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Capillary Blood Glucose and Venous Blood Glucose Measured with Portable Digital Glucometer in Diabetic Dogs

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Abstract

This study aimed to compare the glycemic values obtained with a glucometer with those determined by a colorimetric enzymatic assay in venous blood as well as to evaluate the possibility of using capillary blood samples of dogs with diabetes mellitus. A group with 30 diabetic dogs was formed and from each dog three blood samples were obtained for glycemic evaluations by different methods and blood collection sites. The mean glycemic values showed no significant difference between the different sites of blood collection and methods (P=0.90). Venous, pinna and carpal pad blood glucose showed excellent correlation with the colorimetric enzymatic assay (r=0.98; r=0.95 and r=0.96 respectively) and the obtained values fit properly the clinically acceptable intervals in the error grid analysis. The present study revealed that carpal pad, venous and pinna glucose measurements are clinically acceptable and this method is feasible for use in hospitalized diabetic dogs. The sample attainment of carpal pad proved to be effective and a viable alternative. Further work is necessary to assess the utility of this technique in a home environment.

Key Words: glycemia, venous blood sample, pinna, carpal pad, *diabetes mellitus*.

Introduction

The attainment of information about blood glucose concentration is an important parameter for the establishment of many diagnoses and vital therapeutic decisions in veterinary medicine (5). Situations as monitoring diabetic patients or patients with other endocrine diseases, obese or anorexic animals, hospitalized, intoxicated patients or those in emergency procedures usually require continuous glycemic measurement in small animal clinics.

Historically, before 1975, the routine blood glucose monitoring in patients consisted only in urinary determination (9). Only around 1980 glucose monitoring using blood samples replaced tests that used urine and was recommended as the standard method to be implemented in clinical routine (8).

The glucometers are devices developed to measure glycemia of capillary blood in humans, obtained through digital or heel puncture using a lance or hypodermic needle. They are automatic and easy to use and determine the blood glucose contents by means of chemical reactions calculated from arranging reagent strips impregnated with glucose oxidase, peroxidase and chromogeny (16).

Among the main advantages of a glucometer use are the small blood amounts required (1-5μL of blood), the quickness the results are given (around 5 to 25 seconds) and the low cost. The method's accuracy has been studied and the correlation with traditional methods is generally good once the blood collection and measurement techniques are correct (12, 16).

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The glycemic determination and performance of glycemic curves are important features in the treatment of diabetic dogs. These measurements are used in order to verify the efficiency of insuline therapy and to serve as a base for future treatment modifications. When performed in the veterinary private practice or hospital, it requires that the patient remains in a non-familiar environment for hours, what could affect the glucose levels due to stress and alterations in food intake during the test (4).

Due to the viability, veterinarians have used venous blood to determine glycemia with the glucometer in dogs, although the devices were developed for measuring blood glucose in human capillary blood (19). Alternatively, it has been tried to find different capillary blood sites of easy access for drawing blood and that could also be performed by diabetic dogs owners in order to achieve the best monitoring of those patients (1, 4, 11).

The goals of the present study were to compare blood glucose concentrations measured on samples from venous blood, pinna capillary blood and carpal pad interface of diabetic dogs with a portable blood glucometer. We therefore set out to investigate the clinical value and accuracy of blood glucose measurements with a semi-automated chemistry analyzer obtained by a laboratorial colorimetric enzymatic assay, described by Trinder (18).

Material and methods

The experiment was performed at the Veterinary Teaching Hospital "Governador Laudo Natel" (HVGLN) of São Paulo State University (UNESP), campus of Jaboticabal after approval by the local Ethics and Welfare Committee (CEBEA) (Protocol number 003428/10).

The experimental group was composed of 30 diabetic dogs undergoing treatment at the HVGLN. After the authorization given by the animal's owner or responsible, blood samples were drawn from each patient for glycemic analysis. From the same sample, one drop of blood was used to measure glycemia with the portable digital glucometer. Soon after, capillary blood samples from pinna and carpal pad interface were taken to measure glycemia using the digital glucometer. All the blood samples from the studied groups were obtained by an expert veterinarian in order to avoid possible mistakes during blood collection and measurement using the glucometer.

As a rule, the first glycemic measure using the glucometer (1) would be taken from venous blood (approximately 1.0 µL), before sodium fluoride addition, which was performed by pressing softly the syringe's piston and the resultant drop of blood was put at the proper place of the reagent strip, previously set into the glucometer, according to the fabricant recommendations. Continuously, the venous blood left was put into test tubes containing the enzyme inhibitor sodium fluoride (2) and immediately sent to the Veterinary Clinical Pathology Laboratory of HVGLN and processed for measuring glycemia using the colorimetric enzymatic assay, previously described by Trinder (18).

The other dosages performed from the same dog using the glucometer were from capillary blood samples drawn from the pinna and carpal pad interface (Figures 1A-1D). After the blood drop formation, it was instantly absorbed by the reagent strip and in 5 seconds blood glycemia was measured. As a way to ease the sample collection, a soft pressure in the proximal portion of the pinna was made, as well as a strangulation in the proximal portion of the animal's arm, moments before blood collection was performed (Figures 1A and 1C).

We highlight that the detection range of Accu Check® Go glucometer is from 10 to 600 mg/dL and that the glycemic value at the semi-automatic system LabQuest® is linear to 500 mg/dL. When the obtained values were equal or higher than 500 mg/dL, the sample was diluted in saline (150 mmol/L) and the result was multiplied by the dilution factor (adjusted to fit between 80 and 200 mg/dL). Both systems were properly calibrated previously following the manufacturer recommendations.

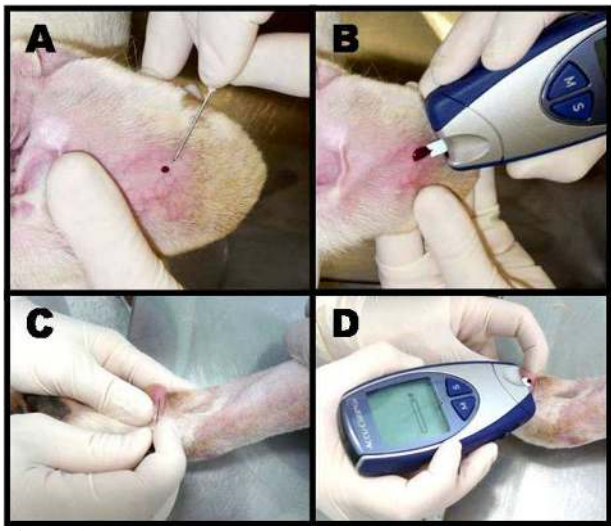


Figure 1. Illustration of the procedures adopted to draw capillary blood through puncture in the pinna and carpal pad interface. **(A)** Puncture of pinna with hypodermic needle 25 x 0.7 mm. **(B)** Drop of blood resultant from the auricular puncture being absorbed by the reagent strip set into the glucometer Accu-Chek® Go. **(C)** Carpal pad's puncture with hypodermic needle 25 x 0.7 mm. **(D)** Capillary glycemic measurement of carpal pad using the digital glucometer Accu-Chek® Go.

The glycemia values obtained by these methods and collection sites were recorded and the means from each group were compared with the one-way analysis of variance or test of Kruskal-Wallis anova on Ranks, according to the test of normality of Kolmogorov-Smirnov for the values that had no Gaussian distribution. The correlations between the different glycemia analysis methods were obtained by Pearson test. In addition, the means were evaluated by Tukey Test when the values of p

were lower or equal to 0.05 and were considered significant (Sigma Stat Program).

Mean percent errors of each blood collection site were calculated from the difference between the mean given by the glucometer and by the colorimetric enzymatic assay for further comparison with the values considered acceptable by the standardized organizations of this measure.

The clinical accuracy of glycemia obtained by the glucometer, in relation to those obtained by the colorimetric enzymatic assay were evaluated by error grid analysis modified by Parkes (15). The obtained values were distributed in zones which represent the risk resulting of an incorrect measure: zone A represents errors without clinical effect (analytic accuracy); zone B represents values that have a deviation more than 20% of the reference values but with no negative repercussion on the treatment; zone C, values that may induce unnecessary treatment; zone D, danger of severe errors in treatment and zone E, errors that may induce the clinical conduction to dangerous consequences

Results

Venous and capillary glycemia from 7 male dogs (23.23%) and 23 female dogs (76.67%) were evaluated (from a group of 30 diabetic dogs), which showed no resistance to the punctures. Fourteen animals (46.67%) were mongrel dogs and the others were Poodle (4), English Cocker Spaniel (4), Labrador retriever (2), Brazilian Terrier (2), Rottweiler (1), Yorkshire Terrier (1), Teckel (1) and Weimaraner (1).

The glycemic means (n=30) showed no significant difference among the different blood collection sites and methods (P=0.90) (Table 1).

The mean percent error values between samples obtained from venous blood, pinna and carpal pad and measured using portable glucometer, when compared to the laboratorial colorimetric enzymatic assay ranged approximately, 9.80%, 4.35% and 11.20%, respectively. Although not significant, the measures of the glucometer were higher than those of the reference values in approximately 90%, 66.67% and 73.33% of the attempts, in venous, auricular and carpal pad measurements, respectively.

The error grid analysis showed that 93.33% (n=28) of the venous blood glycemic values obtained using the glucometer have remained in zone A and 6.67% (n=2) in zone B (Figure 2A), 80% of the auricular capillary blood have remained in zone A and 20% (n=6) in zone B (Figure 2B) whereas 80% of carpal pad glycemic values have remained in zone A, approximately 17% in zone B and 3% in zone C (Figure 2C).

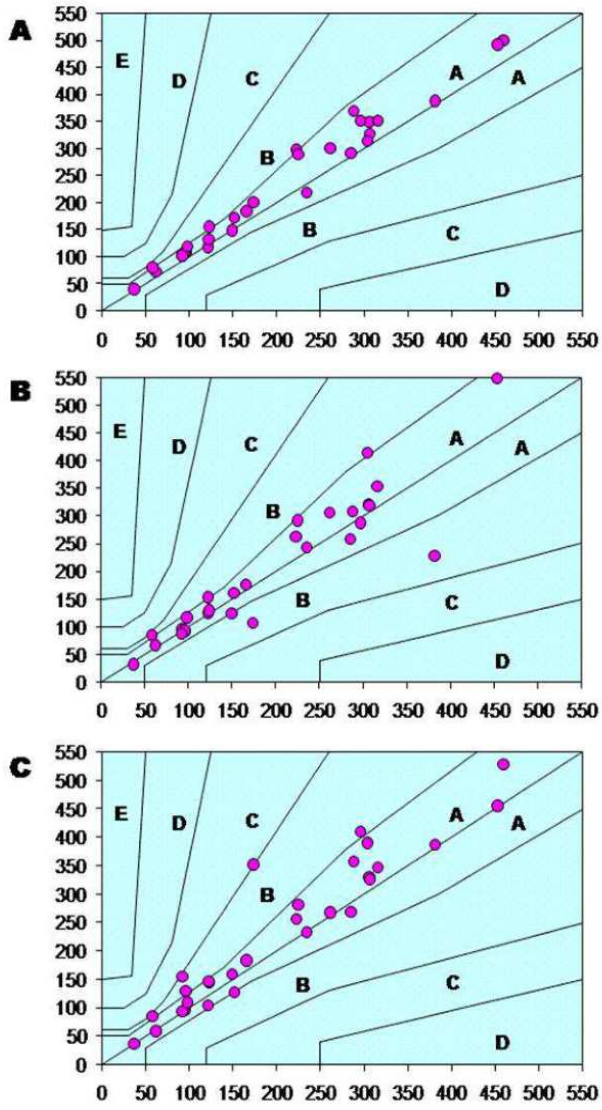


Figure 2. Graphic representations of glycemia obtained by colorimetric enzymatic assay and digital portable glucometer Accu-Chek® Go from diabetic dogs analyzed through error grid modified by Parkes (15). Venous blood glycemic values **(A)**, pinna capillary glycemic values **(B)** and carpal pad interface capillary glycemic values **(C)**.

Discussion

Literature reports have demonstrated that although glucometers are easy handling devices, accuracy may be compromised if the person performing the analysis is not properly trained. In order to avoid this interference, all the samples of this study were obtained by the same veterinarian, contributing for the homogeneity among the blood collections. In addition, this allowed the professional to compare individually the advantages and disadvantages of obtaining capillary blood samples from different sites.

Table 1. Descriptive statistics (mean ± standard error of mean, minimum and maximum values) of glycemia from diabetic dogs by laboratorial colorimetric enzymatic assay (venous) and using digital glucometer whose samples were venous and from pinna and carpal pads.

Samples of diabetic dogs group	Mean ± SE mean	Maximum Value	Minimum Value
Venous Glycemia – colorimetric enzymatic assay	220.40±25.17	618.70	37.28
Venous Glycemia– Glucometer	242.00±25.94	591.00	40.00
Pinna Glycemia – Glucometer	230.00±27.38	591.00	32.00
Carpal pad Glycemia– Glucometer	245.10±26.06	549.00	36.00

The carpal pad puncture was idealized similarly to the main site for obtaining blood in humans, which are the finger tips or heel (7, 8, 13). Besides, we found to validate this site as an option for blood collection, once some animals showed resistance to puncture in the pinna or, eventually, alterations or deformities in these sites, interfering with sample obtainment. Some authors (4) used the pinna puncture to measure capillary glycemia in dogs using the glucometer. But we could not find in the literature any report that had used canine carpal pad as a blood source to measure blood glucose.

Actually, there was no resistance to either pinna or carpal pad punctures, which shows that these sites may be alternative sites for measuring canine glycemia. In addition, the mentioned sites were especially suitable in small size and light color skin dogs, since the skin is softer and the vessels are more visible.

There was no significant difference between venous, auricular capillary and carpal pad glycemias using the different methods, demonstrating not only that those blood collection sites are viable but that the glucometer results had excellent correlation to those obtained by colorimetric enzymatic assay from dogs with diabetes mellitus. Correlation between glycemic results obtained by glucometer and colorimetric enzymatic assay in non-hyperglycemic dogs (10, 19) and in hyperglycemic humans (7) were previously reported. Also, the fact that the auricular capillary glycemia has shown a percent mean error lower than 5% when compared to colorimetric enzymatic assay, adequates the strict recommendations of the American Diabetes Association (ADA), and demonstrates the accuracy of the measurements performed with the glucometer in that sample. The mean percent error of 11.20% of glycemias obtained from carpal pad and 9.80% of venous blood, although is not adequate to the ADA regulations, adequates to the acceptable limit for other associations, as Food and Drug Administration (FDA) (2), International Organization of Standardization (ISO) (6, 14) and National Institutes of Health (NIH) (5). The differences among mean percent errors of the measurements performed with a glucometer have not shown significance and were lower than those found by other authors using similar procedures (10, 11).

Other than the statistical confirmation of the accuracy of the performed measurements using the glucometer, it was also used the grid error modified by Parkes (15) in order to determine the clinical accuracy of this device.

The clinical importance of an incorrect evaluation depends on how the therapy will be changed by the glucometer results, when compared with the decision that would be made based on the colorimetric enzymatic assay results (1) so, adequating the results here obtained to this evaluation manner would add more reliance in the therapy and in the glycemic control of diabetic patients, besides the facility in obtaining blood to have glycemia measured by this technique. It is important to consider that this type of analysis was developed to be applied in humans and, although it has already been used in veterinary medicine (1, 11), it is not validated as a unique method of glycemic evaluation (1). Nonetheless, the arguments for its usage in veterinary medicine are plausible and based on the fact that the tolerable glycemic interval for diabetic humans (90 – 180 mg/dL) recommended by Standards of medical care in diabetes (2005), is narrower than the tolerable interval considered for dogs with diabetes mellitus (100 - 300 mg/dL) (3).

In the error grid, 97% of diabetic canine blood samples were classified in zones A and B (n=29) and only 3% (n=1) of samples of carpal pad capillary blood were in zone C. Similarly in this work when testing two portable devices in diabetic canines, Bluwow et al. (2007) had 99% of glycemia values in zones clinically acceptable whereas Luppi et al. (2007) had 100% of values of venous and capillary glycemia of capuchin monkeys (Cebus apella) placed in zones A and B (9). Comparatively, these studies in diabetic humans showed 97% of samples in zone A and 3% in zone B (17).

Monitoring human diabetic patients associated with modern and more practical diagnostic methods resulted in an increase both in the number of the diagnosed cases and in patient's life expectancy in the last decades (14). Similarly to the human medicine, the search for alternative sites for drawing blood and determining glycemia in companion animals is important for making blood glucose monitoring easier for the owners, besides providing earlier diagnosis and more accurate treatment.

The present study revealed that carpal pad, venous and pinna glucose are clinically acceptable and this method can be of feasibility in hospitalized diabetic dogs. Although the glycemia measured from pinna blood using the digital glucometer has been the closest one to the

colorimetric enzymatic assay using venous blood, the carpal pad blood sample collection presented to be effective and is, thus, a viable alternative to the venous and pinna punctures. Furthermore, these authors recommend the periodical adoption of error grid to certify the accuracy of glycemia obtained using glucometers in order to maintain the clinical reliance of the results.

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