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Phenylalanine Hydroxylase Deficiency: Phenylketonuria and Hyperphenylalaninemia

Joesph R. Wiencek[1]

[1]Division of Laboratory Medicine, Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA 22908-0168.

Correspondence:
Joesph R. Wiencek PhD, Division of Laboratory Medicine, Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA 22908-0168; email: JW3ZB@hscmail.mcc.virginia.edu, Phone: (434)-924-9712, FAX: (434)-924-2107

Running Title: Phenylalanine Hydroxylase Deficiency
Text Word Count: 1837
Reference Count: 7
Slide Count: 17
Conflict-of-interest disclosure: The author declares no competing financial interests. There is no conflict of interest.
Keywords: Hyperphenylalaninemia; Newborn Screening; Phenylalanine Hydroxylase Deficiency; Phenylketonuria

Questions

1. What is phenylalanine hydroxylase (PAH) deficiency?
2. What steps are involved in the diagnosis of PAH deficiency?
3. How is PAH deficiency treated?

TITLE: Phenylalanine Hydroxylase Deficiency: PKU and Hyperphenylalaninemia

PRESENTER: Joesph R. Wiencek, PhD

Slide 1:

Hello, my name is Joesph Wiencek. I am an assistant professor of pathology at the University of Virginia School of Medicine. Welcome to this Pearl of Laboratory Medicine on phenylalanine hydroxylase (PAH) deficiency.

Slide 2:

Let us first begin by discussing the three categories of amino acids (AAs) that the body needs for biosynthesis. Humans and other animals are not able to synthesize all twenty AAs needed for protein synthesis and some of them must come from dietary intake. Essential AAs that come from out diet include histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine and phenylalanine (PHE). The other two categories of AAs are known as nonessential, which are made in our bodies and conditional which are not usually considered essential, except in times of illness and stress.

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PHE is one of nine essential AAs acquired by the diet through protein intake. Dietary intake of AAs does not always match the body's demands. Therefore, our enzymes modify dietary amino acids to provide adequate concentrations of all AAs needed for biosynthesis. PHE is an example of an amino acid in a metabolic pathway that undergoes enzyme modification to produce tyrosine (TYR) and subsequently TYR to its derivatives - dopamine, norepinephrine and melanin etc. Similarly, in the body, there are a significant number of enzymatic pathways for balancing the pool of amino acids both for synthesis and for degradation. The number of enzymes involved in this process creates a great potential for genetic diseases. Disruption through a mutation of one or more enzymes in the metabolism of only one amino acid may cause secondary deficiencies of other AAs and may also have profound consequences for growth and development – some of these genetic diseases are fatal.

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PAH deficiency, also known as phenylketonuria (PKU) due to the characteristic phenylketones that accumulate in the urine, was the first inborn error of metabolism identified through population-based screening. A Norwegian physician by the name of Asbjørn Følling first described the disorder in 1934. However, treatment of patients with PAH deficiency with a low PHE diet did not start until the mid-1950s. Upon clinical treatment, one of the first treated patients did show improvements with a decreased blood concentration of PHE but

unfortunately, the patient had irreversible developmental disabilities. During this same period, decreased hepatic PAH deficiency was determined to be the underlying biochemical defect. Following recent recommendations by ACMG, the use of PAH deficiency to describe the clinical spectrum of phenotypes ranging from PKU to hyperphenylalaninemia will be used in this presentation.

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Deficiency of PAH is an autosomal-recessive disorder due to genetic abnormalities in the long arm of chromosome 12. As of March 2017, over 991 variants have been identified; most of which are point mutations although deletions, duplications and insertions have been reported. Missense mutations typically cause inappropriate protein folding, which can decrease the enzyme's activity. In the Caucasian population, the incidence of disease is approximately 1 in 10,000 live births whereas this rate may increase depending on geographic location such as seen Ireland and Turkey.

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The pathological mechanism for PAH deficiency centers around toxic PHE and PHE metabolite concentrations in the tissues during growth and development, particularly the central nervous system. Infants born with PAH deficiency will have an unremarkable clinical presentation immediately after birth. However, due to the accumulation of these neurotoxins, if left untreated, these children will go on to develop characteristic phenotypic findings such as developmental delay, seizures, ataxia, motor deficits and behavioral problems. An additional and unusual finding is the mousy odor of a patient's urine, due to the accumulation of phenylpyruvic acid.

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The cofactor for PAH tetrahydrobiopterin (BH_4) is essential for the proper activity of the enzyme. Defects in the recycling and synthesis of the cofactor are due to rare genetic mutations that can lead to secondary deficiency of PAH and subsequently increase in PHE. Therefore, all newborns with an elevated PHE by newborn screening (NBS) will commonly undergo additional evaluation for potential disorders of BH_4 synthesis and regeneration.

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The era of newborn screening originated in the early 1960s when Dr. Robert Guthrie developed the first routine screening test for PAH deficiency utilizing dried blood spots. Since then, newborn screening (NBS) for PAH deficiency and, many other inborn errors of metabolism, have spread throughout North American as well as most of the developed world. Over time the techniques to detect an elevated PHE in a dried blood spot has evolved considerably. Although innovative, Dr. Guthrie's semi-quantitative bacterial inhibition assay is limited in detecting only one disease at a time and requires long-manual sample analysis that takes up to 48 hours for

bacterial growth. Following the advent of tandem mass spectrometry in the 1990s, one instrument now can detect multiple biochemical markers for a large number of diseases. As such, mass spectrometry is one of the central components of NBS in public health laboratories due its high sensitivity and specificity.

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Quantification of of PHE, TYR and the complete plasma AAs profile following a positive newborn screen is necessary to confirm the diagnosis. In the clinical laboratory, there are two main types of confirmatory methods used to perform plasma amino acid analysis. The first widely used method utilizes high performance liquid chromatography, or HPLC, with UV detection. Since most AAs do not absorb UV light, other than PHE and TYR, the amine group within a free amino acid is derivatized either before or after column separation. In addition to this method, there are also published procedures that utilize high performance liquid chromatography coupled with a tandem mass spectrometer detector. The mass spectrometry methods are highly sensitive and specific and do not require any derivatization steps. An additional, although less common method, utilizes enzymatic PHE determination using PHE dehydrogenase (NAD-linked) for rapid determination and should also be mentioned.

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In the case of an elevated PHE on a newborn screen, other diagnostic tests are available and used in the work up of this disorder. Measurement of Pterins, a group of cofactors for PAH, in urine or blood is also useful in determining any secondary causes of PAH deficiency. To follow up for abnormal concentrations of pterin, the infant will undergo additional enzyme testing in the pterin generation/regeneration pathway. In addition, mutational analysis of the PAH gene is recommended to improve therapy planning with respect to dietary PHE restriction and the likelihood of response to other available treatments. Lastly, one test that is available but not recommended is PAH enzymatic activity due to its limited location within the patient's body.

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Early diagnosis of PAH deficiency and subsequent dietary and/or pharmaceutical treatment is essential in preventing developmental delays in PAH deficient children. Following a positive newborn screen, it is necessary to have additional diagnostic testing performed to rule out many factors that may affect the initial PHE concentration (e.g. timing of the sample and the dietary protein-load prior to sampling). These results are then immediately shared with the child's family and care provider. This information is important to try and determine the category of blood PHE concentrations and the appropriate treatment plan. Infants with a blood PHE concentration greater than 600 µmol/L at the time of testing will undergo treatment. In the United States, providers will initiate treatment at blood PHE concentration at 360-600 µmol/L although there is

conflicting evidence around this desired goal of therapy. On the other hand, infants in the category of blood PHE concentrations of 120 to 360 µmol/L do not typically undergo treatment but require evaluation on an annual or biennial basis. As the patient ages, close PHE and TYR monitoring becomes less important following nervous system development. However, age dependent requirements are available through primary literature and clinical guidelines

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Dietary treatment of PAH deficiency is a life-long endeavor that requires a low PHE diet in combination with a specialized medical formula deficient of the problematic PHE amino acid. In these patients, TYR becomes an essential amino acid and restriction of dietary protein runs the risk of generating a secondary TYR deficiency. Therefore, both PHE and TYR are often monitored simultaneously in follow-up laboratory studies. Fortunately, to help individuals monitor PHE consumption, manufacturers of food products will label their item if it contains PHE as depicted in the figure on the right. This helps to allow an individual to monitor their PHE intake to avoid unnecessary harm. For each patient with PAH deficiency it is important to consider factors such as PAH activity, patient age, growth rate and other considerations to determine an individual's daily PHE allowance.

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The US Food and Drug Administration approved sapropterin dihydrochloride as the first pharmacologic agent to treat PAH deficiency back in 2007. Sapropterin also known as Kuvan is a synthetic molecule that is identical to the natural occurring cofactor, BH_4. Although the exact mechanism remains unclear, approximately 25-50% of individuals with residual PAH activity increase metabolism of PHE when administered sapropterin with no known serious side effects. As such, the medication remains a first line therapy to all PAH-deficient patients regardless of genotype. An additional therapeutic approach, utilizes large neutral AAs or LNAAs, which block the uptake of PHE in the blood brain barrier and small intestine. PHE is also a large neutral amino acid and in this process, LNAAs compete for associated receptors and block PHE absorption. The use of LNAAs is currently limited to adolescents as well as adults. However, it is not a recommended treatment for pregnant patients due to limited clinical evidence about the effects of fetal growth and the development of the fetus' central nervous system. A third compound that is currently in phase III clinical trials for treatment of PAH deficiency is polyethyleneglycol-conjugated phenylalanine ammonia lyase. The enzyme phenylalanine ammonia lyase is isolated from bacteria and appears to be able to lower PHE concentrations in PAH deficient patients even when the amino acid is not restricted from diet. With respect to either dietary or pharmacologic treatments for these patients, the goal is to lower the patient's blood PHE concentrations and improve any clinical symptoms.

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Phenylalanine embryopathy (maternal PKU) is a pathologic condition characterized by fetal development in the presence of very high concentrations of PHE. The condition leads to physical and cognitive effects on the developing fetus that may result in microcephaly, poor fetal growth, congenital heart defects and intellectual disability. A maternal PHE threshold of less than 360 µmol/L is recommend in fetal development as well as prior to conception. A mother should also avoid LNAAs and undergo close monitoring of fetal growth through routine prenatal care. For optimal maternal/infant outcomes, mothers with PAH deficiency should also restrict and maintain an appropriate PHE concentration through diet or medications such as sapropterin.

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In summary, PAH deficiency is an autosomal recessively inherited disorder of PHE degradation with over 900 identified genetic variants. Fortunately for these patients, routine NBS is capable of detecting elevation in PHE concentrations in dired blood spots and appropriate follow-up can take place before any neurologic problems develop. With currently safe and even newer medications being developed, potentially life-long treatments are expected to become more individualized and helpful for PAH deficient patients in the future.

Slide 16: References

Slide 17: Disclosures

Slide 18: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on phenylalanine hydroxylase deficiency.