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Original Research Article

A study to assess the feasibility of using hemolysis index to predict the corrected potassium in a hemolysed sample

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ABSTRACT

Background: Potassium is one of the most commonly affected analytes in a hemolysed sample. Many formulae have been devised to predict the actual potassium in a hemolysed sample. This study was performed to compare the predicted potassium value in a hemolysed sample to that of potassium value in a non-hemolysed sample of the same patient.
Methods: The hemolytic index (HI) derived equation from the paper by Dimeski et al was used to calculate potassium value in this study. A total of 99 paired samples were evaluated where the first sample in a pair was the hemolysed one and the other sample was a non-hemolysed one.
Results: This study found that the potassium value in a sample and its respective HI have weak positive correlation. However, there was a statistically significant strong positive correlation between the estimated potassium of hemolysed sample to that of the potassium in the non-hemolysed sample.
Conclusions: Hence, we conclude that it is feasible to use HI-derived equation to predict potassium in a hemolysed sample to avoid repetition of each sample.

Keywords: Hemolysis, HI, Estimated potassium, Hyperkalemia

INTRODUCTION

As modern healthcare starts to heavily rely on laboratory medicine, it is important for all clinical laboratories irrespective of the setup to ensure the prevention of errors as much as possible. Apart from the analytical quality, there is increasing evidence that suggests that pre-analytical problems account for 60-70% of total errors in the testing process.[1] *In vitro* hemolysis accounts for approximately 40-70% of samples received in the clinical laboratory that are unsuitable for analysis.[2] Although the measurement of many analytes is affected by hemolysis, the influence on measured potassium concentration is probably most widely recognized. The misdiagnosis of hypokalemia or hyperkalemia based on these spurious results could have devastating consequences in patient management.

Hemolysis is the process of breakdown of RBCs (red blood cells) and release of free hemoglobin and other intracellular contents into the plasma. Free hemoglobin<0.05 g/L can be physiologically present in the plasma while concentrations above 0.20 g/L gives a slightly detectable pink tinge to serum and plasma. Factors like lipemia/turbidity or icterus may hamper the ability to see hemolysis in a sample visually. Hemolysis can occur because of *in vivo* as well as *in vitro* processes.[2] Apart from free hemoglobin, hemolysis leads to release of many other contents like magnesium, potassium, lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and phosphate. These contents can interfere with the analysis of other analytes in a positive or negative way. For example, the measurement of the above-mentioned analytes will be positively affected by hemolysis. On the contrary, the intracellular proteases released by hemolysis may degrade troponin T

from myocardial injury, thus negatively affecting its measurement.[3]

Hence, detection of hemolysis is of paramount importance. Traditionally, it was done by visual inspection of samples and comparison with pictures of specimens with increasing hemolysis. This method was clearly limited by inter-observer variability and poor reproducibility. A method for accurate detection of hemolysis that can be uniformly applied across laboratories was essential. HI is one such technical tool available. HI is a calculation, based on the absorbance measurements performed on serum or plasma at different wavelengths which provides semi-quantitative estimate of hemolysis detected in the sample.[4]

HI is especially advantageous in high-volume laboratories where every sample is subjected to HI calculation and the sample report can be flagged if the HI value is above the detection limit. The laboratory in-charge may then proceed with the protocol for such samples practiced at their institute (adding a comment for the clinician, red flagging the report or rejecting the sample). Despite the availability of this parameter, it is still not the 'gold standard' owing to multiple reasons. Different analytical platforms from different manufacturers measure and report HI in a variable fashion. The variable factors are sample size, diluent used and its volume, read wavelengths, calculations and estimate report, where HI may be expressed in ordinal values (e.g., +1, +2, +3), actual mass concentration units of hemoglobin, hemoglobin molar units or unitless absolute value (absolute number).[5]

This study has been done with the intention to find the feasibility of using HI given by auto-analyzer Vitros 5600 to predict the corrected potassium. This will be done by comparing the serum potassium in a sample that was hemolysed and rejected with serum potassium of a non-hemolysed sample.

METHODS

Retrospective observational study design used in study.

The study conducted for six months from January 2021 to April 2021.

Inclusion criteria

Serum samples submitted for K measurement with a HI more than 50 and serum samples with a normal icterus and lipemia indices were included in the study.

Exclusion criteria

Serum samples with abnormal icterus and lipemia indices and samples from patients with known haemolytic conditions (based on the clinical information provided with the requisition form) were excluded from the study.

Study methodology

The 99 samples submitted for measurement of serum potassium and with HI (haemolytic index) more than 50 were selected from the laboratory information system (LIS). As a part of the institutional policy, fresh samples were asked to be resubmitted within 24 hours for serum potassium measurement. Such 99 paired samples were selected for the study. Serum potassium and HI were measured in both initial and repeat samples in Vitros 5600 auto-analyser. Ortho Vitros uses fresh erythrocyte hemolysate as the interferant material and tests 35 µl of undiluted sample (without consuming the sample) to calculate the HI index. It reads the sample at wavelengths of 522/750 nm and reports the value as concentration units.[5] Corrected potassium was calculated from the first sample (hemolyzed) based on the formula: corrected K^+=Measured K^+= (HI X 0.004). This formula was taken from the experiment conducted by Dimeski et al where samples from 41 volunteers were hemolysed by conditions that resemble artefactual hemolysis. They found that the potassium increase ranged from 0.0029 - 0.0053 mmol/L per unit of HI, with a mean of 0.0036 mmol/L. Thus, 1g/L of free hemoglobin or 100 units of HI will cause a 0.36 mmol/L (rounded off to 0.4 mmol/L) increase in potassium.[6]

Statistical analysis

Corrected K^+ and the potassium measured in the repeat sample were compared and statistical analysis was done to assess the feasibility of using corrected potassium based on correction factor of HI. Statistical analysis was done using SPSS software. Demographic data is expressed as mean and percentages. Pearson correlation coefficient was used to correlate variables. A $p<0.05$ is statistically significant.

RESULTS

The results of the study showed that among 99 participants, 39.4% were females and 60.6% were males (Table 1).

Table 1: Distribution of study participants according to gender (n=99).

Gender	Frequency	Percentage (%)
Female	39	39.4
Male	60	60.6

The mean age of study group was 44±18.3 years. The mean potassium level in the first sample (hemolysed) was 5.16±1.18 mmol/L while the HI for the same was 218±174. The mean potassium level in the repeat sample (non-hemolyzed) was 4.06±1.04 mmol/L while the HI for the repeat samples was below 50. The mean corrected potassium level using the formula mentioned above was 4.29±1.04 mmol/L (Table 2).

Table 2: Descriptive statistics of different parameters.

Variables	Mean	Standard deviation
Age (years)	44	18.3
Potassium 1	5.16	1.18
HI 1	218	174
Corrected K^+	4.29	1.04
Potassium 2	4.06	1.04

The values of potassium in the initial sample (K^+ 1) were correlated with their respective HI values using Pearson coefficient. The Pearson correlation coefficient, R=0.32 while coefficient of determination, $R^{2=}$0.10. This shows that there is a weak positive correlation between the 2 which statistically significant (p<0.0009) (Figure 1).

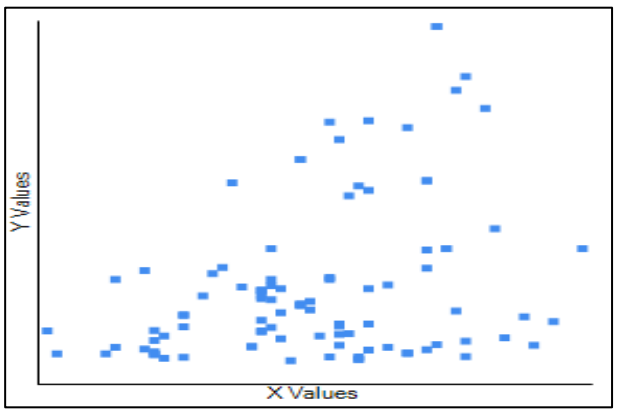

Figure 1: X values=potassium in initial sample, Y values=hemolytic index (HI 1).

The values of potassium in the repeat sample (K^+ 2) were correlated with their respective HI values using Pearson coefficient. Pearson correlation coefficient, R=0.09 while the coefficient of determination, $R^{2=}$0.01. This shows that there is a weak positive correlation between the 2 which was statistically not significant (p=0.32) (Figure 2).

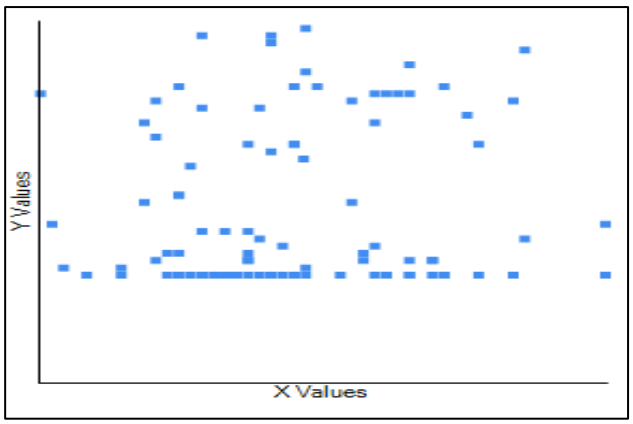

Figure 2: X values=potassium in second sample, Y=hemolytic index (HI 2).

The values of corrected potassium derived by the mathematical formula mentioned in the methodology were correlated with the potassium values in the repeat (second) sample using Pearson coefficient. The Pearson correlation coefficient, R=0.97 while the coefficient of determination, R^2 was 0.94. This shows that there is a strong positive correlation between the two which was statistically significant (p<0.00001) (Figure 3).

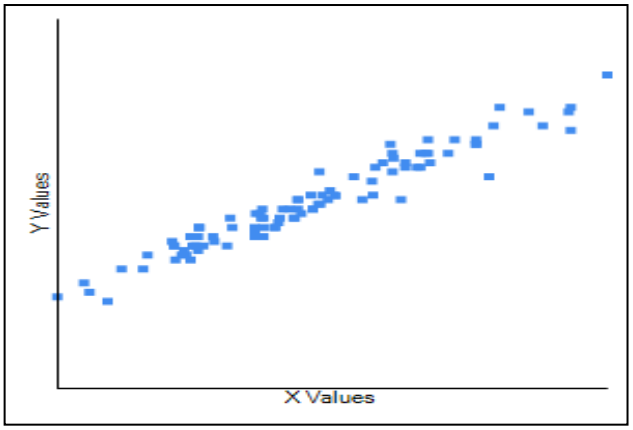

Figure 3: X values=corrected K^+; Y values=potassium in repeat sample.

DISCUSSION

Hemolysis is a common occurrence in clinical laboratory samples and potassium is one of the most commonly affected analytes. The development of methods to estimate the corrected potassium in a hemolysed sample has led to abandonment of the estimation of hemolysis by visual inspection method. Different formulae have been devised based on the manufacturer of the analyser and also based on other parameters like MCHC. The formula developed by Dimeski et al has been used in this study to estimate the potassium value in the initial hemolysed sample.[6] Dimeski et al used a novel method of producing *in vitro* hemolysis that closely mimics true hemolysis.[6] They collected blood sample and divided it into five to eight equal aliquots of 1.5 ml each. The first aliquot was centrifuged and potassium concentration and HI were measured for it. The subsequent samples were passed through 21 G needle to simulate blood collection process. The number of times the blood has to pass through the needle increased with subsequent aliquots producing an increasing range of hemolysis. Analyses for potassium and HI were done on Hitachi modular analysers. The mean potassium increase was 0.0036 mmol/L per unit HI. The equation they developed to estimate corrected potassium was: corrected K^+=measured K^+= (HI×0.004).[6]

CONCLUSION

This study showed that there is a weak positive correlation between the potassium value and hemolytic index in both the samples (hemolysed and non-hemolysed). However, there is a strong and significantly positive correlation between the potassium estimated by equation and the measured potassium in the non-

hemolysed sample. The actual potassium value can be estimated in a hemolysed sample fairly accurately. Thus, we can safely avoid ordering repeat samples if the estimated potassium value is within normal limits. However, more studies with larger sample size and heterogeneous patient cohort are required to validate the above-mentioned formula.

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