THE IMPACT OF DIFFERENT VOLUMES OF HEPARIN ON PH, PCO2 AND HCO3- READINGS DURING BLOOD GAS SAMPLING

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Abstract

Background: Mistakes in blood gas sampling influence the credibility of blood gas readings. Volume of heparin used as anticoagulant may change readings through its dilutional direct binding and compositional actions.

Objective: To evaluate the influence of various volumes of heparin during blood gas sampling on PH, PCO2 and HCO3- readings.

Methods: Our double blind, randomized and prospective investigation included 105 adult patients, of both sexes, aged 34-46 years and admitted at our intensive care unit, King Hussein hospital, King Hussein medical center, Amman, Jordan, during the period Feb.2013-Aug.2015, after obtaining written informed consent from all guardians and approval from the Royal ethical and research committee. Patients were divided randomly into three groups. Group I patients (n=35) received heparinized syringe for blood gas sampling using 25 IU of heparin. Group II patients (n=35) received heparinized syringe for blood gas sampling using 50 IU of heparin. Group III patients (n=35) received heparinized syringe for blood gas sampling using 100 IU of heparin. A total volume of 1 ml blood in each syringe was received and analyzed by blood gas analyzer. Statistical analysis was performed using Friedman’s test and Wilcoxon test.

Results: There was a significant discrepancy regarding the readings of pH, PCO2 and HCO3- in the three syringes (P < 0.05). The PCO2 and HCO3- readings reduced with the increasing volume of heparin.

Conclusion: Least heparin used during blood gas sampling is most suitable for assessing PH, PCO2 and HCO3-. The technique of blood gas sampling should have a standard protocol regarding volume of heparin used.

Introduction

Blood gas analysis has a crucial importance in evaluating changes in respiratory and metabolic elements. Credibility of these findings is highly valuable in the management of critically ill patients. Blood gas sampling performed in the intensive care unit is not collected adequately causing wrong blood gas readings, influencing negatively the management. There are some mistakes during the collection of samples for blood gas analysis (1). Absence of a standard protocol for collection of blood sample for blood gas analysis includes the use of various volume syringes, various blood sample volumes and the use of different volumes of heparin causing different dilution of blood samples. The capillary blood is clinically comparable to arterial blood only if PH and PCO2 are requested. The International Federation of Clinical Chemistry (2) indicates the use of pre-prepared dry-balanced heparin syringes for blood gas analysis. Liquid heparin is used in different intensive care units for financial issue. Liquid heparin as anticoagulant in samples for blood gas may influence blood gas elements by its dilutional, direct binding and
compositional actions (2). It is indicated that the volume of liquid heparin must be 5% (50 mg/ml) or less and obviously less than 10% (100 mg/ml) to avoid dilutional action, as it is well known that 1 mg sodium heparin is equivalent to 100 units heparin (3).

The objective of this investigation was to assess the influence of different doses of heparin during blood gas sampling on PH, PCO2 and HCO3⁻ in intensive care unit in our Jordanian population.

Methods
Our double blind, randomized and prospective investigation included 105 adult patients, of both sexes, aged 34-46 years and admitted at our intensive care unit, King Hussein hospital, King Hussein medical center, Amman, Jordan, during the period Feb.2013-Aug.2015, after obtaining written informed consent from all guardians and approval from the Royal ethical and research committee. Patients were divided randomly into three groups. In group I patients (n=35), a heparinized syringe was used for blood gas sampling using 25 IU of heparin. In group II patients (n=35), a heparinized syringe was used for blood gas sampling using 50 IU of heparin. In group III patients (n=35), a heparinized syringe was used for blood gas sampling using 100 IU of heparin. A total volume of 1 ml blood in each syringe was received and analyzed by blood gas analyzer for PH, PCO2 and HCO3⁻ variables.

Blood gas analysis has multiple parameters and it is not optimum to measure sample size according to a single parameter. Samples were taken only in patients in whom blood gas analysis was clinically indicated. Three blood samples were taken from each patient in pre-prepared heparinized syringes. Samples were taken with 1 ml syringe having the smallest measurable division of 0.02 ml. Sodium heparin solution, containing 1,000 IU/ml of heparin was used for anticoagulation. Syringes were prepared immediately before the sampling. The arterial blood specimens were taken during the single sampling technique to achieve a total volume of 1 ml in each of three syringes. Group I syringe was the control for all comparisons because it had the least volume of heparin and least potential of mistake.

Statistical analysis
Statistical analysis was performed using Friedman’s test and Wilcoxon test. Mean percentage bias (the difference between means of a parameter is divided by the average of two means and multiplied by 100) was determined and P < 0.05 was considered significant.

Results
There were no significant differences between the three groups in terms of age and gender. The overall age range of the whole study group was 34-46 years with a mean age of 41.5 years. The age range was 36-44 years with a mean age of 39.5 years in group I, an age range of 35-45 years with a mean age of 40 years in group II and an age range of 36.5-45.5 years with a mean age of 40 years in group III. Overall males percentage was 48.6%(51) and overall females percentage was 51.4%(54). The percentage of males was 31.4%(16), 37.2%(19) and 31.4%(16) in groups I, II and III, respectively. The percentage of females was 35.2%(19), 29.6%(16) and 35.2%(19) in groups I, II and III, respectively.

Table I. The calculated P values were not significant for PH, PCO2 and HCO3⁻ values while comparing each variable in each group with the other (Table II). The comparison between group I and group II, group I and group III, group II and group III syringes are demonstrated in Table III. Comparison of observed mean % bias with desirable % bias is presented in Table II. The observed bias was more than desirable % bias for PCO2 and HCO3⁻ values. The heparin in vial and syringe are in equilibrium with the air, and analysis of heparin solution by blood gas analyzer showed a pH of 6.85. Before starting the main study, we calculated the Coefficient of variation for PH, PCO2 and HCO3⁻, it was 0.06, 3.99 and 4.39%, respectively.
Discussion

Most causes of mistakes in blood gas analysis occur during sampling (1). Causes include hemolysis, variation of temperature, plastic syringes, air bubble, late sample transport, dilution impact of heparin, various volumes of blood in syringes, micro-clotting in the sample and mistakes in blood gas analyzer machines (1). In our investigation, we used syringes with 25 IU heparin (Group I) and 50 IU heparin (Group II). Using 100 IU of heparin in a syringe was practiced in group III. Increased heparin to blood ratio can cause increased heparin concentration and blood dilution. The minimal volume needed by the blood gas analyzer for the analysis is 0.3 ml. It is a frequent error to use a lesser volume for analysis, having an increased concentration of heparin. Sampling small volume of blood with high volume of heparin caused the same problem as ours (4). Four different sample volumes in plastic heparin balanced syringes were compared and shown that reduced sample volumes were correlated with significant bias in blood gas analysis (5). We varied the volume of heparin and maintained the volume of blood constant. Immediate analysis of samples collected in plastic syringes with no requirement of ice storage and storage of delayed samples in glass syringe was demonstrated in other work (6). Pre-heparinized syringes prepared could be a cause of difference in results (7).

Same plastic syringes and heparin preparation were analyzed immediately after collection in our investigation. For exact measurement of PCO2, frothing and removal of all air bubbles within 2 min of sample collection should be avoided (8). Bubble formation was handled by taking a free flow arterial sample and by removing any formed bubbles immediately.

Taking free flow sample decreased the potential of hemolysis. Micro-clotting was not found in any sample and blood gas machine was adjusted periodically. The only potential cause for bias in our study could be the presence of high volume of heparin. Results of our investigation showed no significant P values for mean PH, PCO2 and HCO3 readings. The PCO2 and HCO3 readings demonstrated progressively increasing bias with the increasing volume of heparin. Hutchison AS, found that PCO2 and HCO3 were inversely associated with the volume of heparin used and more than 10% dilution was correlated with a decrease in PCO2 and HCO3 (9). Changes in PCO2 and HCO3 influence the metabolic and respiratory elements of acid-base readings causing unexplained respiratory alkalosis and metabolic acidosis in the same time (10). The difference in PH between group I and group II was less than desirable bias but significant (Table II). Although heparin is an acidic solution, blood pH was not changed until 40% dilution due to the buffering ability of blood (9). In our investigation, the recorded bias was more than the desirable bias given by Ricos et al. (11) for PCO2 (Table II). Test and retest accurate achievement of the analyzer before commencing the observed bias to effects of heparin was done. The observed significant bias in readings of pCO2 and HCO3 were due to the action of heparin only. The three affected parameters are all important for measurement of acid base balance. Modifications in these parameters may have an important impact on the accuracy of acid base measurements (12). International Federation of Clinical Chemistry indicated blood collection up to 20 times the heparin volume (3).

Exact heparin concentrations in blood gas analysis are important for anticoagulation. Inadequate heparinization may cause clotting leading to wrong test results, machine malfunction and unneeded repeated sampling. Excess heparin can be a cause of mistake by the dilution and direct binding actions of heparin. Optimum volume of blood collection is important because smaller blood volumes can cause mistakes by dilution.

Chhapola et al.(4) showed that there was a significant difference in readings of PH, PCO2 and HCO3 in the three syringes in which they used minimal, 60 IU heparin and 120 IU heparin. They demonstrated that the PCO2 and HCO3 levels reduced with the increasing amount of heparin. There were limitations in our study. First, the group study included relatively small number of adult patients. Other studies should include larger numbers with children. We used 25, 50 and 100 IU heparin in our study. We think that less and higher amounts must be used to clarify our statement. This is the second limitation. Our protocol must be standardized to omit bias and other minor mistakes. This is the third limitation.
Conclusion
Minimal heparin had the least impact on blood gas parameters with no clotting. There is a demand for standardization of the technique of syringe preparation and blood sampling for blood gas analysis. Excess of heparin influences different parameters of blood gas by its dilution and direct binding actions.

References
### Table I. Demographics.

<table>
<thead>
<tr>
<th></th>
<th>G I</th>
<th>G II</th>
<th>G III</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Age (yr) mean/range</td>
<td>39.5/36-44</td>
<td>40/35-45</td>
<td>40/36.5-45.5</td>
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<tr>
<td>Sex (no, %) M</td>
<td>16(45.7%)</td>
<td>19(54.3%)</td>
<td>16(45.7%)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>19(54.3%)</td>
<td>16(45.7%)</td>
</tr>
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</table>

### Table II. Results comparison between the groups.

<table>
<thead>
<tr>
<th>element</th>
<th>Group I</th>
<th>GROUP II</th>
<th>Group III</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPARIN</td>
<td>25 IU</td>
<td>50 IU</td>
<td>100 IU</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PH</td>
<td>7.347+/-0.084</td>
<td>7.36+/-0.078</td>
<td>7.36+/-0.073</td>
<td>&gt;0.05</td>
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<tr>
<td>PCO2(mmHg)</td>
<td>51.39+/-14.99</td>
<td>45.29+/-13.09</td>
<td>42.29+/-10.89</td>
<td>&gt;0.05</td>
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<tr>
<td>HCO3(mmol/l)</td>
<td>27.39+/-7.69</td>
<td>25.69+/-7.19</td>
<td>24.39+/-6.59</td>
<td>&gt;0.05</td>
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</table>

### Table III. Recommended %bias of blood gas elements.

<table>
<thead>
<tr>
<th>element</th>
<th>Desirable bias %</th>
<th>Group I and group II</th>
<th>Group I and group III</th>
<th>Group II and Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean bias %</td>
<td>P</td>
<td>Mean bias %</td>
<td>P</td>
</tr>
<tr>
<td>PH</td>
<td>1.8</td>
<td>0.16</td>
<td>&lt;0.05</td>
<td>0.16</td>
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<tr>
<td>PCO2(mmHg)</td>
<td>1.8</td>
<td>11.33</td>
<td>&lt;0.05</td>
<td>18.4</td>
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<tr>
<td>HCO3(mmol/l)</td>
<td>----</td>
<td>5.3</td>
<td>&lt;0.05</td>
<td>10.48</td>
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