Phenylketonuria in Sohag: A Preliminary Study

Amira Mourad[1], Tahia H. Saleem[2], Nagwa S. Ahmed[3] and Sawsan M.A. Abuhamdah[4,*]

[1]Department of Medical Biochemistry, Faculty of Medicine, Sohag University [2]Department of Medical Biochemistry, Faculty of Medicine, Assiut University,[3] Department of Medical Biochemistry, Faculty of Medicine, Sohag University, Egypt,[4]Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, University of Jordan.

Abstract

Phenylketonuria (PKU) is one of the commonest inborn error of metabolism, it is an autosomal recessive metabolic genetic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional. The diagnosis of this disorder can be confirmed by analysis of urine components. The present study aimed to assess the prevalence of PKU among children aged 6 months to 6 years in Sohag governorate Egypt, its relationship to malnutrition and identifying families with higher predisposition to having children with inborn errors of metabolism. One hundred children were selected from 18,000 patients seen in the pediatric neuropsychiatry clinic of Sohag University hospital over three years, between May 2008 - May 2011. They were presented with clinical symptoms suggestive of probable preliminary diagnosis of PKU. Proper clinical and laboratory investigations, including ferric chloride test in urine, total protein and albumin in serum, were screened to confirm the diagnosis. PKU was diagnosed in two children cases. The diagnosed cases were suffering from mild malnutrition represented by low levels of serum albumin and total protein comparable to cases of Marasmus and kwashiorkor or other deficiencies like rickets. Screening of the newborn with special emphasis on PKU is highly recommended before discharge from the nursery for children delivered in the hospital or on first visit to the clinic for children delivered at home. Early detection would help prevent serious and permanent neurological impairment.

Keywords: Phenylketonuria (PKU), Inborn Errors of Metabolism (IEMs), Sohag Governorate, Egypt, Phenylalanine Hydroxylase, Neurological Impairment, Newborn Screening.

Introduction

Many inborn errors of metabolism (IEMs) are classified as organic acidemia, in which organic acids accumulate in the urine (Kuhara, 2007). Human urine contains numerous metabolic intermediates at a variety of concentrations that can provide clues for diagnosing inborn errors of metabolism and other genetic mutations (Albers *et al*., 2001; Blau *et al*., 1996; Imamura *et al*., 1999; Lee *et al*., 2011).

For most of the cases with IEMs, the clinical presentations are variable and nonspecific and routine laboratory tests do not usually identify the etiology of the disease. Metabolite analysis can comprehensively detect enzyme dysfunction caused by a variety of abnormalities (Kuhara, 2007; Ponzone *et al.*, 1990).

Phenylketonuria (PKU) is an autosomal recessive genetic disorder characterized by a deficiency in the Enzyme phenylalanine hydroxylase.

When this enzyme is deficient, phenylalanine accumulates and is converted to phenyl pyruvate, which is also known as phenylketone, which is detectable in the urine. Other unknown factors also interfere in determining the metabolic profile of PKU (Oh *et al*., 2004; Blau *et al*., 1996). It is important to emphasize that early diagnosis and treatment are critical for patients with IEMs. The present study aims to identify the prevalence of PKU among children aged 6 months to 6 years seen in the pediatric clinic of Sohag University. Relationship of this disease to malnutrition was also investigated through determining the total protein and albumin in the sera of the children. Families predisposed to have children with this IEMs are also identified and counseled. It is important to test the validity and the practicality of using inexpensive biochemical laboratory tests beside the clinical neurophysiologic criteria for diagnosing PKU in an underprivileged locality

Patients and Clinical Examination

The study was approved by the clinical ethics committee of the Sohag Faculty of Medicine. One hundred children, aged 6 months to 6 years, were selected from 18,000 patients examined in the pediatric clinics of Sohag University hospital over 3 years from May 2008 to May 2011. They presented with clinical symptoms suggestive of probable preliminary diagnosis of PKU. Depending on the severity of symptoms they were seen either as outpatients or were admitted as inpatients. These symptoms included delayed milestones of growth, vomiting since birth, blonde hair and eyebrows, jaundice and organomegaly, rickets, diarrhea, pneumonia, convulsions and skeletal deformities. Exclusion criteria from the study included cerebral palsy, myasthenia gravis, primary muscular dystrophies, myotonia congenita, polymyositis, mental retardation, epilepsy, Friedrich's ataxia, hereditary spastic paraplesia, poliomyelitis and cardiostenosis.

For all children participating in the study, informed written consent was obtained from a parent or next of kin. Children and parents were interviewed for full social and clinical history taking, including consanguinity between parents, history of sibling death, congenital anomalies and IEMs , especially PKU. Physical examination of the children covered the general look, hair changes, developmental milestones, head circumference and congenital anomalies. Full neurological examination was carried out for general behavior, muscle power and reflexes. Chest and abdominal examination was done for detection of organomegaly.

2.1 Biochemical methods

A sample of 5 ml blood was withdrawn from each child and allowed to clot at room temperature. The samples were centrifuged at 3,000 rpm and the sera were separated and stored frozen at -20 °C until used for assaying the chosen parameters using commercial kits. Serum total protein was determined by the Biuret method (Henry, 1964) and serum albumin by a modified bromocresol green binding assay (Tietz, 1995). Urine samples were also obtained and screened for PKU using the ferric chloride method (Berry *et al.*, 1958).

Results

Females were predominant in the studied cohort (65%). The ages of 49% of the studied children were two years or less, while 39% were between 2 and 4 years. The remaining 12% aged between 4 and 6 years. The common presentations among the studied children are presented in Table 1. It could be seen that gastrointestinal manifestations constituted most of the complaints. Deficiency manifestations like delayed growth milestones and rickets also ranked high. By reviewing family history, it was found that sibling deaths were reported in 13% of cases and mental retardation in 5%. Nine percent of the families reported consanguinity between parents. Physical examination of the children revealed congenital anomalies in 5 cases, in the form of polydactyle, cleft lip, cleft palate and ventricular septal defect. Three of these cases had consanguinity between parents.

Age	Frequency	Percent	Valid Percent	Cumulative Percent
Less than or Equal 2 years	49	49.0	49.0	49.0
Between 2 and 4 Years	39	39.0	39.0	88.0
More than 4 Years	12	12.0	12.0	100.0
Total	100	100.0	100.0	

Table 1. Age distribution of infants screened

During the course of the study, two cases of PKU were identified, based on laboratory tests, clinical presentation and family history. Both phenylketonuric infants were born in rural areas. Analysis of their family histories revealed that both infants were siblings of consanguineous parents. One of the infants had a family history of sibling deaths and the other had a family history of mental retardation.

Clinical examination of the two positive cases revealed typical presentation of PKU. Both infants suffered mild malnutrition expressed as decreased levels of serum total proteins and albumin comparable to cases of malnutrition of marasmus, kwashiorkor or other deficiencies like rickets (Table 2).

Complaint	Frequency
Diarrhea	19%
Pneumonia	15%
Delayed growth milestones	13%
Rickets	12%
Vomiting	11%
Convulsions	6%
Jaundice and organomegaly	5%
Coarse facial features and skeletal deformities	2%

1. **Table 2.** Common clinical presentations of the studied children

2. Clinical findings

The first diagnosed case was a three year old girl of a consanguineous couple. The child presented with history of delayed growth milestones, repeated vomiting and hyperirritability since birth. She was treated for hypoxic ischemic encephalopathy with seizures. There was family history of mental retardation, but no sibling deaths, convulsions or albinism. The child had frequent myoclonic jerks with drooling of saliva. There was gross microcephaly with head circumference of 37 cm. Overall motor, language and social developmental delays were noted. Brain CT scan was uneventful. Urine was tested for PKU by the ferric chloride method, and gave a positive result.

The second diagnosed case was a three and half years old boy of a sanguineous couple. Family history revealed death of 2 siblings after 1-2 weeks of birth. Clinical examination revealed delayed developmental parameters like walking, speaking and head support (Table 3). CNS examination showed normal higher mental functions. There was generalized hypotonia with normal plantar reflex and lordosis of the lumbar spine. The urine sample which was taken was turbid and foul smelling. Screening for PKU by the ferric chloride method was positive.

	Healthy controls	Cases with Malnutrition	Case 1 PKU	Case 2 PKU
Total protein	6.0-8.0 mg/dl	3.0-5.2 mg/dl	5.0 mg/dl	5.5 mg/dl
Serum albumin	3.5-5.5 mgldl	1.5-2.8 mg/dl	2.5 mg/dl	2.7 mg/dl

Table 3. Serum levels of total proteins and albumin in the diagnosed cases of PKU as compared to ranges of cases presented with malnutrition and healthy controls in the studied cohort.

Discussion

The use of the ferric chloride method for detecting phenyl pyruvate in urine as a screening procedure for PKU has been proven acceptable, easy and cheap. It is suitable for screening the newborn in underprivileged areas without large medical facilities. Out of the 18,000 cases seen in the pediatric clinics of Sohag University hospital over three years, one hundred were suspected and 2 cases were confirmed to have PKU. This prevalence of 1 in 9000 is higher than what was reported by other groups. However, increases in the number of diagnosed cases over time have been reported (Bhatt *et al*., 2008; Choudhuri *et al.*, 2006; Kumta, 2005; Imamura *et al.*, 1999; Schulpis *et al.*, 1991; Wuu *et al.*, 1988). This may be attributed to advances in diagnostic technology, better coverage and reporting, and increased awareness.

Hypoproteinemia has been reported by Hanley and his co-workers (1970) in 5 of 32 infants treated with a special low phenylalanine formula. Generally, a phenylalanine- restricted diet consists of protein hydrolysate, amino acid mixtures, fruits and vegetables with minimal amount of natural animal products, usually milk, to meet the daily requirement of phenylalanine needed for early growth. In children with PKU, diet therapy could influence the immune system, not only by antigenic change, but also by

producing changes in plasma lipids. It has been known to cause a marked reduction of arachidonic acid levels in both plasma total lipids and phospholipids of children with PKU during dietary intervention.

An added finding in the present study is the effect of consanguinity on the incidence of metabolic disorders, as we reported a higher incidence of parental consanguinity than other population studies. Because inborn errors of metabolism are extremely rare and random variation in their incidence is high, the diagnosis of even one extra case over a short time period may have an important effect on the birth prevalence of certain disorders.

In conclusion, we strongly recommend the expanded newborn screening for PKU for every baby born before discharge from the nursery for children delivered in the hospital or on first visit to the clinic for children delivered at home. Early diagnosis is important for treatment and genetic counseling. The ferric chloride method used in the present study for detection of PKU is simple, acceptable, inexpensive and can be used for screening in remote or underserved areas in the countryside.

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