Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Biochemistry

Hemolysis- An Interference or An Influence on Routine Biochemical Parameters

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Original Research Article

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Article History
Received: 31.12.2017
Accepted: 06.01.2018
Published: 30.01.2018

DOI:
10.36347/sjams.2018.v06i01.056

Abstract: Hemolysis is one of the most common reason for inadvertent reports, and sample rejection in clinical laboratory. The aim of this study is to know the effect of hemolysis on routine biochemical parameters. Fifteen healthy volunteers are selected for this study. Venous plasma is collected and grouped into four levels of hemolysis based on hemoglobin concentration : Group I: 0-0.10 g/L, Group II: 0.10-0.50 g/L, Group III: 0.51-1.00 g/L, Group IV: 1.01-2.50 g/L, Group V: 2.51-4.50 g/L. Lysis is achieved by mechanical agitation. The analytes are measured in Automated Biochemistry Analyser. Aspartate aminotransferase (AST) levels are significantly affected even at undetectable levels of hemolysis.Variations of potassium and total bilirubin are observed in moderately hemolyzed samples (hemoglobin >1 g/L). Alanine aminotransferase (ALT), Cholesterol, and Inorganic phosphate (Pi) concentrations are not interfered up to severely hemolyzed levels (hemoglobin: 2.5-4.5 g/ L). Albumin, Alkaline phosphatase (ALP), Amylase, Chloride, HDL-cholesterol, Creatine kinase (CK), Glucose, Total protein, Triglycerides, and Uric acid levels vary significantly but within allowable limits. Hemolysis causes alteration of many parameters in Biochemistry. It is therefore preferable to do hemoglobin estimation in plasma for analytes with potential interference, to avoid wrong reporting of results.
Keywords: Hemolysis, sample rejection, Biochemistry parameters, CLIA, AST.

INTRODUCTION

Prevention of medical errors is a goal of health care. With the advent of automation, the issue of laboratory errors from preanalytical variables has received a great deal of attention.

Among the major preanalytical variables influencing patient results, sample hemolysis exerts a major role. Hemolytic samples are a common occurrence in laboratory practice.

Hemolysis is the most common reason for sample rejection, account for 3.3% of all routine samples and ~ 60% of the rejected samples [1]. Hemolysis can occur in vivo or in vitro. In vivo hemolysis accounts for only 3.2% [2].The incidence of in vitro hemolysis is more and often preventable if due care taken in specimen collection, transportation, & processing [3-5].

Hemolysis can be an interference or an influence factor in the laboratory analysis. Hemolysis causes release of cellular constituents into the plasma which can either falsely elevate the concentration of certain analytes or have a dilutional effect resulting in lower values. Moreover, hemolysis causes spectrophotometric interference of the analytes [6].

Conversely, even if the hemolysis is invisible also, there can be discharge of cellular contents into plasma [7]. So invisible hemolysis can cause false results. Some of the analytes are not affected by hemolysis. Ignorance of the above fact can result in inadvertent rejection of samples.

Since the knowledge of possible effects of hemolysis is important for correct interpretation of the results, the aim of the present work is to evaluate the influence of hemolysis on routinely used biochemical tests.

MATERIALS & METHODS

15 healthy volunteers were enrolled in this study. 6ml of venous blood was collected in 5 different heparinised tubes.

Hemolysis was achieved by mechanical trauma. In 4 of the tubes, hemolysis was achieved by pushing forcefully through the needle 3, 6, 9, 12 times respectively to get varying levels of hemolysis. This

method of cell lysis was almost similar to the mechanical disruption of erythrocytes during blood collection. They were all centrifuged at 1000 x g for 10 min. The supernatant plasma removed and was recentrifuged at 1200 x g for 20 min.

The free hemoglobin of the samples were measured colorimetrically by cyanmethemoglobin method [8]. Absorbance was measured at 540nm.

The samples were categorised into five groups, based on free hemoglobin concentrations as:
Group I - 0-0.1g/L ; no hemolysis (n=10)
Group II-0.1-1.0g/L; slight hemolysis (n=10)
Group III-1.0-2.5g/L; mild hemolysis (n=14)
Group IV-2.5-4.5g/L; moderate hemolysis (n=15)
Group V-4.5-6.5g/L; severe hemolysis (n=11)

The exclusion criteria of a hemolyzed sample was the concentration of its free Hb concentration discordant with the degree of hemolysis determined for each group, so the number of samples were not equal at each group. Reference Range for plasma free hemoglobin: 0-0.1g/L.

Plasma concentrations of glucose, urea, creatinine, AST, ALT, ALP, protein, albumin, bilirubin, amylase, sodium, potassium, calcium, chloride, phosphorus, uric acid, cholesterol, triglycerides, HDL were analysed in all groups of hemolysed samples. The measurement of the analytes were done in Olympus AU480 Automated Biochemistry Analyser.

The effects of hemolysis were evaluated according to the total allowable error recommendations of Clinical Laboratory Improvement Amendments (CLIA'88) [9] (Table-1). CLIA' 88 regulations have established fixed limits for assessing the method and laboratory performance for specific regulated analytes. In practice, the total allowable error for a given analytical method must be less than the limits fixed by CLIA for the analyte in question.

Statistics

To compare the concentration of analytes in the hemolysed and the non hemolysed specimen, percentage of bias was calculated by the formula

$$(Cx-C1/C1) *100$$

Cx- concentration of the analyte in hemolysed sample
C1-concentration of the analyte in non hemolysed sample

All analysis were done using Graphpad software Version 6.0 for windows XP. Wilcoxon matched pair signed rank test was used. p value < 0.05 was considered statistically significant.

RESULTS

The median free hemoglobin concentration of the groups I, II, III, IV, V were 0.05, 0.65, 1.5, 3.1, 5.5 g/L respectively. At free hemoglobin concentration of 0.2 g/L, hemolysis was visible by the red color of the plasma. The results of this study were shown in table 1 and also in figure-1.

The AST values show significant increase in linearity with free hemoglobin concentration. Among the electrolytes, potassium increased gradually with increase in hemolysis.

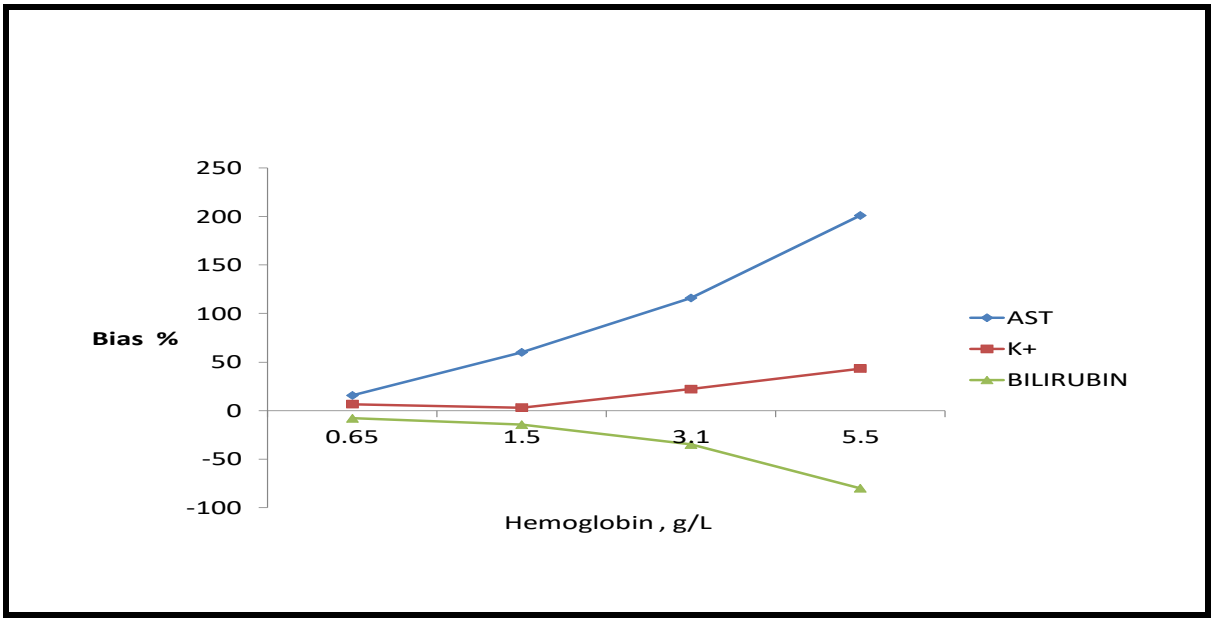

Fig-1: Showing comparison of hemoglobin and measured parameters: AST, potassium, Total Bilirubin

Table-1 shows the method, median value of the analytes and free hemoglobin concentrations for each group, percentage relative bias of the analytes compared to the non hemolysed group, desirable bias ±, CLIA '88 acceptable limits ±.

Table-1: Effects of hemolysis on various clinical chemistry parameters

Analyte	**Method**	**Free Plasma Hemoglobin g/L**					**Desirable Bias ±**	**CLIA ±**
		No lysis	**0.65**	**1.5**	**3.1**	**5.5**		
Albumin (g/L)	BCG method	4.3	4.2 (-3%) P=0.36	4.3 (-0.4%) p=0.88	4.5 (+5.8%) **p=0.04**	4.7 (+8.2%) **p=0.03**	1.3%	10%
ALP (U/L)	PNPP	84.5	82 (+3.6%) P=0.78	69.5 (-6%) p=0.38	66 (-14%) P=0.26	79 (+18%) P=0.57	6.4%	30%
ALT (U/L)	IFCC without P5P	8	11.5 (+3.0%) P=0.52	12 (+4.0%) p=0.12	12 (+5.0%) p=0.24	13 (+18%) **p=0.04**	12%	20%
Amylase (u/L)	CNP triose	45	46.5 (-2.0%) **p=0.04**	46 (-5.3%) **p=0.03**	47 (-10.3%) **p=0.03**	51 (-15.2%) **p=0.03**	7.4%	30%
AST (U/L)	IFCC without P5P	15.5	18.5 (+30%) **P=0.04**	25.5 (+60%) **P=0.009**	35 (+116%) **P=0.003**	50 (+201%) **P=0.002**	5.4%	20%
Bilirubin total (mg/dl)	Diazo, colorimetric	0.45	0.3 (-8%) **P=0.02**	0.43 (-14.5%) **P=0.03**	0.4 (-35%) **P=0.02**	0.3 (-80%) **P=0.019**	10%	20%
Calcium Total (mg/dl)	OCPC method	9.2	9.2 (-2.0%) P=0.49	9.1 (+1.1%) P=0.98	9.1 (-0.4%) P=0.43	9.1 (+1.3%) P=0.86	0.8%	0.25mmol/L
Chloride (mmol/L)	Colorimetric	96.5	96.5 (-1.8%) P=0.84	97 (-6.2%) P=0.48	97 (-4.2%) P=0.28	99 (-2.6%) P=0.26	0.5%	5%
Cholesterol (mg/dl)	Enzymatic, colorimetric	133	136 (-1.9%) P=0.95	147 (+4.1%) P=0.57	151 (+5.5%) P=0.32	149 (+7.6%) **P=0.04**	4.0%	10%

Table-1: Effects of hemolysis on various clinical chemistry parameters (Continuation)

Analyte	Method	Free Plasma Hemoglobin (g/L)					Desirable Bias ±	CLIA ±
		No lysis	0.65	1.5	3.1	5.5		
Creatinine	Jaffe's / alkaline picrate	0.7	0.8 (-1.7%) P=0.68	0.8 (0.0%) P=0.24	0.8 (0.0%) P=0.36	0.8 (-2.3%) P=0.83	3.4%	15%
Glucose	GOD	86	89 (-1.0%) **P=0.03**	79 (-4.2%) **P=0.01**	85 (-0.9%) **P=0.001**	82 (-7.8%) **P=0.003**	2.2%	10%
HDL (mg/dl)	Direct	46	45 (+2.3%) P=0.46	46 (+2.0%) P=0.97	49 (-1.0%) P=0.07	50 (-0.8%) **P=0.04**	5.2%	30%
Phosphorus (mg/dl)	UV Molybdate	3.5	3.7 (+0.4%) P=0.57	3.7 (+1.6%) P=0.98	3.8 (+3.5%) **P=0.04**	4.2 (+10.3%) **P=0.005**	3.2%	10%
#Potassium (mmol/L)	Ion Selective Electrode	3.8	4.3 (+6.7%) **P=0.04**	4 (+3.1%) **P=0.005**	4.5 (+22.1%) **P=0.003**	5.4 (43.4%) **P=0.002**	1.8%	0.5 mmol/L
Protein Total (g/L)	Biuret	8.4	8.5 (+1.9%) P=0.28	8.4 (+0.7%) P=0.64	8.8 (+3.5%) P=0.08	8.5 (+1.45) **P=0.02**	1.2%	10%
*Sodium (mmol/L)	Ion Selective Electrode	142	139 (-1.1%) P=0.15	141 (-0.7%) P=0.13	140 (-0.6%) P=0.08	140 (-0.5%) P=0.31	0.3%	4 mmol/L
Triglycerides (mg/L)	Enzymatic, Colorimetric	67	67 (-1.3%) P=0.94	73 (-1.6%) P=0.69	68 (-0.8%) P=0.56	72 (-18.2%) **P=0.05**	10.7%	25%
Urea (mg/dl)	Urease, UV	22	20 (-6.2%) P=0.57	21 (-1.9%) P=0.48	20 (-1.4%) P=0.43	23 (+3.8%) P=0.82	5.5%	9%
Uric acid (mg/dl)	Uricase, UV	5	4.3 (-14%) P=0.57	5.3 (+1.5%) P=0.62	5.5 (+12%) P=0.37	5 (+1.3%) P=0.03	4.8%	17%

+To compare the values both CLIA'88 allowable limits and Analytical Quality Specifications Desirable bias [11, 20] were given.

*To compare the values of sodium, limits % was converted to mmol/L:1.1%(1.5 mmol/L),0.7%(0.99 mmol/L),0.6%(0.85 mmol/L),0.5%(0.71 mmol/ L).

#To compare the values of potassium, limits % was converted to mmol/L : 6.7% (0.25 mmol/ l), 3.1%(0.11mmol/L), 22.1%(0.83mmol/L),43.4%(1.65mmol/L).

^To compare the values of calcium, limits % was converted tommol/L: 2%(0.18mmol/L),1.1%(0.1mmol/L),0.4%(0.03mmol/L),1.3%(0.11mmol/L).

Table-1 summarises the effects of hemolysis on various clinical chemistry parameters. As expected the AST, Potassium values increase linearly with increase in free hemoglobin. Total Bilirubin shows a gradual decrease shown in figure-1.

Of the analytes evaluated, the bias recorded for albumin, alkaline phosphatase, alanine transaminase, amylase, calcium, chloride, HDL-cholesterol, creatinine, glucose, sodium, total protein, triglycerides, urea and uric acid were lower than the CLIA allowable limits even upto 5.5g/L of free haemoglobin, although some differences were statistically significant ($p \leq 0.05$).

Value of inorganic phosphorus increase significantly with increase in haemoglobin concentrations.

DISCUSSION

Interference of hemolysis is due to the release of hemoglobin and other cellular constituents that may falsely increase many of the analyte concentrations because of large differences between intracellular and extracellular concentrations. In this study, the concentrations of AST, potassium, phosphorus showed significant increase as expected from the previous studies [10-13].

Free hemoglobin with its pseudo-peroxidase activity interferes in the bilirubin procedure by inhibiting the diazonium color formation. In our study, we found statistically low values for all groups as in literature [14].

Some of the analytes have a dilutional effect due to discharge of cellular contents showing a false decrease as is the case of glucose, sodium and calcium in this study [19]. In addition to the dilutional effect, the decrease in glucose can also be attributed to the premature decomposition of hydrogen peroxide by hemglobin.

Chemical interference of free hemoglobin occurs in a variety of analytic reactions and methods and analyte concentration dependent spectrophotometric interference [15], due to an increase of the optical absorbance or a change in the blank value, especially for laboratory tests employing measurements at 415, 540 and 570 nm, where hemoglobin absorbs more strongly [16, 17].

The first step to eliminate interference is recognising its existence. Visually, hemolysis is defined as free hemoglobin concentrations above 0.2 g/L [18], which confers a detectable pink to red hue to serum or plasma. But AST concentrations are affected even before hemolysis is visible. Nowadays serum hemolysis index is a popular solution for interference detection preanalytically. Manufacturers give the list of test-specific serum indices for hemolysis, lipemia and bilirubin interferences.

CONCLUSION

The clinical usefulness of laboratory test results depends on accuracy and precision. The presence of endogenous or exogenous substances in body fluids can adversely affect the determination of many analytes in laboratory practice. We conclude that hemolysis affects many of the laboratory parameters most notably AST, potassium, phosphorus and total bilirubin. For other analytes; albumin, ALP, amylase, chloride, HDL-cholesterol, glucose, total protein, triglycerides, and uric acid, differences were statistically significant, but remained within the CLIA limits.

We therefore recommend routine estimation of free hemoglobin for those analytes which have a significant interference.

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