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Alterations in clinico-biochemical and oxidative stress parameters in diabetic dogs

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Abstract

Diabetes mellitus is a metabolic disorder characterized by high levels of glucose in blood and changes in carbohydrate, lipid and protein metabolism which are caused by reduction in insulin secretion and or insulin inaction. The clinical features of 34 diabetic dogs were encountered loss of body weight, appetite, increased frequency of urination , water intake 73.5%, Increased hunger 61.8%, Sudden weight loss 41%, Obesity 59 %, weakness or fatigue 85.3%, Thinning or dull hair and presence of dermatitis 44% , Cloudy eyes and cataract 47% Ketonuria 35.3%, Vomiting and signs of dehydration 35.3%, Halitosis and dental problems 26.5%, Ascitis 11.8%, Chronic non-healing wounds 29.4% blindness during study period of 2012-15. The diabetic dogs showed hyperglycemia, hypercholesterolemia, hypertriglyceridemia in diabetic dogs in comparison to healthy dogs,. ALP, ALT and AST are markedly increased in diabetic dogs, as compared to health. The renal function of diabetic dogs were also compromised and reflected as increased in creatinine and blood urea nitrogen level as compared to healthy dogs respectively. This study also revealed a significant decreased significantly in the activity of the intra erythrocyte total antioxidant capacity like reduction in GSH (0.44±0.04 µmol MTT formazan/mg Hb), SOD (0.34±0.02 µmol/ml of packed RBC) and Catalase (3.93±0.30µmol H_2O_2 decomposed/min/mg Hb) in RBCs of diabetic dog than normal subject (0.44±0.04 µmol MTT formazan/mg Hb), (0.63±0.04/ml of packed RBC) and (7.23±0.32 H_2O_2 decomposed/min/mg Hb) indicating diabetic dogs were suffering from oxidative stress due to hyperglycemia. There was significantly increased in intraerythrocytic lipid peroxidation level (7.99±0.37 nmol MDA/mg Hb) as compared to healthy dogs (3.37±0.39 nmol MDA/mg Hb) establishes release of reactive oxygen species in the RBCs of diabetic dogs.

Key words : Diabetis mellitus, Hyperglycemia, Oxidative stress

Diabetes mellitus (DM) in dogs is a multifaceted common endocrinopathy and remains a humbling challenge for the clinician and the researcher. Diabetes is an emerging endocrinopathy in dogs and its incidence ranges from 1 in 50 to 1 in 400 (Dey, 2011) and in Odisha the prevalence rate of canine diabetes is 3.5 percent (Jena *et al*., 2016). DM is a metabolic disorder characterized by high levels of glucose in blood and changes in carbohydrate, lipid and protein metabolism which are caused by reduction in insulin secretion and/or insulin inaction (Ahmed and Glodstein, 2006). It is a commonly found that dogs with many concurrent complications like metabolic acidosis nephropathy, hepatic lipidosis and hepatic failure (Mauna, 1995). Dogs suffering from DM may also be associated with other diseases like hepatic necrosis and acute pancreatitis (Cook *et al*., 1993 and Hiblu *et al*., 2015). DM is associated with increased oxidative stress due to elevated glucose levels in the plasma. Glucose promotes glycosylation of both plasma and cellular proteins with increased risk for vascular events. Furthermore, hyperglycemia has been demonstrated to activate lipid peroxidation and induce the overproduction of reactive oxygen species (Fatani *et al*., 2015).

The objective of the following study is to reveal the changes in clinico-biochemical and oxidative stress parameters in dogs suffering from diabetes melitus

Materials and Methods

A total of 34 clinically presented diabetic dogs in teaching veterinary clinical complex (TVCC), Govt. hospitals and private pet clinics of Odisha from October 2013 to December 2015 were studied for hematobiochemical changes. The animals showing blood glucose value more than 140 mg/dl was subjected to further hemato-biochemical examination by collecting blood samples through different commercially available vials containing sodium fluoride (NaF), clot activator, EDTA vials for blood glucose, biochemical parameters and PCV estimation, respectively. Biochemical parameters were estimated

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by autoanalyser (Turbochem 100) using commercial reagent kits in the Department of Preventive Medicine, C.V.Sc & A.H., OUAT, Bhubaneswar.

About 2-3 ml blood samples were collected from diabetic dogs on 0 day, 15th day, 30th day and 45th day and analyzed for biochemical and oxidative stress parameters with autoanalyser (Turbochem 100) using commercial reagent kits as per regular monitoring of blood glucose control.

Assessment of oxidative stress in diabetic in dogs was done by assaying of erythrocyte oxidant – antioxidant status. The lipid peroxides level in the RBC hemolysate was determined by the method of Placer (1967). Reduced glutathione was estimated by DTNB method of Prins and Loos (1969). Catalase activity in hemolysate was estimated by using H_2O_2 as a substrate as per the method of Bergmayer (1983). Superoxide dismutase was estimated as per the method described by Madesh and Balasubramanian (1998).

Statistical analysis

Results are expressed as mean ± SE and comparison was done within the days between groups. The statistical significance of data between groups was analyzed by applying two-way ANOVA using Graph Pad Prism v4.03 software program (San Diego, CA, USA), the differences between the experimental groups were considered statistically significant at $P<0.05$.

Results and Discussion

The clinical features of diabetic dogs with respect to body weight, appetite, frequency of urination, water intake, dullness, cloudy eyes and cataract and blindness was recorded during the study period. Increase in water consumption, increased food intake in spite of gaining weight and sudden weight loss were common occurrence (Table 1). The classical clinical signs of polyuria, polydipsia (PU/PD), polyphagia, poor body condition and weight loss were also recorded previously (Rucinsky *et al.,* 2010, Behrend *et al.,* 2018).

A general sense of lethargy and pants after mild exposure to physical activity in uncomplicated cases, thinning or dull hair and presence of dermatitis, vomiting and signs of dehydration, halitosis and dental problems, ascitis and chronic non healing wounds were also reported by previous workers (Guptill *et al*., 2002; Hess *et al.,* 2003; Caryn and Kirk 2007, Herrtage, 2009; Huang, 2012).

Table 1: Percentage of different clinical signs and symptoms in diabetic dogs

Sl. No.	Clinical signs	Percentage of observation
1	Increase in water consumption	73.5 (25/34)
2	Polyurea	73.5 (25/34)
3	Increased hunger	62 (21/34)
4	Sudden weight loss	41 (14/34)
5	Obesity	59 (20/34)
6	Weakness or fatigue	29 (29/34)
7	Thinning or dull hair and presence of dermatitis	44 (15/34)
8	Cloudy eyes and cataract	47(16/34)
9	Ketonuria	35.3 (12/34)
10	Vomiting and signs of dehydration	35.3 (12/34)
11	Halitosis and dental problems	26.5 (9/34)
12	Ascities	11.8 (4/34)
13	Chronic non-healing wounds	29.4 (10/34)

It is found in the present study that there is hyperglycemia, hypercholesterolemia, hypertriglyceridemia in diabetic dogs in comparison to healthy dogs. Regardless of the underlying etiology, diabetic dogs are hyperglycemic and glycosuric. Increased fat mobilization leads to hepatic lipidosis, hepatomegaly, hypercholesterolemia, hypertriglyceridemia, and increased catabolism (Behrend *et al.,* 2018, Rucinsky *et al.,* 2010; Sridhar *et al*., 2005 and Huang, 2012). These increments may reflect mild liver cell damage that is related to decreased blood flow due to dehydration. Alterations in lipid metabolism because of diabetes may also contribute to increases in these liver enzymes. This altered fat metabolism may be noted by increase in serum cholesterol concentrations (Sridhar *et al*., 2005). Altered lipoprotein metabolism results in the hypertriglyceridaemia that occurs in DM (Iwasaki *et al*., 2007, Huang, 2012 and Jena *et al*., 2019). Metabolic disorder of lipids and hyperlipidemia in old aged dogs may lead to obesity and diabetes (Kawasumi *et al.,* 2014).

Alkaline phosphatase, Alanine transaminase and Aspartate aminotransferase are markedly increased in diabetic dogs, as compared to healthy dogs. These suggest hepatic involvement in DM. Diabetic dogs often show increased alkaline phosphaaise and alanine

Table 2: Level of different biochemical parameters in healthy and diabetic dogs

Biochemical parameters	Healthy	Diabetic
Blood Glucose (mg/dl)	98.44±5.40	347.31±10.43***
Hb1c %	4.47±0.18	9.91±0.16***
Fructosamine (µmol/L)	3.71±0.21	7.99±0.31***
Triglycerides(mg/dl)	148.69±8.58	363.31±12.32***
Cholesterol (mg/dl)	168.13±10.26	440.25±13.32***
ALP (IU/L)	144.56±9.36	514.88±21.36***
ALT (IU/L)	60.69±3.94	169.50±5.60***
AST (IU/L)	75.00±6.49	192.31±10.07***
Creatinine (mg/dl)	0.87±0.05	1.80±0.03***
BUN (mg/dl)	27.56±1.68	57.38±2.07***

Data are expressed as Mean±SE; n=16. Data bearing superscript*** differ significantly at p≤0.001.

aminotransferase (Jena *et al.,* 2019, Rucinsky *et al.,* 2010, Behrend *et al.,* 2018 and Huang, 2012). Diabetic dogs may also be associated with other diseases like hepatic necrosis and hepatic enlargement (Hiblu *et al*., 2015).

Serum creatinine and BUN are insignificantly increased in the diabetic dogs, as compared to healthy. Evidence for renal failure in diabetic dogs reveals azotemia, increased serum creatinine and BUN (Huang, 2012 and Jena *et al*., 2019).

PCV level was not significantly changed in any group of the diabetic dogs. Kothari *et al.* (2012) reported anemia and PCV in diabetic dogs and described it due to dehydration in diabetic dogs (Comazzi *et al.,* 2007).

DM tends to cause oxidative stress in both humans and dogs and increased oxidative stress may play a role in diabetic complications (EL-Seady and EL-Deeb, 2012). The results presented in this study revealed a significant decreased in the activity of the total antioxidant capacity in RBCs of diabetic dog than normal subjects. These reductions were resulted from decreased activities of antioxidant enzymes, Superoxide dismutase, catalase and decreased level of reduced glutathione. Most of the body cells have an enzyme system to eliminate active oxygen species, because some of these active species are toxic. SOD, catalase and GSH comprise a major defence system against oxygen toxicity (Jena *et al.,* 2014). ROS are increased by hyperglycemia which is due to diabetes (both type 1 and type 2) and, to a lesser extent, due

Table 3: Comparison of different oxidative parameters between healthy and diabetic dogs

Oxidative parameters	Healthy	Diabetic
LPO (nmol MDA/mg Hb)	3.37±0.39	7.99±0.37***
GSH (µmol MTT formazan/ mg Hb)	0.68±0.04	0.44±0.04***
SOD (µmol/ml of packed RBC)	0.63±0.04	0.34±0.02***
Catalase (**µ**mol H_2O_2 decomposed/min/mg Hb)	7.23±0.32	3.93±0.30***

Data are expressed as Mean±SE; n=16. Data bearing superscript *** differ significantly at p≤0.001.

to insulin resistance (King and Loeken, 2004). An imbalance resulting from the increased production and or reduced scavenging of these free radicals, leads to a metabolic state of oxidative stress, which consequently leads to tissue damage (Fatani *et al*., 2015).

The exact mechanisms underlying the disease are unknown; however, there is growing evidence that excess generation of reactive oxygen species (ROS), largely due to hyperglycemia, causes oxidative stress in a variety of tissues. Oxidative stress results from either an increase in free radical production, or a decrease in endogenous antioxidant defences or both. ROS are products of cellular metabolism and are well recognized for their role as deleterious species. In diabetic patients, oxidative stress is closely associated with chronic inflammation. Multiple signalling pathways contribute to the adverse effects of glucotoxicity on cellular functions. There are many endogenous factors (antioxidants, vitamins, enzymes, metal ion chelators) that can serve as endogenous modulators of the production and action of ROS (Rochette *et al*., (2014). The level of MDA (malondialdehyde) for lipid peroxidation is markedly increased in diabetic dogs in comparison to healthy. Level of GSH and activities of catalase and SOD is significantly less in diabetic dogs, as compared to healthy (Seghrouchni *et al*., 2002). The increased level of MDA in diabetic in this study reflected the increase in lipid peroxidation due to DM. EL-Seady and EL-Deeb, (2012) approved the increase in lipid peroxidation in diabetic dogs. The results suggest that in diabetic dogs, oxidative stress is related to the severity of diabetic mellitus and RBC-Catalase activity decreases whereas, RBC-TBARS concentration increases (Chansaisakorn *et al*., 2009).

GSH serves as an electron donor to certain

enzymes involved in the metabolism of ROS (Andreyev *et al*., 2005, Slonchak and Obolens'ka, 2009 and Jena *et al*., 2019). In conclusion, diabetic dogs had higher oxidative stress which was related to severity of disease and disturbs the cell volume regulation or oxidative stress affected membrane transport in diabetic dogs (Chansaisakorn *et al*., 2009).

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