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Canine Glycosylated Hemoglobin Stability at Room and Refrigerator Temperatures over Ten Days

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Abstract

Background: Artifactual changes in blood may occur when the analysis time is delayed or an incorrect sample storage method is used. These changes may seriously interfere with biochemical blood test results. In dog studies, glycosylated hemoglobin A1c (HbA1c) is a widely used biomarker for diabetes mellitus (DM) screening. However, the conditions necessary to maintain canine HbA1c stability in dogs have not been well-established.

Methods: The objective of this study was to detect and compare canine sample HbA1c levels before and after canine blood was stored at refrigerator (4°C) and room (25°C) temperatures for 1, 3, 5, 7, 10 and 14 days.

Results: Compared with the baseline values, the HbA1c concentrations remained almost unchanged until 10 days. No significant differences were calculated between the HbA1c values at different time points under the refrigerator temperature condition (P>0.05).

Conclusion: These study indicates that different types of storage at either room or refrigerator temperature and delayed analyses for canine blood samples do not affect HbA1c results

Keywords: Canine diabetes mellitus; Glycosylated hemoglobin; HbA1c

Abbreviations: HbA1c: Glycosylated Hemoglobin A1c; DM: Diabetes Mellitus; ETDA: Ethylenediaminetetraacetic Acid

Introduction

Diabetes mellitus (DM) is a common endocrinopathy in dogs [1-5]. DM is characterized by chronic hyperglycemia and can be diagnosed and monitored using clinical signs (polyuria, polydipsia, polyphagia and weight loss), serum glucose levels, blood glucose curves, serum fructosamine levels and glycosylated hemoglobin concentrations [1].

Glycosylated hemoglobin is a hemoglobin product with a glucose attached to its N-terminal valine, which forms a beta-chain. This process is slow, non-enzymatic and irreversible [6,7]. Three types of glycosylated hemoglobin have been identified in the dogs, including hemoglobin (Hb) A1a, A1b and A1c. HbA1c is the most important glycosylated fraction of the hemoglobin molecule compared with A1a and A1b [8]. HbA1c concentrations in the peripheral blood current depend on the duration and extent of hyperglycemia, as well as the erythrocyte lifespan [9].

Moreover, in human diabetic patients, diagnostic procedures using HbA1c as an indicator for DM have some practical benefits compared with the fasting plasma glucose and oral glucose tolerance test [10], as indicated by the following: i) the subjects do not have to fast; ii) samples can be collected at any time of the day; iii) little biological variability is observed; iv) the results are not altered by acute factors, (e.g., stress and exercise); v) the results reflect the long-term blood glucose concentration; and vi) a single sample can be used (e.g., whole blood without centrifugation).

Unlike blood glucose, which is easily influenced by the time of day, exercise, eating, administration of insulin or stress, the HbA1c structure is stable [10] and dutifully reflects the glucose levels over the previous 10 to 14 weeks in dogs [11]. Due to its stability, HbA1c is the most important blood biomarker for human DM diagnosis [12]. In addition, only chronic alterations in glucose metabolism (chronic hypoglycemia or hyperglycemia) can induce significant changes in dog HbA1c concentrations [13,14]. Thus, HbA1c reflects long-term serum glucose control in dogs.

HbA1c is a biomarker that reflects long-term blood glucose control in dogs and is widely used in veterinary clinical practices [2,8,11,13-22]. Although the *in vivo* characteristics of HbA1c are well-known, information on HbA1c stability *in vitro* is lacking. In veterinary practices, a routine blood glucose concentration measurement is often performed immediately after sampling using a biochemistry analyzer. In certain cases, blood samples are referred to laboratories for HbA1c analyses. The time from sampling to analysis can be up to 2-3 days when samples collected at the end of the week are analyzed the following Monday. In such cases, it is important for the laboratory and the veterinarians to know whether changes may have occurred that could affect the validity of the results.

We hypothesized that canine HbA1c is stable for analyses after sample storage. Therefore, the purpose of this study was to detect and compare the HbA1c levels in individual canine EDTA-anticoagulated

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blood samples before and after storage at room and refrigerator temperatures for 1, 3, 5, 7, 10 and 14 days.

Materials and Methods

Sample collection and HbA1c concentration analyses

Blood samples (1 ml) in ethylenediaminetetraacetic acid (EDTA)-containing tubes (Becton Dickinson, Plymouth, UK) were collected through cephalic venipuncture from 72 dogs (42 non-diabetic and 30 diabetic dogs) presented to the veterinary hospital at the National Pingtung University of Science and Technology or National Taiwan University. The DM criteria were defined as classic clinical signs (polyuria, polydipsia, polyphagia and unexplained weight loss) and hyperglycemia (casual plasma glucose ≥ 200 mg/dl) [23]. The non-diabetic dogs were reported to be in good health by their owners and the results of a physical examination. The HbA1c levels were measured immediately using a point-of-care analyzer in accordance with the manufacturer's protocol (SIEMENS, DCA Vantage® Analyzer). This study protocol was approved by the Animal Care and Use Committee of the National Pingtung University of Science and Technology.

HbA1c stability analyses

Heparin-anticoagulated blood samples were also collected from the 15 non-diabetic dogs (randomly selected from the above 42 non-diabetic dogs). The HbA1c levels in the EDTA- and heparin-anticoagulated blood samples were measured immediately upon collection (day 0). To analyze the HbA1c levels from the different anti-coagulant samples, the HbA1c levels were measured for portions of the EDTA and heparin-anticoagulated samples on day 1 of storage at 25°C. After the first determination, the EDTA-anticoagulated blood samples were divided into 2 equal parts. One part was maintained on the bench in the laboratory, where the room temperature was 25°C, while the other part was stored at 4°C. To analyze HbA1c stability, the HbA1c levels were measured for portions of the 2 samples after 1, 3, 5, 7, 10 and 14 days of storage at either 4°C or 25°C.

Statistical analyses

The results from each parameter for each time-point and group were analyzed using the Student's *t*-test. The changes in HbA1c level upon storage at different temperatures were calculated using a linear regression analysis. The P values <0.05 and <0.01 were considered significant and highly significant, respectively.

Results

HbA1c concentrations compared in healthy and DM dogs

Figure 1 shows the blood HbA1c levels for each dog (42 non-diabetic and 30 diabetic dogs). The average blood HbA1c concentration in the non-diabetic dogs was 3.48 ± 0.28 %, ranging from 3 to 4.1 %, median 3.5 %. The average blood HbA1c value in the diabetic dog group was 5.41 ± 0.84 %, ranging from 3.7 to 7.5 %, median 5.1 %. Compared with the non-diabetic dogs, the diabetic dogs had significantly higher HbA1c levels in their blood (P<0.001) (Figure 1).

The time course for HbA1c concentrations in different anticoagulant samples

For the HbA1c levels in different anticoagulant samples, the HbA1c values in different anticoagulant samples stored at 25°C did not significantly change from 0 through 24 hours of storage (Figure 2). Our data indicate that HbA1c is stable in both EDTA- and heparin-anticoagulated samples.

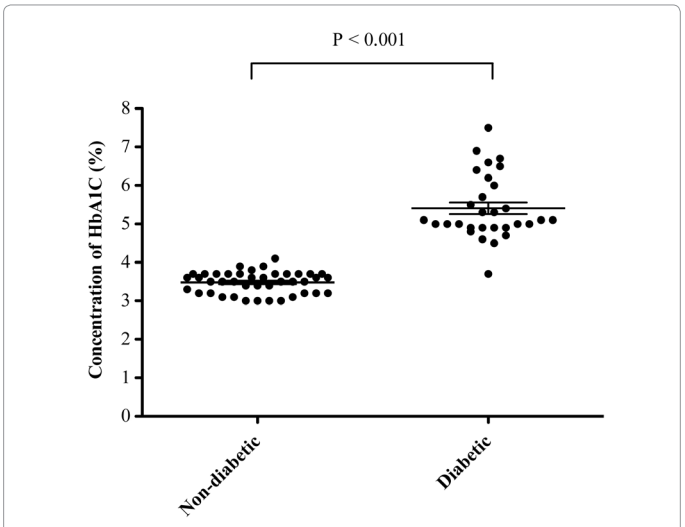

Figure 1: Dot plot of HbA1c levels in 42 non-diabetics and 30 diabetic dogs. The long horizontal lines represent the mean concentrations for each group. The error bars show the SD. An unpaired, 2-tailed Student's *t*-tests was used to compare the HbA1c levels between different healthy and DM groups.

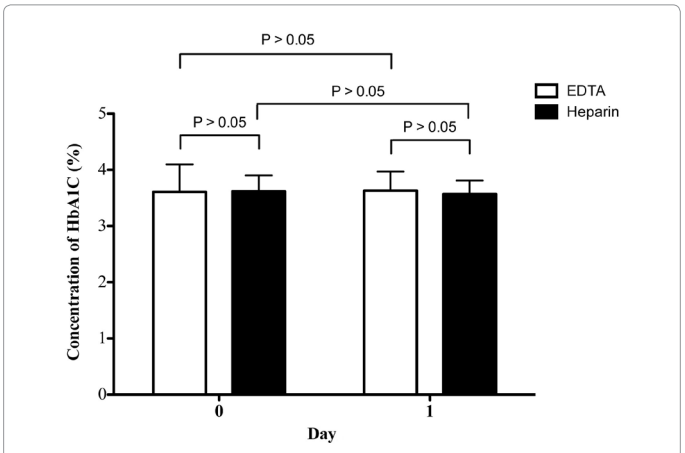

Figure 2: Changes in the HbA1c levels for the EDTA- (white bar) and heparin-anticoagulated (black bar) blood samples. The data are the mean and SD for 15 non-diabetic dogs. An unpaired, 2-tailed Student's *t*-tests was used to compare the HbA1c values between each anticoagulated blood sample and time point. The P values <0.05 and <0.01 were considered significant and highly significant, respectively.

The time course for HbA1c concentrations at different storage temperatures

The changes in HbA1c levels upon storage at different temperature were calculated using a linear regression analysis. The results with the HbA1c stored for different time periods under room temperature conditions exhibited a significant correlation (R^2=-0.628, P<0.05) (Figure 3). A significant difference was not calculated for the HbA1c values at different time points in refrigerator temperatures (Figure 3) (R^2=-0.444, P>0.05). Compared with the baseline values, the HbA1c concentrations remained almost unchanged over 10 days for the samples stored at both 4°C and 25°C. Taken together, our results indicate that the whole blood specimens stored for up to 10 days at room or refrigerator temperature are suitable for HbA1c measurements.

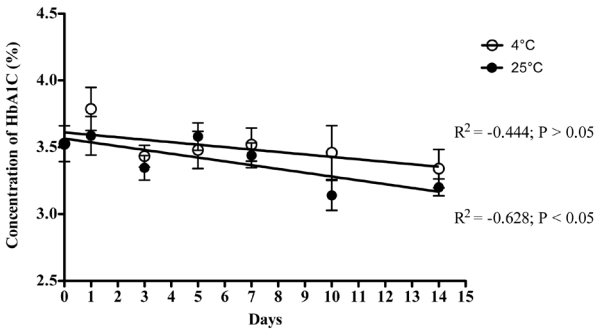

Figure 3: Changes in the HbA1c levels upon storage at refrigerator (open circles) or room (solid circles) temperatures. The data are the mean and SD for 15 non-diabetic dogs. A linear regression analysis was used to calculate the HbA1c values between time points. The P values <0.05 and <0.01 were considered significant and highly significant, respectively.

Discussion and Conclusion

HbA1c is a well-known biomarker for DM diagnosis in both humans and animals [1,10]. In human whole blood samples, HbA1c is stable for 1 week at 4°C [10]. However, few studies have examined HbA1c *in vitro* stability in animals. Our study shows *in vitro* stability for HbA1c from dogs. We found that at both room and refrigerator temperatures, the HbA1c concentration remained stable for up to ten days. In this study, HbA1c was stable in both EDTA- and heparin-anticoagulated samples. These data are useful for veterinary clinical practices and to facilitate an easier inspection process.

Several studies have demonstrated a strong correlation between HbA1c values and canine diabetes. Our results also reinforce previous suggestions that HbA1c levels are significantly greater in diabetic dogs [2,8,11,13-22]. Our results are consistent with previous studies, wherein HbA1c levels were stable in human blood stored at a refrigerator temperature for more than 7 days [10]. For the study herein, delayed analysis (for up to 10 days) of canine blood samples did not produce artifactual changes in the HbA1c levels.

HbA1c is detected through rapid immunomigration, which uses an antibody raised against the N-terminal portion of the human HbA1c beta chain [15]. DCA devices (SIEMENS, DCA Vantage® Analyzer) are the most commonly used point-of care HbA1c devices. Compared with the central laboratory HPLC method, DCA correlates well with the laboratory method and produces acceptable precision in human practice settings [23-25]. Therefore, the HbA1c test can be used as a point-of care when the blood samples are stored at room and refrigerator temperatures for ten days.

Canine blood samples can be stored at room or refrigerator temperature for 10 days and remain suitable for canine HbA1c detection. This finding is valuable for veterinary clinical practices.

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