



EFFECT OF HEMOLYSIS ON BIOCHEMISTRY PARAMETERS IN EMERGENCY BIOCHEMISTRY LABORATORY IN A TERTIARY CARE HOSPITAL IN NEW DELHI.

Biochemistry

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ABSTRACT

Background : Hemolysis is the most common pre-analytical error encountered in biochemistry laboratories. The aim of the present study was to evaluate the effect of visible hemolysis on biochemistry parameters done in the emergency laboratory.

Materials and methods : 150 blood samples (50 highly hemolyzed, 50 slightly hemolyzed and 50 no hemolysis) received in the Emergency Biochemistry laboratory of Safdarjung Hospital, New Delhi, were included in the study .They were analyzed for different biochemical parameters and the results compared.

Results : No significant change is seen samples with slight hemolysis. However, values of potassium, total and direct bilirubin are significantly elevated and ALP is decreased in samples that are highly hemolyzed.

Conclusion: Test values of slightly hemolyzed samples can be reported as there is little interference seen. However, in case of highly hemolyzed samples, values of ALP, potassium, total and direct bilirubin should not be reported.

KEYWORDS

Hemolysis, interference, clinical biochemistry.

INTRODUCTION :

Hemolysis, defined as disruption of the red cell membrane, resulting in the release of hemoglobin, constitutes for nearly 60 % of rejected samples in clinical laboratories.^{1,2} Both in vivo and in vitro hemolysis constitute the most common preanalytical source of error in biochemistry laboratories.^{3,4} The common causes of hemolysis include faulty drawing techniques, agitation, inappropriate transportation, improper storage etc.⁵ In some cases, for instance, during cardiac surgery or cardiac bypass, it might be inevitable to use partly hemolyzed serum.⁶ Different clinical biochemistry laboratories have adopted individual policies regarding rejection of hemolyzed samples. Serum shows visual evidence of hemolysis when the hemoglobin concentration exceeds 200mg/L.⁷ Thus, it is of paramount importance to know what all parameters are affected by hemolysis. The current study aimed to analyze the effect of visual hemolysis on commonly performed parameters in emergency biochemistry lab. The results of the study might be helpful to modify current policies regarding sample rejection.

MATERIALS AND METHODS:

In the emergency biochemistry laboratory of this institute, hemolysis is identified based on visual inspection of samples. Samples with light pink coloured serum are labeled as slightly hemolyzed whereas samples with dark pink to red coloured serum are labeled as highly hemolyzed. Highly hemolyzed samples are rejected except paediatric samples in which bilirubin is reported. Slightly hemolyzed 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 hemolyzed, (slightly or highly) were included in the study. (They were all centrifuged at 3000 rpm for 10 minutes). They were categorized based on visual inspection as follows:

- Group I- No hemolysis (50 samples)
- Group II- slightly hemolyzed (red) (50 samples)
- Group III- highly hemolyzed (pink) (50 samples)

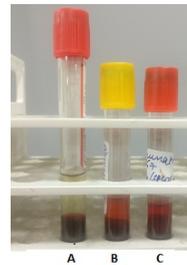


Figure 1 : Picture showing (A) blood sample with No hemolysis (B) Slightly hemolyzed sample (C) Highly hemolyzed sample.

Results were analyzed to determine if visible hemolysis had a significant impact on the analyte concentrations. All analytes were measured with Beckman Coulter AU680 analyzer using proprietary reagents.

Serum concentrations of ten parameters including sodium, potassium, total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, amylase were analyzed.

Statistical analysis was conducted using Microsoft Excel 2016 program. 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:

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 analyzed for different biochemical parameters and the results compared as shown in Table 1.

Analyte	Method	No Hemolysis	Group I (slightly Hemolyzed)	Group II (highly Hemolyzed)	p ₁	p ₂
TOTAL BILIRUBIN(mg/dl)	DPD	5.5	6.3	16.2	0.832	0.002*
DIRECT ILIRUBIN(mg/dl)	DPD	0.9	1.9	3.5	0.623	0.002*
AST(U/L)	IFCC	25	30	145	0.432	0.003*
ALT(U/L)	IFCC	11.3	18.5	12.6	0.875	0.763
ALP(U/L)	IFCC	260	289	206	0.715	0.049*
AMYLASE(U/L)	CNPG ₃	52	55.3	61	0.075	0.765
UREA(mg/dl)	GLDH	95.	85	102	0.654	0.745

CREATININE (mg/dl)	JAFFE'S	3.2	3.5	4.6	0.862	0.812
SODIUM(mEQ/L)	Ion Selective Electrode	144	140	136	0.395	0.901
POTASSIUM mEQ/L)	Ion Selective Electrode	4.7	5.6	12.2	0.900	0.001*

TABLE 1-Table showing methods, median values of the analytes for the 3 groups : no hemolysis,slightly hemolyzed and highly hemolyzed; p_1 , p-value for comparison between no hemolysis and slightly hemolyzed ; p_2 : p-value for comparison between no hemolysis and highly hemolyzed; * statistically significant($p < 0.05$ is significant).

DISCUSSION-

As per current literature, slight hemolysis has been found to have no significant effect on most test values but it is seen that severe hemolysis 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.⁷ Also, it is seen that one hundred mg hemoglobin per liter increases serum LDH activity by 10%and serum potassium concentration by 0.6%.⁷

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 hemolyzed as compared to those which had no hemolysis.(Table 1). On the other hand, in samples which were highly hemolyzed, concentrations of total bilirubin ,direct bilirubin and potassium were significantly increased as compared to samples with no visible hemolysis.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.

Yucel D et al⁸ have reported that hemolysis had the maximum effect on the concentrations of lactate dehydrogenase, acid phosphatase, and potassium. Perovic A et al⁹ 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 al¹⁰ overestimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, creatine kinase (CK), iron, lactate dehydrogenase (LDH), lipase, magnesium, phosphorus, potassium and urea was observed . They also found that the mean values of albumin, alkaline phosphatase (ALP), chloride, gamma-glutamyltransferase (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 hemolysis by visual inspection.

Koseoglu M et al¹¹ observed that hemolysis interference affected lactate dehydrogenase (LD) and aspartate aminotransferase (AST) almost at undetectable hemolysis by visual inspection (plasma hemoglobin < 0.5 g/L). Similar study was done by Du Z et al¹²

in which they found that out of the twenty-eight analytes analyzed, ten analytes were had clinical significant alterations at different degree of hemolysis.

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

CONCLUSION:

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

LIMITATIONS:

Visual assessment is not a reliable method to identify hemolysis, Free hemoglobin concentrations should be measured because invisible hemolysis, can also cause interference in test values. Also, the results need to be confirmed in a bigger sample size including more test parameters.

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