ALKAPTONURIA: BLACK URINE DISEASE

Abstract

Alkaptonuria is one of a rare autosomal recessive genetic disorder, which results from the deficiency of homogentisate 1, 2 dioxygenase (HGD). HGD gene is expressed I' the liver, kidney, prostate, small intestine, and colon. This enzyme plays a role in the metabolism of tyrosine that converts homogentisic acid (HGA) into malate and acetoacetate. In the absence of HGD, homogentisic acid produced in excess by the liver oxidizes into ochronotic pigment polymer. Accumulation of this pigment in various tissues leads to systemic disease. This process is called ochronosis. Alkaptonuria was amongst the first genetic disorders in humans that found to follow the principles of Mendelian recessive inheritance. The term alkaptonuria originated from the Arabic word "alkali."

Keywords: Alkaptonuria, HGD, Epidemiology, HGA

Authors

Kolanupaka Shivani
Department of B pharmacy
Jyothishmathi Institute of Pharmaceutical Sciences
thimmapur, karimnagar,India.
sr3153509@gmail.com

Neelam Shivani
Department of B pharmacy
Jyothishmathi institute of pharmaceutical science
Thimmapur, karimnagar, Telangana, india.
shivanineelam1@gmail.com

Gundi Archana
Department of B.pharmacy
Jyothishmathi institute of pharmaceutical sciences,
Thimmapur, Karimnagar, Telangana,India.
archanagundi999@gmail.com

Jeggula Keerthana
Department of B pharmacy
Jyothishmathi Institute of pharmaceutical science
Thimmapur, karimnagar, Telangana, India
keerthanajeggula@gmail.com

Bandi Sreeja
Department of B pharmacy
Jyothishmathi institute of pharmaceutical sciences
Thimmapur, karimnagar, Telangana, India
sreejabandi7@gmail.com

I. INTRODUCTION

Urine with alkaptonuria, a genetic disorder, when placed in air, turns black .Ochronosis, an accumulation of dark pigment in connective tissues including cartilage and skin, is another feature of the illness [1]. This blue-black hue usually becomes apparent after the age of 30. Early-onset arthritis, particularly in the spine and major joints, is common in patients with alkaptonuria. Additional signs of this condition include potential heart problems, kidney stones, and prostate stones. [2].

Figure 1: A. Normal Urine B. Alkaptonuria Urine

II. EPIDEMIOLOGY

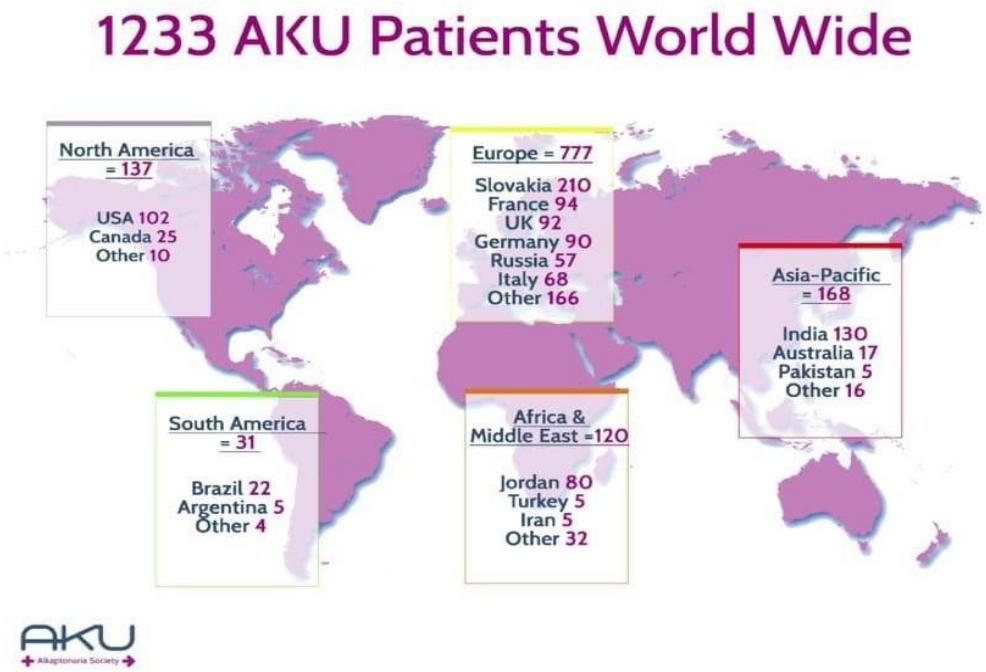

Figure 2: Overview of the Number of Patients with Alkaptonuria Reported World Wide.

One instance of AKU every 250 000–1 000 000 newborns occurs globally. 950 AKU patients have been found thus far in 40 different nations. According to reports, Slovakia, the Dominican Republic, India, and Jordan have higher rates of this illness. In Slovakia, where up to 1 in 19 000 people are affected, the frequency is highest. Analysis of the affected families indicated that they frequently reside in secluded hamlets, which led researchers to the conclusion that the founder effect—the loss of genetic diversity caused by genetic isolation—

was primarily responsible for the disease's high incidence. There is also no genotype-phenotype link; all mutations result in the development of ochronosis, despite the fact that this is challenging to do with such an uncommon disorder [3].

III. ETIOLOGY

1. **Infection:** Untreated infections can lead to eye damage. This includes phthisis bulbi.

2 **Chronic Retinal Detachment:** Retinal detachment is the process in which the retina separates from the choroid. This rare, but serious condition requires an emergency visit to a retinal specialist. A retinal detachment interferes with the blood flow through your eye, depleting necessary components such as oxygen. In turn, your eye tissues may get damaged or can die. Complications from surgery. Any type of surgery carries the risk of adverse effects. This includes eye surgery. Some people with phthisis bulbi may have tissue damage from eye surgery that develops into this condition.

3. **Long-Term Inflammation:** Also called uveitis, long-term inflammation of the eye can damage related tissues. According to British researchers behind a study published in Acta Ophthalmological trusted source, uveitis is the most common cause of end-stage eye.

4. **PHPV, or Persistent Hyperplastic Hyperplastic Primary Vitreous:** is a hereditary disorder that is present at birth. The eyes of newborns with PHPV are deformed. Between weeks 7 and 20 of gestation, fetal eyes begin to form.

5. **Retinoblastoma:** This condition involves the buildup of tissue with calcification on the surface of the eye. The calcification can ultimately grow into a tumor-like mass. It is particularly common in youngsters and is reversible. But for a full recovery, timely therapy is essential.

6. **Injury to the Eye:** Significant injuries to your eye may eventually lead to phthisis bulbi. Even if your eye has healed from traumatic events, such as a car accident, there may be lingering tissue damage that you may not be able to see. Eventually, the tissues can break down and lead to further complications [4].

IV. INHERITENCE

Both copies of the gene in each cell have mutations because this disorder is inherited in an autosomal recessive method. One copy of the defective gene is carried by each parent of a person with an autosomal recessive illness, but often neither parent exhibits the disease's symptoms [5].

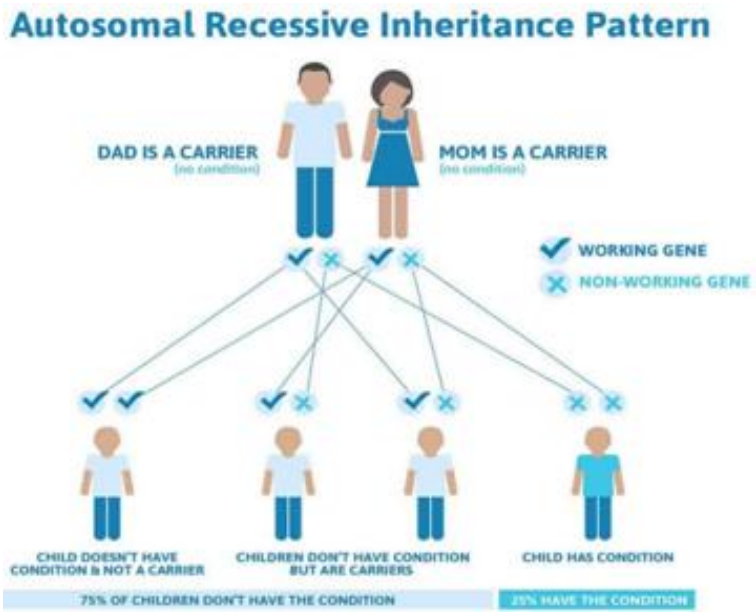

Figure 3: Autosomal Recessive Inheritance Pattern

V. SIGNS AND SYMPTOMS

Across time, homogenisic acid builds up in tissues all across the body. It can build up in almost any area of the body, including the heart, tendons, cartilage, nails, and nails. It disorganizes things and makes the tissues darker. A person with alkaptonuria might start experiencing joint problems in their 30s.Following lower back pain and stiffness are knee, hip, and shoulder pain. Early osteoarthritis symptoms are comparable to these ones. The body's strong, flexible tissue, cartilage, can eventually become fragile and tear, harming the joints and the spine. There may be a need for joint replacement procedures [6].

1. **Joints and Bones:** A person with alkaptonuria may start experiencing joint problems in their 30s.Knee, hip, and shoulder discomfort are usually followed by stiffness in the lower back. These are similar to the early signs of osteoarthritis. A robust, adaptive tissue found throughout the body, cartilage can deteriorate and shatter over time, harming the spine and joint. Surgery for joint replacement may be required.

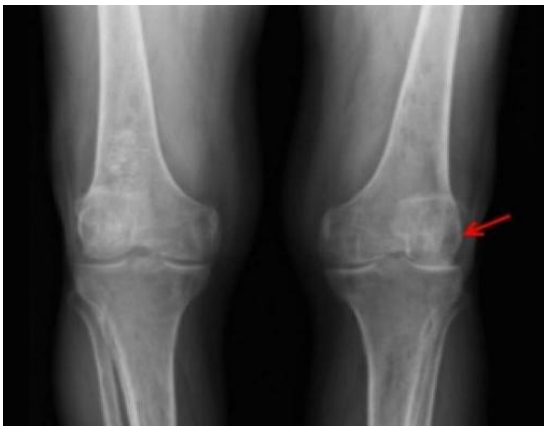

Figure 4: Joints and Bones

2. **Ears and Eyes:** On the white of their eyes, brown or black specks commonly appear in many people. An additional symptom of alkaptonuria in many individuals is thickening of the ear cartilage. In addition, the cartilage can seem blue, grey, or black. Ochronosis is the term for this. Earwax can be either brownish-red or black.

Figure 5: Dark Spots in the Eye and Ear

3. **Skin and Nails:** Sweating with a colored tint can stain clothing and cause some people to develop blue or black spots on their skin due to alkaptonuria. Nails may also change to a brownish or blue hue. The cheeks, forehead, armpits, and genital region are the places where skin color changes are most noticeable and where sweat glands are located.

Figure 6: Discolouration of Skin

4. **Breathing Difficulties:** The muscles and bones surrounding the lungs may stiffen, which will prevent the chest from inflating and cause breathing problems or shortness of breath.

5. **Heart, Kidney and Prostate Problems:** Homogentisic acid deposits can cause heart valves to constrict, stiffen, and become brittle and black. Additionally, blood vessels can stiffen and deteriorate. This can cause heart disease and necessitate the replacement of heart valves. Additionally, the deposits have been linked to kidney, bladder, and prostate stones[7].

VI. PATHOPHYSIOLOGY

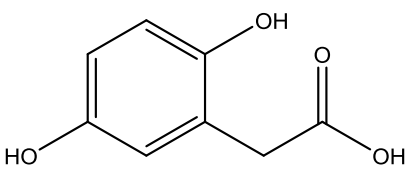

Figure 7: Structure of Homogentisic Acid

Chemical Skeletal Formula of Homogenistic Acid: Alkaptonurics' bodily fluids collect homogentisic acid, which has the following chemical skeleton. The gene homogentisate 1,2-dioxygenase (HGD), which is responsible for producing the enzyme homogentisate 1,2-dioxygenase (HGD), is present in two copies in everyone's DNA (one from each parent). HGD is typically found in many body tissues, including the liver, kidney, small intestine, colon, and prostate. Alkaptonuria patients have defects in both copies of the gene, which prevents the body from producing an enzyme that functions as it should. Exons 6, 8, and 13 are often where HGD mutations are detected, however over 100 anomalies have been reported across the whole gene. The typical HGD enzyme is a hexamer, consisting of six subunits arranged in two trimers of three. It also contains one iron atom. The enzyme's solubility, structure, or activity can all be impacted by various alterations. Rarely, the condition appears to be passed from parent to child in an autosomal-dominant manner, with alkaptonuria being linked to a single faulty copy of the HGD gene; in those circumstances, additional mechanisms or genetic flaws may be at play [8].

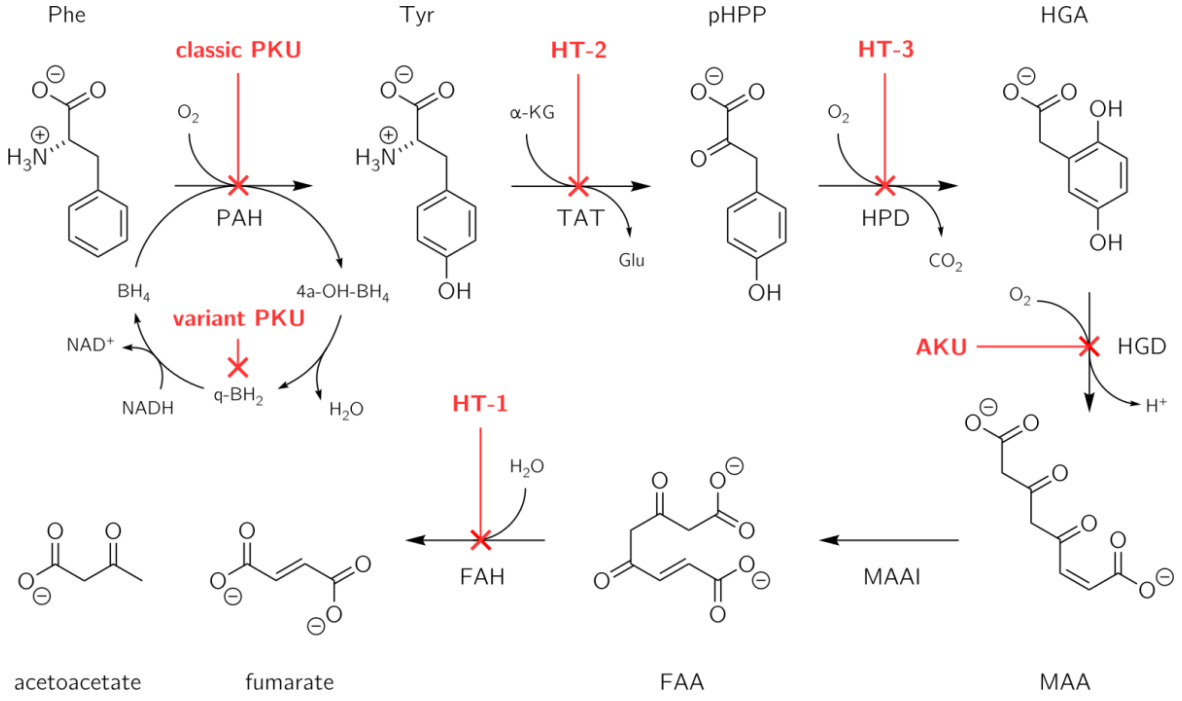

Figure 8: Pathophysiology of Alkaptonuria is Due to the Absence of the Liver Homogentisate Dioxygenase is Functional.

The metabolic (chemical) processing of the aromatic amino acids phenylalanine and tyrosine is carried out by the HGD enzyme. These often enter the bloodstream from protein-rich foods and the body's normal protein turnover. Tyrosine is specifically required for a number of processes, including hormones (such as the thyroid hormone thyroxine), melanin (the dark pigment in the skin and hair), and some proteins. However, the vast majority of tyrosine (more than 95%) is unused and is broken down by a set of enzymes to produce acetoacetate and malate. Although a significant amount of homogentisic acid is excreted into the urine by the kidneys, homogentisic acid levels in the blood are 100-fold higher than would be expected in alkaptonuria because the HGD enzyme cannot convert homogentisic acid (generated from tyrosine) into 4-maleylacetoacetate [8].The homogentisic acid is changed into the chemically similar compound benzoquinone acetic acid, which produces

polymers that resemble the melanin-like pigment of the skin. These are deposited in specific tissues like cartilage's collagen, a connective tissue protein. The condition is known as ochronosis (because the tissue has an ochre appearance), and ochronotic tissue is stiffened and particularly brittle, which impairs its ability to function normally and causes harm [9].

VII. DESCRIPTION (MICROSCOPIC/HISTOLOGIC)

Alkaptonuria and exogenous ochronosis both share several ochronosis-related characteristics. Early alterations in the connective tissue include homogeneity of collagen and elastotic fibers that are degenerating [10], as well as deposits of yellow-brown, banana-shaped, sickled, or spherical ochronotic entities .Exogenous ochronosis, broad solar elastosis-like alteration, and occasional entities in direct continuity with collagen or elastic fibers may all be found in an interfollicular distribution. Ochronotic entities that occasionally have decreased basal keratinocyte pigmentation and are associated with colloid milium are known as pigmented colloid milium. Additionally, granulomatous response has been shown When implicated, comparable ochronotic structures can be detected in the connective tissues of the heart, scleral, and skeletal tissue.

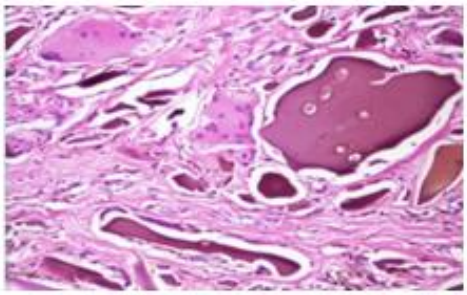

Figure 9: Microscopic Image of Alkaptonuria

VIII. HISTORY

In 1908, Garrod's usage of AKU in the Croonian lectures brought the illness to public attention. However, numerous descriptions of the trio of characteristics linked to AKU go back far further than this. The condition was first recorded in the 16th and 17th centuries. The Egyptian mummy Harwa, which is thought to have existed as early as 1500 BC, contained the earliest known clinical instance of AKU. The Arabic term "alkali" (which means "alkali") and the Greek verb "to suck up oxygen greedily in alkali" are the origins of the name Alkaptonuria. Boedeker came up with the moniker in 1859 after seeing peculiar decreasing characteristics in a patient's urine. Because the HGA pigment looked to be ochre-colored (yellow/brown) under a microscope, Virchow initially identified and termed ochrnosis in 1866.Since gentisic acid, a benzoic acid derivative, and HGA share a tight structural link, HGA was first recognized as the causal element in 1891 and given that name. The genetic flaw was identified, cloned, and located on chromosome 3q21-q23 by 1995.

IX. PHYSICAL FINDINGS

There are various clinical spectrums for alkaptonuria. The trinity of alkaptonuria is made up of ochronosis, homogenistic aciduria, and ochronotic osteoarthropathy. Ochronosis

typically manifests in the third or fourth decade and is caused by the buildup of benzoquinone acetate in connective tissue that is both extracellular and intracellular. Finally, Homogentisinic acid polymer accumulates within hyaline articular cartilages, leading to the development of ochronotic arthropathy [11]. The clinical characteristics result from ochronosis:

1. **General: Sk**in discoloration as well as grey pigmentation in the sclerae or the cartilage of the ears.

2. **Joint And Bone:** joint effusions caused by arthritis and lumbar discomfort (ankylosis), decreased joint mobility and impaired**,** disability may result from spinal and thoracic motion. Additionally, the frequency of fractures brought on by osteopenia is rising.

3. **Respiratory:** Restrictive lung disease and a decreased respiratory reserve.

4. **Cardiac:** An increase in the prevalence of coronary artery disease, as well as valvular heart disease such as aortic stenosis (more prevalent), aortic regurgitation, and mitral valve stenosis.

5. **Neurological:** Increased risk of stroke, peripheral neuropathy, tinnitus, and diplopia. Increase in the prevalence of prostatic, gallbladder, and renal stones.

X. EVALUATION AFTER THE FIRST DIAGNOSIS

If not done as part of the evaluation that resulted in the diagnosis, the following tests are indicated to identify the severity of the disease and the patient's needs in someone with alkaptonuria: Detailed medical history and physical examination, with a focus on the spine's and other main joints' range of motion. Consult a physical therapist if you experience joint pain or restricted range of motion. For those over 40, an electrocardiogram and cardiac echocardiogram are recommended. TSH and free thyroxine levels are evaluated to rule out primary hypothyroidism. Renal ultrasound or helical abdominal CT to look for renal calculi. consultation with a medical geneticist, a genetic counselor, or a licensed advanced genetic nurse to explain the nature, importance, and effects of the problem to the affected people and their families.

Treatment of Manifestations: Individualized symptomatic management of joint pain; physical and occupational therapy support optimum muscular flexibility and strength; Replacements for the knee, hip, and shoulder are available when necessary; if necessary, surgical treatment for kidney and prostate stones; Thyroid hormone replacement with aortic valve replacement may be necessary in cases of stenosis.

1. **Surveillance:** Echocardiography is used in those older than 40 to look for stenosis, aortic dilatation, and calcification of the mitral or aortic valves. Think about CT imaging for people who have symptoms that point to coronary artery calcification.

   Examine thyroid function at the time of the initial diagnosis and continue to do so every one to two years after that.

2. **Agents and Situations to Avoid:** In an effort to slow the progression of severe arthritis, physical strain on the spine and major joints (such as through strenuous manual labor or impact sports) is encouraged.

3. **Risk Analysis of Relatives:** In order to identify those who would benefit from preventive treatments to help preserve overall joint mobility and function as early as feasible, it is appropriate to screen older and younger siblings of an affected person who appear to have no symptoms.

XI. LABORATORY STUDIES

Clinical examination suggests a diagnosis, which is supported by the amount of HGA detected by gas chromatography-mass spectroscopy in the urine. Two orders of magnitude higher elevations than average are seen using spectrophotometric quantification. It could be wise to check for HGA in all patients who have radiographic evidence of osteoarthritis because many people appear without black urine. An x-ray of the spine will show severe calcification and disk degeneration, especially in the lumbar region. The diagnosis is confirmed by genetic testing.

1. **Diagnosis:** Adults who develop black urine when exposed to air may have alkaptonuria. Infants with black streaks in their diapers from dried pee may have alkaptonuria. In-depth family history can also indicate whether a person is more likely to acquire alkaptonuria. Alkaptonuria can only be diagnosed using a few definitive tests [5].

   The following are highlighted:

   - **Detection of Homogenistic Acid in Urine:** Gas chromatography-mass spectrometry can be used to identify and measure homogentisic acid in 24-hour urine samples.

   - **Molecular Genetic Testing:** It is possible to identify HGD gene mutations in urine or blood using molecular methods like the polymerase chain reaction (PCR).

2. **Prognosis:** The illness progresses over time. The patient often has a gradual but steady loss in function. It is possible to develop complications including arthritis in the spine, hips, shoulders, and knees. The Achilles tendon ruptures more frequently, and kidney and prostate stones are more likely to form. The coronary arteries and the aortic and mitral valves may stiffen and harden. The patients' pain levels have increased, and loss of mobility as time as it does, physical mobility aids like crutches or wheelchairs are frequently necessary.

   Despite the prognosis may worsen due to cardiac problems, longevity is not greatly reduced.

XII. MANAGEMENT

- Tyrosine restriction, or a low-protein diet, can reduce HGA generation, but this treatment is difficult to sustain over the long term and does not appear to improve AKU symptoms.

- Nitisinone, a triketone herbicide that prevents the activity of p.HPPH, the second enzyme in the pathway that breaks down tyrosine. Oral nitisinone lowers urinary HGA excretion in a mouse model of AKU by roughly 80%, but it also raises plasma tyrosine levels as a side effect. Nitisinone has the same pharmacological action in humans, however during a three-year randomized treatment trial involving 40 patients with alkaptonuria, one patient experienced keratopathy typical with tyrosine toxicity38. Nitisinone is well tolerated when taken orally at a dose of 2 mg per day, as evidenced by the >95% reduction in urinary HGA excretion as well as the average plasma tyrosine level of 800 M. To avoid ochronosis and related joint arthropathies, nitisinone medication should be started as soon as possible after birth .Nitisinone has recently been demonstrated to entirely block pigment deposition in the chondrocytes within the articular cartilage of the knee in a mouse model of AKU. Reduction of pain by classical analgesic drugs, physiotherapy and/or rest, pain control is crucial in the day-life of AKU patients and is tackled by a wide range of analgesic drugs: paracetamol, non-steroidal anti-inflammatory drugs and opioids. Physiotherapy has also been shown to improve activity.
- Replacement of the knee, shoulder, and/or hip to lessen physical impairments. In older AKU patients, 3 to 5 surgical treatments with short-term success are relatively common. Joint injections with hyaluronic acid may be effective, as was shown in a patient with early ochronotic arthropathy.
- Gene therapy or liver transplantation for enzyme or gene substitution; however, these methods are not currently available [8].
- Offering symptomatic relief, preventative measures, and supportive care are the key priorities. The following list of therapeutic methods is briefly highlighted:

1. **Ascorbic Acid:** Since it stops HGA from building up in the tissues, high dosages of ascorbic acid (vitamin C) have occasionally been advised for the treatment of alkaptonuria(1G/day).However, studies have indicated that ascorbic acid treatment for this illness over a lengthy period of time is typically ineffective.

2. **Nitisinone:** A novel medication called nitisinone is being researched as a possible cure for alkaptonuria. The United States Food and medication Administration (USFDA) has designated nitisinone as an orphan medication and granted approval for the treatment of tyrosinemia, a metabolic condition [12]. Nitisinone therapy can lower plasma and urinary HGA by more than 95%, according to studies. The greatest disadvantage is the buildup of tyrosine, the dangers of which are uncertain over the long run [13]. refers to the following advantages: 5 mg, 10 mg, 20 mg, 4 mg, and 2 mg/ml

   The most common adverse reactions associated with nitisinone use include

   - **Adverse Metabolic Reactions**

     - Elevated tyrosine levels
     - Snimming

   - **Adverse Blood Reactions**

     - Thrombocytopenia, Leukopenia, and Granulocytopenia

- **Adverse Ocular Reactions:** Conjunctivitis, eye discomfort, corneal opacities, photophobia, keratitis, and cataracts

- **Adverse Dermatological Reactions:** Dry skin, pruritis, epistaxis, exfoliative dermatitis, maculopapular rash, alopecia

- **Toxicity:** The majority of people who keep a regular diet without restricting phenylalanine and tyrosine have toxicity as a result of nitisinone medication, which prompts hypertyrosinemia symptoms. Tyrosine poisoning has the potential to mimic the symptoms of the many genetic tyrosinemia subtypes, including mental retardation, hyperkeratotic plaques on the palms and soles, and delays in growth.

3. **Pain Medication:** Ibuprofen, naproxen, and other anti-inflammatory drugs like paracetamol can help to reduce discomfort and swelling. By numbing the spinal cord's nerve terminals, procedures like transcutaneous electrical nerve stimulation (TENS) can significantly reduce pain [14].

4. **Surgery:** If a joint replacement or heart valve replacement is essential, surgery can be required.

    - Hip substitute.
    - Replacement of knee.
    - Replacement of the aortic valve.

5. **Diet:** Dietary protein restriction may aid in lowering phenylalanine and tyrosine intake, decreasing the progression of the disease by lowering HGA synthesis. Here are some foods that typically have low levels of tyrosine and protein[15].

    - **Fruits:** Most fruits are low in both protein and tyrosine. Examples include apples, pears, oranges, berries, and melons. Be cautious with avocados, bananas, and nuts, as they contain moderate amounts of tyrosine.

    - **Vegetables:** Many vegetables are low in protein and tyrosine. Some examples include lettuce, cucumbers, peppers, carrots, and zucchini. However, spinach and broccoli have slightly higher tyrosine content, so they should be consumed in moderation.

    - **Grains:** Most grains like rice, pasta, and bread are low in both protein and tyrosine. Check food labels for any protein or tyrosine content added during processing.

    - **Dairy Substitutes:** If you need a milk substitute, choose low-protein, non-dairy alternatives like rice milk or almond milk. These are typically low in tyrosine as well.

    - **Low-Protein Breads and Pastas:** Some specially formulated low-protein bread and pasta products are available for individuals on restricted diets. These can be found in health food stores or online.

- **Cooking Oils:** Oils such as olive oil, canola oil, and vegetable oil are low in protein and tyrosine and can be used for cooking.
- **Sweets and Desserts:** Some sweets like hard candies, jelly beans, and some non-chocolate-based candies can be low in both protein and tyrosine. However, always check labels, as some may contain gelatin or other protein sources.

- **Beverages:** Most beverages, including water, fruit juices (without added protein), and certain herbal teas, are low in protein and tyrosine. Be cautious with protein shakes, sports drinks, and some nutritional supplements.

- **Condiments:** Mustard, ketchup, and certain low-protein salad dressings can be included in a low-tyrosine diet. However, be mindful of high-protein condiments like soya beans

6. **Exercise:** Exercises can help with some alkaptonuria symptoms, like stiffness and soreness in the joints. Exercise on a regular basis helps strengthen the joints while also helping to build muscles. But you must watch out that your joints are not overworked during the activities. Avoid such exercises that are stressing the joints if you feel there is additional tension. In addition, exercising promotes stress reduction, weight loss, and better posture.

7. **Emotional Help:** It can be emotionally taxing to receive a diagnosis for a chronic ailment like alkaptonuria, but anxiety and sadness can make the symptoms worse. So seeking medical advice is crucial for maintaining mental wellness.(15)

- **Complications**

  - Prostate and renal stones.
  - Gallbladder stone
  - Stones in the salivary glands.
  - Tendon and ligament ruptures.
  - Fractures and osteoporosis.
  - Stenosis and calcification of the aortic valve.
  - Amyloidosis (16).

- **Life Style Modifications:** Refer the patient for genetic counselling. It is important to stress the importance of diligent follow-up with a biochemical geneticist.

XIII. FUTURE PERSPECTIVES

Nitisinone is a safe and efficient treatment for AKU, although it just manages the condition. Patients receiving nitisinone are required to adhere to a strict, low-tyrosine diet and must be closely watched to make sure their circulatory tyrosine levels are not rising excessively. The major step in treating the underlying genetic etiology of single-gene illnesses like AKU would be the creation of medications that precisely target cartilage through gene replacement therapy. Since no molecules are presently available, there has been little advancement in the creation of novel therapeutics and diagnostic imaging techniques. The situation in bone is in striking contrast to this, as the discovery of bisphosphonates

resulted in bone scintigraphy and the most effective family of treatment medicines for bone disease.

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