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Accidental Diagnosis of Alkaptonuria in a Suspected Case of Mucopolysaccharidosis

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Abstract: Alkaptonuria is a very rare genetic disorder characterized by the lack of enzyme homogentisate oxidase in tyrosine metabolism. This leads to the accumulation of homogentisic acid in cells and body fluids. The clinical feature includes arthritis, ochronosis which is the pigmentation of cartilage and darkening of urine on standing. The disease is usually diagnosed by assessment of signs and symptoms of ochronosis and confirmation of suspected diagnosis can be achieved by detection of homogentisic acid in urine with increase in amino acid tyrosine in plasma. In this case study, the disease is diagnosed by silver nitrate test followed by ferric chloride test. Further confirmation was done by amino acid analysis using high performance liquid chromatography (HPLC).
Keywords: Mucopolysaccharidosis, Homogentisic acid, Arthritis, Ochronosis, Black urine.

INTRODUCTION

Inborn Errors of Metabolism (IEM) form a large class of genetic diseases involving congenital disorders of metabolism. The majority are due to defects of single genes that code for enzymes that facilitate conversion of various substances (substrates) into others (products).The term Inborn Errors of Metabolism was coined by a British physician, Archibald Garrard (1857-1936), in the early 20th century (1908).

He is known for work that prefigured the "one gene-one enzyme" hypothesis, based on his studies on the nature and inheritance of Alkaptonuria (AKU) [1]. He reported that patients complain their underwear get blackened. Alkaptonuria is a rare autosomal recessive disease caused by a deficiency of enzyme, homogentisate oxidase in tyrosine metabolism leading to the accumulation of homogentisic acid [2].

Homogentisic acid gets oxidized slowly to benzoquinone acetate which is polymerized to a black colored pigment alkapton. Alkapton is deposited in bones, connective tissue and other organs (nose, pinna of the ear and so on) causing a condition known as Ochronosis (because of the ochre color of the deposits). When HGA binds to collagen, cartilage matrix becomes stiffened, resulting in the aberrant transmission of loading to underlying subchondral bone. Aberrant loading leads to the formation of pathophysiological structures including trabecular excrescences and high density mineralised protrusions (HDMPs). These deposits may be responsible for the occurrence of arthritis in the fourth and fifth decades of life [3].

Alkaptonuria plays a very important role in the history of human and biochemical genetics. Human chromosome 3q21–q23 codes for the AKU gene and an animal model the *aku* mouse is developed. Subsequently, the first gene encoding an HGO enzyme was cloned from the ascomycete fungus *Aspergillus nidulans*. Two missense mutations cosegregating with alkaptonuria in two Spanish pedigrees established HGO as the defective gene in alkaptonuria. The identification of a third missense and a frame shift mutation in Slovakian families further confirmed the role of HGO mutations in the pathogenesis of the disease [4].

The first symptoms, occurring in early adulthood, involve a painful, progressively debilitating arthritis of the spine and large joints. Cardiac valvular disease and renal and prostate stones occur later [5]. The clinical features exhibited by the alkaptonuria patient may also be observed in some other genetic disorders of which the Mucopolysaccharidosis has got the close resemblance to that of alkaptonuria.

Mucopolysaccharidosis are a group of metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down molecules called glycosaminoglycans. These long chains of sugar carbohydrates occur within the cells that help

build bone, cartilage, tendons, corneas, skin and connect ive tissue. Glycosaminoglycan's (formerly called mucopolysaccharides) are also found in the fluids that lubricate joints [6].

The mucopolysaccharidosis share many clinical features but have varying degrees of severity. These features may not be apparent at birth but progress as storage of glycosaminoglycans affects bone, skeletal structure, connective tissues, and other organs. Neurological complications may include damage to neurons (which send and receive signals throughout the body) as well as pain and impaired motor function. Physical symptoms generally include coarse or rough facial features (including a flat nasal bridge, thick lips, and enlarged mouth and tongue), short stature with disproportionately short trunk (dwarfism), dysplasia (abnormal bone size and/or shape) and other skeletal irregularities, thickened skin, enlarged organs such as liver (hepatomegaly) or spleen (splenomegaly), hernias, and excessive body hair growth[7].

CASE REPORT

A 5year old male child, born to the non-consanguineous parents, presented with retarded growth to the department of Pediatrics, Kasturba Hospital, Manipal, India. The clinical examination revealed the features of Short stature and low IQ along with dental abnormalities, Myopia, syndactyly, Osteopenia with curved radius bone. The clinician suspected it to be a case of Mucopolysaccharidosis and to confirm the same; the urine sample was sent to the Biochemistry Lab. The test for Mucopolysaccharide(MPS) was found to be negative. Hence we tested the urine sample for other IEM and found it to be positive for Alkaptonuria. For further investigations we requested for the blood sample of the child and analysed the amino acid profile using High Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS

- Random urine and blood sample was collected

Tests for mucopolysacharides (MPS) in the urine:

- Dimethylene Blue Test and CPC(Cetyl Pyridinium Chloride)[8]

Tests for Alkaptonuria

- Benedict's Test[9]
- Ammonical Silver nitrate test [9]
- Ferric chloride test[9]
- High Performance Liquid Chromatography(HPLC) for plasma amino acid profile[10]

RESULTS

- Tests for MPS were found to be negative which prompted us to go for the tests for alkaptonuria.
- In the Portion of the urine kept open, exposed to air, black color was observed.
- The urine when heated with Benedict's qualitative glucose reagent developed dark greenish black supernatant and a yellow precipitate of cuprous oxide.
- Pitch black color was observed immediately in Ammoniacal Silver Nitrate Test
- Fleeting green color was observed in Ferric Chloride Test
- HPLC of plasma showed 9 fold increases in tyrosine (Figure 2) when compared to the reference value of the same age group children [10].

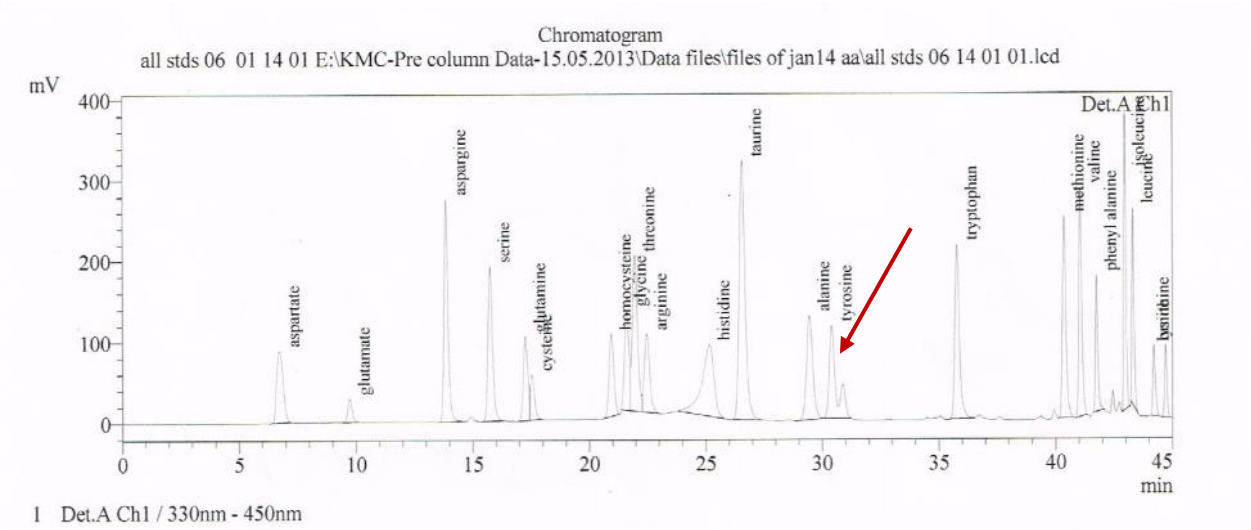

Fig-1: Showing standard amino acid peaks. The arrow mark indicates the standard tyrosine peak

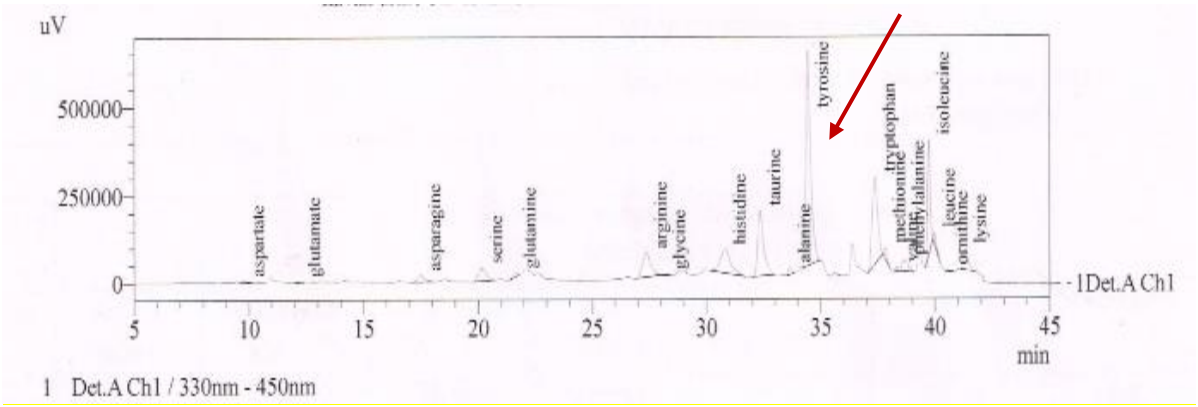

Fig-2: Plasma amino acid profile of the patient. The arrow mark indicates the tyrosine peak

DISCUSSION

In the above mentioned case the child was suspected with mucopolysaccharidosis possessing the skeletal deformities that resembled with that of many other Inborn Errors of Metabolism like Alkaptonuria, Homocystinuria, Gaucher's disease etc. In the absence of positive findings for MPS, tests for other metabolic intermediates were performed and we could arrive at the diagnosis as alkaptonuria.

Alkaptonuria is a rare autosomal recessive disease caused by a deficiency of enzyme, homogentisate oxidase in tyrosine metabolism leading to the accumulation of homogentisic acid [2].

As per the screening programme conducted by the Research Laboratory for Clinical Genetics at Martin in Slovakia by over 611,000 inhabitants (509000 newborns) the world-wide highest incidence of AKU (1 in 19,000) was recorded, and a total of 208 patients (110 children) were registered[11].

Homogentisic acid is a normal intermediate in the metabolism of tyrosine and normal individuals do not excrete HGA because it is converted into maleylacetoacetic acid by the enzyme homogentisate 1, 2 dioxygenase. AKU patients excrete high concentrations of HGA in urine (range 4-8 g/day) [12].Homogentisic acid gets oxidized slowly to benzoquinone acetate which is polymerized to a black colored pigment alkapton. Alkapton is deposited in bones, connective tissue and other organs (nose, pinna of the ear and so on) causing a condition known as Ochronosis (because of the ochre color of the deposits). These deposits may be responsible for the occurrence of arthritis in the fourth and fifth decades of life[13].

In addition to cartilage, homogentisic acid accumulates in other connective tissue including tendons and ligaments and even bone. Over time, affected tissue becomes discolored, brittle and weak. Affected individuals may develop abnormalities affecting the tendons including thickened Achilles tendons and inflammation of the tendons (tendonitis). Affected tendons and ligaments may be particularly susceptible to rupturing. Eventually, discoloration of tendons may become visible on the overlying skin [14].

The initial diagnosis was done on urine getting blackened on exposure to air. Further by Ammoniacal silver nitrate test, black precipitate was obtained on addition of ammonium hydroxide. Ferric chloride test was done which gives fleeting green color immediately on addition of ferric chloride [9]. Further to confirm the above findings, quantification of plasma tyrosine was done by High Performance Liquid Chromatography, which showed 9 fold increases in tyrosine confirming the defect in tyrosine metabolism. Increased level of tyrosine observed in this case is a consequence of metabolic block in the homogentisate oxidase step.

Based on the above laboratory reports, the clinician could analyze the patient's symptoms from the proper angle and could change the treatment modality.

The treatment includes the restriction of phenylalanine and tyrosine in the diet and administration of vitamin C (ascorbic acid) which is used to stall the pigment deposition on collagen [2]. Recently, Nitisinone, a potent inhibitor of the second enzyme in the tyrosine catabolic pathway, is considered as potential therapeutic agent who showed 95% reduction in urinary HGA as reported in the study [5].

CONCLUSION

Alkaptonuria in spite of being known to the clinicians since many years, is still a under diagnosed disease due to the overlapping features with other genetic conditions. However, increase in case reporting and research about alkaptonuria would help in providing the patients the scope for the proper diagnosis and care [15]. Considering the present trend of going in for the advanced technological investigations like Tandem Mass Spectrometry and Gene analysis which becomes a burden to the patient economically, the tests mentioned in our study offer a cost effective and yet reliable diagnostic aid. Increased awareness about the availability of such cost effective and convenient

methodology may encourage the patients as well as the clinicians to approach the laboratory for the diagnostic help and the intervention may be started at the earliest.

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