Alkaptonuria is an inherited disorder of metabolism of aromatic amino acids phenylalanine and tyrosine that is caused due to the lack of activity of the enzyme homogentisate 1,2-dioxygenase (HGD). The homogentisic acid is not metabolised – it accumulates in the body and is excreted into urine. The polymer – the ochronotic pigment – impregnates bradytrophic tissues.

**Clinical Signs**

Alkaptonuria is characterised by:
- homogentisic acid accumulation in the body
- presence of homogentisic acid in urine
- visible, functionally benign symptoms of eyes and ears
- debilitating changes in the locomotor system.

**History**

Scientists found alkaptonuria in the Egyptian mummy Harwa dated to around 1500 BC. The name alkaptonuria was first used in 1859 in a patient whose urine contained a reducing compound (alkapton) later identified as the homogentisic acid. In 1902, Garrod postulated a hypothesis that alkaptonuria is an inherited metabolic disease and that deficiency of the enzyme that metabolises homogentisic acid is a result of a defective gene. This concept was later proved correct. His article about alkaptonuria was published in *Lancet* in 1902 (Garrod, 1902). At that time it was a bold statement when one considers how little was known about enzymes, human genetics and intermediary metabolism. His knowledge was later summarised in his book *Inborn Errors of Metabolism* (Garrod, 1909). In 1958, the study by La Du et al. provided a biochemical evidence of the defect in alkaptonuria (La Du et al., 1958). They demonstrated the absence of homogentisic acid metabolising enzyme activity in liver homogenates from a patient with alkaptonuria. He found that the defect is related to one enzyme – 1,2-dioxygenase of the homogentisic acid. He suggested that the people affected do not synthesise the enzyme. The gene responsible for alkaptonuria was identified in 1993 by Pollak et al. and it was localised to chromosome 3q2 (Pollak et al., 1993). In alkaptonuria, the homogentisic acid is not broken down due to the lack of enzyme activity that metabolises the homogentisic acid; it accumulates in the body and is excreted in urine. The homogentisic acid builds an oxidised polymer in the body, which is stored in the form of blue-black deposits in tissues (ochronosis).
AETIOLOGY AND PATHOGENESIS

Phenylalanine and tyrosine are the simplest aromatic amino acids derived from alanine. Phenylalanine is an essential amino acid that cannot be synthesised by our body. This is not true for tyrosine. The body can synthesise tyrosine, but only if there is sufficient amount of phenylalanine – a precursor of tyrosine and the enzyme involved in the conversion of phenylalanine to tyrosine. Phenylalanine and tyrosine are closely related; phenylalanine is converted to tyrosine in the liver and to phenylpyruvate in the kidneys. Aromatic amino acids have a common intermediary metabolism. The conversion of phenylalanine to tyrosine and further metabolism are controlled by a complex enzymatic system. The failure to convert phenylalanine to tyrosine is known as phenylketonuria – one of the most common recessive inborn diseases (approximately 1 affected in 10 000 newborns). Phenylketonuria is caused by the lack of the enzyme phenylalanine hydroxylase, which converts phenylalanine to tyrosine or its cofactor tetrahydrobiopterine. Alkaptonuria arises as a result of the lack of another enzyme – 1,2-dioxygenase of the homogentisic acid (homogentisate 1,2-dioxygenase; HGD). This enzyme is a part of the degradation pathway of aromatic amino acids phenylalanine and tyrosine. Homogentisate excreted in the urine is then oxidised by oxygen in the air to brownish-black pigment - alkapton. Other symptoms later in life include ochronosis – pigmentation of the connective tissue (cartilage). The mechanism of ochronosis is the oxidation of homogentisate polyphenoloxidase resulting in the production of benzochinone and some other chemicals. Endogenous ochronosis is due to alkaptonuria.

EPIDEMIOLOGY AND GENETIC ASPECTS

The observation of alkaptonuria inheritance described by Garrod was studied closely by Hooben et al. (1932) who confirmed that alkaptonuria is inherited in an autosomal recessive manner. They found that about half of the affected individuals came from kin marriages.

The list of scientists who significantly contributed to the knowledge of the clinical manifestations of alkaptonuria and ochronosis includes Sitaj, Červeňanský, Urbánek, Húttl and others (Sitaj, 1947; Sitaj et al., 1956; Sitaj and Lagier, 1973; Urbánek and Sitaj, 1955; Červeňanský et al., 1959; Húttl et al., 1966). In 1947, Sitaj diagnosed and described the first case of alkaptonuria (Sitaj, 1947). Till 1953, Sitaj and his team collected a sample of 102 patients – members of 15 families – while at that time approximately 100 cases were described in the whole world, mostly isolated case reports. Between 1956 and 1960, they registered other affected families increasing the total number to 28 and the number of patients with alkaptonuria increased to 182, of whom 43 had ochronosis. Slovakia and the Dominican Republic became known in the literature as the countries where the highest incidence of alkaptonuria (1 in 19 000 inhabitants) was found. The world prevalence is one case in 250 000–1 000 000 inhabitants. Increased prevalence in Slovakia is explained by the fact that patients come from mountainous areas where the population evolved as genetic isolates with frequent marriages of parents related to each other. However, it should be noted that the active way of looking for patients, which has been a tradition in Slovakia for more than 50 years and dates back to the first case described by Sitaj, also contributed to the sample size.

Sitaj, Červeňanský and Urbánek described ochronosis, its development in patients with alkaptonuria and the detailed clinical picture as well as manifestations in the joints (Sitaj, 1947; Sitaj and Lagier, 1973; Urbánek and Sitaj, 1955; Červeňanský et al., 1959). They published the results in a monograph titled Alkaptonuria and ochronosis (1956), which was the first in the world literature (Sitaj et al., 1956). Their pioneering work is still being cited. Sršen et al. from the research laboratory of clinical genetics in Martin followed up on their epidemiological studies and continued in the genetic analysis of patients with alkaptonuria in Slovakia (Sršen, 1984; Sršen et al., 1996; Sršen et al., 2002). Institutions participating in the molecular characterisation of mutations in the Slovak population include: Institute of Molecular Physiology and Genetics SAS, Bratislava and Faculty of Natural Sciences of Comenius University in Bratislava (Zaťková et al., 2000a; Zaťková et al., 2000b; Zaťková et al., 2000c). Most of the observed families came from sites in Slovakia studied by Sitaj et al. (Sitaj, 1947; Sitaj et al., 1956; Sitaj and Lagier, 1973). Later work was continued by Rovenský and coworkers (Rovenský and Urbánek, 2000; Rovenský and Urbánek, 2003) and the genetic aspects by Bošák in cooperation with laboratories in Bratislava (Bošák, 2010; Zaťková et al., 2000a; Zaťková et al., 2000b).

In 1958, La Du with co-workers found that the biochemical essence of alkaptonuria is the lack of biological activity of the enzyme HGD in the liver, thus confirming the original assumption of Garrod that it is a metabolic disorder (La Du, 1958). HGD is an enzyme that is involved in the catabolism of phenylalanine and tyrosine. It has a molecular weight of 50 kDa and consists of 445 amino acids. It is specific for homogentisic acid and does not oxidase related substances such as gentisic acid and phenylacetic acid. The gene that encodes the HGD enzyme was cloned in 1996, thereby opening the era of molecular genetics of alkaptonuria (Granadino et al., 1997).

We now know that the HGD gene consists of 14 exons (coding parts of the gene) and 13 introns (non-coding part of the gene). HGD gene has a tissue-specific expression, particularly in the liver, kidneys, small and large intestine, prostate and the brain (Fernandez-Canón, 1996). Increased activity in the liver and kidneys has been attributed to metabolic activity of these organs. In the brain, HGD probably participates in the degradation of amino acids derived from neurotransmitters, which often contain aromatic amino acids. The HGD gene was localised on the long arm of chromosome number 3 in...
The first signs of deposition of the ochronotic pigment can be seen in the newborn. Their urine darkens when exposed to the air and leaves brownish black spots on their diapers. The spots are highlighted and cannot be washed away when alkaline soap is used. The dark earwax is also a characteristic that can be seen after birth. Dark urine and ear wax remained the only clinical symptoms of alkaptonuria for many years. In the meantime, a much more serious process takes place in the organism affected by this metabolic disorder. The ochronotic pigment is produced by oxidative polymerisation of the homogentisic acid; it accumulates in bradytrophic tissues and stains them dark brown. In principle, it is a benign process, which goes on unnoticed for a long time.

The first signs of deposition of the ochronotic pigment can be detected accidentally during professional examination of the anterior segment of the eye. The ochronotic pigmentation of the ocular structures is present in approximately 70% of patients. In addition to the sclera, lumps of the ochronotic pigment can be found in the conjunctiva and cornea. Since similar pigmentation of the cornea is not present in other medical conditions, this finding is regarded as pathognomonic for alkaptonuric ochronosis. Skinsnes described a patient who underwent enucleation of his only eye (the other one was lost due to injury) due to a pigment stain in the sclera deemed as melanomasarcoma (Skinsnes, 1948). After an unrelated death of the patient, however, the autopsy revealed that this was an alkaptonuric ochronosis with ocular pigmentation. Pigment spots on the sclera are mostly visible. They appear usually in the third decade of life in two-thirds of patients with alkaptonuric ochronosis. In the advanced stage, they can be seen with the naked eye. When found, chronic poisoning with phenolic substances, arsenic and lead, Addison’s disease, and blue sclerae in osteopatysis should be excluded. The diagnosis of alkaptonuria is based on the characteristic findings in urine. Alkaptonuria patients do not seek medical help due to difficulties with viewing these spots – they are without subjective complications.

In parallel with the ocular manifestations, ochronotic changes can be found in the hearing organ. Colour changes of the auricle are visible in the 10th to 15th year of life. Detailed histological examination of the temporal bone performed by Brunner revealed accumulation of the ochronotic pigment in the bone and its membranous parts (Brunner, 1929). The changes taking place in the ears are slow and patients are alerted to the blue-grey colour of the ear by their relatives. On the cartilage, painless, hard, rough lumps can be seen firmly connected with the basis and shining through the delicate skin as dark-blue-violet colour. The first rough ridges appear on the lower arm of the anthelix, and later throughout the anthelix, in the fossa triangularis, cymba conchae, cymbal and the tragus. In advanced cases, sometimes auricle deformation can be found. The external auditory canal is without changes, ear wax is dark brown, drum is dark, dull, often inverted, with an atypical reflex, with bluish tint, and in most cases calcium incrustations are present. Patients may also suffer from hearing loss type hypoacusis mixta with a stronger involvement of the perceptive apparatus. Symptoms of alkaptonuria hearing organ are specific and often lead to the diagnosis of this disease.

Also, changes in the skin typical of alkaptonuric ochronosis include mainly brownish or bluish pigmentation of the skin under the arm, in the face, neck and hands, and rarely on the nails. Given their visibility, they may be relevant for the early diagnosis of alkaptonuric ochronosis. Ochronotic pigment is deposited also in the internal organs. In the field of cardiovascular organs, it is the myocardium and blood vessels. Statistically significant myocardial disorders were not found, but earlier atherosclerotic changes in the aorta were observed. Urolithiasis was found in more than half of the patients and rare cases of nephropathy were seen. From a clinical point of view, the most serious process takes place in the joints and is called ochronotic arthropathy. Basically, it is a degenerative process with a known genesis and with an increased risk of disability. The basic clinical manifestation of ochronotic arthropathy from the beginning is related to the spine. The first subjective difficulties appear at the end of the third decade of life. Gender is significant with a predominance of men relative to women 2:1. Objective findings include flattening of thoracic kyphosis and lumbar lordosis, mild rigidity with a tendency to deterioration. Later, in an advanced stage, the contours of the spine worsen with irregular spinous processes and complete ankylosis of the entire lumbar and thoracic spine. The spine is rigid, irregular and the contours do not change when bending forward. Cervical spine maintains its mobility for a relatively long time despite significant sciotic changes. In an advanced stage, dorsal flexing and rotational movements become limited, while the
head moves forward. As a result of degenerative changes to the plates in the narrowing intervertebral space, body height decreases up to 8 cm in 20 years.

Using X-ray of the spine, characteristic calcification of intervertebral discs can be diagnosed. Osteolytic and hyperplastic changes and secondary reactive bone formation can be found on the vertebral bodies. Osteophytes are created, sometimes even massive bone bridges of the type of ankylosising hyperostosis. Calcification of some peripheral bundles of the connective rings may be similar to pseudosyndesmotic bridges. Even in the early stages, hollow formations in the plates are formed; this is called the vacuum phenomenon. In the intervertebral joints, the gap is narrowed and reactive subchondral sclerosis is present. Sometimes, calcification is found in the ligaments between the spinous processes. Occasionally, osteoporotic vertebral fractures are found. On the other hand, thickening of bone structure is not unusual. This resembles Paget osteitis.

While the spine is affected in all patients with ochronotic arthropathy, peripheral joints are often, but not always, affected. Based on the analysis of 26 patients with ochronotic arthropathy, it may be noted that small joints are spared and large joints are affected in the following order: knee (64%), shoulders (42.3%) and hips (34.6%). The finding in the knee is basically of arthritic nature. It differs from genuine osteoarthrosis by an earlier start (average of 39 years), faster progress and larger deformations. Hydrops occurred in 30.4% of our patients. Based on a series of investigations, Hüttl et al. have found that the synovial effusion is of a non-inflammatory, irritating and degenerative nature. The effusion has a yellowish colour that remains unchanged even after prolonged standing in air, suggesting a low concentration of homogentisic acid (Hüttl et al., 1966). From the nosographic point of view, it is important to find histiocytes with brown-violet and blue-black cytoplasmic inclusions, which can be assumed to be the phagocytosed ochronotic pigment.

The finding of histiocytes with pigment inclusions in the cytoplasm was described by Hüttl et al. for the first time in the world literature (Hüttl et al., 1966). The X-ray image in ochronotic arthropathy shows similar changes as in osteoarthritis, and this is often asymmetric. The characteristic sign is the formation of free calcified and ossificated pea sized or even larger bodies of a diverse shape. These are signs of ochronotic chondromatosis. Occasionally, narrow strips of calcified soft tissue parts of the extremities are detected that resemble Thieberg–Weissenbach syndrome. The main difference between genuine arthrosis and ochronosis of the knee is in a more rapid progression and in advanced findings in relation to the age of the patient with ochronosis.

In the shoulder joints in the early stages of ochronosis, there are painful episodes of the type of humeroucapsular periarthrophy that are probably related to the deposition of pigment and lime deposits tendons in the rotator. Gradually, the mobility gets limited due to the retraction of the joint capsule, destruction of the cartilage and the adjacent bone structures.

X-ray image of the shoulder joints shows even at an early stage signs of ossificating enthesopathy. Siťaj proves with an analysis of 42 patients with ochronosis that the calcareous deposits in the shoulder rotators are present in more than 25% of patients (Siťaj et al., 1956). In the next stage, around the 50th year of life, patients develop degenerative changes with exostosis on the bottom of the joint fossa, and later with cystoid translucency, usures and destruction on the humerus head. This finding is completely different from genuine osteoarthrosis and pathognomonic for ochronotic arthropathy of the shoulder.

The hip joints are affected only in later stages of ochronosis and approximately in a third of patients. The course is faster than in coxarthrosis and results in an almost complete restriction of the mobility.

The X-ray examination shows a severe, in some patients, destructive coxarthrosis. Červeňanský et al. describe ochronotic enthesopathy in the hip area and highlight the selective deposition of the ochronotic pigment in the tendons (Červeňanský et al., 1959).

Similarity of alkaptonuric ochronosis with other diseases. The metabolic disorders in ochronotic arthropathy of the spine and large joints of the limbs include osteoporosis. It is assumed that this is a secondary form of osteoporosis due to immobilisation of severely affected individuals. Barel et al. describe an affected family with alkaptonuria, phenylketonuria and congenital cataract (Barel et al., 1960). Occasionally, alkaptonuria occurs concurrently with psoriasis. In 1955, Urbánek and Siťaj described a unique coincidence of alkaptonuric ochronosis and Bechterev’s disease in a 51-year-old man (Urbánek and Siťaj, 1955). The patient came from a family in which four of five siblings had ochronotic arthropathy. Based on an analysis of clinical and X-ray findings in the spine, it could be assumed that ochronotic arthropathy and Bechterev’s disease interact. In our patient, typical ochronotic changes were present, especially calcifications of the intervertebral discs, less marked in comparison with other healthy patients with ochronosis in advanced disease stages. It may be that the premature rigidity of the spine due to the Bechterev’s disease prevented age-related development of ochronotic changes. On the other hand, despite standard Bechterev’s disease symptoms (affected sacroiliac joints, paraspinal ligament ossification and obliteration of intervertebral joints), the patient had disproportionately low pain throughout the course of the disease.

The long-term observation of a large number of patients with ochronosis revealed that the relatively subtle pain is characteristic for ochronotic arthropathy (Siťaj et al., 1956).

Japanese authors Kihara et al. described the coexistence of ochronosis and rheumatoid arthritis in a 64-year-old woman (Kihara et al., 1994). Magnetic resonance imaging of intervertebral discs found the typical changes suggestive for ochronotic arthropathy. At the same time, symptoms of rheumatoid wrist arthritis were identified with positive rheumatoid factor and nodules, which histologically were compatible with the diagnosis of RA. The authors state at the end that the
pre-existing ochronotic arthropathy could have masked the manifestation of RA and made it quite difficult to diagnose. The conclusion of these two case reports is that the process of ochronosis slows down the development of inflammatory symptoms of Bechterev’s disease and rheumatoid arthritis and makes it more benign.

**DIAGNOSIS**

Diagnosis of alkaptonuria is based on the proof of homogentisic acid in urine. It does not occur in a healthy person. Laboratory evidence of homogentisic acid is based on its reducing properties. In practise, the test using the Fehling’s solution, which is used in diabetes, proved valuable. While diabetic urine with Fehling’s solution brings brick-red clot, alkaptonuric urine changes the colour after the addition of the Fehling’s solution to grey-black. For screening, alkalisation of the urine with 10% NaOH can be used. After instillation of NaOH to the tube with the urine, a dark ring is created and then the entire sample of urine darkens. Earlier quantitative methods for determining homogentisic acid in the urine used the ability of the acid to reduce silver, phosphomolybdic acid or iodine. The drawback of these methods was the fact that they may determine also other reducing compounds present in the urine. This bias appeared in lower concentration of homogentisic acid in the urine of patients with alkaptonuria. Certain marked improvement brought the extraction of homogentisic acid into ether and subsequent iodometric determination. In 1961, Seegmiller et al. developed a spectrophotometric enzymatic determination of homogentisic acid in plasma and urine using the purified homogentisic acid oxygenase, which enabled the authors to specifically determine 1 µg of the acid (Seegmiller et al., 1961). The oxidatively produced maleylacetic acid is determined spectrophotometrically at 330 nm. Electrophoretic method of determining homogentisic acid was established by Trnavská (1962). At present, the quantitative determination of the homogentisic acid in urine is based on liquid chromatography and capillary electrophoresis.

The diagnosis of ochronosis is based on finding pigment spots on ocular structures, on the blue-grey discolouration of the auricles and skin in the armpit and on the X-ray findings in the calcified intervertebral discs. In the advanced stage of the disease, irregularly protruding spinous processes of the thoracic and lumbar spine are typical of ochronotic arthropathy and the finding of pigmented inclusions in the cells of the synovial effusion is specific.

Differential diagnosis. The fresh urine of a patient with alkaptonuria has a normal pale yellow colour and after prolonged standing in air or in contact with alkali media (soaps, etc.) darkens to dark grey to black. This sign of alkaptonuria is specific and it will almost always distinguish it from some other diseases associated with changes of urine colour. For example, the urine in the hereditary disease congenital erythropoietic porphyria (m. Günther), which also begins shortly after birth, has a very typical red colour. Similarly, inhaematuria or haemoglobinuria urine has a pinkish red colour. The analysis of the urinary sediment is of decisive importance for the diagnosis. In bilirubinuria, the urine has a reddish-brown colour (like black beer) and in melanuria a dark brown colour. Urine is always coloured when it is fresh, with the exception of alkaptonuria, when the urine darkens in contact with the air after hours or immediately after the addition of alkali. Ochronosis may be endogenous (on the basis of alkaptonuria) and exogenous, caused by the contact with certain chemicals. Several authors have described yellowish pigmentation of the skin and cartilage caused by carbolic acid (phenol), which was previously used to treat leg ulcers and other cases. This exogenous “karbolochronosis” was not associated with spondylitis and arthropathy. More recently, exogenous ochronosis in the skin and sclera associated with myxedema caused by long-term use of resorcinol was described. Anderson described the pigmentation of the eye conjunctiva and cornea due to working in the production of hydroquinone (Anderson, 1947). The sclera was dark and spots on the cornea caused blindness. Sugar and Waddell found a pigmentation of the sclera and cartilage similar to ochronosis after long-term use of atebrin (Sugar and Waddell, 1946). Skinssnes describes a case of incorrect melanomascroma diagnosis in a patient with ochronosis that led to a tragic outcome – enucleation of a single eye of the patient (see above) (Skinssnes, 1948).

Differentiating ochronotic changes in the spine from other spondylopathies is crucial to find calcified intervertebral discs, which are pathognomonic for alkaptonuric ochronosis. Spondylopathies in chondrocalcinosis could cause certain problems in differential diagnosis, but this disease is characterised by painful process with episodes of attacks of inflammatory nature, by calcifications of small joints, especially at the wrists, and the spondylopathy has an easier course with no tendency to ankylosis. In rare cases, a distinction from calcified discs in hemochromatosis can be considered. When in doubt, analysis of the urine for the presence of homogentisic acid is decisive, as well as a comprehensive view of the patient and evaluation of eye, ear and skin. Alkaptonuric ochronosis has a characteristic polytope symptomatology that usually does not cause greater differential difficulties. Rather, it is necessary to exclude other diseases that may require more urgent intervention before the full clinical picture develops.

**THERAPY**

As yet, no causal treatment is available for alkaptonuria; therapeutic interventions essentially are in three directions:
- reduction of the excretion of homogentisic acid into the urine,
- restriction of the onset of ochronosis,
- therapeutic and preventive interventions to influence ochronotic arthropathy.

In recent decades, various dietary interventions have been tried to reduce the formation of homogentisic acid and its excretion.
into urine including vitamins, hormones and other substances with uncertain and in some cases only a transient effect.

When it comes to limiting the production of the ochronotic pigment and reducing the risk of ochronosis with its disastrous consequences especially on the musculoskeletal system, a positive effect is attributed to vitamin C (Morava et al., 2003; Turgay et al., 2009). Some studies, however, did not confirm the positive effect of vitamin C and there is a lack of long-term controlled clinical studies targeting this problem (Phornphutkul et al., 2002). Progress in therapy leads to a direct pharmacological reduction of the homogentisic acid. Theoretically, it would be possible to use nitisinone, a triketone herbicide that quickly and reversibly binds 4-hydroxyphenylpyruvate dioxygenase, which catalyses the formation of homogentisic acid from 4-hydroxyphenylpyruvate. This treatment has, however, important adverse effects such as increased plasma concentrations of tyrosine and subsequent irritation of the cornea (Suwannarat et al., 2005). Application of nitisinone in alkaptonuria has so far been experimental.

Alkaptonuria as a congenital metabolic disease is now well defined. Gene therapy has moved from the phase of model experiments to the phase of clinical application in humans and one can hope that prospectively it will be introduced in the therapy of alkaptonuria. The solution would be the replacement of the missing homogentisate dioxygenase, but the application of recombinant homogentisate dioxygenase requires more careful studies of the distribution of isomerase and other enzymes following the metabolic pathway.

Therapeutic and preventive interventions aimed at influencing ochronotic arthropathy are essentially identical to those used in the treatment of the degenerative disease of the spine and limb joints. These include non-steroidal anti-rheumatic drugs, physical therapy, rehabilitation with balneotherapy, prevention of the creation of deformities and rheumosurgical interventions if needed. The follow-up of all newborn children diagnosed with alkaptonuria, their constant monitoring, diet and life style guidance, proper selection of sports and especially a suitable profession are very important.

In this regard, the research and practise of the workgroup of Prof. Sršeň at the Medical faculty of the Comenius University in Martin are of particular importance. In children with alkaptonuria, mild restriction of daily intake of proteins rich in phenylalanine and tyrosine, as well as the application of ascorbic acid supplemented with vitamins E, A and selenium is recommended. In addition, the recommendations include a daily regimen saving the sites predilected to be affected – large joints and the spine with corresponding selection and implementation of a profession.

Somatic gene therapy is progressing from the phase of experimental models to the stage of clinical application in humans and one can only hope that treatment will be introduced for alkaptonuria in the near future.

References


