Research Article

Is liquid heparin comparable to dry balanced heparin for blood gas sampling in intensive care unit?

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Abstract

Introduction: Blood gas (BG) analysis is required for management of critically ill patients in emergency and intensive care units. BG parameters can be affected by the type of heparin formulations used-liquid heparin (LH) or dry balanced heparin (DBH). This study was conducted to determine whether blood gas, electrolyte, and metabolite estimations performed by using DBH and LH are comparable. **Materials and Methods:** A prospective study was conducted at pediatric intensive care unit (PICU) of a tertiary care hospital. Paired venous samples were collected from 35 consecutive children in commercially prepared DBH syringes and custom-prepared LH syringes. Samples were immediately analyzed by blood gas analyzer and compared for pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Cl^-, and lactate. Paired comparisons were done and agreement was assessed by Bland-Altman difference plots. The 95% limits of absolute agreement (LOA) were compared with the specifications for total allowable error (TEa). **Results:** The *P* values were significant for all measured parameters, with the exception of pCO_2 and K +. Bland-Altman difference plots showed wide LOA for pCO_2, pO_2, HCO_3^-, Na^+, K^+, and Cl^- when compared against TEa. For pCO_2, HCO_3^-, Na^+, K^+, and Cl^-, 40%, 23%, 77%, 34%, and 54% of samples were outside the TEa limits, respectively, with LH. **Conclusion:** Our study showed that there is poor agreement between LH and DBH for the BG parameters pCO2, pO_2, $HCO3^-$, K^+, Na^+, and Cl^- and, thus, are not comparable. But for pH and lactate, LH and DBH can be used interchangeably.

Keywords: Blood gas analysis, dry balanced heparin, liquid heparin, pre-analytic error

Access this article online

Website: www.ijccm.org

DOI: 10.4103/0972-5229.125428

Quick Response Code:

Introduction

Blood gas (BG) analysis is widely used in emergency and intensive care units for the management of critically ill patients. BG analysis performed at point of care or central laboratory requires whole blood samples with anticoagulation to avoid pre-analytic errors in BG parameters. Heparin has been the anticoagulant of choice for BG analysis.[1] Historically, liquid sodium heparin (LH) has been used for BG analysis. However, concerns about dilution effects of LH and increasing repertoire of tests in BG analysis have led to development of commercial syringes with dry balanced. Dry balanced heparin (DBH) of sodium, lithium or zinc formulations DBH should be used everywhere. Custom-prepared LH syringes are still used at our unit and at most of the intensive care units in resource-poor countries.

BG analysis is often used for electrolyte estimation, deducing anion gap, and to make other clinical decisions. In addition to various pre-analytic factors,[2] BG parameters are dependent on the type of heparin formulation, which can be LH or DBH. Both LH and DBH have different performance characteristics. Present-day BG analysis reports include blood gas, acid base, electrolytes, and other metabolites; however, most of the earlier studies[3-5] had compared DBH and LH syringes with respect to pH, pCO_2, and pO_2 only. We conducted this study to determine whether BG, electrolyte, and metabolite estimations performed by

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commercially prepared DBH and custom-prepared LH syringes are comparable without a risk of clinically relevant discrepancies.

Materials and Methods

The prospective study was conducted in consecutive cohort of children admitted to the pediatric intensive care unit (PICU) of a tertiary care hospital, and data collection was done in January 2013. Paired blood samples from 35 children were included in the study. The sampling was done as a part of clinical protocol in PICU in children in whom it was indicated on clinical grounds. Parental consent was taken before sample collection. Two types of syringes were used for collection of samples. Type-1 syringe was commercially prepared electrolyte balanced heparin syringe (PICO50; Radiometer, Copenhagen, Denmark) with 40 IU/ml of DBH. Type-2 syringe was self-prepared with LH. Type-2 syringe was a conventional 1-ml tuberculin syringe (DispoVan plastic Hindustan syringes and medical devices Ltd.; Ballabgarh, India) with needle (26 G; 0.5 inch) containing the smallest measurable division of 0.02 ml. Type-2 syringe was prepared by first filling the barrel of syringe until 1 ml marking and then flushing out all the LH solution and air four times so that no visible LH solution was left in the syringe barrel or hub.

LH solution derived from gut mucosa, containing 1000 IU/ml strength of heparin, was used for the preparation of Type-2 syringe (Gland Pharma Limited, Hyderabad, India). The analysis of LH solution by blood gas analyzer (BGA) revealed pH of 6.85, Na + of 15 mmol/l, and pO_2 of 150 mm Hg, as LH in vial and syringe gets in equilibrium with the air. Paired venous samples were collected in Type-1 and Type-2 syringes in a single sampling procedure. Sample was collected in Type-1 syringe as per the manufacturer's recommendations, and in Type-2 syringe till 1 ml mark. Sample collection was done at the bedside by a single trained resident of PICU. Sampling was done with free flow of blood to prevent hemolysis and formation of bubbles. Bubbles, if formed, were immediately removed and syringes were rolled between palms of hand to ensure uniform mixing of heparin and blood. Samples were analyzed immediately after collection by point-of-care BGA (ABL 800 basic; Radiometer) in PICU. The sequence of sampling and analysis among syringes was rotated. BGA calibrated automatically every four hourly with weekly quality check with control solutions. To check for test-retest precision of BGA, each sample was analyzed in duplicate and the coefficient of variation was calculated (CV %) for both syringe types. BGA showed excellent precision with CV % of < 1% for pH, HCO_3^-, lactate, and K + and < 3% for pCO_2, pO_2, Na^+, and Cl^-. For statistical analysis, we considered Type-1 syringe as reference because it is recommended for reliability by a previous clinical study.[6]

Sample size calculation

BG analysis includes multiple parameters and it is not possible to calculate sample size on the basis of a single parameter; however, sample size was calculated for pCO_2 measurements, because previous studies[3-5] showed that pCO_2 was the parameter most sensitive to dilution. Sample size was calculated based on Altman's normogram[7] assuming alpha of 0.05, power of 0.8, the required difference of 5.7% (total allowable error for pCO_2), and the assumed SD of differences of pCO_2 in paired samples of 5 mm Hg. The effect size came out to be 0.5. The calculated sample size was 33 paired samples. However, we took a convenient sample size of 35 paired samples.

Statistical analysis

BG parameters were expressed as mean and standard deviation (SD). Agreement analysis was done in accordance with Clinical and Laboratory Standards Institute (CLSI) document EP9-A2[8] using Analyse-it Method evaluation software. Paired comparisons were done using the Wilcoxon matched pair signed-rank test and P =< 0.05 was considered significant. Agreement was assessed by Bland-Altman difference plots;[9] mean bias and 95% limits of absolute agreement (LOA) were calculated. LOA of BG parameters were then compared with the specifications for total allowable error (TEa). TEa is the total amount of error in a test that is medically, administratively, or legally acceptable. Two methods are considered to give clinically equivalent results if results measured on the same specimen do not differ by more than TEa. The specifications for TEa [Table 1] were compiled by Ricos *et al.* from data on within-subject and between-subject biologic variation.[10] The proportion of samples with differences beyond TEa% was calculated.

Results

Mean, SD, and P values (Wilcoxon signed-rank test) of pH, pCO_2, pO_2, HCO_3^-, lactate, Na^+, K^+, and Cl^- by Type-1 and Type-2 syringes are shown in Table 1. The P values were significant for all measured parameters, with the exception of pCO_2 and K +. Bland-Altman difference plots showed wide LOA for pCO_2, pO_2, HCO_3^-, Na^+, K^+, and Cl^-, when compared against percentage TEa [Figures 1-7]. The proportion of samples outside TEa% for various parameters is shown in Table 1.

Table 1: Descriptive statistics, mean bias, *P* values, 95% limits of agreement, total allowable error, and proportion of samples beyond TEa%

Analyte	DBH (mean ± SD)	LH (mean ± SD)	Mean bias	P value	95% Limits of absolute agreement	TEa%	Proportion beyond TEa%
pH	7.429 ± 0.05	7.413 ± 0.055	−0.016	<0.0001	−0.057–0.025	3.9	0
pCO_2, mm Hg	39.52 ± 6.4	38.59 ± 5.8	−0.92	0.077	−6.62–4.77	5.7	14 (40%)
Bicarbonate, mmol/l	25.8 ± 4.1	25.3 ± 3.9	−0.55	<0.0001	−3.6–2.5	4.9	8 (23%)
pO_2	42.5 ± 24.11	37.6 ± 23	−4.9	0.001	−21.9–12	NA	-
Potassium, mmol/l	3.71 ± 0.53	3.67 ± 0.53	−0.04	0.109	−0.44–0.35	5.8	12 (34%)
Sodium, mmol/l	132.6 ± 7.9	129.5 ± 7.7	−2.9	<0.0001	−10.92–4.97	0.9	27 (77%)
Chloride, mmol/l	109 ± 9.7	107.1 ± 8.75	−1.9	<0.0001	−6.86–3.06	1.5	19 (54%)
Lactate, mmol/l	2.1±1.15	1.96±1.03	−0.15	0.004	−0.98–0.67	30.4	1 (3%)

Significant *P* values are mentioned in bold letters

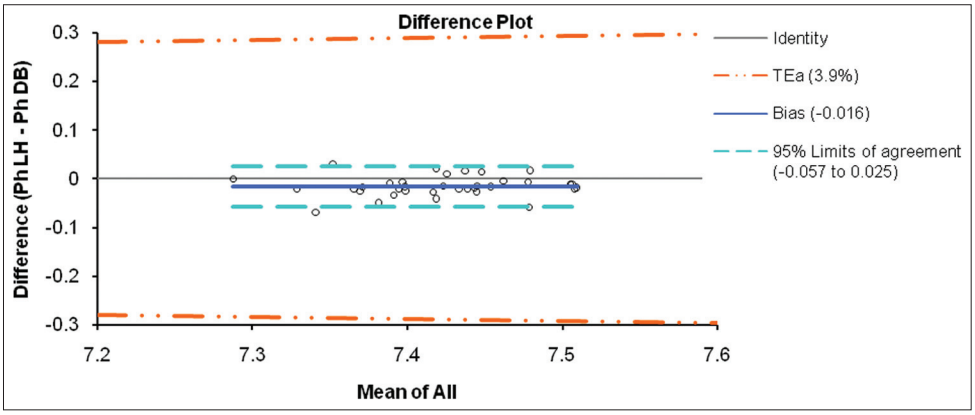

Figure 1: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for pH between liquid heparin and dry balanced heparin syringes

Discussion

Heparin affects BG parameters by direct binding and dilution effects.[1] The amount of heparin needed to prevent coagulation has been stated to be very low (1 IU/ml), but the actual amount needed for BG sampling is higher because of inadequate mixing of heparin with blood.[11] The World Health Organization (WHO) recommends minimum 8-12 IU/ml of LH formulation or 40-60 IU/ml of DBH formulation for adequate anticoagulation[12] LH readily mixes with blood[13] and is cost effective, when compared to commercial DB syringes. However, use of LH is prone to dilutional effects. DBH prevents dilutional effects, but a study on heparin release kinetics has demonstrated slower heparin release in DBH formulation.[13] Consequently, there is risk of micro-clotting leading to clinically significant errors, particularly for pH, pO_2, and pCO_2[14] and BGA malfunction. Although quantification of the exact amount of LH is not possible in custom-prepared syringes, we prepared LH syringe with the smallest achievable volume in a practical situation. We did not observe clotting in any of the samples, and hence, the amount of heparin in both types of syringes was sufficient to provide adequate anticoagulation.

We tested agreement for routine BG parameters between two types of syringes against TEa. Blood pH was statistically different between LH and DBH syringes, but LOA were narrow and no sample was outside TEa limits [Figure 1]. Syringes were comparable for pH estimation. Agreement between Type-1 and Type-2 syringes for both pCO_2 and HCO_3^- was poor with wide LOA; further, 40% and 23% of samples were outside TEa for pCO_2 and HCO_3^-, respectively [Figures 2 and 3]. Ordog *et al.*[3] and Hutchison *et al.*[4] used increasing concentration of LH and showed that pH did not change till 40-50% dilution of blood with pCO_2 and HCO_3^-, indicating an inverse relation with the volume of LH used. Heparin is an acidic solution, but the pH of blood is not affected because of the buffering effect of oxyhemoglobin and plasma proteins.[15] The effect observed on pCO_2 and HCO_3^- is not due to heparin salt *per se*, but represents the effects of fluid portion of heparin solution as addition of normal saline to whole blood has been shown to produce the same effects on pCO_2 and HCO_3^-.[5] While sampling for a BG analysis, LH with a relatively low pCO_2 gets mixed with whole blood having higher pCO_2 concentration. This results in pCO_2 decline which is proportional to the relative differences in the pCO_2 between blood and LH.[16] A

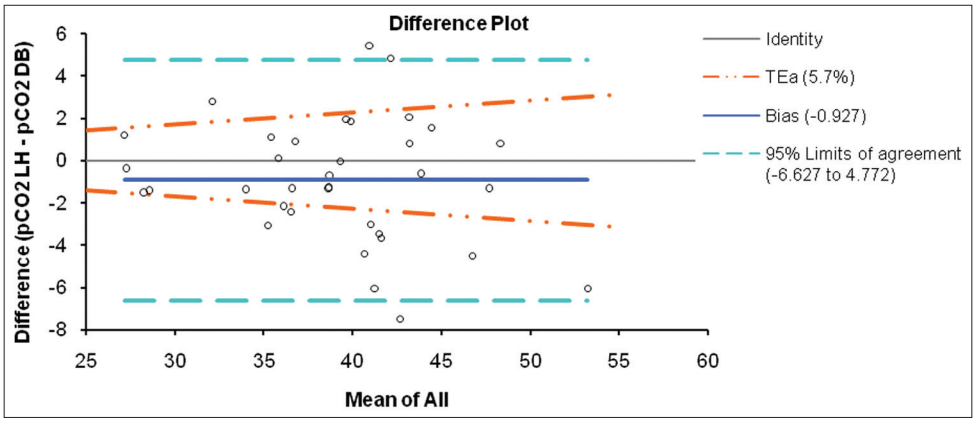

Figure 2: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for pCO_2 between liquid heparin and dry balanced heparin syringes

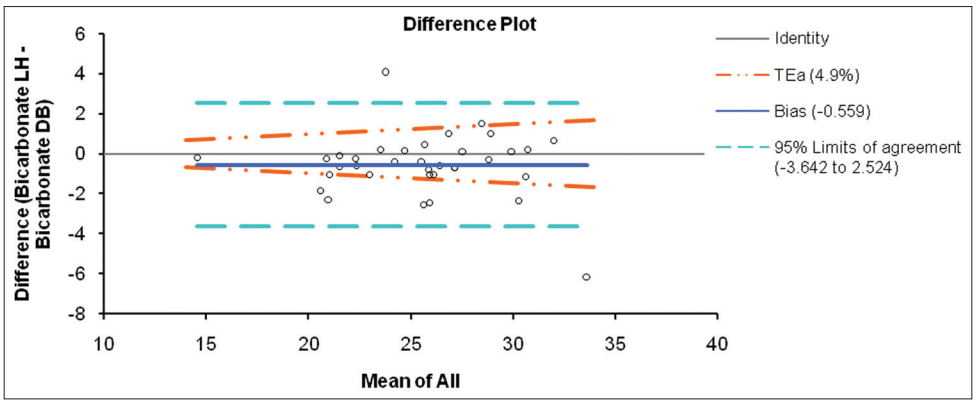

Figure 3: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for bicarbonate between liquid heparin and dry balanced heparin syringes

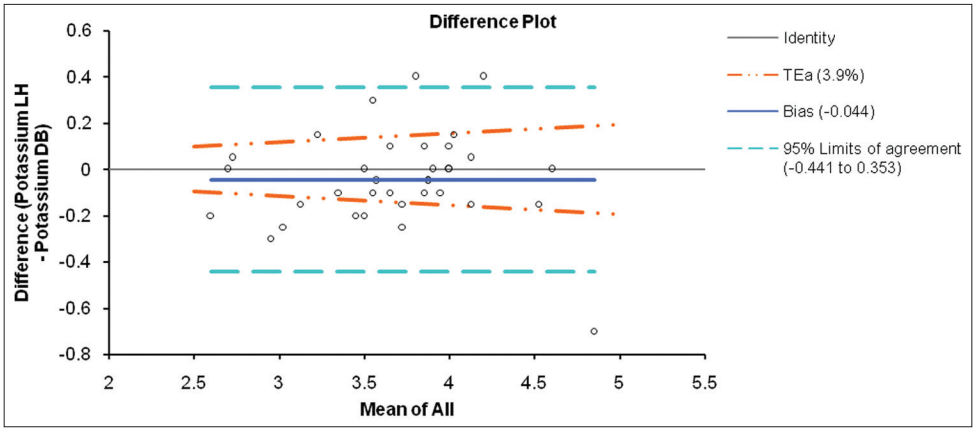

Figure 4: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for potassium between liquid heparin and dry balanced heparin syringes

neonatal study[17] comparing LH and DBH demonstrated that LH produces a reduction in pCO_2 while pH remains unaffected.

We observed very wide LOA for both Na^+ and K^+ and 77% and 34% of the samples were beyond TEa limits for these two parameters, respectively [Figures 4 and 5]. Previous studies[11,18,19] have shown that addition of heparin causes underestimation of positively charged ions due to direct binding and dilution of blood samples. Dilution effect is observed only in syringes with LH. We achieved smallest amount of LH in syringes, but even that amount resulted in error in substantial proportion of samples for both Na^+ and K^+. Literature has shown

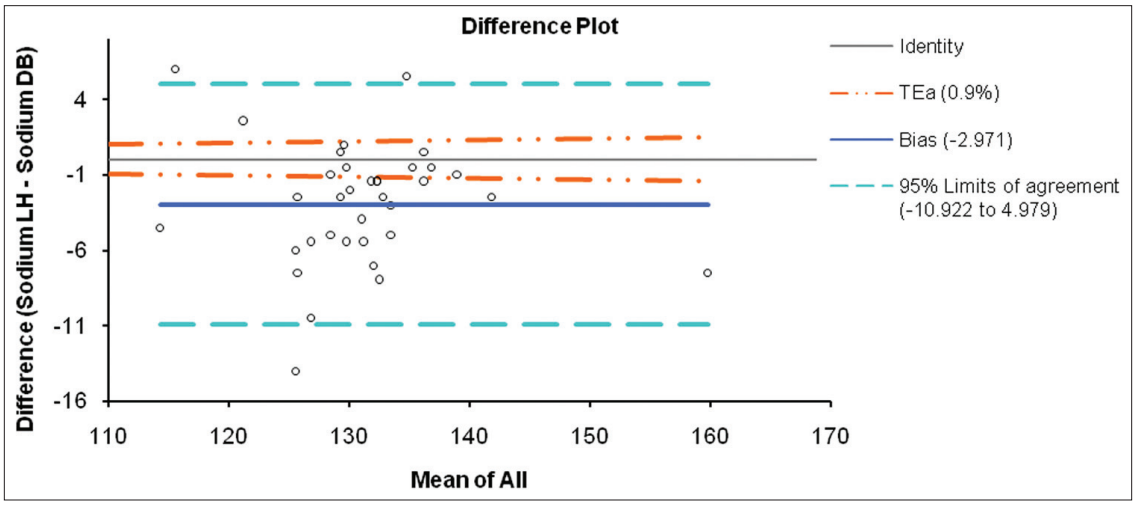

Figure 5: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for sodium between liquid heparin and dry balanced heparin syringes

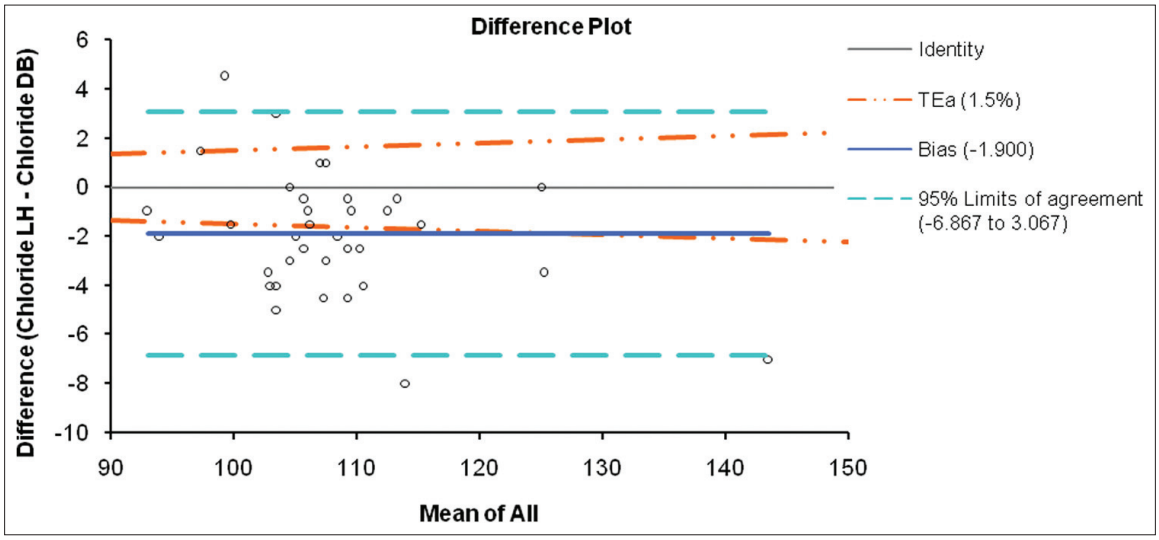

Figure 6: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for chloride between liquid heparin and dry balanced heparin syringes

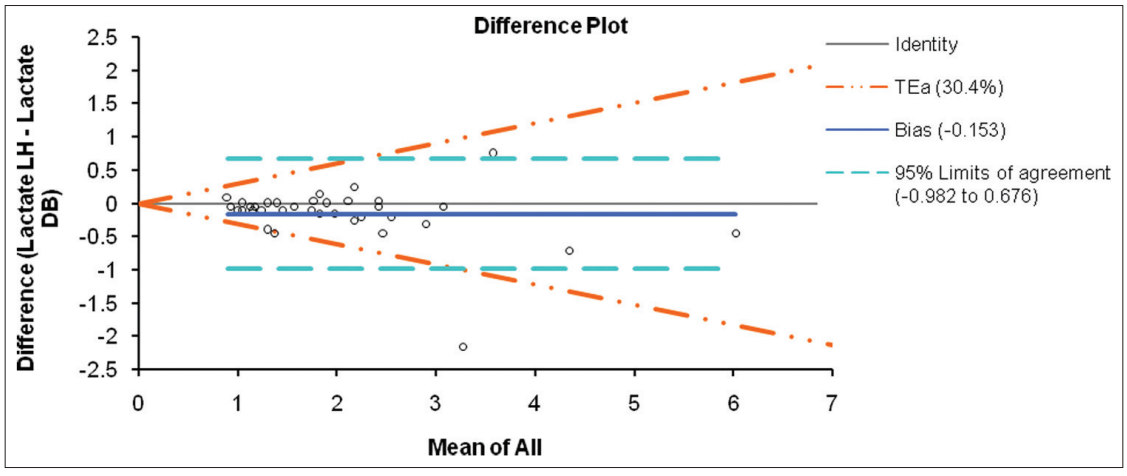

Figure 7: Bland-Altman plot showing mean bias, 95% limits of absolute agreement, total allowable error% for lactate between liquid heparin and dry balanced heparin syringes

significant underestimation for Na^+ and K^+ with LH. In an earlier study,[18] we showed that sampling with LH underestimates Na^+ and K^+. The amount of LH used in that study was higher; however, it reflected prevailing practice of the unit at that point of time. We observed poor agreement for Cl^- among two types of syringes and 54% of samples were outside TEa limits [Figure 6]. Findings of our study suggest that DBH and LH are not comparable for Cl^- estimation. BGA at our center does not have the facility for estimation of calcium and magnesium, so these were not compared in our study. However, as far as calcium and magnesium estimation is concerned, a previous study[19] has shown negative bias on calcium and magnesium estimation by LH. But Chantler *et al*.[20] found good correlation for magnesium between LH and DBH in their study.

Our study did not show any clinically relevant difference in lactate between DBH and LH syringes, with only one sample beyond TEa limits [Figure 7]. However, a delay in the processing of sample can increase the blood lactate levels due to effects of anaerobic metabolism. A delay of > 15 min at room temperature has been shown to cause significant lactate overestimation.[21] We analyzed both samples within 5 min of collection, and rotation of syringe sequence during analysis in BGA eliminated the possibility of time-related error in lactate estimation. The pO_2 is also relatively resistant to the dilution effect.[1] Some studies[17,22] showed no effect of LH on pO_2, while others have demonstrated increase in pO_2 at higher (40-50%) dilutions.[15] Increase in pO_2 noted at higher dilution was due to higher pO_2 levels in LH compared to blood.[16] The amount of LH used in our study was much less compared to earlier studies. We observed very wide LOA and negative bias for pO_2 in LH syringe. Oxygen is known to diffuse across the plastics[23] and both the syringes used in our study are made up of plastic material, so the difference cannot be explained based on dilutional effects of heparin alone.

When compared against TEa specifications for pCO2, $HCO3^-$, Na^+, K^+, and Cl^-, 40%, 23%, 77%, 34%, and 54% of samples were outside TEa limits, respectively, with LH and is not acceptable clinically. Although the mean values of pH and lactate were significantly different, the percentage of samples lying outside TEa was negligible, so they are acceptable clinically. Our study has clearly demonstrated that LH and DBH cause difference in the clinically important BG parameters and cannot be used interchangeably. This is a pilot study and has limitations, as it reflects the difference in one BGA only. Ideally, a multicentric study involving different BGAs could have reflected the difference better and would increase the generalizability of results. Further, we were not able to compare the errors in calcium and magnesium estimations due to nonavailability of estimation of these parameters in BGA at our center.

To conclude, our study shows that there is poor agreement for the BG parameters pCO2, pO_2, $HCO3^-$, K^+, Na^+, and Cl^- between LH and DBH syringes, as substantial percentage of differences was outside TEa limits. So, LH syringes are not comparable to commercially available DB syringes. It can lead to differences in assessment of acid base and electrolytes in individual patients and can confound AG calculations also. If the parameters of interest are pH and lactate, then these syringes can be used interchangeably.

Acknowledgment

We acknowledge the support provided by Radiometer, India for providing DBH syringes.

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How to cite this article: Chhapola V, Kumar S, Goyal P. Is liquid heparin comparable to dry balanced heparin for blood gas sampling in intensive care unit?. Indian J Crit Care Med 2014;18:14-20.

Source of Support: Nil, **Conflict of Interest:** None declared.