30. Simianer S, Krause D, Rau R. [Concomitant manifestation of ochronosis and chronic polyarthritis in a patient]. *Z Rheumatol* 1998;57:50-52.
31. Gemignani G, Olivieri I, Semeria R, Giustarini S, Pasero G. Coexistence of ochronosis and ankylosing spondylitis. *J Rheumatol* 1990;17:1707-1709.
32. Yagan R, Khan MA. The coexistence of ochronosis and ankylosing spondylitis. *J Rheumatol* 1991;18:1639-1640.
33. Weinberger KA. The coexistence of ochronosis and ankylosing spondylitis. *J Rheumatol* 1991;18:1948-1949.
34. Roth A, Schmidt K, Muller KM, Haaker R. [A case report: ochronosis in combination with chondrocalcinosis]. *Z Orthop Ihre Grenzgeb* 1999;137:76-78.
35. Carrier DA, Harris CM. Bilateral hip and bilateral knee arthroplasties in a patient with ochronotic arthropathy. *Orthop Rev* 1990;19:1005-1009.
36. Demir S. Alkaptonuric ochronosis: a case with multiple joint replacement arthroplasties. *Clin Rheumatol* 2003;22:437-439.
37. Di Franco M, Coari G, Bonucci E. A morphological study of bone and articular cartilage in ochronosis. *Virchows Arch* 2000;436:74-81.
38. Aliberti G, Pulignano I, Schiappoli A, Minisola S, Romagnoli E, Proietta M. Bone metabolism in ochronotic patients. *J Intern Med* 2003;254:296-300.
39. Murray JC, Lindberg KA, Pinnell SR. In vitro inhibition of chick embryo lysyl hydroxylase by homogentisic acid. A proposed connective tissue defect in alkaptonuria. *J Clin Invest* 1977;59:1071-1079.
40. O'Brien WM, La Du BN, Bunim JJ. Biochemical, pathologic and clinical aspects of alcaptonuria, ochronosis and ochrotonic arthropathy. Review of world literature (1584-1962). *Am J Med* 1963;34:813-838.
41. Cortina R, Moris C, Astudillo A, Gosalbez F, Cortina A. Familial ochronosis. *Eur Heart J* 1995;16:285-286.
42. Vlay SC, Hartman AR, Culliford AT. Alkaptonuria and aortic stenosis. *Ann Intern Med* 1986;104:446.
43. Gaines JJ Jr, Pai GM. Cardiovascular ochronosis. *Arch Pathol Lab Med* 1987;111:991-994.
44. Dereymaeker L, Van Parijs G, Bayart M, Daenen W, Lauwerijns J, De Geest H. Ochrotonosis and alkaptonuria: report of a new case with calcified aortic valve stenosis. *Acta Cardiol* 1990;45:87-92.
45. Kragel AH, Lapa JA, Roberts WC. Cardiovascular findings in alkaptonuric ochronosis. *Am Heart J* 1990;120(6 Pt 1):1460-1463.
46. Kenny D, Ptacin MJ, Bamrah VS, Almagro U. Cardiovascular ochronosis: a case report and review of the medical literature. *Cardiology* 1990;77:477-483.
47. Helou J, Masters RG, Keon WJ, Veinot JP. Ochrotonosis: an unusual finding at aortic valve replacement. *Can J Cardiol* 1999;15:1013-1015.
48. Zund G, Schmid AC, Vogt PR, Grunenfelder J, Turina MI. Green aortic valve: alcaptonuria (ochronosis) with severe aortic stenosis. *Ann Thorac Surg* 1999;67:1805.
49. Cercek M, Prokselj K, Kozelj M. Aortic valve stenosis in alkaptonuric ochronosis. *J Heart Valve Dis* 2002;11:386-388.
50. Soker Cakmak S, Cevik R, Aksunger A, Unlu K, Ava S. Ocular ochronosis: A case report and clinical findings. *Acta Ophthalmol Scand* 2002;80:340-342.
51. Nikkels AF, Pierard GE. Medical mystery: the answer. *N Engl J Med* 2001;344:1642-1643.
52. Robinson-Bostom L, Pomerantz D, Wilkel C, Mader R, Lerner L, Dufresne R, Flotte T. Localized argyria with pseudo-ochronosis. *J Am Acad Dermatol* 2002;46:222-227.
53. Rausing A, Rosen U. Black cartilage after therapy with levodopa and methyl dopa. *Arch Pathol Lab Med* 1994;118:531-535.
54. Levin CY, Maibach H. Exogenous ochronosis. An update on clinical features, causative agents and treatment options. *Am J Clin Dermatol* 2001;2:213-217.
55. Morava E, Kosztolanyi G, Engelke UF, Wevers RA. Reversal of clinical symptoms and radiographic abnormalities with protein restriction and ascorbic acid in alkaptonuria. *Ann Clin Biochem* 2003;40:108-111.
56. Forslind K, Wollheim FA, Akesson B, Rydholm U. Alkaptonuria and ochronosis in three siblings. Ascorbic acid treatment monitored by urinary HGA excretion. *Clin Exp Rheumatol* 1988;6:289-292.