Analytical Comparison between Spectrophotometer and Portable Glucometer for Measurement of Blood Glucose in Horse

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ABSTRACT

Objective: Analytical methods comparison for the determination of blood glucose are essential in clinical laboratory practice as it improves the quality of health care through accurate and reliable clinical decision making. Therefore, the study was done to assess the analytical performance between point-of-care glucometer named easy touch glucose monitoring system and spectrophotometer named EMP-168 biochemical analyzer for blood glucose determination in horse.

Results: Twenty paired samples from horses visiting SPANA clinic of college of veterinary medicine and agriculture Addis Ababa University was used. Data obtained from both instruments was compared. The results showed that the mean value of blood glucose concentrations were higher in glucometric method (106 mg/dl) than the spectrophotometric (73 mg/dl) with the calculated t-statistic significantly different p-value of 0.000. This study showed the clinical inaccuracy of the glucometer over the spectrophotometer.

Keywords: Glucometer; Glucose; Spectrophotometer

INTRODUCTION

Glucose is the most abundant sugar that serves as principal source of energy for tissues except during prolonged fasting [1]. Glucose can be rapidly increased as a result of the absorption from ingested food [2]. The body naturally tightly regulates blood glucose concentration as part of metabolic hemostasis [3]. In human medicine morbidity and mortality are associated with hypoglycaemia and hyperglycaemia [4]. Comparative examples of hypoglycaemia and hyperglycaemia are reported in veterinary medicine as illustrated in the season of diagnosis in neonatal foals [5]. It is important to have accurate methods of glucose determination to recognize glycaemia. On top of accuracy, rapid monitoring of glucose is of significant importance in glycemic control [1,6]. Point-of-care glucometers (POCG) have now found a wide range of applications in medicine both for diagnostic and management of glycaemia [7].

POCG are used in both animals and humans. The convenience, viability with little blood volumes, and low turnaround time as contrasted with biochemical analyzers settle POCG an advantageous for determining blood glucose concentration [8,9]. For accuracy, glucose levels from the same specimen would ideally be compared by analysis on the glucometer and by comparative method [7]. In veterinary medicine, biochemical analyzers using enzymatic methods like hexokinase or glucose oxidase are considered as the standard method for blood glucose assay [10]. However, these methods require large sample volumes, costly and high turnaround time [11]. It has been claimed that glucose determination with a POCG are helpful to improve glycemic control [8]. Different POCG are advertised in market; in any case, most of them are intended for use in humans [11,12].

There is limited data on analytical comparison of the POCG and spectrophotometers; particularly in Ethiopia there is no data. Therefore the present study is aimed to give baseline information about the analytical performance of POCG as compared to spectrophotometer. In this regard the proposed study together with other similar studies will have implication in veterinary medicine.

MATERIALS AND METHODS

Study design, area and period

A cross-sectional study was conducted, in the SPANA operation area in Adea district, Oromia, Ethiopia. The charities operate under the college of veterinary medicine of Addis Ababa University. The...
study aims to compare the analytical performance of glucometer over spectrophotometer for determination of glucose in horse and it was conducted from April 2019 to May 2019.

Sample size determination and sampling techniques
Most authors recommend forty paired patient samples in the method comparison experiment [13]. However by considering the resources and time only twenty paired samples are included in this study.

Laboratory analysis
Sample collection and processing: Blood sample from jugular vein obtained using plain vacationer tubes from horses brought to charities SPANA veterinary clinic for clinical diagnosis was utilized for the research. Aliquot of sample was used for determination of glucose by glucometers and serum from same sample was utilized for spectrophotometric analysis. Samples taken from animals on any treatment during the study were excluded due to possible impact on glucose measurement.

Determination of glucose by spectrophotometer
The spectrophotometer used for the study was EMP-168 biochemical analyzer Chengdu Empsun medical technology co. LTD China. Glucose in the sample was oxidized by glucose oxidase to produce gluconate and hydrogen peroxide (H₂O₂). H₂O₂ is then oxidatively coupled with 4 amino-antipyrene (4-AA) and phenol in the presence of peroxidase to yield a red quinoneimine dye that is measured at wavelength of 546 nm proportional to concentration of glucose in the sample.

Determination of glucose by glucometer
The glucometer used for the study was Easy touch glucose monitoring system MHC medical products LLC Taiwan. The instrument utilizes test strips impregnated with glucose oxidase. When blood is applied to the area a chemical reaction takes place, then a transient electrical current is formed. This reaction creates a harmless electrical current that the meter interprets as a glucose concentration in mg/dl. The system was calibrated with standard solution containing known glucose concentration.

Quality assurance
Serum was separated within 30 minutes and prior to analysis serum was placed at -20°C. Glucometer and biochemical analyzer were calibrated using calibrator and quality control samples were run before running samples for tests.

Data management and statistical analysis
The data was entered and coded in Microsoft Excel spreadsheets (Microsoft Corporation) then transferred to statistical software for analysis (SPSS Version 20). The results are presented in mean, standard deviation (SD) for both glucometers and spectrophotometric method. T-test was used to compare means and statistical association was assessed by p-value. Correlation coefficients were generated with the Pearson correlation plot to evaluate the relation between glucometers and spectrophotometric method.

RESULT
A total of 20 paired glucose results obtained from horse blood were analyzed in this study. It was found that the mean value of blood glucose concentrations were higher in by the glucometer (106.9 mg/dl) than by the spectrophotometric method (73.92 mg/dl) (Table 1). In addition results of t-test analysis revealed a statistically significant difference (p<0.00) between the mean data pairs of overall measurements between glucose measurement by glucometer and spectrophotometer (Table 2).

In this output, it can be seen that the sample mean of the difference scores is 32.97, with a standard deviation of the differences given by 25.69. The calculated t-statistic (with 19 df) is given by 5.74, which has a p-value of 0.000. When interpreting these results, it is noticed that the mean of the two measurement differences is not zero, which is supportive of the H₀ that μ≠ 0. The p-value given in the computer output is p=0.000. That is, at α=0.05 level, H₀ is rejected and conclude that the glucometer is different from the spectrophotometric measurement. The output also includes a 95% confidence interval on the mean difference. In this case, the confidence interval (20.95, 45.00) doesn’t contain zero. This suggests that μ ≠ 0 (thus the glucometer cannot substitute the spectrophotometric measurement of glucose.

Correlation of glucose measurement by glucometer and spectrophotometer
Pearson correlations also revealed a weak correlation value (r=0.233) between glucose measurement by glucometer and measurements of blood glucose by spectrophotometer with p=0.323 (Figure 1).

The correlation coefficient (r) measures the strength of a linear association between two variables and ranges between -1 (perfect negative correlation) and +1 (perfect positive correlation). A value of 0 indicates no linear relationship.

Table 1: Descriptive statistics of blood glucose by methods.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Glucometer (mg/dl)</th>
<th>Spectrophotometer (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>58</td>
<td>47.19</td>
</tr>
<tr>
<td>Maximum</td>
<td>178</td>
<td>95.36</td>
</tr>
<tr>
<td>Mean</td>
<td>106.9</td>
<td>73.92</td>
</tr>
<tr>
<td>Mean SE</td>
<td>5.78</td>
<td>2.53</td>
</tr>
<tr>
<td>SD</td>
<td>25.85</td>
<td>11.31</td>
</tr>
</tbody>
</table>

Figure 1: Pearson correlation plot for Glucose.
negative correlation) to 1 (perfect positive correlation). Accordingly the results of our study revealed correlation value of 0.233 which is a weak correlation as per Cohen’s the interpretation of a correlation coefficient (0.3 to +0.3=weak correlation) [14].

**DISCUSSION**

This study was designed to evaluate glucometers in a clinical setting. Studies performed in a clinic setting are more informative than those conducted in a controlled laboratory setting because they are more relevant to the point-of-care environment and field conditions [15]. According to our study the mean glucose measured from glucometer was significantly different from the mean glucose measured in serum by the biochemical analyzer (p<0.000). In this study there were higher mean glucose values for glucometer as compared to spectrophotometer. Similar studies found the mean blood glucose concentration measured in whole blood by using in glucometer was greater than that on the biochemical analyzer [16].

Pearson correlation coefficients showed a weak positive correlation between glucose measured by glucometer and by the biochemical analyzer. These r values (0.233) suggest that a correction equation could not be derived for the glucometer as it yielded values significantly different from the biochemical analyzer. A similar study in adult horses admitted on emergency basis, whole blood glucose measurements by glucometer designed for human use were poorly correlated with the biochemical analyzer [5]. On contrary to this study Pearson correlation coefficients (r) for different glucometers (r=0.98; p<0.0001, r=0.96; p<0.0001, r=0.98; p<0.0001, r=0.98; p<0.0001 and r=0.98; p<0.0001) showed strong positive correlation for each glucometer and the biochemical analyzer [16]. The reasons for differences in glucometer accuracy between whole blood and spectrophotometer is that glucometer designed for human has an impact on glucose measurement as the distribution of glucose between plasma and RBC varies between animal species [17].

**LIMITATIONS**

The study had certain limitations. A fundamental limitation is that the left over sample blood sample was taken from animals visiting clinic and was selected on the basis of owner’s willingness to participate in the study. Furthermore, there was shortage of resources for increasing the sample size and other models of glucometers for comparison.

**CONCLUSION AND RECOMMENDATIONS**

This study showed the clinical inaccuracy of the glucometer and concluded that glucometer used in this study was not reliable for clinical decision making. Even though the study does not give reliable result by glucometer as compared to spectrophotometer, we cannot decide glucometer is inaccurate and is not clinically acceptable for monitoring blood glucose concentrations. Veterinarians and laboratory technologists need a certain level of confidence in the results of glucometers, with the high level of agreement between the spectrophotometric methods. Therefore based on the above conclusion large sample size to validate the analytical capacity to of glucometers and also a need to evaluate different models of glucometers against spectrophotometer is recommended.

**DECLARATIONS**

**Ethics approval and consent to participate**

Owner of the study animals visiting for clinical diagnosis were asked for their consent and when agree upon was the leftover sample was utilized for the study.

**Consent to publish**

All authors read and agreed to publish the manuscript

**Availability of Data**

The data that support the finding of the study are available in the manuscript as supplementary materials

**Funding**

The research was funded by Department of Biomedical Sciences, College of Veterinary Medicine, and Addis Ababa University

**Competing Interests**

The authors declare that they have no competing interests.

**Authors’ Contributions**

Study conception and design: YCM; Sample collection: NTJ; Provision of glucometer and strips: YCM; Performing the experiments: YCM and NTJ; Drafting of manuscript: NTJ and YCM; Analysis and interpretation of data: NTJ and YCM.

All authors participated in critical appraisal of the manuscript. All authors read and approved the final manuscript. NTJ used the data for partial fulfillment of the requirements of the BSc degree in Veterinary Laboratory Technology.

**ACKNOWLEDGEMENTS**

We would like to express our sincere gratitude to Department of Biomedical Sciences, College of Veterinary Medicine, and Addis Ababa University for allowing us to use laboratory resource. This study would not have been completed without the support of SPANA especially Mr. Tibebe Ashene for his contribution in specimen and data collection during the fieldwork.

**REFERENCES**