INFLUENCE OF HEMOLYSIS ON THE ANALYTICAL PERFORMANCE OF BS300 IN THE DETERMINATION OF 6 ENZYMES

RANAIVOSOA Miora Koloina[1], RAMAMINIAINA Manevarivo Eddy[2], RAKOTONIAINA Tinà[2], RAHAJANIRINA Valdo[3], RASAMOELINA Tahinamandranto[4], RANDRIAMANANTANY Zely Arivelo[5], RAKOTO ALSON Olivat[6], RASAMINDRAKOTROKA Andry[7]

[1]Biologist. Laboratory of Biochemistry of Joseph Ravoahangy Andrianavalona University Hospital Antananarivo, Madagascar

[2]Medical Biology Student, Laboratory of Joseph Ravoahangy Andrianavalona University Hospital Antananarivo, Madagascar

[3]Medical Biology Student, Laboratory of Joseph Raseta Befelatanana University Hospital Antananarivo, Madagascar

[4] Scientist, Centre d'Infectiologie Charles Mérieux, University of Antananarivo, Madagascar

[5]Professor of Immunology, National Center for Blood Transfusion Antananarivo, Madagascar

[6]Professor of Biological Haematology, Medical Biology Department of the Faculty of Medicine Antananarivo, Madagascar

[7]Professor of Immunology, Laboratory of Training and Research in Medical Biology, University of Antananarivo, Madagascar

Corresponding Author: Miora Koloina Ranaivosoa , Laboratory of Biochemistry of Joseph Ravoahangy Andrianavalona University Hospital Antananarivo, Madagascar,

Contact of corresponding author : +261 34 58 003 28; mathyakris@yahoo.fr

ABSTRACT

Hemolysis is a common source of error in the determination of a large number of biochemical blood parameters. The aim of this study is to determine the hemolysis influence on analytical performances of the BS 300 (Mindray®) on the dosage of 6 enzymes such as lipase, gammaglutamyl transferase, alkaline phosphatase, alanine transaminase, aspartate aminotransferase and lactate dehydrogenase.The secondary aim was to establish a reliable reporting for the clinicians in front of hemolysed samples for the enzymes dosage. To determine an influence of the hemolysis on the measure, the variation limit of 10 % was chosen. Gammaglutamyl transferase, and lipase were negatively influenced by the hemolysis. Alanine transaminase, aspartate aminotransferase and lactate dehydrogenase were positively influenced by the hemolysis.

Keywords: biochemistry, BS 300, enzymes, hemolysis, interference

RESUME

L'hémolyse est une source d'erreur fréquente pour le dosage d'un grand nombre de paramètres biochimiques sanguins. L'objectif de cette étude est de déterminer l'influence de l'hémolyse sur les performances analytiques de l'automate BS300 (Mindray®) sur le dosage de 6 enzymes telles que la lipase, la gammaglutamyl transférase, la phosphatase alcaline, l'alanine aminotransférase, l'aspartate aminotransférase et la lactate déshydrogénase. L'objectif secondaire est d'établir un compte rendu fiable aux cliniciens devant des échantillons hémolysés pour le dosage des enzymes. Le seuil de 10% de coefficient de variation par rapport à la valeur vraie définissant une interférence sur la mesure a été choisi. La saisie et le traitement

des données ont été effectués sur le logiciel Excel. La gammaglutamyl transférase, la phosphatase alcaline et la lipase ont été négativement influencées par l'hémolyse. L'alanine aminotransférase, l'aspartate aminotransférase et la lactate déshydrogénase ont été positivement influencées par l'hémolyse.

Mots clés : biochimie, BS 300, enzymes, hémolyse, interférence

INTRODUCTION

Hemolysis is a common source of error in the determination of a large number of biochemical blood parameters [1]

The term interference is defined as a modification of the measured signal, relating to a concentration determined, due to the presence of a body accompanying the analyte in the medium subjected to the analysis, modifying the accuracy of the results [2]

In fact it is the capacity of a substance contained in the sample to modify the right value of an analyte dosage result expressed in concentration or in activity [3].

The staining intensity varies according to the free hemoglobin concentration [4].

The aim of this study is to determine the hemolysis influence on analytical performances of the BS 300 (Mindray®) on the dosage of 6 enzymes. The secondary aim was to establish a reliable reporting for the clinicians in front of hemolysed samples for the enzymes dosage.

MATERIALS AND METHODS

Constitution of plasma pools

Plasma pools were constituted from the remainders of patients samples collected from laboratory. The plasma was collected after analysis realization. Visibly hemolysed, icterical or lactescent samples including one or several biological results outside the normal range were excluded. The constitution of plasma pools was realized from April 8th to April 12th 2019.

Overloading of plasma samples

Preparation of hemolysat

Plasma pools consisted of a preparation of the hemolysat from full blood pool obtained on sodium heparinate. These blood samples were centrifuged at 2 500 g during 10 minutes at 25°C on a centrifuge GR4i (Jouan®). Supernatants were expelled and red blood cells collected after plasma removal and the Buffy coat were pooled. Three washes volume to volume of these collected cells were thereafter hemolysed by addition of distilled water volume to volume (figure 1). A centrifugation at 4000 g enabled to remove cells debris. Therefore we got hemolysat.

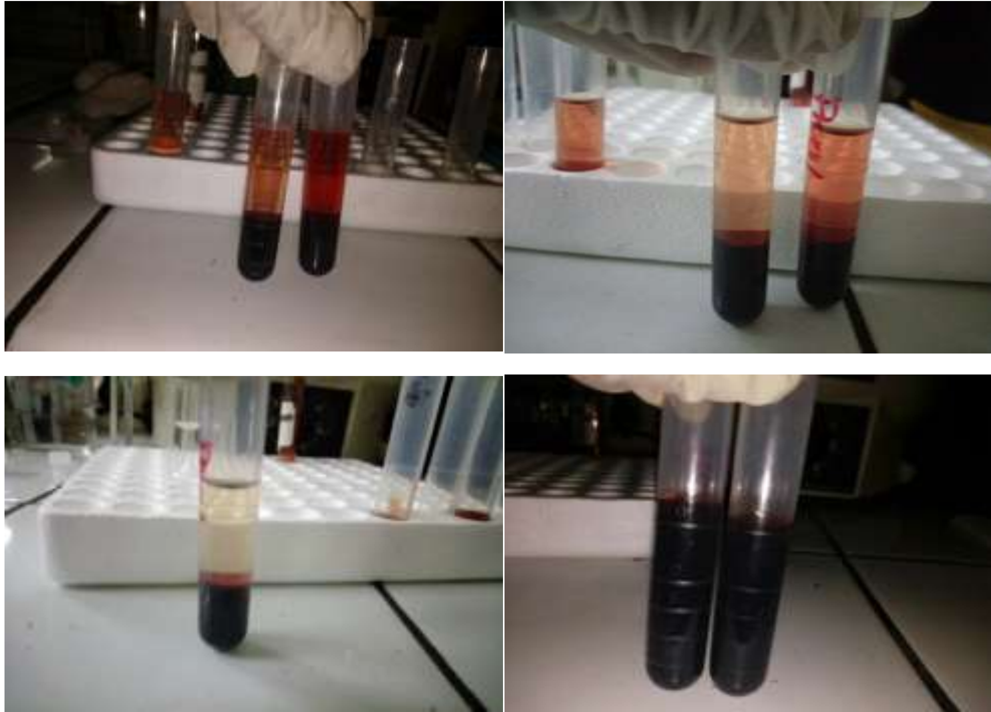

Figure 1: Three volume to volume successive washes of red blood cells and hemolysat process of creation

Source: Biochemistry Joseph Ravoahangy Andrianavalona University Hospital

Then hemoglobin on the hemolysat was measured on semi- automatic spectrophotometer Secomam (BASIC®). The hemoglobin concentration within the hemolysat was adjusted at 120g/L, then a series of 8 dilutions was realized in order to obtain a concentration range of H1 to H9 (H1=120 g/L; H2=60 g/L; H3=30 g/L; H4=15 g/L; H5=7,5 g/L; H6=3,7g/L; H7= 1,87 g/L ;H8=0,93 g/L ; H9=0,46 g/L) (figure 2)

Figure 2: Hemoglobin concentration range

Source: Biochemistry Joseph Ravoahangy Andrianavalona University Hospital

Plasma samples hemoglobin overloading

From the hemolysat concentration range, hemoglobin overloading was realized by mixing volume to volume the hemolysat of each point of range with plasmas for each studied parameter. And then each parameter was dosed on BS300 (Mindray®). The performing automaton of clinical biochemistry was the automaton BS300 (Mindray®) by Shenzhen Mindray Bio-medical Electronics.

It is an open system including:

- A specimen processing system and the reactive plateau
- A management system of the reaction mixture with disposal optical bowls
- A photometric measure system (UV-Visible photometry and an ISE module)

An information processing system (patients data, calibration and control data) which consists of a computer with the Chemistry Analyzer Software BS300 (Mindray®).

The automaton BS300 allows the dosage of routine biochemical parameters such as substrates, electrolytes and enzymatic activities.

The validity of measures was guaranteed by the passage of the calibrator and the control before the parameters dosage.

The multi-calibrator was a freeze-dried human serum (Multi-calibrator CC/H Chromatest®REF1975005). It was diluted with distilled water according to the provider instructions.

The control solutions were freeze-dried. They were diluted with distilled water according to the provider instructions. Only qualified technicians were able to reconstitute them. Two control levels were used, an abnormal one and a pathological one. The choice was made about the 10% threshold of the variation coefficient compared to real value defining a perturbation. The data entry and processing were performed on Excel.

Instrumentation

Parameters for which the influence of hemolysis has been studied on BS 300 (Mindray®). The kinetic enzymatic method is the assay method used.

Seric indexes measure.

Automaton BS300 (Mindray®) was not able to identify the seric index. The correspondence between hemolysis index and hemoglobin concentration was visually determined. This correspondence was carried out by 4 qualified interns in medical biology, two of them were second year interns and another one was a third-year interns, by three laboratory technicians respectively with 10 years, 6 years and 1-year experiences. The achieved results were collected and were as follows:

The hemolysis index [++++] corresponds to a rate of hemoglobin concentration from H1 to H3.

The hemolysis index [+++] corresponds to H4

The hemolysis index [++] corresponds to H5 and H6

The hemolysis index [+] corresponds to H7, H8 and H9.

Its degrees of hemolysis were obtained by the averages of visual evaluations made by all the people involved in the research and then confirmed by a 7 years experienced biologist.

The parameters studied were : gammaglutamyl transferase (GGT), Alkaline phosphatase (ALP), lipase, alanine transaminase (ALAT), aspartate aminotransferase (ASAT) and lactate dehydrogenase

RESULTS

Each overload pool was dosed 10 times. The average of the 10 dosages was taken.

Then, hemoglobin overloading was realized for each parameter.

GGT, lipase and alkaline phosphatase were negatively influenced by the hemolysis (Figure 3,4,5)

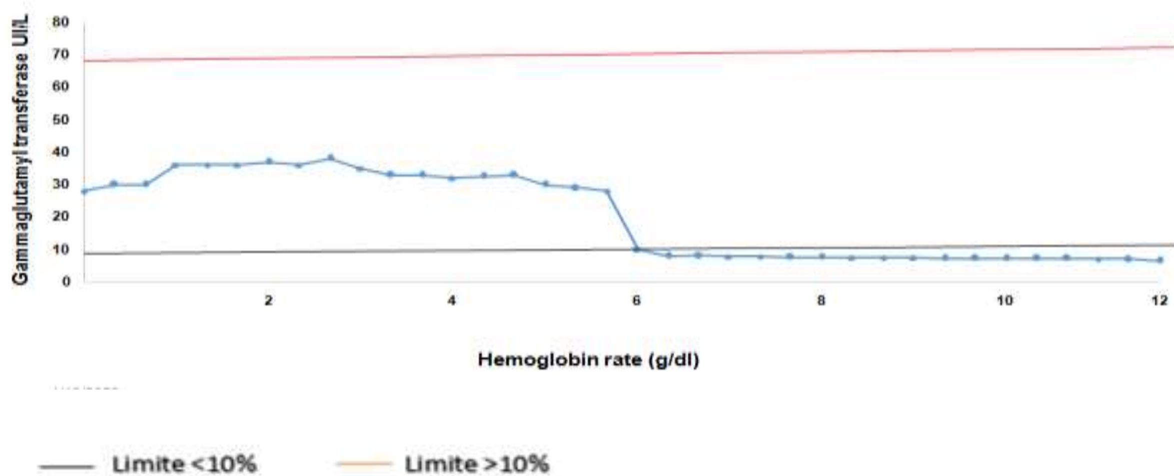

Figure 3. Gammaglutamyltransferase dosage experimental result

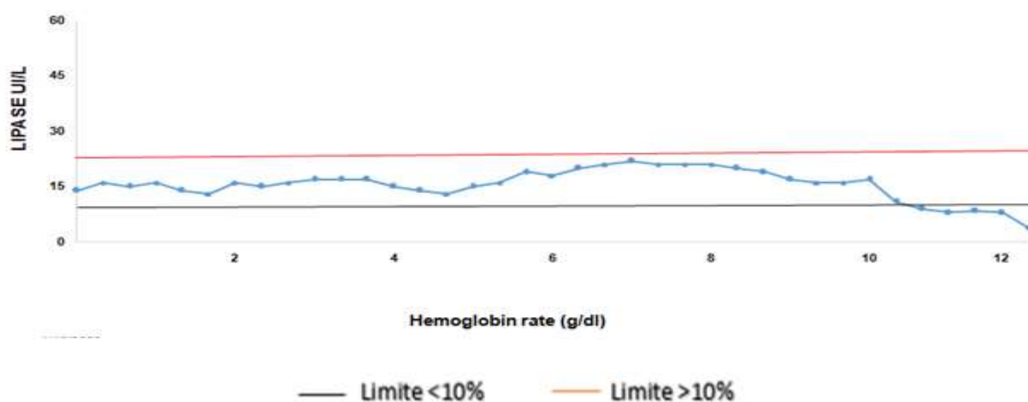

Figure 4. Lipase dosage experimental result

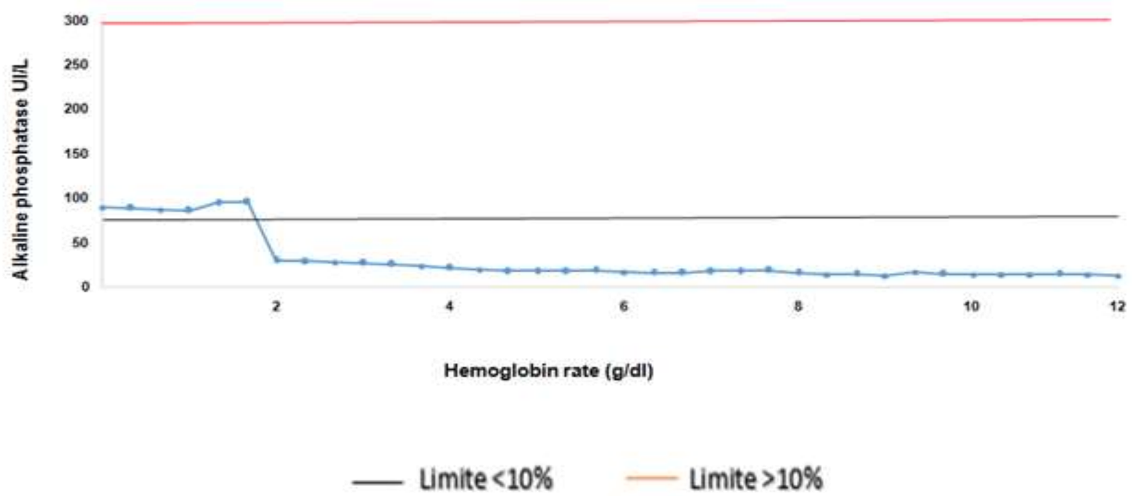

Figure 5. Alkaline phosphatase dosage experimental result

AST, ALT, and lactate dehydrogenase were positively influenced by the hemolysis (figure 6), (figure 7), (figure 8)

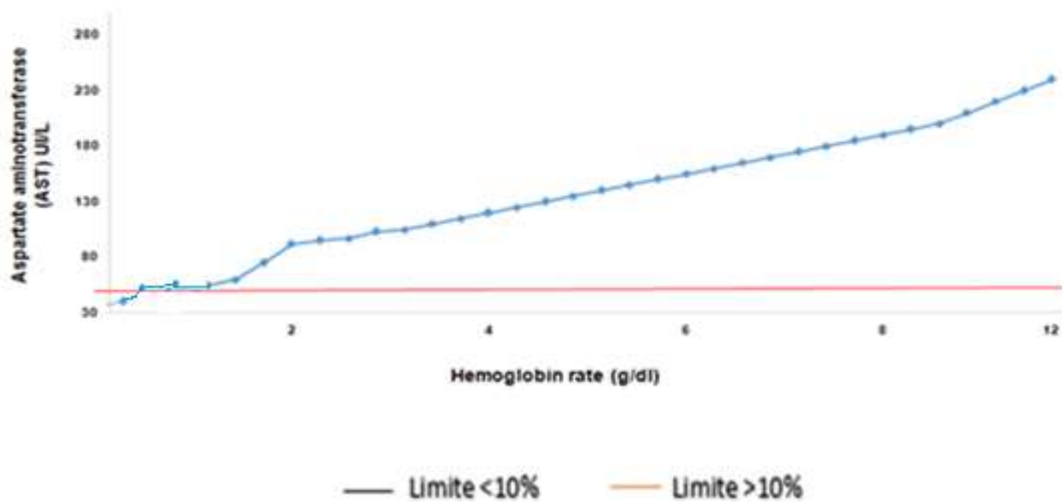

Figure 6: AST dosage experimental result

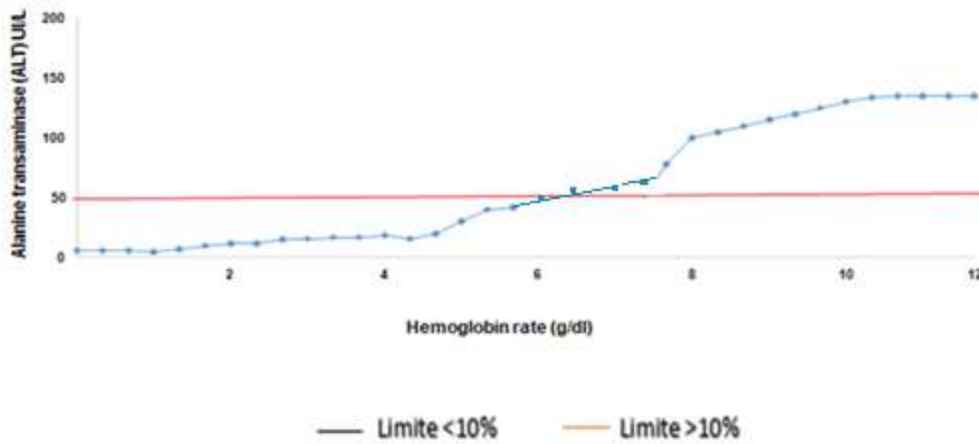

Figure 7 : ALT dosage experimental result

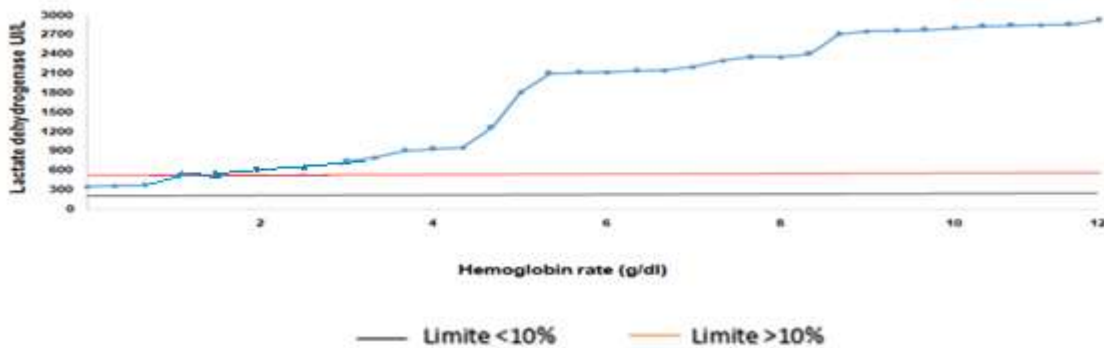

Figure 8 : Lactate dehydrogenase dosage experimental result

DISCUSSION

Parameters negatively impacted by hemolysis

1. GGT

The catalytic concentration of gamma-glutamyl transferase is decreased by higher hemoglobin concentrations of 6.1 g/l (figure 3). This hemoglobin rate corresponds to a degree of hemolysis of [++++].

Therefore, the measurement of GGT activity is negatively impacted by hemolysis only for considerable hemolysis. The GGT assay faces negative interference, proportional to the hemoglobin overload, and this interference, which appears to be primarily physical interference, is relatively well corrected after automatic dilution of the sample.

This result is consistent with that of France in 2012 [5] who reported negative interference from a hemoglobin concentration of 8.1 g/L.

This result is similar to the study performed in Germany in 1999 [6] which found a decrease of about 20 % in the catalytic concentration of -glutamyltransferase in the presence of 5 g/l hemoglobin but a hemoglobin concentration below 3 g/1 does not disturb the reaction.

This is due to the release of glycine from leukocytes and blood platelets. The concentration of glycine in blood platelets is 10 to 15 times higher than in plasma [7]. Glycine is a known inhibitor of the enzyme [6] in the presence of Glycylgycine.

2. Lipase

The measurement of lipase activity is negatively impacted by hemolysis. This work has also shown a negative interference of hemoglobin on the determination of lipase and this, starting from a hemoglobin concentration of 10 g/L (figure 4) (Hemolysis [++++]), leads to a decrease of more than 10% of the measured activity. This result is consistent with that of S.Ben Mohammed et al. in Tunisia [8] who noted a negative interference of hemolysis on the determination of lipase from a hemoglobin value >13.7 g/L. This result is consistent with the study carried out in Italy in 2009, which reported negative interference from 12.4 g/L hemoglobin concentration and thus hemolysis inhibits serum lipase activity [9].

3. Alkaline phosphatase

Alkaline phosphatase (PAL) is an enzyme found in many tissues, especially bone, liver, intestine, kidneys. It hydrolyses organic phosphates and releases insoluble mineral phosphates that are important for the calcification and mineralisation of the skeleton and is also believed to act as a « transporter» of phosphate radicals and other substances (lipids and Ca in the intestine).

The measurement of PAL activity is negatively impacted by hemolysis, but only for normal PAL activity. For normal PAL activity, there is a decrease in activity measured from a hemoglobin concentration of 2.3 g/L (figure 5). This decrease increases proportionally up to a hemoglobin concentration of 12.1 g/L, then becomes less significant beyond that point but remains significant. Same result found in France in 2000, which obtained a negative interference of hemolysis on the determination of PAL activities with a hemoglobin concentration of 3 to 5 g/L [5], this result is comparable to a study carried out in Europe in 2003 showing significant negative interference from a hemoglobin concentration of 2.3 g/L[8]. The dilutive effect caused by the leakage of intracellular components into the surrounding fluid, especially in cases of severe hemolysis, can lead to a decrease in PAL values [10]. In this study, no other solutions were added to the samples, which may also have a dilutive effect on the analysis.

Parameters negatively impacted by hemolysis

1. ASAT

As in the case of aspartate aminotransferase, hemolysis caused an increase in the catalytic concentration of aspartate aminotransferase [11-14]; the enzymatic activity of erythrocytes is 40 times higher than that of plasma [11]. Hemolysis positively interferes with the measurement of AST activity. For a normal AST value, an increase of more than 10% in the measured activity is noted with a hemoglobin concentration of 0.1 g/L, well before the visual limit of detection. This increase is proportional to the degree of hemolysis up to a hemoglobin concentration of 4.8 g/L, above this hemoglobin concentration a plateau is reached. This result is consistent with other studies [12, 15 - 18] which showed a positive influence of hemolysis on the AST assay at a hemoglobin concentration of 0.5 g/L.

Cell contents at higher concentrations of AST penetrate into the surrounding plasma when erythrocyte lysis occurs [15]. This enzyme with an intraerythrocyte concentration 15 times higher than the serum concentration is released during red cell lysis.

As expected in the current study, the AST value showed significant increases as in previous studies [12], [15], [16-23].

Therefore, it is an interference of intake. The interference is thus positive and proportional to the extent of hemolysis.

2. ALAT

This enzyme with an intraerythrocyte concentration 7 times higher than the serum concentration is released during the lysis of the red blood cells. It is therefore an interference of intake.

In this study the measurement of ALT activity is impacted by hemolysis. Hemolysis interferes positively for a hemoglobin concentration between 6 g/L and 12.1 g/l.

3. LDH

Lactate dehydrogenase (LDH) is a ubiquitous intracellular enzyme that catalyzes the reversible conversion of pyruvate to lactate in the presence of NAD+/NADH . In an aerobic environment, it catalyzes the transformation of lactate into pyruvate, which is involved in gluconeogenesis. In an aerobic environment, it participates in glycolysis by hydrolysing glucose. It is a 135 kDa tetramer composed of two types of sub-units. The alpha-hydroxy-butyrate dehydrogenase (HBDH) subunits are found in high concentrations in red blood cells and myocardial tissue. It is an intracellular enzyme contained in most tissues (myocardium, liver, kidney) and blood cells (mainly erythrocytic and myelocytic lines). At LDH, the intraerythrocytic to plasma ratio is 750. Since the catalytic concentration of lactate dehydrogenase in erythrocytes is about 160 times higher than that in serum or plasma [11], even slight hemolysis (0.2 g/l hemoglobin) interferes strongly.

In the present study, lactate dehydrogenase is significantly affected by hemolysis. On a normal LDH value, hemolysis obviously interferes positively on the measurement of LDH activity in a very important way, even in the visible absence of hemolysis. An increase of more than 10% in the measured activity is noted with a hemoglobin concentration of 0.95 g/L(figure 8), i.e. before the visual detection limit. This influence is more pronounced on the activity of LDH, an enzyme whose intraerythrocyte activity is 161 times greater than plasma activity [14]. This increase is proportional to the degree of hemolysis, which is in line with the studies carried out in Europe from 1998-2004[13-22, 23] and another study carried out in the United States in 2000 showed that a significant positive interference is found with LDH [24].

Conclusion

For GGT and lipase, the negative interference of hemolysis only occurs at high hemoglobin levels, i.e. at a degree of hemolysis [++++]. A sample hemolyzed at [++++] rarely, if ever, appears. In any case, [++++] hemolysis requires a request for a second sample.

For alacaline phosphatase, with hemolyzed sample at [+++], the result is conditional; at [+++], a second sample must be requested.

For LDH, even slight hemolysis (0.2 g/l hemoglobin: [+] strongly overestimates the LDH value.

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