Factitious Results in Clinical Chemistry Tests Caused by Common Endogenous Interferents

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Total laboratory processes are comprised of pre-analytical, analytical and postanalytical phases. Quality management must be performed in all phases to yield the accuracy and precision of laboratory results. In spite of large evidence of the benefit in implementing a quality program and the improvement of laboratory automation instruments to reduce all defects within the processes, laboratory errors still occur. The majority of errors lie in the preanalytical phase. Plebani et al, reported that the distribution of mistakes was preanalytical in 68.2%, analytical in 13.3%, and post-analytical in 18.5%.[1] The preanalytical phase involves all the various processes before a sample can be measured. Preanalytical factors are parts of patient-related variables (age, sex, diet, stress, medication, etc.), specimen collection (patient identification, venepuncture techniques, specimen volume, types of anticoagulants, etc.), specimen transport, specimen processing and storage. Analytical factors involve methods and instruments used for measuring analytes in the specimen that currently are performed with a highly advanced technology automation system. Finally, postanalytical factors consist of reporting, comprehension and interpretation of laboratory results. Since the laboratory mistakes are principally detected in the preanalytical phase and may lead to further inappropriate investigations or treatments, detection and management of unsuitable specimens including misidentification, quantity or quality issues play an important role in preventing the errors, thereby reducing unjustified increase in costs. The hemolytic, lipemic or icteric samples which can be detected in the preanalytical phase are considered unsuitable for routine clinical chemistry tests due to biological and analytical interferences. Moreover, the presence of paraproteins interfere in many biochemical measurements. Hemolysis, lipemia, bilirubinemia and paraproteinemia are classified as the most common sources among the endogenous interferents. This article reviews the factitious results in clinical chemistry tests caused by hemolysis, lipemia, bilirubinemia and paraproteinemia and the mechanisms of analytical interferences.

Hemolysis

Hemolysis is defined as the release of intracellular contents of erythrocytes or other blood cells into plasma or serum. Hemoglobin, in particular causes visible pink or red color of serum or plasma after centrifugation if the level of free hemoglobin exceeds 300 mg/L.[2] Hemolysis can occur in vivo or in vitro. In vivo hemolysis occurs in several clinical conditions such as hemolytic anemia and transfusion reactions and is associated with reducing the level of haptoglobin as well as increasing levels of indirect bilirubin or reticulocytes. In vitro hemolysis, the major cause of hemolysis, occurs during the specimen collection or processing such as alcohol contamination with a blood sample during venepuncture, small-gauge needles, difficult blood draws, prolonged tourniquet time, vigorously mixing of the blood samples with anticoagulant or high speed or prolonged centrifugation. Hemolytic specimens were recognized as high as 3.3% of routine samples and up to 40%-70% of unsuitable specimens.[3]

Hemolysis can interfere with many clinical chemistry tests causing a false increase in alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), acid phosphatase, creatinine, creatine kinase (CK), lactate dehydrogenase (LDH), lipase, magnesium, phosphorus, potassium and urea, whereas a false decrease was found in albumin, alkaline phospha-tase (ALP), bilirubin, chloride, γ-glutamyltransferase (GGT), glucose and sodium.[4,5] In general, a mild degree of hemolysis does not cause a clinically significant effect on most test results since the difference between the test value from the sample with interference and the control sample is lower than the maximum allowable deviation of the analytical method or the desirable bias of the test. However, clinically relevant difference was found in AST, chloride, LDH, potassium and sodium even at lower concentrations of hemoglobin that do not show discoloration of samples by visual assessment.[5] In addition to the influence of hemolysis on routine chemistry tests, there have been studies showing evidence of overestimation of serum folate and underesti-mation of insulin and cardiac troponin T (cTnT) from hemolysates.[6-8] The occurrence of interferences depends on the test methods or instruments used.

Mechanisms of hemolytic interference

Hemolytic interference causes spurious results by various mechanisms:

- Release of intracellular components of red blood cells with a high intracellular content of analytes such as potassium, AST, ALT, LDH, acid phosphatase, iron, folate, magnesium, phosphate into plasma or serum produces falsely high results of the analytes. On the contrary, it may cause spuriously low results in some analytes that have lower concentrations in intracellular compared to extracellular compartments such as sodium, chloride or glucose due to dilutional effects.[2,4,9]
- Chemical interference may be caused by some intracellular components that are released from red blood cells. For example, adenylate cyclase might interfere with some analytical methods of CK activity, especially with inadequate inhibitors of adenylate cyclase in the test reagents; peroxidase of free hemoglobin interfere with the diazotization procedures for bilirubin detection, leading to slightly low levels of bilirubin and proteases that are released from blood cells degrade cTnT producing falsely decreased results.[8-10]
- Optical interference may result from the coloring effect of hemoglobin. Hemoglobin begins to absorb around 340 nm and the maximum absorbances are at 540- to 580-nm wavelengths. Many routine clinical chemistry tests use spectrophotometry for detection of the chemical reactions. Interference can be caused by the spectrophotometric property of hemoglobin or modification of the blank value. The alteration depends on the method and analyte concentration.

Lipemia

Lipemia represents as turbidity in the serum or plasma which is visible before the analytical process. It is principally caused by large particles of lipoproteins such as chylomicrons or VLDL, the main lipid component of which is triglyceride. Thus it is usually recognized when triglyceride concentrations are above 300 mg/dL.[2] Lipemia is usually caused by intake of food with high fat content. After fat ingestion, chylomicrons are detected in plasma after about 6-12 hours. Lipemia can occur as a result of the disturbance of lipoprotein metabolism or parenteral nutrition as well.

Lipemic interference is commonly found in routine clinical chemistry tests. Not only can it influence measurements of uric acid, glucose, phosphorus, total bilirubin and total protein, but also causes falsely increasing levels of total cholesterol and HDL-cholesterol.[9,11] Ultracentrifugation can remove the turbidity of lipemic samples and the clear infranatant obtained can be analyzed. This technique will provide more accurate results. Likewise, lipemia can affect the measurement of sodium resulting in pseudohyponatremia.

Mechanisms of lipemic interference

There have been reports suggesting that lipemia interferes with test results by the following mechanisms:

- The turbidity interferes in spectrophotometric, turbidimetric or nephelometric assays by light scattering or absorption. Chylomicrons which are the least dense particles cause a floating creamy layer whereas VLDL induces more homogenous turbidity. The degree of light scattering varies, depending on size, shapes and components of the lipoprotein particles. Turbidity can affect the absorbance in spectrophotometry at nearly all wavelengths and therefore the analytical values may not be correct. The manufacturers commonly use IntraLipid, the synthetic emulsion composed of soybean oil and egg phospholipids, to perform interference studies and the extent of lipids that would not be affected on test values is usually reported in their leaflets. However, this may not correlate with patient's triglyceride levels due to varieties of natural lipoprotein particles in human plasma.
- Hyperlipidemia can cause pseudohyponatremia if serum sodium is measured by indirect ion-selective electrode (ISE) or flame photometry because serum sodium is restricted to serum water and the increment of lipid in serum will displace the water compartment leading to a decrease in proportion of water. This effect is not found if serum sodium is determined by the direct ISE method.[12]

Bilirubinemia

Bilirubin is derived from heme catabolism. Hyperbilirubinemia can be observed in hepatic or hemolytic diseases. The main type of bilirubin in hemolytic jaundice is unconjugated bilirubin whereas the major form in hepatocellular jaundice or obstructive jaundice is conjugated bilirubin. After specimen centrifugation, hyperbilirubinemia can be visible as icteric serum or plasma. However visual assessment is not sensitive and may be unreliable when compared to spectrometric detection.[13] Results from previous studies have indicated that bilirubin interferes in creatinine, glucose, cholesterol, triglyceride, phosphorus, uric acid and total protein measurements.[14] Furthermore, extreme hyperbilirubinemia can cause false positive acetaminophen results.[15]

Mechanisms of bilirubin interference

The analytical interference by bilirubin can be caused by

- Spectral interference:

The interfering effect of bilirubin is due to the spectral properties of bilirubin absorbance in the wavelength between 340 and 500 nm. Bilirubinemia induces high background absorbance which is proportional to its concentration. Thus, it mainly interferes in spectrophotometric assays.[2]

- Chemical interference:

Bilirubin may interact chemically with test reagents. Since bilirubin reacts with peroxidase catalyzed reactions, H_2O_2 generated during the chemical reaction is utilized by bilirubin, thereby causing spuriously low results of creatinine, glucose, cholesterol, triglyceride and uric acid.[15] Bilirubin also causes underestimation in phosphorus measurement that uses a UV method for the detection of phosphate by formation with phosphomolybdate.[16]

Paraproteinemia and hyperimmunoglobulinemias

Circulating paraproteins are found in patients with multiple myeloma or lymphoproliferative disorders. Furthermore, the interferent can be observed in other clinical conditions, previously described mainly as case reports, such as multiple sclerosis or Guillain-Barré syndrome receiving an intravenous infusion of immunoglobulin therapy. Paraproteins differ from other common endogenous interferents since they cannot be detected by discoloration of serum or plasma. Paraprotein interferences can occur in many chemical tests, but the incidence may be underreported due to unawareness of the underlying disease. The occurrence can be identified by a clinician who recognizes that such abnormal labora-

tory results are not in accord with the patient's symptoms. However, the frequency of false laboratory results caused by paraprotein interference is variable and particularly individual. Paraprotein interference also depends on the instruments and reagent methods used.[6]

There have been rare case reports of paraprotein-induced pseudohyponatremia, pseudohypoglycemia, artifactial hyperbilirubinemia, artifactially low HDL-cholesterol, pseudohyperphosphatemia or hypophosphatemia, factitious hypouricemia, factitious hypoalbuminemia, spurious underestimation of urea or creatinine or thyroxine measurements, spuriously increased serum C-reactive protein (CRP) and antistreptolysin-O (ASO). IgM paraproteins as interferents are frequently found in these cases due to their high molecular weight. However, the findings cannot be predicted. Some cases are unfortunately misdiagnosed resulting in further unnecessary investigations or inadvertent therapeutic managements, so clinicians must carefully interpret the laboratory results.[6,17-27]

Mechanisms of paraprotein interference

Paraproteinemia or hyperimmunoglobulinemia can interfere in many clinical chemistry measurements including nephelometry, turbidimetry or immunoassays. The effect of paraproteins or immunoglobulins may be classified according to the following mechanisms:

- Increasing serum or plasma viscosity due to the presence of paraproteins results in a decrease in the water compartment. Consequently, the amounts of water soluble analytes are spuriously underestimated. This mechanism can explain the cause of pseudohyponatremia that is measured by indirect ion selective electrode methods (ISE).[17-19]
- Precipitation of paraproteins with reagents may occur during the test procedures, resulting in turbidity and its interference in nephelometric, turbidimetric or colorimetric assays.[20-26]
- Paraproteins may interfere immunoassays by interaction with the specific antibody reagents, thereby falsely increasing laboratory values.[27]

Recommendation

To improve the quality of laboratory results, common endogenous interferences due to hemolysis, lipemia or bilirubinemia should be visually checked after centrifugation by laboratory personnel and should be documented in laboratory reports. However, visual assessment has been described as unreliable.[13] This may be due to subjective interpretation, laboratory staff workload or inappropriate barcode labelling on sample tubes. Sophisticated automated analyzers have been developed which are able to determine the degree of hemolysis, lipemia or bilirubinemia and reported as "hemolytic index, lipemic index and icteric index respectively". The index results are represented as semiquantitative measurements and correlate well with the amounts of interferents. Many automated instruments were proved that they are efficient to identify and classify the interferents, especially unsuitable hemolytic samples.[28] Practically, laboratory personnel can identify and resolve the problems of interferents after the analyzers indicate the problems by flagging systems as well as validating the process before releasing the test results.

Because the interferents mainly arise from in vitro hemolysis, occurring during blood sampling procedures, standardization of phlebotomy techniques and training play a significant role in the laboratory preanalytical process. Appropiate bore size needles should be used for collecting blood samples and tourniquet time should be less than 1 minute to reduce the risk of hemolysis.[29,30] Since the influences of hemolysis, lipemia, bilirubin and paraproteins on some analytes are dependent on methods and instruments, it is difficult to predict which samples will cause interference. Clinicians should be aware of the factitious laboratory results of clinical chemistry tests that may be affected by the interferents in each patient's circumstances.

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