Original Article

Phenylketonuria: Our Experience in Nine Years at a Tertiary-level Referral Institute

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ABSTRACT

Introduction: Phenyl ketonuria is an inborn error of amino acid metabolism resulting in excessive phenyl alanine levels in blood resulting in a spectrum of neurological defects. Patients and Methods: We retrospectively went through the records of patients diagnosed as Phenyl ketonuria in the last nine years in our team and patients who's data could be accessed were analyzed in detail. Details of laboratory tests, imaging clinical features, course were recorded. Observation: A total of 32 patients were identified in nine years of which data was available only for 15 patients. Age at diagnosis varied from 2.5 years to 7 years. 73% were males. Global developmental delay, Microcephaly, Seizures blond hair, spasticity, regression, Ocular Hypertelorism, low set ears, Seborrhea, Hypotonia, Family history of mental retardation and Consanguinity was common one patient showed a large hypo pigmented area in left arm with eczematous rash. Results of Lab Tests: Urine ferric chloride test and DNPH was positive in all cases. Tandem mass spectroscopy showed elevated phenyl alanine, normal tyrosine and elevated PHE tyrosine ratio in all cases. MRI showed symmetrical Flair hyperintensities in T2 weighted images in the parieto occipital region hypo on T1 with no diffusion restriction in 11 cases and MRS was normal. Genetic testing showed one non consanguineous family having carrier state. Follow up is from 1 year to 5 years. Seizures controlled in all. Regular fallow up shows change in hair color and gain of mile stones. There was no mortality. Conclusion: Phenyl ketonuria is a controllable metabolic disease. However there is considerable delay before diagnosis resulting in persistence of sequelae in children with PKU as well as normal children born to PKU mothers which needs attention to prevent these complications.

Keywords: Newborn screening, phenylketonuria, preventable global developmental delay, pregnant people with PKU need special care

INTRODUCTION

Asbjørn Følling,[1] a Norwegian physician, in 1934, first described 10 children who excreted large amount of phenylpyruvic acid in their urine. That was a very interesting story when a mother of two mentally retarded children came to see Følling and informed her children are not only retarded but their urine had a peculiar smell. Følling in his usual thorough way examined children's urine with all routine methods including ferric chloride test for ketones. Normally, it is brown and turns purple in the presence of ketones. But it turned green in the case of these two children. Folling repeated the test for a few days and was convinced it is indeed not an artifact but the children are excreting something that normal people do not.

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Figure 1: Distribution of age at diagnosis

Figure 2: Distribution of gender

Figure 3: Photograph showing hypotonia

Figure 4: Photograph showing blonde hair and fair skin
and he later detected it to be phenylpyruvic acid when the compound on processing smelt like benzoic acid and he called the disease oligophrenia phenylpyruvica, which later came to be known as phenylketonuria (PKU). Jervis described the enzyme defect and the Canadian physician Robert Guthrie who had an affected son and niece devised the newborn screening test. Jervis described the enzyme defect and the Canadian physician Robert Guthrie who had an affected son and niece devised the newborn screening test. This is a genetic disorder inherited in an autosomal recessive pattern, as a result of mutation in gene coding for the enzyme phenylalanine hydroxylase (PAH). It is localized to chromosome 12q23-2. This is a 90-kb gene with 13 exons and codes for an RNA of approximately 2400 bases; more than 500 mutations are known and therefore the spectrum is variable based on the type of mutation. The mutation decreases catalytic activity in phenylalanine. PAH is a hepatic enzyme and needs tetrahydrobiopterin to convert phenylalanine to tyrosine. About 1 in 10,000 children born in the United Kingdom are detected to have PKU as per newborn screening and applying the criteria of phenylalanine concentration of more than 240 µmol/L, and had their treatment initiated within 20 days. Cofactor defect is found in less than 1% of affected children.

PAH deficiency is an inherited autosomal recessive defect in metabolism of amino acid phenylalanine.
causing significant elevation of phenylalanine to more than 20 mg/dL (1200 µmol/L), which is called classic form. If the levels of phenylalanine are 600–1200 µmol/L, it is called mild form and causes mental retardation by hitherto unknown mechanism. Small percentage of patients have no deficiency of PAH but lack tetrahydrobiopterin synthesizing and recycling which is a cofactor, this causes deficiency of tyrosine precursor of dopamine and tryptophan precursor of serotonin resulting in more serious neurological complications and called malignant PKU. After a protein meal, phenylalanine in the amino acid pool causes a release of glucagon from the pancreas. Hepatic PAH is controlled by dephosphorylation and influence BH4 cofactor interaction with PAH. This condition is more common in Asians and Caucasians. Most common clinical features are mental retardation; vomiting; eczematous skin changes such as rashes and eczema; spasticity; blond hair, fair skin, and blue eyes due to tyrosine deficiency; seizures; self-mutilations; and behavioral abnormalities Cases 1, 2, 3 and 4 mentioned as Figures 3, 4, 5. Urine smells mousy due to the presence of phenylacetic acid.[6]

Neurotoxicity in Phenylketonuria

Neurotoxicity in PKU is not well understood. It presents as hypomyelination and demyelination. There is a possibility of disruption of mechanism involved in the formation of L-DOPA, dopamine, and catecholamines. Large neutral aminoacids are transporters of (LNAA) are the sole transporters of aminoacids across blood brain barrier. Excessive amount of phenyl alanine competitively blocks LNAA. This causes defective transport of essential aminoacids serotonin and catecholamines.[7-9]

Investigations

Urine ferric chloride test is performed by several methods. Urine sample is collected on filter paper and dried. Ferric chloride (10%) is added dropwise, turning the sample-containing filter paper green. The green color fades after 5 min.[10] Modified dip strip test done using ferric chloride with magnesium and cyclohexyl sulfonic acid which shows the color gray to blue.

Guthrie test in newborn screening is performed by soaking a drop of blood into disk of filter paper placed on a plate containing a culture of *Bacillus subtilis*, and β-2-thienylalanine is added. When phenylalanine is present inhibition of bacterial growth is opposed.

![Figure 7: Normal MRS](image-url)
Tandem mass spectroscopy can be used to diagnose PKU, hypothyroidism, galactosemia, and cystic fibrosis, and for TMS blood samples are gathered in filter paper which remains safe for years.[11] Level of phenyl alanine above 150 µmol/L in neonates and above 120 µmol/L in older children is considered abnormal. Diagnosis of other diseases involved in synthesis and recycling of BH4 needs testing using BH4 loading, urine and plasma pterin metabolites assessment, neurotransmitter metabolites assessment, blood spot for dihydrobiopterin reductase measurement. Tests targeting direct enzyme measurements need tissue biopsy.[12-14]

Prenatal screening is done using cloned human PAH gene probe to analyze DNA isolated from cultured amniotic fluid cells.

**Treatment**

The treatment should be started as early as possible. Breastfeeding can be continued. Dietary restriction food materials containing of phenylalanine with supplementation of tyrosine, vitamins, lysine and monitoring blood levels of phenylalanine one to two times a week and later as child grows once a month maintaining levels of phenylalanine at 2–6 mg/dL (120–360 µmol/L) is mandatory. For normal growth, most newborns need 40–60 mg/kg/day of dietary phenylalanine and older children need 200–400 mg/kg/day or less than 700 µmol/L. High proteins such as meat, legumes, dairy, and nuts are avoided. Foods containing aspartame, an artificial sweetener, are avoided. A piece of bread contains 120–150 mg of phenylalanine, and therefore, reduction in the intake of bread is recommended. Starchy vegetables such as potatoes, corn, and beans should be avoided. Fruits, nonstarchy vegetables, and low-protein diets are recommended.

Sapropterin dihydrochloride, the cofactor of tetrahydrobiopterin, can be administered to children with BH4 deficiency. Injectable enzyme replacement therapy with adenoviral vectors of protein fusions targeting the liver through portal vein (enzyme substitution with phenylalanine ammonia lyase, an alternate enzyme) is
being experimented. This is facilitated by additional partial liver or hepatocyte transfer. High doses of LNAAs might competitively inhibit phenylalanine and supplements such as PhenylAde and PreKUnil at doses of 0.4 mg/kg body weight can be used. But these supplements are not recommended for children less than 15 years of age and for pregnant women. Glycomacropeptides, side product of cheese manufacture, are low in phenylalanine and rich in valine, isoleucine, and threonine. For suspected patients with co-factor deficiency who do not respond to diet tetrahydrobiopterin which activates residual PAH and improves oxidative metabolism of phenylalanine is used at 5 to 20 mg/kg/day. Caution is needed while using along with drugs like tetrahydrate folate reductase inhibitors. Patients might need dopamine, carbidopa, and 5 OH tryptophan. Gene therapy using functional PAH gene, targeting liver in to portal vein, using nonviral direct injection of naked plasmid DNA, genetically engineered probiotic which deliver the specific enzymes are all being tried but when retrovirus was used leukemia like syndrome occurred.[15]

**Patients and Methods**

We analyzed 15 patients diagnosed with PKU for clinical, radiological, and biochemical features. Informed consent was obtained from all of them. They all underwent detailed clinical examination. Magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and tandem mass spectroscopy were performed. Urine samples were analyzed for organic acids. Genetic testing was performed when possible. Patients were regularly followed up with recommended diet and other symptomatic measures. Serum phenylalanine levels were not measured regularly as the patients were poor and facility not available in the government sector.

**Observation**

In a tertiary-level referral hospital, we had a total of 32 patients in 9 years. However, data were available only for 15 patients. For the patients, age range varied from 2.5 to 7 years [Figure 1]. Of 15 patients, 27% were females and 73% were males [Figure 2]. Most common abnormality (in 14 of 15 cases) was found to be global developmental delay. Other observations made are as follows: microcephaly was observed in 13 patients; ocular hypertelorism in 2; low-set ears in 2; and seborrhoea in 1 [Figure 3]; hypotonia in 1 [Figure 4]; spasticity in 3; seizure in 9; and regression of milestones in 5. Family history of mental retardation was seen in 7 cases. Consanguinity was reported in 11 cases. Fair skin was seen in 13 cases with blond hair [Figure 5]. One patient showed a large hypopigmented area in left arm with eczematous rash [Figure 6]. In all cases, results of urine ferric chloride test and dinitrophenylhydrazine (DNPH) test were found to be positive. Tandem mass spectroscopy showed elevated phenylalanine, normal tyrosine, and elevated phenylalanine/tyrosine ratio in all cases. MRI showed symmetrical hyperintensities in T2-weighted images, and in fluid-attenuated inversion recovery (FLAIR) images in the parietooccipital region in 11 cases and hypointensities on T1-weighted images with no diffusion restriction was observed [Figure 7]. MRS was found to be normal [Figure 8]. Serum ammonia ranged from 28 to 180 µmol/L. One nonconsanguineous family also showed asymptomatic carrier state. Index case and mother showed mutation in exon 7 of PAH gene (Chr12: 103246708G>G/A; c727C>C/T) [Figures 9 and 10], and father showed mutation in exon 6 of PAH gene (Chr12: 10324897G>G/T) [Figure 11]. The period of follow-up was from 1 to 5 years. Seizures are
controlled in all. Regular follow-up showed change in hair color and gain of milestones. No mortality was reported. Periodic measurements of blood levels of phenylalanine could not be done in any of our patients. Complete adherence to diet was seen in only small children and dietary restriction was difficult in older children as they could not be controlled by parents.

**Figure 10:** Genetic test in father showing exon 6 of PAH gene (Chr12:10324897G>G/T)

**Figure 11:** Distribution of clinical features

**Figure 12:** Photograph showing blonde bald fine hair
Global delay and blond hair are also seen in other conditions such as Menkes kinked hair syndrome and biotinidase deficiency. They are differentiated as follows.

**Biotinidase deficiency**
This enzyme defect is observed mainly in patients with multiple carboxylase deficiency. Seizures, ataxia, optic atrophy, alopecia, and skin rashes are other common features [Figure 12].

**Menkes kinky hair syndrome**
Menkes kinky hair syndrome an X-linked disorder due to abnormal copper metabolism. Hypotonia, seizures, mental retardation, brittle, twisted lanugo hair, and poikilothermia are other common features. Intracranial arteries are twisted and tortuous. Parenteral replacement of copper is being tried but carries a very poor outcome [Figure 13].

**Treatment and outcome**
Except one patient who could afford the special readymade diet, all patients were prescribed modified diet. They also received treatment for seizures. No mortality was reported. Of all the patients, steady improvement in mile stone was observed in 11 and arrest of regression in 7. Hair color became dark in all patients but did not normalize. At 4 year follow up 4 patients are dependent for activities of daily living.

**Discussion**
When there is partial or complete absence of PAH enzyme with probably cofactor BH4 deficiency phenylalanine accumulates in the blood and that is a neurotoxin. It is important to regularly measure blood levels and maintain at 120–360 µmol/L for optimum development. In our series, the most common feature was global developmental delay, followed by blond hair, seizures, regression, dysmorphism, spasticity, hypotonia, and skin changes. With extension of newborn screening to all newborns, early diagnosis can be ensured, which is of great importance in protecting the nervous system and improving quality of life.

**Conclusion**
PKU is common in our country. There is gross delay in diagnosis and initiation of treatment in our group of patients with minimum age at diagnosis being 2.5 years. In this series, we have used low-protein, low-phenylalanine diet with vitamin supplementation, and only one patient could afford PKU formula. None received LNAA or sapropterin dihydrochloride. We could not monitor regular blood level in any of our patients. There is urgent
need for awareness into this disease, as if the treatment is initiated very early, the outcome is good, though diet is the mainstay of treatment in our patients in addition to symptomatic measures.

**Declaration of patient consent**
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**
There are no conflicts of interest.

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