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TITLE: Tyrosinemias: Biochemistry and Clinical Laboratory Investigation

PRESENTER:

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Slide 1:

Hello, my name is Kwabena Sarpong. I am a Clinical Chemistry Fellow in the Division of Lab Medicine and Department of Pathology at the University of Virginia School of Medicine. Welcome to this Pearl of Laboratory Medicine on “Tyrosinemias: Biochemistry and Clinical Laboratory Investigation”

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In this talk, we will discuss the biochemical pathway for tyrosine metabolism, identify enzyme mutations/protein deficiencies leading to tyrosinemias, their management and the clinical laboratory’s approach to diagnosis of tyrosinemias

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Tyrosinemias, just like any inborn error of metabolism, are caused by defects in enzymes needed to metabolize tyrosine. This leads to the accumulation of tyrosine and other harmful metabolites in the blood, skin, eyes, kidneys and other tissues. These clinically manifest as hypertyrosinemia and include three main forms; Tyrosinemia type I or hepatorenal tyrosinemia is the most common with a prevalence of 1:100,000 individuals (with the Saguenay-Lac Saint-Jean region of Quebec having a higher prevalence). Oculocutaneous or type II tyrosinemia has a global prevalence of 1:250,000 individuals and more common in the Arab and Mediterranean populations.

Tyrosinemia type III or 4-alpha hydroxyphenylpyruvic acid oxidase deficiency is the rarest form with very few cases reported in literature. It must be noted that tyrosinemias follow an autosomal recessive inheritance pattern where an affected individual would need one of the mutated gene from each parent to have the disorder. Alkaptonuria, Hawkinsinuria and transient tyrosinemia are also tyrosine metabolism disorders that will be discussed later in this presentation.

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In the body, phenylalanine is enzymatically modified into tyrosine, which can be incorporated into proteins, as a precursor of several neurotransmitters or be broken down via a number of reversible and irreversible reactions using three key enzymes indicated in red in this metabolic pathway shown. I will not be discussing other enzymes involved in this pathway because their deficiencies cause little or no increase in blood levels of tyrosine. In the subsequent pathway, tyrosine undergoes a transamination reaction, catalyzed by tyrosine aminotransferase to produce 4-hydroxylphenylpyruvic acid. This allows the copper containing enzyme, 4-hydroxyphenylpyruvic acid dioxygenase to convert 4-hydroxyphenylpyruvic acid to homogentisic acid via an oxidative decarboxylation/hydroxylation irreversible reaction. Cleavage of a benzene ring on homogentisic acid produces maleylacetoacetic acid which undergoes isomerization to form fumarylacetoacetic acid. The final irreversible step involves the hydrolysis of fumarylacetoacetic acid to form fumaric acid and acetoacetic acid. Deficiencies in any of the three key enzymes result in accumulation of tyrosine and/or some of its metabolites such as succinylacetone that can be harmful to the body.

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Tyrosinemia type I results from deficiency of the enzyme fumarylacetoacetic acid hydrolase (FAH), which is the terminal enzyme in the tyrosine catabolic pathway. The pathophysiology includes the accumulation of fumarylacetoacetatic acid in hepatocytes leading to cellular damage and death. Fumarylacetoacetatic acid is further converted to succinylacetoacetate and succinylacetone, the later metabolite is known to interfere with

hydroxyphenypyruvic acid dioxygenase, leading to high plasma concentrations of tyrosine. Succinylacetone also inhibits porphobilinogen (PBG) synthase, an enzyme that catalyzes the biosynthesis of porphyrin from aminolevulinic acid. This results in reduced heme synthesis and accumulation of aminolevulinic acid to such concentrations that allows the induction of neurologic episodes.

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Tyrosinemia I is usually suspected in individuals with positive newborn screening for the presence of succinylacetone and elevated tyrosine. However, in cases where these metabolites are not detected by newborn screening, untreated tyrosinemia I presents with severe liver disease in young children or with severe liver dysfunction usually in the first 12 months. These children may have episodic neurological crisis which includes altered mental status, peripheral neuropathy, pain in the abdominal regions and sometimes respiratory failure which often requires mechanical ventilation. If this becomes severe and chronic, individuals develop renal tubular dysfunction that involves a Fanconi-like renal syndrome with abnormal excretion of amino acids in urine, renal tubular acidosis and phosphate loss. Hypophosphatemic rickets results with normal serum calcium concentrations. With little to no management of tyrosinemia I, these children stand a higher risk of hepatocarcinoma that often leads to death.

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FAH deficiency in tyrosinemia I results in the diversion of fumarylacetoacetic acid to increased concentration of succinylacetone in the blood and its concomitant increased excretion in urine. There is also elevated plasma concentrations of methionine, phenylalanine and tyrosine while the metabolites of tyrosine including hydroxyphenyllactate, hydroxyphenylacetate and hydroxyphenylpyruvate have significantly higher concentrations in urine. As a result of succinylacetone interference with the hepatic enzyme, PBG synthase, there is increased urinary excretion of aminolevulinic acid. Finally, liver damage and dysfunction results in marked changes in liver function with elevated AFP in serum and prolonged PT and PTT.

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During newborn screening, clinical laboratories require the qualitative measurement of succinylacetone in urine organic acids screen using gas chromatography mass spectrometry. Second-tier confirmation tests can be performed on newborn blood spots using tandem mass spectrometry that would provide the concentration of succinylacetone, where their elevations above a reference interval of 5 µM are indicative of tyrosinemia I. In addition, quantitative analysis by tandem mass spectrometry provides the concentration of tyrosine and methionine in the plasma. It should be noted that an elevated tyrosine outside the reference range can result from a number of conditions including tyrosine I, II, transient tyrosinemia of the newborn or other liver diseases. In the same way, elevated methionine can indicate defects in methionine metabolism, liver problems or homocystinuria.

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Molecular testing of the *FAH* gene can also be used to further diagnose the particular genotype in tyrosinemia I. Of the various variants, four common pathogenic *FAH* variants have been identified. Preliminary molecular testing involves sequence analysis of the *FAH* gene which is followed by targeted gene analysis in populations where those specific genes are common. For example, the proline-leucine enzyme mutant is a pathogenic variant that occurs in ~99% of affected individuals of the Ashkenazi Jewish ancestry while the IVS12+5Guanine>Adenosine gene mutant is 33.7% of all variants worldwide and most predominately found in French Canadians)

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There is currently no cure for tyrosinemia I. As such, children diagnosed must be provided with appropriate management as outlined in the US and European recommendations. With acute liver failure, individuals often require respiratory support, appropriate fluid management and blood products that corrects the tendency to bleed.

Treatment of tyrosinemia I include restricted diet control, liver transplantation and the intake of nitisinone. Liver transplantation was the only available therapy before nitisinone became available for treatment of tyrosinemia I. Currently, liver transplantation is reserved for children with severe liver damage and who do not respond to nitisinone therapy. Nitisinone interferes with the second step of tyrosine catabolism, where it inhibits 4-hydroxyphenylpyruvic acid dioxygenase and prevents the accumulation of fumarylacetoacetic acid and its conversion to succinylacetone. However, nitisinone intake increases the blood concentration of tyrosine and requires patients to have a low tyrosine and phenylalanine diet strategy to prevent the harmful effects of elevated tyrosine concentrations. Nitisinone and dietary management should be initiated as soon as diagnosis of tyrosinemia I is confirmed. Nitisinone treatment and dietary restrictions have been documented to be associated with improved outcomes and some studies suggest much better outcomes when the treatment is started at an asymptomatic stage.

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Effective control of the amount of protein the child consumes is critical for keeping the concentration of tyrosine in the blood within normal limits. High protein-containing foods tend to be high in tyrosine and phenylalanine and thus foods like milk, meat, poultry, fish, eggs, cheese, nuts and beans should be excluded from the low-tyrosine, low-phenylalanine diets, in addition to being on vegetarian diets. Special medical food formulation such as Tyrex-1 and Tyros-1 have been developed as milk substitutes for babies with tyrosinemia to replace high protein foods. These formulas contain all the necessary proteins and other nutrients needed for growth in babies, but are tyrosine- and phenylalanine-free, as indicated on the labels.

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The other types of tyrosinemias are rare and will be discussed briefly. In tyrosinemia II, there is a defect in the *TAT* gene that encodes the tyrosine aminotransferase enzyme,

which is involved in the first step of the tyrosine catabolic pathway. This results in elevated blood concentrations of tyrosine.

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The most distinctive clinical features of individuals affected with tyrosinemia II are the painful hyperkeratotic plaques on the soles and palms, known as palmoplantar keratoderma. Plantar surface of the digits may show marked yellowish thickening associated with hyperkeratosis, which are progressive, non-pruritic and associated with hyperhidrosis. Ophthalmologic involvement includes deposition of tyrosine crystals in the cornea that ultimately leads to ulcers of the cornea, corneal opacity and photophobia. Developmental delay and intellectual disability is common in affected individuals

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Laboratory findings include elevated concentrations of tyrosine in urine and plasma. The metabolites of tyrosine such as 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, 4-hydroxyphenylacetate and N-acetyltyrosine are detected in urine organic acids analysis using GC-MS and they can be quantitated. Further work up might include genetic testing for the *TAT* gene that encodes the tyrosine aminotransferase protein.

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Restricted diet low in tyrosine and phenylalanine have been instrumental in the management of tyrosinemia II, with the concomitant lowering of plasma tyrosine concentrations leading to resolution of the oculocutaneous manifestations seen in these children. Oral retinoids can also be given to treat the associated skin lesions.

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Tyrosinemia III is the rarest form of the three tyrosine disorders, with only a few cases reported in literature. Tyrosinemia III is caused by a deficiency in hydroxyphenylpyruvic acid dioxygenase that converts 4-hydroxyphenylpyruvic acid to homogentisic acid leading to accumulation of tyrosine.

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The few affected individuals are known to have intellectual disabilities, seizures, intermittent ataxia, microencephaly and other skin or ocular changes, with no liver involvement. As with the other two types, restricted diet low in tyrosine and phenylalanine is crucial in the management of these individuals.

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Alkaptunuria and Hawkinsinuria are also genetic disorders that affect tyrosine metabolism. Individuals with Alkaptunuria have a mutation in the *HGD* gene for the enzyme homogentisate 1,2-dioxygenase resulting in the accumulation of homogentisic acid in the blood and tissues. Homogentisic acid and its oxidized form alkapton are excreted in the urine, giving it an unusually dark color. The accumulating homogentisic acid causes damage to cartilage (that leads to osteoarthritis) and heart valves, as well as precipitating as kidney stones and stones in other organs. Hawkinsinuria is an autosomal dominant disorder that occurs when there is partial deficiency of 4-hydroxyphenylpyruvate dioxygenase enzyme. The enzyme produces the reactive intermediate 1,2-epoxyphenyl acetic acid, but is unable to convert this intermediate to homogentisate. The intermediate then spontaneously reacts with glutathione to form hawkinsin. This is characterized by failure to thrive, persistent metabolic acidosis, sparse hair and excretion of Hawkinsin in the urine. Another tyrosine abnormality that is found in newborns is transient tyrosinemia. This is not an inborn error of metabolism because this is not caused by a gene defect or mutation. It occurs as a result of delayed maturation of enzymes involved in the tyrosine catabolic pathway. This often occurs in premature infants with no clinical symptoms. Laboratory findings include

hypertyrosinemia, moderate hyperphenylalaninemia, and tyrosiluria and often resolves spontaneously.

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To summarize, tyrosinemias are autosomal recessive disorders that occur as a result of enzyme defects in the metabolism of tyrosine. Type I is the most common and results in accumulation of succinylacetone and subsequent hepatorenal consequences. Gas chromatography and tandem mass spectrometry have been instrumental in the screening and confirmation of tyrosine metabolites for diagnosis of tyrosinemia. Currently, low tyrosine and phenylalanine diets in combination with nitisinone have been successfully used to manage these individuals. However, gene therapy strategies such as the CRISPR-Cas genome editing approaches holds a lot of future promise in the treatment and management of tyrosinemias.

Slide 20: References

Slide 21: Disclosures

Slide 22: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Tyrosinemias: Biochemistry and Clinical Laboratory Investigation.”