IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 2, Issue 2 (Jan. – Feb. 2016), PP 49-52
www.iosrjournals.org

Hemolytic index – A tool to measure hemolysis in vitro

Usha Adiga[1], Yogish S[2]
[1] *Associate Professor, Department of Biochemistry ,KAiMS, KARWAR*
[2]*Tutor, Department of Biochemistry,KAiMS, KARWAR*

Corresponding author:
Dr. UshaSachidanandaAdiga
Associate Professor, Department of Biochemistry
Karwar Institute of Medical Sciences, Karwar Karnataka, India

Abstract: *Introduction: Hemolysis is the commonest causeof pre analytical 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 -1:Grading of hemolysis based on HI and gross appearance

HI	Appearance of serum	Degree of hemolysis
<20	Clear	No hemolysis
20-100	Pink tinged	Slight hemolysis
100-300	Red	Moderate hemolysis
>300	Dark red	Marked hemolysis

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 to1000 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-2 :Categorization of Hemolytic index

Degree of hemolysis	Minimum	Maximum
H0	0	09
H1	10	199
H2	200	299
H3	300	399
H4	>400	

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 -3:Hemolytic index of patients in Transasia XL -640

	Frequency	Percentage
H0	4	0.58
H1	399	57.4
H2	218	31.36
H3	44	6.33
H4	30	4.31

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 artifactual 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, fist 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 lyse 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 analyteindicating 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:

Our special thanks to DrPoornima RT, Professor &Head ,Biochemistry for her support.

References

[1]. Derberg J, Wallin O, Grankvist K, Brulin C. Is the testresult correct? A questionnaire study of blood collectionpractices in primary health care. J Eval Clin Pract.Inpress 2009.
[2]. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, PalickaV, et al. Haemolysis: an overview of the leading causeof unsuitable specimens in clinical laboratories. ClinChem Lab Med 2008;46:764–72.
[3]. Jones BA, Calam RR, Howanitz PJ. Chemistry specimenacceptability: a College of American Pathologists QProbesstudy of 453 laboratories. Arch Pathol Lab Med 1997;121:19–26.
[4]. Kalra J. Medical errors: impact on clinical laboratories andother critical areas. ClinBiochem 2004;37:1052–62.
[5]. Carraro P, Plebani M. Errors in a stat laboratory: types andfrequencies 10 years later. ClinChem 2007;53:1338–42.
[6]. Wiwanitkit V. Types and frequency of preanalytical mistakes in the first Thai ISO 9002:1994 certified clinical laboratory, a 6-month monitoring. BMC ClinPathol 2001;1:5.
[7]. Burns ER, Yoshikawa N. Hemolysis in serum samples drawn by emergency department personnel versus laboratory phlebotomists. Lab Med 2002;33:378–80.
[8]. Grant MS. The effect of blood drawing techniques and equipment on the hemolysis of ED laboratory blood samples.JEmergNurs 2003;29:116–21.
[9]. Kennedy C, Angermuller S, King R, Noviello S, Walker J,Warden J, et al. A comparison of hemolysis rates using intravenous catheters versus venipuncture tubes for obtaining blood samples. J EmergNurs 1996;22:566–9.
[10]. Fang L, Fang SH, Chung YH, Chien ST. Collecting factors related to the haemolysis of blood specimens. J ClinNurs 2008;17:2343–51.
[11]. Cox SR, Dages JH, Jarjoura D, Hazelett S. Blood samples drawn from IV catheters have less hemolysis when 5-mL (vs. 10-mL) collection tubes are used. J EmergNurs2004;30:529–33.

[12]. Dugan L, Leech L, Speroni KG, Corriher J. Factors affecting hemolysis rates in blood samples drawn from newly placed IV sites in the emergency department. J EmergNurs 2005;31:338–45.
[13]. Carraro P, Servidio G, Plebani M. Hemolyzed specimens:a reason for rejection or a clinical challenge? ClinChem 2000;46:306–7.
[14]. Lippi G, Montagnana M, Salvagno GL, Guidi GC. Interference of blood cell lysis on routine coagulation testing.ArchPathol Lab Med 2006;130:181–4.
[15]. Lippi G, Salvagno GL, Montagnana M, Brocco G, Guidi GC. Influence of hemolysis on routine clinical chemistry testing. ClinChem Lab Med 2006;44:311–6.
[16]. Carraro P, Servidio P, Plebani M. Haemolyzed specimens: a reason for rejection or clinical challenge? ClinChem 2000;46:306–7.
[17]. Lippi G, Plebani M, Di Somma S, Cervellin G. Hemolyzed specimens: a major challenge for emergency departments and clinical laboratories. Crit Rev Clin Lab Sci 2011;48:143–53.
[18]. Mozzi R, Carnevale A, Valente C, Dolci A, Panteghini M. Recording, monitoring, and managing pre-analytical issues in a metropolitan university hospital. Biochim Clin 2013;37:95–9.
[19]. Lippi G, Guidi GC. Risk management in the preanalytical phase of laboratory testing. ClinChem Lab Med 2007;45:720–7.
[20]. Sodi R, Darn SM, Davison AS, Stott A, ShenkinA. Mechanism of interference by haemolysis in the cardiac troponin T immunoassay. Ann ClinBiochem 2006;43:49–56.