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Alkaptonuria, a Rare Genetic Disorder and its Molecular Basis

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ABSTRACT

Alkaptonuria (AKU) involves a one of a noticeable place in the historical background of human hereditary qualities since it was the principal infection to be translated as a Mendelian latent attribute by Garrod in 1902. Alkaptonuria is an uncommon metabolic issue coming about because of loss of homogentisate 1, 2 dioxygenase (HGO) movement. Mutations become the cause of this leads which may be due to loss-of-function mutation and missense mutation. Influenced people gather expansive amounts of homogentisic corrosive, a mediator result of the catabolism of tyrosine and phenylalanine, which obscures the urine and stores in connective tissues bringing on an incapacitating joint inflammation. Dietary confinement of tyrosine and phenylalanine lead to a decrease in the generation of HGA, however, a serious limitation of the mentioned amino acids is harmful in the long run and might be perilous.

Keywords: Alkaptonuria, Homogentisic Acid, Phenylalanine, Tyrosine, Connective Tissue

I. INTRODUCTION

Alkaptonuria is a genetic dysfunction which is due to the deficiency of homogentisate 1,2-dioxygenase which functions by converting homogentisic acid (HGA) into maleylacetoacetic acid in a pathway degrading tyrosine. Indication of HGA in the urine, arthritis affecting spine and larger joints and ochronotic that causes bluish-black pigmentation in connective tissue are the three main significant symptoms of alkaptonuria. When HGA excreted in urine gets oxidized, a product similar to melanin is generated which causes the urine to turn black. Other symptoms may include deposition of pigment, calcification of aortic or mitral valve and sometimes renal stones. Occurrence of alkaptonuria is due to the expression of autosomal recessive genes. Every offspring of a person with a homologous recessive gene for the disease is at a risk of 25% for the disease expressing itself, and an asymptomatic carrier is at a risk of 50% and a person who is not a carrier of the disease and is not affected is at a risk of 25% (Baker et al., 1948).Alkaptonuria or ochronosis is a worldwide disease whose prevalence in Slovakia has been observed to be 1:19,000. Alkaptonuria is diagnosed by checking the intensity of the amount of homogentisic acid in the urine by gas chromatography-mass spectrometry analysis. Alkaptonuria individuals excrete homogentisic acid ranging in between one and eight grams daily. If the alkaptonuria patient is heterozygous, two types of tests are recommended, Molecular genetic testing and Biochemical testing. Biochemical testing is not suitable in case of detection of carrier. Apart from this medical treatment, patients should avoid uplifting heavy objects that cause stress to spine and large joints preventing severe arthritis to take place. Moreover, vitamin C has been found beneficial in reduction and lowering levels of ochronosis and homogentisic acid by attempts of giving a low-protein diet but experiments did not support the role of vitamin C in reducing ochronosis and this idea was disproved later on. The symptoms exhibited by alkaptonuric patients are treated by physical therapy to maintain flexibility and muscle strength, knee, hip and shoulder should be replaced when in need, removing renal and prostate stones by surgical intervention. Recent research introduced a drug named nitisinone which is found to decrease the homogentisic acid levels in and it is under research that whether it can cure the other symptoms caused by disease too. This disease has equal effects on males and females. It is observed that the symptoms develop severe in males as compared to females. It is a rare disease. It may occur in one in every 250,000-1,000,000 births.

Slovakia and Germany are the areas where the increased frequencies of this disorder have been found (Srsen et al., 2002).

Diagnosis

Firstly, ochoronosis is a bluish blackish pigmentation which is due to the accumulation of Homogentisic acid and its oxidation products in the connective tissues. It may occur in the eye tissues, but the pigmentation has no effect on vision. Pigmentation is also observed in thed ear cartilage. An increase of HGA and its oxidation items (e.g., benzoquinone acidic corrosive) in connective tissue stimulates ochronosis. Dark pigmentation of the sclera is seen watched halfway between the cornea and the external and internal canthi at the addition of the recti muscles (Osler's sign). Ear ligament pigmentation is first found in the antihelix and concha, and later in the tragus. The ligament is slate blue or dim and feels sporadic or thickened. Calcification of the ear ligament might be seen on radiographs (Spencer et al., 2004). Color additionally shows up in cerumen and in sweat, creating staining of attire. The sweat organs are rich in ochronotic shade granules, and intradermal infusion of epinephrine into the skin of the axillary vault will yield dark colored dark sweat beads in the follicular a holes. A profound purple staining might be seen on the skin of the hands, comparing to the fundamental ligaments, or in the web between the thumb and the pointer. Broad or quickly dynamic skin pigmentation has been accounted for with impeded renal status, apparently because of diminished renal leeway of HGA. The staining tends to be most articulated in sun-uncovered locales, in ranges of high eccrine organ thickness, for example, the axillae, palms, and soles, on the private parts, and in ligament, particularly of the ear and nose (Millucci et al., 2015). The determination of endogenous ochronosis depends on individual and family history, urine testing, and histology. Expected consequences of demonstrative reviews tests incorporate subjective examines for HGA. The nearness of HGA can be affirmed with gas chromatography and mass spectrophotometry. The measure of HGA discharged every day in people with alkaptonuria is frequently 1-8g. A typical 24-hour pee test contains 20-30mg of HGA.

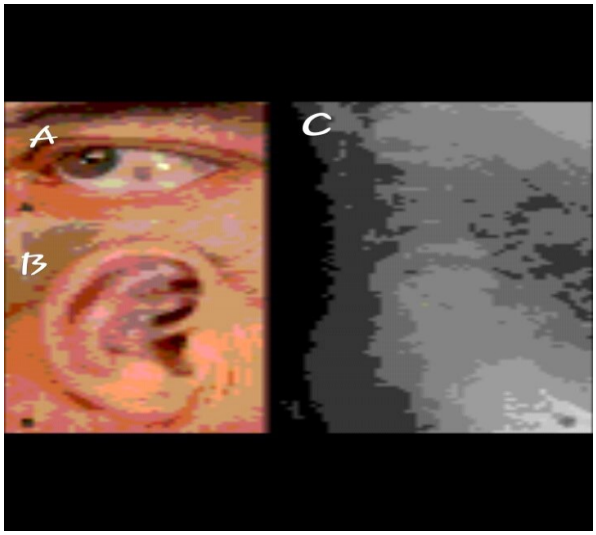

Figure A) Sclera of the eye. B) Antihelix and concha effected by oschronosis. C) Findings of the lumbar spine with disc flattening, calcification, and osteophyte formation

Another way to diagnose alkaptonuria is the presence of dark urine. A normal urine sample contains 20-30 mg of Homogentisic acid, but the patients with alkaptonuria contains between the 1 to 8 grams per day. Oxidation of the HGA discharged in the urine creates a melanin-like item and makes the urine turns dim in the wake of standing. People with alkaptonuria often have dim or that turns dim on standing or introduction to an antacid specialist, in any case, obscuring may not happen for a few hours subsequent to voiding and numerous patients never watch any anomalous shading to their . Diaper staining could be the primary indication of the malady (half). The severity of this disease varies within the members of same family. The studies show that the affected person life span is not disturbed by this disease. Human body functions are so interlinked that if one system is not working properly, we can see its adverse effects on the other systems as well. Pigments may accumulate in the heart valves and blood vessels and cause the valve calcification which causes the backward flow of the blood through a defective heart valve. Joint diseases occur: knees, shoulders and hips are affected. kidneys are responsible for the secretion of Homogentisic acid in a heavy amount in the affected person, due to which kidneys are also affected. The impaired renal function causes the development of ochronosis. A few patients have also a history of the renal and prostate stones which require surgical removal (Zatkova et al., 2003).

Case Report

A European male was diagnosed with Alkaptonuria at the age of 8. At the same time of diagnosis, biochemical testing and analysis confirm the presence of Homogentisic acid in the . Signs of ochronosis were developed at the age of 12 on ears. Before 25 years of age he got shoulder and lower back pain. His shoulder joints had affected very severely cause swelling and limited mobility. His spine become impaired thoracic, cervical and lumber mobility. All the weight bearing joints of knees, vertebral columns and joints of hips showed a severe cartilage damage. Testing shows that muscles due to constant pain become slightly weak. The patient showed a mood disorder by chronic mildly depressed or irritable mood often accompanied by other symptoms like limited mobility. Patient experienced constant pain in the toes and feet. Patient had no trouble with speech, swallowing and vision. Sensory testing was also normal. Patient endured the surgical replacement of the right shoulder. The surgery improved the range of motion. He was stick to a lower protein diet and he was also given steroid injections in both shoulders, to reduce pain, but they had no effect to change his quality of life.

His X-rays studies are as follows:

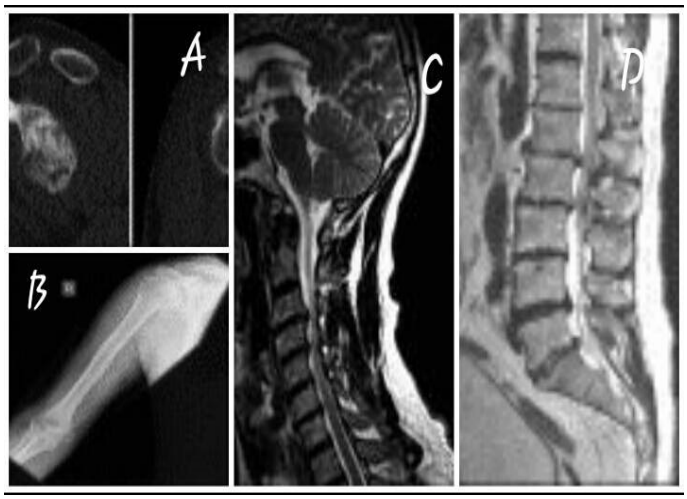

Figure A shows a CT scan of left (left image) and right (right image) shoulder indicating advance arthropathic of glenohumeral joint with aggregate loss of joint space, checked subchondral sclerosis and subchondral blister arrangement. Figure B is a displayed X-ray of right shoulder indicating uniquely progressed degenerative change with considerable narrowing of the glenohumeral joint space, noteworthy sclerosis and subchondral blister arrangement. Figure C shows a sagittal view of T2-weighted MRI of C-spine shown multi-level direct circle space narrowing, diffuse plate swells, osteophyte development, and circle herniations. Figure D contains a A sagittal view of T2-weighted MRI of lumbar spine (Phornphutkul et al., 2002).

Discussion of the case

The patient family history was unremarkable, no one ever had Alkaptonuria and Osteoarthritis, but he got ochronosis at the age of 12 and all the other symptoms like black urine, ear pigmentation, lower back and shoulder pain were present before the age of 25. The numbness of feet and toes were appeared due to the impairment of cervical, thoracic and lumber mobility, causing the hindrance in the normal transmission of spinal signals. Due to the limited mobility patients undergone the depression state and developed psychological issues, due to the inability to do daily activities. They become isolated, anti-social and feels unworthy. This phase is largely ignored by doctors. One more thing which is noticed that the surgical replacement of the shoulder and managing of the diet increased the range of the motion due to which his daily routine activities improved his quality of life. Lifestyle therapies and counselling and psychotherapy effectiveness in treating Alkaptonuria is unknown (Oexle et al., 2008).

Molecular causes of this disease

The basic defect in Alkaptonuria is the deficiency of the homogentisate 1,2- dioxygenase enzyme activity. The body cannot process the amino acids, phenylalanine and tyrosine, which occur in protein. HGD gene causes the mutation for the enzyme homogentisate 1,2-dioxygenase which degrades homogentisic acid. Due to the absence of the enzyme homogentisic acid and its oxidized form alkapton are excreted in urine, giving urine a dark color. Every person contains two copies of HGD gene from their parents that have genetic information for the production of homogentisate 1,2- dioxygenase enzyme. This enzyme is normally found in body tissues such as small intestine, kidney, liver and prostate, colon. People who have alkaptonuria have abnormalities in both of copies, due to which body cannot produce sufficient of this enzyme and as a result homogentisic acid accumulates in the body. The normal HGD enzyme has six subunits which are organized into two trimers. It also

contains an iron atom. Researches have determined that the HGD gene is present on the chromosome 3. As we know human cells have 46 chromosomes carrying different genes. Each chromosome has short arm which is indicated as “p” and long arm indicated as “q”. the HGD gene is present on the long arm of chromosome 3. Due to the missense mutations, HGD gene is mutated and as a result HGA accumulates in various tissues and changes the tissue color to black and damages the tissues (Zouheir Habbal et al., 2014)

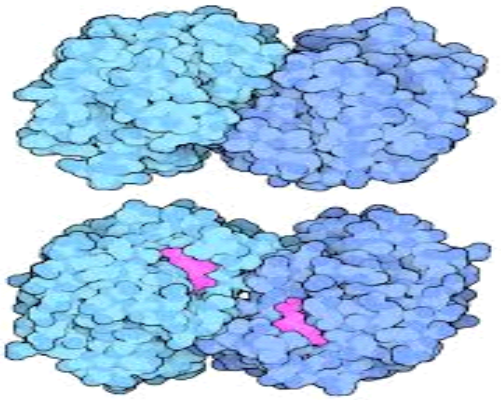

Figure . Two trimers of HGD enzyme

Different mutations happen like missense mutation which leads to this disease. Evidence also shows that one of these missense mutation is a result of the loss-of-function mutation. So that is why different mutations become the cause of Alkaptonuria (Gehrig et al., 1997). These mutations alter the structure, solubility and functioning of the enzyme. Very often this disease also transmitted to the progeny in autosomal dominant way. In this autosomal dominant fashion, only one of the copy from a single parent is linked with the disease. The below diagram shows the pathophysiology of alkaptonuria, which is due to the absence of HGD enzyme in the liver. The cycle is as follows:

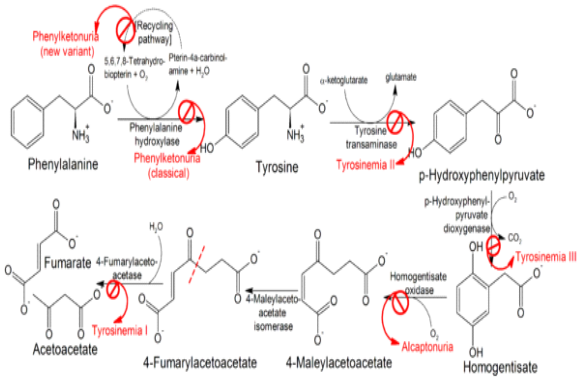

Figure Pathophysiology of alkaptonuria

Tyrosine degradation pathway

Tyrosine and phenylalanine are aromatic amino acids, which enter into the blood stream through food contains foods. They are required for many functions such as regulation of the hormones, melanin pigment, but the 95 percent of these amino acids remain unused and is chemically processed by HGD enzyme. Homogentisic acid is generated by tyrosine which is metabolized by HGD enzyme. As we have discussed in Alkaptonuria, HGD enzyme lost its function due to mutation in the HGD genes and cannot processed the homogentisic acid, which accumulates in the body tissues. Normally HGA acid is metabolized into 4- maleylacetoacetate. Higher levels of HGA in the body forms polymers by the conversion of HGA into benzoquinone acetic acid. These polymers are like melanin and deposits into the connective tissues and results in the ochronosis (Fernández-Cañón et al., 1966).

Therapies

Reduction of phenylalanine and tyrosine allegedly lessened homogentisic corrosive discharge in the urine of a child. In a grown-up, a comparative confinement purportedly had no impact on discharge of the anomalous metabolite. Regardless of whether a mellow dietary limitation from ahead of schedule in life would stay away from or limit later entanglements is not known, but rather such an approach is sensible. Vitamin C, as much as 1 g/d, is suggested for older youngsters and grown-ups. In earliest stages, a background marked by dim recolored diapers ought to caution the doctor to alkaptonuria. Infants, youthful kids, and asymptomatic youthful grown-ups can be assessed with basic urine testing on an outpatient premise. Medical treatment is utilized to improve the rate of shade affidavit. This limits articular and cardiovascular difficulties in later life. Reduction of phenylalanine and tyrosine has apparently diminished homogentisic corrosive discharge. Regardless of whether a mellow dietary limitation from right on time in life would maintain a strategic distance from or limit later difficulties is not known, but rather such an approach is sensible. Vitamin C, as much as 1 g/d, is prescribed for more older youngsters and grown-ups. The gentle cancer prevention agent nature of ascorbic corrosive retards the procedure of transformation of homogentisate to the polymeric

material that is saved in cartilaginous tissues (Kaspar et al., 2005).

Limited utilization of nitisinone, an inhibitor of the catalyst 4-hydroxyphenylpyruvate dioxygenase, which intercedes arrangement of homogentisic corrosive, has been accounted for. Urinary homogentisate discharge was notably decreased, however security of delayed utilize is as yet an open question. Nitisinone is a drug which can be used for the treatment of alkaptonuria. Researches demonstrated that this drug can easily reduce the HGA level in the urine up to 95 percent in AKU patients. Practical's have done on the alkaptonuria mice which was treated with this drug and did not develop ochronosis. However, the untreated alkaptonuria mice developed ochronosis (Introne et al., 1998). No treatment is demonstrated to anticipate or rectify the pigmentary changes of ochronosis. Dietary confinement of phenylalanine and tyrosine has been proposed to decrease the generation of HGA, yet serious limitation of these amino acids is not handy in the long haul and might be perilous. No believable reviews have shown the clinical viability of ascorbic corrosive. Oral bisphosphonate treatment has been recommended to end the dynamic bone misfortune, in any case, a planned investigation of four influenced people neglected to show profit (O'Brien et al., 1962).

II. CONCLUSION

Alkaptonuria is a multidimensional disease with developed neurological, physiological and psychological issues. Alkaptonuria patients having a customary eating routine create ∼5–7 g of HGA/d and significantly more than that on an eating regimen higher in protein. The greater part of this item is efficiently wiped out in the urine, on the grounds that HGA is effectively discharged by the kidneys. Renal discharge of HGA remains the vital line of resistance for the alkaptonuria: it diminishes the buildup of HGA in alkaptonuria tissues and in this way, moderates the improvement of ochronosis and joint inflammation. Giving the missing HGO to alkaptonurics ought to deliver a lot of the normal item maleylacetoacetic corrosive (MAA) (Granadino et al., 1977). This corrosive typically is then con-verted, by the following chemical, an isomerase, to fumarylacetoacetate corrosive (FAA), and the last is part, by a hydrolase, into fumarate and acetoacetate.

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