Effect of Haemolysis Interferences on Emergency Biochemistry Parameters in a Tertiary Care Hospital in New Delhi

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ABSTRACT

BACKGROUND

Haemolysis is the most common pre-analytical error encountered in biochemistry laboratories. Several clinical biochemistry laboratories have adopted individual policies regarding rejection of haemolysed samples. The aim of this study was to evaluate the effect of visible haemolysis on biochemistry parameters done in the emergency laboratory.

METHODS

150 blood samples (50 highly haemolysed, 50 slightly haemolysed and 50 no haemolysis) that were received in the Emergency Biochemistry laboratory of Safdarjung Hospital, New Delhi, were included in the current study. They were analysed for different biochemical parameters and the results compared. Statistical analysis was conducted using Microsoft Excel 2016. Data was presented as, number and median. Mann-U Whitney test was used to test any significant difference between the groups. $p < 0.05$ was considered to be significant.

RESULTS

No significant change was seen in samples with slight haemolysis. However, values of potassium, total and direct bilirubin were significantly elevated (p -value-0.001, 0.002 and 0.015 respectively). Moreover, mean ALP was found to be 290 ± 19.76 U/L in slightly haemolysed samples and 211 ± 16.67 U/L in samples that are highly haemolysed ($p=0.049$). Therefore, there was a heterogeneous and unpredictable response to haemolysis observed for several parameters that prevents the adoption of reliable corrective measures for results on the basis of the visible haemolysis.

CONCLUSIONS

The test values of slightly haemolysed samples can be reported as there is little interference seen. However, in case of highly haemolysed samples, values of ALP, potassium, total, and direct bilirubin should not be reported. Nevertheless, visual assessment is not a reliable method to identify haemolysis, free haemoglobin concentrations should be measured.

KEY WORDS

Haemolysis, Interference, Clinical Biochemistry

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BACKGROUND

Hemolysis, defined as disruption of the red cell membrane, resulting in the release of haemoglobin, constitutes for nearly 60 % of rejected samples in clinical laboratories.^{1,2} Both in vivo and in vitro haemolysis constitute the most common pre analytical source of error in biochemistry laboratories.^{3,4} Previously, many studies have studied the effect of haemolysis on several biochemistry analytes.⁵⁻⁷ In order to achieve reduction of variability and a higher reproducibility, several guidelines have been published including for the performance of such studies, for the in vitro simulation of haemolysis and the handling and processing of haemolysed blood specimens.⁸ It is also fundamental to quantify the degree of haemolysis for the adequate management of samples and test results.⁹ The degree of haemolysis in a sample is frequently assessed by measuring the free haemoglobin in serum or plasma.¹⁰

The common causes of haemolysis include faulty drawing techniques, agitation, inappropriate transportation, improper storage etc.^{11,12} In some cases, for instance, during cardiac surgery or cardiac bypass, it might be inevitable to use partly haemolysed serum.¹³⁻¹⁵ Different clinical biochemistry laboratories have adopted individual policies regarding rejection of haemolysed samples. Serum shows visual evidence of haemolysis when the haemoglobin concentration exceeds 200 mg/L.¹⁶⁻¹⁷ In most of the clinical laboratories haemolysed samples are being rejected on pre-analysis stage according to the visual detection of serum interferences, even if the tests that have been requested may not be interfered with haemolysis.¹⁸⁻²⁰ However, according to current literature, visual assessment of sample haemolysis has been shown to have little agreement with the actual concentration of haemoglobin interference.²¹⁻²³

However, even if there is no visible haemolysis, there can still be discharge of the cell constituents into serum or plasma.²⁴⁻²⁵ So invisible haemolysis is an important cause of inaccurate results and should be detected before the analysis.²⁶⁻²⁸ With increasing number of biochemical tests, number of samples and increased automation it becomes important for laboratory staff to give results faster, so pre-analytical determination of haemolysed sample and determination of interfered analytes before analysis becomes very important.²⁹ The amount of haemolysis needed to affect a test is mostly dependent on the test being performed.³⁰ In general, slight haemolysis has little effect on most tests; but, it will cause increased test results for some tests like potassium and lactate dehydrogenase.³¹ In majority of the labs, samples with slight haemolysis are analysed and the results usually reported with a comment indicating the degree of haemolysis and the effect on the test result.³² On the other hand, grossly haemolysed samples could affect the results of many tests and therefore, a recollection is requested for most grossly haemolysed samples.

Thus, it is of paramount importance to know what all parameters are affected by haemolysis and understand the correct interpretation of the same. The current study, therefore, aimed to analyse the effect of visual haemolysis on commonly performed parameters in an emergency biochemistry laboratory in a tertiary care hospital in New Delhi. The results of the study might be helpful to modify current policies regarding sample rejection.

METHODS

This is a retrospective study conducted in the emergency biochemistry laboratory of Safdarjung Hospital, New Delhi. Haemolysis was identified based on visual inspection of samples. Samples with light pink coloured serum are labelled as slightly haemolysed whereas samples with dark pink to red coloured serum are labelled as highly haemolysed. Highly haemolysed samples are rejected except paediatric samples in which bilirubin is reported. Slightly haemolysed are reported with a comment added. Blood samples which were received in the Emergency Biochemistry laboratory of Safdarjung Hospital, New Delhi, between September 2019 and October 2019 and found to be visually haemolysed, (slightly or highly) were included in the study. The study was approved by Ethics committee and informed consent was obtained. A sample size of convenience was taken including 50 samples in each category as described below. (They were all centrifuged at 3000 rpm for 10 minutes). They were categorized based on visual inspection as Group I- No haemolysis (50 Samples), Group II- Slightly haemolysed (red) (50 Samples), and Group III- Highly haemolysed (pink) (50 Samples).

Results were analysed to determine if visible haemolysis had a significant impact on the analyte concentrations. All analytes were measured with Beckman Coulter AU680 analyser using proprietary reagents. Serum concentrations often parameters including sodium, potassium, total bilirubin, direct bilirubin, alanine aminotransferase, (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, amylase, were analysed.

Statistical Analysis

Statistical analysis was conducted using Microsoft Excel 2016 program. Data was presented as, number and mean and standard deviation. T-test was used to test any significant difference between the groups. $p < 0.05$ was considered to be significant.

RESULTS

Analyte	Method	No Haemolysis	Group I (Slightly Haemolysed)	Group II (Highly Haemolysed)	p ₁	p ₂
Total Bilirubin (mg/dl)	DPD	5.2±0.02	7.1±0.08	21.2±3.4	0.832	0.002*
Direct Bilirubin (mg/dl)	DPD	0.88±0.09	2.7±0.01	5.5±1.2	0.623	0.015*
AST(U/L)	IFCC	22±1.23	35±1.1	148±9.9	0.432	0.003*
ALT(U/L)	IFCC	14.3±3.6	21.9±2.5	15.6±0.09	0.875	0.763
ALP(U/L)	IFCC	265±11.45	290±19.76	211±16.67	0.715	0.049*
Amylase(U/L)	CNPG ₃	50±4.7	58.3±7.56	69±5.6	0.075	0.765
Urea(mg/dl)	GLDH	89±9.4	80±2.6	109±18.7	0.654	0.745
Creatinine (mg/dl)	JAFFE'S	3.6±0.06	3.9±0.03	7.6±0.8	0.862	0.812
Sodium (mEq/L)	Ion Selective Electrode	149±15.1	136±12.76	130±5.5	0.395	0.901
Potassium mEq/L)	Ion Selective Electrode	5.5±0.03	6.6±0.04	16.2±3.6	0.900	0.001*

Table 1. Methods, mean ± SD, of the analytes for the 3 groups: No haemolysis, slightly haemolysed and highly haemolysed; p₁: p-value for comparison between no haemolysis and slightly haemolysed; p₂: p-value for comparison between no haemolysis and highly haemolysed; * statistically significant ($p < 0.05$ is significant).

Total of 150 blood samples (50 from each group) received in the Emergency Biochemistry laboratory of Safdarjung Hospital, New Delhi, between September 2019 and October 2019 were included in the study, based on colour of serum on visual inspection. They were analysed for different biochemical parameters and the results compared as shown in Table 1.

DISCUSSION

As per current literature, slight haemolysis has been found to have no significant effect on most test values but it is seen that severe haemolysis increases the concentration of parameters like lactate dehydrogenase, isocitrate dehydrogenase, potassium, magnesium, phosphorus particularly.⁷ AST activity is found to be increased by 2% for each 100 mg of Hb/liter.³³ Also, it is seen that one hundred mg haemoglobin per liter increases serum LDH activity by 10% and serum potassium concentration by 0.6%.³⁴

In this study it was found that no significant difference was found between the values of any of the parameters in samples which were slightly haemolysed as compared to those which had no haemolysis. (Table 1). On the other hand, in samples which were highly haemolysed, concentrations of total bilirubin, direct bilirubin and potassium were significantly increased as compared to samples with no visible haemolysis. Moreover, concentrations of ALP were significantly reduced. No significant change was found in other test values. These findings are similar to results of previous studies.³⁵

Yucel Det al³⁶ have reported that haemolysis had the maximum effect on the concentrations of lactate dehydrogenase, acid phosphatase, and potassium. Perovic A et al³⁷ found that although no interference was detected for calcium, chloride, creatinine, C-reactive protein (CRP), glucose and sodium, clinically significant difference was found for LDH, CK-MB, AST and potassium, total bilirubin. Also, in a study done by Lippi G et al³⁸ overestimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, creatine kinase (CK), iron, lactate dehydrogenase (LDH), lipase, magnesium, phosphorus, potassium and urea were observed. They also found that the mean values of albumin, alkaline phosphatase (ALP), chloride, gamma-glutamyl transferase (GGT), glucose and sodium were decreased. An important observation was variations of AST, chloride, LDH, potassium and sodium in specimens displaying mild or almost undetectable haemolysis by visual inspection.

Koseoglu M et al³⁸ observed that haemolysis interference affected lactate dehydrogenase (LD) and aspartate aminotransferase (AST) almost at undetectable haemolysis by visual inspection (plasma haemoglobin <0.5 g/L). Similar study was done by Du Z et al.³⁷ In which they found that out of the twenty-eight analytes analysed, ten analytes were had clinical significant alterations at different degree of haemolysis.

Thus, there is heterogeneous and unpredictable response to haemolysis observed for several parameters that prevents the adoption of reliable statistic corrective measures for results on the basis of the visible haemolysis.

No significant change is seen in test values in blood samples with slight haemolysis. However, although values of potassium, total and direct bilirubin are significantly elevated, decreased values of ALP are seen, in samples that are highly haemolysed on visual inspection.

CONCLUSIONS

The test values of slightly haemolysed samples can be reported as there is little interference seen. However, in case of highly haemolysed samples, values of ALP, potassium, total, and direct bilirubin should not be reported. Visual assessment is not a reliable method to identify haemolysis, free haemoglobin concentrations should be measured because invisible haemolysis, can also cause interference in test values.

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