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ORIGINAL ARTICLE

Assessment of Risk for Interference by Circulating Biotin in Samples Received for High Sensitivity Troponin-T, Thyrotropin, and for Prostate Specific Antigen Testing by Immunoassays

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SUMMARY

Background: **Biotin interference in streptavidin-based immunoassays is known and may lead to erroneous results and thus to diagnostic error. The recent increase in reports of biotin interference in immunoassay-based testing has been attributed to increased intake of biotin supplements by the public and to the high dose biotin therapy in patients with neurological and inherited disorders. Circulating biotin levels greater than 20 ng/mL are reported to exhibit interference in high sensitivity troponin T (hs-TnT), thyroid stimulating hormone (TSH), and in prostate specific antigen (PSA) among other assays when using our Cobas® 6000 immunoassay analyzer (Roche Diagnostics, IN, USA). This study aims to examine the risk for biotin interference among our patient population.**

Methods: **Serum and plasma leftover samples from 183 different patients were collected following completion of hs-TnT (53 samples), TSH (45 samples), and PSA (85 samples) testing. Aliquots were stored frozen at -20°C until analysis. Biotin concentrations in these samples were measured using an ELISA (ALPCO, Salem, NH, USA) according to the manufacture's protocol. Samples with biotin levels of 20 ng/mL or greater were considered as high-risk samples (HRS) for biotin interference.**

Results: **The overall concentrations of biotin in our patients' samples ranged from 0.02 ng/mL to 11.38 ng/mL (median 0.42 ng/mL). The median and (range) biotin concentrations in hs-TnT, TSH, and PSA samples were 0.27 ng/mL (0.02 - 6.86 ng/mL), 0.39 ng/mL (0.08 - 11.38 ng/mL), and 0.47 ng/mL (0.09 - 7.73 ng/mL), respectively. Although there was no significant difference between biotin levels in samples for TSH or PSA measurement (p = 0.85), biotin in samples for PSA and for hs-TnT and in samples for TSH and hs-TnT were significantly different (p = 0.049 and 0.089), respectively. None of the samples had biotin levels greater than or equal to 20 ng/mL.**

Conclusions: **Using representative samples with requests for hs-TnT, TSH, and PSA testing, where reliable performance for the selected assays at their lowest measurement range is required for clinical intervention, among our study population the risk was considered minimal as their circulating biotin levels were less than 20 ng/mL. However, educating clinicians and laboratory users regarding the potential of biotin interference is always recommended.**

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KEY WORDS

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INTRODUCTION

Biotin, a water-soluble vitamin B-7 (also known as vitamin H), has many biological activities. It is a cofactor for biotin-dependent carboxylases involved in gluconeogenesis, fatty acid synthesis, amino acid catabolism

as well as regulation of gene expression thought to play a role in neurological dysfunction [1]. Western diet contains 35 - 75 μg of biotin per day [2] with 30 μg considered as the daily nutritional requirement. It is present in a protein bound form and is released during the biotin cycle catalyzed by pancreatic biotinidase enzyme [3]. Cellular uptake of released biotin is mediated by a sodium-dependent transporter [4,5]. High therapeutic dosage of biotin is used in patients with genetic biotin deficiency [1], multiple sclerosis [6,7], and in biotin-thiamin responsive basal ganglion disorder [1], as well as in alleviating muscle cramps in patients undergoing renal dialysis [8].

Biotin-streptavidin based immunoassays are widely used both in automated immunoassay analyzers and in research and clinical enzyme-mediated immunosorbent assays (ELISAs). The strong biotin-avidin binding and ability to easily attach biotin to proteins and antibodies as well as its relative low cost make it a very widely used and preferred detection system. However, biotin interferes with streptavidin-based immunoassays and can cause erroneous laboratory results leading to a risk for inappropriate patient management. In a recent review of the 8 most popular immunoassay analyzers used in the United States, 221 of 374 test methods (59%) used biotin-based assays and 82 (22%) of those had manufacturer-reported interference thresholds of circulating biotin levels of less than 51 ng/mL [9]. Biotin levels greater than 20 ng/mL are known to exhibit interference in high sensitivity troponin T (hs-TnT) assay, 25 ng/mL in thyroid stimulating hormone (TSH) assay, and 30 ng/mL in prostate specific antigen (PSA) assays when using our Cobas® 6000 immunoassay analyzer (Roche Diagnostics, IN, USA).

The recent increase of biotin interference reports in clinical chemistry testing has been attributed to increased intake of biotin supplements by the public and to the high-dose biotin therapy in patients with multiple sclerosis. Although several reported studies examining pharmacokinetics of the biotin intake and clearance indicated that excessive intake resulted in circulating levels that caused interference [10], there is no study to our knowledge that measured representative samples received into clinical laboratories to randomly assess the risk among patient populations. In this study, we selected biotin-streptavidin based assays, namely hs-TnT, TSH, and PSA, that either had critical decision limits or were most commonly ordered to assess the risk for biotin interference.

MATERIALS AND METHODS

Serum and plasma leftover samples from 183 different patients following the completion of hs-TnT (53 samples), TSH (45 samples), PSA (85 samples) testing in our laboratories were stored at -20°C until analysis. Biotin concentrations in these samples were measured using an ELISA-based assay (ALPCO, Salem, NH, USA) according to the manufacture's protocol. In brief, this is a competitive binding assay where the wells are pre-coated with streptavidin, and biotin in the sample competes with an enzyme-labeled biotin conjugate. The concentration of the biotin in the sample is inversely proportional to the color intensity. The analytical measurement range of the assay is 0.048 to 1.1 ng/mL. Samples with concentration higher 1.1 ng/mL were diluted 1:5 with sample dilution buffer and re-assayed as per instructions. Biotin levels of 20 ng/mL or greater are reported to cause interference and such samples will be considered high risk samples (HRS) for interference. The percentage of HRS was assessed. Statistical analysis was performed using Minitab® software (State College, PA, USA).

This project was considered exempt by our research Institutional Review Board.

RESULTS

A total of 183 samples were included in this study. Biotin levels in the study patients' samples ranged from 0.02 ng/mL to 11.38 ng/mL (median 0.42) (Table 1). The median and (range) of biotin concentrations in hs-TnT, TSH, and PSA samples were 0.27 ng/mL (0.02 - 6.86 ng/mL), 0.39 ng/mL (0.08 - 11.38 ng/mL), and 0.47 ng/mL (0.09 - 7.73 ng/mL), respectively, (Table 2). The distribution in biotin levels among the various patient samples is shown in Figure 1. Biotin levels in patients' samples of the population tested were not normally distributed. There was no significant difference between biotin levels in samples for either TSH or PSA tests (Mann-Whitney test) ($p = 0.85$). However, biotin concentrations among PSA and hs-TnT samples and between TSH and hs-TnT were significantly different ($p = 0.049$ and 0.089, respectively). Of the 183 study specimens tested, none were considered HRS as their biotin levels were less than 20 ng/mL.

DISCUSSION

This study measured circulating levels of biotin in samples received into the laboratory for hs-TnT, TSH, and for PSA testing on patients being investigated for acute coronary syndrome, thyroid dysfunction, and those being monitored following total prostatectomy, respectively. Those assays were selected for their clinical utility in our patient population. For instance, we apply a strict acute myocardial infarction rule out protocol using our hs-TnT assay that requires reliable performance at the assay detection limit and adequate assay precision detecting a delta as low as 3 ng/mL [11,12]. Any interference or deterioration of the assay performance will limit the utility of this protocol. Similarly, we rely on a lower modified detection limit (0.05 ng/mL) of the PSA assay to detect early rise in PSA following a total prostatectomy for additional clinical and radiological exami-

Table 1. Biotin levels in serum and plasma samples from all study samples.

All study samples	
Interference threshold (ng/mL)	≥ 20
Sample size	183
Biotin concentration range (ng/mL)	0.02 - 11.38
Median biotin concentration (ng/mL)	0.42

Table 2. Interference threshold (as defined by the manufacturer), respective study sample size, biotin concentrations range and median levels in samples from patients with troponin T (hs-TnT), TSH, and PSA measurements requests.

	hsTnT	TSH	PSA
Interference threshold (ng/mL)	≥ 20	≥ 25	≥ 30
Sample size	53	45	85
Biotin concentration range (ng/mL)	0.02 - 6.86	0.08 - 11.38	0.09 - 7.73
Median biotin concentration (ng/mL)	0.27	0.39	0.47

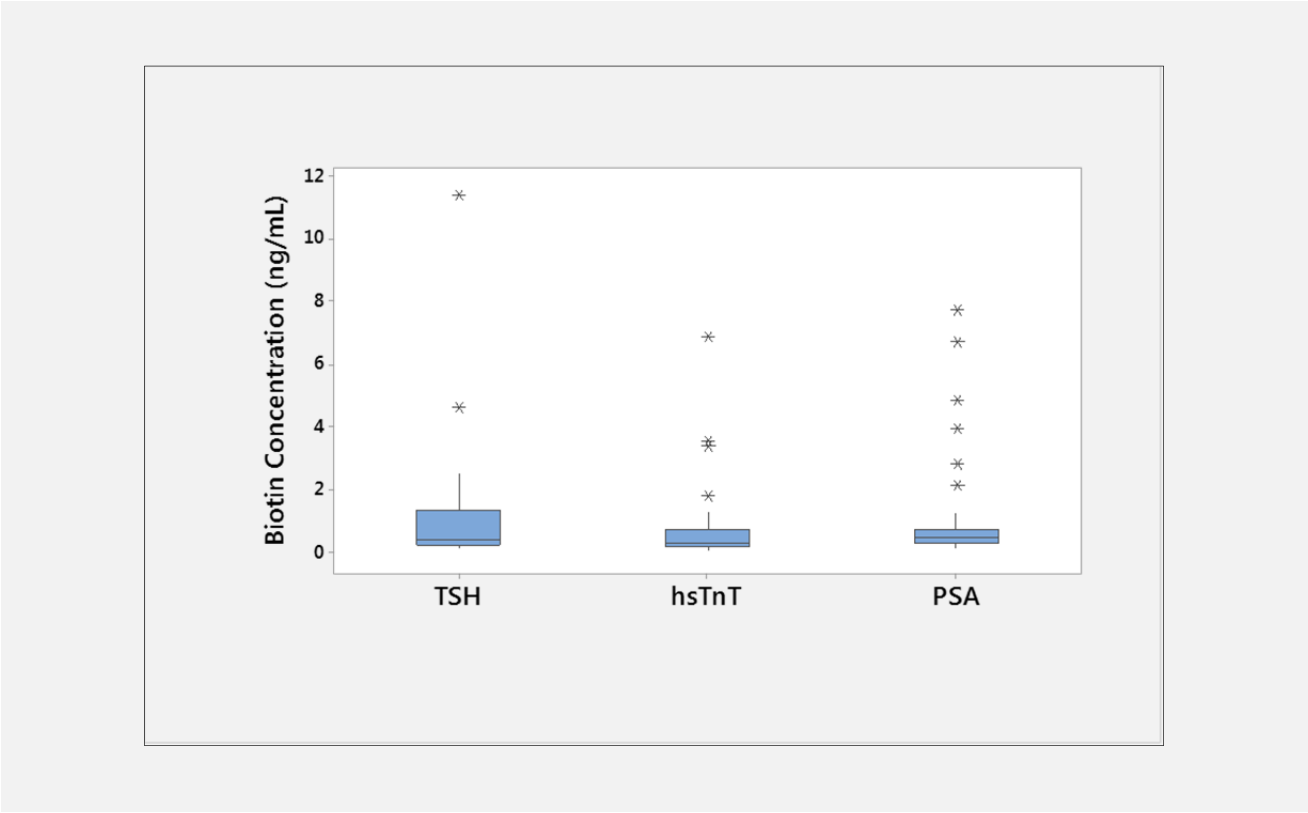

Figure 1. Median, quartile, and outlier distribution of circulating biotin concentration in samples from patients being tested for TSH, hs-TnT, and TSH levels.

nation. TSH is a commonly ordered test and considered a screen in the investigation of thyroid dysfunction. The hs-TnT, TSH, and PSA assays employ two monoclonal antibodies each. One of the antibodies is biotinylated while the other is complexed to a ruthenium detector complex. A streptavidin bound microparticle is used to capture the antibody-antigen sandwich complex. Presence of biotin in the sample will compete with the biotinylated antibody for the streptavidin microparticle causing a reduction in detectable sandwich complex and thus indicating falsely low results.

Many commercially available assays utilize biotin-streptavidin interaction in their methodology, and interference has been reported in 82 assays for biotin [9]. Biotin interference can produce erroneous laboratory results and cause serious clinical consequences. For instance, biotin interference can lead to a combined falsely low TSH and falsely high FT4 mimicking a pattern seen in patients with thyrotoxicosis [13,14]. Although therapeutic prescriptions of biotin are easily identifiable, the difficulty resides with over the counter and vitamin supplementation intake. Biotin has been marketed to improve hair and nail beauty, and this may reflect an increase in consumer purchase of biotin supplements in recent years.

The number of samples tested in this study represented 5.3% (for PSA tests), 1.9% (for hs-TnT), and 1.0% for (TSH) tests received into the clinical laboratory on a monthly basis. Among patient samples in this study, biotin concentrations were far below levels stated by the manufacture to cause interference in the respective assays. The lowest biotin concentration reported by the manufacturer to cause interference is 20 ng/mL, and thus samples containing biotin ≥ 20 ng/mL are considered high interference risk for the purpose of this study. For instance, biotin levels greater than 20 ng/mL are known to exhibit interference in hs-TnT, compared with 25 ng/mL in TSH assays, and 30 ng/mL in PSA assay when using our Cobas® 6000 immunoassay analyzer (Roche Diagnostics, IN, USA).

Circulating biotin half-life is between 8 and 18 hours [15] and the assay manufacturers recommend waiting 8 hours before measuring samples from patients known to take high biotin supplements (> 5,000 μg). TSH and PSA samples are likely to be received from fasting patients attending outpatient clinics, although the extent of fasting cannot be ascertained. In contrast, for hs-TnT, samples are likely to come from non-fasting patients. A recent survey showed that few patients report taking vitamin supplements, and fewer know what dosage levels they are taking [16]. The survey showed that only 7.7% reported taking biotin, and only 8.1% and 14.8% of those reported taking biotin supplements at 10,000 and 5,000 μg, respectively. Approximately 7.4% of 1,442 patients presenting to the ED showed biotin levels > 30 ng/mL likely to interfere with several assays [16]. Based on reported prevalence of biotin intake [16], we would expect at least 3 patients in this study to show biotin levels greater than 20 ng/mL. The highest biotin level among our patient population was 11.3 ng/mL, suggesting a lower risk population. This suggests that our patient population is at low risk for biotin interference. The total number of samples tested represent 2.0% of samples received for those tests which is higher than the estimated 1% of patients reportedly taking 5,000 μg biotin supplementation or greater. In this study, circulating biotin levels were significantly different between hs-TnT and TSH and between hs-TnT and PSA samples. This may be a reflection of the difference in patient population where those with hs-TnT requests represent acute presentation compared with PSA and TSH screening and the chronic nature of presentation. Samples were collected during the months of December and November, and it is not clear if the usage of biotin supplements among our patient population changes with season. A recent survey "personal communication" suggested increased biotin supplements purchase during the months of December and January which may represent a New Year's resolution to beautify the hair, nails, and looks. Commercially available biotin assays are rather expensive and prohibit population screening. Therefore, we limited the study to representative numbers of assays the performance of which at their detection limits provide the basis for interventional protocols at out institution. Future studies include in-house development of biotin assay that will facilitate measurement of a wider and larger number of patient population and wider test menu. In our study, all 183 patient samples tested showed biotin concentrations far less than 20 ng/mL which is our lowest level among our study assays to cause interference. This finding, however, may be a reflection of our patient population or due to the small number of samples which is a limitation of this study. The number of samples selected represented 2% of requests received into the laboratory on a monthly basis. The laboratory serves limited outpatient clinics (urology) as well as specialty services which might not reflect the practice encountered in other clinical laboratory settings.

CONCLUSION

Using representative samples with requests for hs-TnT, TSH and PSA, known to be most affected by circulating biotin levels, the risk for interference by biotin among our population was considered minimal. However, educating clinicians and laboratory users of the potential of biotin interference is always recommended.

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Declaration of Interest:
The authors have no conflict of intertest associated with the content of this manuscript.

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