CASE REPORT

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Interference with Immunoassays of a Neonate on High Biotin: Case Report and Literature Review

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ABSTRACT

Biotin is sometimes administered in mega doses to children to treat certain inborn errors of metabolism. We report the case of a one-week-old newborn who was started on a 'mitochondrial cocktail' that contained a high dose of biotin. On day seven of life, his thyroid function test showed a biochemical picture of primary hyperthyroidism, which was not clinically evident. The suspicion of immunoassay interference with thyroid function test was confirmed when another lab, using a different immunoassay, gave normal results. If the biochemical profile does not match the clinical picture, it is reasonable to doubt the test result and think of assay interference.

As one of the B vitamins, biotin is an essential water-soluble and completely absorbed micronutrient.[1] It 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 with a half-life of around two hours, and in high concentrations, up to 18.8 hours.[4,5]

In children, high-dose biotin therapy is medically indicated for certain pathologies, such as inborn errors of metabolism. Peak serum biotin levels occur 1–3 hrs post-ingestion.[5,6] It is an essential co-factor for five carboxylases involved in fatty acid synthesis and energy production.[7] Of note, it has been reported to cause interference in immunoassays resulting in abnormal thyroid function tests (TFTs). Biotin interference with the TFT can lead to a wrong diagnosis of hyperthyroidism leading to unnecessary treatment.[1]

CASE REPORT

The patient was a one-week-old boy born to healthy consanguineous parents at 38 weeks of gestation via caesarean section, with a birth weight of 2.7 kg, and APGAR scores of 4 and 8 at respectively one and five minutes. Antenatally, he had bilateral ventricular and pelvicalyceal dilatation. On day two of life, he developed poor oral intake and lethargy. His random bedside blood glucose was found to be low (2.1 mmol/L), that was corrected with a 10% dextrose intravenous bolus, followed by a maintenance 10% dextrose infusion. Blood gas results showed severe metabolic acidosis: pH 6.91; pCO_2 13 mmHg; HCO_3 2.6 mmol/L; lactate 11.5 mmol/L; base excess 28 mmol/L. He had a high ammonia at 507 µmol/L, and high lactate at 17 mmol/L. He was started on sodium bicarbonate infusion. Plasma ammonia normalized on day three of life to 49 umol/L, but the high lactate level persisted.

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' comprising carnitine, biotin, coenzyme Q10, and thiamine.

On day seven of life, the baby developed hepatocellular dysfunction with conjugated hyperbilirubinemia and deranged liver enzymes. TFT showed high free thyroxine (FT4) at > 100 pmol/L and low thyroid stimulating hormone (TSH) at < 0.01 mIU/L, despite the patient

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Table 1: Thyroid function test (TFT) results while on biotin supplement, after pausing biotin, and stopping biotin, in two labs that used different immunoassay methods.

Day of life	Immunoassay method used for TFT with biotin status	FT4 (9.7–14.2 pmol/L)*	TSH (0.8–3.9 mIU/L)*	FT3 (3.8–6 pmol/L)*	Anti-TSH-R (0–1.5)*	Anti-TPO antibodies (0–50 IU/mL)*
7	Streptavidin-biotin-based immunoassay while on biotin (lab 1)	> 100	< 0.01			
8	Repeat streptavidin-biotin-based immunoassay – on same dose of biotin (lab 1)	> 100	0.02	9	> 40	< 10
10	Streptavidin-biotin-based immunoassay – 24 hrs after holding biotin (lab 1)	47.4	0.06			
	Acridinium-ester-based assay– 24 hrs after holding biotin (lab 2)	14.7	0.9			
11	Streptavidin-biotin-based immunoassay – 48 hrs after holding biotin (lab 1)	35.9	0.06			
15	Streptavidin-biotin-based immunoassay – seven days after stopping biotin (lab 1)	20.0	0.91			

**reference range; FT4: free thyroxine; TSH: thyroid stimulating hormone; FT3: free triiodothyronine; TSH-R: thyrotropin receptor; TPO: thyroid peroxidase.*

showing no clinical signs of hyperthyroidism or goiter. He was not irritable, was hemodynamically stable, and had normal vital signs (afebrile; heart rate 140/min; blood pressure 89/55 mmHg). A repeat TFT again showed high FT4 and low TSH. The tests were conducted using a biotin-streptavidin-based immunoassay.

The discordance between the clinical picture and biochemical thyroid status raised the suspicion of immunoassay interference with TFT. Therefore, we temporarily stopped the biotin supplementation for about 24 hrs and sent a new sample to another laboratory which used acridinium-ester-based assay. This time the TSH and FT4 results were normal at 0.9 mIU/L and 14 pmol/L, respectively. Another set of samples sent to the two labs also returned the earlier differing pattern of results [Table 1]. Furthermore, biotin interference was confirmed by normalization of the FT4 and TSH seven days after stopping biotin using the biotin based immunoassay. Additionally, investigating the baby for thyrotoxicosis, anti-thyrotropin receptor antibody was performed initially, and it came back very high at > 40 UI/L [Table 1]. The baby's mother was investigated for thyroid disease and found normal. From a metabolic point of view, the baby was confirmed to have pyruvate carboxylase deficiency due to a homozygous variant in the PC gene c.2278C>T *(p.Arg760Trp)*. Informed written consent was obtained from the patient's father.

DISCUSSION

The possibility of laboratory interference should be considered when test results deviate from the expected clinical presentation. 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 our knowledge, this is the first such case report from Oman. Only a few cases have been reported internationally, and still fewer from the Middle East and North Africa region. Holmes et al,[8] in 2017 reviewed 327 instances of interference in immunoassays and found that 59.1% of these were biotin based. With rising use of biotin supplementation, the potential for an increase in occurrence of interference seems high.[9] Other authors have also cases of erroneous TFT results due to biotin-based interference.[10,11] 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 a small molecule capable of covalently attaching to various biological molecules with minimal impact on their functional or antigenic properties. Biotin's high avidity for streptavidin and avidin has rendered the biotin-streptavidin/avidin interaction ideal for immunoassays.[12]

From a clinical standpoint, two types of immunoassays are commonly used to quantify physiological markers: (a) non-competitive or two-site sandwich immunoassays used to evaluate larger molecules such as TSH, and (b) competitive assays used to measure smaller molecules such as thyroid and steroid hormones. Both 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. To address this issue, laboratories issue warnings about the possibility of biotin interference in assays that use biotin-streptavidin, but too often ignored or not shared amongst staff due to high turnover rates and alert fatigue.[13]

When using a competitive immunoassay, if the biotin level is high in serum or plasma, it can cause positive interference, i.e., false elevation of analyte concentration. Conversely, when a sandwich immunoassay format is employed, biotin can cause negative interference. In results indicating hyperthyroidism, 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,[14] which was the biochemical picture given by the initial streptavidin-biotin-based immunoassay of our patient while on biotin [Table 1].

Moreover, the copresence 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.[9,14–19]

There are usually six types of interferences that can affect the reported levels of the thyroid function components (TSH, FT4, and FT3). One of these—biotin interference with streptavidin-biotin-based immunoassays—has already been discussed above as it directly pertains to this case report. Others include macro-TSH interference, anti-ruthenium antibodies, anti-streptavidin antibodies, anti-thyroid auto antibodies, heterophil, and human anti-animal antibodies. These are briefly introduced below.

Macro-TSH interference. Macro-TSH prevalence in tested samples is thought to be 0.6–1.6%.[18] Macro-prolactin is a large circulating form of TSH bound to a monomeric immunoglobulin G antibody. This form of TSH is usually inactive; however, due to its large size, its 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 aforementioned streptavidin-biotin-based assay.[20]

Other commercial immunoassays are also prone to this interference, such as chemiluminescent microparticle immunoassay using chemiluminescent labelled conjugates (e.g., acridinium labels), enzyme-linked immunoassay-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.[21]

Anti-streptavidin antibodies-based interference. Streptavidin is a protein produced by Streptomyces 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 the absence of coinciding clinical signs and symptoms. Mismanagement of cases based on similar erroneous results has been reported.[22] Testing the patient's sample on a different immunoassay 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]

Anti-ruthenium antibodies-based interference. 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.[22] 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.[22,23] 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 < 0.1–0.24% of cases.[20] The manufacturer has since then replaced the method with other immunoassay methods.[20]

Antibodies to thyroid hormones interference. Other reported interferences include antibodies to thyroid hormones (ie.; against T3 and T4) also known as anti-thyroid hormone antibodies. These antibodies are rare in the general population; however, they are found in up to 40% of autoimmune thyroid diseases.[24] Most of these antibodies interfere with one-step immunoassay 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 unnecessarily adjusting or increasing doses of antithyroid medications/thyroxin replacement therapy thereby causing harm or potential harm to patients.[20,24,25]

Heterophil antibodies interference. Heterophil antibodies are weak polyspecific antibodies present usually in low titers and can bind loosely with the fragment crystallizable region of animal immunoglobulins. The rheumatoid factor also behaves like heterophil antibodies binding non-specifically to the fragment crystallizable region of immunoglobulins. On the other hand, human anti-animal antibodies are monospecific, high-affinity antibodies against animal epitopes from goats, rabbits, sheep, horses, or, more frequently, mice (also known as human anti-mouse antibody). 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.[20,26] Solutions to eliminate heterophil antibody interferences include but are not limited to, precipitation of antibodies using polyethylene glycol solution and then retesting the supernatant in the patient's sample for TSH, FT4, and FT3 and testing the patient sample in heterophil blocking tubes containing heterophil blocking reagent.[20]

CONCLUSION

Biotin Interference (as well as other interferences) with the TFT is well documented. If the biochemical profile does not match the clinical picture, it is reasonable to doubt the test results and think of assay interference. Detailed medication history and literature review are necessary when thinking of assay interference. The best practice is that in hospitals with laboratories 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.

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