Alkaptonuria: An Inborn Error of Amino Acid Metabolism

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Background: Alkaptonuria (AKU) is a rare hereditary metabolic disorder that occupies a unique position in the history of medical and biochemical genetics because it was the first human metabolic disorder to be interpreted as genetically determined. This condition is characterized by deficiency of HGAO, an enzyme that is mainly found in hepatocytes. The medical interest in alkaptonuria stems from its association with ochronosis, arthropathy and homogentisicaciduria.

Objectives: The objective of the present study was to screen urine of children, who were less than two years of age for detection of inborn errors of amino acid and carbohydrate metabolism-with particular reference to: 1. Phenylketonuria (PKU), 2. Alkaptonuria (AKU), 3. Galactosemia, 4. Fructosuria and 5. Pentosuria.

Place of study: This study was performed in the department of chemical pathology of Postgraduate Medical Institute, Lahore, which is part of the first author's studies of M. Phil. Degree in Chemical Pathology.

Study Design: A cross sectional, hospital based study.

Methods: In addition to chemical tests, one dimensional descending paper chromatography was used in this study for the detection of hereditary metabolic disorders.

Results: Out of 2,000 children (1194 males and 806 females), only one female child having alkaptonuria was detected. The elder sister of this infant, investigated as part of the family study also revealed the presence of this condition.

Conclusion: The present study has established the fact that hereditary metabolic disorders like AKU also exist in Pakistan and the paper chromatography of the urine furnishes a simple technique to identify HGA / urinary sugars and amino acids.

Keywords: Hereditary metabolic disorders, Alkaptonuria, Homogentisicaciduria, Ochronosis, Arthropathy, Paper Chromatography, Pakistan.

Introduction

Alkaptonuria, an inborn error of aromatic amino acid (phenylalanine and tyrosine) metabolism is caused by a recessively inherited deficiency of the enzyme homogentisic acid oxidase (HGAO) that is normally found in the liver and kidney. This condition is characterized by accumulation in the body and excretion in the urine of homogentisic acid (HGA). The slow accumulation of the bluish-black polymer of HGA in connective and cartilaginous tissues produces a bluish- black discoloration of the cheeks, nose, sclerae and ears called 'ochronosis' which becomes evident by mid-adult life. Degeneration of the pigmented joint cartilages leads to arthritis of spine and peripheral joints.[1-6]

This is a very rare hereditary metabolic disorder (about 1 in 250,000). Although, cases have been reported from all over the world but the precise frequency of alkaptonuria is not known as most estimates depend on ascertainment of ochronosis. It is most common in certain areas of Eastern Europe, particularly in Slovakia, where the frequency is relatively high- one case per 19,000 populations.[2-3,7-8]

AKU, the prototypic inborn error of metabolism, occupies a unique position in the history of medical genetics because it was the first human condition to be interpreted by Garrod, as genetically determined and transmitted in a typical Mendelian recessive manner.[1-3,7-8] At the beginning of the last century, Garrod included three disorders of amino acid metabolism- alkaptonuria, albinism and cystinuria- to illustrate his concept of the inborn errors of metabolism, the rare disorders each characterized by a specific block in biochemical pathway and resulting in the disposal by some alternative route of a metabolite immediately preceding that metabolic block. He then postulated that these abnormalities were due to inheritable enzyme deficiency.[1]

Recent advances in the understanding of the molecular basis of AKU have verified that loss of function mutations in the HGAO gene are responsible for the disease.[7-9] A few mutations have been repeatedly detected in patients from different European countries.[8] Molecular analysis of the HGAO gene, has been mapped to chromosome 3.[10-12]

The clinical interest in alkaptonuria stems from its association with ochronosis.[13-14] During this process, an oxidation product of HGA, also known as 'alkaptone', accumulates slowly in the connective and cartilaginous tissue throughout the body and this in turn leads to a bluish-black melanin-like pigmentation, where it is toxic and harmful to the bones and the cartilage.[13] Ochronosis does not occur in body tissues until there is long exposure to HGA. This acid is derived from metabolism of both phenylalanine and

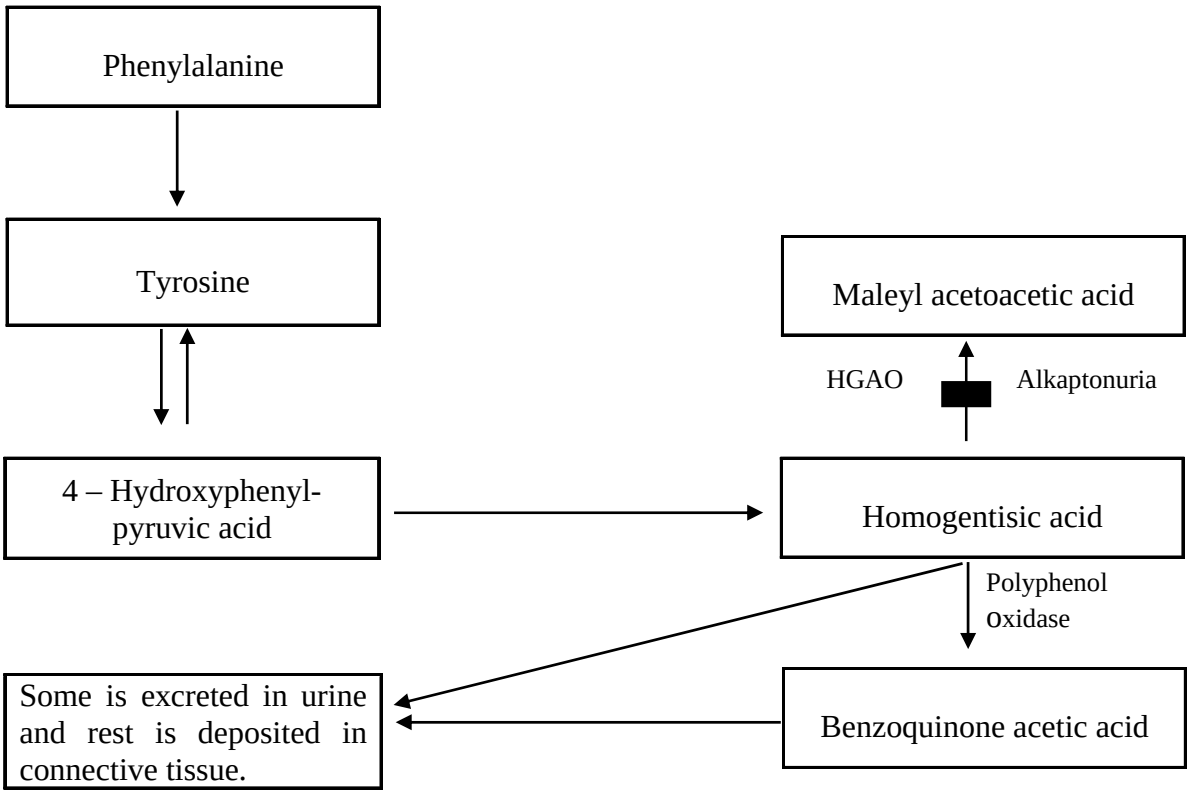

Figure 1: *Showing site of metabolic block responsible for AKU.*

tyrosine. In AKU, this acid (HGA) cannot be further metabolized due to deficiency of the enzyme HGAO that catabolizes the oxidation of HGA (figure 1). In the absence of this enzyme, HGA and benzoquinone acetic acid build up in the body and excreted in large amounts in the urine throughout the patient's life and rest is deposited in connective tissues. This leads to the characteristic features like darkening of urine, ochronosis and arthropathy.[13-19]

Bickel suggested that early detection and treatment of hereditary metabolic diseases must be carried out on large scale. This will not only provide early diagnosis but also will provide a guideline for the management of such cases and will help to develop them normally. [20]

Management of alkaptonuria is usually conservative.[21]

Aims and Objectives

The objective of this study was to screen urine of children who were less than two years of age for detection of inborn errors of amino acid and carbohydrate metabolism- with particular reference to: 1. Phenylketonuria (PKU), 2. Alkaptonuria (AKU), 3. Galactosemia, 4. Fructosuria and 5. Pentosuria.

Subjects and Methods

In this study, two groups of subjects were investigated systematically to detect inborn errors of amino acid and carbohydrate metabolism with particular reference to the above mentioned disorders.

Two thousand (2000) children of less than two-year ages were randomly selected for this study from pediatric departments of various Lahore hospitals. Early morning urine specimens were collected for detection of inherited metabolic disorders.

Chemical screening tests (Fehling's test and Benedict's test) and one dimensional descending paper chromatography were used to identify urinary sugars and amino acids in this pilot study.[22-24]

Paper Chromatography:

Each urine specimen was subjected to one dimensional descending paper chromatography for identification of amino acids and sugars. Urine specimens and standard solutions were applied as a series of spots and loaded paper chromatogram was developed in butanol, acetic acid and water (12:3:5), followed by sequential staining for amino acids and sugars.

Chromatography for HGA: Urine and reference solution were applied to the paper chromatogram and the chromatogram was developed in the solvent (butanol: acetic acid: water; 4:1:5) and the spots were localized by staining with 5% ammoniacal silver nitrate reagent. HGA spots gave an instant black color (Figure 2).[23-24]

Results

The result of this screening program was as follows:

Out of 2,000 children (1194 males and 806 females), only one female child having alkaptonuria was detected. The elder sister of this infant investigated as part of the family study also revealed the presence of this condition.

The history of these cases and chemical examination of the urine revealed very strong features of alkaptonuria. Freshly voided urine was normal in appearance but became dark brown cola after some hours, on standing at room temperature and this reaction was particularly noteworthy when alkali (10% NaOH or 3% $AgNO_3$) was added to the urine specimen.[22,23] The paper chromatography[23-24] and photometry[22] were used to confirm the diagnosis and presence of HGA in the urine (Table 1).

Fig. 2: *Descending paper chromatogram of urine showing HGA spots- (from L to R), 1-Urine of the case detected 2- standard HGA solution and 3- urine of the elder sister of the case.*

Discussion

This documented study on inborn errors of metabolism gave the results of a thorough biochemical screening by means of well proved methods. The study shows that the problem of inborn errors of metabolism does exist in Pakistan and we need to know their nature and incidence by means of national survey. We cannot pick cases of any disease unless we are conscious of its existence and suspect it. The need of his survey is indicated by the fact that the modern medicine has made it possible to prevent and manage this class of disorders.[3,21]

Table I: *Summary of Biochemical Findings in the Alkaptonuric Patients.*

Case no.	Age	Sex	T E S T S					HGA in urine (mg / dl)
			Transient blue – green color with 10% $FeCl_3$ reagent	Greenish-yellow turbidity with benedict's solution	Dark ring at the surface with 10% NaOH solution	Instant black color with 3% $AgNO_3$ reagent	Excretion of HGA in urine (chroma-tography)	
212	1 month	F	+	+	+	+	Confirmed	280
212a	6 years	F	+	+	+	+	Confirmed	312

HGA = Homogentisic Acid

Till recent past, diagnosis of metabolic disorders was made by the positive findings with the non-specific chemical tests. But in view of the other disorders showing false positive with these chemical tests, diagnosis of metabolic disorders is now confirmed with chromatographic methods. Therefore, in addition to chemical tests, one dimensional descending paper chromatography was used in this study for the detection of metabolic disorders[23-24] and proved quite satisfactory. The chromatography has the advantages of being cheap and capable of handling a large number of individual samples simultaneously on a single sheet. Thus the methodology employed in this pilot study, which is both cheap and reliable for detecting the maximum number of cases, can easily be adopted for the national survey program.

In the present study, the diagnosis of AKU was established by demonstrating HGA in the urine, which turned dark brown spontaneously on standing. This phenomenon is due to oxidation and polymerization of HGA. It is also pH-dependent; this reaction is particularly noteworthy if the urine is alkaline or when alkali is added to the specimen.[22,23] The diagnosis of alkaptonuria is further confirmed by detecting and measuring HGA in the urine by chromatography[24] and photometry.[22]

Conclusion:

The detection of a case of AKU during the present study has established the fact that this metabolic disorder does occur in Pakistan. Paper chromatography of the urine furnishes a

simple, reliable and economical technique to identify sugars and amino acids or HGA.

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