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Review

Laboratory Generated Artifacts in Plasma Amino acid Quantitation

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Abstract:

The pace of physicians' involvement in amino acid metabolism has been enormous in the last five decades. With further development of technology to identify and quantitate upto picomoles of amino acids, their metabolites and related peptides, diagnosis and effective medical intervention in cases of inherited metabolic disorders have been well within the reach of the clinician. Automatic amino acid analyzers have become an essential part of major medical and research centers around the world. The technology has come indeed as a boon to physicians who in particular deal with inherited defects of amino acid metabolism. However, the technology comes with the risk of major deviations from the actual results when a few minor variations are not looked into. Trivial variations in basic steps of obtaining the sample, the choice of anticoagulant, hemolysis etc. can cause significant variations in the resulting values, particularly while dealing with inherited defects of amino acid metabolism and their treatment/management. Effects of such factors are revisited here for the benefit of the modern day laboratory personnel.

Introduction

Physicians have been interested in amino acids for a long time after the first amino acid Aspargine was discovered in 1806. After the epic Croonian lecture of Sir Garrod, the awareness of the amino acid associated metabolic disorders has been growing, owing to the relative ease of their quantitation with the existing technologically advanced methods and also for the fact that most of them respond to treatment and / or dietary restriction.

Amino acid quantitation and analysis have been one of the primary necessities in determining the biochemical etiology of inborn errors of metabolism. Several aminoacidopathies are characterized by significant elevations in amino acid concentrations in the plasma. Clinical effects of such elevations and the consequent organic acidemias that generally occur with most of them are well known.(1) Quantitation of amino acids with the automated analyzer on ion exchange columns has been the method of choice for quick, reliable and effective interpretation of the aminoacidogram in suspected cases of metabolic defects. Several trivial factors when not considered lead to significant deviations from the actual picture. A few of them are dealt here.

Choice of anticoagulant: Heparin has been the most commonly used and preferred anticoagulant for preparation of plasma for amino acid analysis. The mucoitin polysaccharide inhibits the formation of thrombin from pro-thrombin. Normal concentration of use for analysis is 2mg/10mL blood. However, its indiscriminate use leads to hemolysis and may introduce artifacts. Another anticoagulant, EDTA (ethylene diamine tetra acetate) chelates calcium ions for anticoagulant effect and is available in two forms viz. dipotassium and dilithium salt. The former is preferred owing to greater solubility. However, artifacts could be generated with the change in the brand/supplier applied. We have detected Ninhydrin positive artifact co-eluting with taurine at our laboratory.

Hemolysis: Hemolysis could occur during phlebotomy, preparation of the sample and transport

of the specimen. Even when hemolysis is not noticeable on naked eye inspection, spectroscopic examination may reveal bands of oxyhemoglobin.(2) In such samples, increases in concentrations of Taurine, Glutamic acid and Glutathione have been noted and decreases of upto 50 % have been noted with respect to Arginine and Cystine in our laboratory.

Deproteinising agent: Picric acid (3) and sulfosalicylic acid (4) have served the purpose of deproteinising the sample prior to application onto the column. Appreciable losses of Tryptophan have been observed with picric acid. Sulfosalicylic acid has the advantage of not altering the amino acid composition and also makes the Dowex treatment, that ensues the use of picric acid, unnecessary after deproteinisation.

Delay in deproteinisation: Effect of delay in deproteinisation can be readily noticed with reference to Cysteine, Homocystine and Cystine, wherein they readily bind to red cell and plasma proteins in the time gap available.(3, 5) Conversion of pyruvate to lactate is also favored during the delay. Formation of urea is also consequent to delay, particularly in contaminated specimens. Errors with respect to Homocystine could prove costly when dealing with a homocystinuric on dietary restriction since actual prognosis may not be indicated.

Buffy coat contamination: This is probably the easiest contaminant while separating plasma. The effort of acquiring maximum plasma is a great temptation to resist. Contamination with leucocytes and platelets leads to a high estimate of Glutamic acid and Aspartic acid since these cells contain much higher levels than plasma itself.(6) Other aminoacids are not significantly affected as their concentration in mature erythrocytes is similar to that in plasma.(7,8) It would be wise enough to leave a 5- 7mm layer above the buffy coat region before aspirating plasma.

Delay in analysis: Deproteinised plasma stored for longer periods, even in a freezer, may not result in the most accurate amino acid determinations. Losses of Glutamine and Aspargine have been noted whilst increases in Glutamic acid and Aspartic acid have been reported. However, it is generally opined that a 90-day freezer storage could still render the sample suitable for amino acid analysis.(9)

Conclusion

Amino acid pool of the plasma is subject to a lot of variations at any given instant of time. Membrane transport against a concentration gradient, nutritional state and circadian rhythm all contribute to the variations. With these factors accounting for only minor variations, a careful attention to the above detailed trivial facts is mandatory if one appreciates a critical and accurate analysis. Such facts when ignored may indicate apparent changes that do not actually reflect the biochemical reality, particularly with reference to restriction therapy in amino acid related disorders. With the awareness about inherited defects of metabolism growing, it now becomes mandatory to highlight such errors and the consequences associated with them to the fresh generation of experimenters so that biochemists, diagnosticians and care-givers for the metabolically "erred" as well as laboratory technicians should be well aware of these trivia.

Table 1. A few inherited amino acid metabolism disorders detectable by amino acid chromatography and quantitation

Disorder	Defective Enzyme
Phenylketonuria	L- Phenylalanine tetrahydrobiopterin: oxygen oxidoreductase (4-hydroxylating)
Hypertyrosinemias	Tyrosine aminotransferase /p– HHPA hydroxylase
Hyperhistidenimas	L-Histidine ammonia lyase
Hyeprlysinemias	Lysine: a ketoglutarate: triphosphopyridine nucleotide oxidoreductase
Hyperglycinemias	Glycine decarboxylase / Propionyl Co A carbon dioxide ligase
Sarcosinemia	Sarcosine: oxygen oxidoreductase
Hyperprolinemias	L-Proline NAD(P) 5 oxidoreductase / D [1] pyrroline 5 carboxylate dehydrogenase
Homocystinuria	Cystathionine synthetase
Cystathioninuria	L-Homoserine hydrolyase
Maple syrup urine disease	Branched chain a keto acid oxidases
Urea cycle disorders (hyperammonemias)	Enzymes of urea cycle

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