Hemolytic index – A tool to measure hemolysis in vitro

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Abstract: Introduction: Hemolysis is the commonest cause of preanalytical error. Hemoglobin released interferes with analyte concentration chemically and optically. Contents of RBCs released might falsely elevate analyte concentration. This is one of the commonest cause of sample rejection which poses problem in fresh sample collection. Inconvenience caused to patients results in non co-operation for further investigations. The aim was to use HI as an automated determinant of hemolysis in venous blood specimens sent to our clinical chemistry laboratory and measure the extent of hemolysis

Methods: The study was conducted in Clinical Biochemistry laboratory in the month of January 2016. Total of 695 samples were collected and hemolytic index (HI) was estimated in autoanalyzer, transasia XL-640. HI values were categorized from H0 to H4. Percentage of sample in each category was calculated.

Results: The majority of the samples were lysed to H1(52.7%) and H2(31.36%)(small to intermediate degree). Percentage of non hemolyzed samples is minimum (0.58%) whereas marked hemolysis was 4.31%.

Conclusion: Hemolytic index estimation is the systematic way of ensuring that the sample is fit for analysis. The use of automated HI estimation overcomes the inherent limitations of classical visual estimation by providing a more objective and accurate estimate of hemolysis.

Key words: hemolysis, interference in analysis, hemolytic index

I. Introduction

Hemolysis is the commonest cause of preanalytical error. Prevention of medical errors is a goal of health care system. The laboratory errors due to pre analytical variables has received a great deal of attention. It has been analyzed that hemolysis of patient specimen may interfere with accurate measurement of analytes. Rate of rejection of sample is highest due to hemolysis and getting fresh sample is a problem.

Kroll and Elin (1) defined interference as the effect of a substance present in the sample that alters the correct value of the result. Medicine lab tests can be affected by endogenous constituents of the sample. Creatinine , triglycerides, glucose, cholesterol, uric acid, iron, total protein, bilirubin are the parameters affected by hemolysis.

Hemolysis can be detected visually, it is essential to estimate it by direct analysis. Hemolytic index is useful in this regard. It is a tool that makes lab professionals aware of interferences. It improves the quality of the sample. Advantage of it is that it minimizes the aberrant test results.

The grading of hemolysis is as shown in Table 1;

<table>
<thead>
<tr>
<th>HI</th>
<th>Appearance of serum</th>
<th>Degree of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>Clear</td>
<td>No hemolysis</td>
</tr>
<tr>
<td>20-100</td>
<td>Pink tinged</td>
<td>Slight hemolysis</td>
</tr>
<tr>
<td>100-300</td>
<td>Red</td>
<td>Moderate hemolysis</td>
</tr>
<tr>
<td>&gt;300</td>
<td>Dark red</td>
<td>Marked hemolysis</td>
</tr>
</tbody>
</table>

Haemolysis is the release of haemoglobin and other intracellular components from erythrocytes into the surrounding plasma following damage of the cell membrane (2). Hemolysis is a common reason for specimen rejection (3), reported to account for 40%–70% of all unsuitable specimens sent to the laboratory (2). The variation is dependent on different methods used for estimation of haemolysis, as well as different cut-off thresholds for analytical interference.

A growing body of evidence indicates that most errors in laboratory testing arise in the pre analytical phase (4,5) as the result of human mistakes (6). In vitro haemolysis is one important example since this is caused primarily by inappropriate specimen collection and handling (2), such as prolonged use of venous stasis (7), delayed separation of blood from plasma (2) and blood collection through intravenous catheters (8, 9). Most
previous studies have used subjective visual assessment (7–9, 10–12) or the analysis of free haemoglobin with laborious manual spectrophotometric techniques (11, 13–15) to evaluate the prevalence of haemolysis. The haemolysis index (HI) in automated analysers is a more efficient method for detecting haemolysis. The hemolysis index, H, is reported in hemolysis units that are linear, up to 1000 mg/dl, and semi-quantitative. For many years now, the HI has been used in laboratories to automatically reject samples that are hemolyzed in order to avoid analytical interference. However, the possible use of all samples with detectable HI as a marker of the overall pre analytical quality of the blood sample has not been reported previously. The aim was to use HI as an automated determinant of hemolysis in venous blood specimens sent to our clinical chemistry laboratory and measure the extent of hemolysis.

II. Methods

This study was conducted in the Department of Biochemistry, Karwar Institute of Medical Sciences. A total of 695 patient samples were collected in the month of January 2016, out of which 268 males and 271 female patients. Blood samples were collected in the clinical laboratory in EDTA bottles, vacutainers or sometimes in syringes. Frequently samples were found to be hemolysed and gave erroneous results. We used to assess the extent of hemolysis by visual assessment which was not accurate. Visual detection of hemolysis is subjective and therefore mostly unreliable since it may over- or under-estimate the actual amount of hemolysis in the specimen. An automated serum index detection by direct measuring hemoglobin concentration photometrically has been implemented. We used Transasia XL-640, automated clinical chemistry analyzer in our laboratory that measures the degree of hemolysis.

Principle of assay:

The assay is based on calculations of absorbance measurements, of diluted samples at different bichromatic wavelength pairs to provide a semi-quantitative representation of levels of hemolysis in serum and plasma samples. The XL-640 analyzer takes an aliquot of the patient specimen and dilutes it with saline (0.9% sodium chloride) to measure the absorbances for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength). From these absorbance values the instrument calculates the serum index value for hemolysis. The extent of hemolysis was represented by HI, ranging from H0-H4.

Classification of degree of hemolysis is as follows:

<table>
<thead>
<tr>
<th>Degree of hemolysis</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>H1</td>
<td>10</td>
<td>199</td>
</tr>
<tr>
<td>H2</td>
<td>200</td>
<td>299</td>
</tr>
<tr>
<td>H3</td>
<td>300</td>
<td>399</td>
</tr>
<tr>
<td>H4</td>
<td>&gt;400</td>
<td></td>
</tr>
</tbody>
</table>

HI is said to be of small degree, if free hemoglobin is up to 50mg/dl. 50-300mg/dl is said to be intermediate degree and more than 300mg/dl is called high degree hemolysis.

Statistical analysis was done by descriptive statistical methods.

III. Results

We found that majority of the samples were lysed to H1 (52.7%) and H2 (31.36%) (small to intermediate degree). Percentage of non hemolyzed samples is minimum (0.58%) whereas marked hemolysis was 4.31%. We have represented HI in the Table 3 as follows.

<table>
<thead>
<tr>
<th>Degree of hemolysis</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0</td>
<td>4</td>
<td>0.58</td>
</tr>
<tr>
<td>H1</td>
<td>399</td>
<td>57.4</td>
</tr>
<tr>
<td>H2</td>
<td>218</td>
<td>31.36</td>
</tr>
<tr>
<td>H3</td>
<td>44</td>
<td>6.33</td>
</tr>
<tr>
<td>H4</td>
<td>30</td>
<td>4.31</td>
</tr>
</tbody>
</table>

IV. Discussion

We have observed a significant mild to moderate hemolysis in our patients' samples with minimal massive hemolysis. Non hemolyzed samples were negligible in our laboratory. Thus it is great problem that has to be dealt with in our laboratory.
Abnormal disruption of erythrocytes may occur in vivo or in vitro, due to clinical or artificial causes, respectively(16). Many problems due to troublesome specimen collections or handling may affect the samples and cause in vitro hemolysis, as thoroughly reviewed by Lippi et al.(17). In vitro hemolysis remains the leading cause of unsuitable specimens both for outpatient and inpatient samples, hemolyzed specimens accounting for 40–70% of all unsuitable specimens, nearly five times higher than the second leading cause of assay interference(18,19).

Hemolysis could be due to patient factors on which laboratory doesn’t have any control. Laboratory factors causing hemolysis are those related to collection, transport, processing etc. IV collection, capillary collection frequently cause hemolysis. Gauge of needle, arm position, location of venipuncture, antiseptic used for phlebotomy, tourniquet time, first clinching, syringe transfer, vigorous mixing are important aspects of phlebotomy that lead to hemolysis. Transport of the samples from collection Centre to laboratory by courier transport can lyme samples. Centrifugation, analysis after a long time, tube mixed prior to analysis, recentrifugation, postanalytical storage temperature, duration of storage are key points causing hemolysis.

Even mild hemolysis can cause clinically meaningful variations in sodium, potassium, chloride, LDH and AST values. Reliability of testing is questioned. The rationale behind this when lysed erythrocytes release potassium, LDH, AST, magnesium and other components which show a false elevation. Analyte results that are falsely increased by hemolysis are: Acetaminophen, ALT, NH₃, AST, Phosphorus, CK, Potassium, Iron, UIBC, cardiac troponin (20). In addition to chemical interference with reagents or analytes, hemoglobin also poses optical interference.

Such erroneous results may mislead patients’ medical conditions. This factor might question authenticity of the laboratory. It is essential to take corrective measures. Procedure for corrective action shall include an investigative process to determine underlying causes of problem. Technical or quality management system shall be identified. Action plan need to be developed, implemented and monitored to reduce the likelihood of occurrence of such nonconformities.

Another problem with hemolysis is risk of sample getting rejected. Getting fresh samples from outpatients is delayed. Patient inconvenience and dissatisfaction are the common problems with this. Erroneous results mislead the diagnosis of clinical conditions. So it is the primary duty of laboratories to systematically quantify hemolysis of patients’ specimen so as to ensure sample integrity.

V. Conclusion

Hemolytic index estimation is the systematic way of ensuring that the sample is fit for analysis. The use of automated HI estimation overcomes the inherent limitations of classical visual estimation by providing a more objective and accurate estimate of hemolysis. HI is above the cut-off for an analyte indicating significant interference, the test report is automatically managed according to the criteria established by the laboratory manager, i.e. a comment to alert the clinician, flagging the result and/or rejecting the sample. In that way, detection of clinically significant hemolysis interference, HI values and corrective actions taken are directly archived.

Acknowledgements:

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References

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