Influence of Measurement Principle on Total Hemoglobin Value

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Abstract

Background: Total hemoglobin measurement is indispensable for determining the stroke type (haemorrhagic vs. ischaemic) and need for blood transfusion. Conductivity- and absorbance-based measurement methods are used for blood gas analysis of total hemoglobin. For conductivity-based measurement, total hemoglobin is calculated after converting blood conductivity into a hematocrit value, whereas absorbance measurement is based on light absorbance after red blood cell hemolysis. We previously reported hemolysis observed after infusion or transfusion during veno-arterial extracorporeal membrane oxygenation, and total hemoglobin differed between conductivity and absorbance measurement methods possibly due to plasma electrolyte changes and hemolysis. Methods: In this study, test samples with controlled electrolyte changes and hemolysis were created by adding sodium chloride, distilled water or hemolysed blood to blood samples collected from healthy volunteers, and total hemoglobin values were compared between both methods. Results: Conductivity-based measurement revealed reduced total hemoglobin value (from 15.49 to 13.05 g/dl) following the addition of 10% sodium chloride, which was also reduced by the addition of hemolysate. Conversely, the addition of distilled water significantly increased total hemoglobin value than the expected value. In the absorbance method, there was no significant change in total hemoglobin value due to electrolyte change or hemolysis. Conclusions: The absorbance method should be used when measuring total hemoglobin in patients with expected blood conductivity changes. However, when using this method, the added contribution of hemoglobin from hemolysed erythrocytes lacking oxygen carrying capacity must be considered.

Background

The total hemoglobin (tHb) value in red blood cells (RBCs) is a critical measure of blood oxygen transport capacity. When the hemoglobin value abruptly declines due to hemorrhage, oxygenation of the hierarchy cannot be properly maintained. Thus, the accurate measurement of tHb in emergency, surgery and intensive care settings is vital for understanding the current condition of patients with hemorrhage and is the most important indicator for determining the need for transfusion of RBC products. We previously experienced a case in which hemolysis was observed after infusion or transfusion during veno-arterial extracorporeal membrane oxygenation (VA ECMO), and tHb value differed between conductivity [1] and absorbance [2] measurement methods. Thus, the tHb value did not match the stroke type and treatment status.

The conductivity method measures the potential difference between two electrodes in a blood specimen. This potential difference is converted into a hematocrit value according to the characteristic inverse relationship between conductance and the number and size of RBCs in the specimen, and tHb is subsequently calculated. However, this method may be influenced by changes in electrolyte concentration in the absence of tHb changes; therefore, there is doubt concerning the reliability of this method in patients with electrolyte imbalances due to stroke type and treatment. Alternatively, the absorbance method is not influenced by electrolytic concentration in the absence of tHb changes. However, in the absorbance measurement method, tHb is measured via light absorbance after RBCs are completely

hemolysed by ultrasonic waves, so it is impossible to distinguish between hemoglobin from non-functional RBCs and that released from functional RBCs.

Thus, both measurement methods have advantages and limitations in the clinical setting. Therefore, to examine how conductance- and absorbance-based methods are influenced by electrolyte balance changes and hemolysis, we compared tHb values of blood samples collected from healthy adult volunteers with controlled changes conferred by adding normal sodium chloride (NaCl) solution, distilled water (DW) or hemolysed blood.

Methods

Sample, Setting and Interventions

Blood samples were collected from five healthy adult male volunteers (mean age, 33.6 ± 7.40 years) who provided informed consent to participate in this study. Approximately 18 mL blood per person was collected from the cubitus vein in a vacuum blood vessel using a 21-gauge needle. NaCl solution, DW and hemolysed blood were added to the collected blood from volunteers to create three models, namely, high conductivity, low conductivity and hemolysis, respectively (Figure 1). Blood tHb value was measured using an ABL 77® (conductivity 77) and ABL 725® (absorbance 725) system for conductivity- and absorbance-based measurements, respectively (both instruments from Radiometer K.K., Tokyo, Japan). For the conductivity change model, 10% NaCl (Otsuka saline 10% solution®; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was added to increase the serum Na^+ level by 30 and 60 mEq/L (Table 1), whereas DW (PL®; Fuso Pharmaceutical Industries, Ltd., Tokyo) was added to reduce the serum Na^+ level by 10% and 20% (Table 2). For the hemolysis model, the collected blood was completely hemolysed by freeze–thawing, and a proportion of this was added to untreated sample. Complete hemolysis was visually confirmed via light microscopy (Table 3).

tHb and Na^+ levels were measured thrice in each specimen, and each specimen was measured using both methods and tHb corrected for dilution ratio.

Statistical Analysis

All measurements are expressed as mean ± standard deviation, and Student's *t*-test was used to compare values between the two measurement method groups and between each measurement method group and untreated controls. The level of significance (*P*) was set at 0.05 (two-tailed).

Results

Mean tHb values of control (untreated) blood samples did not differ between conductivity 77 and absorbance 725 (15.49 ± 1.18 vs. 15.37 ± 0.09 g/dl, $P > 0.05$). Mean tHb values significantly differed

between conductance and absorbance measurement methods for both the +30 mEq/ml Na^+ (14.04 ± 1.13 vs. 15.53 ± 1.02 g/dl, $P < 0.05$) and +60 mEq/l (13.05 ± 0.97 vs. 15.20 ± 0.91 g/dl, $P < 0.05$) samples. Compared with untreated control samples, the conductivity 77 (Figure 2) yielded lower tHb values, whereas the absorbance 725 showed no difference in tHb values (Figure 3). Thus, conductivity-based but not absorbance-based tHb measurements were sensitive to increased sample electrolyte concentration. Conductivity 77 and absorbance 725 values also significantly differed for the 10% (14.53 ± 1.02 vs. 14.06 ± 0.94 g/dl, $P < 0.05$) and 20% (13.41 ± 1.02 vs. 12.46 ± 0.89 g/dl, $P < 0.05$) dilution samples. Compared with untreated control samples, conductivity 77 yielded elevated tHb values (Figure 4) and the magnitude of elevation increased with dilution (20% > 10%), whereas absorbance 725 showed no difference in tHb values (Figure 5). Conductivity 77 and absorbance 725 values also differed for all hemolysis samples (10%: 15.49 ± 1.18 vs. 15.37 ± 0.90 g/dl, 50%: 13.81 ± 0.77 vs. 15.56 ± 0.87 g/dl, 90%: 12.45 ± 0.88 vs. 15.91 ± 1.15 and 100%: 12.09 ± 0.87 vs. 15.96 ± 0.95 g/dl; all $P < 0.05$). Compared with untreated controls samples, tHb values were progressively reduced by the addition of haemolysed blood as measured using conductivity 77, and the values also differed as measured using absorbance 725 (Figure 6). Serum Na^+ levels were approximately equivalent among corresponding samples according to conductivity 77 and absorbance 725 measurements (Figure 7).

Discussion

Conductivity 77 is a portable blood gas measurement device that operates on the basis of the conductivity principle. The measurement duration is short, and manipulation and maintenance are easy, making conductivity 77 optimal for use in emergencies. Moreover, for the absorbance 725 device used in this and other studies. There were no differences between conductivity 77 and absorbance 725 in pH, pCO2, pO2, Na^+, K^+, Ca^{2+}, and hematocrit, suggesting sufficient reliability. Conductivity 77 is also useful compared with absorbance measurement and centrifugal separation methods. However, a dissociation between conductivity- and absorbance-based hematocrit measurements has been reported [3-5]. Using the conductivity-based method, the measured hematocrit value is lower than the absorbance-based value because of the influence of dilution by infusion, cardiopulmonary bypass and electrolyte concentration [6]. Alternatively, we found that absorbance-based measurements did not differ between control samples (untreated) and samples with experimentally altered electrolyte levels and pre-existing hemolysis (after correcting for dilution).

The conductivity measurement method uses two electrodes that are in direct contact with the specimen blood. A current is passed between the electrodes, and the potential difference is measured. The potential difference varies linearly with conductance, which depends on cell density and electrolyte concentration. However, four factors affect conductance-based measurements aside from cell density/size, electrolyte concentration, protein concentration, osmotic pressure and hemolysis [7,8]. Blood is a viscous fluid that contains plasma, cells and proteins. Electrolytes account for most of the conductivity of plasma, whereas 99% of the cells are nonconductive RBCs. The measurable Na^+ level of the conductivity 77 instrument ranges from 80 to 200 mmol/l, and the Th.B. value is calculated using Na^+ correction. In this study, the

Th.B. value was reduced by elevated Na^+ level (high conductance) and vice versa, suggesting that additional Na^+ causes errors in Th.B. measurement because tHb cannot be corrected even within the Na^+ measurement range of the conductivity 77 instrument.

Strengths and Limitations

Proteins account for 1%–7% of the plasma but are nonconductive. The reduces of nonconductive materials increase conductivity due to volume effects. Conductivity 77 measures tHb by assuming that the protein concentration is constant. Therefore, in case of excess replacement fluid or low protein levels, the conductivity of blood is increased, which causes an error in tHb measurement [9]. Total osmotic pressure is conferred by crystalloid (mainly from Na^+) and colloid (mainly from albumin) osmotic pressures. Under an osmotic pressure increase, water leaves RBCs and the cell volume is reduced. Conversely, low osmotic pressure results in RBC swelling. In accordance with the principle of the conductivity measurement method, an error may occur because tHb measurement is inversely proportional to RBC volume. Hemolysis occurs when RBCs (nonconductors) rupture. As the proportion of hemolysed RBCs increases, that of plasma Hb increases. Therefore, our results also suggest that the electrical conductivity of blood increases and measured tHb value decreases with hemolysis.

The absorbance 725 is a desktop-type blood gas measurement device that is based on sample light absorbance. In this case, tHb value is measured according to hemoglobin concentration and optical path length (which is constant).[2] Prior to measurement, ultrasonic vibration is applied to completely hemolyse RBCs in the specimen. Therefore, Hb from non-functional RBCs is added to that from functional RBCs. In our study, electrolytes and hemolysis did not affect absorbance-based tHb measurements. However, fetal hemoglobin (HbF) has been reported to have molecular structure different that is from that of adult hemoglobin, and errors occur in HbF measurement when the total bilirubin concentration is high [10-12]. Both instruments also measure Na^+, but the measurement principle is identical, so there were no differences in the measured values between these instruments.

Based on these findings, when excessive replacement fluid or electrolyte abnormality is suspected, an absorbance-based tHb measurement method should be used. However, absorbance-based measurements should be corrected for Hb from non-functional RBCs to obtain a better estimate of the blood oxygen carrying capacity.

Conclusions

Blood gas analysis devices use different principles for tHb measurement. Therefore, the characteristics of the measuring device must be chosen according to potential errors introduced by the pathological state or treatment. For patients suspected of having excessive replacement fluid or an electrolyte abnormality, blood conductivity changes are possible. In such patients, absorbance measurement should be performed while taking hemolysis and bilirubin levels into consideration.

Declarations

Ethics approval and consent to participate: Okayama Saiseikai General Hospital Institutional Review Board. Approval date: 7 October 2014. Approval (No.: 141001)

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1 High conductivity model

10%NaCl	Blood volume (ml)	10%NaCl (ml)	Total (ml)
30 mEq/L added	1.975	0.025	2.00
60 mEq/L added	1.950	0.050	2.00

Table 2 Low conductivity model

10%NaCl	Blood volume (ml)	10%NaCl (ml)	Total (ml)
30 mEq/L added	1.975	0.025	2.00
60 mEq/L added	1.950	0.050	2.00

Table 3 Hemolysed blood model

Hemolysed blood rate	Blood volume (ml)	Hemolysed blood volume (ml)	Total (ml)
Hemolysed blood 10%	1.80	0.20	2.00
Hemolysed blood 50%	1.00	1.00	2.00
Hemolysed blood 90%	0.20	1.80	2.00
Hemolysed blood 100%	0.00	2.00	2.00

Figures

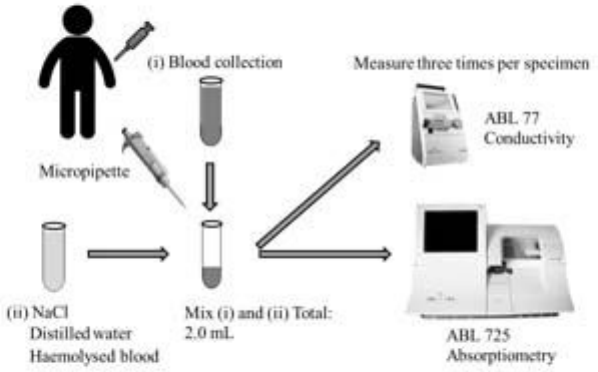

Figure 1

Measured using conductance and absorbance measurements. NaCl solution, DW and hemolysed blood were added to the collected blood.

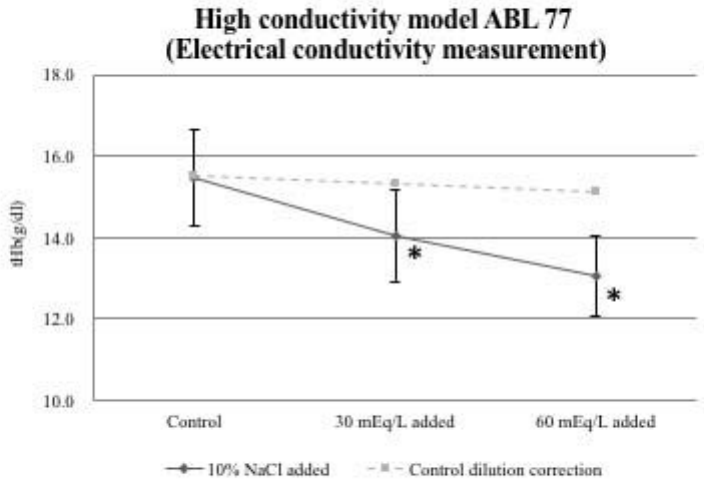

Figure 2

Effect of elevated serum electrolyte concentration (30 and 60 mEq/l Na+) on tHb. Mean tHb values significantly differed between control and 10% Nacl added on the conductivity 77.

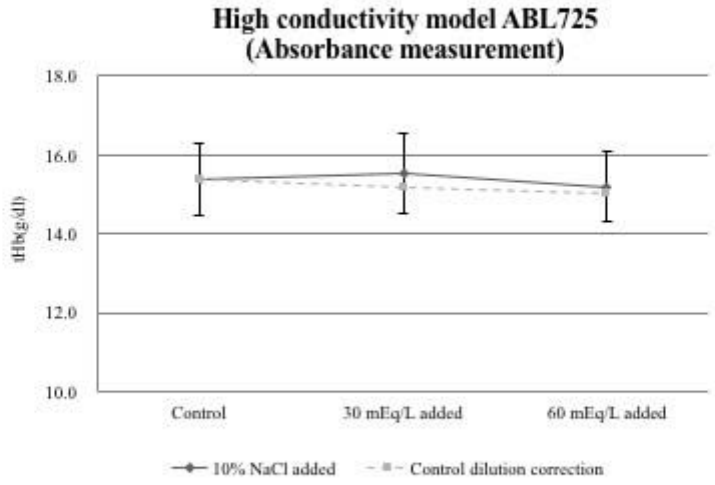

Figure 3

Effect of elevated serum electrolyte concentration (30 and 60 mEq/l Na+) on tHb. The absorbance 725 showed no difference in tHb values.

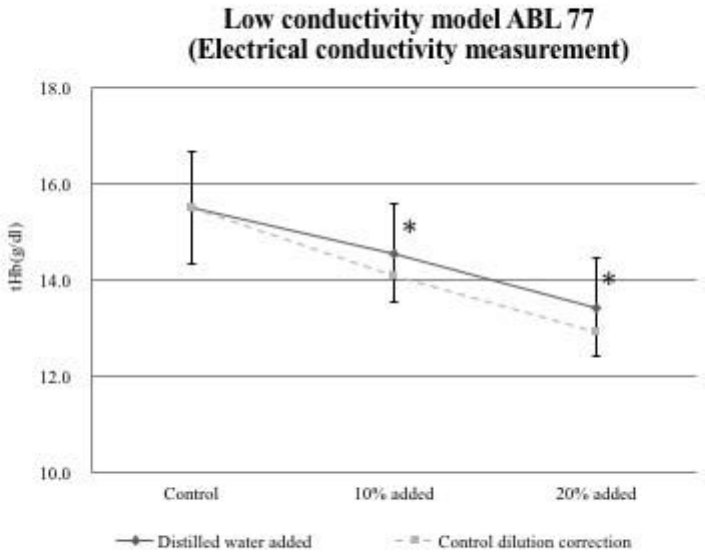

Figure 4

Effect of reduced the serum Na+ level by 10% and 20% on tHb. Mean tHb values differed between control and DW added on the conductivity 77.

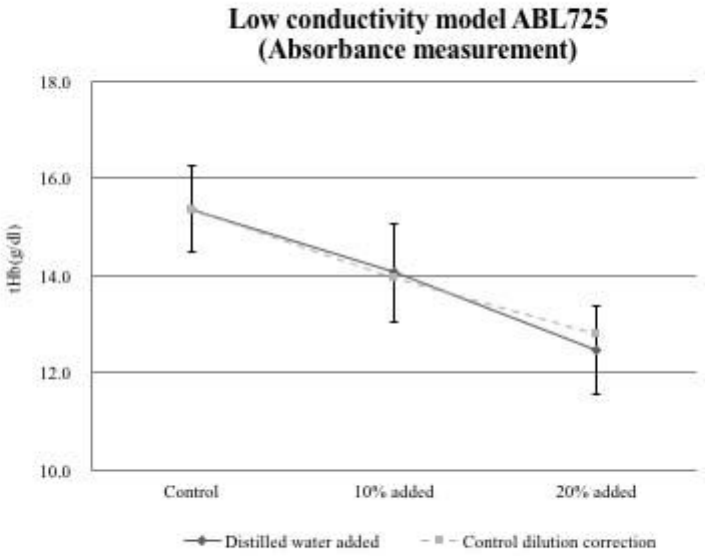

Figure 5

Effect of reduced the serum Na+ level by 10% and 20% on tHb. The absorbance 725 showed no difference in tHb values.

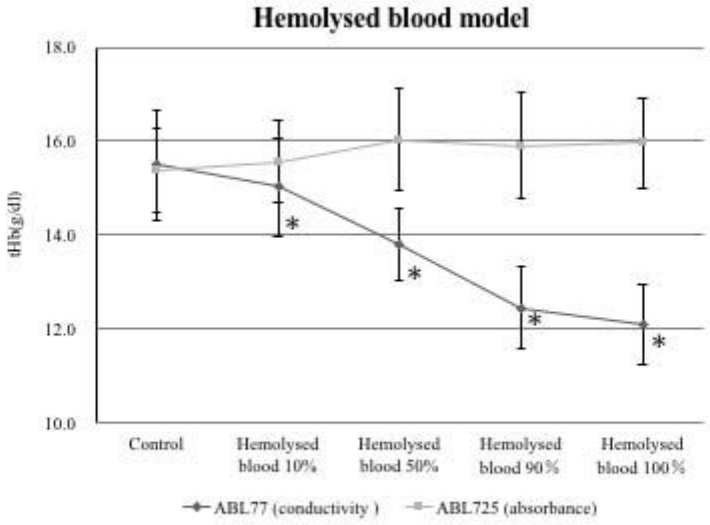

Figure 6

Effect of the hemolysis model (10, 50, 90 and 100% haemolysed) on tHb. Mean tHb values significantly differed between the absorbance 725 and the conductivity 77.

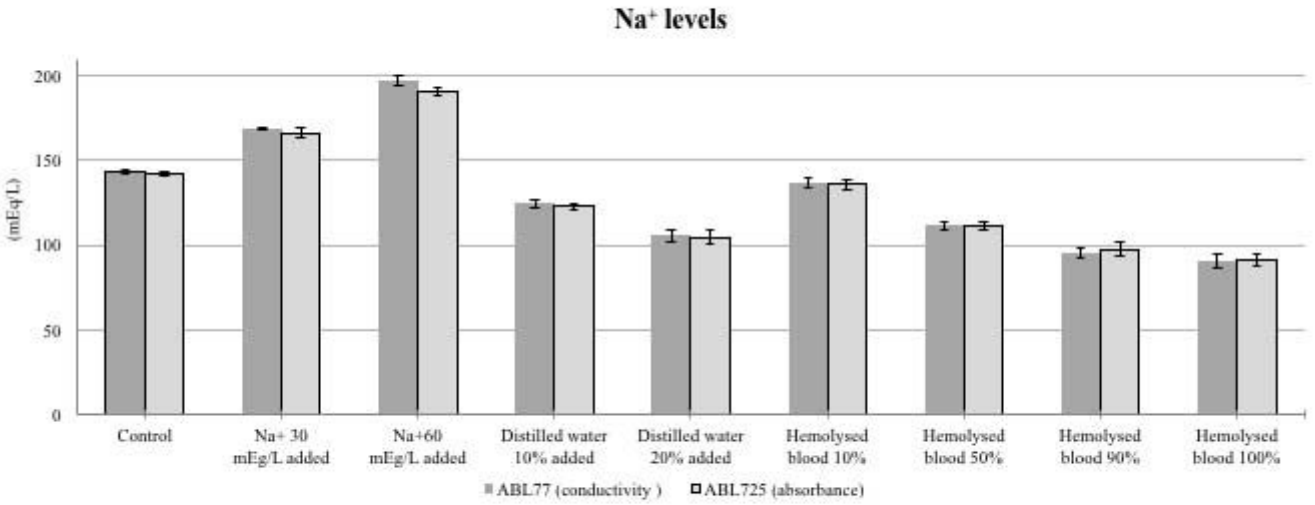

Figure 7

Serum Na+ levels among corresponding samples according to conductivity 77 and absorbance 725. The levels were approximately equivalent measurements in all states.