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HEMATO-BIOCHEMICAL ALTERATIONS IN CANINE DIABETES MELLITUS WITH SPECIAL REFERENCE TO GLYCATED HEMOGLOBIN AS A DIAGNOSTIC TOOL

[a]Shruthi J.S., [a]Ramesh, P.T. and [b]Umesh, K.G.
[a]Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru-24.
[b]Scientific Communication Manager, Waltham Mars India Pvt Ltd.
*Corresponding author e-mail: shruthijsvet@gmail.com

ABSTRACT
Canine diabetes mellitus is a syndrome and, in the majority of cases, the underlying pathogenesis of disease is not established, although insulin deficiency/ resistance is a consistent feature. There are very few studies on diabetes in animals, inspite of upsurge of diabetes in humans in India. The present study was undertaken to study the hemato-biochemical alterations in canine diabetes mellitus with special reference to glycated hemoglobin as a diagnostic tool. Glycated hemoglobin was estimated with whole blood using commercially available kit based on the principle of ion exchange chromatography methodin both healthy and diabetic dogs. Hematological profile of the diabetic dogs revealed mild leukocytosis. The mean concentration of the glycated hemoglobin in healthy dogs was 6.78 ± 0.25%. The glycated hemoglobin concentration was found to be significantly elevated in the diabetic dogs (9.94 ± 0.42) % with values ranging from 8.6 to 14.2 %. Glucose and glycated hemoglobin values were significantly elevated in diabetic dogs and showed positive correlation on linear regression. Thus, HbA1c may be considered as one of the important screening test for diabetes mellitus in dogs provided each laboratory establishes its own standards and with similar analytical procedures.

KEYWORDS: Diabetes mellitus, Dogs, Glucose, Glycated, hemoglobin, linear regression analysis

INTRODUCTION

Diabetes mellitus is a chronic disorder associated with chronic complications including retinopathy, neuropathy, nephropathy and angiopathy. The syndrome diabetes mellitus (DM), commonly referred to as diabetes, has for a long time been known as a frequently occurring disease in humans as well as companion animals especially dogs. Diagnosis of DM has changed over the years (World Health Organisation, 2011). There has been a huge development since the time when diabetes was diagnosed through a sweet taste of the urine. Initially, the diagnosis of DM was mainly based on glycosuria (Banting *et al.*, 1922). Thereafter, diagnosis and treatment of DM have largely been based around measurement of blood glucose concentrations. The glycosylated hemoglobin (HbA_1) assay has gained special importance in the assessment of glycaemic control in diabetic patients (Bunn, 1981). It is widely accepted as an objective time averaged index of blood glucose control over the preceding six to eight weeks.

MATERIALS & METHODS

A total of 200 dogs presented to Veterinary College Hospital, Bengaluru, with a history of polyuria, polydipsia, obesity, rapidly developing bilateral cataracts, rapid weight loss or in combination thereof, were screened. Dogs showing random blood sugar above 140mg/dl, fasting blood sugar above 200mg/dl and glycosuria were included in the study and allocated to diabetic group (Rucinsky *et al.,* 2010).The animals brought for general health check-up which were found to be apparently healthy, formed the healthy group which consisted of 20 dogs.

Hematology

Hemoglobin, PCV, total erythrocyte count, and platelet count was determined using automated blood cell analyzer and the values were recorded in both diabetic and healthy dogs.

Biochemistry

Fasting Blood Sugar was estimated by Glucose-Oxidase-Peroxidase (GOD-POD) method (Kaplan, 1984) and expressed in mg/dl. Glycated hemoglobin was estimated with whole blood using commercially available kit based on the principle of ion exchange chromatography method of Trivelli *et al*. (1971). The concentration of HbA1c was expressed as percentage. Serum creatinine was estimated by Modified Jaffe's Kinetic Method. The results were expressed in mg/dl. ALT was estimated using the kinetic assay method proposed by the International Federation of Clinical Chemistry. The results were expressed in U/L. Cholesterol was estimated by modified Roeschlau's method and the results were expressed in mg/dl. Triglyceride was estimated based on the method of Wako and the modifications by Fossati *et al.* (1961) and McGowan *et al.* (1983).

Urinalysis

Urine sample was collected by catheterization of bladder and was analyzed using urinalysis reagent strips for the following parameters *viz*., glucose, ketone bodies, protein and leucocytes.

Statistical analysis

A linear regression analysis was performed to evaluate the association of glucose and glycated hemoglobin in healthy and diabetic dogs. Descriptive statistics for the different data were analysed as per Snedecor and Cochran procedures (1989).

RESULTS

Epidemiology revealed the incidence of diabetes mellitus to be 0.14% in dogs. Highest incidence was recorded in the age group of 6-9 years in female intact Labrador breeds. Also the highest incidence of diabetes was observed in the month of November and January suggesting a winter predisposition for the disease.

Hematological profile: There is no significant difference between healthy and diabetic group with respect to parameters like TEC, Platelet count, hemoglobin and packed cell volume. However there was a significant increase in the total leucocyte count in the diabetic group when compared to healthy group (Table 1, Figure 1).

TABLE 1: Hematological profile of diabetic and healthy dogs

Parameter	Healthy dogs	Diabetic dogs
TEC ($\times 10^6$/µl)	8.06 ± 0.46^a	7.13 ± 0.37^a
TLC ($\times 10^3$/ µl)	4.92 ± 0.55^a	10.73 ± 1.01^b
Platelet ($\times 10^5$/ µl)	3.09 ± 0.29^a	3.70 ± 0.39^a
Hemoglobin (g/dl)	12.52 ± 0.67^a	11.62 ± 0.44^a
PCV (%)	46.55 ± 2.95^a	39.89 ± 1.57^a

Note: Means bearing same superscript are not significantly different at (P <0.05) in the same row.

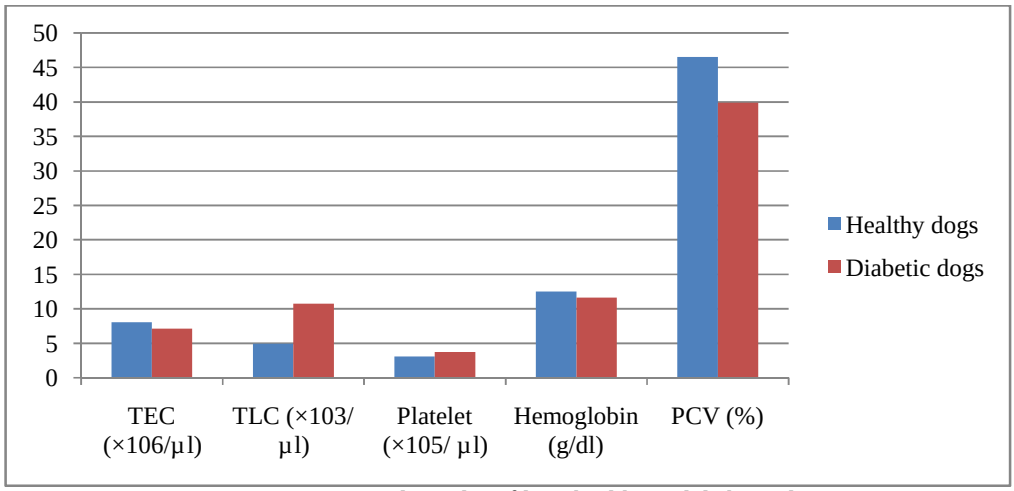

FIGURE 1. Hematological profile in healthy and diabetic dogs

Serum Biochemical profile:

There was a significant increase in the parameters like ALT, Cholesterol, Triglycerides, Fasting blood sugar and Glycated hemoglobin values in Diabetic group when compared to healthy group (Table 2). However the increase in the creatinine value in the diabetic group was not statistically significant.

Table 2: Biochemical profile of diabetic and healthy dogs

Parameter	Healthy dogs	Diabetic dogs
Creatinine (mg/dl)	0.99 ± 0.09^a	1.16 ± 0.08^a
ALT (U/L)	33.40 ± 3.32^a	148.43 ± 35.17^b
Cholesterol (mg/dl)	118.85 ± 5.60^a	230.8 ± 18.28^b
Triglycerides (mg/dl)	65.1 ± 5.08^a	85 ± 12.66^b
Fasting Blood Sugar (mg/dl)	91.1 ± 4.73^a	404.45 ± 40.01^b
Glycated haemoglobin (HbA1c %)	6.78 ± 0.25^a	9.94 ± 0.42^b

Note: Means bearing same superscript are not significantly different at (P<0.05) in the same row.

A linear regression analysis was performed to evaluate the association of glucose and glycated hemoglobin in healthy and diabetic dogs (Fig. 2, 3). This correlation was found to be positive in healthy (r= 0.62, p<0.005) and diabetic (r= 0.92, p<0.0001) dogs.

$$y = 0.03x + 3.72, \qquad r^2 = 0.39$$

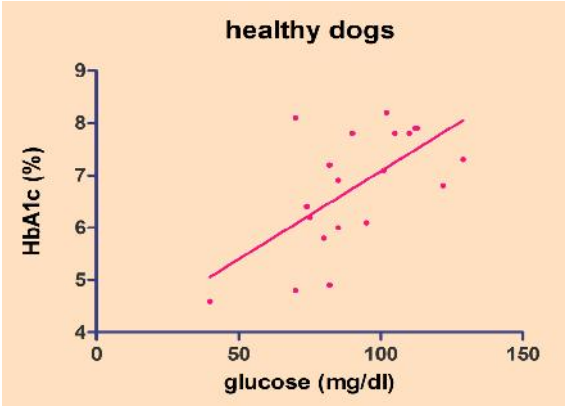

FIGURE 2: Linear regression of HbA1c and glucose concentrations in healthy dogs (r = 0.62, p<0.005)

y = 0.009x + 5.98, r^2 = 0.84

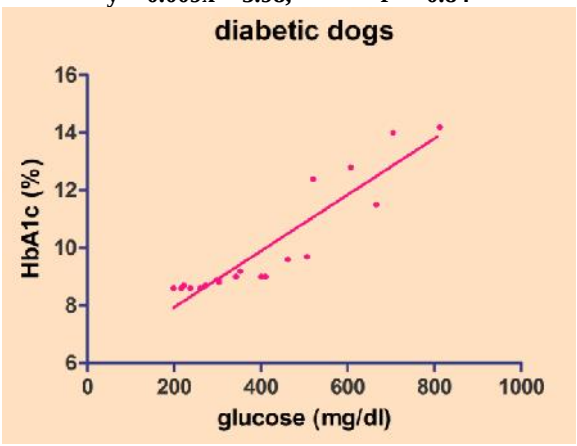

FIGURE 3: Linear regression of HbA1c and glucose concentrations in diabetic dogs (r = 0.92, p<0.0001).

DISCUSSION & CONCLUSION

The prevalence rate of diabetes mellitus in the present study was 0.14 % which is in agreement with the findings of Wilkinson (1960) and Krook *et al*. (1960) who have recorded similar prevalence rate.

Hematological profile

There was a significant increase in the TLC of diabetic dogs (Table 1 and Fig. 1) indicating mild leukocytosis which is in accordance with the findings of Feldman (1983) and Deepa *et al*. (2014). In the present study, two of the diabetic dogs had cystitis and one dog had chronic bilateral otitis externa which would explain the mild increase in total leucocyte counts in these dogs.

Serum Biochemical profile

There was a significant (P<0.05) elevation in the ALT values in the diabetic dogs compared with the healthy group. The above finding is in agreement with Sattar *et al*. (2007) and Doxey *et al*. (1985). Specific elevations in ALT and triglycerides suggest hepatic fat accumulation as a potential contributing factor for conversion to diabetes in humans. If ALT increases and stays high, then there is an association with diabetes risk (Sattar *et al*., 2007). The elevated level of ALT in this study might be due to hepatic cellular damage leading to leakage of this enzyme into the circulation. Cholesterol levels are indicative of the chronicity and severity of the disease with values ranging from 300 mg /dl in early cases to 900 mg /dl in more advanced diabetes (Wilkinson, 1960). The increase of cholesterol levels in diabetic dogs in the present study when compared to healthy dogs are also in agreement with the findings of Doxey *et al*. (1985), Hess *et al*. (2000) & Nelson and Reusch (2014).

The blood glucose concentration was significantly elevated in the diabetic dogs. The high mean FBS values obtained in the present study may be attributed to the fact that most of the dogs were presented in chronic untreated stage. The mean concentration of the glycated hemoglobin in healthy dogs was 6.78 ± 0.25%. This is in agreement with the findings of Prathaban *et al*. (1990) who has also reported the normal value of GHb in healthy dogs as 6.68 ± 0.37%. The glycated hemoglobin concentration was found to be significantly elevated in the diabetic dogs 9.94 ± 0.42% with values ranging from 8.6 to 14.2% which is in concurrence with findings of Deepa *et al.* (2014) who has recorded 9.98% mean HbA1c value in subclinical diabetic dogs. The significantly elevated levels of glycated hemoglobin values have also been recorded by other authors like Hasegawa *et al*. (1991), Haberer and Reusch (1998) and Marca *et al*. (2000).

The significant correlation observed in healthy and diabetic dogs between glucose and glycated hemoglobin in the present study are in agreement with Loste and Marca

(2001) who have reported positive correlation in hypoglycemic ($r = 0.91$, $p < 0.005$) or hyperglycemic dogs ($r = 0.74$, $p< 0.001$).

Present results showed that glycated hemoglobin assays are able to detect chronic changes of blood glucose concentrations in dogs and has a positive correlation with glycemia, mainly in hyperglycemic dogs. Thus, HbA1c may be considered as a screening test for diabetes mellitus in dogs provided each laboratory establishes its own standards and with similar analytical procedures. As the glycated hemoglobin analysis is easily available, cost effective, can be estimated with available human kits and is least affected by acute physiological alterations, it was found to be useful as one of the valuable biomarkers for diagnosis of diabetes in dogs. At the moment, glycated hemoglobin may be the best alternative to validated fructosamine measurement in dogs in India.

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