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Effects of Hemolysis on Serum Markers of Liver Function

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ABSTRACT

Introduction

Samples received in the laboratory are often rejected because of visible hemolysis. Every laboratory has protocols on whether to accept or reject hemolyzed samples based on tests ordered. The objective of the study was to observe and analyze the variations seen in association with hemolyzed samples in liver function test parameters and electrolytes measurement.

Methods

This cross-sectional observational study was carried out in 88 laboratory samples in the Clinical Biochemistry Laboratory. Hemolysis was induced by mechanical mixing. This study compared reported values of bilirubin, protein, albumin, aminotransferases (ALT and AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), sodium and potassium between hemolyzed and non-hemolyzed samples.

Results

Mean values of total bilirubin (TB) and direct bilirubin (TB), ALT, ALP, GGT and sodium (Na) were found to be significantly decreased post hemolysis ($p<=0.001$) with mean variances ($\sigma 2$) at 0.30 (TB), 0.38 (DB), 0.56 (ALT), 0.18 (ALP), 0.01 (GGT) and 0.001 (Na). In contrast, mean values of AST (σ^2= -0.9, $p<0.001$) and potassium (σ^2 = -0.3, $p<0.001$) were found to be significantly elevated post hemolysis. Total protein and albumin were also found to be elevated in hemolyzed samples but the average variance was not statistically significant. Degree of hemolysis had highly varying effects in some parameters like AST and total protein, but less varying and more consistent effects on other measured parameters.

Conclusion

Free hemoglobin estimation should be considered before rejecting a clinical sample because of hemolysis. Mathematical equations cannot be expected to reproduce corrected values of the analytes in question because of the large variances, so these tests need to be repeated on non-hemolyzed samples for the analytes that are affected.

Keywords
Hemolysis; interference; routine chemistry analysis

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INTRODUCTION

Hemolysis of collected serum samples remains a significant determinant of pre-analytical errors in laboratory reporting. While multiple factors, including the phlebotomy procedure, tourniquet use, storage and processing protocols can lead to in-vitro hemolysis, the potential pitfalls related to hemolysis and associated results whenever relevant, cannot be overlooked, as the eventual outcome is that patient diagnosis and treatment are affected. This study aimed to evaluate the effect of hemolysis on serum markers of Liver Function.

Hemolysis accounts for over 60% of blood sample rejections[1,2] in the laboratory.[3-5] It has been associated with varying alterations of biochemical parameters including sodium, potassium, chloride, bilirubin, transaminases (Aspartate Aminotransferase, AST and Alanine Aminotransferase, ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total protein and albumin depending upon the degree of hemolysis.[3-7] The general rule is that samples with mild hemolysis are accepted and samples with gross hemolysis are rejected when it comes to assessing liver function parameters. Conventionally, any indication of hemolysis is grounds for sample rejection when it comes to measuring electrolytes.[8,9]

This study focused on samples with in-vitro hemolysis, as it should be noted that only about 2% of hemolyzed samples received in the laboratory are due to in-vivo hemolysis.[8]

This study was conducted to observe and analyze the variations seen in association with hemolyzed samples in liver function test parameters and electrolytes measurement.

METHODS

This cross-sectional observational study was carried out in the Clinical Biochemistry Laboratory of Tribhuvan University Teaching Hospital from October to December 2023. The study was approved by the Institutional Review Committee (IRC) of Institute of Medicine [164(6-11)/E2 070-080]. Informed consent was obtained from all participating patients. No personal information other than gender of consenting patients was collected. Consenting patients who arrived for biochemical investigations were asked to provide three milliliters of blood that was separately stored in plain vials.

Overall, this cross-sectional observational study was conducted on 88 patient samples collected for routine analysis. A part of each sample was subjected to In-vitro hemolysis using mechanical trauma/vigorous shaking in samples collected in plain vials. Based on free hemoglobin concentration, hemolyzed samples were graded into Mild (Hb: 0.25-3 gm%), Moderate (Hb: 3-5 gm%) and Gross (Hb: >5 gm%). Biochemical parameters were assessed using ABBOT architect® chemistry analyzer and electrolytes were assessed using ZOKOH® ISE analyzer.

RESULTS

Free hemoglobin (gm%) estimation in hemolyzed samples showed the average free hemoglobin concentration to be comparable between genders. The categorical changes in parameters following hemolysis are depicted in Table 1. The statistical significances of median variances were evaluated with a Wilcoxon Rank Sum Test, the p-values for which are tabulated. With average z-scores around -3.0, this significance holds true for up to 99.5% of measured samples. Significant changes (p=0.001 or less) were observed in total bilirubin, direct bilirubin, ALT, ALP (decreased) and in AST (elevated). Changes observed in total protein, albumin and GGT were not significant in this regard.

Patient samples were grouped into pairs as Hemolyzed (H) and Non-Hemolyzed (NH) and paired samples t-tests were carried out to observe the significance of changes in values between patient samples in the two groups for individual assayed parameters.

Table 1. Categorical changes in values following hemolysis

Parameters	Decreased	Elevated	No Change	p-value	z-score
Total Bilirubin	20	2	1	0.001	-3.447
Direct Bilirubin	18	1	4	0.001	-3.403
SGPT / ALT	18	1	0	<0.001	-3.645
SGOT / AST	2	17	0	<0.001	-3.582
Total Protein	9	11	0	0.13	-1.512
Albumin	9	11	1	0.9	-0.187
ALP	17	0	1	<0.001	-3.622
GGT	9	1	0	0.007	-2.705
Sodium	12	0	6	0.002	-3.106
Potassium	1	17	0	<0.001	-3.686

Table 2. Comparison of average values of measured parameters across hemolyzed and non-hemolyzed samples (n=88)

Parameter	Average values		Co-relation (r)	p-value
	Hemolyzed samples (n=88)	Non-hemolyzed samples (n=88)		
Total Bilirubin	20.95 ± 17.8	13.73 ± 13.25	0.915	<0.001
Direct Bilirubin	7.26 ± 8.37	5.26 ± 5.83	0.963	<0.001
SGPT	36.36 ± 29.37	17.94 ± 13.66	0.930	<0.001
SGOT	36.36 ± 22.66	64.68 ± 31.04	0.330	0.17
Total Protein	76.2 ± 3.66	100.3 ± 46.02	0.227	0.34
Albumin	43.61 ± 4.48	44.76 ± 11.06	0.375	0.09
Alkaline Phosphatase	82.05 ± 32	59.5 ± 26.83	0.848	<0.001
GGT	101.6 ± 127.57	87.6 ± 117.58	0.998	<0.001
Sodium	139.94 ± 3.15	133.94 ± 12.91	-0.449	0.06
Potassium	4.21 ± 0.49	5.45 ± 1.48	0.260	0.3

Differences in measured values were seen across all parameters that were assayed. A summary of the parameters compared is shown in Table 2.

Changes in measured variables were significant for laboratory parameters like Bilirubin (both total and direct), SGPT (ALT), ALP and GGT. Although average values for potassium in hemolyzed samples were found to be elevated in hemolyzed samples, the statistical significance wasn't established owing to the high variance in potassium levels across samples that were hemolyzed as described in the later section of the results.

Variances between hemolyzed and non-hemolyzed samples were calculated and compared across different laboratory parameters. Negative variances indicate an increase in average levels of the parameter in question in the hemolyzed sample. This is depicted in Figure 1.

The average changes in parameters based on degrees of hemolysis were also compared between parameters that had samples in different grades of hemolysis. There was a significant difference in variances according to the degree of hemolysis and change in measured parameters for Serum Total Bilirubin, Total Protein and Albumin.

Average values of serum protein and albumin were found to be significantly elevated in patient samples that were moderately or grossly hemolyzed.

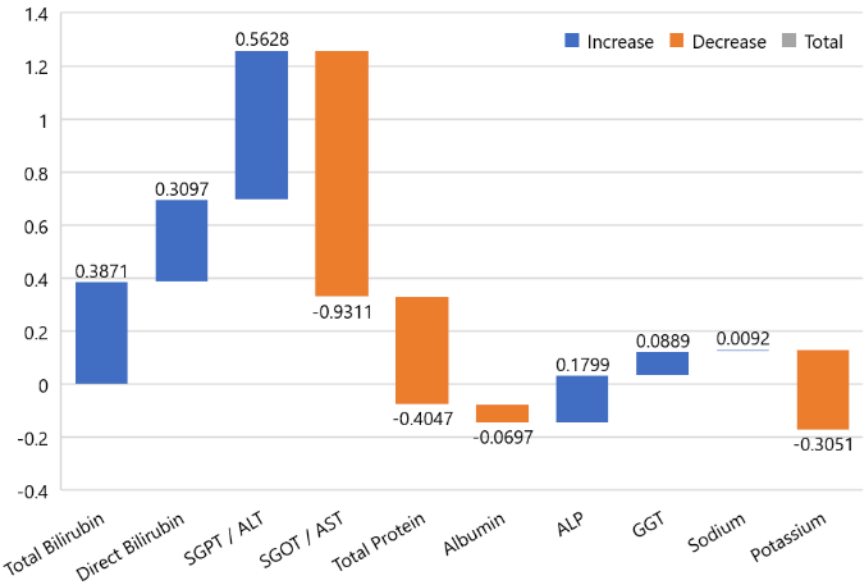

Figure 1. Variances across different parameters between hemolyzed and non-hemolyzed samples

Table 3. Mean changes in variances compared across degrees of hemolysis

Parameters	Mean changes in variances			P-value
	Mild Hemolysis (n=62)	Moderate Hemolysis (n=10)	Gross Hemolysis (n=16)	
Total Bilirubin	-0.33 ± 0.25	-0.61 ± 1.48	-0.7 ± 0.07	0.006
Direct Bilirubin	-0.2 ± 0.22	-0.33 ± 0.47	-0.47 ± 0.13	0.2
Total Protein	0.03 ± 0.21	1.34 ± 0.02	1.46 ± 0.02	<0.001
Albumin	-0.06 ± 0.15	0.46 ± 0.21	0.32 ± 0.04	<0.001

DISCUSSION

Many laboratories have a general rule that mandates rejection of grossly hemolyzed samples that are sent to the clinical chemistry laboratory. We induced hemolysis on our study samples and compared the results in the hemolyzed samples to the values obtained from the same samples when not hemolyzed. Most of our findings are in agreement with conventional understanding of what changes hemolysis makes to laboratory analytes. Individual parameters had different levels of changes, some significant enough to make a difference in clinical decision making, while some not as much.

Gender, generally has no direct influence on in-vitro hemolysis but some studies have shown some gender wise difference in in-vivo hemolysis for susceptible patients with underlying diseases.[10]

Serum Potassium has long been established as a marker extensively affected by hemolysis, owing to its high intracellular concentration. Although serum potassium levels have been utilized in post mortem examinations to evaluate time of death, hemolysis can have significant implications on this as well.[11] Our study samples showed an overall increase in potassium levels in hemolyzed samples, as expected. Serum sodium levels are also expected to change, but the difference seen in our study in hemolyzed samples was not statistically significant. Other studies generally report that the changes contributed for by the intracellular sodium in general is very little given the lower concentration inside cells.[3,6] Overall, as the reproducibility of hemolysate production is relative, the degree of hemolysis in a sample, rather than the concentration of the analyte in question, Sodium or Potassium actually affects the results.[12,13]

Total Protein and Albumin values on an average were increased post hemolysis. This is partly justified by the fact that the intracellular protein components add to the estimated serum protein values after the cells are lysed. The difference was not significant enough to make a difference in mildly hemolyzed samples, but in moderately or grossly hemolyzed samples, the difference observed was significant enough to make a big difference. Most studies have found that protein levels are significantly altered post hemolysis,[1,3,8] and some have even recommended not measuring total protein at all at the slightest visual indication of hemolysis in a laboratory sample.[1] Based on our results, it is inferred that in moderately hemolyzed samples or worse, estimating total protein and albumin can be grossly erroneous.

The variances in alanine aminotransferase (ALT) and Gamma glutamyl transpeptidase (GGT) levels in our study was not very large, but the changes observed in average values post hemolysis was significant. The average values were underreported for these enzymes in the hemolyzed sample. In contrast, aspartate aminotransferase (AST) values were over-reported in hemolyzed samples. As the variances seen in AST values between samples was very high, the average difference was not found to be significant in independent samples. Regardless, AST values are found to be significantly affected by hemolysis in other studies too, and are excluded from analysis in hemolyzed samples.[2-5]

We found a significant decrease in ALP activity in hemolyzed samples with little variance between samples. The activity of ALP is decreased for a number of possible reasons, the most significant being the hemolysate affecting the buffer activity. The decrease in ALP is expected to be different based on whether tris-carbonate, diethanolamine or aminomethylpropanol are used as buffers.[14]

Hemolysis also caused a significant decrease in reported values of Bilirubin in our study, with minimal variance between samples. The extent of interference was more significant in terms of Total Bilirubin, even when degree of hemolysis was taken into account. So, the expected interference is a determinant of both Direct Bilirubin concentration in the sample as well as the degree of hemolysis, as reported in the past.[15,16]

Overall, the effect of hemolysis was highly varied on some parameters that we evaluated, while consistent in others. Practically, none of the parameters that we evaluated were found to be

totally unaffected, it is just a matter of the degree of hemolysis and the extent of the interference seen. This has several additional implications, like in prenatal testing, where samples with free hemoglobin (>4 gm%) were found to interfere significantly with test results.[17]

CONCLUSION

Mildly hemolysed samples do not affect parameters of liver function evaluation as much as moderate or grossly hemolzyed samples. Rather than rely on visual validation of hemolysis, free hemoglobin estimation should be considered before rejecting a clinical sample. As the degree of variance seen so as to the changes seen after hemolysis is large, mathematical equations cannot be expected to reproduce corrected values of the analytes in question.

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CONFLICT OF INTEREST

The author(s) declare that they do not have any conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

AB was primarily involved in Research Concept and design, RT was involved with laboratory wet work and obtaining ethical approval. AN and ET obtained background research, and all authors were involved in laboratory work supervision. MR and RD handled data analysis. Manuscript preparation was done by AB and VS. All authors read and approved the final manuscript.

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