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The hemolysis index as a tool for monitoring mild hemolysis in biochemical assays at the emergency laboratory

Sofiane Bouazza,[1,2] Amine Bouarfa,[3] Ali Khalfa,[4,5] Slimane Brikhou,[1,6] Abbassia Demmouche[1,2]

[1]Department of Biology, Faculty of Natural and Life Sciences, University of Djillali Liabès, Sidi Bel-Abbès; [2]Laboratory of Biotoxicology, University of Djillali Liabès, Sidi Bel-Abbès; [3]Laboratory of Medical Analysis, Specialised Hospital of Gynaecology and Obstetrics, Sidi Bel-Abbès; [4]Department of Agri-Food, Faculty of Science and Technology, University of Ain Temouchent, Ain Temouchent, Algeria; [5]Laboratory of Clinical and Metabolic Nutrition, Oran; [6]Laboratory of Applied Molecular Biology and Immunology, (Biomolim), Tlemcen, Algeria

Correspondence: Ali Khalfa, Department of Agri-Food, Faculty of Science and Technology, University of Ain Temouchent, 46000, Algeria. Laboratory of Clinical and Metabolic Nutrition, Oran, Algeria.

Tel.: +213.553058438.

E-mail: ali.khalfa@univ-temouchent.edu.dz

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Abstract

Hemolysis is a common pre-analytical error that can affect the accuracy of biochemical assay results. The aim of the study is to investigate the impact of hemolysis on the measurement of nine emergency biochemical parameters: glucose, creatinine, calcium, potassium, sodium, Aspartate

Aminotransferase (AST), Alanine Aminotransferase (ALT), total bilirubin, and direct bilirubin. The study included blood samples collected from various units of the maternity hospital, including high-risk pregnancies follow-up, postpartum care, gynecology, neonatology, and intensive care. The study employs a Hemolysis Index (HI) to monitor and assess sample quality, determining their suitability for the analysis of the nine aforementioned biochemical parameters. The results reveal that hemolysis can significantly affect the accuracy of tests results for the studied parameters, particularly for potassium (increased 4-12 times) and AST (increased 1.47-48.55 times). Three of the studied substances showed a significant, negative, and moderate correlation with HI after the osmotic shock was induced, including AST ($r = -0.501$, $p = 0.006$), ALT ($r = -0.516$, $p = 0.004$), and sodium ($r = -0.598$, $p = 0.001$). Conversely, no significant association was found for the other parameters, which are: glucose ($r = 0.079$, $p = 0.639$); creatinine ($r = 0.140$, $p = 0.402$); direct bilirubin ($r = 0.292$, $p = 0.075$); total bilirubin ($r = 0.272$, $p = 0.114$); calcium ($r = 0.215$, $p = 0.196$); and potassium ($r = -0.188$, $p = 0.258$). Our findings indicate that HI may not be helpful for calculating a predicted value for samples with HI ranging between 87.30 and 295.9. Therefore, it is crucial to establish a threshold for the degree of hemolysis beyond which releasing the result would be considered potentially harmful to the patient especially new-borns and premature infants. In conclusion, the appropriate use of HI in clinical laboratories can enhance patient care quality by minimizing the risk of misdiagnosis leading to inappropriate treatment, particularly when requesting a second sample is not feasible.

Introduction

Hemolysis is defined as the expulsion of red blood cells' cellular contents through membrane rupture. It is considered "troublesome" in the field of medical analysis. Some authors use the term

"analytical error",[1] due to hemolysis, and others use the term "analytical interference".[2,3] First of all, it seems more relevant to distinguish between these two terms: an analytical error is a concept linked to a result skewed by different factors affecting a given analyte's assay technique; analytical interference is, as described in the article on technique validation, is “a perturbation: modification of the measured signal, relative to a determined concentration, following the presence of a body accompanying the analyte in the medium subjected to the analysis, modifying the accuracy of the results.”[4] It follows that interference is one of the causes of errors due to a spectral disturbance.

Hemolysis frequently causes errors in laboratory testing. The rate of hemolyzed samples received in clinical laboratories is around 10%;[3] about 60% of these samples, are banned from analysis because they are a frequent reason for rejection on rough visual observation.[2] Even stronger: some clinical laboratories even systematically do not accept-hemolyzed samples.[5] Hemolysis is visually recognised at 0.1 g/L (1.55 µmol/L) for some professionals.[6] or at 0.2 g/L (3.1 µmol/L) for others[7]. But even though it remains invisible, it also constitutes a possible source of erroneous results[2] that can compromise the clinical diagnosis as well as the follow-up of the patient.

There are two types of hemolysis: *in vivo* and *in vitro*, the first being much rarer than the second (3.2% of all hemolyzed samples).[2] The causes of *in vitro* hemolysis are almost entirely related to the preanalytical conditions.[5] These include contact with foreign surfaces, sampling procedures, transport conditions, and improper centrifugation or separation of samples.[7] Regarding *in vivo* intra-vascular hemolysis, there are three common etiologies: hemolytic anemia, cardiovascular surgery, patients under circulatory assistance.

Hemolysis Index (HI) is a reliable predictor of hemolysis.[8] This index is commonly used to assess the presence of hemolysis in serum samples. Different wavelengths have been investigated for measuring HI, with findings indicating that the optimal wavelength is precisely 571 nm.[9] The use of a reference chart aids laboratory personnel in determining if a hemolyzed sample is acceptable for testing and provides guidance on result interpretation.[10] The spectrophotometric assessment of cell-

free hemoglobin (fHb) concentration, correlated with HI, serves as a reliable marker of red blood cell damage and can be used to screen patients suspected of having intravascular hemolysis.[11] The purpose of this work is to clarify the influence of hemolysis on the measurement of nine biochemical parameters involving glucose, creatinine, calcium, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), total bilirubin, and direct bilirubin which are assayed by spectrophotometry and potassium, sodium, assayed by selective electrode and to validate or invalidate the results of these measurements. This is crucial because the prognosis of a patient often depends on the reliability of the values provided by the laboratory to the clinician, especially in emergency structures.

Materials and Methods

Experimental design

Blood samples were collected from the maternity hospital in Sidi Bel Abbès, Algeria. Fifty samples were collected from various units of the maternity hospital, including high-risk pregnancy follow-up, postpartum care, gynecology, neonatology, and intensive care. The contents of each sample were poured into two separate lithium heparin tubes. One of these tubes was subjected to induced hemolysis. Osmotic shock was triggered by adding an equal volume of injectable water to the blood.[12] This causes an increase in the volume of erythrocytes followed by their rupture. All tubes (normal and hemolyzed) were centrifuged at 3500 rpm for 10 minutes. To avoid any potential bias due to hemolysis, only samples with normal serum bilirubin levels were selected and retained. Nine serum biochemical parameters were evaluated before and after hemolysis (Figure 1).

Biochemical parameters assessment

Sodium and potassium levels were determined using an EasyLyte Na/K analyser (Medica Corporation, Bedford, MA, USA). All other serum biochemical parameters were measured before and after hemolysis using an XL-300 biochemistry analyser (Erba Diagnosis, Mannheim, Germany) with its reagents and multi-calibrator. Dosing methods are described as follow: Glucose (Glucose oxydase - Glucose peroxydase (GOD-POD)), creatinine (Jaffe's reaction), calcium (Arsenazo III, complexon), AST and ALT (International Federation of Clinical Chemistry, without pyridoxal phosphate), total and conjugated (direct) bilirubin (Cetrimonium bromide, accelerator).

HI determination

HI level was determined using two points of photometric measurements according to the following formula:

$$HI = \frac{1}{\frac{A}{10}}\left((\gamma)\left(\frac{B}{10^5}\right)(\alpha-\beta)\right)\begin{cases}\alpha = \lambda 600\\ \beta = \lambda 700\\ \gamma = \lambda 570\\ A = 1271\\ B = 12100\end{cases} \quad (1)$$

Where:

α, β and γ represent the absorbance values of each wavelength that are obtained from measurements of samples and phosphoric acid buffer and corrected by water blank, A and B are constants.

Statistical analysis

Scatter plots and box plots were created using Microsoft Office Excel 365. The data were analysed using IBM SPSS version 28 and expressed as mean and standard deviation, the mean difference is presented at 95% confidence interval. The comparison between serum biochemical parameter levels before and after hemolysis was done using a paired Student test and their association with the Pearson correlation test. The association between serum biochemical parameter levels and HI after hemolysis was tested using Pearson correlation test. All p-values less than 0.05 are considered as statistically significant.

Results

HI

HI was slightly and significantly different before and after hemolysis ($p = 0.021$).

Glucose

The comparison between glucose levels before and after hemolysis was significant ($p < 0.001$) with a mean difference of -75.6 mg/dL (Table 1). In addition, serum glucose levels were negatively, strongly, and significantly correlated before and after hemolysis ($r = 0.844$, $p < 0.001$). Moreover, a positive, weak, and non-significant correlation ($r = 0.079$, $p = 0.639$) was recorded between serum glucose levels and HI after hemolysis (Figure 2A).

Creatinine

The measurement of serum creatinine level after hemolysis revealed a significant increase ($p < 0.001$), the mean difference was about -0.5 mg/dL (Table 1). In fact, serum creatinine level was significantly proportional before and after hemolysis ($r = 0.638$, $p < 0.001$). Conversely, a positive, weak and non-significant correlation between serum glucose levels and HI after hemolysis was indicated ($r = 0.140$, $p = 0.402$) (Figure 2B).

Aspartate aminotransferase (AST)

AST assessment before and after hemolysis was markedly different ($p < 0.001$), the mean difference reached -279.6 UI/L (Table 1). Furthermore, a negative weak and non-significant correlation between serum AST levels before and after hemolysis was shown. Besides, a negative moderate, and significant correlation ($r = -0.501$, $p = 0.006$) between AST levels and HI after hemolysis was found (Figure 3A).

Alanine aminotransferase (ALT)

A significant rise in ALT after hemolysis was marked ($p < 0.001$) with a mean difference of -50.2 UI/L (Table 1). Also, a positive, weak and significant correlation between serum ALT levels before and after hemolysis was observed ($r = -0.171$; $p = 0.351$). Moreover, a negative, moderate and significant correlation ($r = -0.516$, $p = 0.004$) between serum ALT levels and HI after hemolysis was marked (Figure 3B).

Direct bilirubin

Higher levels of direct bilirubin were obtained after hemolysis ($p < 0.001$); the mean difference was about -1.0 mg/dL (Table 1). What's more, direct bilirubin levels were significantly and moderately proportional before and after hemolysis ($r = 0.297$; $p = 0.038$). Equally, direct bilirubin concentrations increase moderately and non-significantly ($r = 0.292$, $p = 0.075$) with HI after hemolysis (Figure 4A).

Total bilirubin

Serum total bilirubin values slightly decreased after hemolysis ($p < 0.001$), with a mean difference in the range of 0.5 mg/dL (Table 1). Additionally, total bilirubin values were significantly and moderately proportional before and after hemolysis. Similarly, total bilirubin concentrations increase moderately but not significantly ($r = 0.272$, $p = 0.114$) with HI after hemolysis (Figure 4B).

Calcium

After hemolysis, a considerable change in serum calcium concentrations was found ($p < 0.001$); the mean difference was about 48.9 mg/L (Table 1). This reduction was positive and significant before and after hemolysis. On the other side, calcemia was weakly and non-significantly associated ($r = 0.215$, $p = 0.196$) with HI after hemolysis (Figure 5A).

Sodium

The ionogram revealed a significant decrease in sodium after hemolysis ($p < 0.001$); the mean

difference was around 35.8 mmol/L (Table 1). A positive moderate and significant correlation was shown before and after hemolysis. Serum sodium concentrations were negatively, moderately, and significantly correlated (r = -0.598, p = 0.001) with HI after hemolysis (Figure 5B).

Potassium

The kalemia increase was noticeable after hemolysis (p < 0.001); the mean difference was -37.0 mmol/L (Table 1). What's more, the kalemia levels were not significantly associated (r = 0.069, p = 0.628). Equally, no association (r = -0.188, p = 0.258) was found between kalemia and HI after hemolysis (Figure 5C).

Discussion

The medical laboratory is confronted daily with the influence of hemolysis on clinical chemistry tests. The technical staff involved in the sampling must be attentive to blood quality in order to minimise the number of unsuitable samples.[13,14] There is no yet consensus on the link between analytical error and the value of fHb for a given analyte

In vitro hemolysis interference is a disturbance that causes inaccurate results. It may be due to the release of constituents of the erythrocyte cytoplasm (including hemoglobin) into plasma or serum. On the other hand, the intervention of hemoglobin as pseudo-peroxidase, in the chemical reaction involving the generation of reactive oxygen species can also participate.[15] Another cause is spectral interference.[14]

There are three phenomena caused by hemolysis that affect the measured analytes. The first case is the absence of interference with the parameters regardless of their values (normal, high, or low), such as calcium and urea, for a hemoglobin concentration up to 1000 µmol/L (16.1 g/L) or even

2000 µmol/L (32.2 g/L). The second phenomenon is the elevation of the analyte, depending on HI. The concentrations of certain parameters in erythrocytes are significantly higher than their concentrations in plasma. (Example: HI is ascending and proportional to the increase in AST concentration). The third phenomenon is physical or chemical interference compared to the plasma value of the analyte. Overall, a noticeable influence of hemolysis is observed for normal values but negligible for pathological values. An example of this interference is the overestimation of creatinine for normal or low values; this increase never reaches double the true value. Multiplying AST by 10 or 20 does not affect ALT in the same way, which can only double or triple (ALT is less elevated in erythrocytes than AST).[16-22]

The present study reveals significant alterations in several biochemical parameters following hemolysis, highlighting the impact of this phenomenon on serum levels. Notably, we observed a marked increase in AST (-279.6 ± 247.2 UI/L), potassium (-37.0 ± 8.5 mmol/L), glucose (-75.6 ± 36.2 mg/dL), creatinine (-0.47 ± 0.32 mg/dL), and ALT (-50.2 ± 55.2 UI/L), while a decrease in total bilirubin (0.5 ± 0.5 mg/dL), calcium (48.9 ± 15.4 mg/L) and sodium (35.8 ± 11.6 mmol/L) was observed. This pattern of changes highlights the complex interplay between hemolysis and serum biochemistry.

These findings align with previous studies examining the impact of hemolysis on various biochemical markers. Ercan[23] reported an increase in AST between 51.61-97.85%, ALT between 6.78-10.24%, potassium between 13.27-23.63%, creatinine between 2.5-3.57%, and a decrease in total bilirubin between 7.41-1.85%, calcium between 0.52-0.83%, and sodium between 0.14-0.79%. Koseoglu *et al.*[2] observed similar trends, with increases in ALT between 4.8-20%, AST between 60-163%, potassium between 10.7-35.8%, and decreases in creatinine between 0-2.3%, glucose between 1.2-8.3%, calcium, and sodium between 0-1.4%. While our study shows a decrease in total bilirubin, Ercan[23] reported a decrease between 7.41-1.85% and Koseoglu *et al.*[2] observed a decrease

between 32.7-100%. Additionally, our study shows a decrease in direct bilirubin (-1.0 ± 0.6 mg/dL) whereas Ercan[23] reported direct bilirubin to be 180% or indeterminate.

To address the issues related to the influence of hemolysis, it is necessary to rigorously recognise and report any manipulation that may lead to *in vitro* hemolysis. There are three possible directives for hemolysis. The first is the measurement of fHb.[17] The second is to notify the clinician.[14] The third is to request a new sample if the first two guidelines fail.

Hemolysis can affect the accuracy of blood glucose measurements.[18] It is therefore important to consider the presence of hemolysis when interpreting blood glucose results, as it can lead to a decrease in blood glucose. This decrease can be explained by two hypotheses: premature decomposition of hydrogen peroxide (a chemical component in the GOD-POD method for blood glucose measurement), or a dilution effect caused by the release of erythrocyte fluid content.[19]

Studies have shown that hemolysis can interfere with the measurement of creatinine in clinical laboratory tests. In one study, samples with significant hemolysis were not accepted for creatinine analysis.[10] Another study found that the log (RBC-Hb/RET-Hb) ratio, which is a measure of red blood cell survival and hemolysis, is significantly correlated with an overestimation of serum creatinine.[20] Furthermore, a study comparing various methods of blood sample transport found that samples transported by a pneumatic tube system had a greater occurrence of hemolysis and higher median creatinine concentrations.[21] According to Poupon *et al*.,[22] the effect of hemolysis on creatinine varies depending on the two common analytical techniques available on the market (Jaffe's method at 510 nm, with an increase limited to twice the original value) or (PAP method at 340 nm, with no influence). These results indicate that HI can affect blood creatinine assessment if the Jaffe's method is used, further highlighting the importance of considering hemolysis when interpreting creatinine results in clinical practice.

Studies have demonstrated that AST levels are particularly elevated in the presence of hemolysis. In sickle cell disease, AST is released via intravascular hemolysis, leading to higher AST levels

compared to ALT.[23] Furthermore, another study found that HI can provide information on analytes affected by hemolysis, and that AST was one of the analytes influenced by hemolysis.[24] In patients with chronic hepatitis C virus (HCV) infection, liver damage is less severe in those with normal serum ALT levels compared to those with elevated ALT levels.[25,26] However, some patients with normal ALT levels may still have advanced liver disease.[27] These findings suggest that ALT levels can be influenced by the hemolytic status and can reflect the severity of liver disease in HCV infection. Therefore, the presence of hemolysis can impact AST levels, and the AST:ALT ratio can be used as a marker for hemolysis.

The concentration of total bilirubin in hemolyzed samples decreases proportionally to the degree of hemolysis.[28] It is absolutely clear that the cause of the interference of hemolysis is not due to physiologic conversion of plasma/serum heme groups to bilirubin *in vitro*.[29] The likely cause is the pseudo-peroxidase activity of hemoglobin, which interferes with the formation of the diazonium molecule (a product of the Pauly reaction, essential for the color development needed for measurement).[15] This leads to an underestimation of total bilirubin.[30]

There is a relationship between electrolytes and the serum hemolysis index. Studies have shown that hemolysis can affect the measurement of electrolyte concentrations in blood samples.[31] Delgado *et al*. found that sodium-selective electrodes showed a decrease in results for this cation with increasing hemolysis.[31]

According to Wang and Felsher, there was no difference in the phosphorus content of serum and plasma, whether or not the blood is hemolyzed.[32] However, the calcium content of non-hemolyzed serum was slightly higher than that of plasma.[32]

Hemolysis can affect various parameters, but there are mechanisms by which certain parameters are not affected or only slightly affected, such as albumin, uric acid, calcium, C-reactive protein, myoglobin, NT-pro BNP, S100 protein, and urea.[16] Furthermore, chloride and total protein are also not significantly altered, even in heavily hemolyzed samples.[33]

The systematic implementation of serum indices, including the hemolysis index, in clinical laboratories equipped with continuous flow analyzers can be beneficial for identifying hemolyzed samples and ensuring reliable test results.[34] Overall, the evidence suggests that the serum hemolysis index is a reliable predictor of hemolysis and can be used to monitor and assess sample quality. Possible causes of pre-analytical hemolysis include: the use of intravenous catheters and vacuum sampling techniques, inappropriate puncture sites, complicated blood sampling, prolonged tourniquet application, insufficient filling of tubes, and excessive agitation of samples.[35] Hemolysis can also occur during centrifugation if it is too long or repeated.[12] Samples transported via Pneumatic Tube Systems (PTS) are more prone to hemolysis than samples transported by hand. Speed, distance, and impact forces during transport can affect the extent of hemolysis.[10]

The limitations of HI include the difficulty of performing rigorous analysis to evaluate interference in routine laboratories.[12] The use of HI alone for monitoring phlebotomy performance may not be straightforward, as specimens drawn from patients with intravascular hemolysis can be identified and excluded from the analysis.[10] The routine use of the hemiglobincyanide method, the current reference method for measuring free hemoglobin (fHb), is unsuitable for a timely diagnosis of intravascular hemolysis.[36] The wavelength used for HI measurement varies in each laboratory, leading to a lack of standardization.[37] Additionally, HI may not be able to accurately detect hemolysis at low levels, as visually undetectable hemolyzed samples can be identified by other methods.[8]

Table 1 could be used as a guide for clinicians and laboratory staff when resampling is not possible for the nine studied biochemical parameters in hemolyzed samples. Specifically, it provides the appropriate comment to use when dealing with samples exhibiting hemolysis indicated by an HI ranging from 87.3 to 295.9. This table helps ensure consistent and informative documentation when encountering these situations, ultimately aiding in accurate interpretation of results and patient care.

Several strategies can be adopted to mitigate the impact of hemolysis on laboratory test results, optimising their accuracy for medical decision-making. These strategies include: proper blood collection and handling; appropriate storage and transportation, and the use of HI, especially automated statistical methods that correct results for a certain degree of hemolysis (although it is generally advisable to repeat the test with a fresh sample when possible);[2] Other strategies include: communication with clinicians regarding critical relationships between pre-analytical errors like hemolysis and test results and alerting them for hemolyzed,[5] and continuing education and training of phlebotomists and laboratory staff. Finally, establishing an acceptable threshold of hemolysis for various tests based on the level of interference can be helpful.[4]

Conclusions

Hemolysis can significantly alter the accuracy of biochemical assay results, particularly for certain parameters such as AST and potassium. HI is a useful tool for monitoring and assessing sample quality and can be used in clinical laboratories specially in those equipped with continuous-flow automation.

The establishment of an acceptable threshold of hemolysis for various tests based on the level of interference can be advantageous in interpreting the results, especially for moderate hemolysis. Rigorous reporting of any manipulation that may cause *in vitro* hemolysis is essential to reduce the impact of hemolysis on assay outcomes. However, in cases of acceptable hemolysis, the results could be released with the comment: "results subject to confirmation".

It is essential that further research is needed to examine the impact of hemolysis on other biochemical parameters and assay with multiple materials. The advancement of more robust analytical techniques that are resistant to interferences from hemolysis can enhance the accuracy and reliability of biochemical assay results. The use of serum indices, including HI, in clinical

laboratories can improve the quality of patient care and reduce the risk of misdiagnosis or improper treatment.

References

1. Pontet F. Hémolyse et protéines sériques (Hemolysis and serum proteins). Ann Biol Clin (Paris) 2000;58:637-8.
2. Koseoglu M, Hur A, Atay A, Cuhadar S. Effects of hemolysis interferences on routine biochemistry parameters. Biochem. Medica 2011;21:79–85.
3. Sapin R. Interférence de l'hémolyse et de l'hémoglobine : exemple du dosage de l'insuline (Interference of hemolysis and hemoglobin: example of insulin assessment). Ann Biol Clin (Paris) 2003;59:113-4.
4. Benlakehal MT, Le Bricon JP, Feugeas BB. Influence de l'hémolyse sur le dosage et l'électrophorèse des protéines sériques (Influence of hemolysis on serum protein measurement and electrophoresis). Ann Biol Clin (Paris) 2000;58:367-71.
5. Lippi G, Blanckaert N, Bonini P, et al. Hemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med 2008;46:764-72.
6. Dhondt JL, Hayte JM, Demouveaux A. Les prélèvements « hémolysés » ("Hemolyzed" samples). Ann Biol Clin (Paris) 2000;58:639.
7. Yücel D, Dalva K. Effect of *in vitro* hemolysis on 25 common biochemical tests. Clin Chem 1992;38:575-7.
8. Ishiguro A, Nishioka M, Morishige A, et al. What is the best wavelength for the measurement of hemolysis index? Clin Chim Acta 2020;510:15-20.
9. Karabulut B, Arcagok BC. A neglected and promising predictor of severe hyperbilirubinemia due to hemolysis: carboxyhemoglobin. Fetal Pediatr Pathol 2020;39:124-31.
10. Hyder MA, Hasan M, Moheildien AM. Visual HI for routine clinical chemistry laboratory parameters. Int J Clin Biochem Res 2018;5:217-21.
11. Atakan EF, Aykal G, Yalçınkaya HM, et al. The effect of automated hemolysis index measurement on sample and test rejection rates. Turk J Biochem 2019;44:630-4.
12. Meites S. Reproducibly simulating hemolysis, for evaluating its interference with chemical methods. Clin Chem 1973;19:1319.

13. Ünlü B, Küme T, Emek M, et al. Effect of blood cell subtypes lysis on routine biochemical tests. J Med Biochem 2018;37:67-77.
14. Ali D, Sacchetto É, Dumontet E, et al. Interférence de l'hémolyse sur le dosage de vingt-deux paramètres biochimiques (Hemolysis influence on twenty-two biochemical parameters measurement). Ann Biol Clin (Paris) 2014;72:297-311.
15. Shuangshuang H, Xiaofeng L, Dan H, et al. Ferrous hemoglobin and hemoglobin-based oxygen carriers acting as a peroxidase can inhibit oxidative damage to endothelial cells caused by hydrogen peroxide. Artificial Organs 2021;45:1229-39.
16. Lippi G, Salvagno GL, Blanckaert N, et al. Multicenter evaluation of HI in automated clinical chemistry systems. Clin Chem Lab Med 2009;47:934-9.
17. Goyal T, Schmotzer CL. Validation of HI thresholds optimizes detection of clinically significant hemolysis. Am J Clin Pathol 2015;143:579-83.
18. Verfaillie CJ, Delanghe JR. Hemolysis correction factor in the measurement of serum neuron-specific enolase. Clin Chem Lab Med 2010;48:891-2.
19. Dupuy AM, Bargnoux AS, Kuster N, et al. Determination of hemolysis cut-offs for biochemical and immunochemical analytes according to their value. Clin Chem Lab Med (CCLM) 2020;28:1232-41.
20. Maier-Redelsperger M, Lévy P, Lionnet F, et al. Strong association between a new marker of hemolysis and glomerulopathy in sickle cell anemia. Blood Cells Mol Dis 2010;15;45:289-92.
21. Şaşmaz Mİ, Ayvaz MA, Dülger AC, et al. Aspartate-aminotransferase to platelet ratio index score for predicting HELLP syndrome. Am J Emerg Med 2020;38:459-62.
22. Poupon C, Lefèvre G, Ngo-François S, et al. Hemolysis interferences on frequently required stat analysis: a French multicentric study. Ann Biol Clin 2015;73:705-16.
23. Ercan, Ş. The evaluation of hemolysis index thresholds for significant hemolysis interference on routine biochemistry analytes. Turkish J Biochem 2016;41:223-31.
24. Dor-Mohammadi T, Daryani NE, Bashashati M, et al. Relationship between serum alanine aminotransferase levels and liver histology in chronic hepatitis C-infected patients. Indian J Gastroenterol 2005;24:49-51.
25. Ebrahimi Daryani N, Azmoudeh Ardalan F, Dormohammadi T, et al. The relationship between serum alanine aminotransferase level and liver biopsy findings in chronic hepatitis C. Govaresh J 2004;9:232-36.
26. Ding Z, Zhang J, Deng CY, et al. Association of body mass index with serum alanine aminotransferase in Chinese adolescents: a school-based cross-sectional study. Gastroenterol Rep 2020;8:119-24.

27. Jalali-Nadoushan M, Vaez Mahdavi MR, Soroush MR, et al. Relationship between serum bilirubin concentration and inflammatory cytokines in victims exposed to sulfur mustard. Trauma Mon 2017;22:e64931.
28. Greene DN, Holmes DT, Lin MJ, et al. Development of an equation to correct for hemolysis in direct bilirubin measurements. Clin Chim Acta 2014;429:194-7.
29. McDonagh AF, Lo SF, Maisels MJ, Watchko JF. Serum bilirubin measurements in hemolyzed samples. Clin Chim Acta 2011;412:1275.
30. Azhar M, Galgalkar S, Chakraborty I, et al. Hemolysis detection in sub-microliter volumes of blood plasma. IEEE Trans Biomed Eng 2020;67:1243-52.
31. Delgado JA, Morell-Garcia D, Bauça JM. Hemolysis interference studies: the particular case of sodium Ion. EJIFCC 2019;30:25–34.
32. Wang CC, Felsher AR. The effect of hemolysis on the calcium and inorganic phosphorus content of serum and plasma. J Lab Clin Med 1925;10:269-72.
33. Lippi G, Salvagno GL, Montagnana M, et al. Influence of hemolysis on routine clinical chemistry testing. Clin Chem Lab Med (CCLM) 2006;44.3:311-16.
34. Heireman L, Van Geel P, Musger L, et al. Causes, consequences and management of sample hemolysis in the clinical laboratory. Clin Biochem 2017;50:1317-22.
35. Pasqualetti S, Szőke D, Panteghini M. Heparinate but not serum tubes are susceptible to hemolysis by pneumatic tube transportation. Clin Chem Lab Med (CCLM) 2016;54:785-9.
36. Lippi G, Favaloro EJ, Franchini M. HI for the screening of intravascular hemolysis: a novel diagnostic opportunity? Blood Transfus 2018;16:433-7.
37. Douhal Y, Benito Zamorano V, Rivas Chacón LDM, et al. Study of interference produced by hemolysis in 73 analytical tests. Biomed. J Sci Tech Res 2020;24:18362-7.

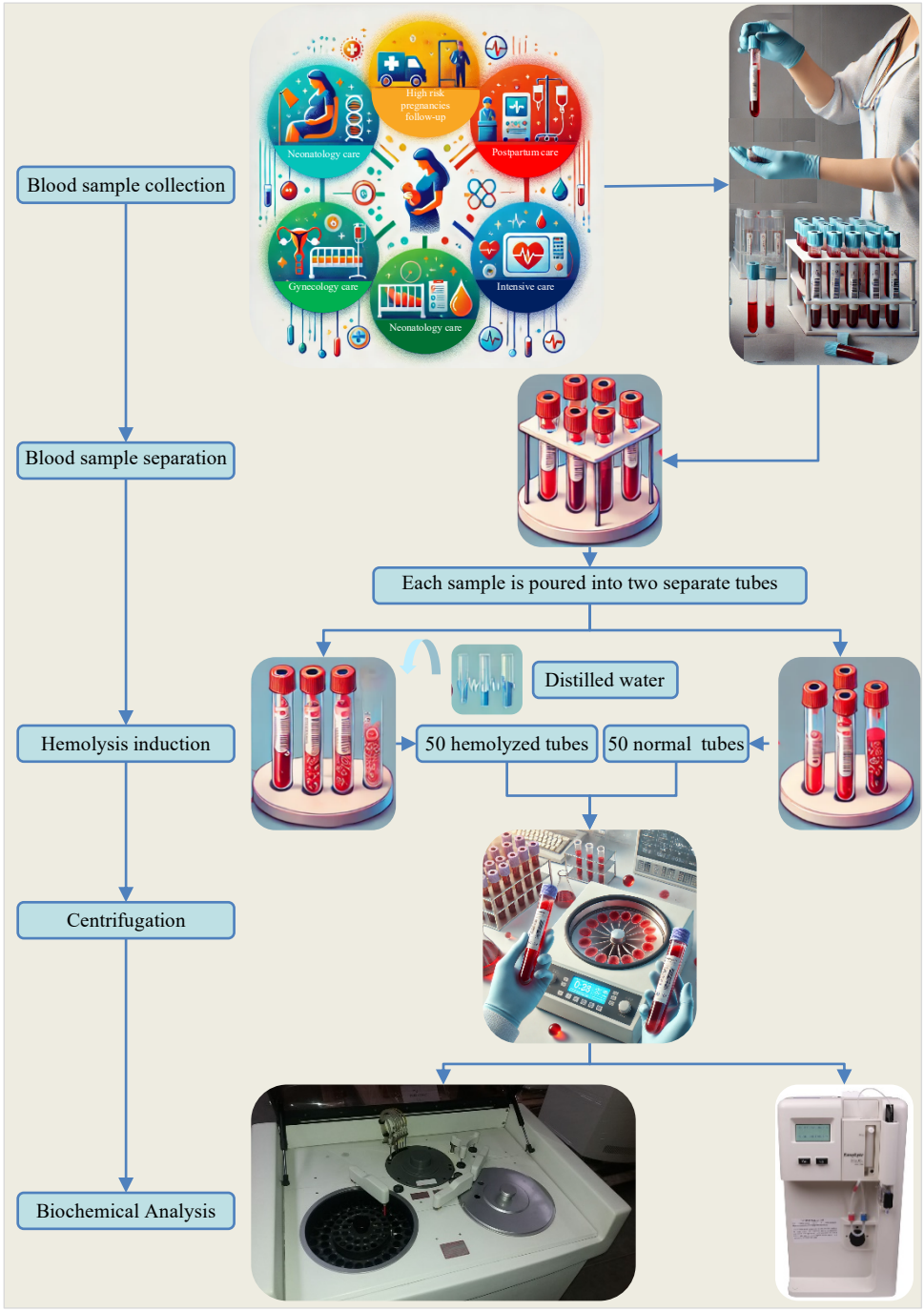


Figure 1. Experimental design.

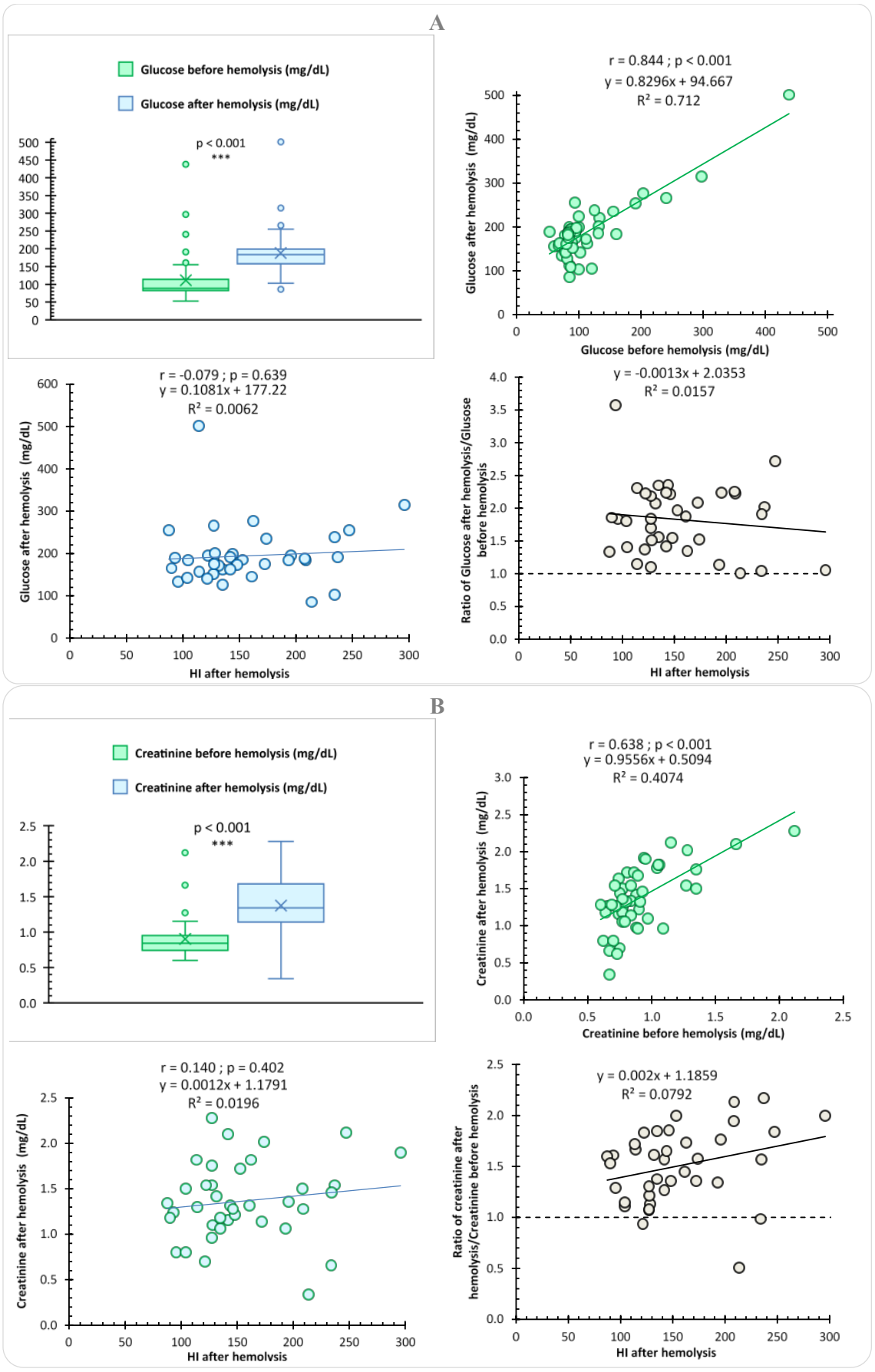


Figure 2. Variation of serum glucose (A) and creatinine (B) levels and correlation with HI after hemolysis.

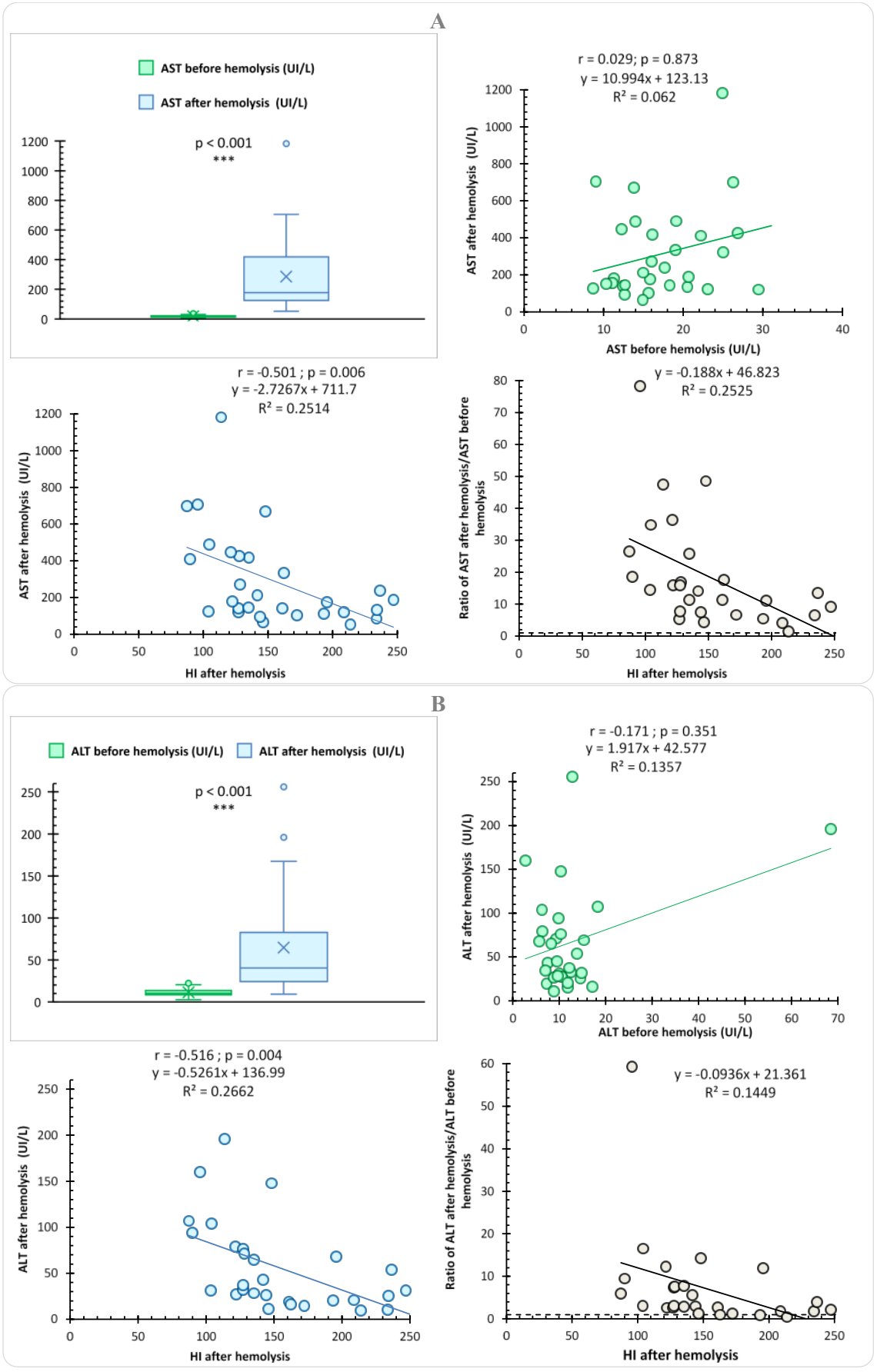


Figure 3. Variation of serum AST (A) and ALT (B) levels and correlation with HI after hemolysis.

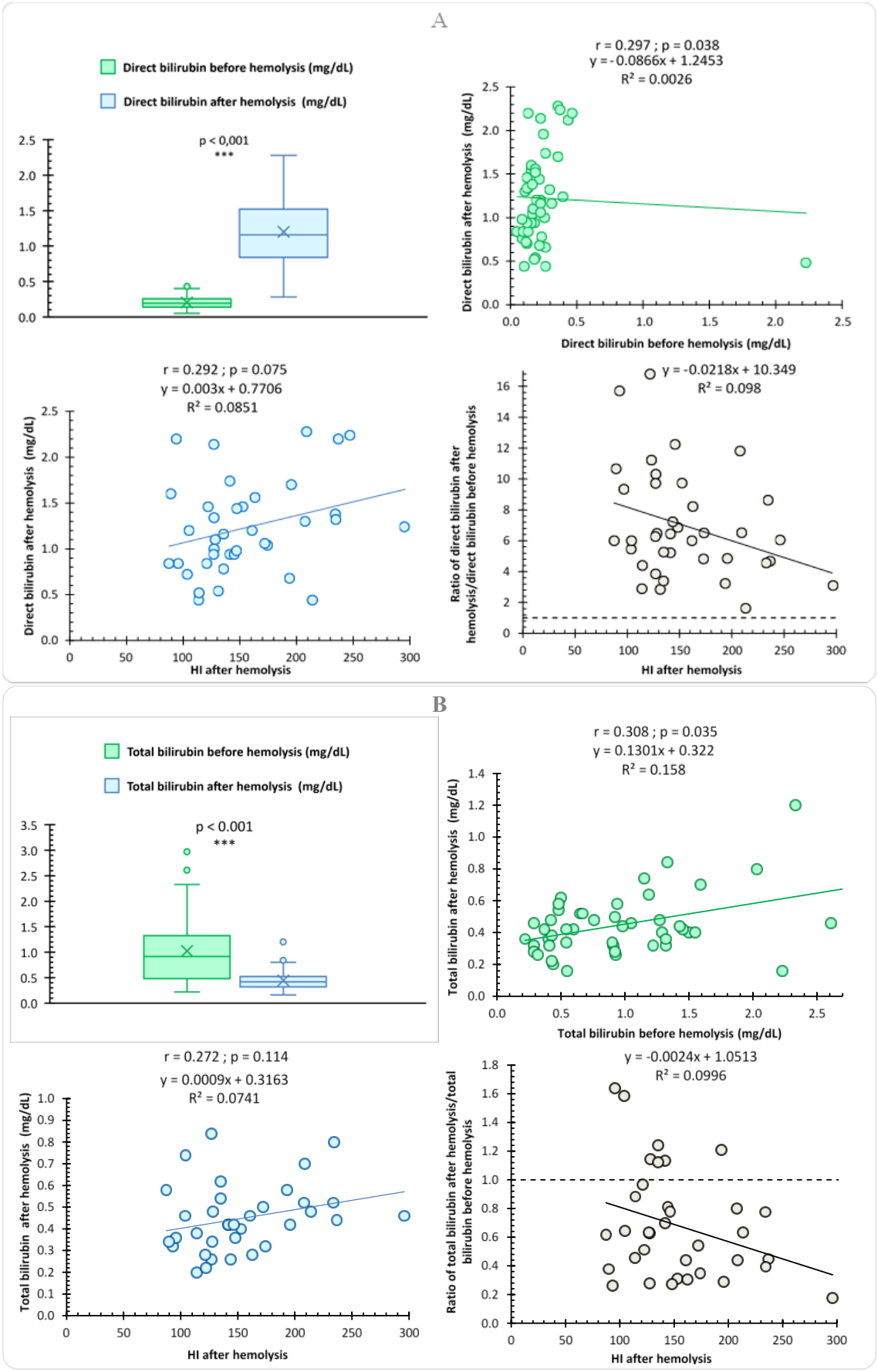
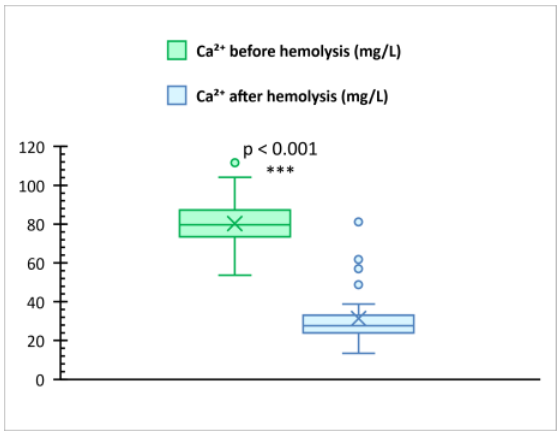
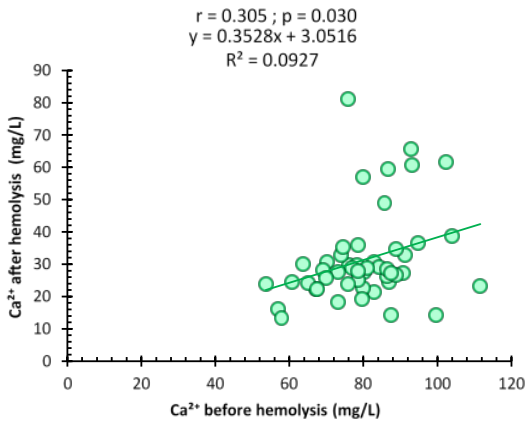
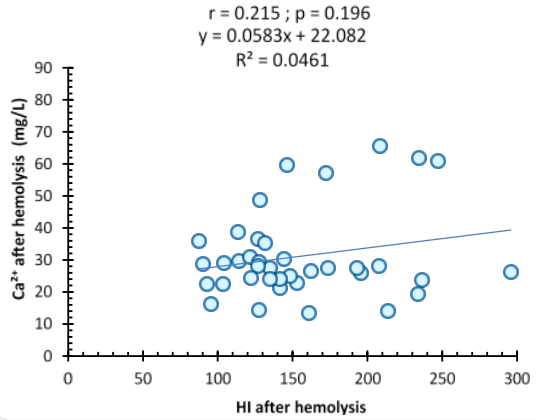


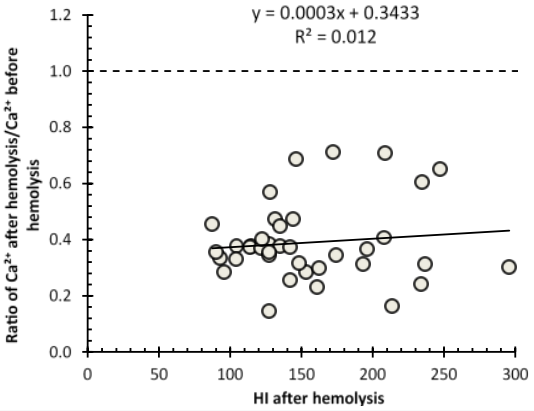
Figure 4. Variation of serum direct (A) and total bilirubin (B) levels and correlation with HI after hemolysis.

A

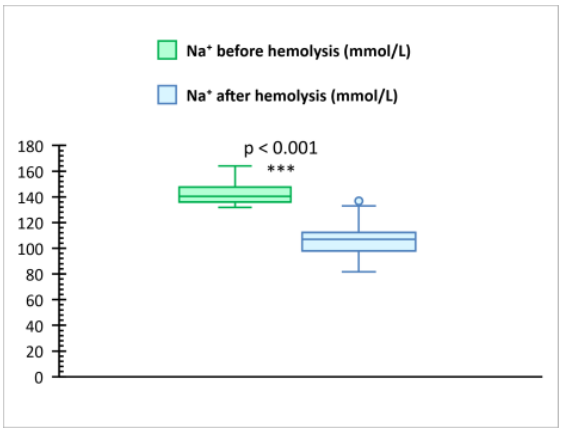

Ca²⁺ before hemolysis (mg/L)
Ca²⁺ after hemolysis (mg/L)
p < 0.001
***

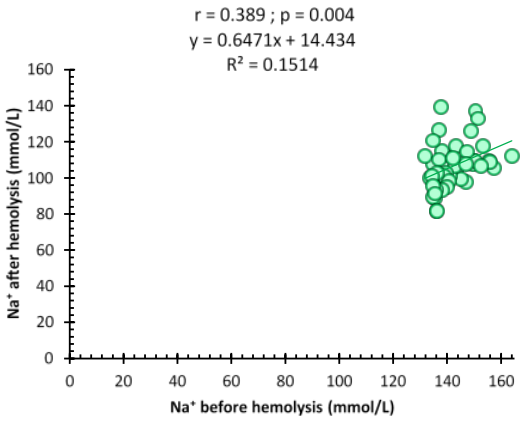

r = 0.305 ; p = 0.030
y = 0.3528x + 3.0516
R² = 0.0927
Ca²⁺ after hemolysis (mg/L)
Ca²⁺ before hemolysis (mg/L)

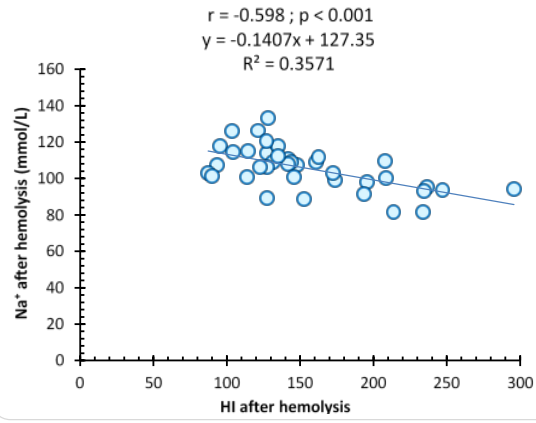

r = 0.215 ; p = 0.196
y = 0.0583x + 22.082
R² = 0.0461
Ca²⁺ after hemolysis (mg/L)
HI after hemolysis

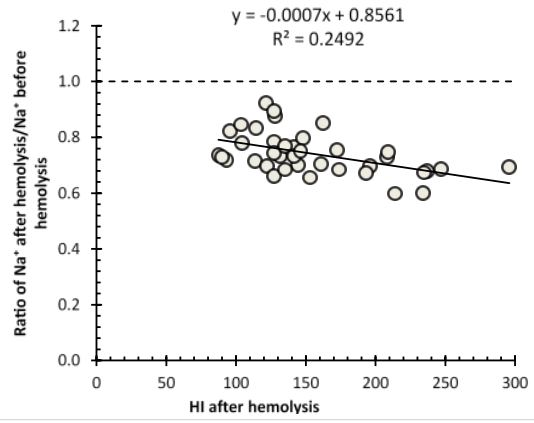

y = 0.0003x + 0.3433
R² = 0.012
Ratio of Ca²⁺ after hemolysis/Ca²⁺ before hemolysis
HI after hemolysis

B


Na⁺ before hemolysis (mmol/L)
Na⁺ after hemolysis (mmol/L)
p < 0.001
***


r = 0.389 ; p = 0.004
y = 0.6471x + 14.434
R² = 0.1514
Na⁺ after hemolysis (mmol/L)
Na⁺ before hemolysis (mmol/L)


r = -0.598 ; p < 0.001
y = -0.1407x + 127.35
R² = 0.3571
Na⁺ after hemolysis (mmol/L)
HI after hemolysis


y = -0.0007x + 0.8561
R² = 0.2492
Ratio of Na⁺ after hemolysis/Na⁺ before hemolysis
HI after hemolysis

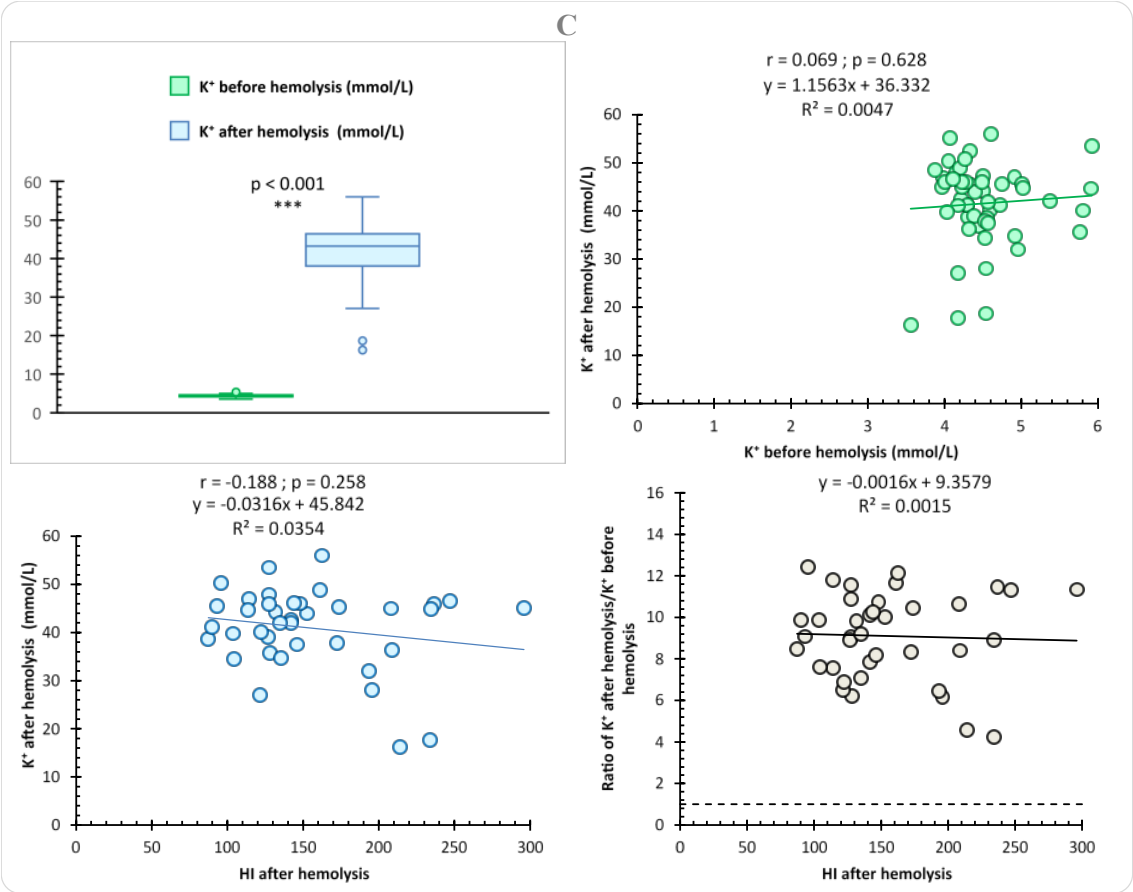


Figure 5. Variation of serum calcium (A), sodium (B) and potassium (C) levels and correlation with HI after hemolysis.

Table 1. Variation of the studied parameters using HI in the range of [87.30 - 295.9].

Parameters	**Before hemolysis**	**after hemolysis**	**Mean difference with 95% confidence interval**	**P value**
Na^+ (mmol/L)	142.5±7.4	106.6±12.2	35.8±11.6 [32.6; 39.1]	< 0.001
K^+ (mmol/L)	4.5±0.5	41.5±8.5	-37.0±8.5 [-39.4; -34.7]	< 0.001
Glucose (mg/dL)	111.2±64.9	188.0±63.7	-75.6±36,2 [-85.9; -65.3]	< 0.001
Ca^{2+} (mg/L)	80.3±11.9	31.4±13.9	48.9±15.4 [44.6; 53.3]	< 0.001
Creatinine (mg/dL)	0.9±0.3	1.4±0.4	-0.5±0.32 [-0.6; -0.4]	< 0.001
AST (UI/L)	18.2±5.8	286.5±244.3	-279.6±247.2 [-368.7; -190.5]	< 0.001
ALT (UI/L)	11.3±4.3	64.7±59.4	-50.2±55.2 [-70.1; -30.3]	< 0.001
Direct bilirubin (mg/dL)	0.2±0.1	1.2±0.5	-1.0±0.6 [-1.15; -0.8]	< 0.001
Total bilirubin (mg/dL)	1.0±0.7	0.4±0.2	0.5±0.5 [0.3; 0.7]	< 0.001