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CLINICAL, HEMATOLOGICAL AND BIOCHEMICAL ASPECTS ASSOCIATED WITH CANINE DIABETES MELLITUS

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ABSTRACT: In this study, the clinical, hematological and biochemical features associated with diabetes mellitus were recorded. This work was done on twenty adult dogs (7 – 13 years old) of both sexes selected from private clinic in Alexandria province and divided into two groups according to clinical and laboratory findings. The study revealed the following:

- Clinical signs of diabetic dogs were polyuria, polydipsia, polyphagia associated with progressive loss of body weight.
- Hematological changes were significant increases in the total leucocytic count, neutrophil %, monocyte %, eosinophil % and total thrombocyte count. Lymphocyte % was significantly decreased in diabetic dogs in comparison with healthy ones.
- Serum biochemical analysis revealed significant increases in the values of glucose, cholesterol, urea, alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP) and potassium. Meanwhile, the values of creatinine, bilirubin and sodium were significantly decreased in diabetic group in comparison with healthy one.
- Significant increase ($P<0.01$) in the value of serum fructosamine in diabetic dogs when compared with healthy ones.
- Urine analysis showed significant increases in specific gravity, glycosuria, acetonuria and bilirubinuria while the pH value was significantly decreased.

INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by an absolute or relative insulin deficiency and glucagon excess, which result in persistent and unregulated hyperglycemia (**Stogdale, 1986**). It occurs most frequently in adult or older dogs and cats (**Martin and Capin, 1975**). The most common cause of diabetes mellitus is pancreatitis. In acute pancreatitis, hyperglycemia results from the release of an excessive amount of glucagon from the damaged pancreas, while in chronic pancreatitis the pancreas may atrophy resulting in decreased numbers of islet cells to release insulin to meet a carbohydrate challenge (**Karen and Kenneth,**

1984). Unlike cat. a transient or reversible form of insulin dependent diabetes mellitus (IDDM) is uncommon in dogs **(Macintire, 1993)**. The most common cause of transient IDDM in dogs is the correction of a concurrent insulin-antagonistic disease (e.g. diestrus in the bitch). Resolution of the insulin antagonism may result in resolution of the carbohydrate intolerance if a considerable population of β cells remains functional. Serum fructosamine refers to glycosylated proteins (albumin and other plasma proteins) that have been linked to a glucose molecule by a non-enzymatic reaction. The serum fructosamine concentration is affected by the serum protein concentration and its composition **(Thomas and Müller, 1990)** and by the average blood glucose concentration during the lifetime of plasma proteins. In dogs the average life of albumin is 8.2 days **(Jain, 1986)** and the serum fructosamine concentration can be used to evaluate serum glucose concentration over a period of one to three weeks.

The purpose of this study was to determine the following points:

Clinical signs specific for canine diabetes mellitus.

Hematological and biochemical changes associated with canine diabetes mellitus.

The relationship between serum fructosamine and serum glucose concentrations in diabetic dogs.

MATERIALS AND METHODS

1. Animals:

A total number of twenty adult dogs (7 – 13 years old) of both sexes were selected from private clinic at Alexandria governorate, and divided into two groups. Group I included 10 diabetic dogs diagnosed according to the clinical and laboratory findings **(Feldman and Nelson, 1987)**. Group II consisted of 10 apparently clinically healthy dogs used as a control group.

2. Samples:

a. Blood samples:

Two blood samples were collected from each dog under investigation by puncture of a cephalic vein following a 12 – 15 hours fasting period and collected into vacuotainers containing clot activator and EDTA. The first sample was used to obtain clear serum for the biochemical analysis. The second sample was used for hematological examination.

b. Urine samples:

Urine samples were collected during natural urination or by using dog catheter following a 12 – 15 hrs fasting period, and examined by strip test (Boehringer, Mannheim-Germany).

3. Hematological and biochemical examination:

Determination of hemtological values including total leucocytic

count, lymphocyte %, neutrophil %, monocyte %, eosinophil %, erythrocytic count, hemoglobin content, packed cell volume and thrombocyte count, were made according to **Sastry (1985)**. The serum values of glucose, cholesterol, urea, creatinine, alanine transferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphates (ALP), sodium, potassium and bilirubin were measured colorimetrically by using commercially available test kits according to the methods described by **Trinder (1969), Watson (1961), Wybenga et al. (1971), Husdan and Rapopost (1968), Reitman and Frankel (1957), Szasz (1969), King and King (1954), Trinder (1951), Terri and Sesin (1958)** and **Sherlock (1951)**, respectively. The fructosamine was measured using a spectrophotometric assay previously validated for measurement of fructosamine concentration in canine serum according to the method described by **Jensen (1992)**.

4. Urine analysis:

Urine specific gravity was determined using urinometer according to **Delmar (1989)**. pH, glucose, acetone and bilirubin were examined by strip test supplied by (Boehringer, Mannheim-Germany).

5. Statistical analysis:

Statistical analysis of the results was performed using the t-test procedure in the Statistical Analysis System (SAS, 1987).

RESULTS

Clinical signs:

Diabetic dogs showing varying degree of signs including polyuria, polydipsia, polyphagia and weight loss (Fig. 1).

The blood picture values including total leucocytic count, differential leucocytic counts (lymphocyte, neutrophil, monocyte and eosinophil percentage), erythrocytic count, hemoglobin concentration, packed cell volume and thrombocyte count in diabetic and healthy dogs are given in Table (1).

The results of serum biochemical analysis including glucose, cholesterol, urea, creatinine, alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), sodium, potassium, bilirubin and fructosamine in diabetic and healthy dogs are presented in Table (2). While the urine analysis including specific gravity, pH, glucose, acetone and bilirubin in diabetic and healthy dogs are given in Table (3).

DISCUSSION

Most changes in diabetes mellitus are a consequence of insulin deficiency. In our study, clinical examination of diabetic dogs revealed that the main signs were polydipsia, polyphagia, lethargy, weakness and weight loss. Such clinical signs were in agreement with those obtained by **Philip (1994)** and **Wong et al.**

(1999). These clinical signs can be explained as follows, high urinary glucose concentration produce an osmotic diuresis and therefore polyuria, cerebral cellular dehydration due to hyperosmolality, secondary to hyperglycemia causes thirst (polydipsia). A prolonged osmotic diuresis may cause excessive urinary electrolyte loss and weight loss (**Philip, 1994, Miller, 1995** and **Briggs et al., 1998**).

Hematological studies as recorded in Table (1) revealed that diabetic dogs showed a highly significant ($P<0.01$) increase in the values of total leucocytic count, neutrophil, monocyte percentage, and significant ($P<0.05$) increase in the percentage of eosinophil, while lymphocyte percentage was highly significantly ($P<0.01$) decreased. These results (leucocytosis, lymphopenia, neutrophils and monocytosis) were attributed to stress which may be resulted from diabetic ketoacidosis (**Coles, 1986**), or due to the presence of infection.

Concerning the biochemical findings of diabetic dogs, significant increases in the values of serum glucose, cholesterol, urea, ALT, GGT, ALP, potassium, bilirubin and fructosamine were recorded. While, serum creatinine and sodium values were significantly decreased in diabetic dogs in comparison with their values in apparently clinically healthy dogs (Table 2). Hyperglycemia in diabetic dogs was in agreement with the results obtained by **Holeek et al. (1995), Miller (1995), Briggs et al. (1998) Struble et al. (1998)** and **Wong et al. (1999)**. This can be explained by the hypoinsulinemia resulted from loss of β cells which leads to impaired transport of circulating glucose into most cells and accelerated hepatic gluconeogenesis and glycogenolysis with subsequent development of hyperglycemia. Hypercholesterolemia in diabetic dogs was consistent with the findings of **Philip (1994)** and **Rebecka et al. (1998)**. This result could be explained by low activity of lipoprotein lipase which is necessary for the very low density lipoprotein (VLDL) catabolism and requires insulin for optimal activity and when insulin deficiency is very severe the rate of cholesterol synthesis is increased (**Philip, 1994**)

Serum urea concentration was high in diabetic dogs, and this result coincided with those obtained by **Philip (1994)** and **Abou El-Enean (1997)**. This significant increase is mainly attributed to renal complication or nephropathy resulted from increased glycation of structural proteins in the arterial wall supplying the glomerular basement membrane (**Philip, 1994; Richard and Quillermo, 1998**). Highly significant ($P<0.01$) increases in the activities of serum ALT, GGT and ALP in diabetic dogs were in agreement with the findings obtained by **Vajdovich et al. (1993)**. This result could be attributed to the hepatocellular damage caused by fatty liver infiltration (**Cornelius, 1970** and **Allen et al., 1997**). Hyperkalemia

recorded in our study may be secondary to the diabetic ketoacidosis, because of failure of glucose entry into cells in the absence of insulin and because of the low glomerular filtration rate **(Philip, 1994)**.

The highly significant ($P<0.01$) increase of bilirubin in diabetic dogs was attributed to the impaired hepatic excretion. **Philip (1994)** stated that conjugated bilirubinemia is one of the earliest manifestations of impaired hepatic excretion. Hyponatremia in diabetic dogs was in agreement with the result obtained by **Philip (1994)**. This hyponatremia could be attributed to the osmotic effect of the high extracellular glucose concentration which draws water from the cells and dilutes the sodium. A marked ($P<0.01$) decrease in serum creatinine level was observed in diabetic group. This result disagreed with the result obtained by **Abou El-Enean (1997)**, while, **Philip (1994)** concluded that, if renal dysfunction is caused by a reduction in glomerular filtration rate, plasma urea concentration tends to rise faster than that of creatinine and tends to be disproportionately higher with respect to the reference limits.

Serum fructosamine concentration in diabetic dogs was highly significantly ($P<0.01$) increased. This result was correlated to the results obtained by **Thorensen and Lorenzen (1997), Coppo and Coppo (1997)** and **Loste and Marco (1998)** who stated that fructosamine concentration reflects long-term glucose concentration and used to diagnose persistent hyperglycemia and monitor the treatment in diabetic patients. **Reusch et al. (1993)** concluded that measurement of the serum fructosamine concentration can be used to evaluate the average serum glucose concentration over a period of 2 to 3 weeks. Because serum fructosamine is not affected by acute changes in blood glucose concentration, but reflects the average blood glucose concentration during the previous 2 to 3 weeks. it can be used for long-term monitoring of glucose and to detect chronic hyperglycemia **(Loste and Marco, 1998)**. Also, our result was consistent with **Coppo and Coppo (1997)** who found that fructosamine concentrations were increased in diabetic dogs while in hyperglycemic, non-diabetic dogs fructosamine values were normal.

Regarding the urine analysis, in diabetic and healthy dogs (Table 3), diabetic dogs showed marked increase in specific gravity, in spite of polyuria due to increased glucose concentration (glycosuria). Decreased pH could be attributed to metabolic acidosis **(Rebecka et al., 1998)**. Glycosuria observed in our study was parallel to the result obtained by **Imamura et al. (1988)** which occurs only when the plasma and therefore glomerular filtrate concentration exceeds the tubular reabsorptive capacity. **Richard and Quillermo (1998)** proved that glycosuria usually does not develop in animals with stress hyperglycemia

because the transient increase in the blood glucose concentration prevents glucose from accumulating in urine to a detectable concentration. Acetonuria, recorded in diabetic dogs was consistent with the results obtained by **Imamura et al. (1988), Rebecka et al. (1998), Ruiz Moreno et al. (1997)** and **Richard and Quillermo (1998)**. This result can be explained by increased lipid and protein breakdown in the absence of insulin resulting in production of excessive amount of ketone bodies (ketonemia) and ketonuria. Bilirubinuria, in diabetic dogs, could be attributed to impaired hepatic excretion function resulting in conjugated bilirubinemia which is water soluble and therefore can be excreted in urine. Similar results were obtained by **Philip (1994)** and **Ruiz Moreno et al. (1997)**.

In conclusion:

1. It is important to document both hyperglycemia and glycosuria to establish a diagnosis of diabetes mellitus.

2. The plasma potassium concentration may fall rapidly once insulin is injected and therefore it should be monitored frequently, and potassium given as soon as the plasma concentration starts to fall.

3. Determination of the serum fructosamine concentration can be used to detect the persistent unregulated hyperglycemia in canine diabetes mellitus and it distinguishes favorably between dogs with diabetes mellitus and dogs with diseases that on a clinical basis resemble diabetes mellitus.

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Fig. (1).: Diabetic dog showing loss of weight

Table (1): Blood picture values (mean ± SE) of diabetic and clinically healthy dogs.

Groups	WBC (x $10^3/\mu l$)	Lymphocyte %	Neutrophil %	Monocyte %	Eosinophil %	RBCs (x $10^6/\mu l$)	HB	PCV	Thrombocyte (x $10^3/\mu l$)
Diabetic	10.60 ± 0.64**	17.12 ± 1.0**	73.36 ± 1.22**	6.12 ± 0.48**	2.88 ± 0.29*	6.73 ± 0.14	15.94 ± 0.36	45.80 ± 0.77	438.24 ± 39.81**
Control	5.58 ± 0.19	31.22 ± 0.40	62.88 ± 0.73	2.88 ± 0.30	2.22 ± 0.44	6.85 ± 0.14	15.66 ± 0.21	40.33 ± 3.21	251.44 ± 17.38

** Highly significant (P<0.05). * Significant (P<0.05).

Table (2): Serum biochemical values (mean ± SE) of diabetic and clinically healthy dogs.

Groups	Glucose mg/dl	Fructosamine µmol/L	Cholesterol mg/dl	Urea mg/dl	Creatinine mg/dl	Bilirubin mg/dl	ALT U/L	GGT U/L	ALP U/L	Na mmol/L	K mmol/L
Diabetic	433.20± 31.38**	560.36± 15.20**	378.56± 24.43**	40.24± 5.56*	0.546± 0.06**	0.177± 0.006**	42.72± 4.37**	38.07± 6.31**	1604.4± 457.62**	146.80± 0.87*	4.44± 0.09*
Control	101.44± 1.94	288.55± 10.38	215.66± 5.22	25.33± 0.89	0.846± 0.03	0.052± 0.010	19.66± 0.92	2.42± 0.12	77.88± 13.81	149.77± 0.49	4.04± 0.16

** Highly significant (P<0.05). * Significant (P<0.05).

Table (3): Urine analysis in diabetic and clinically healthy dogs.

Groups	Specific gravity	pH	Glucose	Acetone	Bilirubin
Diabetic	1037.91*	5.20	+++	++	++
Control	1033.33	5.61	-	-	-

* Significant (P<0.05).