

Glycosylated haemoglobin in dogs: study of critical difference value

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SUMMARY

Measurement of glycosylated proteins can be of use in diagnosis and monitoring of diabetic dogs. Its use in monitoring can be facilitated by comparison of results with a reference interval derived from levels in normal dogs. In this study, a commercial immunoturbidometric assay was used to measure glycosylated haemoglobin in 15 normal dogs over a 5-week period. Following statistical analysis of the results a critical difference value of 0.38 per cent was obtained. © 2001 Harcourt Publishers Ltd

DIABETES mellitus is a relatively common endocrine disease in dogs. It can be suspected from clinical signs of polyuria, polydipsia, polyphagia and weight loss and detection of persistent hyperglycaemia and glycosuria. Following stabilisation with daily insulin injections, diabetic dogs are often monitored by means of periodic blood glucose estimations and urine analysis. However, this practice may lead to error because it is likely to be unrepresentative; an individual glucose measurement reflects the glucose concentration at the time the sample is taken and significant fluctuations may occur during a 24-hour period.

In an attempt to overcome this situation, measurement of plasma levels of the glycosylated proteins fructosamine and glycosylated haemoglobin is recommended (Rijnberk 1996, Feldman and Nelson 1996). Glycosylated haemoglobin (HbA_{1c}) is formed by an irreversible and nonenzymatic reaction between glucose and haemoglobin in the red blood cells. The glycosylation rate is directly related to the plasma glucose concentration and to the lifespan of the circulating erythrocytes. In human medicine the assay of HbA_{1c} is commonly used to monitor diabetic patients. The presence of glycosylated haemoglobin in canine blood was first reported by Wood and Smith (1980) and since then several studies have demonstrated its usefulness in monitoring diabetic dogs (Smith et al 1982, Mahaffey and Cornelius 1982, Dennis 1989, Jensen 1995). Glycosylated haemoglobin can be considered as an index of the average plasma glucose concentration over the preceding 2 to 3 months (Mahaffey et al 1984, Willms and Lehmann 1990).

In consequence, periodic assay of HbA_{1c} can be used to monitor progress in diabetic dogs treated with insulin. Each result can be compared either with a reference interval in normal dogs or with previous results from the same patient. However, while the latter may facilitate

monitoring disease progression in an individual animal, it is possible that changes in HbA_{1c} could be the result of other circumstances, either physiological or related to concurrent disease, which are not directly related to diabetes mellitus. For this reason it would be helpful to know the critical difference (d_k) value (Stamm 1982, Costongs et al 1985). If two consecutive results differ by less than the critical difference value, it can be concluded that the difference is probably due to physiological variation. However, when the difference is greater than the critical difference value, other factors, either related to progression of the disease or the presence of concurrent disease, are more likely to be involved.

In this study results obtained from glycosylated haemoglobin assays in normal dogs, conducted over 5 consecutive weeks were used to calculate the critical difference value.

MATERIALS AND METHODS

Animals and sample collection

Fifteen clinically healthy adult Beagle dogs, male (6) and female (9), with an age range of 3 to 8 years, were supplied by the Animal Experimentation Service of the Veterinary Faculty for use in this study.

Each dog was sampled once per week for 5 consecutive weeks. Following a 12 hour fast, blood samples were obtained by jugular venipuncture from each dog between 08.00 and 09.00 hours 2.5 ml of blood was transferred to EDTA tubes and samples of whole blood were stored at -20°C prior to analysis. It had been demonstrated previously that glycosylated haemoglobin could be satisfactorily stored at -20°C for up to 77 days (Loste and Marca 1999).

Throughout the study all dogs were monitored and none showed signs of disease or unusual stress factors which could have interfered with the final results. At the end of the study the dogs were returned to the Animal Experimentation Service.

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Analytical procedure

Glycosylated haemoglobin was assayed using a commercial immunoturbidometric assay employing a monoclonal antibody against HbA_{1c} (Tinaquant[®] @ Hemoglobin A_{1c} II. Ref. 1488414. Boehringer, Mannheim). Glycosylated haemoglobin reacts with the anti-HbA_{1c} antibodies to give a soluble immunocomplex. The excess antibodies are then bound by polyhapten and the resulting agglutinated complex is measured turbidimetrically. The assays were performed in an automated analyser (Technicon RA-500). Previously this method had been evaluated and the usefulness of glycated haemoglobin measurement in canine samples demonstrated (Marca and Loste 2000). All samples were assayed together on the same working day to reduce between-day variability. Each sample was run in duplicate, with normal and pathological commercial controls (Boehringer, Mannheim) included in each run.

Statistical analysis

First the set of results was evaluated statistically for approximate normality of distribution. Then, the d_k value was calculated as described by Jensen and Aaes (1992) and Jensen et al (1992) from $d_k = 2\sqrt{2}\sigma$. The d_k value depends on the total variance (σ_t^2) which included the weekly variance in the same dog (σ_s^2) and the analytical (residual) variance (σ_e^2). The variances were statistically calculated from an analysis of variance (ANOVA). Thus, $d_k = 2\sqrt{2(\sigma_s^2 + \sigma_e^2)}$.

RESULTS

Means and absolute ranges for glycosylated haemoglobin concentrations are presented in Figure 1. First the normal distribution for the entire data set on all animals was subjected to statistical analysis, followed by an ANOVA. Weekly differences in the same dogs were not statistically significant although variation between dogs was noted with mean values ranging from 0.68 ± 0.13 per cent to 1.96 ± 0.18 per cent. The critical difference was calculated to be 0.38 per cent.

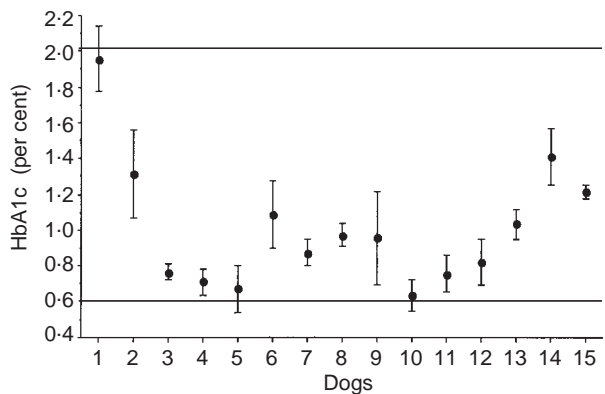


FIG 1: Mean and absolute range values for glycosylated haemoglobin concentration (per cent) in 15 clinically healthy dogs measured at weekly intervals over a 5-week period. The two horizontal lines in bold represent the normal reference range for canine glycosylated haemoglobin concentration.

DISCUSSION

A method for determining human glycosylated haemoglobin has been described using monoclonal antibodies against the last four amino-terminal groups of the haemoglobin chain (Karl et al 1993). Because of the structural similarity between canine and human haemoglobin (Brimhall et al 1977) the method can be applied to canine samples. The authors have used this procedure to assay HbA_{1c} levels in healthy and diabetic dogs and results obtained have been in accord with data previously reported by Jensen (1995), although in the present study within-run precision was notably lower (Marca and Loste 2000).

The glycosylated haemoglobin assay is an important aid in diagnosis and monitoring the long-term management of diabetic dogs treated with insulin. Periodic assay is recommended (Mahaffey et al 1984, Willms and Lehmann 1990) and consecutive analytical results compared using the critical difference value (Stamm 1982, Costongs et al 1985). This value can be an aid to ascertaining whether the difference between two consecutive results is due to physiological variation or to other factors.

Glycosylated haemoglobin results are presented in Figure 1. All 15 dogs had mean analytical results for HbA_{1c} within the reference interval previously established for the authors' laboratory. The variation interval for the 5 consecutive weeks was also within these limits, although in three dogs (1, 5 and 10) some values outside the reference interval were recorded. This was to be expected, because only 95 per cent of the values from the general population were included in determination of the reference interval.

The d_k value calculated in this study was 0.38 per cent. This figure can be used to help in deciding whether the difference between two consecutive analytical results can be ascribed to natural variation or not. Thus, if the difference between two consecutive HbA_{1c} results is below 0.38 per cent it is most likely due to physiological or natural variations. However, if it is above 0.38 per cent, other factors, such as concurrent illness or stress may be involved, or the dose of insulin may be inadequate, in which case a re-evaluation is recommended.

The d_k value for canine glycosylated haemoglobin has not been reported previously. Therefore comparisons cannot be made. However, in this study, all samples for glycosylated haemoglobin were assayed in the same run, using the same calibration, reagents and controls, in order to reduce the between-run variability. Had the samples been assayed over several working days, it is likely that a higher d_k value would have been obtained.

In conclusion, glycosylated haemoglobin assay has a place in supporting the diagnosis and therapeutic monitoring of canine diabetes mellitus. Although further investigations should be undertaken in normal and diabetic dogs to identify the physiological or pathological factors that affect this parameter, the d_k value is an important aid to correctly interpret results obtained during the management and ongoing monitoring of diabetic dogs.

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