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CASE REPORT

Causes and Management Strategies for Unusual Hypoglycemia: a Case Series

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SUMMARY

Background: **Glucose levels are vital for indicating the body's sugar content, with imbalances leading to diseases like diabetes or hypoglycemia-related symptoms such as palpitations and fatigue.**
Methods: **This case series describes three cases of hypoglycemia identified in recent years, utilizing multiple glucose measurement methods and exploring strategies to eliminate interferences.**
Results: **Two cases of pseudo-hypoglycemia induced by PEGylated recombinant human granulocyte colony-stimulating factor (PEG-rhG-CSF) and Evolocumab injections, and one case of true reactive hypoglycemia following a glucose tolerance test in a patient post-gastric bypass surgery.**
Conclusions: **For unusual hypoglycemia, collaboration with clinicians and multiple methods is crucial for accurate analysis to differentiate true from pseudo-hypoglycemia, ensuring precise diagnosis and optimal clinical service.**
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INTRODUCTION

Glucose is an essential energy source, and maintaining balanced blood glucose levels is critical to health. With diabetes, characterized mainly by elevated glucose, world-wide prevalence in 2021 was estimated to be approximately 537 million and is forecast to reach 783 million by 2045 [1]. While the perils of hyperglycemia are well-known, the risks associated with hypoglycemia should not be underestimated, potentially leading to myocardial infarction, arrhythmias, and in extreme cases, coma or death. Hypoglycemia is a significant hazard for diabetic patients, underlining the necessity of accurate detection and distinction between true and false cases to mitigate risk to patients.
Glucose measurement in serum primarily including glucose oxidase, glucose dehydrogenase, and glucose hexokinase. The glucose oxidase method can yield falsely low results due to interference from reducing

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substances like vitamin C, uric acid, glutathione. The glucose dehydrogenase method is inferior in specificity to the glucose oxidase method and may cross-react with other sugars, including maltose. The hexokinase method is widely accepted as the reference standard for glucose measurement due to its high specificity. This methodology is predicated on the enzymatic reaction where glucose and adenosine triphosphate combine to form glucose-6-phosphate, catalyzed by hexokinase. Subsequently, glucose-6-phosphate dehydrogenase facilitates the reduction of NADP+ to NADPH. The quantity of NADPH produced, measured at a wavelength of 340 nm, is directly proportional to the glucose concentration present in the sample [2]. Additionally, point-of-care testing glucose meters are widely used in clinical practice, with their rapidity and convenience.

Despite technological advances in glucose detection, pseudo-hypoglycemia persists. Immunoglobulins, particularly IgM, can interfere with the hexokinase method due to the antibody activity of the monoclonal protein against an antigen in the hexokinase reagents [3]. Other factors such as sample storage time and increased red or white blood cell counts can also cause pseudo-hypoglycemia [4,5]. Additionally, confirming whether patients have ingested hypoglycemic agents before sample collection and obtaining a comprehensive medical history are critical to interpreting results accurately.

As clinical laboratory professionals, we must ensure the analytical and clinical validity of test outcomes. In cases of unusual hypoglycemia, identifying interference and assessing glucose result authenticity are crucial for delivering dependable laboratory data for clinical application. Next, we will discuss three recent cases of unusual hypoglycemia identified using the hexokinase method.

CASE PRESENTATION

Case 1

In recent work, we identified three consecutive patients in the breast surgery department with critical fasting glucose values, each below the threshold of 2.5 mmol/L. This observation deserves special attention because such low blood glucose results are often consistent with significant symptoms, including dizziness, sweating, and cognitive impairment. Notably, the individuals in these cases did not exhibit any of these symptom indicators. Upon ruling out laboratory errors, it was found that each patient had undergone blood biochemistry rechecks three days post-chemotherapy, which showed hypoglycemia. Their initial glucose levels were within normal ranges upon admission, and there was no diabetes or use of hypoglycemic agents recorded. In the comparison with others who had similar diseases and underwent equivalent chemotherapy regimens, the distinguishing factor for these individuals was injection of pegylated recombinant human granulocyte colony stimulating factor (PEG-rhG-CSF) the preceding day to avert leukopenia induced by chemotherapy. Post-injection, their white blood cell counts rose dramatically to 84.63, 66.14, and 54.57 x 10^9/L, respectively.

It had been found that patients with chronic myeloid leukemia are prone to pseudo-hypoglycemia due to significant increases in leukocytes, causing *in vitro* glucose consumption [6]. This phenomenon also occurred in lymphocytic leukemia patients, indicating that the hypoglycemic effect correlates more with total leukocyte count rather than cell type [7,8]. Additionally, the timing of sample processing impacts glucose results. Without prompt separation from blood kept at room temperature after collection, the concentration of blood glucose will decrease over time [5]. This rate of decline is directly influenced by the count of leukocytes. In this case, these three patients had blood drawn at approximately 6 a.m., and samples reached our laboratory by 8 a.m., allowing sufficient time for glycolysis in numerous white blood cells. That makes sense!

Case 2

During a routine biochemical assessment at our outpatient clinic on January 11, 2024, a 50-year-old male patient's glucose result was unexpectedly negative at -0.87 mmol/L. His other results were as usual and upon retesting, the glucose remained negative. The patient did not exhibit any hypoglycemic symptoms, a discrepancy with the laboratory findings. Internal quality control metrics for that day were normal, and no aberrant glucose results were reported for other patients in the same batch, indicating this was an isolated incident.

Upon initial analysis, the patient's sample appeared turbid due to elevated triglyceride (TG) levels of 12.76 mmol/L, prompting us to consider whether turbidity was causing interference. Diluting the specimen 3-fold and 5-fold showed effective reduction in TG levels but did not resolve the negative glucose result. Centrifugation at 10,000 rpm for 20 minutes to mitigate the effects of chylomicron dilution also did not correct the negative glucose result, ruling out turbidity as a factor.

Further investigation into the biochemical reaction curve for glucose revealed an abnormal increase in absorbance after reagent 1 (R1) was mixed with the sample (Figure 1). A subsequent drop in absorbance followed the addition of reagent 2 (R2), leading to the negative glucose result. The reaction continued to occur after R2 addition, suggesting interference by a serum substance with R1.

To ascertain the patient's actual glucose level, we used an Abbott FreeStyle Optium Neo blood glucose meter (electrochemical method) to test whole blood samples collected at the same time as the serum specimen, which indicated a glucose concentration of 5.0 mmol/L. Serum glucose measured by the glucose oxidase method showed a concentration of 5.55 mmol/L, confirming the interference in the hexokinase method-based glucose detection previously encountered.

Given that monoclonal IgM can interfere with the hexokinase assay and finding no evidence of gammopathy in the patient’s history, leaving us uncertain about the

source of interference in the patient's glucose testing [9, 10]. For subsequent research, the patient's serum sample was preserved at -80℃.

The patient's fasting blood glucose level was reassessed on January 21st, returning to a normal value of 5.8 mmol/L. This event further compounded our confusion, suggesting that the interfering substance had disappeared after 11 days, hinting at the possibility of an exogenous origin for the interference. Further investigation into the patient's medical history, Evolocumab injection had caught our attention. Evolocumab is a human monoclonal immunoglobulin G2 that inhibits the binding of proprotein convertase subtilisin kexin 9 (PCSK9) to low-density lipoprotein receptor by binding to PCSK9, thus lowering low-density lipoprotein levels. Given its molecular weight of approximately 144 kDa [11], this protein could theoretically interfere with glucose measurement. The patient received an injection of Evolocumab on January 8 and subsequently underwent a blood glucose test on January 11. At this juncture, the concentration of the drug in the blood was at its peak, which resulted in interference with the glucose assay. A follow-up blood glucose test was conducted on January 22, 14 days after the last injection of Evolocumab, coinciding with the drug's half-life. By this time, the impact of Evolocumab on the glucose measurement had disappeared, providing a plausible explanation for the transient interference observed.

To validate this finding, the patient’s serum sample from January 11 was subjected to polyethylene glycol (PEG) treatment to precipitate any macromolecular proteins that might interfere. The post-treatment glucose level measured 5.08 mmol/L, confirming the efficacy of the PEG precipitation and supporting the hypothesis that Evolocumab interfered with the hexokinase glucose assay.

Case 3

A 71-year-old male patient was admitted to the hospital due to "dizziness", and routine testing after admission revealed a fasting blood glucose of 6.23 mmol/L, indicating impaired fasting glucose. The subsequent day, a "postprandial 2-hour blood glucose" was ordered. The patient received a glucose tolerance test at 6:00 a.m. (150 mL of 50% glucose solution), and had blood samples drawn at 8 a.m. These samples were centrifuged at 9 a.m. and analyzed, yielding a blood glucose result of 1.84 mmol/L. The finding was consistent on retest, and all quality control measures, specimen conditions, and reaction curves were verified as normal. An urgent critical value was reported, with a nurse immediately testing the patient's glucose using a rapid glucose meter, resulting in 5.9 mmol/L.

Considering the time lapse between the tests, it was postulated that the critical value may not have accurately represented the patient's glucose levels at 8 a.m. Notably, the patient had a history of gastrectomy performed 50 years prior for a gastric ulcer. Historically, patients with gastric or duodenal ulcers often suffered from perforations due to the lack of effective antacids during that era. The prevailing treatment was a subtotal gastrectomy with subsequent gastric bypass surgery (GB). Recent studies increasingly report that patients who have undergone GB surgery are at risk of developing severe hyperinsulinemic hypoglycemia, often years after the procedure [12]. This condition stems from the accelerated transfer of nutrients from the stomach to the intestine and the enhanced intestinal insulinotropic effect [13,14]. Therefore, hypoglycemia observed in the patient is highly likely to be reactive hypoglycemia. Acute hypoglycemic episodes in such cases are typically precipitated by the swift gastric emptying of sugary fluids into the small intestine, prompting compensatory insulin release. However, by noon, following food consumption or the metabolic conversion of glycogen to glucose, the patient’s blood glucose levels normalized. This observation suggests that reactive hypoglycemia may be the underlying cause of the patient’s persistent dizziness.

DISCUSSION

As clinical laboratory professionals, we occasionally confront experimental results that significantly differ from clinical symptoms, challenging our ability to promptly assess their accuracy. We describe three unusual cases of hypoglycemia, delving into their causes and seeking strategies for future rapid and accurate resolution, thus ensuring the timely feedback of clinically reliable results.

In the first case, PEG-rhG-CSF exhibits a larger molecular weight, longer half-life, stable concentration, and reduced immunogenicity compared to granulocyte colony-stimulating factor [15], justifying its widespread clinical use. We advise immediate serum separation post-collection in patients treated with drugs that may significantly elevate leukocyte counts. If not feasible, sodium fluoride as an anticoagulant is recommended to inhibit glycolysis and preserve glucose levels without compromising other assays.

The second case, the brief processing period for outpatient samples in our laboratory, spanning no more than 60 minutes from blood collection to the initiation of testing, essentially negates the impact of glycolysis on glucose measurements. With normal red and white cell counts, the cause of unusual hypoglycemia remained elusive.

Faced with false hypoglycemia or paradoxically "negative" blood glucose levels caused by unknown interferences, we have delineated strategies to mitigate such interferences. First, dilution or high-speed centrifugation can be employed. In most cases, the pseudo-hypoglycemic phenomenon disappears because the interfering source is diluted or separated. Second, employing alternative methodologies for glucose testing, such as glucose oxidase or glucose dehydrogenase method, is recommended for reevaluation of glucose levels. Finally,

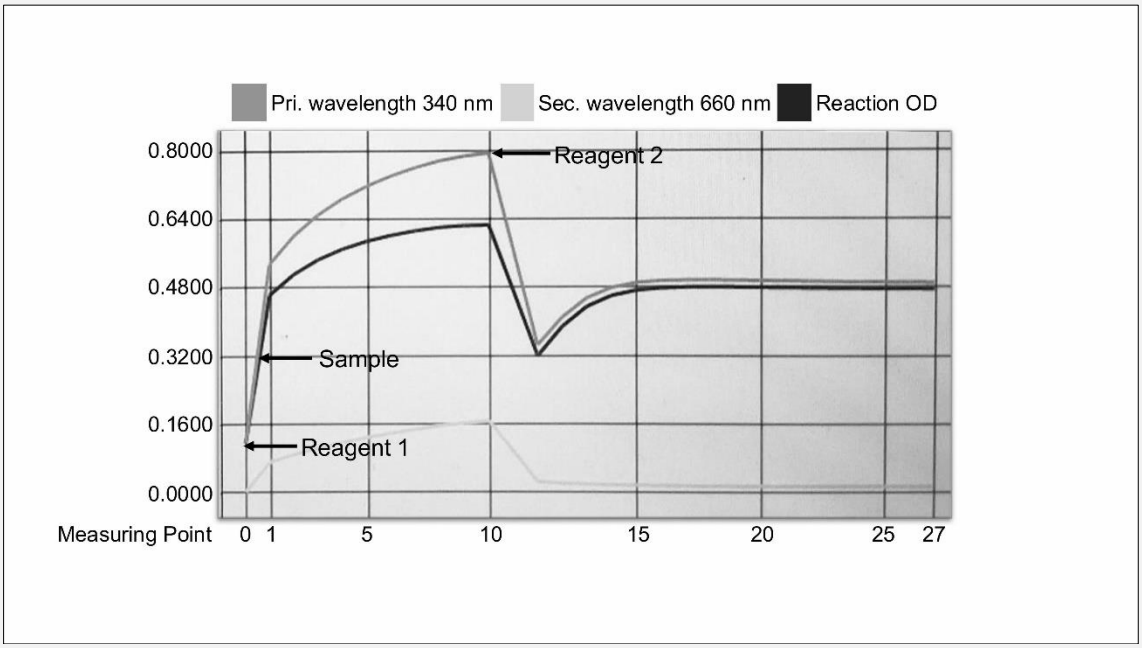

Figure 1. Blood glucose reaction curve for the patient in case 2.

the use of immunofixation electrophoresis and PEG precipitation is advised. Immunofixation electrophoresis is conducted to detect immunoglobulins, while PEG precipitation is utilized to remove large protein molecules, thus preventing errors in glucose measurement.

As for reactive hypoglycemia, which differs from hypoglycemia due to medications or tumors, often resolves spontaneously through endogenous regulatory mechanisms without food intake. True hypoglycemia, once extrinsic influences are excluded, must be promptly communicated to clinicians, aiding in swift diagnosis and treatment. Moreover, individuals with prior upper gastrointestinal surgeries may experience adverse reactions to glucose tolerance tests due to the high osmotic load, which can precipitate severe dumping syndrome. Alternative tests using a balanced meal are preferable in such contexts and any provocative testing that may induce hypoglycemia should be conducted under medical supervision in a secure setting [12].

In summary, it is vital for laboratory staff to distinguish between authentic and spurious glucose results. Although the glucose hexokinase method for serum glucose measurement is highly specific and exhibits substantial resistance to interference, it is not infallible and should not solely replace other methods in practice. When test results significantly diverge from clinical symptoms, it is imperative to promptly consult with clinicians and judiciously employ various methods for cross-comparison and verification, thereby ensuring the accuracy of the measurement outcomes.

Declaration of Interest:

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References:

1. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. 2021. https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf

2. Lijo B. Glucose Oxidase vs Glucose Hexokinase. 2022. https://www.agappe.com/swiss_en/blog-details/glucose-oxidase-vs-glucose-hexokinase.html

3. Dalal BI, Brigden ML. Factitious biochemical measurements resulting from hematologic conditions. Am J Clin Pathol. Feb 2009; 131(2):195-204. (PMID: 19141380)

4. Janssen K, Delanghe J. Importance of the pre-analytical phase in blood glucose analysis. Acta Clin Belg 2010 Sep-Oct;65(5):311-8. (PMID: 21128557)

5. Lippi G, Salvagno GL, Lampus S, et al. Impact of blood cell counts and volumes on glucose concentration in uncentrifuged serum and lithium-heparin blood tubes. Clin Chem Lab Med 2018 Nov 27;56(12):2125-31. (PMID: 29935113)

6. Ghrewati M, Singh B, Maroules M. Hyperleukocytosis, Artifactual Hypoglycemia, and Massive Hepato-Splenomegaly: A Case of Chronic Myelogenous Leukemia. Am J Med Sci. Oct 2020;360 (4):e3. (PMID: 32448496)

7. Field JB, Williams HE. Artifactual hypoglycemia associated with leukemia. N Engl J Med. Nov 9 1961;265:946-8. (PMID: 13892768)

8. Jiang M, Ahmet A. Hypoglycemia during treatment of acute lymphoblastic leukemia. Paediatr Child Health. Aug 2023;28(5):305-306. (PMID: 37484034)

9. Masson P, Chutova E, Oris C, Dannus LT, Sapin V, Bouvier D. [Waldenstrom's macroglobulinemia highlighted by turbidity interference: a case report]. Ann Biol Clin (Paris) 2022 Sep 1;80(5): 487-93. (PMID: 36453737)

10. Bossard V, Sauvageon Y, Fraissinet F, Dreyfus B, Thuillier R, Hauet T. [False paraprotein-induced hypoglycemia in the measurement of glucose by the hexokinase method]. Ann Biol Clin (Paris) 2019 Aug 1;77(4):439-45. (PMID: 31418706)

11. REPATHA (evolocumab) injection, 2021. https://www.pi.amgen.com/-/media/Project/Amgen/Repository/pi-amgen-com/Repatha/repatha_pi_hcp_english.pdf

12. Salehi M, Vella A, McLaughlin T, Patti ME. Hypoglycemia After Gastric Bypass Surgery: Current Concepts and Controversies. J Clin Endocrinol Metab 2018 Aug 1;103(8):2815-26. (PMID: 30101281)

13. Laferrère B, Teixeira J, McGinty J, et al. Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. J Clin Endocrinol Metab 2008 Jul;93(7):2479-85. (PMID: 18430778)

14. Nguyen NQ, Debreceni TL, Bambrick JE, et al. Rapid gastric and intestinal transit is a major determinant of changes in blood glucose, intestinal hormones, glucose absorption and postprandial symptoms after gastric bypass. Obesity (Silver Spring) 2014 Sep; 22(9):2003-9. (PMID: 24829088)

15. Jiang Y, Zhang J, Zhong J, et al. Efficacy and safety of PEG-rhG-CSF versus rhG-CSF in preventing chemotherapy-induced-neutropenia in early-stage breast cancer patients. BMC Cancer 2023 Jul 26;23(1):702. (PMID: 37495949)