Interference with Immunoassays of a Neonate on High Biotin

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Abstract

Biotin is an easily available over-the-counter supplement. Its main use in mega doses in pediatrics is almost limited to treating inborn errors of metabolism. We are reporting a one-week-old neonate who was suspected to have an inborn error of metabolism on day two of life due to hypoglycemia, metabolic acidosis, high lactate and ammonia. He was started on a high dose of biotin as part of a so-called "mitochondrial cocktail "which contains Carnitine, Biotin, Co-Enzyme Q10, and Thiamine. His thyroid function test (TFT) was checked at day seven of life investigating hepatocellular dysfunction. The result showed a biochemical picture of primary hyperthyroidism, which was not clinically evident. Immunoassay interference with the thyroid function test was suspected due to discordance between the clinical picture and biochemical thyroid status. The interference was investigated by analyzing the sample in another lab using a different immunoassay. Furthermore, immunoassay biotin interference was confirmed by normalization of the TFT seven days after stopping biotin using the same initial immunoassay. This case report clearly demonstrates that if the biochemical profile does not match the clinical picture, it is reasonable to doubt the test result and think of assay interference.

Keywords: Biotin; immunoassay; interference; neonate, FT4, TSH; Anti-Thyroid receptor antibody; thyroid function test; biotin- streptavidin based immunoassay

Introduction

As one of the B vitamins, Biotin is an essential water-soluble and completely absorbed micronutrient. It is readily available naturally from a wide variety of plant and animal-based foods with a minority supplied by gut bacterial synthesis (1). Biotin is also marketed as a supplement either part of the vitamin B-complex or alone. It is available over-the-counter under a variety of names, including vitamin B7, vitamin H, and coenzyme R, and sometimes maybe an un-named supplement advertised as an aid to improve hair and nail health. This water-soluble vitamin is a cofactor of multiple carboxylases that catalyze critical steps in the metabolism of fatty acids, glucose, and amino acids (2). Biotin also plays key roles in gene methylation and cell signaling (3). In healthy individuals, biotin in low concentrations is eliminated from the circulation relatively quickly with a half-life of around 2 hours. (4) On the other hand, when high doses of biotin are administered, experiments suggest that the elimination half-life could be up to 18.8 hours.(5)

In the pediatric age group, high-dose biotin therapy is medically indicated for certain pathologies, such as inborn errors of metabolism (for example: biotinidase deficiency, and biotin–thiamine responsive basal ganglia disease). Peak

serum biotin levels occur 1–3 hours post-ingestion (6, *7*). It is an essential co-factor for five carboxylases involved in the fatty acid synthesis and energy production (8). Of note, it has been reported to cause interference in immunoassays resulting in abnormal thyroid function tests. Biotin interference with the Thyroid function test (TFT) can lead to a wrong diagnosis of hyperthyroidism leading to unnecessary treatment. (9)

Case Report

A one-week-old neonate was born to healthy consanguineous parents. Antenatally, he was noted to have bilateral ventricular and pelvicalyceal dilatation. He was born term at 38 weeks of gestation via lower segment caesarean section. APGAR score was 4 and 8 at 1 & 5 minutes respectively. His birth weight was 2.7 kg (50th percentile), length 50 cm (90th percentile), and head circumference 35.5 cm (90th percentile). On day two of life, he developed poor oral intake and lethargy, his random bedside blood glucose was found to be low (2.1mmol/L), that was corrected with a 10% dextrose intravenous bolus, followed by a maintenance 10% dextrose infusion. Blood gas result showed severe metabolic acidosis: pH 6.91, pCO_2: 13 mmHg, HCO_3: 2.6 mmol/L, lactate 11.5 mmol/L, BE -28 mmol/L. He had high ammonia and lactate; 507 µmol/L (16 – 60) , and 17 mmol/L(0.5-2.2) respectively. He was started on sodium bicarbonate infusion. Plasma ammonia was repeated at day 3 of life, which normalized to 49umol/L, but he had persistent high lactate.

The initial working diagnoses were organic acidemia, pyruvate metabolic defect, and Biotinidase deficiency. Given the fact of persistent lactic acidosis, mitochondrial disease was also considered and the patient was started on the mitochondrial cocktail: Carnitine (85mg four times daily , Biotin (10 mg once daily) , coenzyme Q10 (60mg twice daily, and Thiamine 100mg once daily.

On day 7 of life, the patient developed hepatocellular dysfunction with conjugated hyperbilirubinemia and deranged liver enzymes. Therefore, a thyroid function test (TFT) was done as part of the evaluation, which showed high free thyroxine (FT4), (> 100 pmol/L) and suppressed thyroid stimulating hormone (TSH) (< .01 mIU/L). Clinically, the newborn was not showing any signs of hyperthyroidism. He was not irritable and continued to be hemodynamically stable and with normal vital signs; afebrile, heart rate 140 beat per minute, and blood pressure 89/55 mmHg. Clinical examination revealed no goiter. However, despite the lack of any signs or symptoms of hyperthyroidism, his repeated TFT showed persistent high FT4 and low TSH.

Immunoassay interference with thyroid function was suspected due to discordance between clinical picture and biochemical thyroid status. The first TFT test was done using a biotin- streptavidin based immunoassay(test/platform) . The TSH measurement is based on a non competitive method whereas the FT4 and free triiodothyronine (FT3) measurements require competitive assays. Due to suspicion of Immunoassay interference , TFT sample was sent to another laboratory using acridinium-ester based assay method after biotin supplementation was stopped temporarily for about 24 hours before retesting the thyroid function. The results were as follows ; the TSH was normal at 0.9 mIU/L (0.35-8.5) as was the FT4 : 14 pmol/L (10-27.5) . The acridinium-ester based assay using anti mouse monoclonal antibodies does not use any biotin-based labels. For further confirmation a second sample was obtained and split between the two assays **,** the biotin-streptavidin based assay and the acridinium-ester based assay . The results were as follows : TSH [0.06 (0.72-11.0) mIU/L, FT4: 47.4 (11.5-28.3) mIU/L] which was discordant with the clinical picture using the first assay. In contrast, the result was normal using the second non-biotin based assay [TSH : 0.9 (0.35-8.5) mIU/L, FT4: 14.7 (10-27.5) pmol/L] in concordance with the clinical picture. Furthermore, biotin interference was confirmed by normalization of the FT4 and TSH seven days after stopping biotin using the same initial (the biotin based) immunoassay. Additionally, investigating the baby for thyrotoxicosis, anti- thyrotropin receptor antibody (anti-TSH-R) was performed initially, that came back very high: >40.00 UI/L (0.00-1.50) Table1. The baby's mother was investigated for thyroid disease as clinically indicated in babies with thyrotoxicosis. Her investigation including FT4, TSH, anti-TPO antibodies, and anti-thyrotropin receptor antibody, all came to be within the normal range. From a metabolic point of view, the baby was confirmed to have pyruvate carboxylase deficiency due to homozygous variant in the *PC gene c.2278C>T (p.Arg760Trp).*

Table 1: Thyroid function test on biotin supplement and at different timing after stopping biotin and using different immunoassay method.

	TFT-Strep on high dose biotin	TFT-Strep confirmed on same dose of biotin	TFT –Strep 24hrs after holding biotin	TFT using acridinium-ester based assay	TFT-Strep 48hrs after holding biotin	TFT-Strep 7 days after holding biotin
FT4 (9.7-14.2 pmol/L)	>100	>100	47.4	14.7	35.9	20.6
TSH (0.8-3.9 mIU/L)	<0.01	0.02	0.06	0.90	0.06	0.91
FT3 (3.8-6 pmol/L)	9.0					
Anti-Thyroid receptor antibody	>40 (0-1.5)					
Anti TPO antibodies	<10 IU/ml (0-50)					

Thyroid function test (TFT), TFT using streptavidin-biotin based immunoassays (TFT-Strep), free thyroxine (FT4), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), anti TPO antibodies :Thyroid peroxidase antibodies

Discussion

Laboratory interference is a concern in clinical practice when laboratory test results deviate from the expected clinical presentation. However, in cases where the endocrine profiles obtained from these results appear to be clinically plausible, there is a risk of initiating further diagnostic testing and/or inappropriate treatment prior to investigating the possibility of erroneous results. Automated immunoassay platforms are prone to interferences from many sources. The usual indication that an interference exists is the clinical and biochemical profile discrepancies. Here we report a case of high dose biotin therapy causing false results and leading to clinical confusion. To the best of our knowledge, this is the first case report of its type to be reported from the Sultanate of Oman. Furthermore, although there have been some reports, far and few in between, from various regions around the world, however, to the best of our knowledge, there are very few similar cases reported from the Middle east and North Africa region (MENA). Holmes etal in 2017 reviewed 327 available immunoassays and found that 59.1% of these were biotin based. Even if the scale of the problem is unknown the potential for interference , especially with rising biotin supplementation is huge (10, 19). Similarly, other authors such as Picketty et all in 2017 reported a case of false biochemical hyperthyroidism due to biotin based interference.(11). Suleiman Ra from Saudi Arabia also reported erroneous thyroid function test results in relation to Biotin therapy (12) Alerting physicians to the possibility of biotin interference with commonly ordered and used biotin based-immunoassays is very important. Interference within laboratory tests maybe an old problem, but it is a recurring problem. Pathologists and physicians alike need to be aware of these interferences and how to identify and correct them whenever they are encountered in clinical practice.

Biotin is an essential, water-soluble vitamin that falls under the B-complex family and is involved in critical carboxylation reactions that underpin fatty acid metabolism, amino acid metabolism, and gluconeogenesis. Biotin is a small molecule that can undergo biotinylation, whereby it covalently attaches to various molecules, including proteins, peptides, and nucleic acids, with minimal impact on their functional or antigenic properties. The avidity of biotin for streptavidin and to a lesser extent, avidin, is exceptionally high, making the biotin-streptavidin/avidin interaction an attractive tool for various biomedical techniques, including western blotting, flow cytometry, immunoassays, and immunohistochemistry, due to its durability and robustness. (*13*).

From a clinical standpoint, immunoassays are commonly used to quantify a variety of physiological markers, including protein and steroid hormones, tumor markers, micronutrients, and therapeutic drugs. In general, non-competitive or two-site "sandwich" immunoassays are used to evaluate larger molecules, such as thyroid-stimulating hormone (TSH) and cardiac troponin, while competitive assays are used to measure smaller molecules, such as thyroid

and steroid hormones. Both types of assays may use biotin-streptavidin linkage, which can be hindered by the introduction of exogenous biotin, resulting in a decrease in signal and potentially leading to inaccurate results, depending on the assay setup. To address this issue, laboratories can issue warnings about the possibility of biotin interference in assays that use biotin-streptavidin, but these warnings are often ignored or not shared amongst staff due to high turnover rates and alert fatigue, a common phenomenon in modern medical practice. (14).

The coexistence of positive and negative biotin interferences can create a condition that mimics a pathological state and poses potential harm. If biotin levels are high in serum or plasma, it can result in false elevation of analyte concentration when a competitive immunoassay is used, which is called positive interference. Conversely, when a sandwich immunoassay format is employed, biotin can cause negative interference. These types of interferences can lead to diagnostic errors, particularly in cases of hyperthyroidism. In such cases, biotin may cause positive interference in competitive format assays for FT3 and FT4, while causing negative interference in the TSH assay that uses the sandwich format (15) which explain the biochemical picture of our patient. Moreover, the co-presence of falsely detected thyrotropin receptor antibodies or thyroid peroxidase can further complicate the diagnosis by leading to a misattribution of Graves' disease. This phenomenon has been extensively documented in various case reports, emphasizing the critical importance of vigilance in laboratory testing protocols to prevent such erroneous diagnoses. (*15*- 21). Other types of interferences with thyroid function tests also exist. There are usually six types of interferences that can be affect thyroid function tests (namely TSH, FT4, FT3), one of them has already been discussed in detail in this case report ie: biotin interference with streptavidin-biotin based immunoassays. Others include, Macro-TSH interference, anti-ruthenium antibodies, antistreptavidin antibodies, anti-thyroid auto antibodies and heterophil / and Human anti animal antibodies (HAAA) . Macro-TSH prevalence in tested samples is thought to be between 0.6%-1.6%. (20). Macro-prolactin is a large circulating form of TSH bound to a monomeric IgG antibody. This form of TSH is usually inactive , however, due to its large size it's renal clearance is slow resulting in a prolonged half life and is usually detected in two-site sandwich immunoassays as "regular TSH" by most immunoassays , including the afore mentioned streptavidin-biotin based assay. (22) .Other commercially immunoassays are also prone to this interference, such as chemiluminescent microparticle immunoassay using chemiluminescent labelled conjugates (for example acridinium labels), Enzyme linked immunoassay (ELISA) based methods and electro- chemiluminescent immunoassays are all prone to macro-TSH interference. These TSH assays cross react with macro-TSH causing falsely high TSH levels , with normal FT4 and FT3 levels , thereby mimicking subclinical hypothyroidism clinically. This can erroneously cause unnecessary follow-up investigations and treatment. Macroprolactin is detected accurately by gel filtration chromatography. Samples from suspected cases should be pre-treated with polyethylene glycol and re-tested on the immunoassay platform for correction of falsely elevated TSH values.(23) . Streptavidin is a protein produced by *stroptomyces avidinii,* and binds very specifically to biotin. Some patients have significant levels of circulating anti-streptavidin antibodies, which can interfere with streptavidin-biotin based TSH immunoassays usually causing falsely low levels of TSH and falsely high levels of FT4 and FT3 resulting in a biochemical picture consistent with primary hyperthyroidism, despite absence of coinciding clinical signs and symptoms. Mismanagement of cases based on similar erroneous results have been reported. (22). Testing the patient's sample on a different immunoassays platform, performing serial dilutions, pre-treating the sample with anti-streptavidin antibody blocking agents and retesting the sample are all ways to confirm the presence of this interference. (22). Ruthenium is a rare chemical element and is used in commercial products such as chip-resistors, platinum alloys . It is also used as a label in electrochemiluminescence based immunoassays. Samples that contain circulating Anti-Ruthenium antibodies can cause falsely low TSH levels as well as falsely high FT3 and FT4 levels; giving a biochemical picture consistent with primary hyperthyroidism.(24) In some cases, anti-ruthenium antibodies caused falsely elevated levels of TSH and falsely low FT4 and FT3 results, causing a biochemical picture of primary hypothyroidism. (22, 24, 25). Anti-ruthenium antibody based interferences are usually heterogenous and more complex in their presentation making them more challenging to recognize than biotin, macro-TSH and anti-streptavidin interferences. Luckily, Ruthenium based interferences are rare ; presenting in less than 0.1%-0.24%of cases.(22). The manufacturer has since then replaced it's method with other immunoassay methods. (22).

Other reported interferences include antibodies to thyroid hormones (ie against T3 and T4) alsoknown as Anti thyroid Hormone antibodies (THAAbs).These antibodies are rare in the general population , however they are found in up to 40% of autoimmune thyroid diseases (26). Most of these antibodies interfere with one-step immunoassays giving falsely elevated FT4 and FT3 results in the presence of normal TSH levels, thereby confusing the clinical picture. This is especially problematic in cases of autoimmune hype/ hypothyroidism like grave s disease or Hashimoto's disease where patients are on treatment and follow up. This might result in un-necessarily adjusting or

increasing deses of antithyroid medications/ thyroxin replacement therapy thereby causing harm or potential harm to patients. (22, 26, 27). Heterophil antibodies are weak polyspecific antibodies present usually in low titers and can bind / cross react loosely with the FC region of animal immunoglobulins. The rheumatoid factor also behaves like heterophil antibodies bnding non specifically to the Fc region of immunoglobulins. On the other hand , human anti-animal antibodies (HAAA) are monospecific, high-affinity antibodies against animal epitopes from goats, rabbits, sheep, horses, or, more frequently, mice (also known as Human Anti Mouse antibody, HAMA). Heterophilic antibodies can cause both falsely elevated and falsely low analytes (ie : TSH, FT4 and FT3) . Mostly, heterophilic antibodies have been reported to cause falsely elevated analyte levels , especially TSH levels (22,28). Solutions to eliminate heterophil antibody interferences include but are not limited to , precipitation of antibodies using polyethylene glycol (PEG) solution and then retesting the supernatant in the patient's sample for TSH, FT4 and FT3 and testing the patients sample in heterophil blocking tubes (HBT) containing heterophil blocking reagent (22).

Conclusion

From the literature review, it is clear that biotin Interference (as well as other interferences) with the thyroid function test is well documented. If the biochemical profile does not match the clinical picture, it is reasonable to doubt the test result and think of assay interference. Detailed medication history and literature review are necessary when thinking of assay interference. The best practice that in hospitals with labarotory conducting a biotin-based immunoassay, it is important to exercise caution and obtain a history of biotin supplement intake from the individual at the sample collection station.

Disclosure

The authors declared no conflicts of interest. Written consent was given by the father of the patient.

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