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TITLE: Vitamin A and E Testing

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Slide 1
My name is Margaret Lo. I am a Clinical Chemistry Fellow at the University of Washington. Welcome to this Pearl of Laboratory Medicine on "Vitamin A and E Testing."

Slide 2:
Vitamins are organic compounds required in small quantities for the normal growth and activity of the body. They are classified based on their relative solubility. Specifically, vitamins A, D, E, and K are grouped together as fat-soluble vitamins, while vitamins B and C are grouped together as water-soluble vitamins. Many vitamins, such as A and E, cannot be synthesized in the body and are acquired through dietary intake. An exception is vitamin D, where the functional needs are partly satisfied by the vitamin D generated from 7-dehydrocholesterol in the skin after UV radiation exposure from sunlight. Deficiency of all vitamins can lead to the development of diseases, and overconsumption of many vitamins, including A and E, can be toxic. Hence, vitamin status is often examined when either extreme of the nutritional spectrum is suspected. To improve the assessment of vitamin intake and metabolic demand, clinicians frequently depend on laboratory testing of plasma or serum vitamin concentrations in addition to clinical symptoms and dietary history.

Slide 3:
Although vitamins A and E are clinically distinct, measurement of their serum or plasma concentrations is frequently performed together using high-performance liquid chromatography (HPLC) coupled to UV-VIS detection system. HPLC-based methods for separation of vitamins A and E in serum or plasma were developed as early as the late 1970s and quickly replaced thin-layer chromatography and open column chromatography. Since then, a plethora of literature has described the numerous advancements to the HPLC separation methodology as well as measurements of various combinations of vitamin A and E forms in a variety of specimen types. Here, an example of chromatographic separation between vitamin A and E standards on a reversed-phase HPLC column detected using UV-VIS detector is shown on the left. This well-defined separation allows convenient quantification of both vitamin A and E concentrations in a patient from a single analytical run shown on the right. Compared to earlier analytical methods, HPLC-based assays have increased specificity, reduced lower limit of detection, and improved accuracy and reproducibility.

In addition to the chromatographic separation of vitamins A and E, the detection systems have also been improved. In some assays, a diode array detector (DAD) has been implemented instead of the conventional single channel UV/VIS detector. In contrast to conventional UV/VIS detection systems where the diffraction grating is placed in front of the sample to select for a single wavelength input, the DAD system places the diffraction grating behind the sample to allow detection of a wide range of wavelengths simultaneously by a photodiode array. The advantage of the DAD system is that it can provide information beyond retention time such as peak purity, which can be advantageous for analyzing multiple analytes. More recently, many clinical laboratories are developing and transitioning to mass spectrometry-based detection systems to further improve upon the sensitivity and specificity of existing vitamin A and E assays.

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While the HPLC-based assays have improved the analytical ability of clinical laboratories to quantify vitamins A and E, there are a few pre-analytical issues that could affect interpretation of the results. For example, vitamins A and E serum/plasma concentrations may be altered by intake of certain foods. Many laboratories, therefore, implement a collection policy that includes fasting for 12-14 hours and abstaining from alcohol consumption for 24 hours. Furthermore, since vitamins A and E are photolabile, light protection during sample collection, transport, and storage is often recommended to prevent falsely decreased results. Next, we will discuss the clinical utility of vitamin A testing and then, vitamin E testing.

Slide 5:

Vitamin A is the general term that describes a group of compounds with a β-ionone ring and an isoprenoid side chain known as retinoids. There are 4 naturally occurring retinoids in animals and their structures are shown here. Please note that while the trans isomers are depicted here, retinoids can also exist in cis isomers. Unless otherwise specified, retinol mentioned throughout this presentation indicates all-trans retinol. Retinol is the dominant circulatory form and can be oxidized to form retinal and retinoic acid. Retinol is also the predominant form of vitamin A quantified to evaluate vitamin A status. Retinyl esters, particularly retinyl palmitate depicted here, are the principle storage forms. While not ubiquitous, some clinical laboratories do quantify retinyl palmitate with retinol to measure vitamin A liver reserves. Vitamin A is acquired from 2 sources: animal products or fruits and vegetables. Animal products provide retinoids or preformed vitamin A, with retinyl esters as the major form. Fruits and vegetables provide carotenoids or provitamin A, with β-carotene as the major form. Carotenoids, which are retinoid precursors, have no vitamin A activity until biologically cleaved into retinoids.

Slide 6:

Once ingested, emulsified preformed vitamin A and provitamin A undergo 2 different initial pathways. To the left, we have absorption of preformed vitamin A, or retinoids, which have absorption efficiency around 70-90%. Inside the intestinal cell, preformed vitamin A is hydrolyzed to retinol and then re-esterified and transported to the liver for storage via chylomicrons. To the right, we have absorption of provitamin A, or carotenoids, which have absorption efficiency around 9-22%. Inside the intestinal cell, provitamin A must be converted to retinol before it can be utilized. This conversion is highly regulated and depends on the vitamin A status.

Once delivered to the liver, vitamin A is stored within stellate cells primarily as retinyl palmitate. About 50-85% of vitamin A is stored in the liver, and in healthy adults, these stores may represent 1-2 years of supply. Vitamin A can also be mobilized from the liver to other tissues to meet metabolic demands. To prevent loss by glomerular filtration during this transport step, retinol secreted by the liver is bound to both retinol binding protein (RBP) and transthyretin (TTR) in a 1:1:1 complex. Lastly, vitamin A is excreted through the body via feces and urine after conjugation or oxidation.

Slide 7:

Vitamin A has a variety of functions but its participation in the vision cycle is the best characterized. In the retina, 11-cis-retinal can complex with the membrane protein opsin to generate photosensitive pigments, specifically, rhodopsin in rod cells and iodopsin in cone cells. Light illumination then causes photoisomerization of 11-cis-retinal to all-trans-retinal, leading to a large conformational change in opsin that results in phototransduction. Beyond this major role, vitamin A is also known to regulate growth and differentiation of epithelial tissue, reproduction, and embryonic development. These functions are mediated through binding of retinoic acid with retinoic acid receptors (RARs) or retinoid X receptors (RXRs) in the nucleus. Once activated, these receptors control targeted gene expression by binding to specific DNA sequences that encode structural proteins, enzymes, extracellular matrix proteins, receptors, and RBP. Furthermore, Vitamin A has also been shown to play a role in bone remodeling and immunity.

Slide 8:

Vitamin A deficiency due to malnutrition is very common in resource-limited countries but rarely observed in the United States. In the United States, populations at risk of deficiency include preterm infants, as hepatic accumulation of vitamin A does not occur until the third trimester, and individuals with fat malabsorption due to celiac disease, irritable bowel syndrome, Crohn's disease, and chronic pancreatitis. Individuals with liver disease are also at risk due to diminishing RBP synthesis. This condition can be further exacerbated with alcohol abuse, leading to both liver injury and blockage of retinoic acid formation, which requires both alcohol dehydrogenase and aldehyde dehydrogenase.

There are many clinical symptoms of deficiency and most of them can be reversed through vitamin A supplements. Among the clinical symptoms, night blindness is frequently the first indicator of deficiency followed by the development of Bitot's spots, which are small light-gray, foamy lesions on the conjunctiva that signify ocular changes that may lead to blindness.

In addition to deficiency, ingesting excess preformed vitamin A can be toxic. Acute toxicity is rare and the majority of vitamin A toxicity cases are due to chronic ingestion. Treatment for vitamin A toxicity consists of stopping supplements and restricting preformed vitamin A rich food as well as other supportive care when indicated. Because metabolism of provitamin A is tightly regulated, ingesting large amounts of carotenoids can cause carotenemia or yellowing of the skin that is thought to be non-toxic except in some rare circumstances. For example, supplemental use of the carotenoid canthaxanthin is associated with retinopathy. Additionally, the supplemental use of beta-carotene initially thought to be beneficial for cancer prevention is not recommended in smokers and asbestos workers as it increases the risk of lung and gastric cancer.

Slide 9:
As mentioned in the beginning of this presentation, quantifying serum or plasma retinol concentration is the most common laboratory method used to examine vitamin A status. However, retinol concentrations are not ideal or sensitive indicators of deficiency because circulating retinol does not decline until liver stores become critically depleted. To account for this, dose response tests have been utilized. For this test, two samples are collected from the patient before and 5 hours after administrating a physiologic dose of vitamin A. Individuals with vitamin A deficiency will have a rapid, large, and sustained rise in circulating retinol concentrations detected at the 5 hour time point. This is in contrast with patients who have sufficient vitamin A stores, where a much lower and shallower rise is observed. This test is based on the principle that apo-RBP accumulates in the liver when vitamin A stores become low. Thus, when challenged with vitamin A, accumulated RBP will complex with retinol and be rapidly released into the serum. In addition to retinol, measurement of RBP and TTR has also been suggested as more cost-effective surrogate markers to assess vitamin A status. However, since both RBP and TTR are negative acute phase reactants, C-reactive protein measurement may be necessary to distinguish between inflammatory and nutritional causes of reduced circulatory protein levels. Furthermore, RBP testing may be confounded by inadequate dietary protein, energy, or zinc that are all necessary for RBP synthesis.

Slide 10:
Vitamin E is the general term that describes naturally occurring tocopherols and tocotrienols with a substituted chromanol ring attached to a long phytyl side chain. The principle sources of dietary vitamin E are oil and fats, grains, and nuts. While γ-tocopherol is the major dietary form of vitamin E, circulating γ-tocopherol, in contrast, is very low. Therefore, instead of γ-tocopherol, α-tocopherol is the form quantified by the majority of vitamin A and E assays.

Slide 11:
Vitamin E, unlike vitamin A, is stored primarily in adipose tissue and not the liver. Once ingested, emulsified vitamin E is absorbed non-selectively and delivered to the liver via chylomicrons. In the liver, α-tocopherol is selectively incorporated in very low-density lipoproteins by α-tocopherol transfer protein (α-TTP) and secreted into the circulation for functional needs or storage, while other forms are metabolized and excreted via bile or in urine. Consequently, α-tocopherol is considered the primary bioactive form of vitamin E. However, it is worth noting that increased consumption of γ-tocopherol can shift the equilibrium of γ-tocopherol from excretion to lipoprotein incorporation. Hence, some laboratories offer testing for γ-tocopherol as part of a vitamin E panel with α-tocopherol since the contribution from γ-tocopherol to the overall vitamin E pool can still be significant.

Slide 12:
To date, our understanding about the function of vitamin E is incomplete. The most currently recognized function of vitamin E is as an antioxidant and free-radical scavenger that protects polyunsaturated phospholipids from peroxidation. This function, however, requires synergistic action with vitamin C, which regenerates vitamin E from vitamin E radicals. Vitamin E is also important to maintain normal neurological function and in preventing red blood cell hemolysis. While vitamin E status can be evaluated using functional methods such as protection of erythrocyte hemolysis by peroxide and inhibition of lipid peroxidation products, this testing approach is seldom implemented in the clinical laboratory.

Slide 13:
Vitamin E deficiency is uncommon. Individuals with fat malabsorption caused by celiac disease, Crohn's disease, chronic pancreatitis, cystic fibrosis, and chronic cholestasis are at risk for deficiency. In addition, individuals with inherited genetic disorders such as mutations in α-TTP or lipoprotein B genes can develop deficiency due to inability to transport absorbed vitamin E from the liver. Premature and low birth weight infants are particularly at risk for deficiency because they have less adipose tissue. Symptoms of vitamin E deficiency include anemia, muscle weakness, and vision problems.

Vitamin E toxicity is typically due to overuse of supplements, with the most significant effect being impaired blood clotting. It should be noted that characterization of vitamin E toxicity is not well established.

Slide 14:
To conclude this presentation, this slide shows an example of established reference intervals for retinol, retinyl palmitate, α-tocopherol, and γ-tocopherol in serum or plasma. Overall, the expected serum or plasma concentration of vitamin E is generally 10-fold higher than vitamin A. Since many pediatric reference studies have reported a positive correlation between age and retinol or α-tocopherol, age-specific reference intervals are often established by laboratories. While no specific guideline provides the serum or plasma cutoff for what constitutes a deficient vitamin E concentration, the World Health Organization recommends classification of vitamin A deficiency when the serum retinol concentration is below 200 ng/mL.

In summary, Vitamin A and E testing is indicated when symptoms of deficiency or toxicity arise and can also be used to aid in the evaluation of lipid malabsorption.

Slide 15: References

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Vitamin A and E Testing."