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A statistical model for restoration of serum potassium level disturbed by hemolysis

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ABSTRACT

Background: Blood sample hemolysis affects pre-analytical quality and may cause pseudohyperkalemia. We established a statistical model to estimate the corrected potassium (K^+) in serum.
Methods: Serum K^+ and H index were analyzed, and blood cell index was obtained from the examined Full Blood Examination (FBE) results. A linear-regression model was developed using hemolysis (H) index, K^+ and covariates of blood cell index from 139 cell lysates of blood samples. The model was then validated against 26 *in vitro* physically hemolyzed serum samples.
Results: The final model selected H index, hemoglobin concentration (HGB), and hematocrit (HCT) as important predictors in estimating the K^+ content. The model was validated against artificially hemolyzed serum samples, which returned a correlation of 0.942 between observed and predicted net K^+ increase by hemolysis. The predictors H index, HCT, and HB contributed 93.7%, 3.5% and 2.8% to the model R^2, respectively.
Conclusion: In vitro hemolysis induced pseudohyperkalemia could be accurately predicted and restored by our model for clinical application.

1. Introduction

Lysis of blood cells causes cytoplasmic inclusions to be released into non-cell compartment of blood producing many false diagnostic results which may not be able to represent the pathophysiological conditions. Hyperkalemia is commonly detected in hemolysed blood samples due to intracellular potassium (K^+) concentrations that are about 22 times greater than that in serum/plasma at the normal condition [1,2], and hemolysis becomes one of the most common reasons for sample rejection in clinical pathology laboratories. It has been estimated that about 40–70% of rejection of clinical samplers are due to *in vitro* hemolysis [3–5].

Apart from *in vivo* hemolysis, the *in vitro* hemolysis could be induced by improper technique during collection of blood samples or blood processing, or microorganism action in cultured blood *etc.* Hemolysis causes Red Blood Cells (RBC) to rupture, releasing intracellular hemoglobin (HGB) and K^+ simultaneously. The released HGB in serum/plasma can be detected as an analytical hemoglobin or hemolysis index (H index) with most chemistry analysers in many clinical laboratories around the world. Using hemolysis index for K^+ correction in hemolysed samples becomes possible practically [6], but has been reported with a greater variation of K^+/H index ratio ranging from 0.21 to 0.51 mmol/L per 100 mg/dL soluble HGB [7,8]. Therefore, H index of serum/plasma itself as a reference may not sufficiently be able to precisely correct hyperkalemia caused by hemolysis. The rationale behind this seems that a great variation of RBC index among populations or during the RBC maturation stages, especially with the concentration of HGB in blood, makes H index being incomparable in humans. *In vitro* lysis of white blood cells [3,9] or thrombocytosis [10,11] or tissue cells could also release K^+ causing hyperkalemia without increase of H index. As such, we have examined whether any full blood examination (FBE) characteristics including WBC (white blood cell count), HGB (hemoglobin concentration), RBC (red cell count), HCT (hematocrit), MCV (mean cell volume), MCHC (mean corpuscular hemoglobin concentration), RDW (red cell distribution width) and PLT (platelet count), play a significant role alongside H index for serum K^+ correction using lysates of human washed blood cells. A regression model of K^+ against the above-mentioned FBE characteristics was fit, and model selection tools used to establish the most suitable model. The final model was then validated by artificially hemolysed blood samples for clinical use.

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2. Materials and methods

2.1. Laboratory procedures

a. To determine the influence of cell indices on K^+ and H index caused by hemolysis, we used one whole blood sample and treated it progressively with artificial and mechanical hemolysis by repeated aspiration and expiration through a 22 gauge needle (details see below), made a set of 7 aliquots collected at various times during the treatment. They were then tested on Roche Cobas 501 chemistry analyzer for K^+ (analytical range: up to 4.59 mmol/L net increase) and H index (analytical range: up to 922 mg/dL HGB net increase). The results could demonstrate a pure correlation between K^+ and H Index without the influence of various cell indices changes during the time and among individuals, therefore, setting up the base for our study on the influence of various cell indices of the ratio between K^+ and H index. The results had also set up for the valid analytical ranges in this study.

b. 139 whole blood samples from patients who requested FBE in our laboratory were used for model construction. After separation by centrifugation at 3000 rpm for 5 min, blood cells were washed three times with isotonic saline, and the supernatant was aspirated and discarded. The final packed blood cells were lysed by distilled water. The individual lysate was then spiked into a pooled serum (assuring all results within the instrument analytical ranges with a similar analytical matrix) which had been analyzed for H index and K^+.

c. The model established by the blood lysate samples was validated for its predictive ability against 26 full blood samples of healthy donors. All blood donors were given informed consent prior to the procedures performed which complied with the Declaration of Helsinki. Data privacy was guaranteed by anonymization of all clinical samples, as neither patient identifiers attached to the data nor are the individual results revealed. Original statement of formal ethics committee approval was, therefore, not required. The artificial and mechanical hemolysed samples were made according to Zou, Nolan et al. [12]. In brief, blood samples were divided into aliquots and then hemolysed by repeated aspiration and expiration through a 22 gauge needle 0 to 50 times. Samples were analyzed for K^+ and H index on a Roche Cobas 501 analyzer. K^+ and H index results together with their respective FBE parameters were analyzed to compare with the results predicted from the model.

2.2. Statistics and data analysis

A linear regression model was used to describe the relationship between the H index together with FBE characteristics, and the outcome, K^+. In order to satisfy modelling assumptions, the K^+ and H index variables were log-transformed. A saturated model was fit to these samples, including (log) H Index, RBC (x10^12/L), WBC (x10^9/L), HBG (g/L), HCT, MCV (fl), MCH (pg), MCHC (g/L), PLT (x10^9/L) and RDW (%), as covariates. Backwards model selection was performed according to Akaike's Information Criterion (AIC) [13], to generate the simplest model that best explains these data. Modelling assumptions were checked prior to, and following, the model selection procedure.

3. Results

Within the ranges of serum K^+ (up to 10.1 mmol/L net increase) and H index (equivalent to up to 1245 mg/dL HGB net increase), a greater linearity ($R^2 = 0.99$) is shown (Fig. 1). The result suggests that the degree of K^+ released from cellular compartment could be accurately estimated by H index within an individual subject.

A saturated linear regression model was fit to 139 independent samples, including each of (log) H Index, RBC (x10^12/L), WBC (x10^9/L), HBG (g/L), HCT, MCV (fl), MCH (pg), MCHC (g/L), PLT (x10^9/L) and RDW (%). Details of the final regression model following backwards selection are detailed in Table 1. In addition to H index, HGB and HCT were retained in the model as important and statistically significant predictors.

The final regression model has a corresponding $R^2 = 0.942$, with relative contributions of 93.7%, 2.8% and 3.5%, for H index, HGB and HCT, respectively. The R^2 value indicates that only 5.8% of the variation in the log potassium measurements was not explained by the linear relationship with the specified predictors. The relative contribution of each variable to the model R^2 value was evaluated using the *calc. Relimp* function (with simple unweighted averages (lmg)) in the R package *relaimpo* [14].

In order to validate the predictive ability of the model, subsequent 26 samples were analyzed. The net hemolysis released K^+ was predicted according to the final model specified in Table 1, and these measurements compared to the observed K^+. Fig. 2 shows the predicted K^+ levels against the observed K^+ levels for the 26 hemolysed validation samples. The correlation between the predicted and measured values is 0.9896 and thus $R^2 = 0.9793$, suggesting that this model based on the H index, HGB and HCT can adequately predict the K^+ contained in serum, for blood samples with characteristics within the ranges specified in Table 2.

4. Discussion

Using improper means of venepuncture may cause blood cell lysis and pseudohyperkalemia. As predicted, we demonstrated that the major contribution of K^+ increase in hemolysed samples is from RBC lysis indicated by H index which is proportional to HGB, and could be used for precise prediction in individual subject (with same blood cell indices) as indicated in our study (Fig. 1). However, analysis on the prediction power with our model established based on the contents of blood cells *via* lysis, we found H index contributes about 94% of prediction power, while the relevant HGB and HCT covariates also play significant role with 2.8% and 3.5% of prediction power respectively in the estimation of K^+ release. The significance of our results may suggest that H index together with other instant cellular indices can be co-used to enhance accuracy in estimation of the hemolysis induced net K^+ release, different from the previous studies which did not recommend for using H index in serum/plasma K^+ correction [7]. The discrepancy behind seems that the variabilities of HGB [7] and HCT among individuals could significantly contribute to the inaccuracy by solely using H index for K^+ correction. Compared to the within-subject biological variation of 2.44% for both, the between-subject biological variations of HCT and HGB could be as high as 10.34% and 11.25% respectively [15], accurate adjustments are therefore necessary for a "true" ratio of inter-individual K^+ and H index. The lower within-subject biological variations may directly participate in forming a great linear correlation between K^+ and H index in individual subject showing in our study with a single sample (Fig. 1).

Total HGB as well as HCT, two of the components in FBE, are usually closely related clinically. They could represent HGB and RBC portions in blood. A study on plasma K^+ upon *in vitro* hemolysis was also found that HCT is negatively associated with K^+ release [4]. It would not be surprising that HGB and HCT are both related to the available HGB to be released per RBC in *in vitro* hemolysis. Our analytical results have shown they are significantly correlated with H index should all FBE indexes be the same (e.g in one subject at the same time) under the same condition of *in vitro* hemolysis (Fig. 1), therefore, increase of either would reduce the estimated K^+ concentration in serum/plasma. We could not find any significant correlation with either WBC or PLT, probably due to the samples used in this study did not have a significantly high level with either as indicated with our FBE results, presumably they present far less proportion than RBC in blood at normal ranges and contribute insignificant amount of serum K^+

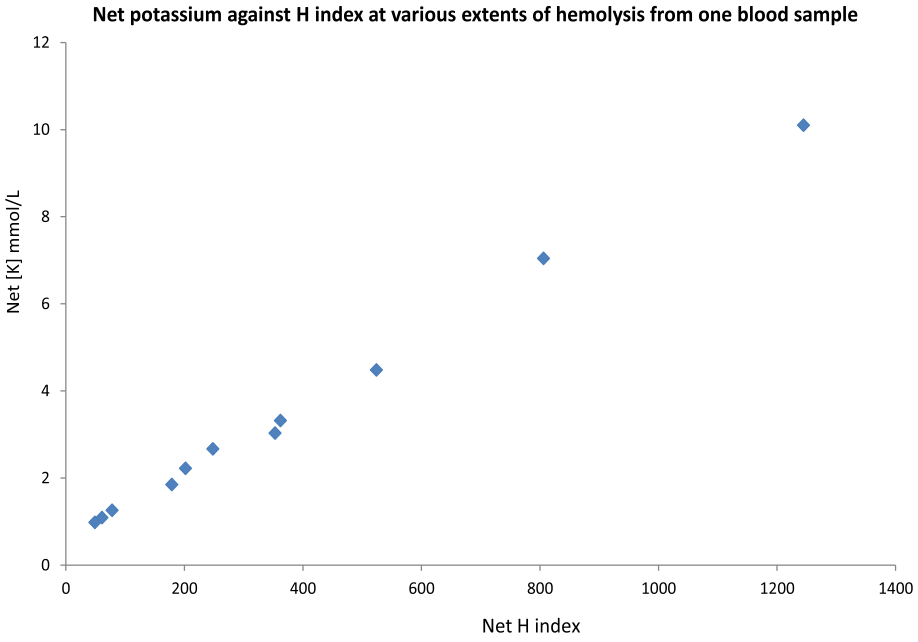

Fig. 1. Serum K^+ against H index in one individual sample. A set of 7 aliquots from one blood sample were consequentially treated by repeated aspiration and expiration through a 22 gauge needle to form hemolysis. A strong correlation of serum K^+ and H index are demonstrated ($R^2 = 0.99$).

Table 1
Summary of the linear regression model fitted to the 139 training samples, after backwards model selection according to the AIC.

	Estimate	Std. Error	t-value	*P*-value	R^2 contribution (%)
Intercept	−5.1571	0.1247	−41.37	< 0.001	
log H Index	1.0082	0.0227	44.38	< 0.001	0.8825(0.9369)
HGB	−0.0127	0.0018	−6.90	< 0.001	0.0263(0.0279)
HCT	3.4217	0.6357	5.38	< 0.001	0.0331(0.0352)

increase upon cell lysis under the shear stress.

Neither RBC, or RDW, or MCV or MCHC could demonstrate a significant correlation in the model, suggesting the sheer stress used on *in vitro* hemolysis might be evenly on RBCs regardless their number, shape and size in our study, despite it has been suggested that any variability of membrane integrity at different maturation stages (with different numbers, shapes and sizes) could cause a significant variation in K^+ release from RBC [16].

The model presented here gives a simple means by which *in vitro* hemolysed samples could be corrected for presudohyperkalemia by H index, HGB and HCT which are typically available concomitantly with most requesting episodes and could be programed for K^+ restoration *via* a laboratory information management system (LIMS). However, the samples of this study have not been included in any cases of

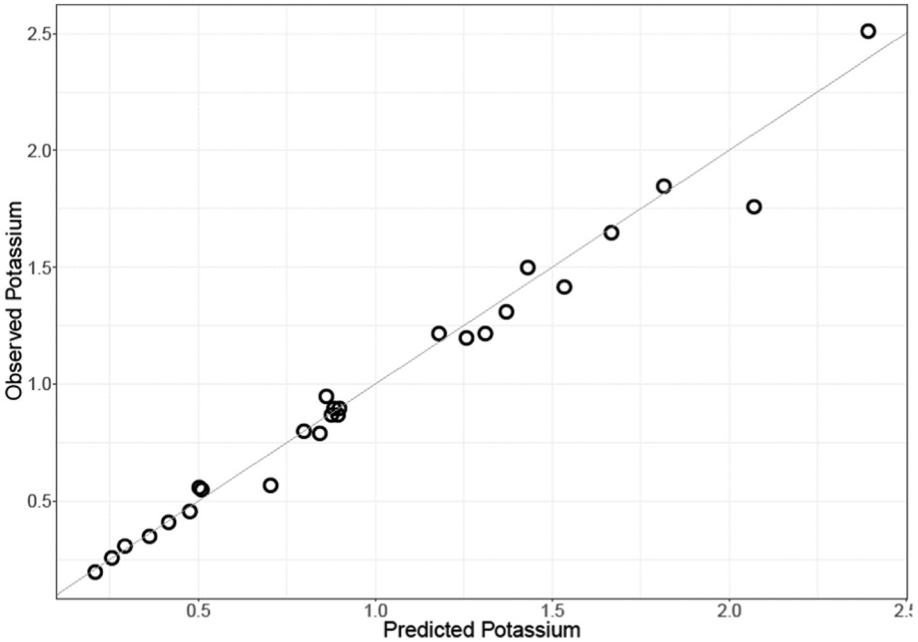

Fig. 2. Validation of the statistical model with 26 blood samples to artificially formed hemolysis. The predicted K^+ levels against that of the measured K^+ levels shows a reliable model for K^+ prediction under hemolysis ($R^2 = 0.9793$).

Table 2
The cell index ranges of the analyzed samples.

Cell indices	Value ranges
RBC [K] (mmol/L)	0.52–1.74
Index H (mg/dL)	138–408
WBC (10^9/L)	3.1–15.1
RBC (10^12/L)	2.15–7.03
HGB (g/L)	109–186
HCT (l/l)	0.338–0.54
MCV (fL)	69–122
MCH (pg)	21.5–40
MCHC (g/L)	298–373
RDW (%)	7.4–15.5
PLT (10^9/L)	76–438

pseudohyperkalemia caused by *in vivo* hemolysis, unjustified H index associated with hemoglobin variants, intracellularly abnormal RBC [17] or any abnormal FBE results beyond the ranges of this study including higher WBC/PLT counts, tissue cell lysis, other sample type(s) (other than serum) and prolonged storage on cell. All of them should be excluded from this model.

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