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Review Article

A Century of Alkaptonuria: Evolving Insights into a Rare Metabolic Disorder

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ABSTRACT

Alkaptonuria is believed to be a rare autosomal recessive disease caused by an imbalance in homogentisate 1,2 dioxygenase (HGD). HGA builds up in the body when the HGD enzyme isn't working correctly, despite the kidneys' ability to frequently remove it. The diagnosis of AKU may not occur until the ochronotic arthropathy manifests, which usually happens in adulthood. There is a paucity of information on the condition's early stages and infancy. The development of a dark color formation in urine, dried urine spot (DUS), immunofluorescence, Thioflavin T (Th-T) staining, Congo Red (CR) staining, and transmission electron microscopy (TEM) may all be used to detect this. Ascorbic acid, phenylalanine and tyrosine diet restrictions, and the only medication approved by the European Medicine Agency as the first disease-modifying treatment for individuals with AKU is nitisinone, which has demonstrated promise as an additional treatment for alkaptonuria.

Keywords: alkaptonuria; homogentisic acid; nitisinone; ochronosis; renal stones; osteoatropahty.

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Introduction

Sir Archibald Edward Garrod recognized alkaptonuria (AKU) in 1908 as being among the four known congenital metabolic disorders [1]. In addition, this was the first disease to be classified as matching Mendelian recessive inheritance. There is a historical background to the illness, with many reports describing people displaying symptoms of AKU. In 1584, Scribonius reported about a young boy whose urine looked like ink. Research says that AKU affects 1 to 8 out of every 1,000,000 newborn babies[2]. 1 in 19,000 is the highest rate found in the Dominican Republic and in the Piestany area in Slovakia [3]. It is believed that AKU is a rare autosomal recessive illness caused by too little homogentisate 1,2 dioxygenase (HGD). Through genetic research on families of Slovak patients in 1993, scientists discovered that the HGD enzyme gene resided at 3q13.33 on chromosome 3 [4].

HGD is an enzyme that converts homogentisic acid (HGA) into maleylacetoacetic acid during the break down of phenylalanine and tyrosine. Biochemically, dysfunction of HGD causes HGA to remain in the body, despite the kidneys removing it at their regular rate [5]. Large quantities of HGA are oxidized by a benzoquinone acetate intermediate, turning into pigments called polymers. In ochronosis, HGA-pigment accumulates in several tissues, mainly in connective tissues, joints and spine cartilage, tendons and ligaments. Due to increased weakness and susceptibility, the connective tissues in ochronosis allow decay to occur which may result in discoloration of the ears, eyes and skin, together with the production of calculi in the kidneys, prostate, gall bladder and salivary glands [6]. In many situations, those with this condition experience renal failure, ruptures in tendons, ligaments and muscles, fractures, reduced bone density (osteopenia), spinal conditions leading to reduced stability (spondylarthropathy), unusual spine curvatures such as kyphosis, scoliosis and the need for joints replacement surgery [7], as illustrated in **Fig 1**. Therefore, several degenerative processes, inflammation and calcification may develop, causing the disorder to progress into severe spondyloarthropathy and osteoarthritis.

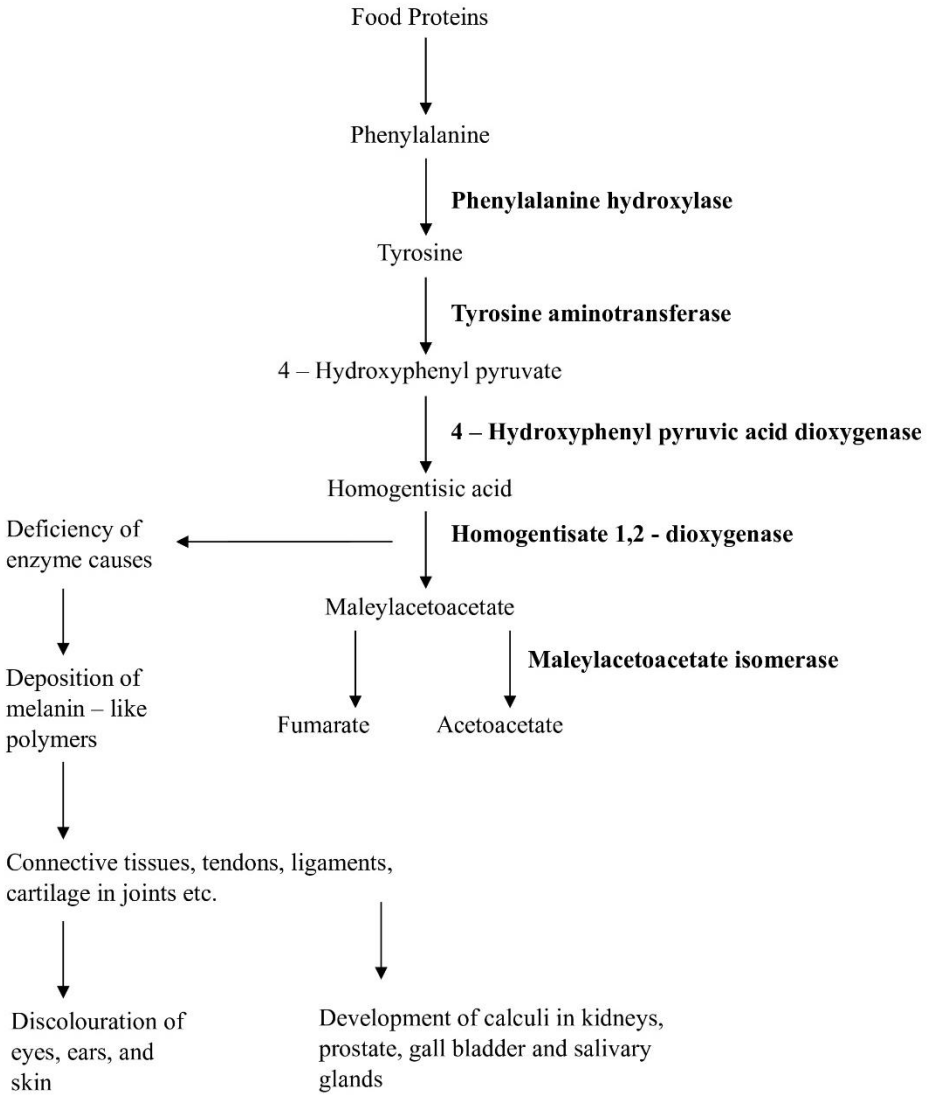

Fig 1 – Pathophysiology of alkaptonuria

Research has found that X-ray changes in the joints usually start around age 30 and people most often have their first joint replacement at age 55 [8]. At the age of 54, heart valve issues are common, unlike coronary artery calcification which is seen most often at 59 years of age. Early diagnosis is difficult because only a few patients are discovered in infancy, so the illness is often not diagnosed until the child starts having back, joint and muscle pain. Prior to 2011, treatment for AKU was limited and involved only pain-relieving drugs and arthroplasty [8, 9]. Studies where vitamin C acts as an antioxidant lowered the amount of benzoquinone acetic acid but had no impact on HGA levels [10]. Taking a low-protein diet is suggested and could serve a purpose,

but difficulties complying are common. A study has introduced a treatment using a drug called nitisinone (NTBC) that blocks 4-OH phenylpyruvate dioxygenase and proved its success in treating the disease [11].

Molecular Genetics of Alkaptonuria (AKU)

The HGD gene, located on chromosome 3q21-q23, encodes the HGD enzyme. Mutations in this gene result in the lack of enzyme function, contributing to the clinical signs of AKU [12]. There has been significant research into the genetics of AKU and many types of changes in the HGD gene have been found in patients worldwide. They consist of missense, nonsense, splicing, small deletion/insertion and large-scale rearrangement mutations.

Figuring out and describing the HGD mutations in AKU has enabled us to better understand the underlying genetic causes of AKU symptoms. Several changes in the HGD gene can cause diverse levels of enzyme deficiency which lead to a wide range of symptoms, from acute to chronic forms of the disease [13]. As a result, genetic testing and counseling programs are now offered to those with AKU and their family members through research at the molecular level. The frequency of AKU varies among different ethnic groups, with increased incidence rates seen in some populations, such as the Dominican Republic and Slovakia. Mutations from the founders and mutation hotspots have been noticed in these populations which help explain the rising AKU cases in some regions [14]. AKU has also been studied in Slovakia due to its more widespread presence than in various other nation. In Slovakia, researchers have studied both the genetic details of HGD mutations and the medical and biochemical features of AKU among the population. Through this research, scientists have gained useful information on the genetic background of AKU in Slovakia and have identified distinctive data for genotype-phenotype comparisons in this group [15]. Understanding the molecular aspects of AKU can suggest novel opportunities for treatment. It is vital to understand the HGD mutations that happen in AKU patients in order to develop special molecular treatments including gene therapy or tiny interventions aimed at repairing the HGD enzyme. This area of research may lead to hopeful treatments for AKU in the future. All in all, HGD mutations have been discovered and studied, leading to correlations between the genotype and the condition, changes in Alkaptonuria mutations depending on population and hopes for new therapeutic methods [16]. Researchers are

making steady progress towards finding out more about AKU and uncovering suitable therapies for this uncommon metabolic disorder.

Diagnosis

Diagnosis may take time, since the arthropathy usually develops when someone reaches adulthood and the earliest details of AKU are not widely known [17]. The delay in forming dark urine is based on how acidic the urine is and such delays may be mistaken for another condition [18]. There is no clear connection between the amount of HGA in the blood and symptoms, age or sex [8, 9]. This can be diagnosed by dried urine spot (DUS), Congo Red (CR) staining, Thioflavin T (Th-T) staining, immunofluorescence, as well as transmission electron microscopy (TEM) as stated in **Fig 2**.

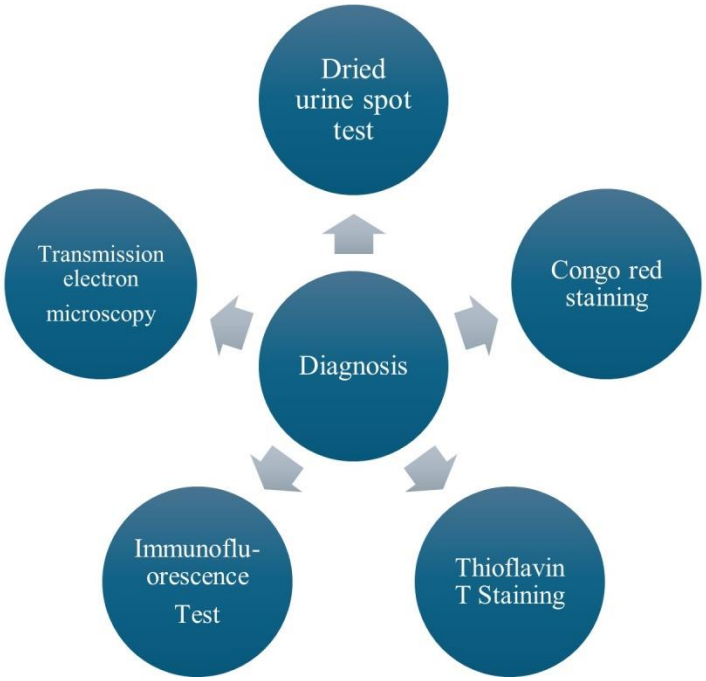

Fig 2 – Diagnosis of Alkaptonuria

DUS are utilized in the detection of hereditary diseases, including AKU as a sporadic occurrence, mainly as a qualitative diagnostic tool. A dark brown hue is widely known to emerge by adding alkali (e.g. sodium hydroxide) to either HGA solutions or AKU urines [18]. Early detection of AKU would help researchers better understand the disease and relate it to physiological processes. HGA in AKU a reducing agent with 2 phenolic groups changes into benzoquinone acetic acid that then acts as an oxidising agent to form a pigment like melanin [9].

Identifying secondary amyloidosis in AKU may prove difficult, but it is critical for rapidly recognizing and treating the condition. According to a study authored by Millucci et al. and published in Diagnostic Pathology in 2014, the selection of relevant specimens is critical for correct diagnosis. The researchers observed that amyloid was detected in just one specimen of the abdominal fat pad, although all patients had amyloid accumulation in salivary glands and other organ biopsies. It proves that salivary glands are the best choice for detecting amyloid during the early stages of AKU. The authors discussed the importance of performing Congo Red (CR) staining, Thioflavin T (Th-T) staining, immunofluorescence and TEM to detect amyloid deposits in different parts of the body [19]. People with AKU vary in their clinical symptoms. The study gives a detailed outline of the patients involved in the examination. The features involved cover peripheral neuropathy, orthostatic hypotension, enlarged ventricles and atria, chronic bacterial infection, spondyloarthropathy, swollen tongue and surgeries in the past [20]. SAA, HGA in plasma and urine and amyloid plaque occurrence were checked for each patient. Assessments were carried out to find out which specimens are important for quick detection of amyloid in AKU patients.

Gabriella Jacomelli's report, called Quick Diagnosis of AKU by Homogentisic Acid, was used for this study [21]. Two approaches for easy AKU testing using HGA measured in dried urine spots are the main focus of Determination in Urine Paper Spots. In the first method, alkaline conditions cause the appearance of color quickly and reliably and allow for some numerical measurements. The second method uses the sensitive and quantitative approach of HPLC, a well-known Liquid Chromatography system. With these approaches, AKU patients can rely on accurate screening [21-23].

Treatment

As stated in the **Fig 3,** the therapies that have been examined for AKU includes:

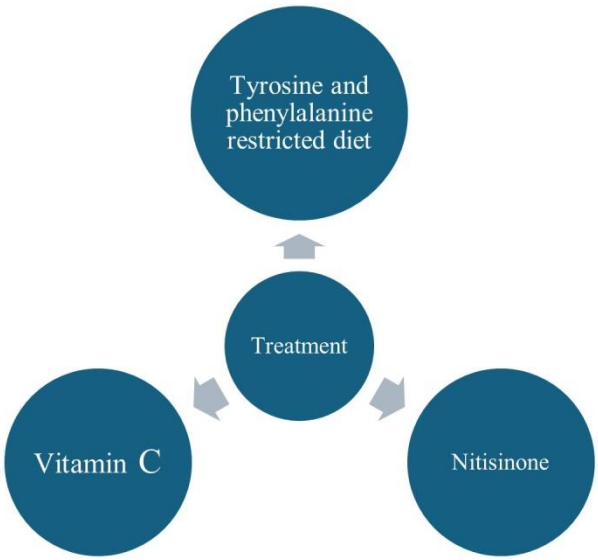

Fig 3 – Treatment of Alkaptonuria

1. **Tyrosine and phenylalanine-restricted diet**

   A diet limited in protein has been recommended as a viable way to minimize the production of HGA. This therapeutic strategy has shown success in treating numerous genetic metabolic illnesses and has exhibited good effects on neurological outcomes and overall survival [24].

2. **Vitamin C (ascorbic acid)**

   Ascorbic acid has been examined for its effects on alkaptonuria, particularly its potential to block the binding of homogentisic acid in connective tissue [25]. Studies have also explored the effects of ascorbic acid on HGA excretion in urine.

3. **Nitisinone**

Nitisinone has exhibited potential as a special treatment for alkaptonuria. It has been tested in people with AKU and has been associated with positive effects, including the suppression of ochronotic osteoarthropathy [26]. The way nitisinone works is by decreasing the presence of HGA produced by an enzyme called HPPD. Reducing the activity of this enzyme with nitisinone will lower the amount of HGA which helps combat ochronosis and its connected symptoms. According to studies including SONIA 2, nitisinone can decrease HGA levels by almost 100 percent and help improve symptoms in those with AKU. Since HGA levels have dropped, the disease progresses more slowly, as seen by the slower increase in the AKU

Severity Score Index (AKUSSI) [23]. The European Medicine Agency recently authorized nitisinone as the first therapy that can slow the progress of AKU in patients. Research has determined that nitisinone works to prevent ochronosis in mouse models of AKU and can also slow the development of the disease when given to human patients with clear signs of AKU [27].

Clinical trials of Nitisinone

SONIA 1

A major research study called the Suitability of Nitisinone in AKU (SONIA 1) is examining how effective and safe nitisinone is for patients with AKU. The researchers tested different doses of nitisinone in two different groups to observe their impact on urine HGA excretion. In the SONIA 1 trial, 40 patients with AKU took part and were randomly assigned into one of five groups which included receiving either no therapy or doses of nitisinone each day at 1, 2, 4 or 8 mg. The main reason for studying this was to assess how nitisinone changed urine HGA excretion after 4 weeks of use. Both urine and serum were taken from each subject for HGA testing [28]. During the experiment, attention was given to negative occurrences and indicators of risk. The experiment showed that the amount of nitisinone a person gets affects urine HGA excretion. Nitisinone at the highest dose eliminated 98.8% of HGA from the urine, compared to baseline levels. Large doses resulted in a bigger drop in HGA levels than usual doses. However, the research team found a rise in tyrosine with all doses, although the impact was less defined than the effect on HGA. In the 4 weeks the participants received nitisinone, no serious issues or side events were identified. Studying the safety and effectiveness of nitisinone after four weeks was not included in the trial [28, 29].

SONIA 2

The SONIA 2 trial investigated the safety and effectiveness of nitisinone in the treatment of AKU. In the study, 138 patients with an inborn error of metabolism received either nitisinone or no manufacturing drug in a randomized manner. The main finding, after a year, was that HGA is regularly excreted in urine. Other outcomes of interest were issues of safety and how the patient was assessed clinically. Based on the findings, the urine HGA excretion was reduced by 99.7%

in patients who took nitisinone compared to the control group. The low level of homogentisic acid found in alkaptonuria also corresponds with its main cause which is the body's reduced ability to accumulate homogentisic acid [30]. Nitisinone consistently showed effective results when used with different patient groups and at different study locations. Besides, the research tracked the progress of the illness by using the AKUSSI. Unlike the control group, the group treated with nitisinone had a much less noticeable improvement in the AKUSSI score over the 48-month period. Nitisinone treatment appears to have slowed AKU and improved the main symptoms of the disease. However, the investigation also examined the safety of using nitisinone. Three out of ten patients in the nitisinone group developed an adverse event, while only one out of ten patients in the control group experienced one. The reason behind discontinuation in the nitisinone group was most often due to side effects. Notably, there were no deaths associated with the treatments during the study. Special attention was given to the development of eye issues related to tyrosine in certain individuals on nitisinone treatment. Because tyrosine was found in nitisinone, it caused some of these side effects to influence corneal eye tests [31]. Although the symptoms could often be controlled and reversed, they still point out the need for careful care of patients treated with nitisinone.

Alkaptonuria concerning other disorders

Relation of AKU to Kidney stones

A study was conducted that seeks to find the chemical composition of renal stones in alkaptonuria patients, delivering information on the genesis and pathophysiology of these stones. The researchers collected renal stones from a 48-year-old alkaptonuria patient and compared them with stones from a 48-year-old non-alkaptonuria patient. Inductively coupled plasma-mass spectrometry (ICP-MS) and Fourier transform infrared spectroscopy (FTIR) were used to investigate the stones [32]. On the report of the findings, the alkaptonuria stones had 33 times more sulfur than the non-alkaptonuria sample. The results suggest that the initial growth and progression of renal stones from COM crystals in people with alkaptonuria depend majorly on sulfur-rich proteins. It unveiled a theory about the development and worsening of ochronotic stones in people living with alkaptonuria. The theory depends on the interaction between benzoquinone acetate and sulfur-containing proteins which helps COM clusters to accumulate

[33]. The theory holds that kidney stone formation in alkaptonuria is more related to sulphur-containing proteins.

The identification of ochronotic pigment constituents in alkaptonuria renal stones is challenging, and further research is required to understand how circulating HGA in alkaptonuria patients can interact with biological molecules, uncovering the exact molecular mechanism underlying renal stone formation. Understanding the nature of calculus is vital, as it helps physicians discover acceptable treatment techniques or devise more efficient stone prevention strategies to avoid the reoccurring development of calculi in alkaptonuria patients [34].

Relation of Alkaptonuria to osteoarthropathy

Osteoarthropathy (OA) is defined by early start and quick development of degenerative alterations like those of osteoarthritis [35]. One of the most frequent causes of OA is chromosomal damage to the joint cartilage.

Early osteoarthropathy in AKU results mainly from alterations in the composition of the cartilage. Histological images in the study reveal that ochronotic pigment is often linked to cartilage problems in alkaptonuric joints, as well as collagen or matrix proteins. Apart from the loss of calcified cartilage and bone beneath the joint, changes in the bone demonstrate a different pattern of mechanical forces. Morphological irregularities probably result from alterations in the extracellular matrix [36]. The results indicate that the found changes in cartilage material might not relate to specific changes seen under the microscope in the articular cartilage and subchondral bone plate. This is caused by HGA accumulating in regions that were previously occupied by GAG which aid in the function of healthy cartilage and have changed in structure. The mechanism probably results in accelerated cartilage aging and changes in its composition in AKU. In particular, the stiffness of the pigmented cartilage in AKU is superior to that seen in OA and regular cartilage. The rigidity is believed to be caused by changes in the ochronotic pigment and the shape of the matrix [37]. Additionally, the results showed that GAG levels in AKU cartilage were lower which encourages more extracellular protein movement. In turn, AKU samples had greater amounts of total protein and more matrix crosslinks. In AKU, cartilage grows faster because of an altered matrix composition with less GAG and more proteins.

The scientists also analyzed samples of cartilage from individuals with AKU, OA and non-OA. Results showed that total protein extracted from AKU cartilage was higher than found in OA or non-OA samples, but the amount of extractable GAG was considerably less in AKU cartilage. Both deamidated COMP (D-COMP) and aged cartilage oligomeric matrix protein (COMP) display the same disease-related changes [38]. These results suggest that less repair of AKU joint cartilage and the slow release of certain cell compounds contribute to early osteoarthropathy in these patients. These essential molecules are virtually never replaced, because they disappear too quickly or decrease slowly and they do not have much time to regenerate due to rapid aging, injuries, changes in the environment or fast molecular degradation. Because of this, AKU patients often see quick development of the disease. The matrix surrounding cells is permanently tied to these structures through the presence of ochronotic pigment in cartilage [39]. The interaction within the ochronotic matrix is likely due to a polymerization of the cartilage's water gaps. In this way, these methods lead to an initial matrix of cartilage that marks AKU patients as having conditions that stimulate premature osteoarthropathy.

Patient perspective

In 2020, a patient survey took place to discover the ways in which AKU-related symptoms were affecting patients' daily lives [9]. Based on the research, the most important symptoms for patients with AKU are pain, loss of capacity and difficulty completing everyday tasks. According to another ScienceDirect study, although AKU does not lower life expectancy, it does negatively impact a person's quality of life [40]. When AKU is advanced, it leads to more diseases in patients that change their lifestyle and work habits for the worse.

To assess how patients affect the disease, researchers use the Alkaptonuria Severity Score Index (qAKUSSI). The healthcare literature suggests that both male and female patients have higher scores on the qAKUSSI as they get older, suggesting their disease is getting worse [40, 41]. Goals of AKU therapy are to improve daily living and to lessen the symptoms of the disease. AKU sufferers have several treatments available such as joint replacement surgeries, physical therapy and pain relief methods, to address damage in bones and joints, control discomfort and preserve mobility. However, there is not yet any particular treatment approved for this condition.

Challenges and future directions

Alkaptonuria is a rare illness that affects several systems, causing difficulties in research, identifying it and providing suitable treatment. Achieving a large enough sample size for research necessitates global cooperation and presents challenges regarding patient recruitment and specialist expertise [42]. There are many areas where it is challenging for patients to receive therapy early due to the absence of practitioners with experience. As a result, many AKU issues are missed, delaying or giving an incorrect diagnosis to the patient. It can be difficult to make targeted drugs and treatment plans for AKU patients because the disease impacts many different organs and systems. In the early days, little was known about AKU, but with recent advances, researchers are finding hope for new approaches. Nevertheless, progress in treatments for AKU was recently acknowledged with regulatory approval of the first drug for the condition [30]. Even though there have been many developments, it is still difficult to make sure all affected people can access these treatments in places with limited medical equipment. Ultimately, unique challenges exist in researching, diagnosing and treating alkaptonuria because it is both a unique disease and affects many areas of the body [9]. While discoveries have opened doors to more research and innovative treatments, the community is just as committed to solving problems in research methods, better diagnosis and making efficient care affordable. Those who study AKU, medical practitioners and patient communities have to work together to tackle these issues and enhance the wellbeing of people with AKU [43]. Patient organizations such as the AKU Society have played a critical role in raising awareness, providing support, and facilitating access to specialized care for those with AKU.

Conclusion

The rare disorder of AKU is caused by a missing enzyme called HGD that stems from mutations in the HGD gene on chromosome 3q21-q23. As a result of this enzyme deficiency, HGA builds up and leads to ochronosis, a disease where dark pigments form in connective tissues and joints, causing swollen joints, early wear and tear on joints and heart valve problems. After Sir Archibald Garrod described the disorder over a century ago, extensive research into it and its genes has taken place, mostly where it occurs more frequently such as Slovakia and the Dominican Republic. By understanding the disease at the molecular level, scientists have

introduced genetic testing and counseling and found that how severe the disease is often depends on certain types of genetic changes. Traditionally, AKU has been managed mostly by supportive and palliative care, with few or no benefits from eating specific diets or taking vitamin C. Many recent advances have brought about the use of nitisinone as the first treatment that can modify AKU symptoms. Nitisinone prevents the work of HPPD, an enzyme that comes before tyrosine breakdown, resulting in a fall in HGA levels. Trials such as SONIA 2 have found that using nitisinone can lower HGA levels by more than 99%, thus significantly slowing the disease. Nitisinone has been given the approval by the European Medicines Agency for treating AKU, representing a big step in its management. Overall, much progress has been made in understanding and treating AKU thanks to breakthroughs in genetics and clinical studies. Introduction of nitisinone into treatment has changed the situation, as patients now have a greater chance for improvement and enjoy higher overall quality of life.

Abbreviations:

- 4-Hydroxyphenylpyruvate Dioxygenase (HPPD)
- Alkaptonuria (AKU)
- Alkaptonuria Severity Score Index (AKUSSI)
- Cartilage Oligomeric Matrix Protein (COMP)
- Congo Red (CR)
- Deamidated COMP (D-COMP)
- Dried Urine Spot (DUS)
- Fourier Transform Infrared Spectroscopy (FTIR)
- homogentisate 1,2 dioxygenase (HGD)
- Homogentisic Acid (HGA)
- Inductively Coupled Plasma-mass Spectrometry (ICP-MS)
- Osteoarthropathy (OA)
- Suitability of Nitisinone in AKU (SONIA)
- Thioflavin T (Th-T)
- Transmission Electron Microscopy (TEM)

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Bintoo Sharma are major contributor in writing and drafting the manuscript. All authors read and approved the final manuscript.

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