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TITLE:The Enigma of Biotin Interference

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Slide 1: Title Slide

Hello, my name is **Saswati Das**. I am a **Specialist biochemist** working **in Central Government Health Services India**. Welcome to this Pearl of Laboratory Medicine on "**The enigma of Biotin Interference**."

Slide 2: What is Biotin?

Biotin is a water-soluble vitamin of the B Complex group and is also known as VitaminB7. Biotin, is an essential coenzyme involved in carbon dioxide transfer in carboxylase reactions. Dietary sources of Biotin are Egg yolk, soybeans, yeast, liver and kidney, nuts and cereals. Dietary supplementation with biotin is very common and is increasingly becoming a threat to patient safety as immunoassays that use streptavidin –biotin binding mechanisms have the potential to be affected by high circulating biotin concentrations.Biotin interference is of increasing concern due to the marketing of high dose biotin supplementation (up to 10 mg in single-ingredient preparations) and trials of experimental multiple sclerosis treatments containing very high biotin doses (up to 300 mg daily)

Slide 3: **The " Biotin" problem.**

All biotin/streptavidin-based immunoassays are susceptible to biotin interference. The interaction of streptavidin and biotin has been utilized for the development of robust and highly sensitive immunoassays by many manufacturers. Biotin in patient samples can cause falsely high or falsely low results. Biotin interference is particularly dangerous for patients in emergency situations who are unaware that they are taking high doses of biotin or when the treating physician does not know the patient is taking high doses.

Slide 4: **Biotin supplementation**

In Western populations, dietary biotin intake is estimated to be 35 to 70 μg daily, a level in line with the recommended dietary allowance. Most multivitamin pills contain about 30 μg of biotin. High-dose supplementation (doses greater than 1 mg/d) plays a role in therapy for several diseases, including biotinidase deficiency, mitochondrial metabolic disorders, and multiple sclerosis. Doses up to 10 mg a day are frequently encountered in nutritional supplements taken to improve hair, skin, and nail health.

Slide 5: Serum Biotin concentration following 5.10.20 mg dosing.

The relationship between biotin intake and blood biotin concentration is an important consideration. Peak blood biotin concentrations occur 1–2 h after biotin ingestion and then rapidly decrease. In healthy volunteers, median (minimum–maximum) peak serum biotin concentrations 1 h after the ingestion of 5, 10, and 20 mg biotin were 41 (10–73), 91 (53–141), and 184 (80–355) ng/mL, respectively.(P. Grimsey, et al)

Slide 6: Role of Biotin in Immunoassays

Biotin is a small molecule that can be attached by covalent bond to a variety of targets without effecting their biological activity. Biotin thus makes the target easy to capture because it forms a strong, stable, and specific non-covalent bond with avidin, streptavidin, or NeutrAvidin proteins.

Slide 7: What effect does biotin interference have?

The risk of patient misclassification due to biotin interference can vary considerably between assays, and is dependent on the biotin and analyte concentrations present in the sample, as well as the specific assay in question. All biotin–streptavidin-based immunoassays are susceptible to biotin interference. The direction of interference

depends on the design of the assay. Some results are falsely elevated, some falsely lowered. Two of the most common immunoassay designs are the sandwich assay and the competitive assay.

Slide 8: Platforms Using Biotin based detection.

Some of the common platforms using biotin based detection are Roche Elecsys , Ortho Clinical Diagnostics Vitros, Beckman Coulter Access, Beckman Coulter DXI, Siemens Centaur, Siemens Immulite , Siemens Dimension.

Slide 9: How Biotin Interferes (sandwich assays)

Depending on the biotin dose, assay design, and assay interference threshold, biotin interference can cause falsely low (sandwich immunoassays) or falsely high (competitive immunoassays). In sandwich / non-competitive immunoassays, the analyte is bound by the signal and biotinylated antibodies. A biotinylated antibody then links the analyte–antibody sandwich complex to a streptavidin-coated solid phase . The signal increases as the analyte concentration increases. In the presence of high biotin concentrations, excess biotin saturates the streptavidin binding sites, preventing linking with the analyte–antibody sandwich complex and leading to falsely low assay results.

Slide 10: How Biotin Interferes (competitive assays)

In competitive immunoassays, endogenous and labeled (signal) analytes compete for a single biotinylated antibody binding site. The biotinylated antibody is then bound to the streptavidin-coated solid phase. The signal decreases as the analyte concentration increases. In the presence of high biotin concentrations, excess biotin binds to the solid phase and prevents the binding of antibody-endogenous and labeled analytes and unbound antibodies are removed in the wash step, leading to a falsely decreased signal and thus falsely high assay results.

Slide 11: Examples

Some examples of Sandwich and Competitive Immunoassays (Roche) prone to biotin interference:

a)TSH (Sandwich assay): Biotinylated monoclonal anti-TSH Ab (mouse) and monoclonal anti-TSH Ab (mouse/human) labeled with ruthenium complex.

b) FT4 (Competitive assay): Polyclonal anti-T4-Ab (sheep) labeled with ruthenium complex competes with biotinylated T4.

c) FT3(Competitive Assay): Monoclonal anti-T3-Ab (sheep) labeled with ruthenium complex competes with biotinylated T3.

Slide 12: Assays commonly affected by Biotin interference

ACTH, AFP, Anti TPO, Anti-TG, Ca125, Ca15-3, Ca19-9, CEA, Cortisol , C-peptide, DHEAS, Digoxin, Estradiol , Folate, Free PSA, FSH, FT3, FT4, betaHCG, Hs TnT , IgE , Insulin , LH, Progesterone, Prolactin, PSA, PTH, SHBG, Testosterone, Total B12 , TSH. Many more analytes are constantly being added to the list.

Slide 13: Detecting and Mitigating Biotin Interference

Detecting biotin interference is indeed a challenge. Some strategies suggested to mitigate the same are:

a) Serial dilution study reduces the concentration of biotin and the analyte concentration adjusted for dilution will provide the correct result.

b) Repeating tests after allowing sufficient time for biotin clearance in the patient and/or using alternative assays/methods e.g., non-biotin– streptavidin-based immunoassays or liquid chromatography– tandem mass spectrometry.

c) Confirmation of the presence of biotin using depletion protocols. Depletion protocols involve the addition of materials to bind and remove any biotin in the sample prior to testing; for example, adding streptavidin agarose beads to the sample (10% sample volume) followed by incubation for 1 h with intermittent mixing.

d) Direct measurement of biotin concentrations.

e) Using streptavidin-agarose beads to remove the biotin before the sample is run on the affected analyzer

Slide 14:Precautions:

Withhold biotin for at least 3 days before test. If the patient is taking higher doses it might required to wait for a few more days. Other interferences in immunoassays like Heterophilic antibodies, Human anti-animal antibodies, High-dose hook effect etc.are to be ruled out.

Slide 15: Best practice recommendations (R. Bowen et al.)

Recommendations for stakeholders to help reduce the risk of biotin interference with immunoassays. For manufacturers it is important to determine biotin interference thresholds and include in product inserts. Increase awareness of biotin interference and provide guidance via bulletins or online resources. For laboratory staff it is pertinent to provide key contact information with assay results/request forms, advise healthcare providers on how to minimize the risk of inaccurate test results and develop internal algorithms to investigate inaccurate test results. For healthcare providers it is necessary that they ask patients about supplement/biotin use, including dosage, disseminate proper patient instructions to prepare for blood tests several days prior to appointment and contact laboratory if biotin interference suspected. The patient also needs to be aware and check the supplement labels for biotin, report all supplement intake to healthcare provider and not take biotin prior to undergoing blood tests.

Slide 16: In Conclusion

Always think about assay interference if there is discrepancy between test result and clinical picture. Assay interference has to be ruled out. If patient is on biotin

supplement, stop the supplement before blood tests done by immunoassay. If in doubt a dialogue with the clinician is recommended. Patient symptoms, clinical examination findings, laboratory results and imaging should be evaluated in concert to gain a holistic clinical picture and avoid misdiagnosis. Proper patient information, awareness and communication with the physician is a key factor in mitigating the risk of biotin interference.

Slide 17: References

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Slide 18: Disclosures

No potential Conflict of Interest

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**The enigma of biotin interference**."