Ochronosis

Fahad Abdulla Ibrahim
Mohammed Mohy El-din Selim *
Hamda Al Ansari *

Common

Ochronosis is an unknown condition characterized by melanin like pigment deposits in many connective tissues and the skin. The pigment in tissue appears yellow brown or OCHRE colored when stained with hematoxylin-eosin. The disease is called ochronosis from the Greek words for yellow disease. There are two types of ochronosis:

I) Endogenous Alkaptonuric Ochronosis

II) Exogenous Ochronosis

I) Endogenous Alkaptonuric Ochronosis:- ochronosis is the clinical hallmark of the metabolic disorder Al Kaptonuria.

Alkaptonuria is a rare inherited autosomal recessive inborn error of metabolism characterized by absent or deficient activity of renal and hepatic enzyme homogentisic acid oxidase [1], thus resulting in accumulation of Homogentisic acid in plasma and is deposited as oxidized melanin like pigment in various connective tissues as in cartilage and dermis. Large amounts of homogentisic acid is excreted in urine as 2,5 hydroxyquinone acetic acid (ALKAPTON).

Homogentisic acid is produced during normal metabolism of phenylalanine and tyrosine [2,3].

Phenylalanine and Tyrosine is catabolized to Homogentisic acid (HGA). In normal persons HGA is acted upon by HGA oxidaze and is transformed into MALOYL ACETO ACETATE [4,5] (Fumaric and aceto-acetic acid)

In alkaptonuric patient phenyl alanine and tyrosine is catabolized to homogentisic acid and because homogentisic acid oxidase enzyme is deficient there is accumulation of homogentisic acid which is acted upon by the enzyme polyphenol oxidase whose highest activity is in cartilage and skin leading to formation of benzoquinone acetic acid which is the ochre pigment that binds irreversibly to collagen [5].

Homogentisic acid excreted in urine is gradually oxidized after exposure to air for sometimes and the urine becomes black and this change in color is enhanced if the urine is alkalinized by adding 10% Potassium Hydroxide [2, 6, 7]. Alkaptonuria was the first disease to be interpreted as an inborn error of metabolism in 1902 (AE-Garrod, Lancet 1902, 2:616-20).

The Human gene for alkaptonuria is cloned and mapped to the long arm of chromosome 4q23 [8, 9]. The incidence of alkaptonuria varies from one in a million[10] to one in 250-000[11] or one in 25000 [12,13] or one in 19000 in Slovak and Dominican Republic [8].

A wide range of clinical manifestations is described in ochronosis and is associated with multi-system disorder related to blue-black discoloration of cartilage and skin due to deposition of oxidized homogentisic acid in connective tissue [14, 15].

Homogentisic acid is oxidized by the enzyme polyphenol oxidase, which is present in connective tissue with the highest activity in cartilage and skin into reactive benzoquinone acetic acid (Ocre pigment or alkapton). Alkapton is inturn bound irreversibly to collagen fibers in the form of a polymer [15, 16, 17] especially in tissues rich in mucopolysaccharide of ground substance [18].

Electron microscopy shows normal collagen as well as masses of degraded collagen. The ochronotic pigment surrounds individual collagen fibrils [19] and finally the collagen fiber becomes entirely replaced by ochronotic pigment. This process is more prominent on sun-exposed areas [16, 20] where actinic damage could initiate degenerative process and provide more foci for pigment deposition [16, 21]. Both damage by pigment deposition and by sun exposure are likely to be involved in pathogenesis of the disorder.

The alkapton ochronotic pigment has some similarity to eumelanin as shown by electron spin response signals and absorption spectra data [22].

The presence of trace metal as zinc in damaged ochronotic tissue lead to formation of free radical components of melanin but it is different from normal melanin in being resistant to bleaching by hydrogen peroxide[18, 19, 23].

Alkaptonuria does not become manifest before the third to fourth decade of life[16], because of effective renal excretion of homogentisic acid. With advance of age renal excretion decreases resulting in more elevated level of homogentisic acid and hence clinical evident disease [7].

* *Consultant Dermatologist*
Hamad Medical Corporation
Dermatology & Venereology Department
P.O. Box: 3050
Doha – Qatar.

Clinical manifestations of Alkaptonurea include:

-

- Homogentisic aciduria: homogentisic acid is markedly elevated in urine (6.7 gram per liter while the normal level is 0.1 gram per liter[7].

- The most disabling manifestation of the disease is ochronotic Arthritis [11, 24], which affects mainly large joints and intervertebral disks. The hands, feet are usually spared [23] or rarely affected [25]. Ochronotic pigment destroys the cartilage of joints and intervertebral disks. Knee involvement usually occurs at an early age and could be quite disabling [3]. Extensive ochrocalcinosis of cartilage contribute further to its degeneration and cartilage fragments contribute to sinovitis [25]. Also hip joint may be affected and the affection could be severe leading to terminal degeneration of the joint that necessitate total hip replacement [26, 27, 28, 29, 30]. Ochronosis should be considered as a cause of low backache in young individuals and the changes could be detected by x-ray and MRI [31, 32, 33].

The cause of Alkoptonuric arthritis is thought to involve increased oxidative stress. Oxygen radicals are suspected to cause inflammation and cellular damage in arthropathy [34]. It is clear that accumulation of homogentisic acid in the connective tissues leads directly or indirectly to arthritic changes. It is possible that other more common types of arthritis develop secondary to the metabolic disturbances leading to less obvious and less easily detected chemical mediators that are involved in the arthropathy [18].

There is also a possible role of immunological response in the evolution of ochronotic arthropathy. Study of cell mediated and humoral response on synovial fluid and peripheral blood in a patient with ochronotic arthropathy showed raised percentage of CD3 positive and CD8 positive and HLA-DR positive and CD2 positive T-cells and the presence of TNF and enhanced levels of immunoglobulins and low levels of C3 in synovial fluid and a higher rate of HLA DR positive and CD 25 positive T lymphocytes in peripheral blood [35]. Affection of spine intervertebral disks causes low back ache [36], thoracic and lumbosacral pain [37] and development of bone spurs further contribute to pain, spine fracture [38], calcifications of the intervertebral disks [6, 39, 40, 41], spondylosis [42, 43] high radicular compression [44] root canal stenosis [45] and affection of dura mater [46].

Skeletal scintigraphy and ochronotic arthrosis imaging using Technicium – 99m dicarboxypropane diphosphonate (99-m Tc-DPD) shows marked accumulation of radio activity in large joints with higher uptake during episode. The intervertebral disk shows higher uptake extending laterally from the axial vertebral column giving the impression of a 'Whisker' and so 'Whisker sign' is characteristic of intervertebral disk ochronosis [47].

Tendon affection in homocystinuria represents 1% of chronic tendon complaints, which is reported to occur in other inherited diseases as Ehler's Danlos and Marfan syndrome [48].

Other clinical manifestations of ochronosis include cardiovascular involvement, which occurs approximately in 50% of cases [49]. The most frequent presenting features of cardiovascular involvement is stenosis of aortic valve [10, 49, 50, 51, 52, 53, 54, 55].

Calcified aortic valve secondary to ochronosis may necessitate aortic valve replacement [56]. A possible link between ochronosis and coronary artery disease has been postulated. The intima of the coronary arteries and the aorta were stained black by ochronotic pigment during coronary bypass surgery.

A link between peripheral vascular disease and ochronosis may be present [58]. Pigment may be found within macrophages in atherosclerotic plagues [58] but no clear evidence for the occurrence of premature arteriosclerosis in these patients[59].

Valvular heart disease with recurrent bacterial infection and marked calcification detected by two dimensional color – Doppler echocardiography was reported and immunological analysis of the patient showed reduced number of T cells with compensatory expansion of CD 56 positive, CD 57 positive Natural killer cell (NK) population and impaired functions of cellular immunity such as phytohemagglutinin response, antibody dependant cellular cytotoxicity NK activity and interleukin-2 production and this report documents immunological abnormality in Alkaptonuria [60].

- Many other organs and systems of the body are affected in ochronosis. Prostate is reported to be affected by ochronotic stones [61], renal stones and obstruction of the urinary tract [62], ochronotic nephropathy [63] and renal failure rarely occur in late stages of the disease [64] and is associated with diffuse cutaneous pigmentation[65] and renal biopsy shows pigment deposition in interstitial tissue and many tubular cells [64].

- Ochromotic hyperpigmentation affects eyelids, forehead, cheeks, axillae, genital region, nail bed, buccal mucosa, larynx.

Dark brown sweat that stains clothes [11, 66] and dark

cerumen and pigmented tympanic membrane and oscicles and may result in hearing loss or tinitus [67, 25].

Rarely palmo-plantar pigmentation and thickening and pitting occurs with ochronosis [6, 68]. Bluish pigmentation of the auricles where the skin is thin [7] with discoloration of the cartilage [69, 70]. Bluish hyperpigmentation of the sclera on medial and lateral aspects (Oster's sign) [7, 12], also amber colored oil globulation within Bowman's membrane of the cornea [71]. Scleral pigmentation usually preceeds the arthropathy and is evident by early fourties and vision is not affected (53).

Hereditary ochronotic skin pigmentation may be distributed in sun-exposed areas and appears as blue-black pigmentation especially of nose, molar eminence, ears, V-line of neck and dorsum of hands and forearms [12]. The clinical features of alkaptonuria is related to age [8]. At birth there is dark urine and dark cerumen. At puberty axillary pigmentation occur. Between age of 20 to 40 ear lobe skin pigmentation and scleral pigmentation appears. Ochronotic arthropathy is seen between age of 30 to 40. Rare organ manifestations occur after the age of 40-years such as affection of upper respiratory tract whose affection is usually asymptomatic [18] however hoarseness, dryness and dysphagia were reported [18]. Ochronotic pigment was found in breasts, thyroid, lymph nodes and bone marrow [65]. Unusual sites involved include teeth [72], CNS [73], and endocrine glands [74].

Alkaptonuric ochronosis in a patient was reported to show intracranial aneurism presenting with subarachnoid hemorrhage and a potentially casual relationship is suggested between cerebral aneurism and alkaptonuric ochronosis [75].

Alkaptonurea is diagnosed by:

1- The clinical finding and family history
2- Urine becoming dark on standing or with alkalinization. This is characteristic of alkaptonuria [2, 6]
3- Benedict's reagent containing copper is used in testing for sugar routinely. Homogentisic acid reduces copper and gives a yellow orange precipitate and a brown black supernatant [6, 76]. The yellow orange precipitate may be misreported as positive for glucosuria, so a negative oxidase test for glucose supports the diagnosis of alkaptonurea [77]. Homogentisic acid is directly identified in urine by gas chromatography mass spectrometry [60] and Ferric Chloride turns alkaptonuria urine blue[68].
4- Screening test for alkaptonuria in infants using high performance liquid chromatography to detect homogentisic acid in urine and plasma [10, 78].
5- Alkaptonuria can be detected by magnetic resonance spectroscopy to quantify urinary homogentisic acid level [79].
6- Ochronotic arthritis can be diagnosed by radiographs [80]

7- Needle biopsy of joint cartilage and the histology of synovial membrane help establish the diagnosis of achronosis [81] articular cartilage shows erosions of the surface, pigment accumulation in chondrocytes and intercellular matrix. Bones are diffusely osteoparotic from limb disuse[82].

8- Characteristic histologic finding can confirm the diagnosis of ochronosis. The reticular dermis shows irregular break up, swelling and homogenization of collagen bundles. Ochrocolored yellow brown pigment lye within collagen bundles and also freely in the deeper dermis[6] and benzoquinone acetic acid is irreversibly bound to collagen as a polymer and by electron microscopy the ochronotic pigment surrounds individual fibrils [83]. The entire collagen fiber degenerates and fibrils loose its periodicity thus giving additional sites for pigment deposition which finally replaces the entire collagen fiber. Pigment is also deposited in the macrophages and free in the dermis [84]. It is also deposited in endothelial cells, basement membrane and secretory cells of glands and within elastic fibers.
9- The diagnosis of ochronosis may be confirmed by measuring homogentisic acid plasma levels that sometimes may be low (in the range of 3mg/dL) because of high renal clearance (upto 4-8 gram / L)[67].
10- In infancy there is discoloration of diapers. However a pH less than 7 or in presence of reducing substances like ascorbic acid the urine will not change color [12]. For this reason diaper staining may be more prominent after the diaper is cleaned with soap which alkalinizes the urine. Normally urine contains no homogentisic acid so any amount in urine is diagnostic [77].
11- When alkalinized urine is placed on photographic paper the paper turns dark and this is known as positive Fishberg test [85].
12- The differential diagnosis includes
- exogenous ochronosis
- other skin condition that cause skin pigmentation as Addison's disease, hemochromatosis, pellagra[12]

- Occular ochronosis from melanoma
- Diseases that result in dark urine as melanuria, porphyria cutanea tarda and hematuria [12]
- Tetracylines, phenothiazines and heavy metal pigmentation [86]
- Arthropathy from ankylosing spondylitis [72], hermated disk [87] and idiopathic osteoarthritis [88].
- Preexisting existing ochronotic arthropathy might mask rheumatoid arthritis manifestations thus making the diagnosis of rheumatoid arthritis difficult [89].

Treatment:

The disease does not affect the life span. The following treatment lines are recommended:

1- Restrict protein intake to 1gram per kg per day with low phenylalanine and tyrosine in diet alters the course of the disease [25].

2- Long term ascorlic acid can reduce excretion of homogentisic acid and can reduce late sequences by diminishing serum concentration of the metabolite benzoquinone acetic acid [26, 44, 90]. It inhibits oxidation and polymerization [76] and also binding of homogentisic acid to collagen [91]. A dose of 100mg per kg per day is advised [90, 91].

3- The drug 2 [2-nitro-4-Trifluor-methyl benzoyl-1, 3-cyclohexanedione (NTBC)] is a potent inhibitor of p-hydroxyphenyl - pyruvate dioxygenase which catalizes the formation of homogentisic acid from p-hydroxyphenyl pyruvic acid when NTBC was given to murine model of alkaptonuria – reduced the urinary homogentisic acid. This experimental finding suggest that NTBC may be the first potent pharmacotherapeutic agent for alkaptonuria [34].

4- Possible substitution therapy by recombinantly obtained homogentisic acid oxidase [7].

5- Systemic steroid in arthritic patients but osteoporosis may be aggravated[76, 91, 92].

6- Surgical operation for disk herniation [12] and valve replacement.

7- Dermabrasion [9]

8- Q-switched laser [11]

II Exogenous achronosis [35, 8, 17, 64, 71, 72] is clinically similar to endogenous ochronosis but differs from it in being not inherited and has no systemic manifestation [9]. It is characterized by ochronotic hyperpigmentation of the face, sides and back of the neck, back and extensor surface of extremities [37, 93]. One case of exogenous ochronosis was reported showing multiple maliary osteoma cutes of the face which was originally described as a sequence of long standing acne[8]. Exogenous ochronosis typically affect dark skinned patients who use hydroquinone preparations in high concentration or even in low concentration leading paradoxically to darkening of the skin.

It is stressed that extended use of the bleaching agent and not its concentration may cause it [11]. The skin first becomes red then displays mild macular pigmentation followed by dark pigmentation usually associated with dark papules or black colloid milia and scanty atrophy and may simulate melasma [94] and also displays papulo nodules with or without erythema [95]. The hyperpigmentation generally occurs after 6-months of the use of the bleaching cream [96] and is usually limited to areas that were exposed to the cream and the change is irreversible and is difficult to treat [97, 98].

Topical agents containing hydroquinone [90, 100], phenol, mercury or picric acid quinine injection and, antimalarial drugs [8, 100, 101], resorsinol [102, 103] may be responsible. Systemic absorption of topical phenal may result in pigmentation of skin and cartilage because of its oxidation to hydroquinone [103]. The mechanism of pigmentation is not clear. It may be caused by local inhibition of homogentisic acid oxidase by hydroquinone resulting in polymerized pigment. Another hypothysis is that hydroquinone by-products increase tyrosinase activity and the pigment may be melanin rather than melanin like prcursor [104]. Systemic absorption of hydroquinone lead to pigmentation of cartilage, sclera and exposed skin [105]. It is reported that a patient with Vitiligo by using hydroquinone to lighten remaining dark areas on his face developed dramatic darkening of the normal skin treated and biopsy showed trace of ochronotic pigment and no melanocytes in vitiligenous area and this may imply that functional melanocytes are important in pathogenisis of exogenous ochronosis[106]. In both endogenous and exogenous ochronosis there are phenolic intermediates that can be converted into melanin like precursors. Histologically exogenous ochronosis shows histologic findings similar to those seen in Alkaptonuria early in its course [35]. Later ochronoid eosinophilic colloid milia may be seen. Granulomatous reaction with sarcoid like granuloma surrounding ochronoid material [35, 56]. The pathology also shows pigment in macrophages or free in the dermis [107], pigment in endothelial cells and basement membrane and

secretory cells of sweat glands [84] as well as pigment of elastic fibers [84] antimalarial ochronosis shows classical ochronosis plus melanin and hemosideren deposition [101, 105]. There is homogenization and swelling of collagen bundles which stains black with Fontana and blue black with methylene blue [108,109]. Sarcoid like granulomas with multinucleated giant cells engulfing ochronotic particles have been noted [110]. Transfollicular elimination of ochronotic fibers has been described [111].

Ultra structural examination reveal homogeneous electron dense irregular structures embedded in an amorphous granular material infiltrating adjacent collagen fibril bundles.

Pigmented particles may be elastic or collagen fibers [112, 113] the pigment may be also melanin [105].

Lightening agents containing hydroquinone may cause serious pigmentation of the eye and in a small number of cases permanent corneal damage. Hydroquinone is a high commodity chemical used in reducing agents, antioxidant, polymerization inhibitor and chemical intermediate. It is a natural ingredient in many plant products including vegetables, fruits, grains, coffee, tea, beer and wine [32].

Topical steroid in combination with hydroquinone were suspected to cause hypertension or D.M. from prolonged use of the steroid. Exogenous ochronosis has to be differentiated from minocycline pigmentation which may affect thyroid, cardiac valve and coronary vessels[114] localized argyria may present with slate gray or blue macules resembling blue nevi and histopathologically shows homogenized collagen bundles resembling ochronosis [6].

Therapy with levodopa and methyl dopa produce pigment metabolite bound to matrix of rib cartilage and differ from endogenous and exogenous ochronosis and the condition is harmless but irreversible [61].

Treatment:

The condition is usually irreversible but it may fade over time [11] cryotherapy and trichloroacetic acid are ineffective [11]. Tretinoin 0.05% topically was effective [115] with sunscreen. Dermabrasion [11, 116] and CO2 laser [117] also Q-switched Ruby laser [11] were successfully tried.

References:

1- Odabas AR; Karakuzu A; Sekuk Y; et al: Alkaptonuria: a case report .

J. Dermatol 2001; 28(3): 158-60

2- Adonis-Koffy L; Conzales E; Nathason S; et al: L'Alkaptonuria: une cause rare de coloration anormale des urines. A propos d'un cas chez le nourrisson. Alkaptonuria: a rare cause of urine discoloration. Report of a case in a newborn.

Arch-Pediatr. 2000; 7(8): 844-6.

3- Aydoghu S; Cullu E; Ozzoy MH; et al: Cementless total knee arthroplasty in ochronotic arthropathy: a case report with a 4-year follow-up.

J. Arthroplasty. 2000; 15(4): 539-43

4- La Du BN; Zannoni VG; Laster L; et al: the nature of the defect in tyrosine metabolism in alkaptonuria.

J. Biol Chem. 1958; 230:251-62.

5- Ramsberger R; Lubinus P; Lubinus HH: Alkaptonurie und ochrontische arthropathie – arthroskopischer und intraoperativer Befund bie Implantation eirier oberflachener neuernden knieendoprothese.

Arthroscopic and intraoperative finding in implantation of a knee joint surface replacing prosthesis.

Chirurg. 1994; 65(11): 1061-5.

6- Vijaikumar M; Thappa DM; Strikanth S; et al: Alkoptonuric ochronosis presenting as palmoplantar pigmentation.

Clin. Exp. Dermatol 2000; 25(4): 305-7.

7- Gutzmer R; Herbst RA; Kiehl P; et al: Alkaptonuric ochronosis: report of two affected brothers.

J. Am. Acad Dermatol. 1997; 37(2pt2): 305-7.

8- Janocha-S; Wolz W; Srsen S; et al: The human gene for alkaptonuria (AKU) maps to chromasome 3q.

Genomics 1994; 19(1): 5-8.

9- Pollak MR; Chou Y HW; Cerda JJ; et al: Homozygosity mapping of the gene for alkaptonuria to 3q2.

Nat-Genet. 1993; 5: 201-4.

10- Kocyigit H; Gurgan A; Terzioglu R; et al: Clinical, radiographic and echocardiographic findings in a patient with ochronosis.

Clin. Rheumatol. 1998; 17(5): 403-6.

11- Kramer KE; Lopez A; Stefanato CM; et al: Exogenous ochronosis.

J. Am.Acad. Dermatol. 2000; 42(5pt2): 869-71.

12- Lubics A; Schneider I; Sebok B; et al: Extensive bluish gray skin pigmentation and severe arthropathy. Endogenous ochronosis (Alkaptonuria).

Arch. Dermatol. 200o; 136(4): 548-9, 551-2.

13- Srsen S: Alkaptonuria John Hopkins Med. J. 1979; 145: 217-26.

14- Hamdi N; Cooke TD; Hassan B: Ochronatic arthropathy – case report and review of literature.

Int. Orthop 1999; 23(2): 122-5.

15- Tourat DM; Sau P: Cutaneous deposition diseases Part II

J. Am. Acad.Dermatol. 1998; 39: 527-44.

16- Quarterman MJ; Hall J M Jr; Gourdin FW; et al: Photodistributed hereditary achronosis.
Arch. Dermatol. 1992; 128(12): 1657-8.

17- Zannoni VG; Malawista SE; La Du BN: Studies on ochronosis II: Studies on benzoguinone acetic acid, a probable intermediate in the connective tissue pigmentation of alkaptonuria.
Arthritis Rheum. 1962; 5; 547-56.

18- La Du BN Jr: Alkoptonuria and ochronotic arthritis.
Mol. Biol. Med. 1991; 8:31-8.

19- Attwood HD; Clifton S; Mitchel RE: A histotogical, histochemical and ultrastructural study of dermal ochronosis.
Pathology. 1971; 3: 115-21.

20- Entwisle BR; Muirden KD; Adam WR; et al: A case of alkaptonuria, ochronosis and ochronotic arthropathy.
Med.J. Aust. 1969; 2:96-97.

21- Kutty MK; Igbal QM; Teh EC: Ochronotic arthropathy.
Arch. Pathol. 1973; 96:100-103.

22- Menon IA; Persad SD; Haberman HF; et al: Characterization of the pigment from homogentisic acid and urine and tissue from an alkaptonuria patient.
Biochem. Cell. Biol 1991; 69: 269-73.

23- Martin JP; Batkoff B: Homogentisic acid autooxidation and oxygen radical regeneration: implication for the aetiology of alkaptonuric arthritis.
Free Radic Biol Med. 1987; 3:241-50.

24- Simianer S; Krause D; Rau R: Gemeinsames Auftreten Von Ochronose und chronischer Polyarthritis bei einer patienten (Concomitant manifestation of ochronosis and chronic polyarthritis in a patient)
Z-Rhematol. 1998; 57:50-2.

25- Janes JJ Jr: The pathology of alkaptonuric ochronosis Hum. Pathol. 1989; 20:40-46

26- Aynaci O; Onder C; Turhan AU: Bilateral hip arthroplasty for ochronotic arthropathy.
Clin. Rheumatol 2000; 192-150:2

27- Dom-K; Pittevils T: Ochronotic arthropathy: the black hip. Case report and review of the literature.
Acta-Orthop-Belg. 1997; 63(2): 122-5.

28- Lequesn M: Les coxopathies rapidement destructrices (rapidly progressing destructive disease of the hip).
Ann. Radiol (Paris). 1993; 36(1): 62-4.

29- Carrier DA; Harris CM: Bilateral hip and bilateral knee arthroplasties in a patient with ochronotic arthropathy.
Orthop-Rev. 1990; 19(11): 1005-9.

30- Warren NP; Coombs RR: Bilateral hip arthropathy in alkaptonuria.
J. R. Coll. Surg. Edinb. 1990; 35(2): 116-117

31- Sahin-G; Milcan-A; Bagis S; et al: a case of ochronosis: upper extremity involvement. Rheumatol Int. 2001; 21(2): 78-80.

32- Choudhury R; Rajamani SS; Rajshekhar V: a case of ochronosis: MRI of lumbar spine Neuroradiology 2000; 42(12): 905-7.

33- Rayan SJ; Smith CD; Slevin JT: Magnetic resonance imaging in ochronosis, a rare cause of back pain.
J. Neuroimaging 1994; 4(1): 41-2.

34- Hegedus ZL; Nayak U: Homogentisic acid and structurally related compounds as intermediates in plasma soluble melanin formation and in tissue toxicities.
Arch. Int. Physiol. Biochim – Biophys. 1994; 102(3): 175-81.

35- Aliberti G; Pulignano I; Proietta M; et al: Contribution of immunological mechanism in a case of ochronotic arthropathy. Panminerva-Med. 1997; 39(3): 237-9.

36- Griep EN; terBor EJ: Een Zeldzame Corzaak Van Chronische rugklachten: Ochronose (an unusual cause of chronic back symptoms: ochronosis).
Ned-Tijdschr – Geneeskd. 1992; 136:184-7.

37- Hortobagyi J; Sebok B; Zombai E; et al: Alkaptonuria Ochronosis.
Orv-Hetil. 1992; 133: 167-8, 173.

38- Millea TP; Segal LS; Liss RG; et al: Spine fracture in ochronosis. Report of a case.
Clin. Orthop. 1992; 281: 208-11.

39- Emel E; Karagoz F; Aydin IH; et al: Alkaptonuria with lumbar disk herniation, a report of two cases.
Spine 2000; 25(16): 2141-4.

40- Aquaron R; Fayet G; Barthet C; et al: Maladie de Parkinson et alkaptonurie: association fortuite ou ochronose striato-nigrale? (Parkinson disease and alkaptonuria: fortuitous association or striatonigral ochronosis?)
Rev-Neural-(Paris). 1995; 151(1): 63-6.

41- Bontoux D; Azais I; Lambert-de-Cursay G: Calcinosis discales (Disk calcinosis).
Rev-Prat, 1994; 44(2): 193-6.

42- Kabasakal Y; Kiyici I; Ozmen D; et al: Spinal abnormalities similar to ankylosing spondylitis in a 58-year-old woman with ochronosis.
Clin. Rheumatol. 1995; 14(3): 355-7.

43- Gemignani G; Olivieri I; Semeria R; et al: Coexistance of ochronosis and ankylosing spondylitis .
J. Reumatol. 1990; 17(12): 1707-9.

44- Marsile C; Menozzi C: un rara caso di compressione radiculo-midollare dorsale alta in soggetto con atropatia ocronotica. Osservazioni clinico-radiologiche (a rare case of high dorsal radicular medullary compression in a patient with ochronotic arthropathy –clinico-radiological features).
Minerva-Med. 1995; 86(1-2): 61-6.

45- Koh KB; Low EH; Ch'ng SL; et al: a case of alkaptonuria with root canal stenosis.
Singapore-Med-J. 1994; 3591): 106-7

46- Liu-W; Prayson RA: Dura mater involvement in ochronosis (alkaptonuria).
Arch. Pathol. Lab. Med. 2001; 125(7): 961-3.

47- Paul R; Ylinen SL: The whisker sign as an indicator of ochronosis in skeletal scintigraphy.
Eur-J-Nucl-Med. 1991; 18(3): 222-4.

48- Jarvinen M; Jozz L; Kannus P; et al: Histopathological findings in chronic tendon disorders.
Scand J. Med. Sci. sports. 1997; 7(2): 86-95.

49- Levine HD; Parisi AF; Holdsworth DE; et al: Aortic valve replacement for ochronosis of aortic valve.
Chest 1978; 74: 466-7.

50- Helou J; Masters RG; Keon WJ; et al: Ochronosis: an unusual finding at aortic valve replacement.
Can J. Cardiol 1999. 15(9): 1013-5.

51- Casselman F; Herijgers P; Meyns B; et al: Aortic sterosis in endogenous ochronosis.
J. Heart Valve Dis. 1999; 8(4): 445-6.

52- Zund G; Schmid AC; Vogt PR; et al: Green Aortic valve: alkaptonuria (Ochronosis) with severe aortic stenosis.
Ann- Thorac- Surg. 1999; 67(6): 1805.

53- Van-Offel JF; De-Clerck LS; Francx LM; et al: The clinical manifestations of ochronosis – a review.
Acta-Clin-Belg. 1995; 50(6): 358-62.

54- Cortina R; Moris C; Astudillo A; et al: Familial ochronosis.
Eur. Heart J. 1995; 16(2): 285-6

55- Kim YI; Daenen W: aortic valve replacement in cardiac ochronosis.
Eur. J. Cardiothorac.
Surg. 1992; 6(11): 625-6.

56- Dereymaeker L; Vanparijs G; Bayart M; et al: Ochronosis and alkaptonuria: report of a new case with calcified aortic valve stenosis.
Acta Cardiol. 1990; 45(1): 87-92.

57- Paul DE; Picone AC; Baisden CE: The black aorta: Alkaptonuria diagnosed during coronary artery bypass.
South. Med. J. 1991; 84: 1416-17.

58- Kenny D; Ptacin MJ; Bamrah VS; et al: Cardiovascular ochronosis: a case report and review of the medical literature Cardiology. 1990; 77(6): 477-83.

59- Gaines JJ; Pai GM: Cardiovascular ochronosis.
Arch. Pathol. Lab. Med. 1987; 11; 991-4.

60- Mori S; Kawaguchi T; Kakinuma H; et al: Alkaptonuria a case complicated with valvular heart disease and immunodeficiency.
Intern. Med. 1994; 33(8): 512-6.

61- Concepcion – Masip T; Banares- Baudet F; Traba ML; et al: Alkaptonuria, litiases prostatica Y ureter ectopico (alkaptonuria, prostatic calculi and ectopic uterer).
Actas-Urol-Esp. 1997; 21 (2); 167-70.

62- Sener RN: Prostatic and renal stones and unilateral obstruction of the urinary tract caused by ochronosis.
AJR. Am. J. Roentgenol. 1992; 158 (1): 214-15.

63- Jagose JT; Bailey RR; Rothwell AG: Alkaptonuria with ochronotic nephropathy and multiple joint replacement for ochronotic arthropathy.
N. Z. Med. J. 1997; 110(1046): 235-6.

64- Venkatasseshan VS; Chandra B; Graziano V; et al: Alkaptonuria and renal failure: a case report and review of the literature.
Mod-Pathol. 1992; 5(4): 464-71.

65- Wyre HW: Alkaptonuria with extensive ochronosis.
Arch. Dermatol 1979; 115: 461-3.

66- Gaines JJ; Tom GD; Khankakhanian N: an ultra structural and light microscopic study of the synovium in ochronotic arthropathy. Hum. Pathol. 1987; 18: 1160-4.

67- Micali G; Di-Stefano AG; Nasca MR; et al: A 46-year old man with a 4-year history of diffuse brownish black pigmentation.
Endogenous ochronosis (alkaptonuria).
Arch. Dermatol. 1998; 134(1): 98, 100-1.

68- Cherian S : Palmoplantar pigmentation: a clue to alkaptonuric ochronosis.
J. Am. Acad. Dermatol. 1994; 30(2 pt1): 264-5.

69- Turiansky GW; Levin SW: Bluish patches on the ears and axillae with dark urine: ochronosis and alkaptonuria.
Int. J. Dermatol. 2001; 40(5): 333-5.

70- ter-Borg EJ: Diagnose in beeld (45). Ochronose diagnostic image (45).
Ochronosis. Ned. Tijdschr-Geneeskd. 2001; 145(27): 1295.

71- Carlson DM; Helgeson MK; Hiett JA: Ocular ochronosis from alkaptonuria.
J. Am. Optom. Assoc. 1991; 62(11): 854-6.

72- Siekert RG; Gibilisco JA: Discoloration of teeth in alkaptonuria ochronosis and parkinsonism.
Oral Surg. 1970; 29: 197-9.

73- Cooper JA; Moran TJ: Studies on ochronosis: report of a case with death from ochronotic nephrosis.
Arch. Pathol Lab. Med. 1957; 64: 46-53.

74- Galdston M; Steele JM; Dobriner K: Alkaptonuria and ochronosis with a report of three patients and metabolic studies in two.
Am. J. Med. 1952; 13: 432-52.

75- Kaufmann AM; Reddy KK; West M; et al: Alkaptonuric ochronosis and multiple intracranial aneurysms.
Surg. Neurol. 1990; 33(3): 213-6.

76- Khadagawat R; Techchandani R; Garg P; et al: Alkaptonuria: early detection.
Ind. J.Pediatr. 1994; 31: 593-5.

77- O'Brien WM; La Du BN; Bnim JJ: Biochemical, pathologic and clinical aspects of alkaptonuria, ochronosis and ochronotic arthropathy.
Am. J. Med. 1963; 34: 813-38.

78- Sakura N; Kato Y; Hamakawa M; et al: Alkaptonuria detected by neuroblastoma screening system.
Eur. J. Pediatr. 1992; 151: 388-90.

79- Yamaguchi S; Koda N; Ohashi T: Diagnosis of alkaptonuria by NMR urinlysis of homogentisic acid.
Tahoku J. Exp. Med. 1986; 150: 227-8

80- Mestan MA; Bustin GL; Wagner LA: Chiropractic care and ochronotic arthropathy.
J. Manipulative – Physiol- Ther. 1999; 22(7): 473-7.

81- Lindoskova M; Hrba J: Vyky dal M; et al: Needle biopsy of joints – its contribution to the diagnosis of ochronotic arthropathy (alkaptonuria).
Clin-Rheumatol 1992; 11(4): 569-70.

82- Di-Franco M; Coari G; Bonucci E: A morphological study of bone and articular cartilage in ochronosis.
Virchows – Arch. 2000; 436(1): 74-81.

83- Kusz D : Emiany Ochronotyczne W: Alkaptonurii – opis przypadka ochronotic changes in alkaptonuria: a case.
Chir. Narzadow-Ruchu – Ortop-Pol. 1999; 64(6): 677-82.

84- Lever WF; Schaumberg – Lever G: Metabolic disorders in : Histopathology of the skin: metabolic disease, 8th edition hildelphia.

JB Lippincott. 1997: 384-86.

85- Garett E: Occular ochronosis with alkaptonuria.

Am J. Ophthalmol. 1963; 55: 617-20.

86- Granstein RD; Sober AJ: drug and heavy metal induced hyperpigmentation.

J. Am. Acad Dermatol. 1981; 5: 1-18.

87- Eisen-berg H: Alkaptonuria, ochronosis, arthritis and ruptured intervertebral disk complicated by homologous serum reaction.

Arch. Intern. Med. 1950; 86: 79-86.

88- Tanner KE; Warren NP; Coombs RR: Ochronosis of the hip joint.

Scand J. Rheumatol. 1991; 20: 63-4.

89- Kihara T; Yasuda M; Watanabe H; et al: Coexistance of ochronosis and rheumatoid arthritis.

Clin. Rheumatol. 1994; 13(1): 135-8.

90- Kamoun P; Coude M; Forest M; et al: Ascorbic acid and alkaptonuria.

Eur. J. Pediatr. 1992; 151 (2): 149.

91- Wolff JA; Barshop B; Nyhan WL; et al: Effects of ascorbic acid in alkaptonuria: alteration in benzoquinone acetic acid and an ontogenic effect in infancy.

Pediatr. Red. 1989; 26: 140-4.

92- Konttinen YT; Hoikka V; Landtman M; et al: Ochronosis a report of a case and a review of literature.

Clin. Exp. Rheumatol. 1989; 7: 435-44.

93- Snider RL; Thiers BH: exogenous ochronosis.

J. Am. Acad. Dermatol. 1993; 28: 662-4.

94- Jordaan HF; Van Niekerk DJT: Transepidermal elimination in exogenous ochronosis.

Am J. Dermatopathol. 1991; 13: 418-24.

95- Dogliotte M; Liebowitz M: granulomatous ochronosis – a cosmetic induced skin disorder in blacks.

S. AF. Med. J. 1979; 56:757-60.

96- Hardwick N; Van Gelder LW; Van Der Merwe CA; et al: Exogenous ochronosis an epidermiological study.

Br. J. Dermatol 1989; 120: 229-38.

97- Findlay-GH: Ochronosis.

Clin. Dermatol 1989; 7: 28-35.

98- Albers SE; Brozena SJ; Glass LF; et al: Alkaptonuria and ochronosis: case report and review.

J. Am. Acad Dermatol. 1992; 27: 609-14.

99- Lawrence N; Bligard CA; Reed R; et al: Exogenous ochronosis in the united states.

J. Am. Acad. Dermatol. 1988; 18: 1207-11.

100- Brauer EW: Safety of over the counter hydroquinone bleaching creams (letter).

Arch Dermatol. 1985; 121: 1239.

101- Tuffanelli D; Abraham RK; Du Bais EI: pigmentation from antimalarial therapy.

Arch. Dermatol. 1963; 88: 113-20.

102- Cullison D; Abele DC; O'Quinn JL: Localized exogenous ochronosis.

J. Am Acad, Dermatol. 1983; 8: 882-9.

103- Howard KL; Furner BB: exogenous ochronosis in a Mexican American woman.

Cutis 1990; 45: 180-2.

104- Engasser PG: Ochronosis caused by bleaching creams (letter).

J. Am. Acad. Dermatol. 1984; 10:1072-3.

105- Bruce S; Tschen JA, Chow D: exogenous ochronosis resulting from quinine injection.

J. Am. Acad Dermatol. 1986; 15: 357-61.

106- Hull RB; Proctor PR: The melanocyte: an essential link in hydroquinone induced ochronosis.

J. Am Acad Dermatol. 1990; 22: 529-31.

107- Attwood HD; Clifton S; Mitchel RE: A histological and histochemical and ultrastructural study of dermal ochronosis.

Pathology 1971; 3: 115.

108- Tidman MJ; Horton JJ; Mac Donald DM: Hydroquinone induced ochronosis – light and electron – microscopic features.

Clin. Exp. Dermatol. 1988; 11: 224-8.

109- Connor T; Braunstein B. Off Center fold: hyperpigmentation following the use of bleaching creams. Localized exogenous ochronosis.

Arch. Dermatol. 1987; 123: 105-6.

110- Jacky WK : Annular granulomatous lesion in exogenous ochronosis are manifestations of sarcoidoses.

Am. J. Dermatopathol. 1995; 17: 18-22.

111- Jordaan HF; Van Niekerk DJT: Transepidermal elimination in exogenous ochronosis.

Am. J. Dermatopathol. 1991; 13: 418-24.

112- Phillips JI; Isaacson C; Carman H: Ochronosis in black South Africans who used skin lightners.

Am J. Dermatopathology. 1988; 8: 14-21.

113- Ho shaw RA; Zimmerman KG; Menter A: Ochronosis like pigmentation from hydroquinone bleaching creams in American Blacks.

Arch. Dermatol 1985; 121: 105-8

114- Sant'Ambrogio S; Connelly J; Di Maio D: Minocycline pigmentation of heart valves.

Cardiovasc-Pathol. 1999; 8(6): 329-32.

115- Camarasa JG; Serra Baldrich E: Exogenous ochronosis with allergic contact dermatitis from hydroquinone.

Contact Dermatitis. 1994; 31: 57-8.

116- Lang PG : Probable co-existing exogenous ochronosis and mercurial pigmentation managed by dermabrasion.

J. Am. Acad. Dermatol. 1988; 19: 942-6.

117- Diven DG; Smith EB; Pupo RA; et al: Hydroquinone induced exogenous ochronosis treated with dermabrasion and CO2 laser.

J. Dermatol Surg. Oncol. 1990; 16: 1018-22.