Clin. Lab. 2024;70:XXX-XXX

CASE REPORT

Hypertriglyceridemia Interferes with Hemoglobin Detection and Calibration Methods

Xiaodi Shi [1, 2], Kangle Feng [1, 2]

[1] *Department of Laboratory Medicine, Shaoxing Central Hospital, Shaoxing, Zhejiang, P.R. China*
[2] *The Central Hospital of Shaoxing University, Shaoxing, Zhejiang, P.R. China*

SUMMARY

Background: **Hemoglobin (HGB) is a pigment protein found in human red blood cells. Laboratories usually measure hemoglobin using a colorimetric method. The factor that causes the increase of blood turbidity (hypertriglyceridemia) can lead to the false increase of HGB, and also cause a significant increase of MCH and MCHC.**
Methods: **By means of a case of hypertriglyceridemia, plasma exchange and formula substitution methods were used to establish a reliable calibration method for hemoglobin (HGB) determination.**
Results: **After calibration, the corrected final values of HGB and its related indexes MCH and MCHC differ greatly from the instrument values. We reported the calibrated results to clinicians.**
Conclusions: **When using a commonly used clinical hematology analyzer to detect hemoglobin, when encountering high TG samples, plasma exchange and formula substitution methods can be used. It can quickly help us correct the HGB, MCH, and MCHC values in blood lipid samples and provide clinicians with accurate reports.**
(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240316)

Correspondence:
Kangle Feng
Department of Laboratory Medicine
Shaoxing Central Hospital
Shaoxing, Zhejiang 312030
P.R. China
Phone: +86 15257584548
Email: 847275496@qq.com

KEYWORDS

hemoglobin, hypertriglyceridemia, plasma exchange, formula substitution

INTRODUCTION

Hemoglobin (HGB) is a binding protein containing pigment cogroups synthesized in human nucleated red cells and reticulocytes, and is a transport protein in red blood cells [1,2]. Laboratory hemoglobin is measured by colorimetry. While hypertriglyceridemia is a major interfering factor, blood samples with high triglycerides (TG) contain chylomicrons (CMs) and very low-density lipoprotein (VLDL) particles, which scatter and absorb light, creating turbidity in the blood sample [3]. The TG in the blood also replaces the plasma volume, so high TG inevitably leads to a false increase in the HGB value [4]. The following is a list of cases to analyze how to calibrate hemoglobin results and calibration methods for patients with high triglycerides.

Case Report accepted March 24, 2024

CASE PRESENTATION

A 50-year-old man was admitted to the hospital due to fractures of the left tibia and fibula. He denied any history of blood transfusions or exposure to blood products. The clinician sent a blood sample to the lab and the patient's blood routine results: red blood cell count (RBC): 5.22 x 10^{12}/L [reference interval (ref): 4.0 - 5.5]; hematocrit (HCT): 0.46 L/L (ref: 0.40 - 0.54); hemoglobin (HGB): 192 g/L (ref: 120 - 160); mean corpuscular hemoglobin concentration (MCHC): 417 g/L (ref: 320 - 360); mean corpuscular hemoglobin (MCH): 37 pg (ref: 27 - 34).

The staff on duty found that the result was abnormally high, and the daily quality control and instrument operation were not abnormal, but the detection instrument Mindray BC-5180 showed a warning message of "turbidity/HGB interference". It was immediately suspected that the condition of the specimen was abnormal, so the specimen was placed in a centrifuge for 3,500 r/minute and centrifuged for 5 minutes. After centrifugation, it was found that the specimen had severe lipids, as shown in Figure 1A. After checking the serum biochemical report of the patient, the triglyceride (TG) was 15.08 mmol/L. He was a high triglycerides patient obviously. Then the staff on duty carried out the following two steps:

1) Formula substitution method:

Complete blood count analysis was performed with Mindray BC-5180 analyzer to obtain HGB concentration of blood lipid samples (HGB_{LB}). Then blood lipid samples were centrifuged at 550 g for 3 minutes, and then plasma hemoglobin concentration (HGB_{LP}) was analyzed. The corrected HGB level was then calculated using the following formula:

$$HGB_{CORRECTED} = HGB_{LB} - (HGB_{LP} - HGB_{LP} \times HCT_{LB})$$
$$MCH_{CORRECTED} = HGB_{CORRECTED}/RBC_{LB}$$
$$MCHC_{CORRECTED} = HGB_{CORRECTED}/HCT_{LB}$$

According to the above formula, we detected plasma hemoglobin HGB_{LP} with a value of 57 g/L, and the result is $HGB_{CORRECTED}$ = 161 g/L, $MCH_{CORRECTED}$ = 31 pg, $MCH_{CCORRECTED}$ = 348 g/L.

2) Plasma exchange method:

Take 4 test tubes numbered 1, 2, 3, 4, and the following steps were taken: 1) Routine blood tube centrifugation, 5 minutes, 3,000 revolutions, quantitative absorption of plasma layer with sample gun, and discard in tube No. 1. Add equal amount of normal saline to blood routine tube with sample gun, mix gently, and continue centrifugation. 2) The plasma layer is absorbed quantitatively with the sample gun and discarded in tube No. 2. The equal amount of normal saline is absorbed with the sample gun and added to the routine blood tube, gently mixed, and centrifugation is continued. 3) Repeat 4 times, the color of the chylo/creamy plasma layer is gradually replaced by a lighter color (Figure 1B). At this time, shake the blood routine tube and test it on the same instrument. The result is HGB 161 g/L, MCH 32 pg, MCHC 348 g/L. The results are basically consistent with the formula substitution method. Finally, we report the above results to clinicians.

DISCUSSION

One of the most common analytical interferences in the clinical laboratory is lipemia [5]. Lipemia is defined as clouding of the sample caused by the accumulation of lipoproteins, primarily very low-density lipoproteins (VLDL) and chylomicrons. These particles are high in triglycerides. It is very common for the laboratory to encounter lipid-blood specimens. For routine blood specimens, lipid-blood has a greater impact on HGB, and lipid-blood will cause a false increase in HGB. When reviewing reports as inspectors, in addition to referring to instrument data, they also need to pay more attention to instrument alarm information, pay attention to the ratio relationship between red blood cell count and hemoglobin when reviewing routine blood reports, pay attention to the interference of lipid blood samples on the methodology, timely use of correction formulas to correct or use plasma exchange method for HGB detection, so as to issue accurate reports. In clinical hematology laboratories, hemoglobin is not tested using plasma or serum samples, and it is difficult to discover hyperlipidemia samples. We can find blood lipid samples by the following methods: 1) TG>4 mmol/L; 2) difference between HGB, MCH, and MCHC values and historical data exceeds 20%; 3) MCHC ≥ 365 g/L; 4) HGB value is inconsistent with RBC count; 5) a large number of unstained fat globules of varying sizes were observed in blood smears; 6) hematology analyzer alarm "turbidity/ HGB interference"; 7) visible turbidity of plasma. When any of these are present in the blood sample, the HGB, MCH, and MCHC values should be corrected. At the same time, when detecting blood lipid samples, inspectors should not only fully understand the interference mechanism, but also choose appropriate procedures to eliminate interference. At present, there are many methods to eliminate the interference of lipemia, such as plasma exchange, normal saline dilution, high-speed centrifugation, freezing high-speed centrifugation, vacuum high-speed centrifugation, polyethylene glycol method, ether extraction method, etc. [6-8]. Among them, plasma exchange method has the advantages of strong anti-interference ability, simple operation, low cost and high efficiency [8], which can meet various requirements of clinical laboratory in general hospitals. But the calibration formula method is fast and simple, high efficiency, and the accuracy rate can meet the requirements.

In short, some pathological or physiological conditions can result in changes in the nature of the blood specimen. In the process of blood cell detection, the interference factors of the specimen itself cannot be ignored.

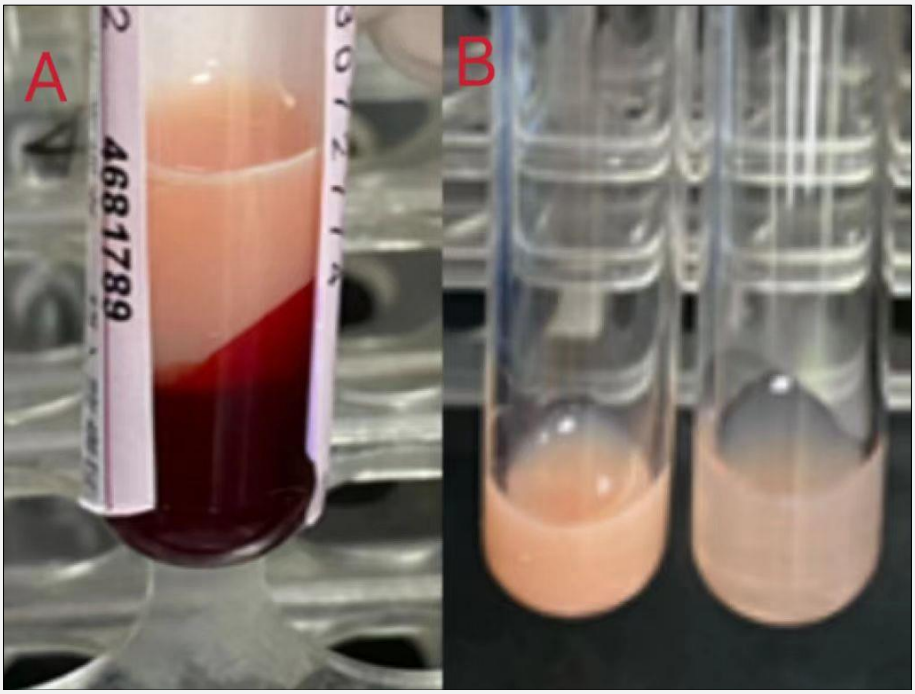

Figure 1. A represents the patient's plasma lipid turbidity. B represents the before and after comparison of plasma after plasma exchange.

This requires the examiner to find the correct solution, strive for accurate results, and provide a reliable diagnostic basis for the clinic.

Sources of Support:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Interest:
All authors declare that they have no competing interests.

References:

1. Ahmed MH, Ghatge MS, Safo MK. Hemoglobin: Structure, Function and Allostery. Subcell Bioche 2020;94:345-382. (PMID: 32189307)

2. Drvenica IT, Stancic AZ, Maslovarić IS, Trivanovic DI, Ilic VL. Extracellular Hemoglobin: Modulation of Cellular Functions and Pathophysiological Effects. Biomolecules 2022 Nov 17;12(11): 1708. (PMID: 36421721)

3. Zeng SG, Zeng TT, Jiang H, et al. A simple, fast correction method of triglyceride interference in blood hemoglobin automated measurement. J Clin Lab Anal 2013 Sep;27(5):341-5. (PMID: 24038218)

4. Fernandez Prendes C, Castro Castro MJ, Sánchez Navarro L, Rapun Mas L, Morales Indiano C, Arrobas Velilla T. Handling of lipemic samples in the clinical laboratory. Adv Lab Med 2023 Feb 20;4(1):5-27. (PMID: 37359904)

5. Mainali S, Davis SR, Krasowski MD. Frequency and causes of lipemia interference of clinical chemistry laboratory tests. Pract Lab Med 2017 Feb 3;8:1-9. (PMID: 28856220)

6. Soleimani N, Mohammadzadeh S, Asadian F. Lipemia Interferences in Biochemical Tests, Investigating the Efficacy of Different Removal Methods in comparison with Ultracentrifugation as the Gold Standard. J Anal Methods Chem 2020 Feb 12:2020: 9857636. (PMID: 32104611)

7. Chen F, Maccubbin D, Yan L, et al. Lipid-altering efficacy and safety profile of co-administered extended-release niacin/laropiprant and simvastatin versus atorvastatin in patients with mixed hyperlipidemia. Int J Cardiol 2013 Jul 15;167(1):225-31. (PMID: 22305632)

8. Agarwal S, Vargas G, Nordstrom C, Tam E, Buffone G J, Devaraj S. Effect of interference from hemolysis, icterus and lipemia on routine pediatric clinical chemistry assays. Clin Chim Acta 2015 Jan 1:438:241-5. (PMID: 25128720)