Management of Phenylketonuria: Current and Future Perspectives

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Abstract

Phenylketonuria (PKU) is an inborn error of phenylalanine (phe) and tyrosine (tyr) metabolism. It is an autosomal recessive disease occurred due to deficiency of liver enzyme phenylalanine hydroxylase (PAH). Hence, phe is not converted to tyr and phe is accumulated in the body. Phe thus channeled to alternative routes of metabolism and forms Phenylketones excreted in urine. Early treatment is essential to prevent mental retardation and other intellectual disabilities. Dietary treatment remains the main cornerstone to manage PKU since last 3-4 decades. A diet low in Phe supplemented with special amino acids formulas must be started soon after diagnosis within seven days of life. Inspite of good results obtained from dietary treatment in PKU, still there are some issues with palatability of the dietary formulations. There are also issues of nutritional deficiencies of vitamins like calcitriol and cobalamin (B12). Poor cognitive and executive functions have been observed in patients who do not follow proper dietary treatment. Attempts have also been made to increase the palatability of food under dietary management. Role of large neutral amino acids (LNAAs) and glycomacropeptides (GMP; found in bovine milk) as a newer dietary management have also been explored. In recent era, advances occurred in terms of genetic therapy and enzyme replacement therapy which opened a new door towards management of PKU. In this review, various treatment aspects of PKU are discussed and explored.

Keywords: *Phenylketonuria, phenylalanine hydroxylase, phenylketones*

Introduction[1-6]

Phenylketonuria (PKU) is an inborn error of phe and tyr metabolism. It is caused mainly due to mutations in the gene coded for phenylalanine hydroxylase (*PAH*) which is located on long arm of chromosome 12. This mutations lead to decrease catalytic activity of PAH which affects the catabolism of phe (*Figure 1*). PAH is located in liver and requires tetrahydrobiopterin (BH4) as a co-factor to convert Phe to tyrosine (Tyr) (*Figure 1*). In PKU, accumulation of phe is due to deficiency of either PAH or its cofactor BH4. Accumulated Phe take alternative routes to form phenylketones like phenylpyruvate, phenyllactate, phenylacetate which are excreted in urine. These ketone metabolites lead to severe central nervous system disability which if left untreated cause irreversible damage. Other clinical features associated with untreated PKU may include sensory and motor dysfunction, skin manifestations and seizures. Changes in behavior and psychic disturbances can become apparent as the age progresses.

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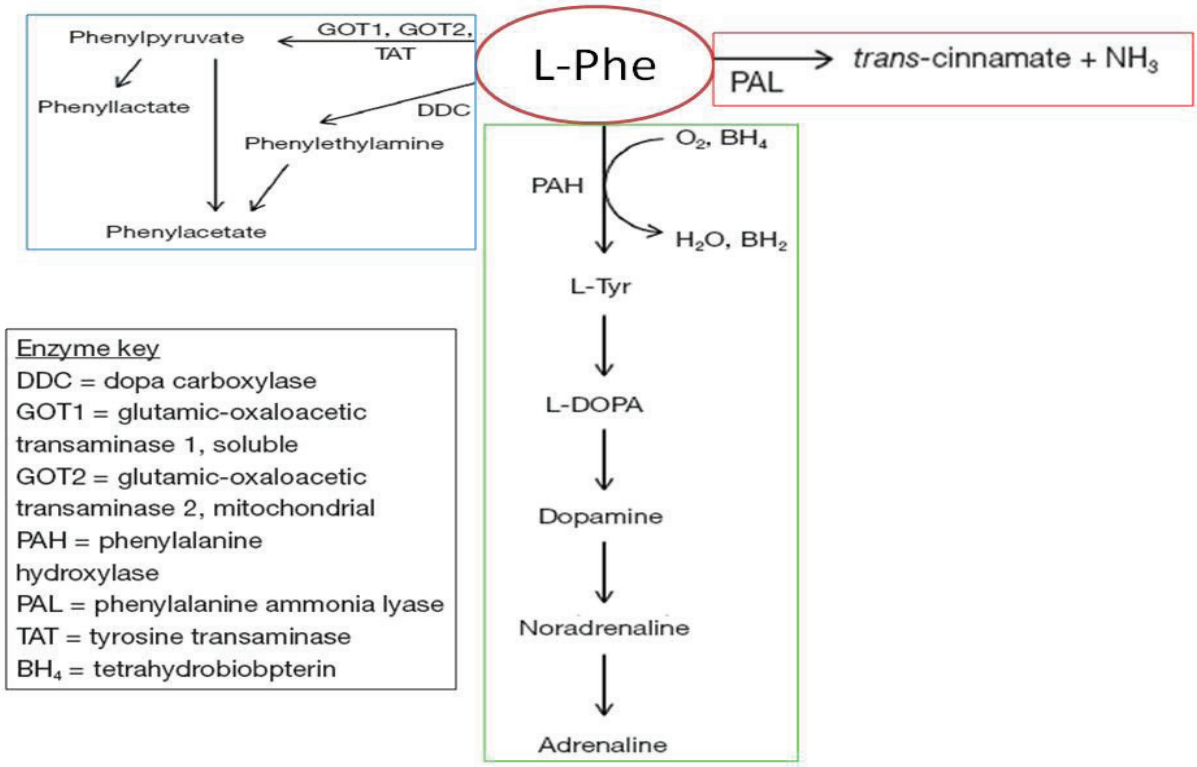

Figure-1: Phe metabolic pathway. Green box shows main catalytic pathway of conversion of phe to tyr by phenylalanine hydroxylase (PAH). Blue box shows alternative route which produces phenylketones in PKU. Red box shows pathway which occurs in plants and yeast with the help of enzyme PAL (adopted from Ho G 2014)[3]

Early diagnosis is essential in PKU to prevent irreversible damage to brain. Currently newborn screening (NBS) program is undertaken for initial diagnosis in the first week of life and further screening can be done for BH4 responsiveness in case of atypical PKU. The incidence of PKU varies widely around the world. The average incidence is 1:10000 live births worldwide. India has lower incidence (1:25000 live births) as compared to western countries (1:1500 live births). At present, there is no cure for PKU. However, the cornerstone of the management of PKU is dietary therapy. Diet low in Phe supplemented by amino acids formulations is recommended. Early diagnosis by NBS along with commencement of dietary therapy has played a role in prevention of intellectual impairment but still neurophysiological and neuropsychological impairments persist in already treated patients of PKU. Moreover, some issues are also there with vitamin deficiencies like calcitriol and B12. Nowadays attempts have been made to cope up with issues occurring with dietary therapy. Advanced food and formulas which increase the compliance towards dietary therapy for example incorporation of large neutral amino acids (LNAAs) and glycomacropeptides (GMP) have been studied in detail and introduced. Still further studies are also required in this direction to establish their full impact. Cofactor BH4 responsive patients respond well to BH4 therapy with success rate of only 30%. Recently, newer modalities came into existence and under research like enzyme replacement/ substitution therapy and gene therapy. In this review, we aim to summarize current and future perspectives of management of PKU.

Current Treatment

Diet therapy

Phe is an essential amino acid. Apart from protein synthesis, phe helps in synthesis of tyr and in turn helps in synthesis of important metabolic substances derived from tyr. In PKU, defect in the conversion of Phe to Tyr makes Tyr an essential amino acid. Therefore, the technical challenge of the dietary treatment is to

formulate a phenylalanine-controlled diet that allows the reduction of systemic Phe concentration, satisfactory tyr provision, and optimal growth and development. Diet low in Phe remains the mainstay of the treatment in PKU. This should be supplemented with special amino acids formula so that proper growth can be ensured. During infancy, Phe free milk lowers the blood levels of Phe. As levels approach the therapeutic range, phenylalanine is added using measured amounts of normal milk and then adjusted until serial blood controls have stabilized. When child puts on solid diet, the diet is progressively adapted with the following main principles: High-protein foods (meat, fish, eggs, dairy, and wheat products) are excluded. Foods with low protein content (milk, vegetables, and fruits) are used to meet the required amount of Phe. Diet restriction is somewhat easier in infancy as it is mainly controlled by parents but it is difficult as the child gets older due to his/her likes and dislikes with the food items. Other important factor that affects the dietary management is the blood Phe concentrations which varies from country to country. Consequently, compliance with the diet is often poor, especially when the patient reaches adolescence, as evidenced by poor control of blood Phe concentrations in this age group. Long-term maintenance of the diet is important for proper management, because it has been observed that patients find it difficult to return to the normal dietary regimen after a long period of unrestricted dietary intake. [7-10]

Earlier it was believed that continuation of dietary therapy after childhood has no role because excess phe has no any role after brain development. But some studies showed poor scholastic performance after discontinuation of diet therapy. Moreover, females having PKU when reached to their reproductive age have higher risk of having baby with maternal PKU syndrome. This can be manifested as small brain size and poor brain development, growth retardation in-utero, cardiac defects and a characteristic facial appearance in the affected infant. As a result of these studies, it was recommended that diet therapy should be continuing life-long. [11, 12]

Although Phe restricted diet is successful in curtailing intellectual impairment in PKU patients, there are certain issues (non-compliance) still remain with current diet therapy. These are poor compliance to synthetic diet formula, persistence of neurological or psychosocial issues and poor quality of life inspite of early intervention, deficient nutritive supply, financial burden of special diet, difficult dietary regimen to follow, psychosocial and emotional factors, knowledge of disease and its outcomes, attitudes towards health-care providers, no reimbursement of food supplements in some health care systems. There is an existence of problem in the diet therapy in PKU patient when provided with aspartame (L-aspartyl-L-Phe methyl ester). It is commonly used as an artificial sweetener and it releases Phe, L-aspartic acid and methanol when gets metabolized. Hence, aspartame should be avoided. Stringent diet therapy leads to nutritional deficiencies. The reported deficiencies are vitamin D, B12, calcium and iron which further aggravate the symptoms in PKU patients. [3, 8, 13-16] These issues stimulated mankind to have other alternatives to be made in diet therapy.

Tetrahydrobiopterin (BH4, Sapropterin) treatment

BH4 is a natural cofactor required by PAH for the conversion of Phe to Tyr. Some mutations are also associated with BH4-sensitive phenotype of PKU. The benefit of administering synthetic preparation of BH4 in patients with non-classical (atypical) PKU was first studied by Schaub *et al*. Pharmacological doses of BH4 may reduce the blood Phe levels was first demonstrated by Kure *et al*. Thus the patients were called BH4-responsive. Two formulations of BH4 have been studied clinically (6R-BH4 dihydrochloride and sapropterin dihydrochloride) but only sapropterin dihydrochloride is approved by the US Food and Drug Administration, the European Medicines Agency, and in Japan for therapeutic use. A single daily dose of 10-20 mg/kg is sufficient to maintain stable blood Phe level over 24 hours. There are other benefits of BH4 treatment which were also observed like improvement in Phe tolerance, improvement in long-term neurological outcomes, decreased in depression and panic attacks especially in females. It was also observed that BH4 supplementation can be appropriate for patients with milder biochemical phenotype. It is less useful in classical PKU. [3, 17-20]

Newer dietary approaches

Large neutral amino acids (LNAAs)

Phe, Tyr, tryptophan, and the branched-chain amino

acids (BCAAs) are considered as LNAAs. They share the common amino acid transporter to cross blood brain barrier. In PKU, high Phe levels compete with entry of other LNAAs which can lead to deficient formation of important neurotransmitters and hence neurological symptoms develops. Keeping this fundamental aspect into consideration, supplementation with LNAAs other than Phe could provide another potential treatment approach. LNNAs exert its effect by competing with phe for transportation at two different sites that is across the blood-brain barrier and across the intestinal mucosa. A double-blind, placebo-controlled study also showed a reduction in blood phenylalanine from baseline of 39% during short-term treatment with LNAAs.[13, 21-23]

Glycomacropeptides (GMP)

During making of cheese, GMP produced as a by-product. It is a 64 amino peptide with no Phe residues and rich in valine, Isoleucine and threonine. Therefore, food made from GMP can be a rich source of protein for PKU patients. Study showed that GMP diet significantly decreases urea production, helped in maintenance of protein concentration and phe utilization. It has also been found that taking diet made from GMP for breakfast promoted satiety and improves compliance.[6, 24-26]

FUTURE (NEWER) TREATMENT

Gene therapy

PAH is only expressed in liver. So gene therapy involves production of recombinant PAH gene which is targeted to hepatocytes. Viral vectors like adenoviral and adeno-associated virus (AVV) vectors have been used in various studies to know their role for the transfer of this gene in PKU mouse model. Recombinant AAV vectors have been used to deliver PAH gene to the liver in a mouse PKU model, allowing correction of PAH up to one year. But over the time, the vector lost due to continual turnover of hepatocytes and in turn developed immunity. Yagi *et al* used an AAV8-pseudotype vector with a self-complementary AAV genome and they had achieved excellent liver transduction and expression of PAH with complete reliving of symptoms and normal blood phe for over one year. Skeletal muscle is preferred over liver for gene therapy due to easy accessibility and longer cell life. However, new strategies are still required to extend gene transfer efficacy. [3, 13, 27-29]

Enzyme replacement/substitution therapy

PAH-based fusion proteins (Enzyme replacement)

PAH-based fusion proteins which specifically target PAH to the liver have been explored. Mice with PKU were injected PAH-based fusion proteins intravenously. Decreased in Phe level within several hours has been noted. But this intervention may be less tolerable clinically due to frequent injections of fusion proteins. Moreover model used in this experiment was not homologous to human PKU but still it can be the alternative if homologous model is used. [3, 13, 30]

Phenylalanine ammonia-lyase (PAL, Enzyme substitution)

PAL is used as enzyme substitution therapy. PAL an enzyme normally found in plants and fungi. It catalyzes the removal of amino group of phenylalanine to ammonia and transcinnamic acid, the latter of which is then quickly converted into hippuric acid and excreted in urine *(Figure-1 red box)*. Study done in mouse model by injecting PAL showed decreased Phe level but due to repeated injections immune response was developed which decreases its half-life. To overcome the issue, recombinant PAL (with polyethylene glycol polymers so-called PEGylation) is used to avoid the immune-mediated degradation of the enzyme and thus longer half-life is achieved. Oral route is preferred than the Parenteral route to achieve compliance to therapy. But again, to maintain enzyme stability is a big issue which can be overcome by using encapsulation or using live microorganism as a delivery systems. Use of probiotics like lactobacillus and genetically modified probiotics in transporting and delivering PAL have also been gained attention and tried. [3, 8, 13, 31-34]

Conclusion

Prompt initiation of low-Phe diet immediately after diagnosis made by newborn screening maximally prevents intellectual impairment in early treated PKU patients. Compliance to the diet therapy is the main issue especially in adolescence and adulthood which in turn lead to impaired blood Phe control and poor outcome with respect to psychosocial and cognitive assessments. BH4 supplementation can be appropriate for patients with milder biochemical phenotype (Non-

classical PKU) as compared to Classical PKU. Newer dietary approaches have been introduced to increase compliance to diet therapy. Data suggested that use of LNAAs is recommended only in adult patients who have non-compliance to stringent diet regimen. Addition of GMP into low-Phe diet has shown to improved palatability and some beneficial effects but still further studies should be undertaken to establish its safety and efficacy. Newer and future treatment modalities for PKU are now under development. They are mainly focused on the basic defect of PKU i.e. catalytic activity of PAH. Gene therapy by using suitable vectors to transfer PAH gene to liver and skeletal muscle have been tried. Under enzyme replacement and substitution therapy role of PAH associated fusion proteins and PAL (PEGylated form) have been explored respectively. The latter has reached phase III clinical trials but still its efficiency is the major issue. Studies related to the use of probiotics to produce and deliver PEGylated form of PAL are still ongoing to establish its safety and efficacy. Nevertheless, enzyme replacement/substitution therapy has been found efficacious in mouse model of PKU but still they all require in depth research and clinical testing. These treatment modalities can also be applicable to other inborn error of metabolism and data from this study highlighted the need for development of alternative therapies for PKU patients irrespective of genotype, phenotype or gender.

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Search Strategy

We searched Google and PubMed with the terms: "phenylketonuria", hyperphenylalaninemia", and "PKU" in combination with "diagnosis", "treatment", "diet", "tetrahydrobiopterin", "sapropterin", "pharmacotherapy", "gene therapy", "enzyme replacement therapy", "large neutral amino acids", "glycomacropeptide", "nutritional status", "management", "adult", and "maternal". We gave preference to papers published within the past 10 years, but did not exclude some important less recent publications.

Ethical Approval : This study did not warrant institutional review board review as no human subjects were involved.

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