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Case Report Open Access

Evidence for Elevated Endogenous Urinary Gamma-Hydroxybutyric Acid Levels: Two Case Reports

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Abstract

Gamma-hydroxybutyric Acid (GHB) is a naturally occurring metabolite of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) that is found in every human cell. Exogenously synthesized in 1960 for possible use as a surgical anaesthetic, GHB exhibits a weak analgesic effect and the potential for seizure-like activity. Since its brief use as an anaesthetic, GHB has been abused as an illicit drug for its euphoric, sedative, and anabolic effects. GHB is now a popular drug-facilitated sexual assault (DFSA) drug, or a "date rape" drug, due to its colourless, odourless, and tasteless properties. Various street names include "G," "Georgia home boy," "liquid ecstasy," "scoop," and "soap." Due to GHB's recreational popularity, the metabolite is now included in the urine toxicology panels of patients undergoing rehabilitation for drug and alcohol addiction. However, exogenous GHB is indistinguishable from naturally produced GHB, which is an important factor to consider when evaluating the urine toxicology panels of patients. Two recent cases exist regarding patients undergoing outpatient rehabilitation who have tested positive for urinary GHB while denying any substance use. The subjects tested positive according to currently accepted cut-off levels on gas chromatography/mass spectrometry analysis (GC-MS) from a purely analytical perspective. From a forensic and indeed clinical perspective, it was found that the subjects had not used GHB. It can be said that these were false positives. Positive urine tests for GHB not only hinder the rehabilitative process, but can also put a patient's employment status in jeopardy, and may result in legal implications as well. Providers, as well as their patients, depend on the most up-to-date clinical data to render appropriate treatment and to protect against unnecessary repercussions. Therefore, the cut-off level of urinary GHB, as well as any factors that may affect increased endogenous production, must be thoroughly examined in order to facilitate accurate identification of drug abusers and to avoid false positives.

Keywords: Gamma hydroxybutyric acid; GHB; Drugs of abuse; Gas chromatography; Mass spectrometry; GC-MS

Introduction

Drug and alcohol addiction is a prevalent and significant health problem. In 2012, the Substance Abuse and Mental Health Services Administration's (SAMHSA) National Survey on Drug Use and Health determined that 23.1 million persons of age 12 or older required treatment for the use of alcohol and illicit drugs [1]. The recovery process for drug and alcohol addiction involves a commitment to change by the patient and the provision of appropriate guidance by the clinician. Additionally, random urine screening is required to support adherence to the treatment plan. The urine toxicology panel regularly screens for substances such as cocaine, benzodiazepines, amphetamines, alcohol, opiates, tetrahydrocannabinol, and gamma-hydroxybutyric acid (GHB). GHB was synthesized in 1960 for anaesthetic purposes; however, the substance achieved popularity as a recreational drug and as a muscle growth supplement to bodybuilders for its anabolic effects [2]. Side effects of GHB include euphoria, dizziness, hypotonia, and amnesia. GHB is abused as an illicit "club drug" due to its euphoric effects. Additionally, due to its sedative properties, the substance is often slipped into an unknowing victim's drink to facilitate sexual assault. GHB is a naturally occurring metabolite of the neurotransmitter gamma-aminobutyric acid (GABA). GHB is synthesized in the brain from GABA by the enzymes GABA aminotransferase and succinic semi aldehyde reductase [3]. GHB is metabolized through the Krebs cycle, with the majority being excreted as carbon dioxide [3]. Therefore, through the metabolism of naturally occurring GHB, only a small amount is expected to be excreted in the urine.

Urinary GHB levels are used to determine if an individual is abusing exogenous GHB. However, multiple patients with increased urinary GHB levels deny exogenous use and in all other aspects appear to be abstaining from drug use. Researchers are currently investigating what factors may cause certain individuals to be more susceptible to increased endogenous production of GHB. One area of interest is the effect of storage time and temperature regarding in vitro GHB production. Whether or not specific substances such as nicotine, alcohol, and antiepileptic drugs may increase endogenous GHB production is also an area of interest [4,5]. The possible effect of diet on endogenous GHB production has also been studied; however, the results suggest that food intake appears to have no effect on GHB urine concentration [6]. Additionally, GHB aciduria is a rare genetic disorder that should be considered. This disorder involves an accumulation of GHB due to a deficit of succinic semi-aldehyde dehydrogenase [6]. Primarily, effort is being put forth to accurately determine a urinary GHB cut-off level at which exogenous consumption can be accurately assumed.

Case Reports

Two patients undergoing outpatient treatment, one male and one female, have tested positive for urinary GHB but convincingly deny exogenous use. Urine sample collections were witnessed and chain of custody was secured. The samples were analyzed by a certified commercial laboratory. Patient "A" is a 37-year-old white male whose medical history is essentially non-contributory. Undergoing treatment for cocaine dependence, his treatment plan includes the completion of 24 group sessions as well as random urine toxicology panels. His toxicology report was negative for all tested substances except GHB. The sample was sent to a specialized facility for gas chromatography/mass spectrometry (GC-MS) analysis. The results, received in 7 days, indicated a GHB level of 4.2 mcg/mL; the laboratory's reporting limit is 2.0 mcg/mL. The patient has been participating actively in treatment and showing no behavioural indication of relapse. Reports from family that were obtained with his consent are indicative of increasing stability in terms of interpersonal relationships and employment. Furthermore, the patient denies taking any over-the-counter or prescription medications. In all aspects, with the exception of the urinary GHB level, the patient appears to be benefitting from treatment and successfully abstaining from drug use.

Patient "B" is a 48-year-old white female being treated for alcohol dependence. She has a comorbid history of asthma, bipolar disorder, and bulimia nervosa, though the latter has been fairly stable. Her medications include albuterol, buproprion, lamotrigine, ibuprofen, and diphenhydramine. After a positive urinary test for GHB, the patient's employment in the allied health professions was placed in jeopardy. A urine specimen was sent for GC-MS analysis, and the results showed a GHB level of 2.9 mcg/mL. Similarly, to patient "A," patient "B" appears to be deriving benefit from treatment and using insight appropriately. Behaviourally, she exhibits signs of increasing stability which were collaborated by her counsellor, psychiatrist, and significant others. In both cases, the GHB results led the practitioners to question the success of the treatment plan, resulting in unnecessary repercussions in the lives of the patients.

Discussion

Research is currently being conducted to establish what factors may cause certain individuals to be more apt to produce increased endogenous GHB, especially in the urine. Variations in urinary GHB levels among GHB-free individuals have already been noted by multiple researchers [7,8]. The determination of a cut-off level in the blood and urine to differentiate exogenous from endogenous GHB is a major objective at this time.

Understanding the pharmacokinetics of GHB helps to better understand and differentiate positive urinary exogenous vs. endogenous GHB levels. After ingestion, peak serum levels of GHB occur in 20-42 minutes, depending on the dose [3]. Therapeutic doses, 12.5-50 mg/L, are almost completely eliminated from the body within 2-8 hours [3]. Therefore, exogenous GHB is practically undetectable 12 hours after ingestion.

In a study to investigate the urine concentration-time profile of GHB, Haller et al. [9] used a moderate, controlled oral dosing of 50 mg/kg of Xyrem, which is the prescription form of GHB indicated for the treatment of narcolepsy. Haller et al. determined that the duration of detection of urinary GHB is actually less than 12 hours. The moderate dose of 50mg/kg resulted in an average peak urine concentration of 150-200 mcg/mL, which occurred within 3 hours of ingestion [9]. The urinary levels measured after moderate, controlled dosages were significantly greater than those of the patients outlined in the case reports. Average doses associated with abuse result in significantly increased urine and serum levels, as well as clinical manifestations. Sporer et al. [10] assessed the GHB levels of 16 patients brought into an emergency department after reported GHB overdose. They determined that the urinary levels of GHB after overdose ranged from 432 to 2,407 mcg/mL [10]. These levels differ greatly from the levels of the patients outlined in the case reports as well as the levels that result from the average therapeutic dose.

Toxicologists are currently attempting to determine an accurate cut-off level in urine that would differentiate endogenous GHB from exogenous GHB. Proposed cut-off levels are 5 and 10 mcg/mL. Any level of GHB above the proposed cut-off level would allow investigators to accurately assume exogenous GHB ingestion. LeBeau et al. [7] observed intra and interindividual variations of urinary endogenous GHB levels. The researchers concluded that significant variations exist among urinary concentrations of endogenous GHB between individuals, but no individual's endogenous GHB concentration approached 10 mcg/mL in the urine. Thus, the results support the proposed cut-off level of 10 mcg/ml to identify exogenous GHB ingestion. Paul et al. [11] also studied endogenous urinary GHB. Through their research, they discovered that endogenous concentrations of GHB ranged from 0.3 to 6 mcg/mL in the urine, which further supports the proposed cut-off value of 10 mcg/mL. A later study by LeBeau et al. [7] was designed to augment previous attempts at determining an appropriate range of normal endogenous GHB concentrations in urine samples of exogenous GHB-free subjects. In the results of the study, GHB concentrations of 207 subjects ranged from 0.00 to 2.70 mcg/mL, with a median concentration of 0.28 mcg/mL [7]. Despite the fact that no urine sample had a GHB level of greater than 2.70 mcg/mL, LeBeau et al. [12] offer support of the 10 mcg/mL cut-off due to the possibility of in vitro production. A study by Elliott [6] also investigated the proposed cut-off level. Of 119 GHB-free subjects, only 2 had urinary GHB levels greater than 2.5 mcg/mL, with both being 3 mcg/mL [6]. Therefore, the researchers concluded that it is not possible to determine the source of GHB at urinary levels below 10 mcg/mL. Shima et al. [8] added support to the 10 mcg/mL cut-off, after a study that resulted in endogenous urinary GHB concentrations ranging from 0.16 mcg/mL to 2.14 mcg/mL.

However, there is still a slight lack of agreement regarding the GHB cut-off value. Crookes et al. [13] developed a unique method for analysis of low levels of GHB in urine. This method consisted of liquid-liquid extraction, silyl-derivatization, and GC-MS analysis to analyze the urine samples of 50 sexually active women between the ages of 14 and 52 years. They determined the upper limit of normal urinary GHB to be 1.46 mcg/mL. These results lead Crookes et al to propose the cut-off level of 5 mcg/mL as an accurate measure to determine exogenous use, which is significantly lower than the alternative 10 mcg/mL cut-off. However, the majority of research supports a 10mcg/mL urinary cut-off level for accurate detection of exogenous GHB use.

The effect of urine sample storage conditions has also been studied with regard to GHB levels. Kerrigan [14] observed in vitro production of GHB over an eight-month period. Drug-free urine specimens were stored at 21, 4, and -20°C. Although preliminary results demonstrated that spontaneous in vitro GHB production was seen, the increases were less than 5 mcg/mL. The most rapid increases in GHB production were seen at elevated temperatures. Therefore, Kerrigan concluded that samples should be stored at –20°C to minimize in vitro production.

This study also addresses the proposed cut-off level of 10 mcg/mL. Concentrations as high as 7 mcg/mL were measured, but 95% of the samples contained less than 5 mcg/mL, and 100% contained less than 10 mcg/mL. LeBeau et al also studied the effect of storage over time on in vitro GHB production. With comparable results to Kerrigan, LeBeau et al. concluded that in order to minimize the risk of in vitro GHB production, the samples should be stored in a refrigerated or frozen condition and analysed as soon as possible after collection [15].

Another area of interest that may affect endogenous GHB production is nicotine use. Moriya et al. [4] suggest that the stimulatory effects of nicotine on the central nervous system may increase the nocturnal formation of GHB. Moriya et al. discovered that the urinary levels of endogenous GHB among male volunteers were the highest among smokers; furthermore, the concentrations among smokers were higher in the morning than in the evening. However, no statistical differences were observed among smokers and nonsmokers. Additionally, the sample population for the study was lacking. Of the 20 participants, 15 were male and none of the females were smokers. Therefore, Moriya et al. were only able to analyse the effects of smoking on GHB production in regards to males. The results of this research are preliminary and represent possible future areas of study.

GHB aciduria is a rare genetic disorder that affects endogenous GHB production. An accumulation of GHB due to a deficit of succinic semi aldehyde dehydrogenase occurs [8]. Patients suffering from this disorder were found to have very high concentrations of GHB in the urine, plasma, and cerebrospinal fluid. Urinary concentrations reported were typically greater than 200 mcg/mL. Patients with urinary GHB levels of 10 mcg/mL or more should undergo a second urine sample to obtain individual basal levels of GHB to rule out GHB aciduria.

Reviewing these factors as they relate to the case studies outlined above, the patients' claims of abstinence from drug use are further supported. Taking into account the timing of these patients' appointments and a urine elimination time of less than 12 hours, patients would have had to have used GHB earlier in the day when other interested parties would have noticed. The patients' urinary GHB levels of 4.2 mcg/mL (patient "A") and 2.9 mcg/mL (patient "B") are also significantly lower than ranges established for therapeutic use (150-200 mcg/mL) [9] and abuse (432-2,407 mcg/mL) [10]. It should be further noted that the patients' urine samples were stored at 2.22°C for approximately 8 hours before being transported to the lab for GHB quantitation via GC-MS. This would make in vitro production of GHB fairly insignificant, since elevations in GHB of stored samples were seen over an eight-month period in both Kerrigan and Lebeau's studies [14]. Furthermore, Patient B is currently taking lamotrigine for Bipolar Disorder. However, lamotrigine has antiepileptic capabilities. Some researchers have investigated the effects of antiepileptic medication on increasing GHB concentrations due to interaction of their metabolic pathways [5,15]. No conclusion has been drawn regarding the effects of antiepileptic medication on urine or blood GHB levels; therefore, further research is necessary.

Conclusion

As a drug of abuse, GHB must be considered in urinary toxicology reports. However, as urinary GHB levels vary greatly, the cut-off level must be considered with great care. Additionally, practitioners must be sure to consider more than just the lab value when determining the success of treatment in a patient being treated for drug abuse. In the case of positive urine toxicology of GHB, care must be taken to note levels on GC-MS analysis, the handling and storage of samples, and any possible interactions with other substances; furthermore, a thorough review of the patient's behaviour and progress must be conducted before confirming that the patient has abused GHB. Based on experience and research, we would like to humbly add our support for a suggested cut-off level of 10 mcg/mL.

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Conflict of Interest

The authors report no conflict of interest.

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